Final addendum to the

Draft Assessment Report

- public version -

Risk assessment provided by the rapporteur Member State
The Netherlands
for the new active substance

FLUPYRADIFURONE

as referred to in Regulation (EC) No 1107/2009

February 2015

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European Commission

Flupyradifurone

VOLUME 1

Rapporteur Member State: The Netherlands

January 2014 November 2014 December 2014

Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of flupyradifurone in Regulation (EU) 1107/2009.

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LEVEL 1

Flupyradifurone

STATEMENT OF THE SUBJECT MATTER AND PURPOSE OF THE MONOGRAPH

1 Statement of subject matter and purpose for which the monograph was prepared

1.1 Purpose for which the monograph was prepared

The monograph for the active substance flupyradifurone and the formulated product Sivanto (plus suffix) was prepared for evaluation under Regulation (EC) No 1107/2009 to support the approval of the new active substance.

1.2 Summary and assessment of information relating to the collective provision of dossiers

Bayer CropScience AG is the sole notifier for flupyradifurone. The question of steps taken to collectively present the dossier does therefore not arise.

1.3 Identity of the active substance (Annex IIA 1)

1.3.1 Name and address of applicant(s) for inclusion of the active substance in Annex I (Annex IIA 1.1)

Applicant:
Bayer CropScience AG
Alfred-Nobel-Strasse 50
40789 Monheim
Germany

Contact person :
[redacted]
BCS-D-Global Regulatory Affairs
Tel. ++49-2173-[redacted] (office)
Fax ++49 2173-383580
e-mail: [redacted]@bayer.com

1.3.2 Common name and synonyms (Annex IIA 1.3)

Flupyradifurone (ISO), no synonyms

1.3.3 Chemical name (Annex IIA 1.4)

IUPAC: 4-[(6-chloro-3-pyridylmethyl)(2,2-difluoroethyl)amino]furan- 2(5H)-one

CA name: 2(5H)-furanone, 4-[[(6-chloro-3-pyridinyl)methyl](2,2- difluoroethyl)amino]-

1.3.4 Manufacturer's development code number (Annex IIA 1.5)

BYI 02960 (applicant's code number)

1.3.5 CAS, EEC and CIPAC numbers (Annex IIA 1.6)

CAS number:	951659-40-8
EU index number:	not allocated
EC (EINECS/ELINCS):	not allocated
CIPAC number:	not allocated

1.3.6 Molecular and structural formulae, molecular mass (Annex IIA 1.7)

Molecular formula: C12 H10Cl F2 N2 O2
Structural formula:

Molecular mass: 288.68 g/mol

1.3.7 Manufacturer or manufacturers of the active substance (Annex IIA 1.2)

Please refer to the confidential annex C (volume 4).

1.3.8 Method or methods of manufacture (Annex IIA 1.8)

Please refer to the confidential annex C (volume 4).

1.3.9 Specification of purity of the active substance (Annex IIA 1.9)

Minimum purity: 960 g/kg

1.3.10 Identity of isomers, impurities and additives (Annex IIA 1.10)

Please refer to the confidential annex C (volume 4).

1.3.11 Analytical profile of batches (Annex IIA 1.11)

Please refer to the confidential annex C (volume 4).

1.4 Identity of the plant protection product(s) (Annex IIA 3.1; IIIA 1)

1.4.1 Current, former and proposed trade names and development code numbers (Annex IIIA 1.3)

Trade name or proposed trade name:

Sivanto plus suffix

Manufacturers code number(s):
Flupyradifurone SL 200 (200 g/L)
BYI 2960 SL 200 G (chemical internal code)
79718845 (material No.)
0113814-001 (internal code)

102000021884-03 (specification and version No.)

1.4.2 Manufacturer or manufacturers of the plant protection product (Annex IIIA 1.2)

Please refer to the confidential annex C (volume 4).

1.4.3 Type of the preparation and code (Annex IIIA 1.5)

Soluble concentrate; code: SL

1.4.4 Function (Annex IIA 3.1; Annex IIIA 1.6)

Insecticide

1.4.5 Composition of the preparation (III 1.4)

Please refer to the confidential annex C (volume 4).

1.5 Uses of the plant protection product(s) (Annex IIA 3.2 to 3.4; Annex IIIA 3.1 to 3.7, 3.9 and 12.1)

1.5.1 Field of use (Annex IIA 3.3; Annex IIIA 3.1)

Intended for agricultural use as an insecticide.

1.5.2 Effects on harmful organisms (Annex IIA 3.2; Annex IIIA 3.2)

Flupyradifurone interacts with insect nicotinic acetylcholine receptors, a class of neurotransmitter-gated cation channels which are involved in excitatory neurotransmission, a target also known for neonicotinoid insecticides. Blockage of these receptors leads to paralysis and death of the target insects.

1.5.3 Summary of intended uses applied for (Annex IIA 3.4; Annex IIIA 3.3 to 3.7, 3.9)

Crop and/ or situation (a)	Country	Product Name	F G or I (b)	Pests or Group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)	Remarks: (m)
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	kg as/hL min max	water L/ha min max	kg as/ha min max		
Hops	N-EU (residue zone)	Sivanto	F	Aphids, *Phorodon humuli,*	SL	200	spray	BBCH 31-75 May July.	1	n.a.	0,0045-0,0075	2000-3300	0.150	21	~~Biannual~~ biennial application proposed
Lettuce	N/S-EU (residue zone)	Sivanto	F	Aphids, *Nasonovia ribisnigri*	SL	200	spray	BBCH 12-49 May--Oct	1	n.a.	0,0125-0,0250	500-1000	0.125	10	Max 1 application per **24** months
Lettuce	N/S-EU (residue zone)	Sivanto	F	Aphids, *Nasonovia ribisnigr*	SL	200	spray	BBCH 41-49 May--Oct	1	n.a.	0,0125-0,0250	500-1000	0.125	3	Max 1 application per **12** months
Lettuce (soil bound)	N-EU S-EU (residue zone)	Sivanto	G	Aphids, *Nasonovia ribisnigri,*	SL	200	spray	BBCH 12-49	2	10	0,0125-0,0250	500-1000	0.125	3	Max 2 applications per 12 months

Remarks:
a) in case of group of crops the Codex classification should be used
b) Outdoor of field use (F), or glasshouse application (G)
c) e.g. biting and sucking in insects, soil born insects, foliar fungi
d) e.g. wettable powder (WP), emulsifiable concentration (EC), granule (GR)
e) Use CIPAC/FAO Codes where appropriate
f) All abbreviations used must be explained
g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench
h) Kind, e.g. overall, broadcast aerial spraying, row, individual plant, between the plants
i) g/l or g/L
j) Growth stage at last treatment
k) The minimum and maximum number of application possible under practical conditions of use must be provided
l) PHI - Pre Harvest Interval
m) Remarks may include: Extent of use/ economic importance/restrictions

1.5.4 Information on authorisation in EU Member States (IIIA 12.1)

Flupyradifurone is a new active substance. No authorizations currently exist in any of the EU Member States.

LEVEL 2

Flupyradifurone

OVERALL CONCLUSIONS

2 Reasoned statement of the overall conclusions drawn by the Rapporteur Member State

2.1 Identity, physical and chemical properties, details of uses, further information, and proposed classification and labelling

2.1.1 Identity

Flupyradifurone has a minimum purity of 960 g/kg.

2.1.2 Physical and chemical properties

Flupyradifurone

Flupyradifurone is an insecticide. Its vapour pressure and volatility are very low. The solubility in water is about 3.2 g/L at a range of pH between 4 and 9. With a log Pow of 1.2 it has no bioaccumulative potential in lipophilic matrices. Flupyradifurone is hydrolytically stable under acidic, neutral and under alkaline conditions at ambient temperature. The hydrolytic degradation is negligible under acidic, neutral and alkaline conditions. Flupyradifurone is rather susceptible for reactions with hydroxyl radicals with photochemical degradation process to contribute significantly to the overall degradation of the substance in case of its occurrence in the atmosphere.
Its flammability, explosive and oxidizing properties are not critical.

Representative product

Flupyradifurone (BYI 2960) SL 200(200 g/L) is not explosive and has no oxidizing properties. Its pH is within the range that naturally occurs *e.g.* in soil. Its stability allows storage under practical and commercial conditions. Its technical properties indicate that no particular problems have to be expected, when it is used as recommended. The shelf life is 2 years in HDPE and shows no degradation.

2.1.3 Details of uses and further information

The representative formulation of Flupyradifurone is Sivanto SL 200 g/L, (orflupyradifuone SL200), it is a soluble concentrate (SL) containing 200 grams per litre (g/L) of Flupyradifurone. Respresentative uses are control of lettuce aphid (*Nasonovia ribisnigri*) in lettuce and hop aphid (*Phorodon humili*) in hop.

Flupyradifurone interacts with insect nicotinic acetylcholine receptors, a class of neurotransmitter-gated cation channels which are involved in excitatory neurotransmission, a target also known for neonicotinoid insecticides. Blockage of these receptors leads to paralysis and death of the target insects.

After uptake into the plant, Flupyradifurone is translocated acropetally in the xylem, in direction of the transpiration stream and is translaminarily distributed into adjacent plant cells.

2.1.4 Classification and labelling

Classification and labelling for the active substance

Physical and chemical properties

No classification is required.

Human health effects

Flupyradifurone (BYI 02960) needs to be classified as Acute oral toxic Cat. 4 H302 according to Regulation (EC) 1272/2008. Flupyradifurone does not need to be classified on the basis of its acute dermal, and inhalation toxicology in rats (Category 5/ Unclassified). Flupyradifurone is considered not irritating to skin and eyes (Category 5/ Unclassified). Flupyradifurone does not have sensitising properties in a LLNA (Category 5/ Unclassified).

Ecotoxicological properties

Category:	Acute 1 and Chronic 1
GHS picogram:	GHS09
Signal word:	Warning
Hazard statement:	H400 “Very toxic to aquatic life”
	H410 “Very toxic to aquatic life with long lasting effects “
Precautionary statements:	(the following may be considered):
	P273 “ Avoid release to the environment”,
	P391 “ Collect spillage”
	P 501 “ Dispose of contents/container to … [in accordance with national/international regulations (to be specified)].

Explanation:

The standard species fish, daphnids and algae are not sensitive to flupyradifurone (L/EC50 >70 mg/L). However, aquatic insects are much more sensitive. The classification is based on the lowest acute L/EC_{50} of flupyradifurone, which is <1 mg/L (*C.riparius* EC50 = 0.0617 mg/L). Therefore, flupyradifurone is classified in Category Acute 1.

Flupyradifurone is considered not ready biodegradable (see LoE fate and behaviour). There are adequate chronic toxicity data available. The NOECs for fish and daphnids are >1 mg/L, but as said above, daphnids are not sensitive to flupyradifurone and it is therefore necessary to take into account

other, sensitive, species. The lowest NOEC is <0.1 mg/L (0.010 mg/L for *C. riparius*). Therefore, flupyradifurone is classified in Category Chronic 1.

The M-factor for flupyradifurone is 10 based on both acute and chronic toxicity.

The precautionary statements may be chosen according to national regulations.

Classification and labelling for the plant protection product

Physical and chemical properties

No classification is required.

Human health effects

The formulation BYI 02960 SL 200 g/L does not need to be classified on the basis of its acute oral, dermal toxicology. According to Regulation (EC) 1272/2008 formulation BYI 02960 SL 200 g/L is classified with H332, Harmful if inhaled (R20, Harmful by inhalation according to Directive 67/548/EEC). BYI 02960 SL 200 g/L is considered not irritating to skin and eyes.
BYI 02960 SL 200 g/L has sensitising properties in a LLNA and is classified with H317 (may cause an allergic skin reaction)/R43 (may cause sensitization by skin contact).

Ecotoxicological properties

Category:	Acute 1 and Chronic 1
GHS picogram:	GHS09
Signal word:	Warning
Hazard statement:	H400 “Very toxic to aquatic life”
	H410 “Very toxic to aquatic life with long lasting effects “
Precautionary statements:	(the following may be considered):
	P273 “ Avoid release to the environment”,
	P391 “ Collect spillage”
	P 501 “ Dispose of contents/container to … [in accordance with national/international regulations (to be specified)].

Explanation:

Toxicity studies with the preparation on the standard species fish, daphnids and algae are available and it is clear that these species are not sensitive to Sivanto (BYI 02960 SL 200) (L/EC50 >100 mg formulation/L). However, aquatic insects are much more sensitive to the a.s.. There is only a chronic study available on aquatic insects for the preparation but not an acute one. Therefore, it would underestimate the acute toxicity to base the classification of the preparation on the available studies.

Instead, the acute classification is based on the summation method. Flupyradifurone is classified as H400 based on its lowest acute L/EC_{50}, which is 0.0617 mg/L, and has an M-factor of 10. The concentration of flupyradifure in the preparation (ca.20%) multiplied by the M-factor (10) is greater than 25%. Therefore, the preparation is also classified in Category Acute 1 (H400). Since this is the worst-case acute classification, the other components of the product do not need to be discussed.
The lowest chronic NOEC for the preparation is ca. 0.06 mg preparation/L (for *C. riparius)*. Therefore, the preparation is classified in Category Chronic 1 (with H410).
The precautionary statements may be chosen according to national regulations.

2.2 Methods of analysis

Analytical methods for analysis of the active substance as manufactured
The HPLC-method AM008809MP1 for the determination of Flupyradifurone (BYI 02960) in technical grade active substance can be used for determination of Flupyradifurone.

Analytical methods for formulation analysis
The HPLC method AM012609MF1for the determination of Flupyradifurone (BYI 2960) can be used for determination of flypyradifurone in the formulation Flupyradifurone (BYI 2960) SL 200.

Analytical methods for residue analysis
The submitted methods are suitbale of monitoring in the relevant matrices.

Residues in body fluids and tissues
A method of analysis for body fluids and tissues is not required, since the active substance is of low acute toxicity (GHS category 4).

2.3 Impact on human and animal health

2.3.1 Effects having relevance to human and animal health arising from exposure to the active substance or to impurities contained in the active substance or to their transformation products

2.3.1.1 Toxicokinetics

Absorption, distribution, excretion and metabolism of flupyradifurone was investigated using three different labelling positions. The active substance was labelled with ^{14}C in the pyridinylmethylene bridge, in the 4-position of the furanone ring and in the 1-position of the ethyl side chain:

Pyridinylmethyl-^{14}C flupyradifurone | Furanone-4-^{14}C flupyradifurone | Ethyl-1-^{14}C flupyradifurone

The pyridinylmethyl-labelled compound was used in an ADME-study in which male and female rats were orally administered with a single low dose of 2 mg/kg bw or a high dose of 200 mg/kg bw. Due to the high water solubility of flupyradifurone, male rats were also given an intravenous dose of 2 mg/kg bw. In this study, the excretion via urine and faeces was investigated as well as the distribution in the plasma and the radioactivity concentration in organs and tissues at sacrifice. The metabolism was investigated in urine and faeces.

A quantitative whole body autoradiography study was conducted also using the pyridinylmethyl-labelled compound following a single oral dose of 5 mg/kg bw to male and female rats. In this study the excretion of radioactivity was determined in urine, faeces and expired air, as well as the distribution in the plasma and the radioactivity concentration in the organs and tissues at various time points.

The furanone-4-labelled compound was used in an ADME-study in which male and female rats were orally administered with a single dose of 2 mg/kg bw. In this study, the excretion via urine and faeces was investigated as well as the distribution in the plasma and the radioactivity concentration in the organs and tissues at sacrifice. The metabolism was investigated in urine and faeces.

A quantitative whole body autoradiography study was also conducted using the furanone-4-labelled compound following a single oral dose of 5 mg/kg bw to male and female rats. In this study the excretion of radioactivity was determined in urine, faeces and the expired air as well as the distribution in the plasma and the radioactivity concentration in the organs and tissues at various time points.

In an organ metabolism study, male and female rats were orally administered with a single dose of 3 mg/kg bw [furanone-4-^{14}C] flupyradifurone. Animals were sacrificed 6 hours after dosage and the metabolism was investigated in urine, plasma, and in extracts of liver, kidney, muscle and fat tissues.

The ethyl-1-labelled compound was used in an ADME-study in which male rats were orally administered with 2 mg/kg bw. In this study, the excretion via urine, faeces and expired air was investigated as well as the distribution in the plasma and the radioactivity concentration in the organs and tissues at sacrifice. The metabolism was investigated in urine and faeces.

In an organ metabolism study, male and female rats were orally administered with a single dose of 3 mg/kg bw [ethyl-1-^{14}C] flupyradifurone. The animals were sacrificed at 1, 6 or 24 hours after dosing. The total radioactivity was determined over the different time periods in urine, while in plasma, liver, kidney, muscle and fat tissue at different time points after sacrifice. The metabolism was investigated in urine, plasma, and in extracts of liver, kidney, muscle, and fat tissue.

Absorption

Following oral administration of a single low dose of flupyradifurone to male and female rats, the gastrointestinal absorption of radioactivity was high. It accounted for >80 % of the dose independent of the labelling position used. The value of >80% can be used for further toxicological risk assessment.

Distribution

The maximum plasma concentration was reached in most cases within 1 or 2 hours after administration of low doses. Only after administration of the high dose of 100 mg/kg bw the peak plasma concentration was observed between 2 and 4 hours after dosage. After reaching the peak concentration, the radioactivity levels in plasma declined steadily by several orders of magnitude in all studies independent of sex or labelling position of the test compound.

Quantitative whole body autoradiography revealed a fast absorption and distribution of the test compound with peak values observed already 1 hour after administration. At this time, the concentrations in liver and kidney were significantly higher than in blood, suggesting a preferred clearance from blood and distribution mainly to these organs which are mainly responsible for metabolism (liver) and excretion (kidney). Higher concentrations than in blood were also detected in the myocardium (heart) and in several glands. Very low levels were found in the brain, spinal cord and renal fat tissue. These results were similar in male and female rats, and independent of the labelling position of the test compound.

Excretion

Excretion was very fast, mainly renal and almost completed after 24 hours. No radioactivity was detected in the expired air after dosing of the pyridinylmethyl- and ethyl-1-labelled compounds, proving

the stability of these labelling positions in the molecule. Only after administration of [furanone-4-^{14}C] flupyradifurone between 1% and 3% of the administered radioactivity was exhaled. This demonstrated that for a small portion of the dose (higher in male than in female rats) the furanone ring of the molecule obviously was opened and was subject of biotransformation to C-1 fragments.
A fast decline of radioactivity concentrations was observed for all organs and tissues in male and female rats during the entire test period. Concentrations fell for most organs and tissues below 5% of the maximum concentration after one day. After seven days, only very low concentrations were found in a few organs and tissues of rats dosed with the pyridinylmethyl-labelled test compound. In the study using the furanone-4-labelled compound, low radioactive residues were measured in almost all organs and tissues due to the incorporation of C1- or C2-fragments into the endogenous carbon pool. The residues in male rats were higher by a factor of 1.4 to 4.7 as compared to female rats. A similar ratio of approximately 3 (male/female) was also found for the formation of $^{14}CO_2$, and originates presumably from sex related differences in metabolism leading to more C1- and C2-fragments and also higher incorporation of these components into the endogenous carbon pool in male rats. In general males and female rats exhibited very similar absorption, distribution and excretion behaviour. The results of these studies demonstrate that there is no indication of any accumulation or significant retention of radioactivity in male and female rats. This observation is supported by the low P_{OW} of 1.2.
Concentrations of radioactivity detected in tissues and organs at sacrifice were either very low or below the limit of detection

Metabolism

Flupyradifurone was intensively metabolised in the rat. Numerous metabolites were formed, most of them being minor ones. The parent compound represented the predominant part of the radioactivity in urine of male and female rats. In faeces of male rats, the metabolite BYI 02960-OH was more prominent than the parent compound. Two metabolites, BYI 02960-6-CNA and BYI 02960-hippuric acid were also prominent in male but not in females rats. The organ metabolism study using the ethyl-1-^{14}C label showed that in the 24 hours samples of plasma, and organs and tissues BYI 02960-DFA was by far the dominating metabolite accounting for more than 50% of the radioactivity. In general, the metabolic profiles in urine and faeces were very similar for both sexes but male rats showed a higher rate of metabolite formation as compared to female rats. 6-CNA is considered a major metabolite in male rat but not in females.

The principal metabolic reactions of flupyradifurone in rats were:

- Hydroxylation followed by conjugation with glucuronic acid or sulfate,
- cleavage of the difluoroethyl group forming BYI 02960-des-difluoroethyl, and difluoroacetic acid (BYI 02960-DFA),

- cleavage of the molecule at the pyridinylmethylene bridge forming BYI 02960-6-CNA, which was further conjugated with glycine to BYI 02960-hippuric acid and BYI 02960-difluoroethyl-amino-furanone.
- The figure below schematically shows the sites of the molecule, which are involved in the metabolic reactions:

Summarizing, the results of the metabolism studies conducted in the rat, a proposed metabolic pathway of flupyradifurone can be described as shown in this figure:

2.3.1.2 Toxicodynamics

Acute toxicity

Flupyradifurone (BYI 02960) needs to be classified as Acute oral toxic Cat. 4 H302 according to Regulation (EC) 1272/2008. Flupyradifurone does not need to be classified on the basis of its acute dermal, and inhalation toxicology in rats (Category 5/ Unclassified). Flupyradifurone is considered not irritating to skin and eyes (Category 5/ Unclassified). Flupyradifurone does not have sensitising properties in a LLNA (Category 5/ Unclassified).

Table 6.11.2-1 Classification

Type of study	Species	Results	Classification (proposed)
Oral route	Rat	Mortalities observed at 2000 mg/kg; none at 300 mg/kg	Category 4 H302 (LD_{50} cut off >300-2000 mg/kg)
Dermal route	Rat	LD_{50} > 2 000 mg/kg	Category 5 / Unclassified
Inhalation	Rat	LC_{50} at 4 hours > >4.7 mg/L (nose only) ~~4671 mg/m^3~~	Category 5 / Unclassified
Primary skin irritation	Rabbit	Non irritating	Category 5 / Unclassified

Eye irritation	Rabbit	Slight redness of the conjunctivae, reversed within 48 hours	Category 5 / Unclassified
Skin sensitization	Mouse	Not sensitizing	Category 5 / Unclassified

Short-term toxicity

Oral

Four subacute oral range-finding studies were performed, with rat, mouse and dog.

In a 28-day study with rats exposed to 0, 75, 200 or 350 mg/kg bw/d via gavage, toxicologically relevant effects were clinical chemical findings, increased organ weights and pathological findings in the 200 mg/kg bw/d group. The NOAEL in this study is set at 75 mg/kg bw/d.

In a 28-day study with rats exposed to 0, 33.6 or 385 mg/kg bw/d via diet, toxicologically relevant effects were clinical chemical findings, increased liver and thyroid weights and associated pathological findings in the 385 mg/kg bw/d group. The NOAEL in this study is set at 33.6 mg/kg bw/d.

In a 28-day study with mouse exposed to 300, 600 and 1200 ppm (equating approximately to 50, 98 and 207 mg/kg bw/day in males and 59, 122, 240 mg/kg bw/day in females) via diet, no toxicologically relevant effects were observed. ~~Given the instability of BYI 02960 in rodent diet the actual concentration is considered to be in the region of 960 to 1080 ppm (166 to 186 mg/kg body weight/day for the males and 192 to 216 mg/kg body weight/day for the females, respectively).~~ The NOAEL in this study is set at 98 ~~166~~ ~~33.6~~ mg/kg bw/d.

In a 28-day study with dogs exposed to 0, 500, 2000 or 4000 ppm (equating approximately to 0, 16, 62, 118 mg/kg body weight/day in males and 0, 18, 77, 131 mg/kg body weight/day in females) via diet, toxicologically relevant effects were body weight loss and reduced body weight gain at the 4000 ppm group. The NOAEL in this study is set at 2000 ppm (62 mg/kg bw/d).

Four semichronic oral studies were performed, with rat, mouse and dog.

In a 90-day toxicity study with rat exposed to 0, 100, 500, 2500 ppm (equating to approximately 0, 6.0, 30.2, 156 mg/kg bw/day in males and 0, 7.6, 38.3, 186 mg/kg bw/day in females) via diet, toxicologically relevant effects were clinical chemical findings, increased relative liver and thyroid weight and associated pathological findings in the 2500 ppm group, and thyroid weight effects in the 500 ppm males. The NOAEL in this study is 100 ~~500~~ ppm (6.0 ~~30.2~~ mg/kg bw/d).

In a 90-day toxicity study with mouse exposed to 0, 100, 500, 2500 ppm (equating approximately to 0, 16, 81, 407 mg/kg bw/day in males and 0, 19, 98, 473 mg/kg bw/day in females) via diet, toxicologically relevant effects were clinical chemical findings, increased relative liver and decreased kidney weights and associated pathological findings in the 2500 ppm group. The NOAEL in this study is 500 ppm (81~~80.6~~ mg/kg bw/d).

In a 90-day toxicity study with dog exposed to 0, 400, 1200 or 3600/2400 ppm (equating approximately to 0, 12, 33 and 102/85 mg/kg bw/ day for males and 0, 12, 41 and 107/78 mg/kg bw/day for females) via diet, toxicologically relevant effects were reduced body weight, reduced body

weight gain, clinical chemistry, significant increase in kidney weight and focal myofiber atrophy/degeneration in the 1200 ppm group. The NOAEL in this study is 400 ppm (12 mg/kg bw/d).
In a 1-year toxicity study with dog exposed to 0, 150, 300 and 1000 ppm (equating approximately to 0, 4.6, 7.8 and 28.1 mg/kg bwd/day for males and 0, 4.1, 7.8 and 28.2 mg/kg bw/day for females) via diet, toxicologically relevant effects were micropathology (degeneration of the skeletal muscle (*gastrocnemius* and *biceps femoris* (atrophy, necrosis and/or presence of inflammatory cells)) findings in the 1000 ppm group. The NOAEL in this study is 300 ppm (7.8 mg/kg bw/d).

Dermal

Male and female rats, 10 rats/sex/group (one control and three test substance-dosed groups) were administered BYI 02960 (50, 150, or 500 mg/kg/day) daily by dermal application for at least 28 consecutive days (minimum of 6 h/day) and euthanized one day following the last dose. Based on the lack of adverse findings observed in the study, the 28-day dermal exposure NOAEL in male and female rats dosed with BYI 02960 was 500 mg/kg bw/day.

Genotoxicity

BYI 02960 was not mutagenic in two Ames tests.
BYI 02960 did not induce structural chromosome aberrations in V79 cells when tested up to and including cytotoxic concentrations and showed no mutagenic potency in vitro in the CHO/HPRT assay.
BYI 02960 did not induce micronuclei in mouse bone marrow cells in two separate in vivo studies.
Overall it can be concluded that BYI 02960 has no mutagenic or clastogenic potential.

Long-term toxicity and carcinogenicity

In a two-year toxicity/carcinogenicity study with rats, effects in the liver and in a less extend in the thyroid and lung (females) were observed in the 2000 ppm dose group. The NOAEL for general toxicity was set at 400 ppm in diet, equal to the average daily intake of 15.8 mg/kg bw/d. No neoplastic lesions were observed in the study at any dose level.
In an 18-months carcinogenicity study with mice, effects in the liver and in de kidney (male) were observed in the 1500 ppm dose group. The NOAEL for general toxicity was set at 300 ppm equal to the average daily intake of 45 mg/kg bw/day. The NOAEL for carcinogenicity was set at the highest dose level of 1500 ppm, equal to the average daily intake of 224 and 263 mg/kg bw/day over 18 months for male and female mice, respectively.

Reproductive and developmental toxicity

In the rangefinder one generation rat reproduction study, BYI 02960 was administered continuously in the feed to Wistar rats (10 animals/dose/sex) at nominal dietary concentrations of 0, 200, 700, and 2000 ppm. The parental systemic LOAEL is 700 ppm (60.0 mg/kg bw/day females), based on

decreased body weight, decreased body weight gain, alterations in food consumption during premating, and decreased spleen weights in females. The parental systemic NOAEL is 200 ppm (17.5 mg/kg bw/day).
The reproductive NOAEL is 147.5 mg/kg bw/day based on no reproductive findings observed at the highest dose tested.
The offspring LOAEL is 700 ppm (60.9 mg/kg bw/day). The LOAEL is based on maternal effects leading to secondarily-mediated effects on pup weight, pup weight gain, and organ weight changes (brain). The offspring NOAEL is 200 ppm (17.5 mg/kg bw/day).

A significant decrease in body weight, but <10% was observed in the mid-dose of the F1 females and a non significant decrease in body weight gain was found in the parental animals (21 % in females) and the F1 animals (16 % in females). Therefore the parental systemic LOAEL is considered 500 ppm (32 mg/kg bw/d) based on decreased body weight gain in females. The parental systemic NOAEL is 100 ppm (6.4 ~~7.7~~ mg/kg bw/d). ~~Therefore the parental systemic LOAEL is considered 1800 ppm (117.4 mg BYI 02960/kg bw/day) based on increased liver and thyroid weights, and minimal centrilobular hypertrophy in males decreased body weight (>10%)(premating, gestation, and lactation), body weight gain (premating; P and F_1). The parental systemic NOAEL was 500 ppm (32.0 mg BYI 02960/kg bw/day).~~The reproductive NOAEL was 500 ppm (34.3 mg BYI 02960/kg bw/day females) based on decreased estrous cycle number, litter size, and the number of implants observed in the F_1 generation at the highest dietary level tested (LOAEL = 1800 ppm (134.0 mg BYI 02960/kg bw/day in females).
A significant decrease in body weight, but <10% was observed in the mid-dose of the F2 pups. The offspring LOAEL is therefore 1800 ppm (134.0 mg BYI 02960/kg bw/day) based on maternal effects leading to secondary effects on pup weight, and physical development. The offspring NOAEL is 500 ppm (34.3 mg BYI 02960/kg bw/day).

In a rat developmental study, BYI 02960 was administered daily by gavage to groups of 25 pregnant Sprague-Dawley female rats per dose-group at 15, 50 and 150 mg/kg/day from gestation day (GD) 6 to 20. Minimal maternal toxicity was observed at the high-dose level, manifested as decreased body weight gain and reduced food consumption. The mean absolute liver weight was increased in the high dose group.Based on these findings the Noael vor maternal toxicity was set at 50 mg/kg bw/day. The increased incidence of parietal incomplete ossification showed no dose response, the unossified cervical centrum and the incomplete ossification of the sternebra was within the historical background. A increase of a short costal cartilage was seen at the high-dose. Based on these findings, the NOAEL for developmental toxicity was set at 50 mg/kg bw/day.

In a complementary study, where groups of 23 sperm-positive female Sprague-Dawley rats were exposed to BYI 02960 by oral gavage from gestation day (GD) 6 to 20 at 20 and 30 mg/kg/day, no maternal toxicity was

observed up to 30 mg/kg/day. Therefore, based on these two studies, it can be concluded that the NOEL for maternal toxicity was 30 mg/kg/day.

In a rabbit developmental study, groups of 23 time-mated pregnant female New Zealand White rabbits were administered BYI 02960 by oral gavage from gestation day (GD) 6 to 28 at 7.5, 15 and 40 mg/kg/day. The effects on (corrected) body weight gain and food consumption were slight and mainly transient, however considered adverse (>10%).The NOAEL for maternal toxicity and developmental toxicity was set at 15 mg/kg bw/day.~~There were no treatment-related findings for maternal and developmental toxicity therefore the NOAEL for maternal toxicity and developmental toxicity was set at 40 mg/kg bw/day.~~

Neurotoxicity

In an acute neurotoxicity study, BYI 02960 was administered by gavage in a single dose to non-fasted young adult Wistar rats at 0, 50, 200 and 800 mg/kg. Compound-related effects were observed at all dose concentrations in both sexes. The only treatment-related effects at 50 mg/kg were limited to higher incidences of piloerection in both sexes and dilated pupils in females only. A follow-up study was performed in order to establish a clear NOAEL for findings observed at all dose levels in the initial study. In this follow-up study, females only were used as they were equal or more sensitive than males at higher dose levels and were administered BYI 02960 at 20 or 35 mg/kg. No treatment-related effects were evident at either dose tested. The dose level of 35 mg/kg of BYI 02960 was considered to be the overall NOAEL for both sexes.

In a 90-day neurotoxicity study, through approximately 13 weeks of continuous dietary exposure to BYI 02960 at 100, 500 or 2500 ppm, there were no neurotoxic treatment-related findings apparent at any dietary level in either sex. Based on these findings, a NOAEL of 2500 ppm was established for the rat (equating to 143 mg BYI 02960/kg bw/day for males). Body weight and food intake effects were seen at the highest dosage. The systemic NOAEL is therefore 29.4/34.8 mg/kg bw/d for males and females respectively.

In a developmental neurotoxicity study, dietary exposure to BYI 02960 from GD 6 through LD 21 to mated female Wistar rats resulted in treatment-related effects (maternal: decreased body weight gain. Offspring: decreased body weight gain, slightly increased in motor and locomotor activity, slightly increased auditory startle habituation) evident only at 1200 ppm (102 mg/kg bw/day) in maternal animals and offspring. ~~Therefore, the NOAEL for both maternal animals and offspring is 500 ppm (42.4 mg/kg bw/day) in this developmental neurotoxicity study.~~

The maternal NOAEL was 500 ppm (42.4 mg/kg bw/d) based on decreased body weight and body weight gain. The developmental NOAEL was 500 ppm (42.4 mg/kg bw/d) based on decreased offspring body weight and body weight gain. The developmental neurotoxicity NOAEL was 500 ppm (42.4 mg/kg bw/d) based on increased startle amplitude in females only on PND 60 and increased motor and locomotor activity on PND 13 in males only.

Further toxicological studies

Biokinetic study

Plasma concentration of BYI 02960 was assessed at three time points after continuous dietary administration for seven days. Groups of 5 male and 5 female Wistar rats received the diet admixed with the test substance at a concentration of 400 ppm (equating approximately to 22.6 mg/kg body weight/day in males and 32.4 mg/kg body weight/day in females).

During the first 15 days of acclimatization, the daily light/dark rhythm was 7 a.m. - 7 p.m. Rats being known to preferably eat during the dark period, the daily light/dark rhythm of rats was changed from November 25, 2009 (pre-study Day 16, *i.e.* 14 days prior to first day of treatment) onwards, *i.e.* dark period was from 2 a.m. to 2 p.m. and light period was from 2 p.m. to 2 a.m., in order to allow the blood sampling during the working day. Clinical signs were observed daily. Body weights were measured just prior to the start of admixed food administration (Study Day 1) and on Study Day 8. Food consumption was recorded on Study Days 1 - 4, 4 - 7 and 7 - 8.

Whole blood was collected on heparin for plasma preparation from treated animals at three time points on Study Day 8 (8 a.m., 2 p.m. and 5 p.m.). The day after the end of the treatment period, animals were sacrificed without necropsy. Dietary administration of BYI 02960 induced no mortalities or treatment-related clinical signs.

The results obtained indicated a small sex difference in the plasma concentrations of BYI 02960, values being slightly higher for females than for males.

In male rats, a Cmax of 8.3 mg/L was measured at the time of collection of 8 a.m. In female rats, a Cmax of 9.4 mg/L was measured at the time collection of 2 p.m. However, in view of the inter-individual variability, the concentrations of BYI 02960 in plasma were considered similar between the three times of blood collection for both sexes (between 7.8 and 8.3 mg/L for males and between 8.8 and 9.4 mg/L for females). In conclusion, in the environmental conditions of the long-term studies (light: 7 a.m. to 7 p.m. & dark period: 7 p.m. to 7 a.m.) and assuming that animals are starting eating food when the light is switched off, the blood collection can be performed between 1 a.m. and 10 a.m. in order to measure the maximum concentration of BYI 02960.

28-Day immunotoxicity study

BYI 02960, was administered continuously via dietary administration to separate groups of female Wistar rats (10/group) at concentrations of 125, 600 and 3000 ppm (equating approximately to 10, 50, 230 mg/kg body weight/day) for at least 28 days. BYI 02960 was considered not to have any immunotoxic potential in female Wistar rats when given in the diet at dose levels up to 3000 ppm (corresponding to approximately 230 mg/kg/day) for at least 28 days.

Toxicity of metabolites

Difluoroacetic acid (DFA, BCS-AA56716) is a major soil, water and plant metabolite of BYI 02960; in the rat ADME study, it was found in urine at around 6% of the administered dose. This metabolite is devoid of genotoxic potential; the acute oral LD50 is between 300 and 2000 mg/kg, similar to parent compound. In a 14 day repeat dietary administration range finding study in the rat, the most significant findings were decreased mean glucose concentration in both sexes and an increase in urea concentration was observed in females only. In a 90-day rat study, DFA was administered in the diet to Wistar rats (10/sex/group) at concentrations of 200, 1000 and 6000 ppm. Lower mean glucose concentrations, lower total bilirubin and slightly higher mean urea concentrations were observed in both sexes at all doses. At 6000 and 1000 ppm dose levels, mean body weight, overall body weight gain and food consumption were reduced in both sexes. Lower hemoglobin concentration and lower mean corpuscular volume were observed in females, together with lower mean corpuscular hemoglobin and lower hematocrit, and higher ketone levels were noted in both sexes. A few black foci were also noted in the glandular part of the stomach in both sexes (including one control female), in correlation with a few cases of focal glandular erosion/necrosis observed at the microscopic examination. The minor changes noted in the clinical chemistry determination at the low dose are considered not to be adverse effects of the test substance as they do not represent any functional impairment in the test organism. Therefore, the dose level of 200 ppm (equating to 12.7 and 15.6 mg/kg body weight/day in males and females, respectively) was considered to be a No Observed Adverse Effect Level (NOAEL) in the male and female Wistar rats. When the NOAEL is expressed in BYI 02960 equivalents, it equates to 38 and 47 mg/kg/day in males and females, respectively. Therefore, Difluoroacetic acid was not more toxic than BYI 02960 after subchronic administration to the rat. The metabolic changes observed with DFA are also observed with BYI 02960. The decrease in glucose was reversible and appeared to be adaptative as it was no longer significant during the second part of the rat carcinogenicity study.

BYI 02960-difluoroethyl-amino-furanone (BCS-CC98193, BYI 02960-DFEAF) is a minor plant metabolite of BYI 02960 and was also observed in the rat ADME study. It accounted for less than 10% of the administered dose in the rat urine. Since the confined rotational crop study with [furanone-4-14C]BYI 02960 indicated rather high residue levels of BYI 02960-difluoroethyl-amino-furanone in leafy crops and the absence of other suitable markers, BCS decided to include this metabolite in the residue definition for the data collection method for target and rotational crops (see residue section). The subsequent residue studies revealed that BYI 02960-difluoroethyl-amino-furanone is only a minor plant metabolite. However, based on the results of the confined rotational crop study, additional tox studies (acute toxicity testing, genotoxicity testing and subacute rat study) were conducted to show that the tox profile of the metabolite is covered by the endpoints derived from the parent compound. In the *in vitro* genotoxicity package, the Ames and HPRT tests were negative and the chromosome aberration test was positive. Therefore, two *in vivo* studies (*in vivo* micronucleus test and *in vivo* unscheduled DNA synthesis) were conducted, both of which were negative. Based upon the overall

findings, BYI 02960-difluoroethyl-amino-furanone can be considered not genotoxic. The acute oral LD_{50} in rats was higher than 2000 mg/kg. The NOAEL of the 28-day rat study was 3000 ppm equating to 243 and 273 mg/kg/day in males and females, respectively based on body weight effects.

Several toxicology studies exist for two plant metabolites BYI 02960-CHMP ((6-chloro-3-pyridyl) methanol) and BYI 02960-6-CAN (6-chloronicotinic acid), which are metabolites common to other pesticides. For BYI 02960-CHMP, the Ames test was negative. The acute oral rat LD_{50} was 1842 mg/kg in males and 1483 mg/kg in females. In a 90-day rat study, BYI 02960-CHMP administered continuously via dietary administration to Sprague Dawley rats (10/sex/group) at concentrations of 160, 800, 4000 and 20 000 ppm resulted in decreases in mean body weights and mean food consumption in both sexes and a statistically significant increases in serum alkaline phosphatase activity in the 20000 ppm group females at study termination only. Histologically, dose-related eosinophilic intranuclear inclusions were seen in the proximal tubular epithelium of kidneys for 20000 ppm males and females and 4000 ppm males. The no observed effects level (NOEL) was 800 ppm (48.9 mg/kg/day) in males, and 4000 ppm (275.9 mg/kg/day) in females. When the NOEL is expressed in BYI 02960 equivalents, it equates to 97.8 and 551.8 mg/kg/day. Therefore, BYI 02960-CHMP was less toxic than BYI 02960 after subchronic administration to the rat.

For BYI 02960-6-CAN (6-chloronicotinic acid) an acute oral rat toxicity study and an Ames test were performed for the registration of acetamiprid. BYI 02960-6-CAN was not genotoxic and not acutely toxic.

The reference values of the parent flupyradifurone are applicable to both metabolites DFA and DFEAF; the experts considered that there was sufficient evidence to conclude that DFEAF is unlikely to have genotoxic potential.

ADI

The calculation of the ADI is based on the highest dose at which no adverse effect is observed in the most appropriate study in the most sensitive species. BYI 02960 was tested in several short-term studies in dogs, rats, and mice, in long-term toxicity studies in rats and mice, and in reproduction and developmental toxicity studies in rats and rabbits, providing the basis for the establishment of the ADI. ~~The lowest NOAEL (7.8 mg/kg/day) was obtained from a 1-year study in dog. Similar critical effects (myofiber degeneration, similar number of effected animals) were seen in the 90-day study with dog. It is therefore suggested to use both the 1-year dog study and the 90-day dog study to set the NOAEL of 12 mg/kg/d used as a starting point for the establishment of the ADI, also taking into account the sufficient dose spacing between the 90-day dog NOAEL and the 1-year LOAEL. Application of a safety factor for inter- and intraspecies differences of 100 results in an ADI of 0.12 mg/kg bw/day.~~

~~The lowest NOAEL (7.7 mg/kg bw/d) was obtained from the 2-generation study in rat based on body weight effects in the parent animals. Application of a safety factor for inter- and intraspecies differences of 100 results in an ADI of 0.08 mg/kg bw/day (rounded from 0.077 mg/kg bw/day).~~

Considering the parental and offspring NOAEL in the multigeneration study, the experts agreed to set the ADI at 0.064 mg/kg bw per day, applying an uncertainty factor of 100.

Table 6.11.3-1 Studies considered for the establishment of the ADI

Study	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Reference
Semichronic toxicity studies			
90-d oral rat	6.0 ~~30.2~~	30.2 ~~156~~	Odin-Feurtet, M. 2009
90-d oral mouse	81	407	Odin-Feurtet 2009
90-d oral dog	12	33	Odin-Feurtet, M. 2008
1-yr oral dog	7.8	28.1	Cady, A. 2012
Chronic toxicity studies			
24-m oral chronic/carcino rat	15.8	80.9	J.C. Garcin, 2012
18-m oral carcino mouse	43	224	P. Kennel, 2012
Reproduction and teratogenicity studies			
1-generation rat			A. D. Milius, B.S., 2010
- Parental	17.5	60	
- Developmental			
- Reproduction	147.5	-	
	17.5	61	
2-generation rat			A. D. Milius, B.S., 2011
• Parental	**6.4** ~~**7.7**~~ ~~32~~	32 ~~117~~	
• Developmental			
• Reproduction	34	134	
	34	134	

ARfD (acute reference dose)

The calculation of the acute reference dose is based on the highest dose at which no adverse effect is observed in the most appropriate acute toxicity study in the most sensitive species. The maternal NOAEL of 15 mg/kg bw/d in the developmental toxicity study in rabbit is based on a reduced body

weight of 12% during the first days of treatment which is an applicable effect to set the ARfD. Application of a safety factor for inter- and intraspecies differences of 100 results in an ARfD of 0.15 mg/kg bw/d.

~~Only slight body weight gain effects were observed at the top dose in the developmental toxicity studies in both rats and rabbits. Acutely toxic effects were seen only in the acute neurotoxixity study, where typical signs of nicotinergic insecticides have been observed. Therefore, it seems appropriate to set up the ARfD using the NOAEL of the acute neurotoxicity study. Application of a safety factor for inter- and intraspecies differences of 100 results in an ARfD of 0.35 mg/kg bw.~~

Table 6.11.4-1 Studies considered for the establishment of the ARfD

Study	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Reference
Reproduction and teratogenicity studies			
Teratogenicity study rat - Maternal - Developmental	50 50	150 150	A. D. Langrand-Lerche, 2010
Maternal tolerability study rat - Maternal	30	-	C. Langrand-Lerche., 2012
Teratogenicity study rabbit - Maternal - Developmental	**15** ~~40~~ 40	40 - -	P. Kennel, 2012
Neurotoxicity studies			
Acute neurotoxicity study in the rat	35	50	JC Garcin, 2011
90-day Neurotoxicity study in the rat - Systemic - Neurotoxicity	29.4 143	143 -	JC Garcin, 2011

AOEL

The calculation of the AOEL is based on the highest dose at which no adverse effect is observed in the most appropriate study in the most sensitive species. BYI 02960 was tested in several short-term studies in dogs, rats, and mice, in long-term toxicity studies in rats and mice, in reproduction and

developmental toxicity studies in rats and rabbits, and neuro and immuno toxicity studies with rats providing the basis for the establishment of the short-term and long-term AOEL.

The lowest NOAEL (6.4 ~~7.7~~ mg/kg bw/d) was obtained from the 2-generation studie in rat based on body weight effects in the parent animals. A correction factor for oral absorption is not necessary since the oral absorption is >80%. Application of a safety factor for inter- and intraspecies differences of 100 results in an ADI of 0.08 mg/kg bw/day (rounded from 0.077 mg/kg bw/day).

~~The lowest NOAEL (7.8 mg/kg/day) was obtained from a 1-year study in dog. Similar critical effects (myofiber degeneration, similar number of effected animals) were seen in the 90-day study with dog. It is therefore suggested to use both the 1-year dog study and the 90-day dog study to set the NOAEL of 12 mg/kg/d used as a starting point for the establishment of the AOEL, also taking into account the sufficient dose spacing between the 90-day dog NOAEL and the 1-year LOAEL. A correction factor for oral absorption is not necessary since the oral absorption is >80%. Application of a safety factor for inter- and intraspecies differences of 100 results in an AOEL of 0.12 mg/kg bw/day for both short-therm and long-therm.~~

Table 6.11.5-1 Studies considered for the establishment of the AOEL

Study	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Reference
Semichronic toxicity studies			
90-d oral rat	6.0 ~~30.2~~	30.2 ~~156~~	Odin-Feurtet, M. 2009
90-d oral mouse	81	407	Odin-Feurtet 2009
90-d oral dog	12	33	Odin-Feurtet, M. 2008
1-yr oral dog	7.8	28.1	Cady, A. 2012
Chronic toxicity studies			
24-m oral chronic/carcino rat	15.8	80.9	J.C. Garcin, 2012
18-m oral carcino mouse	43	224	P. Kennel, 2012
Reproduction and teratogenicity studies			
1-generation rat			A. D. Milius, B.S., 2010
- Parental	17.5	60	
- Developmental			
- Reproduction	147.5	-	
	17.5	61	
2-generation rat			A. D. Milius, B.S., 2011
• Parental	6.4 ~~7.7~~ ~~32~~	32 ~~117~~	
• Developmental			
• Reproduction	34	134	
	34	134	

Neurotoxicity studies			
Acute neurotoxicity study in the rat	35	50	JC Garcin, 2011
90-day Neurotoxicity study in the rat - Systemic - Neurotoxicity	 29.4 143	 143 -	JC Garcin, 2011
Immunotoxicity studies			
28-day immunotoxicity in the rat - Systemic - Immunotoxic	 50 230	 230 -	Repetto M, 2011

2.3.5 Drinking water limit

According to Council Directive 97/57/EC, exposure to BYI 02960 through drinking water should account for not more than 10% of the ADI. If it is assumed that the average daily consumption of water amounts to 2 litre per person of 60 kilogram, a potential drinking water limit of ((60x0.064 ~~0.08~~ ~~0.12~~)/10)/2 mg/l, i.e.0.192 ~~0.24~~ ~~0.36~~ mg/l can be established.

According to Document 8064/VI/79 of the European Commission, the EU drinking water limit for pesticides of 0.1 µg/l is applicable for BYI 02960.

2.3.6 Impact on human or animal health arising from exposure to the active substance or to impurities contained in it

Internal exposure and risk assessment

Risk assessment for operators

Risk assessment was performed using the 75th percentile from the UK-model (UK-75th), the geometric mean from the German model (DE-GM) and the 90th percentile from the Dutch model (NL-90th).

Table 2.3.6-1 Operator internal exposure and risk assessment

Model	Route	Estimated internal exposure (mg a.s./day)		AOEL Systemic * (mg a.s/day)	% AOEL	
		without PPE	with PPE		without PPE	with PPE
Mechanical upward spraying on hop (field)						

Model	Route	Estimated internal exposure (mg a.s./day)		AOEL Systemic * (mg a.s/day)	% AOEL	
		without PPE	with PPE		without PPE	with PPE
UK- 75th	Respiratory	0.0225	0.0225	3.84	0.6	0.6
	Dermal	1.6253	1.0903	3.84	42.3	28.4
	Total	**1.6478**	**1.1128**	**3.84**	**42.9**	**29.0**
DE- GM	Respiratory	0.0288	0.0288	4.48	0.6	0.6
	Dermal	2.3575	2.2047	4.48	52.6	49.2
	Total	**2.3798**	**2.2271**	**4.48**	**53.1**	**49.7**
Mechanical downward spraying on lettuce (field)						
UK- 75th	Respiratory	0.015	0.015	3.84	0.4	0.4
	Dermal	1.9259	0.2821	3.84	50.2	7.3
	Total	**1.9409**	**0.2971**	**3.84**	**50.5**	**7.7**
DE- GM	Respiratory	0.004	0.004	4.48	0.1	0.1
	Dermal	0.891	0.77074	4.48	19.9	17.2
	Total	**0.895**	**0.7113**	**4.48**	**20.0**	**15.9**
Manual downward spraying on lettuce (field) **						
UK- 75th	Respiratory	0.03	0.03	3.84	0.8	0.8
	Dermal	4.5510	0.8077	3.84	118.5	21.0
	Total	**4.5810**	**0.8377**	**3.84**	**119.3**	**21.8**
Manual spraying on lettuce (indoors) ***						
Dutch-90th	Respiratory	0.1250	0.1250	4.48	2.8	2.8
	Dermal	4.2500	0.4250	4.48	94.9	9.5
	Total	**4.3750**	**0.5500**	**4.48**	**97.7**	**12.3**

* Assuming a body weight of 60 kg for UK-POEM and 70 kg for other models
** No suitable module available in German modell
*** No suitable module available in UK-POEM and German model

Risk assessment for bystanders

Risk assessment was performed using the EUROPOEM II model.

Table 2.3.6-2 Bystander internal exposure and risk assessment

Route	Estimated internal exposure (mg a.s./day)	AOEL systemic * (mg a.s./day)	%AOEL

Exposure during mechanical upward spraying on hop (field)			
Respiratory	0.0056	4.48	0.1
Dermal	0.255	4.48	5.7
Total	**0.261**	**4.48**	**5.8**
Exposure during downward spraying on lettuce (field)			
Respiratory	0.0094	4.48	0.2
Dermal	0.021	4.48	0.5
Total	**0.031**	**4.48**	**0.7**

* Assuming a body weight of 70 kg

Resident exposure

Risk assessment was performed according to the German guideline published in 2008 (Martin et al., 2008).

Table 2.3.6-3 Resident internal exposure and risk assessment

Route	Estimated internal exposure (mg/kg bw/day)	AEL systemic * (mg/kg bw/d)	%AEL
Resident exposure during mechanical upward spraying on hop (field)			
Adult			
Dermal	0.000179014	0.064	0.28
Respiratory	0	0.064	0
Total	0.000179014	**0.064**	**0.28**
Child			
Dermal	0.000236874	0.064	0.37
Respiratory	0	0.064	0
Oral (hand to mouth)	0.000107183	0.064	0.17
Oral (object to mouth)	2.67957E-05	0.064	0.04
Total	**0.000370852**	**0.064**	**0.58**
Resident exposure during mechanical downward spraying on lettuce (field)			
Adult			
Dermal	7.49771E-06	0.064	0.01
Respiratory	0	0.064	0

Total	**7.49771E-06**	**0.064**	**0.01**
Child			
Dermal	9.92105E-06	0.064	0.02
Respiratory	0	0.064	0
Oral (hand to mouth)	4.48916E-06	0.064	0.01
Oral (object to mouth)	1.12229E-06	0.064	<0.01
Total	**1.55325E-05**	**0.064**	**0.02**

Risk assessment for workers

Risk assessment was performed using the EUROPOEM II model (outdoor) and EUROPOEM II and Dutch model (indoor).

Table 2.3.6-4 Worker internal exposure and risk assessment

Model	Route	Estimated internal exposure (mg a.s./day)		AOEL Systemic * (mg a.s/day)	% AOEL	
		without PPE	with PPE		without PPE	with PPE
Re-entry exposure after mechanical upward spraying on hops						
EURO-POEM II	Respiratory	-	-	-	-	-
	Dermal	2.066	0.413	4.48	46.1	9.2
	Total	**2.066**	**0.413**	**4.48**	**46.1**	**9.2**
Re-entry exposure after downward spraying on lettuce outdoors						
EURO-POEM II	Respiratory	-	-	-	-	-
	Dermal	0.956	0.191	4.48	21.3	4.3
	Total	**0.956**	**0.191**	**4.48**	**21.3**	**4.3**
Re-entry exposure after spraying on lettuce indoors						
EURO-POEM II & Dutchs model	Respiratory	0.041	0.004	4.48	0.9	0.1
	Dermal	0.956	0.191	4.48	21.3	4.3
	Total	**0.998**	**0.195**	**4.48**	**22.3**	**4.4**

* Assuming a body weight of 70 kg
- The inhalation exposure after field application is not quantifiable with this method. However, it can be expected to be lower

than respiratory exposure during application.

2.4 Residues

2.4.1 Definition of the residues relevant to MRLs

Plant products

The residue definition for post-registration monitoring is proposed as: ~~the sum of~~ parent compound and DFA, expressed as DFA ~~in flupyradifurone equivalents~~, separately.

The residue definition for risk assessment is proposed the sum of parent compound flupyradifurone and its metabolites DFA ~~and difluoroethyl-amino-furanone (DFEAF)~~, expressed in flupyradifurone equivalents. ~~A conversion factor is not required to cover the difference between the residue definition for monitoring and risk assessment as both DFA and flupyradifurone are part of the residue definition for monitoring and as DFEAF is found in levels below <0.01 mg/kg in supervised residue trials and in rotational crops.~~

Animal products

The residue definition for post-registration monitoring ~~and risk assessment~~ is proposed as: ~~the sum of~~ parent compound and DFA, expressed as DFA ~~in flupyradifurone equivalents~~, separately.

The residue definition for risk assessment is proposed as: the sum of parent compound and DFA, expressed in flupyradifurone equivalents.

2.4.2 Residues relevant to consumer safety

Residues in products of plant origin

Protected Lettuce

Nine acceptable residue trials performed in accordance with the cGAP for greenhouse use have been provided. In the greenhouse trials a highest residue value (HR) of 6.04 mg/kg was determined and an the STMR of 2.12 ~~2.0~~ ~~2.2~~ mg/kg for the sum of flupyradifurone and DFA, expressed as flupyradifurone.

Unprotected Lettuce

For the envisaged outdoor use in Northern and Southern Europe with a 10d PHI the required number of trials are available (nine acceptable trials for each region).

For the envisaged agricultural outdoor use in Northern and Southern Europe with a 3d PHI, the residue trials performed with lettuce in Northern and Southern Europe are not acceptable, except for two trials with sampling 2 days after the 1st treatment. No samples were taken 3 days after the first application. However, the results from the trials performed with outdoor lettuce in Northern and Southern Europe at 3 days after the 2nd treatment were valid to determine that the intended use on protected lettuce can be identified as the worst case residue situation, based on a comparison of the

residue values from field and greenhouse testing using the most critical use pattern (2x125 g as/ha, int. 10d, PHI 3d).

Moreover, the tested GAP with two applications and a pre-harvest interval of 3 days is the "home and garden" use, which was submitted in parallel and is envisaged for registration shortly after the agricultural use (see the Evaluation Report for Flupyradifurone as an appendix to Vol. 3, B7). Since this field use is more critical than the agricultural field use, it was agreed with the RMS to conduct all field residue trials according to this GAP to identify the most critical consumer exposure and to avoid re-evaluation of MRLs due to this use. Hence, the trials in the DAR are not in accordance with the cGAP in the DAR, but with the cGAP supported in EU for MRL setting.

Thus, the open field trials in lettuce in Northern and Southern Europe are in compliance with the envisaged "home and garden" use. In addition, sufficient trials were performed providing evidence that the envisaged greenhouse can be identified as the worst case residue situation. Therefore no further trials are required.

Hops

In hops, eight valid trials were conducted in the northern European residue region in the years 2010-2011. Flupyradifurone was applied once as an SL 200 formulation at an active substance rate of 120 - 150 g/ha. All applications were at acceptable rates, and all trials were conducted according to GLP. in dried cones, residue levels also tended to decline with time. When evaluating the highest residues at relevant sampling intervals (either day 21 or, in three trials, day 28), residues ranged from 0.34-2.3 ~~0.61-2.4~~ mg/kg, with a median of 1.05 ~~1.15~~ mg/kg for the sum of flupyradifurone and DFA, expressed as flupyradifurone.

Residues in rotational crops

The metabolism of BYI 02960 residues in the rotational crops wheat (small grain), Swiss chard (leafy crop) and turnip (root crop) was investigated at a nominal application rate of 400 g a.s./ha. The plant back intervals were 29, 135 and 296 days for all crops. The studies were conducted with [furanone-4-^{14}C]BYI 02960 and [pyridinylmethyl-^{14}C]BYI 02960. A study with [ethyl-1-^{14}C]BYI 02960 was not conducted and therefore non-radiolabelled difluoroacetic acid was determined in all extracts obtained in the CRC study conducted with [furanone-4-^{14}C]BYI 02960 to gain information on the fate of the difluoroethane moiety of the active substance.

Corresponding TRR values showed similar residue levels when comparing the studies performed with [furanone-4-^{14}C]BYI 02960 and [pyridinylmethyl-^{14}C]BYI 02960. Generally, the TRR values were slightly higher in the crops of 1st and 2nd rotation of the study performed with [pyridinylmethyl-^{14}C]BYI 02960, except for wheat grains, where it was the opposite way round due to the selective transport of glucose as a label specific metabolite into the RAC.

The levels of parent compound decreased with increasing plant back intervals but nevertheless parent formed a main portion of the residues in all of the RACs in all rotations, except for wheat grains. Other major metabolites (accounting for >10% of the TRR in at least one RAC in one rotation) were

- DFA, which was the main metabolite in wheat grains (all rotations) and in turnip roots (1st rotation) and a major metabolite in all other RACs
- 6-CNA-glucerol-gluA (3 isomers), which were major in and specific for all wheat commodities and accounted in sum for 11% to 33% of the TRR considering all rotations
- BYI 02960-difluoroethyl-amino-furanone, which was a major metabolite in all Swiss chard samples in all rotations and accounted for up to 17% of the TRR
- BYI 02960-OH-glyc, which was major in Swiss chard and turnip leaves in all rotations
- BYI 02960-glyoxylic acid, which was only a major metabolite in wheat forage, hay and straw and in turnip roots of the first rotation, in subsequent rotations it was detected in minor amounts, only
- BYI 02960-bromo-amino-furanone, which was only major in wheat hay of the 3rd rotation
- BYI 02960-OH, which was major in wheat grains of the 1st rotation, only
- Glucose/carbohydrates, which were identified as the main portion in wheat grains after exhaustive extraction and as a major metabolite in turnip roots after conventional extraction.

The proposed metabolic pathway is very similar to the one of primary crops. As observed in target plants the metabolic reactions involved were hydroxylation and oxidative cleavage of the active substance followed by additional oxidation and conjugation processes. No additional metabolic routes were detected, only additional metabolites were identified, e.g. additional conjugates or existing metabolites were subjected to further oxidation reactions.

Four multi-plantback multi-crop rotational crop trials were conducted in Europe (2 each in the northern and southern residue regions) in the years 2010-2011. Flupyradifurone was applied once as an SL 200 formulation either to bare soil or to a target crop (lettuce) at an active substance rate of 200 g/ha, the target crop was then harvested, and crops representing 3 different botanical groups (roots, leafy veg., cereals) were planted on the plots at 3 intervals thereafter. All applications were at the required rates, and all trials were conducted according to GLP.

Quantifiable residues were measured in all rotations. Total residues of BYI 02960 in all rotational crops tended to be highest in the first, earliest rotation with a plant back interval of 25-33d.

Residues ranged from 0.05-0.14 mg/kg (median 0.06 mg/kg) in early harvest root crops, from 0.06-0.21 mg/kg (median 0.08 mg/kg) in early harvest leaf crops, 0.05-0.41 mg/kg (median 0.10 mg/kg) in cereal green forage, <0.07-0.39 mg/kg (median 0.12 mg/kg) in barley straw and from 0.11-0.65 mg/kg (median 0.35 mg/kg) in cereal grain grown in the first rotation, residues as the sum of flupyradifurone and DFA, expressed as flupyradifurone.

Up to the third rotation, residues above the LOQ were detected.

Additionally, field rotational crop trials were performed representative for each type of crop at short plant back intervals (PBI 30 days) and in accordance with the supported dose rate of 125 g as/ha. Potatoes, leek, cucumber, onion, bean (fresh), peas (dry) and winter rape was grown at two locations in Northern and at two locations in Southern Europe. BYI 02960 levels were always low (≤0.05 mg/kg), levels of DFEAF were always <0.01 mg/kg while soil metabolite DFA was present in all trials except in all French bean trials and one leek trial. Residues at harvest showed considerable variation which could not be attributed to geographical zone, soil type, pH, organic matter content, and temperature during treatment, rainfall after treatment or growth period (results not shown). Therefore, results for Northern and Southern Europe are considered as one data set with considerable variation.
HRs of DFA, expressed as DFA were 0.083, 0.076, 0.136, 0.053, 0.365, 0.764 and 0.05 ~~0.26, 0.24, 0.42, 0.17, 1.1, 2.3, and 0.16~~ ~~0.27, 0.25, 0.43, 0.18, 1.1, 2.3 and 0.17~~ mg/kg in potatoes, leek, cucumber, onion, bean (fresh), peas (dry) and winter rape, respectively.

Processing studies

Flupyradifurone stable under all conditions of high temperature hydrolysis for simulation of food processing. The following conditions were tested:

- pH 4 / 90°C (20 min) - pasteurisation
- pH 5 / 100°C (60 min) - baking, brewing, and boiling
- pH 6 / 120°C (20 min) - sterilisation

Metabolite DFEAF is a substructure of the parent compound. All functional groups, chemical moieties and their constitution of DFEAF are also present in the chemical structure of the parent compound. Therefore the chemical stability DFEAF is covered by studies with the parent compound. As parent compound was proved to be stable under the processing conditions, it is concluded that this is also true for DFEAF. In addition, in almost all uses worldwide – and certainly in all EU-relevant uses – DFEAF does not play any significant role in the field residues, generally comprising <5% of the total residue.
Due to the similarity of the chemical structures, the properties of DFA (= difluoroacetic acid) can be interpolated from the properties of TFA (= trifluoroacetic acid) and HOAc (acetic acid). The latter compounds are very common in organic chemistry, have often been used in chemical synthesis and many properties have been investigated. It can be deduced from literature data that these two compounds are stable under the conditions to be tested in a processing study. By interpolation of these properties, it is concluded that DFA is also stable under the processing conditions.

Household preparation of lettuce heads, i.e. removing outer leaves and washing the remaning head (separated in inner and outer head) resulted in processing factors of 1.8 for outer leaves, 0.72 ~~0.73~~ for inner head parts and 0.87 ~~0.76~~ for inner leaves.

Using hops in the production of beer, the transfer factors was determined to be 0.01. In the intermediates in the production of beer, the average processing factor was <0.1.

Residues in products of animal origin

Flupyradifurone was administered orally to laying hens for 29 consecutive days at average dose rates of 1.5 mg/kg feed (1.2N) 6.5 mg/kg feed (5.4N ~~4.3N~~ ~~4.9N~~), 19.4 mg/kg feed (16N ~~13N~~ ~~14.7N~~), and 65.1 mg/kg feed (54N ~~43N~~ ~~49N~~). After the final dose, the animals were sacrificed and the key edible tissues were analyzed for the relevant residues of flupyradifurone. The combined residues of flupyradifurone + DFA in poultry tissues at sacrifice in the 1.2N dosing group were 0.093 mg/kg in muscle, 0.039 mg/kg in fat, and 0.11 mg/kg in liver, expressed in parent compound equivalents. Prior to sacrifice, residues in eggs were measured at various intervals in the high-dose group, and on days 24 and 28 in the three lower dose groups. In the 1N dose group, residues (flupyradifurone + DFA) in eggs amounted to 0.061 and 0.057 mg/kg, respectively.

Residue analysis of the high-dose eggs showed that levels reached a plateau in eggs. Though residues were highest at day 28, residue data from the 54N ~~43N~~ ~~49N~~ egg samples, when evaluated against the dose rate increases calculated as mg a.s./kg bw, suggest that flupyradifurone residues actually reached a plateau between day 4 and day 7; increases in residue levels were only due to increased dose rates.

Depuration occurred quickly. Total flupyradifurone residues in eggs, fat, liver, and muscle from the 54N ~~43N~~ ~~49N~~ dose level hens declined from 1.722, 1.230, 3.480, and 2.410 mg/kg, respectively, to <LOQ at 14 days after cessation of dose administration (=day 42 of the study).

DFA residues represented 65% to 74% of the total flupyradifurone residues in the excreta samples, suggesting significant exposure of the laying hens to DFA residues following the administration of BYI 02960 for 29 days.

Flupyradifurone was administered orally to dairy cows for 29 consecutive days at average *actual* dose rates of 4.8 mg/kg feed (1.6N ~~1.8N~~ ~~2N~~ dose), 23 mg/kg feed (8N ~~9N~~ ~~8N~~), 50 mg/kg feed (17N ~~19N~~ ~~18N~~), and 135 mg/kg feed (44N ~~52N~~ ~~49N~~).

After the final dose, the animals were sacrificed and the key edible tissues were analyzed for the relevant residues of BYI 02960.

The combined residues of BYI 02960 + DFA in bovine tissues at sacrifice in the 1.6N ~~1.8N~~ ~~2N~~ dosing group were 0.063 mg/kg in muscle, 0.041 mg/kg in fat, 0.18 mg/kg in kidney, and 0.17 mg/kg in liver, expressed in parent compound equivalents. Prior to sacrifice, residues in milk were measured at various intervals in the high-dose group, and on day 28 in the three lower dose groups. In the 1.6N ~~1.8N~~ ~~2N~~ dose group, the residues (BYI 02960 + DFA) amounted to 0.043 mg/kg.

Residue levels reached a plateau in milk. Highest residue were determined at day 17 in the 44N ~~52N~~ ~~49N~~ milk samples, remaining at similar levels for the remainder of the study.

Depuration occurred quickly. Total BYI 02960 residues in milk, fat, liver, kidney, and muscle from the 44N ~~52N 49N~~ dose level cows declined from 0.81, 1.40, 1.59, 5.39, and 1.91 mg/kg, respectively, to <LOQ at 6-7 days after cessation of dose administration (=day 35-36 of the study).

Livestock will be exposed to DFA and flupyradifurone through feed as these are the major residues in plants. Based on the data from the feeding studies in which only parent flupyradifurone was dosed, transfer factors were proposed to estimate consumer exposure and to calculate MRLs for the sum of DFA and flupyradifurone. The feeding studies were designed to allow "material balancing" in order to evaluate levels and calculate transfer factors for both the total residue (parent BYI 02960 and DFA) and DFA, despite feeding parent compound only. Since this not an accepted approach, it was concluded in the expert meeting thatfeeding studies with DFA are required. In the meantime, the available study will be used for provisional MRL setting.

Residue intakes by livestock animals and humans

Intake by livestock

A dietary burden calculation was performed for ~~the sum of~~ flupyradifurone and DFA separately, resulting in a maximum dietary burden of 0.06, 0.20 and 0.19 ~~1.52, 2.61 and 2.57~~ ~~1.32, 2.73 and 2.50~~ mg BYI 02960/kg feed and 1.27, 2.40 and 2.36 mg DFA/kg feed for poultry, cattle and pigs, respectively.

Intake by humans

Chronic and acute exposure for the consumer was estimated using the proposed MRLs, the ADI of 0.064 ~~0.08~~ ~~0.12~~ mg/kg bw/d, the ARfD of 0.15 ~~0.35~~ mg/kg bw and EFSAs PRIMo model rev.2. The IEDI ~~TMDI~~ uses maximally 15.1% ~~47.3~~ ~~75.6~~% of the ADI for the WHO Cluster diet B. All other European (national) diets use less of the ADI.

The NESTI uses maximally 82.3 ~~69.2~~% ~~of the ARfD UK infants for oranges (import tolerance) and 69.2%~~ of the ARfD for German children for lettuce after refinement with the HR-P. All other commodities use less of the ARfD in other consumer diets.

2.4.3 Residues relevant to worker safety

Exposure of the worker is discussed under 2.3.4.

2.4.4 Proposed MRLs and compliance with existing MRLs

Flupyradifurone is a new active substance, and is not on the market yet. No MRLs have been set.

2.5 Environmental fate and behaviour

2.5.1 Definition of the residues relevant to the environment

Detailed guidance to define the environmental residue is available in SANCO/10329/2002 rev 2. final (Oct 2002) and SANCO/3268/2001 rev 4 final (Oct 2002). All metabolites accounted for >10% in aerobic soil treated with BYI 02960.

The components of the environmental residue are therefore as follows:

Residue definition for risk assessment:

Soil:	Flupyradifurone (BYI 02960), difluoroacetic acid (DFA), 6-chloronicotinic acid (6-CNA)
Surface Water:	BYI 02960, DFA, 6-CNA, BYI 02960-succinamide, BYI 02960-azabicyclosuccinmaide
Sediment:	BYI 02960, DFA, 6-CNA
Groundwater:	BYI 02960, DFA, 6-CNA
Air:	BYI 02960

Residue definition for monitoring:

Soil:	BYI 02960
Surface Water:	BYI 02960
Groundwater:	BYI 02960
Air:	BYI 02960

2.5.2 Fate and behaviour in soil

Route of degradation

Aerobic soil degradation

The route of Flupyradifurone (BYI 02960) degradation in soil was studied using different radiolabel positions, [pyrindinyl-methyl-^{14}C = PYM], [pyridine-2,6-^{14}C = PYR], [furanone-4-^{14}C = FUR] and [ethyl-1-^{14}C = ETH] BYI 02960. The data gathered in the aerobic soil metabolism studies demonstrated that BYI 02960 is degraded in soil.

When using the PYM label in four different European aerobic soils no major metabolites were detected. All formed metabolites were regarded as transient, which was confirmed by the high mineralization rate to $^{14}CO_2$, i.e. up to 58.6% of AR from [PYM-^{14}C] BYI 02960. The portions of not extractable residues (NER) were comparatively low throughout the study (max 16.8% of AR) in case of [PYM-^{14}C] BYI 02960. As minor transformation products BYI 02960-chloro (max. 1.8%) and BYI 02960-des-difluoroethyl (max. 0.4%) were identified (for structures see Figure 2.5.2-01).

In studies with the FUR label (four European and two US soils) similar results were obtained with the formation of NER (maximum 34.1% of AR) and extensive mineralization to $^{14}CO_2$ (up to 38.9% of AR). With the exception of $^{14}CO_2$ no major metabolites were formed in any of the soils. Degradation, amount of NER as well as of $^{14}CO_2$ formed in sterilized was significantly less than in non-sterile soils, indicating a biological component to the degradation/mineralization and formation of non-extractable residues from BYI 02960. Additionally, soil fractionation showed that even with extraction using strong base, BYI 02960 related residues remain bound to the solid (humin) fraction indicating very strong and irreversible binding to soil.

When using [ETH-^{14}C] BYI 02960 in three aerobic soils one major metabolite, identified as difluoroacetic acid (DFA; max. 33.9% of AR), and one very minor metabolite was detected and quantified. Again, significant amounts of $^{14}CO_2$ (up to 42.3% AR) were measured, indicating that mineralization of the test item and/or its metabolites occurred. The maximum amount of NER was relatively low at 17.9% of AR.

The biotransformation of [PYR-^{14}C] BYI 02960 was studied in one aerobic EU and two US soils. The mineralization to $^{14}CO_2$ was significant (max. 57.4, 20.2 and 36.1% of AR) with the formation of minor metabolites in two soils. However, in one US soil, one major metabolite, which was identified as 6-chloronicotinic acid (6-CNA), was formed at maximum of 17.1%. NER formation was in the range of max. 11.3 to 25.5% of AR in the three soils.

Considering the results from laboratory soil metabolism studies the major routes of degradation of BYI 02960, under aerobic conditions, are:

- cleavage of the difluoroethyl group producing difluoroacetic acid (DFA),
- cleavage of the molecule at the pyridinylmethylene bridge with subsequent oxidation to 6-CNA
- mineralization to CO_2 and formation of non-extractable residues.

Degradation under anaerobic conditions and due to photolysis on the soil are not relevant for BYI 02960.

The biotransformation of BYI 02960 was studied in several EU and US soils under standardized aerobic and anaerobic laboratory conditions, as well as in a terrestrial field dissipation study performed on six different sites in Europe. BYI 02960 was found to be moderately degradable in aerobic soil under laboratory as well as under field conditions. The clear bi-phasic degradation kinetics indicates that the compound is less available for biotransformation with time, probably due to a time-dependent sorption behavior in soil. The BYI 02960 residues remain stable under anaerobic conditions.

Figure 2.5.2-01 Proposed degradation pathway of BYI02960 in soil

Note: The degradates observed and the maximum values are those for all label positions and soils

Anaerobic soil degradation

Three studies investigating the degradation of [PYR-^{14}C], [FUR-^{14}C] and [ETH-^{14}C] BYI 02960 in soils under aerobic, then flooded anaerobic conditions showed that it can be expected that the amounts of BYI 02960 and its major aerobic soil metabolites DFA and 6-CNA remain stable under flooded field conditions. Degradation would be expected to continue according to the proposed overall pathway of degradation of BYI 02960 (see Figure 2.5.2-01) whenever the conditions in soil turn aerobic again.

Soil photolysis

Phototransformation of [PYM-^{14}C] and [FUR-^{14}C] BYI 02960 was studied on a loam soil. From the results it is concluded that direct phototransformation of BYI 02960 is not regarded as a relevant degradation process in the environment. The soil photolytic DT_{50} of BYI 02960 was 99.6 and 109.3 days.

Rate of degradation

Laboratory studies

The following Table 2.5.2-01 summarises the findings in the aerobic laboratory studies. The kinetics data for the metabolite 6-CNA indicate that it is very rapidly degraded in soil with a mean DT_{50} of < 1 week. The metabolite DFA indicated a slightly longer DT_{50}, i.e. it was calculated to be in the range of approx. 2 months.

Table 2.5.2-01 Trigger and modelling endpoints for BYI 02960 and its major soil metabolites

Soil	Dossier position of kinetic evaluation	DT_{50} (d) Trigger values	DT_{50} (d) Modelling input	Formation Fraction
Parent BYI 02960				
AX (Sandy loam)	IIA 7.2.1/01 IIA 7.2.1/07	63.4	169.1	-
AX (Sandy loam)	IIA 7.2.1/02 IIA 7.2.1/08	62.2	141.5	-
AX (Loamy sand)	IIA 7.2.1/04 IIA 7.2.1/09	62.0	210.2	-
AX geometric mean		**62.5**	**171.3**	
HF (Silt loam)	IIA 7.2.1/01 IIA 7.2.1/07	52.4	54.4	-
HF (Silt loam)	IIA 7.2.1/02 IIA 7.2.1/08	33.2	40.5	-
HF (Silt loam)	IIA 7.2.1/04 IIA 7.2.1/09	34.1	43.0	-
HF (Silt loam)	IIA 7.2.1/05 IIA 7.2.1/10	33.0	90.0	-
HF geometric mean		**37..4**	**54.0**	
HN (Loam)	IIA 7.2.1/01 IIA 7.2.1/07	120.0	157.5	-
HN (Silt loam)	IIA 7.2.1/02 IIA 7.2.1/08	98.3	157.5	-
HN geometric mean		**108.6**	**157.5**	
DD (Clay loam)	IIA 7.2.1/01 IIA 7.2.1/07	56.4	60.1	-
DD (Silty clay)	IIA 7.2.1/02 IIA 7.2.1/08	49.3	55.1	-
DD (Clay loam)	IIA 7.2.1/04 IIA 7.2.1/09	33.9	38.6	-
DD geometric mean		**45.5**	**50.4**	
Springfield (Silt loam)	IIA 7.2.1/03	228	164.4#	-
Springfield (Silt loam)	IIA 7.2.1/06	242	179.7#	-
Springfield geometric mean		**234.9**	**172.9**	
Sanger (Sandy loam)	IIA 7.2.1/03	58.3	55.5#	-
Sanger (Sandy loam)	IIA 7.2.1/06	56.3	58.8#	-
Sanger geometric mean		**56.8**	**57.1**	
Overall geometric mean *		**73.2**	**94.8**	**-**
Overall median *		**59.7**	**107.3**	
6-CNA				
Aldham's Farm (Sandy loam)	IIA 7.2.3/02	2.9	2.9	-
Flint Halls Farm (Clay)	IIA 7.2.3/02	2.2	2.2	-

B. Barns Farm (Loam)	IIA 7.2.3/02	5.3	5.3	-
HF (Silt loam)	IIA 7.2.3/03	3.1	3.0	0.266
Sanger (Sandy loam)	IIA 7.2.3/04	36.6	22.4#	0.694
Overall geometric mean		**5.2**	**4.7**	
Overall arithmetic mean				**0.480**
DFA				
DD (Clay loam)	IIA 7.2.3/05	44.9	32.0	0.9087
AX (Loamy sand)	IIA 7.2.3/05	73.6	73.6	0.5896
HF (Silt loam)	IIA 7.2.3/05	67.4	37.8	1.000
Overall geometric mean		**60.6**	**44.7**	
Overall arithmetic mean				**0.833**

normalised to pF2

* Geometric mean and median of the geometric means of the different soils

Field studies

Soil dissipation of BYI 02960 under European field conditions was investigated after application of BYI 02960 SL 200 on bare soil plots at sites in Monheim, Burscheid and Hanscheider Hof (all Germany), Great Chishill (United Kingdom), Albaro (Italy), and Vilobi d'Onyar (Spain). An application was also made in Chilly (Northern France), however the trial was aborted due to heavy rain shortly after application. The sites are located in the ecoregions Northern and Southern Europe.

In general, BYI 02960 residues remained in the upper 0-20 cm soil layer and only very small amounts (<LOQ) were translocated to a maximum depth of 30 cm. At one site (Vilobi d'Onyar, Spain), BYI 02960 residues just above the LOQ were found at day 13 up in the 20-30 cm soil layer. BYI 02960 dissipated from soil at all test sites with DT_{50} values ranging from 8.3 to 251 days.

The metabolite BYI 02960-DFA appeared in the upper soil layer 0-20 cm in amounts of up to 17.4 g/ha soil. However, the metabolite declined to values of max. 7.9 g/ha soil towards the end of the study (in Burscheid, Germany). An overview of the results for parent compound is given in Table 2.5.2-02.

The field data is not different from the laboratory data in that it does not result in different DT_{50}-values on average. The average DT_{50}-value for the non-normalized field studies is 38.4 days (geometric mean) and 40.0 days (median) comaperd to the laboratory DT_{50}-values of 72.2 and 59.7 days. A normalization might be conducted, since these values are not required for modelling this is not conducted.

Table 2.5.2-02 Results for BYI 02960 in field dissipation studies

Location: Trial No.	Soil type (USDA)	Kinetic model	DT50 [d]	DT90 [d]
Monheim (Germany): 09-2702-01	Sandy loam (0-100 cm)	DFOP	41.0	749
Great Chishill (United Kingdom): 09-2702-02	Clay loam (0-100 cm)	DFOP	251	>1000
Burscheid (Germany):	Silt loam (0-75 cm),	DFOP	42.8	484

09-2702-03	Loam (75-100 cm)			
Albaro (Italy): 09-2702-05	Clay loam (0-30 cm), Loam (30-75 cm), Sandy loam (75-100 cm)	DFOP	8.3	279
Vilobi d'Onyar (Spain): 09-2702-06	Loam (0-30 cm), Sandy loam (30-75 cm), Sandy clay loam (75-100 cm)	DFOP	22.6	215
Hanscheider Hof (Germany): 09-2702-07	Loam(0-100 cm)	DFOP	39.0	579
		Gemetric mean	**38.4**	**482.3**
		Median	**40.0**	**531.5**

PECsoil estimations

Calculations were based on a simple first tier approach (Excel sheet) assuming even distribution of the compound in upper 0-5 cm soil layer. A standard soil density of 1.5 g/cm^3 was assumed. Crop interception data which correspond to the intended growth stages were taken from the FOCUS groundwater guidance paper (FOCUS 2010).

Crop interception will reduce the amount of a compound reaching the soil and therefore this has been taken into account depending on the growth stage at application. The interception rates follow the recommendations of the FOCUS groundwater guidance paper (FOCUS 2010) provided in Table 2.5.2-03. As hop and lettuce have not been defined in the FOCUS groundwater guidance paper, vines and cabbage were chosen as surrogate crops. As there is currently no guidance on calculation of PEC_{soil} for greenhouse crops no specific calculation was performed and it is assumed that the glasshouse use is covered by the outdoor use on lettuce.

Flupyradifurone (BYI 02960)

The predicted environmental concentrations in soil (PEC_{soil}) of BYI 02960 were estimated using a simple first tier approach (Excel sheet). Detailed application data used for simulation of PEC_{soil} are compiled in Table 2.5.2-03. PEC soil were not calculated for the glasshouse uses of the product.

PEC_{soil} calculations were based on the DT_{50} of 0.2 days for the fast and 462 days for the slowly degrading compartments (DFOP, worst case of field dissipation studies, k_1=34.66 and k_2=0.0015 and g =0.27).

Table 2.5.2-03 Application pattern used for PEC_{soil} calculations of BYI 02960

Individual Crop	FOCUS Crop Used for Interception	Application				Amount Reaching the Soil per Season application [g a.s. /ha]
		Rate per Season [g a.s. /ha]	Interval [days]	Plant Interception [%]	BBCH Stage	
Hops, GAP – identical with simulation	Vines	1 x 150	-	60	31-75	1 x 60

Lettuce 1 (F), GAP – identical with simulation [1)]	Cabbage	1 x 125	-	25	12-49	1 x 94
Lettuce 2 (F), GAP – identical with simulation [2)]	Cabbage	1 x 125	-	70	41-49	1 x 37.5

[1)] Max 1 application per 24 months (biennial)
[2)] Max 1 application per 12 months (annual)
F = field use

To account for potential accumulation of BYI 02960 in soil (worst-case non-normalised DFOP DT_{90} > 365 days), long-term soil concentrations were calculated.

The PEC_{soil} and the time weighted average values (TWA_{soil}) of BYI 02960 are summarised in Table 2.5.2-04 for hops and in for lettuce Table 2.5.2-05.

Table 2.5.2-04 PEC_{soil} (actual) and TWA of BYI 02960 in hops in the upper 5 cm, DFOP decay

	Time [days]	BYI 02960 Hops, 1 x 150 g/ha	
		PEC_{soil} [mg/kg]	TWA_{soil} [mg/kg]
Initial	0	0.080	-
Short term	1	0.058	0.069
	2	0.058	0.066
	4	0.058	0.063
Long term	7	0.058	0.061
	14	0.057	0.059
	21	0.057	0.058
	28	0.056	0.058
	50	0.054	0.057
	100	0.050	0.054

Table 2.5.2-05 PEC_{soil} (actual) and TWA of BYI 02960 in lettuce in the upper 5 cm, DFOP decay (1st or 2nd cropping per year)

	Time [days]	BYI 02960 Lettuce 1 [1)], 1 x 125 g/ha (field use, interception 25%)		BYI 02960 Lettuce 2 [2)], 1 x 125 g/ha (field use, interception 70%)	
		PEC_{soil} [mg/kg]	TWA_{soil} [mg/kg]	PEC_{soil} [mg/kg]	TWA_{soil} [mg/kg]
Initial	0	0.125	-	0.050	-
Short term	1	0.091	0.108	0.036	0.043
	2	0.091	0.102	0.036	0.041
	4	0.091	0.098	0.036	0.039
Long term	7	0.090	0.095	0.036	0.038
	14	0.089	0.093	0.036	0.037
	21	0.088	0.091	0.035	0.037
	28	0.087	0.091	0.035	0.036
	50	0.085	0.089	0.034	0.035
	100	0.079	0.085	0.031	0.034

[1)] Max 1 application per 24 months (biennial)
[2)] Max 1 application per 12 months (annual)

Accumulation in soil

The potential accumulation of BYI 02960 in soil was calculated considering the following approaches:

- Maximum soil residue in first year: maximum soil residue calculated for one season.
- Long-term plateau concentration C_{min}: maximum of the lower saw tooth curve, which can be considered as background concentration after multiple year use.
- Long-term maximum concentration C_{max}: maximum of the upper saw tooth curve after multiple year use.
- Background C_{min} + maximum of one year in 5 cm depth: to the long-term background concentration C_{min} in a certain depth (e.g. 5, 10 or 20 cm), the maximum residue of one year (distributed in 5 cm) will be added, to take into account a conservative shallow distribution just after an annual application.

Table 2.5.2-06 Long-term soil concentrations of BYI 02960 following multi-year use

	Residues distributed over … [cm]	Seasonal $PEC_{s, max}$, max. soil residue in 1st year [mg/kg]	Long-term plateau / background conc. C_{min} [mg/kg]	Long-term maximum conc. C_{max} [mg/kg]	Background C_{min} + max. of 1 year in 5 cm [mg/kg]
Hops 1 x 150 g/ha	**5**	0.080	0.080	**0.160**	-
	10	0.040	0.040	0.080	0.120
	20	0.020	0.020	0.040	0.100
Lettuce 1 [1)] 1 x 125 g/ha	5	0.125	0.046	0.171	-
	10	0.063	0.023	0.085	0.148
	20	0.031	0.011	0.043	**0.136**
Lettuce 2 [2)] 1 x 125 g/ha	5	0.050	0.050	0.100	-
	10	0.025	0.025	0.050	0.075
	20	0.013	0.013	0.025	**0.063**

[1)] Max 1 application per 24 months (biennial)
[2)] Max 1 application per 12 months (annual)
In bold: Generally, for long-term assessments the substance distribution in soil for annual crops with tillage should be assumed over a depth of 20 cm (lettuce) and for perennial crops without tillage over a depth of 5 – 10 cm (e.g. hops).

Metabolites

Predicted environmental concentrations in soil were calculated for the major soil metabolites DFA and 6-CNA. These metabolites are not automatically "relevant" with regard to their environmental, biological, eco-toxicological or toxicological properties.

PEC_{soil} for the metabolites were calculated using the approach, scenarios and application rates described for the calculations for the parent compound in study IIIA, 9.4/01. Compound specific parameters are summarised in Table 2.5.2-07.

Table 2.5.2-07 Input parameters for PEC_{soil} for metabolites of BYI 02960

Compound	Max. DT_{50} [days]	Max. occurrence in soil [%]	Molar mass [g/mol]	Molar mass correction factor
Difluoroacetic acid	73.6	33.9	96.03	0.333
6-Chloronicotinic acid	36.6	17.1	157.56	0.546

The maximum PEC_{soil} values of metabolites of BYI 02960 are summarised in Table B.8.3-06.

Table 2.5.2-08 Hops and Lettuce: PEC_{soil} (max) of BYI 02960 metabolites

Crop	DFA $PEC_{soil, max}$ [mg/kg]	6-CNA $PEC_{soil, max}$ [mg/kg]
Hops 1 x 150 g/ha of parent	0.009	0.007
Lettuce 1, 1 x 125 g/ha of parent [1)]	0.014	0.012
Lettuce 2, 1 x 125 g/ha of parent [2)]	0.006	0.005

[1)] Max 1 application per 24 months (biennial)
[2)] Max 1 application per 12 months (annual)

Sorption parameters

Batch adsorption studies BYI 02960

Batch adsorption studies were performed for BYI 02960 and two major soil metabolites. The equilibrium sorption BYI 02960 was studied in two batch equilibrium studies in the laboratory at 20°C with six different soils and one time dependent sorption experiment. The data of the batch experiments were evaluated to derive Freudlich isotherms. The resulting arithmetic mean value for 1/n, Kf (ads) and Koc was 0.866, 2.263 (L/kg) and 91.8 (L/kg; Table 2.5.2-09). The desorption Kf (des) and the normalized K_{OC}(des) values were significantly higher (i.e. approx. 2 times) than those obtained for the adsorption phase, indicating that the test item once adsorbed to soil is not readily desorbed. BYI 02960 can be classified as intermediate mobile for adsorption and low mobile for desorption.

Table 2.5.2-09 Adsorption measurements for BYI 02960

Soil	Soil Type (USDA)	pH ($CaCl_2$) [-]	K_f [mL g^{-1}]	1/n [-]	$K_{f,oc}$ [mL g^{-1}]
Laacherhof AXXa	Sandy loam	6.2	2.077	0.8445	98.9
Hoefchen am Hohenseh 4a	Loam	6.6	2.213	0.8682	92.2
Hanscheiderhof	Loam	5.3	2.354	0.8643	107.0
Dollendorf II	Loam	7.2	3.822	0.8648	74.9
Sanger	Sandy Loam	6.8	0.597	0.9021	85.2
Springfield	Silt loam	6.5	1.554	0.8505	132.2
Arithmetic mean		**-**	**-**	**0.8657**	**98.4**

* $K_{oc,EQ}$ restults of the TDS included in the average caluclation

Time dependent sorption study BYI 02960

Time dependent sorption (TDS) of BYI 02960 was studied in four soils a maximum period of 120 days under aerobic conditions in the dark at ca. 20°C and 55% WHCmax. At the beginning of the study the distribution coefficient values RTDS were 1.46, 1.91, 3.35 and 4.66 mL/g for soils AX, HF, HN and DD, respectively. Based on results from the ageing time of 120 days, the RTDS values increased by a factor of 4.4, 2.9, 3.2 and 2.6 (mean of all four soils = 3.3).

A more detailed evaluation showed that the before-mentioned experimental data can be well described by a kinetic-sorption model, with excellent fits and reliable parameter estimates. Such time-dependent sorption data received for BYI 02960 constitute the prerequisite to adequately address the obvious TDS process in higher tier regulatory modelling, e.g. related to a potential groundwater contamination. For BYI 02960 a fitted mean K_{oc} of 80.2 L/kg (K_{om} of 46.5 L/kg) and the Freundlich

exponent 1/n of 0.8605 can be used in higher tier simulation runs (arithmetic mean of the 1/n of the four soils used for the batch sorption and identiacal as the soils used for TDS).

The RMS intended to use both the batch adsorption and time dependent sorption (TDS) K_{oc} and $K_{oc,eq}$ to obtain a arithmetic mean K_{oc}. The RMS did this on basis of the EFSA opinion on the science behind the guidance for scenario selection and scenario parameterisation for predicting environmental concentrations of plant protection products in soil (ESFA Journal 2012;10(2)2562). It is advised to use in each tier, all available relevant scientific information. The RMS was aware that in the draft guidance on how aged sorption studies for pesticides should be conducted, analysed and used in regulatory assessments (Beulke S. and Van Beimum W. 2012, DEFRA projects PS2235 and PS2244) a different opinion is presented, and not to combine the TDS and batch adsorption/desorption data. This UK guidance on TDS is not yet adopted in Europe and also the opinion is not adopted. After a discussion with the applicant and external experts, the RMS decided to not include the TDS $K_{oc,EQ}$ data in the first tier data set for the K_{oc}. The arithmetic mean K_{oc} value is 98.4 L/kg, this is the average of the six batch adsorption values.

Batch adsorption studies for metabolites

The equilibrium sorption of 6-chloronicotinic acid (6-CNA) was studied in experiments using five soils and an aquatic pond sediment. The arithmetic mean 1/n value for soil was 0.9495, adsorption Koc values in soils ranged from 70 to 258, averaging 88.0 (L/kg). Similar Kf and Kdes values for all soils and the pond sediment indicated a reversible equilibration between adsorption and first desorption phases. 6-CNA can be classified as medium to high mobile for adsorption and desorption.

The equilibrium sorption of difluoroacetic acid (DFA) was studied in experiments using five soils. The arithmetic mean 1/n value for soil was 0.8348, adsorption Koc values ranged from 1.7 to 9.5, averaging 6.8 (L/kg). The Koc(des) values were 12.7 to 57.1 times higher than the Koc(ads) values, indicating a strong binding of the test item to the soil. BYI 02960-DFA can be classified as very mobile for adsorption and low mobile for desorption.

The results of the batch adsorption analyses performed for the metabolites are reported below (see Table 2.5.2-10 and Table 2.5.2-11).

Table 2.5.2-10 Adsorption measurements for 6-chloronicotinic acid

Soil	Soil Type (USDA)	pH ($CaCl_2$) [-]	K_f [mL g^{-1}]	1/n [-]	$K_{f,oc}$ [mL g^{-1}]
Clayton	Loamy Sand	6.2	1.027	1.0069	70
Leland	Silt loam	6.6	0.569	0.9706	129
Leland	Clay	7.5	0.833	0.8941	70
San Juan Bautista	Clay loam	8.3	0.690	0.9262	84
Arithmetic mean		**-**	**-**	**0.9495**	**88.3**

Table 2.5.2-11 Adsorption measurements for BYI 02960-DFA

Soil	Soil Type (USDA)	pH ($CaCl_2$) [-]	K_f [mL g^{-1}]	1/n [-]	$K_{f,oc}$ [mL g^{-1}]
Hoefchen am Hohenseh 4a	Silt loam	6.5	1.74	0.9053	9.5
Hanscheiderhof	Loam	5.8	1.07	0.8013	7.8
Dollendorf II	Clay loam	7.4	1.78	0.9579	8.2
Sanger	Sandy Loam	6.0	0.594	0.6902	6.7
Springfield	Silty clay loam	6.5	0.274	0.8194	1.7
Arithmetic mean		**-**	**-**	**0.8348**	**6.8**

PECgw estimations

The predicted environmental concentrations in groundwater (PEC_{gw}) for BYI 02960 and its metabolites were calculated using the simulation model FOCUS PEARL (version 4.4.4) and FOCUS PELMO (4.4.3). Detailed application data used for simulation of PEC_{gw} are compiled in Table 2.5.2-12 to Table 2.5.2-16. Simulations were conducted for annual applications as well as applications performed every second year.

Although there is no currently agreed model for simulating groundwater concentrations following the use in greenhouses as a conservative approach the simulation was performed as an outdoor application considering two applications in accordance with the GAP.

Table 2.5.2-12 Comparison of simulated and actual use pattern

Individual Crop	FOCUS Crop Used for Interception	Application				Amount Reaching the Soil per Season application [g a.s. /ha]
		Rate per Season [g a.s. /ha]	Interval [days]	Plant Interception [%]	BBCH Stage	
Hops, GAP	-	1 × 150	-	60	31 – 75	1 × 60.00
Tier 1, Tier 2b (TDS)						
Hops (every ear), Simulation 1	Vines	1 × 150	-	60	31 – 75	1 × 60.00
Hops (every 2nd year), Simulation 2	Vines	1 × 150	-	60	31 – 75	1 × 60.00
Tier 2a (DFOP)						
Hops (every ear), Simulation 1	Vines	1 × 150	-	60	31 – 75	171 [s] / 129 [f]
Hops (every 2nd year), Simulation 2	Vines	1 × 150	-	60	31 – 75	171 [s] / 129 [f]

[f] used for fast compartment
[s] used for slow compartment

Table 2.5.2-13 Comparison of simulated and actual use pattern (outdoor use)

Individual Crop	FOCUS Crop Used for Interception	Application				Amount Reaching the Soil per Season application [g a.s. /ha]
		Rate per Season [g a.s. /ha]	Interval [days]	Plant Interception [%]	BBCH Stage	
Lettuce 1 (GAP, every year)	-	1 × 125	-	70	41 - 49	1 × 37.5
Lettuce 1 (GAP, every 2nd year)	-	1 × 125	-	25	12 - 49	1 × 93.75
Tier 1, Tier 2b (TDS)						

Lettuce 1 (every year) (early and late), Simulation 1	Cabbage	1 × 125	-	70	41 - 49	1 × 37.5
Lettuce 1 (every 2nd year) (early and late), Simulation 2	Cabbage	1 × 125	-	25	12 - 49	1 × 93.75
Tier 2a (DFOP)						
Lettuce 1 (every year) (early and late) Simulation 1	Cabbage	1 × 125	-	70	41 - 49	42.75[s] / 32.25[f]
Lettuce 1 (every 2nd year) (early and late) Simulation 2	Cabbage	1 × 125	-	25	12 - 49	106.875[s] / 80.625[f]

F = Field use (GAP: "Lettuce 1" = 1 x 125 g/ha)
[f] used for the fast compartment
[s] used for the slow compartment

Table 2.5.2-14 Comparison of simulated and actual use pattern (glasshouse use)

Individual Crop	FOCUS Crop Used for Interception	Application				Amount Reaching the Soil per Season application [g a.s. /ha]
		Rate per Season [g a.s. /ha]	Interval [days]	Plant Interception [%]	BBCH Stage	
Lettuce 2 (every year), GAP	-	2 × 125	10	2 x 25	12 - 49	2 × 93.75
	Tier 1, Tier 2b (TDS)					
Lettuce 2 (every year) early	Cabbage	2 × 125	10	2 x 25	12 - 49	2 × 93.75
Lettuce 2 (every year) late	Cabbage	2 × 125	10	2 x 25	12 - 49	2 × 93.75
	Tier 2a (DFOP)					
Lettuce 2 (every year) ealy	Cabbage	2 × 125	10	2 x 25	12 - 49	2×106.875[s] / 2× 80.625[f]
Lettuce 2 (every year) late	Cabbage	2 × 125	10	2 x 25	12 - 49	2×106.875[s] / 2× 80.625[f]

G = Glasshouse use (GAP: "Lettuce 2" = 2 x 125 g/ha)
[f] used for the fast compartment
[s] used for the slow compartment

Application dates for the simulation runs were defined following the crop event dates of the respective crop and scenario as given by FOCUS (2009).

Table 2.5.2-15 First application dates and related information for BYI 02960 as used for the simulation runs (offset is relevant only for relative application dates, two sets of data are provided for crops with two seasons)

Individual crop	Hops	Hops
Repeat Interval for App. Events	Every Year	Every 2nd Year
Application Technique	Spray	Spray
Absolute / Relative to	Emergence	Emergence
Scenario	1st App. Date/(Julian day)/Offset to crop event	
Chateaudun	29 Apr (119) 28	29 Apr (119) 28
Hamburg	29 May (149) 28	29 May (149) 28
Jokioinen	-	-
Kremsmuenster	29 May (149) 28	29 May (149) 28
Okehampton	-	-
Piacenza	29 Apr (119) 28	29 Apr (119) 28

Individual crop	Hops	Hops
Repeat Interval for App. Events	Every Year	Every 2nd Year
Application Technique	Spray	Spray
Absolute / Relative to	Emergence	Emergence
Scenario	1st App. Date/(Julian day)/Offset to crop event	
Porto	12 Apr (102) 28	12 Apr (102) 28
Sevilla	28 Apr (118) 28	28 Apr (118) 28
Thiva	12 Apr (102) 28	12 Apr (102) 28

Table 2.5.2-16 First application dates and related information for BYI 02960 as used for the simulation runs (two sets of data are provided for crops with two seasons)

Individual crop	**Lettuce 1, 1st cropping**	**Lettuce 1, 2nd cropping**	**Lettuce 1, 1st cropping**	**Lettuce 1, 2nd cropping**	**Lettuce 2, 1st cropping**	**Lettuce 2, 2nd cropping**
Repeat Interval for App. Events	**Every Year**	**Every Year**	**Every 2nd Year**	**Every 2nd Year**	**Every Year**	**Every Year**
Application Technique	**Spray**	**Spray**	**Spray**	**Spray**	**Spray**	**Spray**
Absolute / Relative to	**Emergence**	**Emergence**	**Emergence**	**Emergence**	**Emergence**	**Emergence**
Scenario	**1st App. Date (Julian day)**	**1st App. Date (Julian day)**	**1st App. Date (Julian day)**	**1st App. Date (Julian day)**	**1st App. Date (Julian day)**	**1st App. Date (Julian day)**
Chateaudun	25 Jun (176)	21 Sep (264)	25 Apr (115)	05 Aug (217)	25 Jun (176)	04 Aug (216)
Hamburg	25 Jun (176)	21 Sep (264)	25 Apr (115)	05 Aug (217)	25 Jun (176)	04 Aug (216)
Jokioinen	22 Aug (234)	-	27 May (147)	-	22 Aug (234)	-
Kremsmuenster	25 Jun (176)	21 Sep (264)	25 Apr (115)	05 Aug (217)	25 Jun (176)	04 Aug (216)
Okehampton	-	-	-	-	-	-
Piacenza	-	-	-	-	-	-
Porto	02 Jun (64)	12 Oct (285)	07 Mar (66)	05 Aug (217)	02 Jun (153)	05 Aug (217)
Sevilla	10 May (130)	17 Aug (229)	06 Mar (65)	20 Jun (171)	10 May (130)	19 Jun (170)
Thiva	05 Nov (309)	-	21 Aug(233)	-	05 Nov (309)	-

Further input parameters for PEC_{gw} modelling of BYI 02960 are summarised in Table 2.5.2-17 for BYI 02960 and in Table 2.5.2-18 for the metabolites. Parameters used for degradation pathway in PEARL and PELMO are depicted in Table 2.5.2-19.

For Tier 1, a geometric mean of half-lives derived from SFO and from the slow compartment of the DFOP model was used to obtain a conservative model input.

For Tier 2a, (DFOP) according to FOCUS (2009), DFOP degradation kinetics was considered in leaching modelling based on the procedure described in FOCUS (2006).

To obtain common DFOP parameters over all soils the following procedure was applied: Firstly, all degradation curves following SFO kinetics on Tier-1 were converted to an equivalent DFOP model where the Tier-1 SFO-DT_{50} was assigned equally (g=0.5) to the slow and fast degrading compartment. For those soils where the slow compartment of DFOP was already used for modelling at Tier-1, the corresponding shorter DT_{50} of the fast compartment and g (fraction of total amount applied to the compartment) of the DFOP fit were additionally considered. Finally, the DFOP parameters were calculated as mean over all soils.

For leaching modelling the application rate was doubled and assigned to both compartments according to g of 0.43. Then, two separate leaching simulations were performed: one for the fast compartment using DT_{50fast} of 33 days and one using DT_{50slow} of 95 days. Both PEC_{gw} values were summed up and divided by two to get the final result.

Tier 2b (TDS): Time dependent sorption (TDS) data of BYI 02960 on four soils were derived via curve fitting (study IIA, 7.4.1/05). These parameters constitute the prerequisite to adequately address TDS processes in regulatory exposure modelling (Beulke, S. and van Beinum, W. 2012)[1]. Experimental soil data were re-calculated to fit the TDS model, resulting in a geometric mean DT_{50_eq} of 57.9 days, an arithmetic mean K_{OM_eq} of 47.5 L/kg, an arithmetic mean f_ne of 0.5821, a geometric mean $k_{_des}$ of 0.0309 and an arithmetic mean Freundlich exponent of 0.8605 from the optimized parameters of the four data sets. The PECgw calculations given in this part are based on the average/geometric mean parameters obtained from the individual soil. At the end of this paragraph a comparison with the PECgw from each separate soil data set is given as was proposed in the draft guidance on TDS (Beulke, S. and van Beinum, W. 2012).

Table 2.5.2-17 Substance specific and model related input parameters for PEC_{gw} calculation of BYI 02960

Parameter	Unit	BYI 02960		
		Tier 1	Tier 2a (DFOP)	Tier 2b (TDS)
Common				
Molar Mass	[g/mol]	288.7	288.7	288.7
Solubility	[mg/L]	3200	3200	3200
Vapour Pressure	[Pa]	9×10^{-7}	9×10^{-7}	9×10^{-7}
Freundlich Exponent		0.8657	0.8657	0.8605
Plant Uptake Factor *		0.5	0.5	0.5
Walker Exponent		0.7	0.7	0.7
PEARL Parameters				
Substance Code		BUTn	BUTf[f] / BUTs[s]	BUTk
DT_{50}	[days]	94.8	33.4[f] / 94.8[s]	~~58.0~~ 57.9
Molar Activ. Energy	[kJ/mol]	65.4	65.4	65.4
K_{om}	[mL/g]	57.1	57.1	~~46.5~~ 47.5
Desorp. Rate Coeff.	[1/days]	-	-	0.0309
Equ. Factor		-	-	0.5821
PELMO Parameters				
Substance Code		AS	AS	AS
Rate Constant	[1/day]	0.00730	0.0207[f] / 0.0073[s]	0.01195
Q_{10}		2.58	2.58	2.58
K_{oc}	[mL/g]	98.4	98.4	80.2

[f] DT_{50} used for the fast compartment
[s] DT_{50} used for the slow compartment
* systemic active substance (see mode of action volume B.3.10)

[1] Beulke, S. and van Beinum, W. (2012). Guidance on how aged sorption studies for pesticides should be conducted, analysed and used in regulatory assessments. The Food and Environment Research Agency. Sand Hutton, YO41 1LZ, UK.

Table 2.5.2-18 Substance specific and model related input parameters for PEC_{gw} calculation of BYI 02960 metabolites

Parameter	Unit	Difluoroacetic acid	6-Chloronicotinic acid
Common			
Molar Mass	[g/mol]	96	157.6 [a)]
Solubility	[mg/L]	500000	1430
Vapour Pressure	[Pa]	1.00×10^{-10}	1.00×10^{-10}
Freundlich Exponent		0.835	0.949
Plant Uptake Factor *		~~0.5~~ 0.0	~~0.5~~ 0.0
Walker Exponent		0.7	0.7
PEARL Parameters			
Substance Code		DFA	CNA
DT_{50}	[days]	44.7	4.7
Molar Activ. Energy	[kJ/mol]	65.4	65.4
K_{om}	[mL/g]	3.9	51
Desorp. Rate Coeff.	[1/days]	0	0
Equ. Factor		0	0
PELMO Parameters			
Substance Code		A1	B1
Rate Constant	[1/day]	0.01551	0.14748
Q_{10}		2.58	2.58
K_{oc}	[mL/g]	6.8	88

[a)] The sum of formation fractions of both metabolites is > 1. In order to run the modelling with PELMO, the molar mass of 6-CNA was set to 451 g/mol, resulting from $M_{auxiliary}$ (6-CNA) = M (6-CNA) × 0.478 / (1-0.833) = 451 g/mol

* Data provided to justify that the plant uptake factor for the metabolites was 0.5 was not accepted by the RMS (Schriever et al, 2013 [2])

Table 2.5.2-19 Degradation pathway related parameters for BYI 02960 and its metabolites

Tier 1, Tier 2a (DFOP), Tier 2a (TDS)	Degradation fraction from → to (FOCUS PEARL)	0.83 BUT -> DFA 0.48 BUT -> CNA
Tier 1, Tier 2a (DFOP, slow compartment)	Degradation rate from → to (FOCUS PELMO)	0.0060780 Active Substance → A1 0.0012180 Active Substance → B1 0.0155070 A1 → <BR/CO_2 0.1474780 B1 → <BR/CO_2
Tier 2a (DFOP, fast compartment)	Degradation rate from → to (FOCUS PELMO)	0.017287 Active Substance → A1 0.003466 Active Substance → B1 0.015507 A1 → <BR/CO_2 0.147478 B1 → <BR/CO_2
Tier 2a (TDS)	Degradation rate from → to (FOCUS PELMO)	0.0099550 Active Substance → A1 0.0019960 Active Substance → B1 0.0155070 A1 → <BR/CO_2 0.1474780 B1 → <BR/CO_2

Flupyradifurone in hops

The 80th percentile groundwater concentrations of BYI 02960 in hops are given in Table 2.5.2-20 (Tier 1), Table 2.5.2-21 (Tier 2a; DFOP) and Table 2.5.2-22 (Tier 2a; TDS).

[2] Schriever, C., Gottesbüren, B., Ebert, D., Sur, R. Schmitt, W. (January 2013). ECPA background paper. Plant uptake of compounds via roots sytem – A hydroponic test system to determine plant uptake as input parameter for leaching models. Study not accepted by the RMS.

Table 2.5.2-20 Tier 1 - PEC_{gw} of BYI 02960 – Use in Hops

Scenario	BYI 02960			
	Hops (every year), 1 x 150 g/ha, 60 % interception		Hops (every 2nd year), 1 x 150 g/ha, 60 % interception	
	PEARL PEC_{gw} [µg/L]	PELMO PEC_{gw} [µg/L]	PEARL PEC_{gw} [µg/L]	PELMO PEC_{gw} [µg/L]
Châteaudun	0.457	0.409	0.197	0.169
Hamburg	0.567	0.591	0.271	0.230
Kremsmuenster	0.409	0.461	0.169	0.189
Piacenza	0.377	0.458	0.162	0.210
Porto	0.214	0.275	*0.091*	0.115
Sevilla	0.219	*0.060*	*0.090*	*0.022*
Thiva	0.178	0.154	*0.073*	*0.058*

In italics: values pass the trigger of 0.1 µg/L

Considering the Tier 1 simulations predicted concentrations of BYI 02960 in groundwater are below the trigger of 0.1 µg/L in the scenario Sevilla (model PELMO) when applied every year and in scenarios Sevilla and Thiva (both PEARL and PELMO) and Porto (PEARL), when applied every second year.

Higher tier calculations have additionally been performed at Tier 2a using DFOP simulations and TDS.

Table 2.5.2-21 Tier 2a (DFOP) - PEC_{gw} of BYI 02960 – Use in Hops

Scenario	BYI 02960			
	Hops (every year), 1 x 150 g/ha		Hops (every 2nd year), 1 x 150 g/ha	
	PEARL PEC_{gw} [µg/L]	PELMO PEC_{gw} [µg/L]	PEARL PEC_{gw} [µg/L]	PELMO PEC_{gw} [µg/L]
Châteaudun	0.275	0.247	0.119	0.103
Hamburg	0.338	0.358	0.164	0.140
Kremsmuenster	0.246	0.277	0.102	0.114
Piacenza	0.224	0.275	*0.097*	0.126
Porto	0.130	0.166	*0.055*	*0.069*
Sevilla	0.134	*0.037*	*0.055*	*0.014*
Thiva	0.108	*0.094*	*0.045*	*0.036*

In italics: values pass the trigger of 0.1 µg/L

When considering the use of DFOP kinetics predicted concentrations of BYI 02960 in groundwater are below the trigger of 0.1 µg/L in the scenario Sevilla and Thiva (model PELMO) when applied every year and in scenarios Porto, Sevilla and Thiva (both PEARL and PELMO) and Piacenza (PEARL), when applied every second year.

Table 2.5.2-22 Tier 2b (TDS) - PEC_{gw} of BYI 02960 – Use in Hops

Scenario	BYI 02960			
	Hops (every year), 1 x 150 g/ha, 60 % interception		Hops (every 2nd year), 1 x 150 g/ha, 60 % interception	
	PEARL PEC_{gw} [µg/L]	PELMO PEC_{gw} [µg/L]	PEARL PEC_{gw} [µg/L]	PELMO PEC_{gw} [µg/L]
Châteaudun	0.112	0.094	*0.042*	*0.032*
Hamburg	0.156	0.144	*0.061*	*0.053*
Kremsmuenster	0.117	0.134	*0.042*	*0.047*
Piacenza	0.109	0.152	*0.040*	*0.063*
Porto	*0.048*	*0.075*	*0.017*	*0.025*
Sevilla	*0.039*	*0.006*	*0.012*	*0.002*
Thiva	*0.026*	*0.020*	*0.008*	*0.006*

In italics: values pass the trigger of 0.1µg/L

When considering TDS behaviour the predicted concentrations of BYI 02960 in groundwater are below the trigger of 0.1 µg/L in the scenario Porto, Sevilla and Thiva (models PEARL and PELMO) when applied every year and in all scenarios (both PEARL and PELMO), when applied every second year.

A safe use has been demonstrated as the trigger of 0.1 µg/L is met at tier 1, considering application every year, for the scenario Sevilla (PELMO). For the higher tier calculations applying DFOP the trigger was met in Sevilla and Thiva for annual applications. When considering higher tier calculations with TDS the trigger was also met in several scenarios for annual uses and all scenarios for use every second year.

Flupyradifurone in lettuce

The PEC_{gw} values for the metabolites for the different EU scenarios are presented for glasshouse uses in the following tables. The glasshouse uses (multiple applications) are presented solely since these will result in the highest PECgw.

Table 2.5.2-23 Tier 1 PEC_{gw} of BYI 02960 – Use in Lettuce 2, multiple applications (glasshouse use)

Scenario	BYI 02960 (glasshouse use / every year)			
	Lettuce 2, 1st cropping, every year, 2 x 125 g/ha		Lettuce 2, 2nd cropping, every year, 2 x 125 g/ha	
	PEARL PEC_{gw} [µg/L]	PELMO PEC_{gw} [µg/L]	PEARL PEC_{gw} [µg/L]	PELMO PEC_{gw} [µg/L]
Châteaudun	1.132	0.786	1.543	1.073
Hamburg	2.179	1.879	2.843	2.540
Jokioinen	0.985	0.769	-	-
Kremsmuenster	1.503	1.330	1.819	1.654
Porto	0.877	0.980	1.691	1.787
Sevilla	*0.078*	*0.018*	0.116	*0.024*
Thiva	0.997	0.717	-	-

In italics: values pass the trigger of 0.1µg/L

For the greenhouse uses, considering the tier 1 approach the trigger value of 0.1µg/L is met for the scenario Sevilla for early season, both models and for late season one model even considering the conservative approach for the greenhouse uses.

Table 2.5.2-24 Tier 2a (DFOP) PEC_{gw} of BYI 02960 – Use in Lettuce 2, multiple applications (glasshouse use)

Scenario	BYI 02960 (glasshouse use / every year / DFOP)			
	Lettuce 2, 1st cropping, every year, 2 x 125 g/ha		Lettuce 2, 2nd cropping, every year, 2 x 125 g/ha	
	PEARL PEC_{gw} [µg/L]	PELMO PEC_{gw} [µg/L]	PEARL PEC_{gw} [µg/L]	PELMO PEC_{gw} [µg/L]
Châteaudun	0.682	0.474	0.927	0.684
Hamburg	1.310	1.125	1.706	1.534
Jokioinen	0.598	0.473	-	-
Kremsmuenster	0.898	0.797	1.089	0.991
Porto	0.524	0.576	1.008	1.070
Sevilla	*0.050*	*0.012*	*0.076*	*0.016*
Thiva	0.606	0.438	-	-

In italics: values pass the trigger of 0.1µg/L

For the greenhouse use in lettuce, using the Tier 2a approach (DFOP) the trigger is met for the scenario Sevilla for annual application even considering the conservative use of the outdoor model.

Table 2.5.2-25 Tier-2b (TDS) PEC_{gw} of BYI 02960 – Use in Lettuce 2, multiple applications (glasshouse use)

Scenario	BYI 02960 (glasshouse use / every year / TDS)			
	Lettuce 2, 1st cropping, every year, 2 x 125 g/ha		Lettuce 2, 2nd cropping, every year, 2 x 125 g/ha	
	PEARL PEC_{gw} [µg/L]	PELMO PEC_{gw} [µg/L]	PEARL PEC_{gw} [µg/L]	PELMO PEC_{gw} [µg/L]
Châteaudun	0.245	0.155	0.334	0.214
Hamburg	0.677	0.520	0.905	0.784
Jokioinen	0.185	0.145	-	-
Kremsmuenster	0.475	0.404	0.568	0.502
Porto	0.259	0.355	0.508	0.627
Sevilla	*0.006*	*0.001*	*0.007*	*0.001*
Thiva	0.181	0.119	-	-

In italics: values pass the trigger of 0.1µg/L

For the greenhouse use in lettuce, using the Tier 2b approach (TDS) the trigger is met for the scenario Sevilla for annual applications, even considering the conservative use of the outdoor model. For all the uses considered safe use can be demonstrated in at least 1 scenario even considering tier 1 calculations. When higher tier (Tier 2) simulations are considered the calculated PEC_{gw} is less than the trigger in several scenarios.

Flupyradifurone metabolites in hops

PEC_{gw} for the metabolites were calculated using the approach, scenarios and application described for the calculations for the parent compound in studies IIIA 9.6.1. Compound specific input data for the metabolites are summarised together with the data of the parent compound in Table 2.5.2-18.

Findings: The 80th percentile concentrations for BYI 02960 metabolites for the different EU scenarios are presented in Table 2.5.2-26 for Tier 1.

Table 2.5.2-26 Tier 1 PEC_{gw} of BYI 02960 metabolites (after application of 150 g BYI 02960/ha) in hops

Scenario	Difluoroacetic acid (DFA) PEC_{gw} [µg/L]				6-Chloronicotinic acid (6-CNA) PEC_{gw} [µg/L]			
	every year		every 2nd year		every year		every 2nd year	
	PEARL	PELMO	PEARL	PELMO	PEARL	PELMO	PEARL	PELMO
Châteaudun	1.554	1.377	0.754	**0.697**	*0.010*	*0.009*	*0.005*	*0.004*
Hamburg	1.699	1.830	0.793	0.852	*0.011*	*0.012*	*0.006*	*0.005*
Kremsmuenster	1.064	1.228	**0.525**	**0.596**	*0.009*	*0.010*	*0.004*	*0.004*
Piacenza	1.059	0.957	**0.511**	**0.448**	*0.008*	*0.009*	*0.004*	*0.005*
Porto	**0.696**	0.758	**0.318**	**0.350**	*0.006*	*0.007*	*0.002*	*0.003*
Sevilla	0.976	0.776	**0.479**	**0.428**	*0.005*	*0.002*	*0.002*	*0.001*
Thiva	0.912	0.916	**0.450**	**0.426**	*0.004*	*0.004*	*0.002*	*0.002*

In italics: values pass the trigger of 0.1µg/L
In bold: values pass the trigger of 0.75 µg/L

PEC_{gw} values for the metabolite DFA are above 0.1 µg/L for all scenarios (both PEARL and PELMO), and for applications taking place every year or every 2nd year, respectively. PEC_{gw} values for the metabolite 6-CNA are below 0.1 µg/L for all scenarios (both PEARL and PELMO) for applications taking place every year or every 2nd year, respectively. There are no concerns for groundwater for this metabolite.

Additionally, for difluoroacetic acid (DFA), higher tier have been performed, the results are summarized below Table 2.5.2-27 and Table 2.5.2-28.

Table 2.5.2-27 Tier 2a (DFOP): PEC_{gw} of difluoroacetic acid (DFA) in hops (1 x 150 g/ha of parent)

Scenario	Difluoroacetic acid (DFA) PEC_{gw} [µg/L]			
	every year		every 2nd year	
	PEARL	PELMO	PEARL	PELMO
Châteaudun	1.459	1.299	**0.722**	**0.681**
Hamburg	1.631	1.771	0.772	0.836
Kremsmuenster	1.020	1.189	**0.515**	**0.592**
Piacenza	0.953	0.904	**0.473**	**0.439**
Porto	**0.608**	**0.691**	**0.277**	**0.315**
Sevilla	0.916	**0.715**	**0.459**	**0.424**
Thiva	0.817	0.838	**0.415**	**0.395**

In italics: values pass the trigger of 0.1µg/L
In bold: values pass the trigger of 0.75 µg/L

Table 2.5.2-28 Tier 2b (TDS): PEC_{gw} of difluoroacetic acid (DFA) in hops (1 x 150 g/ha of parent)

Scenario	Difluoroacetic acid (DFA) PEC_{gw} [µg/L]			
	every year		every 2nd year	
	PEARL	PELMO	PEARL	PELMO
Châteaudun	1.475	1.295	**0.709**	**0.667**
Hamburg	1.637	1.777	0.756	**0.827**
Kremsmuenster	1.025	1.208	**0.498**	**0.569**
Piacenza	0.970	0.946	**0.481**	**0.429**
Porto	**0.641**	**0.723**	**0.288**	**0.330**
Sevilla	0.898	**0.732**	**0.439**	**0.408**
Thiva	0.809	0.841	**0.394**	**0.387**

In italics: values pass the trigger of 0.1µg/L
In bold: values pass the trigger of 0.75 µg/L

The groundwater concentration of the metabolite 6-CNA did not exceed the trigger in any scenario at Tier 1. The PEC in groundwater for the metabolite DFA may exceed the 0.1 µg/L and also the 0.75 µg/L trigger, the concentration was < 10 µg/L in all scenarios.

Flupyradifurone metabolites in lettuce

The PEC_{gw} values for the metabolites for the different EU scenarios are presented for glasshouse uses in the following tables.

Table 2.5.2-29 Tier 1 PEC_{gw} of BYI 02960 metabolites (glasshouse use in lettuce 2, 1st cropping)

Scenario	Glasshouse use Lettuce 2, 1st cropping, 2 x 125 g/ha of parent			
	Difluoroacetic acid (DFA) PEC_{gw} [µg/L]		6-chloronicotinic acid (6-CNA) PEC_{gw} [µg/L]	
	PEARL	PELMO	PEARL	PELMO
Châteaudun	3.792	3.026	*0.022*	*0.016*
Hamburg	6.542	5.246	*0.038*	*0.036*
Jokioinen	6.606	5.918	*0.021*	*0.018*
Kremsmuenster	3.748	3.411	*0.028*	*0.026*
Porto	2.145	1.863	*0.020*	*0.022*
Sevilla	1.257	0.949	*0.002*	*0.001*
Thiva	2.875	2.419	*0.019*	*0.015*

In italics: values pass the trigger of 0.1 µg/L
In bold: values pass the trigger of 0.75 µg/L

Table 2.5.2-30 Tier 1 PEC_{gw} of BYI 02960 metabolites (glasshouse use in lettuce 2, 2nd cropping)

Scenario	Glasshouse use Lettuce 2, 2nd cropping, 2 x 125 g/ha of parent			
	Difluoroacetic acid (DFA) PEC_{gw} [µg/L]		6-chloronicotinic acid (6-CNA) PEC_{gw} [µg/L]	
	PEARL	PELMO	PEARL	PELMO
Châteaudun	4.287	3.547	*0.029*	*0.021*
Hamburg	6.976	6.277	*0.050*	*0.048*
Kremsmuenster	3.992	3.664	*0.034*	*0.031*
Porto	2.893	2.692	*0.035*	*0.038*
Sevilla	1.739	1.356	*0.003*	*0.001*

In italics: values pass the trigger of 0.1 µg/L
In bold: values pass the trigger of 0.75 µg/L

Table 2.5.2-31 Tier 2a (DFOP) PEC_{gw} of difluoroacetic acid (DFA) (glasshouse use in lettuce 2)

Scenario	Difluoroacetic acid (DFA) PEC_{gw} [µg/L]			
	Glasshouse use Lettuce 2, 1st cropping, 2 x 125 g/ha of parent (DFOP)		Glasshouse use Lettuce 2, 2nd cropping, 2 x 125 g/ha of parent (DFOP)	
	PEARL	PELMO	PEARL	PELMO
Châteaudun	3.511	2.857	4.267	3.627
Hamburg	6.305	5.095	7.008	6.341
Jokioinen	6.390	5.751	-	-
Kremsmuenster	3.573	3.331	3.970	3.761
Porto	1.863	1.703	2.959	2.773
Sevilla	1.098	0.827	1.669	1.283
Thiva	3.020	2.649	-	-

In italics: values pass the trigger of 0.1 µg/L
In bold: values pass the trigger of 0.75 µg/L

Table 2.5.2-32 Tier 2b (TDS): PEC_{gw} of difluoroacetic acid (DFA) (glasshouse use in lettuce 2)

Scenario	Difluoroacetic acid (DFA) PEC_{gw} [µg/L]			
	Glasshouse use Lettuce 2, 1st cropping, 2 x 125 g/ha of parent (DFOP)		Glasshouse use Lettuce 2, 2nd cropping, 2 x 125 g/ha of parent (DFOP)	
	PEARL	PELMO	PEARL	PELMO
Châteaudun	3.605	2.894	4.127	3.474
Hamburg	6.353	5.297	6.836	6.244
Jokioinen	6.230	5.829	-	-
Kremsmuenster	3.685	3.402	3.948	3.647
Porto	2.059	1.833	2.760	2.650
Sevilla	1.185	0.917	1.589	1.280
Thiva	2.727	2.416	-	-

In italics: values pass the trigger of 0.1 µg/L
In bold: values pass the trigger of 0.75 µg/L

Considering the use in the glasshouses the PEC_{gw} values for the metabolite DFA are above 0.1 µg/L for all scenarios (both PEARL and PELMO), and for applications taking place every year, at Tier 1 and Tier 2. PEC_{gw} values for the metabolite 6-CNA are below 0.1 µg/L for all scenarios (both PEARL and PELMO) at tier 1, and for applications taking place every year, respectively. There are no concerns for groundwater for this metabolite.

The groundwater concentration of the metabolite 6-CNA did not exceed the trigger in any scenario. The PEC in groundwater for the metabolite DFA may exceed the 0.1 µg/L and also the 0.75 µg/L trigger, the concentration was < 10 µg/L in all scenarios The relevance of the metabolite has been assessed in accordance with Sanco/221/2000–rev.10 (2003): “Guidance Document on the Assessment of the Relevance of Metabolites in Groundwater”, and the metabolite was not relevant in terms of efficacy (see IIA, 8.14.1), genotoxicity and toxicity (see IIA, 5.8/01 - 05) and has also been considered in the dietary risk assessment (see IIA, 6.9). Please note: DFA was considered in the dietary risk assessment since it is a constituent of the plant residue definition.

2.5.3 Fate and behaviour in water

Hydrolysis

BYI 02960 proved to be stable under sterile hydrolytically conditions at all pHs.

Photo degradation in water

Sterile buffer

[^{14}C] BYI 02960 photolytically degraded in sterile buffer (pH 7) with a half-life of 13.8 experimental hours. Based on this finding, BYI 02960 should degrade within a few days in the aqueous environment if exposed to sunlight. The major degradates (>10% of applied radioactivity) were identified as [^{14}C] BYI 02960-succinamide and [^{14}C] BYI 02960-azabicyclosuccinamide.

Natural waters

BYI 02960 photolytically degraded in sterile natural water with a half-life of 14.0 experimental hours. The major degradates (>10% of applied radioactivity) were identified as BYI 02960-succinamide and BYI 02960-azabicyclosuccinamide. A minor degradation product was identified as BYI 02960-deschlorohydroxy-succinamide. In the dark controls BYI 02960 was stable. Based on the experimental half-life of 14.0 hours, the half-life of BYI 02960 under environmental conditions in Tokyo, Japan was calculated to be 3.8 days. The phototransformation study in natural water is regarded more relevant for the aqueous environment as the pure and sterile buffer study.

Biodegradability

Ready biodegradability

A respective study was not performed. The parent compound BYI 02960 is considered to be “Not Readily Biodegradable (as shown by the studies IIA, 7.8.2/01; IIA, 7.8.3/01 and IIA, 7.8.3/02).

Water/sediment studies

Five different water/sediment studies were submitted in the dossier. An anerobic water sediment study, two aerobic water sediment studies (one with one label and one with two labels). The fourth water sediment study was with the metabolite DFA. The final study was an outdoor microcosm study.

Anaerobic conditions

BYI 02960 dissipated from aqueous phase to sediment with a half-life of 7.2 and 9.6 days for Lawrence and Pikeville test system, respectively. With respect to the entire test system, BYI 02960 is regarded stable under anaerobic condition, no major metabolite were formed.

Aerobic conditions

[PYR] BYI 02960 is microbially degraded in aerobic water/sediment systems. The DT_{50} values for BYI 02960 in the entire water/sediment systems were 193.1 and 246.9 days for Hönniger Weiher and Angler Weiher, respectively. The only major transformation products were to carbon dioxide and formation of NER .

[FUR] and [ETH] BYI 02960 is translocated from the water phase into the sediment where it is slowly degraded and mineralized. Major transformation products were mineralization to carbon dioxide, formation of NER and one metabolite, DFA, (max. 6.9% of AR). The DT_{50} values for BYI 02960 in the entire water/sediment systems were 208.2 [FUR], 202.4 [ETH] and 246.1 [FUR], 285.0 [ETH] days for Hönniger Weiher and Angler Weiher, respectively.

The dissipation of DFA from the supernatant water phase was characterized by translocation into the sediment and by slowly degradation. With the exception of mineralization to carbon dioxide and formation of NER no major metabolite was found. The DT_{50} values for DFA in the entire water/sediment systems were 106.5 (persistence), 109.0 (modelling) and 967.1 (persistence) 567.2 (modelling) days for Hönniger Weiher and Angler Weiher, respectively.

Microcosm study

BYI 02960 dissipated from the supernatant water phase with a mean DT_{50} of 81 days due to translocation into the sediment and by degradation. The overall degradation (mean of 95.1 days) was faster under the prevailing outdoor conditions in comparison to the laboratory water sediment studies. Considering that BYI 02960 is rapidly degraded by photolysis there may be an enhanced degradation effect of sunlight under the outdoor test conditions.

Table 2.5.3-01 Selected models and endpoints for BYI 02960 (anaerobic dark experiment)

System	kinetic model	chi^2 error	r^2	DT_{50} [days]	DT_{90} [days]
total system					
Lawrence, KS	DFOP	4.1	0.0576	> 1000	> 1000
Pikerville, NC	DFOP	0.66	0.9821	415.0	> 1000
			geometric mean	**664**	**>1000**
water					
Lawrence, KS	DFOP	6.0	0.9867	7.2	> 1000
Pikerville, NC	DFOP	9.0	0.9827	9.6	> 1000

Table 2.5.3-02 Selected models and endpoints for BYI 02960 (aerobic dark experiment)

System	kinetic model	chi² error	r²	DT50 [d]	DT90 [d]
total system					
Hönniger Weiher [PYR] label	SFO	1.2	0.9577	193.1	641.3
Angler Weiher [PYR] label	SFO	1.3	0.9721	246.9	820.1
Hönniger Weiher [FUR] label	SFO	1.5	-	208.2	691.6
Angler Weiher [FUR] label	SFO	1.3	-	246.1	817.4
Hönniger Weiher [ETH] label	SFO	1.6	-	202.4	672.2
Angler Weiher [ETH] label	SFO	1.0	-	285.0	946.9
			geometric mean	**228.1**	**757.7**
water					
Hönniger Weiher [PYR] label	FOMC	1.6	0.9888	8.5	174.6
Angler Weiher [PYR] label *	DFOP	4.4	0.9886	34.5	181.8
Hönniger Weiher [FUR] label *	DFOP	4.1	-	48.5	161.0
Angler Weiher [FUR] label *	DFOP	2.9	-	123.8	411.2
Hönniger Weiher [ETH] label *	DFOP	4.9	-	50.2	116.9
Angler Weiher [ETH] label *	DFOP	2.4	-	117.5	390.3

* DT_{50} and DT_{90} value of the slow degradation phase as a worst case scenario for risk assessment

Table 2.5.3-03 Selected models and endpoints for DFA (aerobic dark experiment)

System	kinetic model	chi² error	r²	DT50 [days]	DT90 [days]
total system					
Hönniger Weiher	SFO	2.9	0.9692	109.0	362.2
Angler Weiher	SFO	3.0	0.5323	567.2	> 1000
			geometric mean	**248.6**	**601.8**
water					
Hönniger Weiher *	DFOP	1.6	0.9949	75.3	250.3
Angler Weiher	SFO	4.8	0.5088	371.5	> 1000

* DT_{50} and DT_{90} value of the slow degradation phase as a worst case scenario for risk assessment

Table 2.5.3-04 Selected models and endpoints for BYI 02960 in microcosm systems

System	kinetic model	chi² error	r²	DT50 [d]	DT90 [d]
total system					
10 ug/L microcosm	SFO	-	-	74.1	
100 ug/L microcosm	SFO	-		116	
			geometric mean	**92.7**	
water					
10 ug/L microcosm	SFO	-	-	62.1	
100 ug/L microcosm	SFO	-	-	99.1	

The degradation route of BYI 02960 in aqueous environments is shown in Figure B.8.4.4-01.

Figure 2.5.3-01 Proposed route of degradation of BYI 02960 in water/sediment systems (BYI 02960 rapidly partitioned into the sediment phase, DFA is mainly present in water phase)

Note: The degradates to be observed as well as the given maximum values are highly dependent on radiolabel and kind of study considered; Ph = photo-transformation

PECsw estimations

Calculation of PEC values for the active substance and metabolites according to FOCUS

$FOCUS_{sw}$ is a four step tiered approach:

Step 1: In this, the most conservative step, all inputs are considered as a single loading to the water body and a worst-case PEC_{sw} and PEC_{sed} is calculated.

Step 2: A refinement is made whereby individual loadings into the water body from different entry routes are considered. Scenarios are also considered for Northern and Southern Europe separately but no specific crop scenarios are defined.

Step 3: An exposure assessment using realistic worst-case scenarios is made. The scenarios are representative of agricultural conditions in Europe and consider weather, soil, crop and different water-bodies. Simulations use the models PRZM, MACRO and TOXSWA.

Step 4: PEC values are refined by considering mitigation measures or specific scenario descriptions on a case-by-case basis.

The PEC values in water and sediment were calculated at Step 1, 2, 3 and 4 for the parent substance. Only global maximum values were calculated, which can be used as conservative estimates of the actual and time-weighted average values assuming no degradation. Study design, compound characteristics and application were the same as for the PEC_{sw} calculations as for the PEC_{sed}.

Note: There are currently no European guidelines for the assessment of exposure of surface water from the use in glasshouses, therefore it was assumed that the use in glasshouses on lettuce is covered by the outdoor field use even considering the different use pattern.

Flupyradifurone (BYI 02960)

Predicted environmental concentrations in surface water and sediment (PEC_{sw} and PEC_{sed}) of BYI 02960 have been calculated for the use in hop and lettuce in Europe employing the tiered FOCUS Surface Water approach. All relevant entry routes of a compound into surface water (principally a combination of spray drift and runoff/erosion or drain flow) were considered in these calculations.

Details of the parameters used in the calculations are summarised in Table 2.5.3-05.

As there is currently no established model for the assessment of exposure of surface water following application in greenhouses the use is assumed to be covered by the field application even considering the higher use rate in greenhouses as the route of entry into surface water would be expected to be much lower following the use in greenhouses.

Table 2.5.3-05 Comparison of actual and calculated use pattern (for FOCUS step 1 & 2)

Individual Crop	FOCUS Crop Used for Interception	Application				Amount Reaching the Soil per Season application [g a.i. /ha]
		Rate per Season [g a.i. /ha]	Interval [days]	Plant Interception [%]	Growth Stage	
Hops, GAP	hops	1× 150	-	-	31-75	-
Hops, simulation		1× 150	-	50 (average crop cover)	31-75	1 x 75
Lettuce 1 (F), GAP	leafy vegetables	1 x 125	-	-	12-49	-
Lettuce 1 (F), simulation [1)] *		1 x 125	-	25 (minimal crop cover)	12-49	1 x 93.75
Lettuce 2 (F), GAP	leafy vegetables	1 x 125	-	-	41-49	-
Lettuce 2 (F), simulation [2)] *		1 x 125	-	50 (average crop cover)	41-49	1 x 63.5

[1)] Maximal 1 use per 24 months (minimal crop cover)
[2)] Maximal 1 use per 12 months (average crop cover)
* First or second cropping per year
F = field use

At FOCUS Step 3 actual application dates were determined by the PAT (pesticide application timer) included within SWASH. Details on application timing are summarised in Table 2.5.3-06.

Table 2.5.3-06 Application dates of BYI 02960 (FOCUS Step 3)

Parameter	Hops		Lettuce 1		Lettuce 2	
PAT start date rel./absolute	Absolute		Absolute		Absolute	
Appl. Method (appl. Type)	ground spray (CAM 2)		ground spray (CAM 2)		ground spray (CAM 2)	
No of appl.	1		1		1	
PAT window range	98		var. Range		30	
Appl. Interval	1		1		1	
Application Details	PAT Start Date (Julian Day)	Appl. Date	PAT Start Date (Julian Day)	Appl. Date	PAT Start Date (Julian Day)	Appl. Date
D1 (1st)	-	-	-	-	-	-
D2 (1st)	-	-	-	-	-	-
D3 (1st)	-	-	30-Apr (120)	04-May	30-Jun (181)	08-Jul
D3 (2nd)	-	-	09-Aug (221)	17-Sep	02-Oct (275)	01-Oct
D4 (1st)	-	-	18-May (138)	18-May	24-Aug (236)	27-Aug
D5 (1st)	-	-	-	-	-	-
D6 (1st)	-	-	21-Aug (233)	25-Aug	05-Nov (309)	06-Nov
D6 (2nd)	-	-	-	-	-	-
R1 (1st)	01-May(121)	02-May	25-Apr (115)	26-Apr	25-Jun (176)	29-Jun
R1 (2nd)	-	-	04-Aug (216)	20-Aug	27-Sep (270)	06-Oct
R2 (1st)	-	-	07-Mar (66)	22-Mar	02-Jun (153)	04-Jun
R2 (2nd)	-	-	06-Aug (218)	06-Aug	21-Oct (294)	24-Oct
R3 (1st)	-	-	06-Mar (65)	10-Mar	10-May (130)	18-May
R3 (2nd)	-	-	20-Jun (171)	25-Jun	24-Aug (236)	28-Aug
R4 (1st)	-	-	06-Mar (65)	06-Mar	10-May (130)	27-May
R4 (2nd)	-	-	20-Jun (171)	23-Jun	24-Aug (236)	27-Aug

Compound specific input data are summarised in Table 2.5.3-07.

Table 2.5.3-07 Substance specific and model related input parameter for PEC_{sw} calculation of BYI 02960

Parameter	Unit	BYI 02960
Molar Mass	g/mol	288.7
Water Solubility	mg/L	3200
Vapour pressure	Pa	9.1×10^{-7}
K_{oc}	mL/g	98.4
Freundlich Exponent (1/n)		0.8657
Degradation		
Soil	days	94.8
Total System	days	228
Water	days	228
Sediment	days	228 / 1000*
Max Occurrence		
Water / Sediment	%	100
Soil	%	100

* used for FOCUS STEP 3 and 4

Step 1 and 2

The maximum PEC values for Steps 1 and 2 are given in Table 2.5.3-08.

Table 2.5.3-08 Maximum PEC_{sw} and PEC_{sed} values for BYI 02960 at Step 1 & 2

Crop Application rate	FOCUS Step	$PEC_{sw, max}$ [µg/L]	$PEC_{sed, max}$ [µg/kg]
Hops 1 x 150 g/ha	1	53.86	43.49
	2 (N-EU)	13.07	12.49
	2 (S-EU)	17.36	16.70
Lettuce 1 1 x 125 g/ha (F) [1)] *	1	37.89	36.24
	2 (N-EU)	6.410	6.249
	2 (S-EU)	11.78	11.51
Lettuce 2 1 x 125 g/ha (F) [2)] *	1	37.89	36.24
	2 (N-EU)	3.191	3.091
	2 (S-EU)	5.337	5.196

[1)] Maximal 1 use per 24 month
[2)] Maximal 1 use per 12 month
* First or second cropping per year

Step 3

The maximum PEC_{sw} and PEC_{sed} values for relevant FOCUS Step 3 scenarios are given in Table 2.5.3-09 and 2.5.3-10. Time dependent PEC values or time-weighted average concentrations are not included in this summary, because they were not used in the risk assessment. However, all values are given in the report.

Table 2.5.3-09 Maximum PEC_{sw} and PEC_{sed} of BYI 02960 for relevant scenarios at Step 3 following application to hops

Step 3 Scenario	BYI 02960: Hops, 1 x 150 g/ha		
	Entry route	$PEC_{sw, max}$ [µg/L]	$PEC_{sed, max}$ [µg/kg]
R1, pond	Spray drift	0.394	0.795
R1, stream	Spray drift	5.531	0.362

Table 2.5.3-10 Maximum PECsw and PEC_{sed} values of BYI 02960 for relevant scenarios at Step 3 following application to lettuce, field applications

Step 3 Scenario	BYI 02960: Lettuce 1 [1)*], 1 x 125 g/ha (F)			BYI 02960: Lettuce 2 [2)*], 1 x 125 g/ha (F)		
	Entry route	$PEC_{sw, max}$ [µg/L]	$PEC_{sed, max}$ [µg/kg]	Entry route	$PEC_{sw, max}$ [µg/L]	$PEC_{sed, max}$ [µg/kg]
D3 (ditch, 1st)	Spray drift	0.830	0.380	Spray drift	0.813	0.339
D3 (ditch, 2nd)	Spray drift	0.840	0.460	Spray drift	0.811	0.321
D4 (pond, 1st)	Drainage	1.035	4.545	Drainage	0.983	3.755
D4 (stream, 1st)	Spray drift	0.794	1.772	Drainage	0.969	1.395
D6 (ditch, 1st)	Drainage	1.268	1.766	Drainage	1.206	1.959
R1 (pond, 1st)	Run-off	0.060	0.162	Run-off	0.337	0.751
R1 (stream, 1st)	Run-off	0.858	0.211	Run-off	2.827	0.698
R1 (pond, 2nd)	Run-off	0.097	0.254	Run-off	0.139	0.411
R1 (stream, 2nd)	Run-off	1.186	0.334	Run-off	2.189	0.415
R2 (stream, 1st)	Run-off	1.586	0.521	Run-off	1.215	0.513
R2 (stream, 2nd)	Run-off	0.940	0.342	Run-off	1.044	0.246
R3 (stream, 1st)	Run-off	2.226	0.469	Run-off	3.698	0.796
R3 (stream, 2nd)	Run-off	3.570	1.011	Run-off	3.297	0.964
R4 (stream, 1st)	Spray drift	0.522	0.054	Run-off	0.830	0.241
R4 (stream, 2nd)	Run-off	4.808	1.255	Run-off	2.954	0.803

[1)] Maximal 1 use per 24 month
[2)] Maximal 1 use per 12 month
* First or second cropping per year
F = Field use

Step 4

The maximum PEC_{sw} and PEC_{sed} values for relevant FOCUS Step 4 scenarios with mitigation options are given in the following tables.

Table 2.5.3-11 Maximum PEC_{sw} and PEC_{sed} values of BYI 02960 at Step 4 including drift reduction (without buffer) – Hops (1 x 150 g/ha)

Step 4		BYI 02960: Hops, 1 x 150 g/ha							
Buffer Width & Type	FOCUS Scenario	PECsw [µg/L] Drift Reduction				PECsed [µg/kg] Drift Reduction			
		25%	50%	75%	90%	25%	50%	75%	90%
0m (drift)	R1 (pond, 1st)	0.296	0.197	0.099	0.039	0.607	0.415	0.218	0.094
	R1 (stream, 1st)	4.149	2.766	1.383	0.553	0.273	0.184	0.093	0.072

Table 2.5.3-12 Maximum PEC_{sw} and PEC_{sed} values of BYI 02960 at Step 4 including buffer zones and drift reduction – Hops (1 x 150 g/ha)

Step 4		BYI 02960: Hops, 1 x 150 g/ha							
Buffer Width & Type	FOCUS Scenario	PEC_{sw} [µg/L] Drift Reduction				PEC_{sed} [µg/kg] Drift Reduction			
		0%	50%	75%	90%	0%	50%	75%	90%
5m (drift)	R1 (pond, 1st)	0.445	0.223	0.111	0.045	0.891	0.465	0.244	0.105
	R1 (stream, 1st)	4.515	2.258	1.129	0.452	0.297	0.151	0.076	0.071
10m (drift & run-off)	R1 (pond, 1st)	0.253	0.126	0.063	0.025	0.522	0.273	0.142	0.061
	R1 (stream, 1st)	2.354	1.177	0.589	0.235*	0.157	0.080	0.04	0.031
15m (drift & run-off)	R1 (pond, 1st)	0.141	0.071	0.035	0.014*	0.302	0.158	0.083	0.036
	R1 (stream, 1st)	1.554	0.777	0.388	0.155*	0.104	0.053	0.032	0.03
20m (drift & run-off)	R1 (pond, 1st)	0.078	0.039	0.02*	0.008*	0.174	0.091	0.048	0.021
	R1 (stream, 1st)	0.708	0.354	0.177*	0.071*	0.048	0.025	0.017	0.016

* reduction of the overall drift percentage is >95%, compared to STEP3, in case drift leads to PECsw max.

The mitigation from 10 m onwards includes spray drift and concurrent run-off buffer. However, as can be seen from the linear decrease of PEC_{sw} values with increasing drift reduction, the PEC_{sw} is always drift dominated and the run-off buffer does not drive the PEC_{sw}.

The step 4 PEC_{sw} for the pond scenario with 5 m buffer and 0% drift reduction is higher compared to the step 3 value. This is due to the default buffer width of 6 m already included in step 3 calculations resulting in a lower drift percentage of 2.63% compared to 2.97% in step 4.

Table 2.5.3-12 Maximum PEC_{sw} and PEC_{sed} values of BYI 02960 at Step 4 including drift reduction (without buffer) – Lettuce 1 (1 x 125 g/ha, field use)

Step 4		BYI 02960: Lettuce 1, 1 x 125 g/ha (field use)							
Buffer Width & Type	FOCUS Scenario	PECsw [µg/L] Drift Reduction				PECsed [µg/kg] Drift Reduction			
		25%	50%	75%	90%	25%	50%	75%	90%

0m (drift)	D3 (ditch, 1st)	0.632	0.434	0.235	0.117	0.335	0.334	0.333	0.332
	D3 (ditch, 2nd)	0.643	0.446	0.249	0.130	0.419	0.399	0.398	0.397
	D4 (pond, 1st)	1.034	1.034	1.033	1.033	4.538	4.531	4.524	4.520
	D4 (stream, 1st)	0.721	0.721	0.721	0.721	1.771	1.771	1.771	1.771
	D6 (ditch, 1st)	1.268	1.268	1.268	1.268	1.766	1.765	1.765	1.764
	R1 (pond, 1st)	0.055	0.050	0.045	0.043	0.148	0.134	0.119	0.111
	R1 (stream, 1st)	0.858	0.858	0.858	0.858	0.210	0.208	0.207	0.206
	R1 (pond, 2nd)	0.092	0.087	0.082	0.079	0.240	0.225	0.210	0.201
	R1 (stream, 2nd)	1.186	1.186	1.186	1.186	0.333	0.332	0.330	0.330
	R2 (stream, 1st)	1.586	1.586	1.586	1.586	0.520	0.520	0.519	0.518
	R2 (stream, 2nd)	0.940	0.940	0.940	0.940	0.342	0.341	0.341	0.340
	R3 (stream, 1st)	2.226	2.226	2.226	2.226	0.466	0.462	0.459	0.456
	R3 (stream, 2nd)	3.570	3.570	3.570	3.570	1.006	1.000	0.995	0.991
	R4 (stream, 1st)	0.392	0.261	0.131	0.074	0.041	0.027	0.025	0.024
	R4 (stream, 2nd)	4.808	4.808	4.808	4.808	1.253	1.251	1.249	1.248

Table 2.5.3-13 Maximum PEC_{sw} and PEC_{sed} values of BYI 02960 at Step 4 including buffer zones and drift reduction – Lettuce 1 (1 x 125 g/ha, field use, 5 and 10m buffer zones)

Step 4		BYI 02960: Lettuce 1 , 1 x 125 g/ha (field use)							
Buffer Width & Type	FOCUS Scenario	PEC_{sw} [µg/L] Drift Reduction				PEC_{sed} [µg/kg] Drift Reduction			
		0%	50%	75%	90%	0%	50%	75%	90%
5m (drift)	D3 (ditch, 1st)	0.252	0.145	0.091	0.059*	0.333	0.332	0.332	0.332
	D3 (ditch, 2nd)	0.265	0.158	0.105	0.073*	0.398	0.397	0.397	0.396
	D4 (pond, 1st)	1.035	1.034	1.033	1.033	4.541	4.529	4.523	4.520
	D4 (stream, 1st)	0.721	0.721	0.721	0.721	1.771	1.771	1.771	1.771
	D6 (ditch, 1st)	1.268	1.268	1.268	1.268	1.765	1.764	1.764	1.764
	R1 (pond, 1st)	0.057	0.049	0.045	0.042	0.154	0.130	0.117	0.11
	R1 (stream, 1st)	0.858	0.858	0.858	0.858	0.208	0.207	0.206	0.206
	R1 (pond, 2nd)	0.094	0.086	0.082	0.079	0.247	0.221	0.208	0.201
	R1 (stream, 2nd)	1.186	1.186	1.186	1.186	0.331	0.330	0.33	0.329
	R2 (stream, 1st)	1.586	1.586	1.586	1.586	0.519	0.518	0.518	0.518
	R2 (stream, 2nd)	0.940	0.940	0.940	0.940	0.341	0.341	0.34	0.34
	R3 (stream, 1st)	2.226	2.226	2.226	2.226	0.460	0.458	0.456	0.455
	R3 (stream, 2nd)	3.570	3.570	3.570	3.570	0.997	0.993	0.991	0.99
	R4 (stream, 1st)	0.191	0.095	0.074	0.074	0.025	0.025	0.024	0.024
	R4 (stream, 2nd)	4.808	4.808	4.808	4.808	1.250	1.248	1.247	1.247
10m (drift and run-off)	D3 (ditch, 1st)	0.151	0.094	0.066*	0.049*	0.332	0.332	0.332	0.332
	D3 (ditch, 2nd)	0.165	0.108	0.080*	0.063*	0.397	0.397	0.396	0.396
	D4 (pond, 1st)	1.034	1.033	1.033	1.033	4.534	4.526	4.522	4.519
	D4 (stream, 1st)	0.721	0.721	0.721	0.721	1.771	1.771	1.771	1.771
	D6 (ditch, 1st)	1.268	1.268	1.268	1.268	1.764	1.764	1.764	1.764
	R1 (pond, 1st)	0.029	0.022	0.019	0.018	0.083	0.064	0.055	0.049
	R1 (stream, 1st)	0.389	0.389	0.389	0.389	0.097	0.097	0.097	0.096
	R1 (pond, 2nd)	0.043	0.037	0.034	0.032	0.122	0.103	0.094	0.088
	R1 (stream, 2nd)	0.540	0.540	0.540	0.540	0.157	0.157	0.157	0.156
	R2 (stream, 1st)	0.716	0.716	0.716	0.716	0.228	0.228	0.228	0.228
	R2 (stream, 2nd)	0.422	0.422	0.422	0.422	0.151	0.151	0.151	0.151
	R3 (stream, 1st)	1.009	1.009	1.009	1.009	0.219	0.217	0.216	0.216
	R3 (stream, 2nd)	1.630	1.630	1.630	1.630	0.452	0.450	0.449	0.448
	R4 (stream, 1st)	0.101	0.051	0.034	0.034	0.012	0.012	0.012	0.011
	R4 (stream, 2nd)	2.184	2.184	2.184	2.184	0.585	0.584	0.584	0.584

* reduction of the overall drift percentage is >95%, compared to STEP3, in case drift leads to PECsw max.

Table 2.5.3-14 Maximum PEC_{sw} and PEC_{sed} values of BYI 02960 at Step 4 including buffer zones and drift reduction – Lettuce 1 (1 x 125 g/ha, field use, 15 and 20m buffer zone)

Step 4	BYI 02960: Lettuce 1, 1 x 125 g/ha (field use)

Buffer Width & Type	FOCUS Scenario	PEC_{sw} [µg/L] Drift Reduction				PEC_{sed} [µg/kg] Drift Reduction			
		0%	50%	75%	90%	0%	50%	75%	90%
15m (drift and run-off)	D3 (ditch, 1st)	0.115	0.076*	0.057*	0.045*	0.332	0.332	0.332	0.332
	D3 (ditch, 2nd)	0.129	0.090*	0.071*	0.059*	0.397	0.396	0.396	0.396
	D4 (pond, 1st)	1.034	1.033	1.033	1.033	4.531	4.524	4.521	4.519
	D4 (stream, 1st)	0.721	0.721	0.721	0.721	1.771	1.771	1.771	1.771
	D6 (ditch, 1st)	1.268	1.268	1.268	1.268	1.764	1.764	1.764	1.764
	R1 (pond, 1st)	0.018	0.013	0.011	0.009	0.055	0.039	0.032	0.027
	R1 (stream, 1st)	0.204	0.204	0.204	0.204	0.053	0.052	0.052	0.052
	R1 (pond, 2nd)	0.025	0.021	0.018	0.017	0.076	0.060	0.052	0.047
	R1 (stream, 2nd)	0.283	0.283	0.283	0.283	0.086	0.085	0.085	0.085
	R2 (stream, 1st)	0.375	0.375	0.375	0.375	0.122	0.122	0.122	0.122
	R2 (stream, 2nd)	0.220	0.220	0.220	0.220	0.081	0.081	0.081	0.081
	R3 (stream, 1st)	0.528	0.528	0.528	0.528	0.119	0.118	0.117	0.117
	R3 (stream, 2nd)	0.856	0.856	0.856	0.856	0.245	0.243	0.242	0.242
	R4 (stream, 1st)	0.069	0.035	0.018	0.018	0.008	0.006	0.006	0.006
	R4 (stream, 2nd)	1.144	1.144	1.144	1.144	0.318	0.317	0.317	0.316
20m (drift and run-off)	D3 (ditch, 1st)	0.097	0.067*	0.052*	0.045*	0.332	0.332	0.332	0.332
	D3 (ditch, 2nd)	0.110	0.081*	0.066*	0.057*	0.397	0.396	0.396	0.396
	D4 (pond, 1st)	1.033	1.033	1.033	1.033	4.529	4.523	4.520	4.518
	D4 (stream, 1st)	0.721	0.721	0.721	0.721	1.771	1.771	1.771	1.771
	D6 (ditch, 1st)	1.268	1.268	1.268	1.268	1.764	1.764	1.764	1.764
	R1 (pond, 1st)	0.016	0.012	0.010	0.009	0.050	0.037	0.03	0.026
	R1 (stream, 1st)	0.204	0.204	0.204	0.204	0.052	0.052	0.052	0.052
	R1 (pond, 2nd)	0.024	0.020	0.018	0.016	0.071	0.057	0.05	0.046
	R1 (stream, 2nd)	0.283	0.283	0.283	0.283	0.086	0.085	0.085	0.085
	R2 (stream, 1st)	0.375	0.375	0.375	0.375	0.122	0.122	0.122	0.122
	R2 (stream, 2nd)	0.220	0.220	0.220	0.220	0.081	0.081	0.081	0.081
	R3 (stream, 1st)	0.528	0.528	0.528	0.528	0.118	0.117	0.117	0.117
	R3 (stream, 2nd)	0.856	0.856	0.856	0.856	0.244	0.243	0.242	0.242
	R4 (stream, 1st)	0.053	0.026	0.018	0.018	0.007	0.006	0.006	0.006
	R4 (stream, 2nd)	1.144	1.144	1.144	1.144	0.317	0.317	0.317	0.316

* reduction of the overall drift percentage is >95%, compared to STEP3, in case drift leads to PECsw max.

Table 2.5.3-15 Maximum PEC_{sw} and PEC_{sed} values of BYI 02960 at Step 4 including buffer zones and drift reduction – Lettuce 2 (1 x 125 g/ha, field use, 5 and 10m buffer zones)

Step 4		BYI 02960: Lettuce 2 , 1 x 125 g/ha (field use)							
Buffer Width & Type	FOCUS Scenario	PEC_{sw} [µg/L] Drift Reduction				PEC_{sed} [µg/kg] Drift Reduction			
		0%	50%	75%	90%	0%	50%	75%	90%
5m (drift)	D3 (ditch, 1st)	0.234	0.127	0.073	0.040*	0.188	0.187	0.187	0.186
	D3 (ditch, 2nd)	0.234	0.127	0.073	0.041*	0.177	0.173	0.172	0.172
	D4 (pond, 1st)	0.982	0.978	0.976	0.975	3.750	3.732	3.723	3.718
	D4 (stream, 1st)	0.969	0.969	0.969	0.969	1.395	1.394	1.394	1.394
	D6 (ditch, 1st)	1.206	1.206	1.206	1.206	1.958	1.958	1.958	1.958
	R1 (pond, 1st)	0.334	0.325	0.320	0.317	0.744	0.723	0.712	0.706
	R1 (stream, 1st)	2.827	2.827	2.827	2.827	0.694	0.693	0.692	0.692
	R1 (pond, 2nd)	0.137	0.130	0.127	0.125	0.404	0.379	0.367	0.359
	R1 (stream, 2nd)	2.189	2.189	2.189	2.189	0.411	0.410	0.409	0.409
	R2 (stream, 1st)	1.215	1.215	1.215	1.215	0.510	0.509	0.508	0.508
	R2 (stream, 2nd)	1.044	1.044	1.044	1.044	0.243	0.242	0.242	0.241
	R3 (stream, 1st)	3.698	3.698	3.698	3.698	0.780	0.775	0.772	0.771
	R3 (stream, 2nd)	3.297	3.297	3.297	3.297	0.949	0.945	0.942	0.941
	R4 (stream, 1st)	0.830	0.830	0.830	0.830	0.239	0.238	0.238	0.238
	R4 (stream, 2nd)	2.954	2.954	2.954	2.954	0.797	0.796	0.795	0.794

Step 4		BYI 02960: Lettuce 2 , 1 x 125 g/ha (field use)							
Buffer Width & Type	FOCUS Scenario	PEC_{sw} [µg/L] Drift Reduction				PEC_{sed} [µg/kg] Drift Reduction			
		0%	50%	75%	90%	0%	50%	75%	90%
10m (drift and run-off)	D3 (ditch, 1st)	0.133	0.076	0.048*	0.031*	0.187	0.187	0.186	0.186
	D3 (ditch, 2nd)	0.133	0.077	0.048*	0.031*	0.173	0.172	0.172	0.172
	D4 (pond, 1st)	0.980	0.977	0.976	0.975	3.740	3.727	3.720	3.717
	D4 (stream, 1st)	0.969	0.969	0.969	0.969	1.394	1.394	1.394	1.394
	D6 (ditch, 1st)	1.206	1.206	1.206	1.206	1.958	1.958	1.958	1.958
	R1 (pond, 1st)	0.143	0.135	0.132	0.130	0.339	0.323	0.314	0.309
	R1 (stream, 1st)	1.270	1.270	1.270	1.270	0.294	0.293	0.293	0.292
	R1 (pond, 2nd)	0.060	0.055	0.052	0.051	0.196	0.177	0.168	0.162
	R1 (stream, 2nd)	0.954	0.954	0.954	0.954	0.193	0.193	0.192	0.192
	R2 (stream, 1st)	0.553	0.553	0.553	0.553	0.207	0.206	0.206	0.206
	R2 (stream, 2nd)	0.469	0.469	0.469	0.469	0.108	0.108	0.108	0.108
	R3 (stream, 1st)	1.683	1.683	1.683	1.683	0.367	0.365	0.363	0.362
	R3 (stream, 2nd)	1.506	1.506	1.506	1.506	0.403	0.400	0.399	0.398
	R4 (stream, 1st)	0.379	0.379	0.379	0.379	0.112	0.112	0.111	0.111
	R4 (stream, 2nd)	1.345	1.345	1.345	1.345	0.373	0.372	0.372	0.372

* reduction of the overall drift percentage is >95%, compared to STEP3, in case drift leads to PECsw max.

Table 2.5.3-16 Maximum PEC_{sw} and PEC_{sed} values of BYI 02960 at Step 4 including buffer zones and drift reduction – Lettuce 2 (1 x 125 g/ha, field use, 15 and 20m buffer zone)

Step 4		BYI 02960: Lettuce 2 , 1 x 125 g/ha (field use)							
Buffer Width & Type	FOCUS Scenario	PEC_{sw} [µg/L] Drift Reduction				PEC_{sed} [µg/kg] Drift Reduction			
		0%	50%	75%	90%	0%	50%	75%	90%
15m (drift and run-off)	D3 (ditch, 1st)	0.097	0.058*	0.038*	0.027*	0.187	0.186	0.186	0.186
	D3 (ditch, 2nd)	0.098	0.059*	0.039*	0.028*	0.173	0.172	0.172	0.172
	D4 (pond, 1st)	0.979	0.977	0.976	0.975	3.734	3.724	3.719	3.716
	D4 (stream, 1st)	0.969	0.969	0.969	0.969	1.394	1.394	1.394	1.394
	D6 (ditch, 1st)	1.206	1.206	1.206	1.206	1.958	1.958	1.958	1.958
	R1 (pond, 1st)	0.140	0.134	0.131	0.129	0.332	0.319	0.313	0.309
	R1 (stream, 1st)	1.270	1.270	1.270	1.270	0.293	0.293	0.293	0.292
	R1 (pond, 2nd)	0.058	0.054	0.052	0.051	0.188	0.173	0.166	0.161
	R1 (stream, 2nd)	0.954	0.954	0.954	0.954	0.193	0.193	0.192	0.192
	R2 (stream, 1st)	0.553	0.553	0.553	0.553	0.207	0.206	0.206	0.206
	R2 (stream, 2nd)	0.469	0.469	0.469	0.469	0.108	0.108	0.108	0.107
	R3 (stream, 1st)	1.683	1.683	1.683	1.683	0.366	0.364	0.363	0.362
	R3 (stream, 2nd)	1.506	1.506	1.506	1.506	0.401	0.399	0.398	0.398
	R4 (stream, 1st)	0.379	0.379	0.379	0.379	0.112	0.111	0.111	0.111
	R4 (stream, 2nd)	1.345	1.345	1.345	1.345	0.373	0.372	0.372	0.372
20 m (drift and run-off)	D3 (ditch, 1st)	0.078	0.049*	0.034*	0.025*	0.187	0.186	0.186	0.186
	D3 (ditch, 2nd)	0.079	0.049*	0.035*	0.026*	0.172	0.172	0.172	0.172
	D4 (pond, 1st)	0.978	0.976	0.976	0.975	3.731	3.723	3.718	3.716
	D4 (stream, 1st)	0.969	0.969	0.969	0.969	1.394	1.394	1.394	1.394
	D6 (ditch, 1st)	1.206	1.206	1.206	1.206	1.958	1.958	1.958	1.958
	R1 (pond, 1st)	0.074	0.069	0.067	0.066	0.185	0.173	0.167	0.164
	R1 (stream, 1st)	0.662	0.662	0.662	0.662	0.156	0.155	0.155	0.155
	R1 (pond, 2nd)	0.032	0.028	0.027	0.026	0.111	0.097	0.091	0.087
	R1 (stream, 2nd)	0.500	0.500	0.500	0.500	0.104	0.104	0.104	0.104
	R2 (stream, 1st)	0.290	0.290	0.290	0.290	0.109	0.108	0.108	0.108
	R2 (stream, 2nd)	0.244	0.244	0.244	0.244	0.058	0.057	0.057	0.057
	R3 (stream, 1st)	0.882	0.882	0.882	0.882	0.199	0.198	0.197	0.196
	R3 (stream, 2nd)	0.790	0.790	0.790	0.790	0.214	0.212	0.212	0.211
	R4 (stream, 1st)	0.199	0.199	0.199	0.199	0.060	0.060	0.060	0.060
	R4 (stream, 2nd)	0.705	0.705	0.705	0.705	0.202	0.202	0.202	0.202

* reduction of the overall drift percentage is >95%, compared to STEP3, in case drift leads to PECsw max.

BYI 02960 metabolites

For BYI 02960, the metabolites difluoracetic acid (DFA), 6-chloronicotinic acid (6-CNA) and BYI 02960-succinamide and BYI 02960-azabicyclosuccinamide were assessed.

Materials and Methods: PECsw for the metabolites were calculated using the approach, scenarios and application rates described for the calculations for the parent compound in study IIA, 9.7/01. Input parameters for the metabolites are described in Table 2.5.3-17.

Table 2.5.3-17 Substance specific and model related input parameters for PECsw calculation

Parameter	Unit	DFA	6-CNA	BYI 02960-succinamide	BYI 02960-azabicyclo-succinamide
Molar Mass	g/mol	96.03	157.6	306.7	288.3
Water Solubility	mg/L	500000	1430	120000	180000
K_{oc}	mL/g	6.8	88	0	0
Degradation DT_{50}					
Soil	days	44.7	4.7	0.1*	0.1*
Total System	days	249	1000	1000	1000
Water	days	249	1000	1000	1000
Sediment	days	249	1000	1000	1000
Max Occurrence					
Water / Sediment	%	6.9	0	39.6	25.9
Soil	%	33.9	17.1	0	0

* The DT50 soil was set to 0.1 d as the model STEP2 does not accept 0 d.

Step 1 and 2: The maximum PEC values for the metabolites of BYI 02960 at Step 1 and Step 2 are given in Table 2.5.3-18. Time dependent PEC values or time-weighted average concentrations are not included in this summary, because they were not used in the risk assessment. However, all values are given in the report.

Table 2.5.3-18 Maximum PECsw and PECsed values for metabolites of BYI 02960

Crop	FOCUS Step	Difluoroacetic acid		6-Chloronicotinic acid		BYI 02960-succinamide		BYI 02960-azabicyclo-succinamide	
		PECsw [µg/L]	PECsed [µg/kg]	PECsw [µg/L]	PECsed [µg/kg]	PECsw [µg/L]	PECsed [µg/kg]	PECsw [µg/L]	PECsed [µg/kg]
Hops 1 x 150 g/ha	1	5.810	0.380	4.177	3.675	4.065	<0.001	2.499	<0.001
	2 N-EU	0.743	0.050	0.232	0.204	4.065	<0.001	2.499	<0.001
	2 S-EU	1.268	0.086	0.463	0.408	4.065	<0.001	2.499	<0.001
Lettuce 1 1 x 125 g/ha (F) [1)] *	1	4.683	0.317	3.481	3.063	0.484	<0.001	0.297	<0.001
	2 N-EU	0.682	0.046	0.289	0.255	0.484	<0.001	0.297	<0.001
	2 S-EU	1.339	0.091	0.579	0.509	0.484	<0.001	0.297	<0.001
Lettuce 2 1 x 125 g/ha (F) [2)] *	1	4.683	0.317	3.481	3.063	0.484	<0.001	0.297	<0.001
	2 N-EU	0.551	0.037	0.193	0.170	0.484	<0.001	0.297	<0.001
	2 S-EU	1.076	0.073	0.386	0.340	0.484	<0.001	0.297	<0.001

F = Field use
[1)] Maximal 1 use per 24 month (minimal crop cover)
[2)] Maximal 1 use per 12 month (average crop cover)
* First or second cropping per year

2.5.4 Fate and behaviour in air

Based on the estimation method according to structure-activity relationship (SAR) methods developed by Roger Atkinson and co-workers the chemical lifetime of the BYI 02960 in the air was assessed by the program AOPWIN^{TM}, version 1.92a (U.S. EPA, 2008). The half-life time ($t_{1/2}$) was estimated within a range of 4.4 hours (short-term scenario) to 13.1 hours (long-term scenario), depending on the mean concentration of hydroxyl radicals present in the troposphere.

In addition, BYI 02960 is susceptible to reactions with ozone, however, that attack and its resulting chemical half-life is considered to be slower by a factor of 2 to 10.

As a consequence of the short half-life in air, no long-range transport of BYI 02960 in the atmosphere is likely to occur nor an accumulation of BYI 02960 in the environmental compartment air. From the low vapor pressure of the substance it is concluded that very low, if any, quantities of BYI 02960 are expected to enter the atmosphere from volatilization.

PECair estimations

As a consequence of the short half-life in air, no long-range transport of BYI 02960 in the atmosphere is likely to occur nor an accumulation of BYI 02960 in the environmental compartment air. From the low vapor pressure of the substance it is concluded that very low, if any, quantities of BYI 02960 are expected to enter the atmosphere from volatilization of soil residues.

2.6 Effects on non-target species

2.6.1 Effects on terrestrial vertebrates

2.6.1.1 Birds

The risk assessment procedure follows the EFSA Guidance Document on Risk Assessment for Birds & Mammals (2009).

Routes of exposure

The formulation is to be sprayed on hops in the field and on lettuce in glasshouses and in the field.

The following routes are considered:

- direct dietary exposure: scenarios for hop and leafy vegetables – field uses only
- direct exposure via drinking water: field and glasshouse uses
- indirect exposure by consumption of earthworms and fish containing residues of BYI 02960 field and glasshouse uses

Acute, short-term and (sub-)chronic dietary toxicity data

Toxicity studies with BYI 02960 (flupyradifurone) were performed with Bobwhite quail (*Colinus virginianus*), canary (*Serinus canaria*) and mallard duck (*Anas platyrhynchos*). The acute oral toxicity of the formulated product was determined in studies on Bobwhite quail (*Colinus virginianus*) and in Chicken (*Gallus gallus domesticus*). There is no indication that the formulation is more toxic than expected based on the active substance content.

Endpoint selection was done according to the recommendations of the Guidance Document on Risk Assessment for Birds & Mammals, EFSA 2009[3]:

Acute risk assessment: where acute tests for more than one species are available the geometric mean may be used for the refined assessment, except when the endpoint for the most sensitive species is more than a factor 10 below the geometric mean of all the tested species. For BYI 02960, in a worst-case approach the lowest LD50 of 232 mg/kg bw is used for Tier 1 acute risk assessment.

Short-term risk assessment; According to the risk assessment scheme a short-term risk assessment is not required. However, the endpoint from short-term dietary studies, e.g. 5-day dietary study in birds (OECD 205) should be used in an acute risk assessment when indicating a higher toxicity via the dietary exposure route (lower LDD50). In the case of BYI 02960 there is no indication that 5-day exposure via dietary route would provoke higher toxicity than one application via gavage in acute study and therefore the short-term toxicity is not considered in the acute risk assessment.

Reproductive risk assessment: The acute oral LD50 value used in the acute avian assessment should

[3] EFSA (2009): Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. The EFSA Journal (2009), 7(12):1438.

be divided by 10 for comparison with the lowest NOAED from the reproduction study (studies) ignoring purely parental effects (e.g. changes in parental body weight and food consumption). The lower of the values should be used in the tier 1 risk assessment.
For BYI 02960 the endpoint from the reproduction study (14 mg/kg bw/d) is lower than the 1/10th of the LD50 of 232 mg/kg bw that has been proposed for use in the Tier 1 acute risk assessment. Therefore, the endpoint of 14 mg/kg bw will be used in a Tier 1 reproductive risk assessment for birds.

An overview of accepted toxicity data for a.s. and formulation is given in Table 2.6.1.1-01. Endpoints selected for first tier risk assessment are marked **bold.**

Table 2.6.1.1-01 Overview of accepted toxicity data for BYI 02960 and BYI 02960 SL 200

Study	Substance	Species	Route	Criterion	Value [mg/kg fd]	Value [mg a.s./kg bw·d]
IIA 8.1.1/01	BYI 02960	*Colinus virginianus*	acute, oral intubation	LD_{50}		**232**
IIA 8.1.1/02	BYI 02960	*Serinus canaria*	acute, oral intubation	LD_{50}		330
IIIA 10.1.6/01	BYI 02960 SL 200	*Colinus virginianus*	acute, oral intubation	LD_{50}		431
IIIA 10.1.6/02	BYI 02960 SL 200	*Gallus domesticus*	acute, oral intubation	LD_{50}		>342
IIA 8.1.2/01	BYI 02960	*Anas platyrhynchos*	5-day-feeding	LC_{50}	> 4741	> 825
IIA 8.1.2/02	BYI 02960	*Colinus virginianus*	5-day-feeding	LC_{50}	> 4876	> 470
IIA 8.1.4/01	BYI 02960	*Anas platyrhynchos*	20-week feeding chronic, reproduction	NOEC	845	83
IIA 8.1.4/02	BYI 02960	*Colinus virginianus*	23-week feeding chronic, reproduction	NOEC	111	**14**

Risk assessment

Exposure via feed

Calculation of Toxicity Exposure Ratio (TER)

According to the EFSA Guidance Document on Risk Assessment for Birds & Mammals (2009), the calculation of acute and long-term Toxicity to Exposure Ratio (TER) is defined as follows:

Acute risk: TERa = LD50 [mg as/kg bw] / DDDac

Long-term risk: TERlt = [lowest of NOEL and LD50/10] [mg as/kg bw] / DDDmean

Calculation of Daily Dietary Dose (DDD)

Acute exposure DDDac:

The daily dietary dose for a single application per crop is given by the following equation:

DDDACac = application rate [kg/ha] × shortcut value (SV90)

Long-term exposure DDDmean:

For a single application the daily dietary dose is given by the following equation:

DDDmean = application rate [kg/ha] × shortcut value (SVm) × fTWA

Where

DDD Daily dietary dose

fTWA Time weighted average factor ftwa based on a default time window of 21 days and a DT50 of 10 days leading to a value of 0.53

90 90th percentile values for acute exposure

m mean values for reproductive/long-term exposure

Standard exposure scenario for Tier 1 risk assessment

The main potential exposure route for birds is expected to be consumption of contaminated feed. This is only relevant for field uses.

The Tier 1 risk assessment is based on generic focal species associated with specific crop scenarios. Default ("shortcut"-) values for the exposure estimate will be used as provided in Appendix A of the EFSA Guidance Document on Risk Assessment for Birds & Mammals (2009) representing a worst case assessment.

In the Tier 1 risk assessment it is assumed that

- animals satisfy their entire food demand in the treated area (PT = 1),
- over an acute time frame (hours) the animals feed on items containing maximum residues (90th percentile), whereas they would ingest food containing mean residues over a long-term period (days to weeks),
- long-term predicted environmental concentrations to be compared with chronic endpoints can be calculated as the time-weighted average concentration. Default assumptions are a time window of 21 days and a DT50 of 10 days leading to a time weighted average factor (= ftwa) of 0.53. This factor is equally valid for feed items consisting of vegetation and for arthropods.

Avian generic focal species for Tier 1 risk assessment

In the field, the product is intended to be used in hops at BBCH 31-75 and in lettuce at BBCH 12-49. According to the EFSA Guidance Document on Risk Assessment for Birds & Mammals (2009) the following generic focal species are addressed in Tier 1 risk assessment.

Table 2.6.1.1-02: Relevant generic avian focal species for Tier 1 risk assessment

Crop	Growth stage (BBCH)	Generic focal species	Representative species	Shortcut value	
				For long-term RA based on RUD_m	For acute RA based on RUD_{90}
Hops	≥ 20	Small insectivorous bird "finch"	Chaffinch	**10.6**	**25.3**
	20-39	Small granivorous bird "finch"	Goldfinch	**5.7**	**12.3**
	≥ 40	Small granivorous bird "finch"	Goldfinch	3.4	7.4
Lettuce (Leafy vegetables)	10-19	Medium herbivorous/granivorous bird "pigeon"	Wood pigeon	**22.7**[1]	**55.5**[1]
	10-49	Small granivorous bird "finch"	Serin	**12.6**	**27.4**
	10-49	Small omnivorous bird “lark”	Woodlark	**10.9**	**24.0**
	10-19	Small insectivorous bird “wagtail”	Yellow wagtail	**11.3**	**26.8**
	≥ 20	Small insectivorous bird “wagtail”	Yellow wagtail	9.7	25.2

Bold values were used for Tier 1 risk assessment. Where the same focal species is representative for different BBCH stages, only the worst-case SV values were chosen for the Tier 1 risk assessment.

[1] The long-term and acute SV-values for this scenario reported in the EFSA GD are 37.0 and 90.6, respectively. However, these were mistakenly based on a FIR/bw of 1.29 (calculated for a passerine species) instead of 0.79 (for non-passerine species, which is correct for the pigeon). The corrected values are in line with those reported for the “pigeon” in early stages of legume forage, maize and oilseed rape.

Tier 1 acute toxicity exposure ratio for birds

Table 2.6.1.1-03: Tier 1 acute DDD and TER calculation for birds

Crop (BBCH)	Generic focal species	LD50 [mg/kg bw]	DDD			DDD	TERA	Trigger
			Appl. rate [kg/ha]	SV90	MAF90			
BYI 02960								
Hops (≥ 20)	Small insectivorous bird "finch"	232	0.150	25.3	1	3.80	61	10
Hops (20-39)	Small granivorous bird "finch"			12.3		1.85	126	
Lettuce (10-19)	Medium herbiv./ granivorous bird	232	0.125	55.5	1	6.94	33	10

	"pigeon"							
Lettuce (10-49)	Small granivorous bird "finch"			27.4		3.43	68	
Lettuce (10-49)	Small omnivorous bird "lark"			24.0		3.0	77	
Lettuce (10-19)	Small insectivorous bird " wagtail"			26.8		3.35	69	

The TER values for both crops are above the trigger of 10 for acute exposure. Hence, no unacceptable acute risk to birds from the use of the product according to the intended use pattern is to be expected.

Tier 1 long-term toxicity exposure ratio for birds

Table 2.6.1.1-04: Tier 1 long-term DDD and TER calculation for birds

Crop (BBCH)	Generic focal species	NO(A)EL [mg/kg bw/d]	DDD				DDD	TERLT	Trigger
			Appl. rate [kg/ha]	SVm	MAFm	fTWA			
BYI 02960									
Hops (≥ 20)	Small insectivorous bird "finch"	14	0.150	10.6	1.0	0.53	0.84	16.6	5
Hops (20-39)	Small granivorous bird "finch"			5.7			0.45	31.9	
Lettuce (10-19)	Medium herbiv./graniv. bird "pigeon"	14	0.125	22.7	1.0	0.53	1.50	9.3	5
Lettuce (10-49)	Small granivorous bird "finch"			12.6			0.83	16.8	
Lettuce (10-49)	Small omnivorous bird "lark"			10.9			0.72	19.4	
Lettuce (10-19)	Small insectivorous bird " wagtail"			11.3			0.75	18.7	

The TER values for both crops are above the trigger of 5 for long-term exposure. It is noted that targeted residue decline data are available in the section on wild mammal risk assessment, which indicate that the TER values for (partly) herbivorous birds are even underestimated.

Hence, no unacceptable risk to birds from the use of the product according to the intended use pattern in lettuce and hops is to be expected.

Metabolites

In the residue section of the DAR, metabolites DFA and DFEAF are considered relevant:

Plants

Residue definition for monitoring:

Results from supervised residue trials show that flupyradifurone and difluoroacitic acid (DFA) are, by far, the most prominent components of the residues in target plants and rotational crops. The residues of metabolite difluoroethyl-amino-furanone (DFEAF) were below the LOQ of 0.01 mg/kg in virtually all samples. Considering these results as well as those from the metabolism studies, the proposed residue definition for monitoring for target plants and rotational crops is:

The sum of parent compound and DFA, expressed in flupyradifurone equivalents.

Residue definition risk assessment:

For flupyradifurone residues in target plants (primary crops) and rotational crops (succeeding crops), the proposed residue definition for data collection and risk assessment is:

The sum of parent compound flupyradifurone and its metabolites DFA and difluoroethyl-amino-furanone (DFEAF), expressed in flupyradifurone equivalents. A conversion factor is not required to cover the difference between the residue definition for monitoring and risk assessment as bith DFA and flupyradifurone are part of the residue definition for monitoring and as DFEAF is found in levels below <0.01 mg/kg in supervised residue trials and in rotational crops.

In the human toxicology risk assessment, the following is concluded:

Toxicologically relevant compounds (animals and plants)	Parent compound, DFA
Toxicologically relevant compounds (environment)	Parent compound, DFA

Based on the above, metabolite DFA is considered to be relevant for the terrestrial vertebrate risk assessment.

No avian toxicity study is conducted with DFA but mammalian toxicity data suggest no higher toxicity of the metabolite than from the parent.

Furthermore, the residue data from trials in lettuce and cereals (as surrogate for ground vegetation in hops) demonstrate little difference in the decline DT50 whether or not DFA is included in the residue concentrations. The trials indicate mostly faster dissipation on foliage than the default DT50 of 10 days.

The first tier risk assessment is therefore based on initial maximum concentrations of the a.s. and the

generic DT50 of 10d and this is considered to cover metabolite DFA.

Exposure via drinking water

An assessment of the risk potentially posed by consumption of contaminated drinking water is required according to the EFSA Guidance Document for Birds and Mammals (2009).
Two scenarios were identified as relevant for assessing the risk of pesticides via drinking water to birds and mammals:

- Leaf scenario, only relevant for birds possibly drinking water from puddles in leaf whorls after application of a pesticide to a crop and subsequent rainfall or irrigation. This scenario is only relevant for acute exposure and only for certain crop types. When the product is applied in hops, the formation of pools in leaf axils where an acute exposure possibly might occur can be excluded. However, for lettuce, the risk of birds drinking from puddles in leaf whorls cannot be excluded. Hence, a corresponding risk assessment has been performed below.
- Puddle scenario. Birds and mammals taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil. This scenario is relevant for all types of applications that may cause contamination of soil and only for long-term exposure.

<u>Risk assessment for the leaf scenario (field use in lettuce):</u>
The exposure of birds to drinking water from pools in leaf whorls as outlined above cannot be excluded from the use of the product in lettuce.
The respective calculations for birds have to be performed only for acute exposure. Generic focal species is a small granivorous bird (body weight 15.3 g) with a DWR (daily drinking water rate) of 0.46 L/kg bw/d. It should be mentioned that DWR, unlike FIR in the case of food intake exposure assessment, is already related to body weight.
According to EFSA Guidance Document for Birds and Mammals (2009), the PECpool calculates as follows:

PECpool = Cspray / 5 [mg/L]

Cspray = 0.025 kg a.s./hL = 25 g a.s./hL = 0.25 g a.s./L = 250 mg a.s./L

The PECpool (= PECdw) is 250 / 5 = 50 mg a.s./L for BYI 02960. The calculation of DDD (here: daily drinking water uptake) and TER are as follows:

DDD = DWR * PECdw

TERA = LD50 [mg as/kg bw/d] / DDD [mg as/kg bw/d]

Table 2.6.1.1-05: Tier 1 acute DDD and TER calculation for exposure via drinking water from pools in leaf axils or on leaves

Compound	DWR [L/kg bw/d]	PECdw [mg/L]	DDD [mg/kg bw/d]	LD50 [mg/kg bw]	TER_A	Trigger
BYI 02960	0.46	50	23	232	10.1	10

The TER_{AC} value for drinking water exposure exceeds the a-priori acceptability trigger of 10 in a worst case risk assessment (highest concentration in spray water volume, lowest LD50). Hence, no unacceptable risk is to be expected from the use of the product according to the intended use pattern.

Risk assessment for the puddle scenario (field use in hop and lettuce):

An “escape clause” recommended in the EFSA Guidance Document for Birds and Mammals (2009) allows for screening the need for a quantitative risk assessment by a comparison between the application rate and the toxicity of the respective substance. This escape clause specifies that “*due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals …, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (= application rate x MAF) (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≥ 500 L/kg).*” [4].

Table 2.6.1.1-06: Evaluation of potential concern for exposure of birds drinking water from puddles (escape clause)

Compound	Koc [mL/g]	Application rate * MAF [g a.i./ha]	LD50 or NO(A)EL [mg a.i./ kg bw/d]	Ratio (Application rate * MAF) / NO(A)EL	“Escape clause” No concern if ratio	Conclusion
BYI 02960	98.4 1)	150 2) x 1	232	0.64	≤ 50	No concern
			14	10.7	≤ 50	No concern

1) Arithmetic mean of ten K_{OC} values (see B.5)

2) Worst-case application rate in hops

This evaluation confirms that the risk for birds from drinking from puddles that may contain residues from the use of product is acceptable.

[4] EFSA (2009): Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA, p. 66

Indirect exposure via contaminated earthworms or fish

Substances with a high bioaccumulation potential could theoretically bear a risk of secondary poisoning for birds if feeding on contaminated prey like fish or earthworms. For organic chemicals, a log P_{OW} > 3 is used to trigger an in-depth evaluation of the potential for bioaccumulation.

Table 2.6.1.1-07: Log Pow values of BYI 02960 and its metabolites

Substance	Maximum Log Pow	Reference
BYI 02960	1.2	Vol.3 B.2.1.13
BYI 02960-succinamide	0.6 at pH 5	Vol.3 B.2 Table B.2.1-2 IIA 7.13b
BYI 02960-azabicyclosuccinamide NA-salt	-1.3 at pH 5	Vol.3 B.2 Table B.2.1-2 IIA 7.13b
DFA	- 3.1 at pH5	Vol.3 B.2 Table B.2.1-2 IIA 7.13b
6-CNA	1.52 at pH 1.98	Vol.3 B.2 Table B.2.1-2 IIA 7.13b

As neither the active ingredient nor any of its metabolites has a log Pow above 3, the potential of bioaccumulation is considered to be low. Hence, there is no need to evaluate potential effects of secondary poisoning of birds.

2.6.1.2 Mammals

The risk assessment procedure follows the EFSA Guidance Document on Risk Assessment for Birds & Mammals (2009).

Routes of exposure

The formulation is to be sprayed on hops in the field and on lettuce in glasshouses and in the field.

The following routes are considered:

- direct dietary exposure: scenarios for hop and leafy vegetables – field uses only
- direct exposure via drinking water: field and glasshouse uses
- indirect exposure by consumption of earthworms and fish containing residues of BYI 02960 field and glasshouse uses

Acute and chronic toxicity data

The summary of the toxicity profile of the active substance BYI 02960 to mammals is provided in Table 2.6.1.2-01. Details of the studies concerned are provided in B.6 of this DAR.

Table 2.6.1.2-01 Toxicity of BYI 02960 to mammals

Test species	Test design	Ecotoxicological endpoint	Reference
Acute risk assessment endpoint:			
Rat	acute oral, toxic class method	$LD_{50, cut-off}$ [1] 2000 mg a.i./kg bw (4/6 dead at 2000, 0/6 at 300)	[redacted] (2009) B.6.2.1 Study 1
Rat	acute oral neurotoxicity study	LD_{50} > 800 mg a.i./kg bw (2/24 dead at 800, 0/24 at 200 and below)	Garcin (2009) B.6.7.1 Study 1
Combined LD_{50} calculation (TOXCALC, probit): 1607 mg/kg bw			
Reproductive risk assessment endpoint:			
Rat reproduction	Dietary exposure over 2 generations	$NOAEL_{maternal}$ 6.~~47.8~~ [1] (100 ppm) mg a.i./kg bw/d $NOAEL_{repro}$ 34 ~~39.2~~ [2] (500 ppm) mg a.i./kg bw/d $NOAEL_{offspring}$ 6.~~47.8~~ [1] (100 ppm) mg a.i./kg bw/d	[redacted] (2011) B.6.6.1 Study 2
Rat developmental toxicity	Gavage over GD 6-20 (gestation days)	$NOAEL_{maternal}$ 50~~15~~ mg a.i./kg bw/d $NOAEL_{offspring}$ 50 mg a.i./kg bw/d	[redacted] (2010)
Rabbit developmental toxicity	Gavage over GD 6-28 (gestation days)	$NOAEL_{maternal}$ 15 mg a.i./kg bw/d $NO(A)EL_{offspring}$ 40 mg a.i./kg bw/d	[redacted] (2012)
Reproductive risk assessment endpoint proposal of notifier		**Tier 1:** 6.~~47.8~~ **mg/kg bw/d**	
		Tier 2 (refined): 34 ~~39.2~~ **mg/kg bw/d**	

1) Mean achieved dose of P & F1 females at 100 ppm (Table 5.6-14 of KIIA 5.6.1/02, [redacted], 2011) In B.6, the NOAEL is based on the lowest intake by either males or females during a particular study phase, in this case intake by males F1-gen during premating, see Table 6.6.1-8 of Vol3 B.6.

2) Mean achieved dose of P & F1 females at 500 ppm (Table 5.6-14 of KIIA 5.6.1/02, [redacted], 2011) In B.6, the NOAEL is based on the lowest intake by either males or females during a particular study phase, in this case intake by males F1-gen during premating, see Table 6.6.1-8 of Vol.3 B.6.

There are no indications that the formulated product enhances the toxicity of the a.s., see the Table below.

2.6.1.2-02 Mammalian toxicity data of the formulated product BYI 02960 SL 200

Test species	Test design	Ecotoxicological endpoint	Reference
Rat	acute, oral	LD50 >2000 mg/kg bw [1]	[redacted] (2010) B.6.12.1 Study 1

1) 0 out of 6 animals died. According to OECD Guideline 423, the LD50 cut-off of BYI 02960 SL 200 is >5000 mg/kg body weight (equivalent to Category 5 (unclassified) of the GHS).

The proposed **acute endpoint of 1607 mg/kg bw** (derived by combining the mortality data from the two acute studies with oral exposure with the a.s.) is accepted.

As long-term endpoint, the notifier proposes a maternal and offspring NOAEL of 100 ppm (7.8 mg/kg bw/d) for tier 1 risk assessment. ~~As a worst-case approach is acceptable but RMS notes that in the mammalian toxicology section of the DAR the endpoints are set at a higher dose level. Also for the developmental studies the endpoints in B.6 of the DAR are higher. See Table B.9.3.3-2.~~

~~Table B.9.3.3-2 Endpoints from 2-generation study in rats and developmental studies in according to B.6.6.1~~

~~Study type~~	~~Reproductive endpoint determined in B.6 :~~	~~Dose (ppm):~~	~~Dose (mg/kg bw/d):~~	~~Based on intake during study phase:~~
~~Rat reproduction~~	~~Relevant parental NOAEL~~	~~500 ppm~~	~~32 mg/kg bw/day~~	~~premating, P-gen, males~~
~~Rat reproduction~~	~~Relevant reproductive NOAEL~~	~~500 ppm~~	~~34 mg/kg bw/day~~	~~gestation, P-gen, females~~
~~Rat reproduction~~	~~Relevant offspring NOAEL~~	~~500 ppm~~	~~34 mg/kg bw/day~~	~~gestation, P-gen, females~~
~~Rat developmental~~	~~Relevant maternal NOAEL~~		~~50 mg/kg bw/day~~	
~~Rat developmental~~	~~Relevant offspring NOAEL~~		~~50 mg/kg bw/day~~	
~~Rabbit developmental~~	~~Relevant reproductive NOAEL~~		~~40 mg/kg bw/day~~	
~~Rabbit developmental~~	~~Relevant offspring NOAEL~~		~~40 mg/kg bw/day~~	

~~Based on the above, the endpoint proposed by the notifier for the first tier is considered to be very conservative.~~ For a tier 2 assessment, the notifier reconsidered this endpoint. Their statement is presented below, with minor modifications by RMS, regarding references to Tables and dossier sections. RMS also incorporated the changes made following Pesticide Peer Review meeting 122 of mammalian toxicology.

According to the refinement options provided in the EFSA GD (Section 4.4, Module 4: Reproductive

risk assessment for mammals, step 9), the Tier 1 endpoint can be refined after re-examination of the reproductive studies for endpoints relevant for reproductive performance. Effects on other endpoints can be disregarded. In this context the EFSA GD specifies a number of potentially relevant endpoints to be evaluated, including body weight, behavior and reproductive performance of adults, and observations on offspring (number, weight, development). A corresponding evaluation for BYI 02960 is presented in the table below, based on the toxicological studies summarized in more detail B.6 of this dossier. If such refinement of the Tier 1 endpoint is considered, the EFSA GD recommends that additional mammalian toxicity studies should also be examined in order to check whether they contain lower NOAELs for relevant endpoints.

Approach taken in this evaluation:

In order to ensure a high level of protection, all repeated dose oral toxicity studies conducted with BYI 02960 in rodents (rat, mouse), rabbits or dogs were evaluated for potentially relevant endpoints. This evaluation included also neurotoxicity studies, in order to account for non-lethal behavioral effects that could become relevant under field conditions.

Since the protection goal of the reproductive risk assessment is the sustainability of potentially exposed wild mammal populations, these studies were evaluated with regard to population level relevant endpoints, i.e. survival, growth, development, behavior and reproductive performance. Other endpoints (e.g. organ weights, physiology, blood chemistry etc.) were not included in the evaluation. Furthermore, neither very high doses (more than 3-fold above the proposed refined endpoint), nor chronic studies of extreme exposure duration (≥ 1 year) were included in the tables.

Since effects on females are typically more relevant for small mammal populations than effects on males, the results of this evaluation are presented in Table 2.6.1.2-04 in the order of increasing achieved doses for females over all evaluated studies. In order to facilitate the comparison of effects at the different dose levels of each study, the letter code in the first column indicates the study ID (letters A-N, as presented in the following overview table), with the respective dosing group indicated by numbers 1-3 (low, mid and high dose level).

Table 2.6.1.2-04 Repeated oral dosing toxicity studies evaluated for selection of the Tier 2 wild mammal reproductive risk assessment endpoint in Table 2.6.1.2-05

Study ID	Study type	Report reference
A	Rat 28-d gavage	B.6.3.1 Study 1, KIIA 5.3.1/01
B	Rat 28-d dietary	B.6.3.1 Study 2, KIIA 5.3.1/02
C	RAT 90-d	B.6.3.4 Study 1, KIIA 5.3.2/01
D	RAT 90-d NT (neurotoxicity)	B.6.7.2 Study 1, KIIA 5.7.4/01
E	RAT 1-gen (one generation reproduction)	B.6.6.1 Study 1, KIIA 5.6.1/01
F	RAT 2-gen (two generation reproduction)	B.6.6.1 Study 2, KIIA 5.6.1/02

G	RAT DT (developmental toxicity)	B.6.6.2 Study 1, KIIA 5.6.10/01
H	RAT DT (suppl.)	B.6.6.2 Study 2, KIIA 5.6.10/02
I	RABBIT DT (developmental toxicity)	B.6.6.2 Study 3, KIIA 5.6.11/01
K	MOUSE 28-d	B.6.3.1 Study 3, KIIA 5.3.1/03
L	MOUSE 90-d	B.6.3.4 Study 2, KIIA 5.3.2/02
M	DOG 28-d	B.6.3.1 Study 4, KIIA 5.3.1/04
N	DOG 90d	B.6.3.4 Study 3, KIIA 5.3.3/01

Results of this evaluation:

Most prominent in the evaluation of all these repeated oral dosing studies with BYI 02960 (see Table 2.6.1.2-04) were observations of moderate body weight effects. Severity of effects was seen to increase with dose and exposure time.

Within reasonable timeframes of environmental exposures (weeks to months), effects of ecological relevance on wild mammal population did not occur at dose levels of at least 30-50 mg/kg bw/d.

In contrast, pronounced reproductive effects of clear ecological relevance did occur at dose levels significantly exceeding 100 mg/kg bw/d (e.g., 14.8% litter size reduction and reduced body weight of > 10% at 120-150 mg/kg bw/d (1800 ppm) in the rat 2-generation study), and only after prolonged exposure in a second generation (F1 females or F2 offspring).

Moderate effects of bodyweight can usually be considered less relevant in wild mammal risk assessments. Moreover, the results of the 90-d rat study with BYI 02960 showed that bodyweight effects approaching 10% (e.g., 8.9% in males and 9.5% in females at 500 ppm) were reversible after cessation of treatment (recovery phase). Potential exposure of wild mammals to BYI 02960 is transient and short-lived (single application, DT50 on foliage ≤ 5 days). Therefore an effect magnitude of ≤ 10% on bodyweight can be considered ecologically acceptable. Body weight effects exceeding this magnitude were not observed at dose levels below 140 mg/kg bw/d (P females: 15.9%), even after prolonged exposure.

Reproductive effects like reduction of litter size were only moderately pronounced and were only observed after long-term exposure in F1 parents and F2 litter (note: not observed in the 1-generation rat reproduction study nor in the first generation of the 2-generation study), indicating reduced risk for reproductive effects under environmentally relevant conditions (low application rate, single application, DT50 on foliage < 5 days).

Overall, this evaluation suggests that a dose level of up to 39.2 mg as/kg bw/d (achieved dose of females at 500 ppm in the 2-gen rat reproduction study) can be proposed as relevant and sufficiently conservative risk assessment endpoint for wild mammal populations:

- no ecologically relevant effects in the parental generation or the F1 litter
- only slight reductions of bodyweight (< 10%) in F1 parents and F2 litter
- body weight reduction by BYI 02960 of ca. 10% was reversible after cessation of dosing.

Severe effects only occurred at dose levels which were 3-4 times higher than 39.2 mg/kg bw/d.

Therefore the refined wild mammal reproductive risk assessment endpoint proposed in this dossier is 39.2 mg as/kg bw/d, the achieved dose of females at 500 ppm in the 2-gen rat reproduction study.

Table 2.6.1.2-05 Potentially relevant wild mammal reproductive risk assessment effects of BYI 02960 in repeated oral dosing studies

F: females; M: males; ↑: increase (higher than control); ↓: reduction (lower than control);
P: parental generation M+F; F1: offspring produced by P M+F, F2: offspring produced by F1 M+F
#: number; bw: body weight; FC: food consumption
n.e.: not evaluated (specific study type without reproductive endpoints); empty fields: no effect observed. Blue shading indicates the NOAELs as determined by RMS in B.6 of the original DAR. Green shading indicates the NOAELs as determined in the updated version of B.6 of December 2014 following Pesticide Peer Review meeting 122.

ID	Species & study type	Dose ppm	mg/kg bw/d* males	mg/kg bw/d* females	Potentially relevant findings in adults	Potentially relevant findings on reproduction and offspring
D1	RAT 90-d NT	100	5.7	6.9		n.e.
C1	RAT 90-d	100	6.0	7.6		n.e.
I1	RABBIT DT			7.5		
F1	RAT 2-gen	100	6.4-6.6	7.0-7.8		
N1	DOG 90d	400	12	12		n.e.
I2	RABBIT DT			15		
G1	RAT DT			15		
E1	RAT 1-gen	200	14.5	15.8-17.5		
M1	DOG 28-d	500	16	18		n.e.
L1	MOUSE 90-d	100	16	19		n.e.
H1	RAT DT (suppl.)			20		n.e.
H2	RAT DT (suppl.)			30		n.e.
B1	Rat 28-d	500	33.6			n.e.
D2	RAT 90-d NT	500	29.4	34.8		n.e.
C2	RAT 90-d	500	~~30.2*~~	38.3		n.e.
F2	RAT 2-gen	500	~~32*~~-32.5	**35.5-39.8**	bw ↓ ≤ 7.8% (F1 F: D_{gest} 0)	bw ↓ ≤ 7.4% (F2 pups)
I3	RABBIT DT			40	FC ↓ ≤ 20%, bw ↓ ≤ 1.7 %	
N2	DOG 90d	1200	33	41	bw ↓ ≤ 9% (M), minimal/slight muscle atrophy	n.e.
G2	RAT DT			50	FC ↓ ≤ 8%, bw ↓ ≤ 1.6 %	
K1	MOUSE 28-d	300	50	59		n.e.
E2	RAT 1-gen	700	50.1	48.8-60.9	bw ↓ ≤ 6.7% (F D_{lact} 14)	bw ↓ ≤ 9.9 % (F1 pups)
A1	RAT 28d		75	75		n.e.
L2	MOUSE 90-d	500	81	96		n.e.
K2	MOUSE 28-d	600	98	122		n.e.
G3	RAT DT			150	FC ↓ ≤ 27%, bw ↓ ≤ 4.2%	bw ↓ 2-3%, indications of slightly delayed fetal development

ID	Species & study type	Dose ppm	mg/kg bw/d* males	mg/kg bw/d* females	Potentially relevant findings in adults	Potentially relevant findings on reproduction and offspring
F3	Rat 2-gen	1800	117.4-122.1	140.2-151.4	bw ↓ ≤ 9.6% (M), ≤ 15.9% (F)	litter size ↓ 14.8% (F2) bw ↓ ≤ 12.5% (F1 & F2 pubs) # estrous cycles ↓ (F1 F)
C3	RAT 90-d	2500	156	168	bw ↓ ≤ 8.9% (M), 9.5% (F), reversible in recovery phase	n.e.
D3	RAT 90-d NT	2500	143	173	FC ↓ ≤ 29% (F, w1); bw ↓ ≤ 10% (M), ≤ 9% (F);	n.e.
E3	RAT 1-gen	2000	147.5	164.4-182.3	bw ↓ F (≤ 12% D_{lact} 13)	bw ↓ ≤ 9.9% (F1 pups)

*NOAEL set by RMS in original DAR but not accepted in peer review.

~~RMS agrees with the notifier that the ecologically relevant endpoint for wild mammals is the mid-dose of the two-generation study in rats (500 ppm). Based on the mammalian toxicology assessment (see table B.9.3.3-2) this corresponds to **34 mg/kg bw/d,** based on intake by females of the P-generation during the gestation period. This is considered to be the relevant endpoint for the ecotoxicological risk assessment.~~

Expert consultation during Pesticide Peer Review meeting 124, ecotoxicology, December 2014:

The 1-year dog study concluded an endpoint of 7.8 mg/kg bw (mammalian toxicity section, original DAR) and it was questioned whether this should be taken into account.

Furthermore, the endpoint from the 2-generation rat study has changed to ‘parental‘ and ‘offspring‘ NOAEL of 6.4 mg/kg bw per day (100 ppm) and ‘reproductive’ NOAEL of 32 mg/kg bw per day (500 ppm). The ‘parental‘ NOAEL of 6.4 mg/kg bw per day is based on significant decrease in body weight (<10% observed in the mid-dose of the F_1 females) and a non-significant decrease in body weight gain found in the parental animals (21% in females) and the F_1 animals (16% in females). The ‘offspring‘ NOAEL of 6.4 mg/kg bw per day is based on reduced body weight/body weight gain in the F_2 pups. These were agreed by the meeting of experts for mammalian toxicology (PESTICIDES PEER REVIEW MEETING 122).

Discussion:

The 1y-dog study was considered as not relevant for ecotox for reproductive risk assessment. Some observed effects (degeneration of skeleton muscle and other muscle degeneration) from this study might be considered as relevant but not for the reproduction. It was noted that in general such type of study is not considered robust by tox experts (few test individuals).

The discussion was focussed on the 2-generation rat study. Three endpoints are available from this study, NOAEL of 500 ppm for reproduction and 100 ppm for offspring and parental toxicity. A dose response relationship was noted for the pup body weight decrease. At 500 ppm the decrease was statistically significant but < 10%. It was questioned if this should be considered as biologically

relevant. At 1800 ppm (the next higher dose) the decrease was >10%. Some experts considered that the statistical significance in this case should not be disregarded considering also the dose response relationship and preferred the lowest NOAEL of 100 ppm.

Overall, the majority of the experts considered that a NOAEL of 100 ppm should be used for risk assessment (**6.4 mg a.s./kg bw/d**).

Risk assessment

Exposure via feed

The first tier risk assessment calculation of is performed in the same way as for birds. The risk assessment procedure follows the EFSA Guidance Document on Risk Assessment for Birds & Mammals (2009).

Mammalian generic focal species for Tier 1 risk assessment

In the field, the product is intended to be used in hop at an application rate of 1 x 0.150 kg a.i./ha, BBCH 31-75, and in lettuce at an application rate of 1 x 0.125 kg a.i./ha, BBCH 12-49. According to the EFSA Guidance Document on Risk Assessment for Birds & Mammals (2009) the following generic focal species have to be addressed in the risk assessment.

Table 2.6.1.2-06 Relevant generic mammalian focal species for Tier 1 risk assessment

Crop	Growth stage (BBCH)	Generic focal species	Representative species	Shortcut value	
				For long-term RA based on RUD mean	For acute RA based on RUD 90th perc.
Hops	≥20	Small insectivorous mammal “shrew”	Common shrew	**1.9**	**5.4**
	≥ 40	Small herbivorous mammal "vole"	Common vole	**21.7**	**40.9**
	20 - 39	Small omnivorous mammal “mouse”	Wood mouse	**3.9**	**8.6**
	≥ 40	Small omnivorous mammal “mouse”	Wood mouse	2.3	5.2
Lettuce (Leafy vegetables)	10-19	Small omnivorous mammal “shrew”	Common shrew	**4.2**	**7.6**
	≥20	Small insectivorous mammal “shrew”	Common shrew	1.9	5.4
	40-49	Small herbivorous mammal "vole"	Common vole	**72.3**	**136.4**
	≥ 50	Small herbivorous mammal	Common vole	21.7	40.9

		"vole"			
	All season	Large herbivorous mammal "lagomorph"	Rabbit	**14.3**	**35.1**
	10-49	Small omnivorous mammal "mouse"	Wood mouse	**7.8**	**17.2**
	≥ 50	Small omnivorous mammal "mouse"	Wood mouse	2.3	5.2

Bold values were used for Tier 1 risk assessment. Where the same focal species is representative for different BBCH stages, only the worst-case SV values were chosen for risk assessment.

The acute and long-term risk are calculated in the tables below.

Table 2.6.1.2-07 Tier 1 acute DDD and TER calculation for mammals

Crop	Generic focal species	LD_{50} [mg/kg bw]	DDD			DDD	TER_A	Trigger
			Appl. rate [kg/ha]	SV_{90}	MAF_{90}			
BYI 02960								
Hops (≥20)	Small insectivorous mammal "shrew"	1607	0.150	5.4	1	0.81	1984	10
Hops (≥ 40)	Small herbivorous mammal "vole"			40.9		6.14	262	
Hops (20 – 39)	Small omnivorous mammal "mouse"			8.6		1.29	1246	
Lettuce (≥20)	Small insectivorous mammal "shrew"	1607	0.125	5.4	1	0.68	2381	10
Lettuce (40-49)	Small herbivorous mammal "vole"			136.4		17.05	94	
Lettuce (All season)	Large herbivorous mammal "lagomorph"			35.1		4.39	366	
Lettuce (10-49)	Small omnivorous mammal "mouse"			17.2		2.15	747	

All TER values for both crops are well above the trigger of 10 for acute exposure. Hence, no unacceptable risk is to be expected from the use of the product according to the intended use pattern.

Table 2.6.1.2-08 Tier 1 long-term DDD and TER_{LT} calculation for mammals

Crop	Generic focal species	NOAEL [mg/kg bw/d]	DDD				DDD	TER_{LT}	Trigger
			Appl. rate [kg/ha]	SV_m	MAF_m	f_{TWA}			
BYI 02960									
Hops (≥20)	Small insectivorous mammal "shrew"	~~34~~ 6.4	0.150	1.9	1	0.53	0.151	~~225~~ 42	5
Hops (≥ 40)	Small herbivorous mammal "vole"			21.7			1.73	~~20~~ **3.7**	
Hops (20 – 39)	Small omnivorous mammal "mouse"			3.9			0.31	~~110~~ 21	
Lettuce (≥20)	Small insectivorous mammal "shrew"	~~34~~ 6.4	0.125	1.9	1	0.53	0.13	~~270~~ 51	5
Lettuce (40-49)	Small herbivorous mammal "vole"			72.3			4.79	~~7.1~~ **1.3**	
Lettuce (All season)	Large herbivorous mammal "lagomorph"			14.3			0.95	~~36~~ 6.8	
Lettuce (10-49)	Small omnivorous mammal "mouse"			7.8			0.52	~~66~~ 12.4	

Bold values do not meet the trigger

All TER values for both crops are above the trigger of 5 for long-term exposure, except for the generic focal species the "vole". ~~Hence, no unacceptable risk is to be expected from the use of the product according to the intended use pattern.~~

Based on the first tier endpoint of 7 mg/kg bw/d proposed originally by the notifier, a risk would be identified for small herbivorous mammals. Therefore, the notifier has proposed two further refinements:

I. measured residue data that allow refinement of the time-weighted average residue concentrations (21-d fTWA) of BYI 02960 on foliage as potential diet of small herbivorous mammals
II. a more realistic evaluation of the focal species exposure scenario (relevance of hop yards and lettuce fields for voles), based on general knowledge from literature and field study results.

<u>I) Refined DT50 of BYI 02960 on foliage</u>

According to the recommendations of the EFSA GD (2009), the Tier 1 risk assessment has been conducted with a “generic” DT50 of 10 days of BYI 02960 on all food items. However, measured residue decline data (Vol. 3, B.7.6.2 study 1 to 4 KIIIA1 6.3.1/01-04 for lettuce, and Vol. 3, B.9.3.2 Study IIIA 10.3.3/02 for cereals) are available that allow the calculation of compound-specific DT50 values for BYI 02960.

From the available residue decline data, it is proposed to select substrates and application types that can be considered representative for foliage as food of small herbivorous mammals in lettuce fields (i.e., spray application residue trial results) and in hop yards (spray application residue trials in young cereals as surrogate for wild undergrowth).

With these data, specific DT50 values for BYI 02960 were calculated by Scherr & Ellerich (2013),; lettuce: KIIIA 10.3.3/03; and Sur & Ellerich (2012), cereals: KIIIA 10.3.3/04) according to best practice in environmental modelling. The residue decline data were analysed with the software package KinGUI, which is a standard tool used for kinetic evaluations. In the first place, fits using the single first order (SFO) model were performed. Their quality was assessed visually and also based on statistical significance of the individual parameters. In case of clear biphasic behaviour in the respective residue data, a DFOP fit (double-first order in parallel) was also performed. Also here, visual acceptability and statistical significance were evaluated.

Residue decline data were analysed both for flupyradifurone only and for total residues of flupyradifurone including also metabolites DFA and DFEAF.

In all trials residues were determined after the second treatment, but in four trials residues were also determined after the first application and DT50 values were estimated for each decline interval.

As a result of KinGUI evaluation, 13 trials in lettuce provided SFO – DT50 values and 5 trials provided a DFOP-DT50. One trial provided an SFO-DT50 value after the first application and an DFOP-DT50 after the second application.

In young cereals, 3 trials provided SFO – DT50 values and 1 trial provided a DFOP-DT50.

Half-live (DT50) values from SFO and DFOP cannot be simply mathematically averaged in order to generate an overall DT50 to be used in time-weighted average (TWA) calculations. Since the (single) DT50 from SFO fits cannot simply be averaged with the (double) DT50 from DFOP fits, calculations of the 21-d fTWA were conducted for each of the trials, and the 17 resulting 21-d fTWA values were combined afterwards. With this approach the full set of available data can be included in the refined exposure assessment.

The TWA values themselves were thus calculated in a second step as follows. First, numerical evaluation of predicted residues in time (with timestep of 0.1 days) was performed, employing the respective kinetic type and the relevant degradation parameter(s) - DT50 value(s) and g-factor (for DFOP). These predicted residues are then used for the 21-d fTWA evaluation per data set and averaged afterwards.

Lettuce: overall, 18 residue dissipation trials with BYI 02960 were available for lettuce. In 13 of these trials, the residue dissipation could be considered according to SFO, whilst the remaining 5 trial results needed fitting with DFOP. Furthermore, one trial provided an SFO-DT50 value after the first application and an DFOP-DT50 after the second application. Since the (single) DT50 from SFO fits cannot simply be averaged with the (double) DT50 from DFOP fits, calculations of the 21-d fTWA were conducted for each of the trials, and the 17 resulting 21-d fTWA values were combined afterwards (for each trial the value after the second application was taken). Due to the number of trials in the evaluation it is proposed to use the geometric mean 21-d fTWA = 0.17 for the refined long-term exposure assessment for small herbivorous mammals in lettuce fields (Table 2.6.1.2-09).

Table 2.6.1.2-09 DT_{50} and 21-d f_{TWA} for residue dissipation of total flupyradifurone on lettuce

Trial code	Trial description	SFO DT_{50}	DFOP DT_{50fast}	DFOP DT_{50slow}	21-d f_{TWA}
R01	10-2223-01, N-EU	2.08	-	-	0.14
R02	10-2223-02, N-EU	-	0.06	7.69	0.14
R03	10-2223-03, N-EU	3.58	-	-	0.24
R04	10-2223-04, N-EU		0.100	9.850	0.35
R05	10-2223-05, N-EU	-	0.19	11.73	0.16
R06	11-2082-01, N-EU	3.25	-	-	0.22
R07	11-2082-02, N-EU		0.22	5.53	0.041
R18 (1st)	11-2082-03, N-EU	5.04			0.33
R18	11-2082-03, N-EU	6.15			0.38
R08 (1st)	11-2082-03, N-EU		1.09	6.51	0.12
R08	11-2082-04, N-EU	-	0.26	4.51	0.093
R09	10-2213-01, S-EU	-	0.06	3.95	0.077
R10	10-2213-02, S-EU	5.55	-	-	0.35
R11	10-2213-03, S-EU	1.68	-	-	0.12
R12	10-2213-04, S-EU	5.22	-	-	0.34
R13	10-2213-01, S-EU	1.91	-	-	0.13
R14	11-2071-01, S-EU	4.40	-	-	0.29
R15	11-2071-02, S-EU	2.67	-	-	0.18
R16 (1st)	11-2071-03, S-EU	2.24	-	-	0.15
R16	11-2071-03, S-EU	-	1.01	9.11	0.13
R17 (1st)	11-2071-04, S-EU	-	0.09	4.33	0.074
Geometric mean 21-d f_{TWA} (only ftwa values after second application included)					**0.17**

However, some uncertainty remains on whether this value is applicable to the full application period. In the GAP, application in lettuce is proposed between BBCH 12-49. The notifier states that the inclusion of the evaluation of some residue decline after the first application and the fact that there is no large difference between the decline after the first and the second application, addresses the question of potential impact of the application stage on the decline curves. To investigate this statement, RMS checked the lettuce trials to see at which BBCH-stages they were performed. See 2.6.1.2-10.

Table 2.6.1.2-10 BBCH stages at application in lettuce trials (shaded trials were analysed both after the first and after the second application)

Trial code	Trial no.	BBCH at first	BBCH at second

		application	application
R01	10-2223-01	41	48
R02	10-2223-02	42	48
R03	10-2223-03	42	48
R04	10-2223-04	43	48
R05	10-2223-05	46	48
R06	10-2082-01	46	48
R07	10-2082-02	43	48
R18	10-2082-03	47	48
R08	10-2082-04	43	48
R09	10-2213-01	41	48
R10	10-2213-02	48	49
R11	10-2213-03	42	46
R12	10-2213-04	44	49
R13	10-2213-05	47	49
R14	10-2071-01	47	49
R15	10-2071-02	17	46
R16	10-2071-03	43	48
R17	10-2071-04	17	49

The table shows that only one of the four doubly analysed trials was performed at a relatively early BBCH stage. In the view of the RMS, this is not enough proof that the residue decline trials really cover also early application stages (from BBCH 12). If these data are used in a refined risk assessment, this issue should be addressed further.

Cereals as surrogate for undergrowth in hop yards: overall, 4 residue dissipation trials with BYI 02960 were available for young cereals. In 3 of these trials, the residue dissipation could be considered according to SFO, whilst in the remaining trial results needed fitting with DFOP. Since the (single) DT50 from SFO fits cannot simply be averaged with the (double) DT50 from DFOP fits, calculations of the 21-d fTWA were conducted for each of the trials, and the 4 resulting 21-d fTWA values were combined afterwards. Due to the number of trials in the evaluation it is proposed to select the median 21-d fTWA = 0.15 for the refined long-term exposure assessment for small herbivorous mammals feeding on undergrowth in hop yards (Table 2.6.1.2-11). The trials were performed at BBCH 25-29, meaning that the cereal plants were in the tillering stage. RMS considers this reasonably representative for the grass that is expected to form the undergrowth vegetation of hop plantations.

Table 2.6.1.2-11a DT$_{50}$ and 21-d f$_{TWA}$ for residue dissipation of total flupyradifurone on young cereals

Trial code	Trial description	SFO DT$_{50}$	DFOP DT$_{50\ fast}$	DFOP DT$_{50\ slow}$	21-d f$_{TWA}$
R01	11-2958-01, N-EU	-	0.145	3.555	0.0649
R02	10-2958-02, N-EU	1.44	-	-	0.10
R03	10-2958-03, N-EU	4.22	-	-	0.28
R04	10-2958-04, S-EU	3.15	-	-	0.21
Median 21-d f$_{TWA}$					**0.15**

The two residue data sets result in very similar 21-d f$_{TWA}$ values (0.17 and 0.15).
~~However, the residue decline data are not used, because based on the NOEAL of 34 mg/kg bw/d, a refined risk assessment is not necessary.~~

Expert consultation during Pesticide Peer Review meeting 124, ecotoxicology, December 2014:

The approach described above, for deriving overall ftwa values from the lettuce and cereal trials, was discussed in the expert meeting. It was questioned whether the approach taken by the RMS was reasonable as a refinement. The studies performed on lettuce were questioned as food items could be weeds including monocots. The RMS highlighted that trials on cereals were also available and the difference in the fTWA was minor indicating not too dissimilar speed of degradation/dissipation. It was noted that 18 trials were available for lettuce and only 4 for cereals. It was discussed whether the scenarios in the GD cover mammals consuming the lettuce crop plants in addition to weeds. Experts noted that different FIR would be needed for a lettuce scenario. The experts agreed that, when refining the assessment, the food items taken by the focal species should be considered.

The representativeness of the studies used was discussed noting that the studies were not performed at the correct growth stage. The lettuce trials were performed at BBCH 17 to 46 whereas the GAP is BBCH 12 to 49 (lettuce). The RMS considered this in the DAR and noted that the lettuce trials do not cover the entire GAP for lettuce. Furthermore, the RMS noted during the meeting that it could be that at later growth stages the dissipation is slower due to slower growth. Some experts did not consider the BBCH stage of the crop to be an issue as the studies should represent weeds in addition to the crop. Furthermore, the DT50 in the two types of trials were not too dissimilar.

The cereal trials were performed at BBCH 25 to 29 and therefore were at a growth stage palatable to birds and mammals. The RMS considered that this was reasonable to represent weeds. The fTWA from both trials were comparable regardless of the growth stage further supporting the use of the studies.

Overall the experts agreed that the available studies were suitable to be used for the refined assessment for the representative uses.

It was discussed how the fTWA values should be used in the risk assessment (geomean, pooled, lower, two assessments). The geomean was questioned as it does not result in a worst case value but then the RMS noted that there were more than ten values indicating that the geomean was reasonable (NL approach). The RMS also noted that the studies needed to cover the entire EU. Some studies were performed in NEU and SEU, no differences were noted. Overall, the experts considered that the data were sufficient for N and SEU. The RMS suggested that the data could be pooled and then the geomean from the pooled data used. The experts noted that pooling data should be done with care and therefore it is difficult to agree on the basis of the available raw data. It was agreed that the RMS should pool the data but check statistically significant differences between the two data sets (lettuce crop vs. cereals and N vs. SEU) whether the pooled data are ok. If so, then the geomean can be taken. If not, then the experts considered that separate risk assessments may be required.

It was agreed that the data was suitable for all weed types and lettuce crop plants given that the data cover monocots and dicots and that the DT50 may not depend on the structure of the plant.

On the basis of the above, the RMS needs to update the risk assessment using the appropriate fTWA.

It was noted that the approach in which the 21-d ftwa is calculated from measurements which started at the last application (in trials with two applications) is acceptable for the current GAP, since this has only one application per year. It was discussed that it may not cover GAPs which have multiple applications, as the 21-d exposure window may be higher at an earlier stage. Therefore, for other GAPs it needs to be carefully considered how to use these data.

The differences between the data sets were checked by the RMS (t-test, unequal variance, two-sided): lettuce N-lettuce S; cereal N- cereal S (note that only four trials were available here so this exercise is less useful); all trials lettuce-cereals; all trials N-S. Result: P>0.1 in all cases. Therefore, the data can be pooled and the overal geometric mean can be taken for all weed types and lettuce crop plants. Thus, in the refined risk assessment for the small herbivorous mammal “vole”, an ftwa of 0.164 can used for the representative uses.

Table 2.6.1.2-11b Ftwa values for the different residue trials and mean values

trial no.	**N/S**	**ftwa**
Lettuce		
R01	N	0.14
R02	N	0.14
R03	N	0.24
R04	N	0.35
R05	N	0.16

R06	N	0.22	
R07	N	0.041	
R18	N	0.38	
R08	N	0.093	
R09	S	0.077	
R10	S	0.35	
R11	S	0.12	
R12	S	0.35	
R13	S	0.13	
R14	S	0.29	
R15	S	0.18	
R16	S	0.13	
			geometric mean lettuce trials: 0.17
Cereals			
R01	N	0.0649	
R02	N	0.1	
R03	S	0.28	
R04	S	0.21	
			median cereal trials: 0.16
			overall geometric mean: 0.164

The shortcut value which was used in the first tier calculation is calculated as SV = FIR/bw * deposition factor * RUD. In the first tier table from the EFSA GD (Appendix A), the shortcut value for the “vole” in “leafy vegetables” at BBCH 40-49 is 72.3. This is derived as follows:

FIR/bw = 1.33 (based on 100% grass intake and 25 g body weight)

Deposition factor = 1 for lettuce (no interception), 0.3 for hops (70% interception)

RUD = 54.2 (default RUD from “grass + cereal” database).

In the refined risk assessment, it needs to be considered if these values are still appropiate.

The deposition values are dependent on crop stage and therefore still relevant. Note that for lettuce, interception should not be taken into account at any stage for the part of the diet that consists of crop plants.

The “vole” foraging in lettuce fields may feed on all types of plants, not only grass. The FIR/bw for a 25 g mammal feeding 100% on ‘non-grass herbs’ is 1.61 (calculated according to Appendix G). Therefore, the first tier FIR/bw of 1.33 may slightly underestimate the risk. However, the default RUD value for the food category “non-grass herbs” is 28.7, which is almost half that of “grass”. Therefore, the calculations based only on 100% “grass” intake are clearly worst-case even though the diet composition of the “vole” in lettuce fields is not known, and as long as the default RUDs are used it is not necessary to use a different FIR/bw in the refined calculations.

Table 2.6.1.2-11c Refined long-term DDD and TER_{LT} calculation for the "vole"

Crop	Generic focal species	NOAEL [mg/kg bw/d]	DDD				DDD	TER_{LT}	Trigger
			Appl. rate [kg/ha]	SV_m	MAF_m	f_{TWA}			
BYI 02960									
Hops (≥ 40)	Small herbivorous mammal "vole"	6.4	0.150	21.7	1	0.164	0.53	12	5
Lettuce (40-49)	Small herbivorous mammal "vole"	6.4	0.125	72.3	1	0.164	1.48	**4.3**	5

Bold values do not meet the trigger

The refined TER value for the use in hops is above the trigger, indicating acceptable risk. For the use in lettuce the TER is below the trigger. The applicant provided a statement to address the risk.

Relevance of the "vole" scenario in hops and lettuce fields

The notifier submitted a statement which is presented without modifications below. Please note that the TER values are based on the worst-case endpoint of 7.8 mg/kg bw/d.

"*The risk to wild mammals from the use of BYI 02960 has been evaluated according to the Tier 1 scenarios provided in the EFSA GD. These scenarios include small insectivorous mammals ("shrew"), small omnivorous mammals ("mouse"), large herbivorous mammals ("lagomorph"), and small herbivorous mammals ("vole").*

*Whilst all other scenarios demonstrated adequate margins of safety, even with the worst case settings of a Tier 1 assessment, the scenario "vole" resulted in Tier 1 TERLT values of 4.5 and 1.6 for the uses in hop and lettuce, respectively. These Tier 1 TERLT values for voles exceed a threshold of 1, indicating that some margins of safety exist even under the worst case settings applied at Tier 1. Quantitative refinement elements (refined reproductive risk assessment endpoint 39.23 mg/kg/bw/d; measured residue decline DT50 resulting in 21-d fTWA = 0.19) that have been introduced above allow a re-calculation of the Tier 2 TERLT values that include margins of safety clearly in excess of the a-priori acceptability trigger of 5 (*values not shown here*).*

Thus, a quantitative refinement at the level of individual exposure and risk can be conducted to demonstrate acceptable risk at the level of individual voles.

However, the protection goal in ecotoxicology aims ultimately at protection of populations rather than individuals. Therefore the section below is intended to additionally evaluate the risk to vole populations from exposure to BYI 02960 after application in hop or lettuce fields. This evaluation is based on the well known biology and ecology of Common voles (Microtus arvalis) as the representative species "behind" the generic focal species scenario "vole", and complemented with a targeted field study in hop yards.

Exposure of Populations

The optimum or prime habitat of the common vole requires permanent vegetation cover and includes grassland or perennial crops such as alfalfa and clover fields. Common voles also occur in sub-optimal (secondary) habitats such as arable land. However, only prime habitats harbor permanent vole populations and are essential for the survival of common vole populations. Secondary habitats are only transiently populated, with regular population declines up to extinction during agronomic operations (e.g., ploughing or harvest), or over winter.

Wherever the species occurs; the population densities of Common voles vary seasonally, with regular mass occurrences being followed by a population break-down which can even lead to local extinction.

Spring crops like lettuce fields are not populated during winter, would not provide much cover against predation and could thus be colonized only from adjacent prime habitats at times of high population density. Spring sown vegetable fields would therefore not belong to the potential prime habitats of Common voles (e.g. Spitz, 1977[5]).

A field study conducted in hop yards in Germany (KIIIA1 10.3.3/01) also confirms that the treated area does not harbor significant proportions of the local vole populations.

Thus, any effect from exposure of voles in areas treated with BYI 02960 would be of low, if any, importance on the local population level which depends on the voles living outside of the lettuce fields or the hop yards.

Vulnerability of populations:

The failure to demonstrate TERLT > 5 for voles is not a rare event, due to the worst case combination of factors involved in the scenario. Actually, in regard of theoretical exposure of individual animals, the vole scenarios are nearly always the most critical all over the EFSA GD (2009). However, with regard of the vulnerability of vole populations, the vole scenario is probably the least critical.

The Joint Working Group on the Guidance Document on Risk Assessment for Birds & Mammals (SANCO 10997/2009) has already raised the question on the "need for the vole scenario… given the resilience of the vole populations"; i.e. well-known fact that voles are able to recover after large population breakdowns, or despite eradication programs with targeted rodenticide use (for recent evaluations see e.g.. Jacob & Tkadlec 2010[6], or Jokic et al. 2010[7]).

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[5] Spitz F (1977), Le campagnol des champs (*Microtus arvalis* (Pallas)) en Europe. EPPO Bulletin 7:156-175. BCS Edition no. M-228600-01-1

[6] Jacob & Tkadlec (2010), Rodent outbreaks in Europe: dynamics and damage. In:Singleton et al. (Eds): Rodent outbreaks: Ecology and Impacts.,p: 217-233. BCS Edition no: M-427173-01-1

This potential for recovery of Common voles is extraordinarily large, due to the ability of Common voles to achieve high population numbers within short periods of time thanks to the reproduction biology of the species (e.g. Lauenstein 1979[8], Jacob 2003[9]):

- *the potentially highest number of young per litter ranges between 12 and 13. The weight of a litter is about 53% of the females weight*
- *average number of young per litter in the field is about 7*
- *gestation period is about 20 days in the field*
- *successful mating occurs when young females are not yet weaned*
- *long reproduction period including occasional winter reproduction*

The most straightforward application of the conclusion by the Joint Working Group on the Guidance Document on Risk Assessment for Birds & Mammals (SANCO 10997/2009) (questioning the "need for the vole scenario… given the resilience of the vole populations)" would be that the failure to meet the a-priori acceptability trigger for long-term exposure of individual voles is not to be considered as problematic as long as the TER trigger for the other wild mammal scenarios reach the respective TER trigger.

This is clearly the case for exposure of wild mammals to BYI 02960.

Small herbivorous mammals in hops

A targeted field study has been conducted in hop yards in Germany (KIIIA1 10.3.3/01).

Live trapping revealed that hop yards do not harbor significant proportions of small herbivorous mammals (Common vole, Field vole or Bank vole), as only 0.09% (3 of 3272) of the individuals of the local vole populations was recorded in-field. Radio-tracking confirmed that voles make very little use of hop yards as feeding habitat. Only one of 10 voles (1 of 5 common voles) potentially spent foraging time in a hop yard. This individual was only trapped once, thus considered a disperser rather than part of a local in-field population.

Overall the results of the study do not confirm that the generic focal species scenario of "small herbivorous mammals (vole)" provided in the EFSA GD 2009 would be relevant for hop yards. To the contrary, a very low number of individual voles (< 0.1%) would be exposed on hop yards and the risk on local populations from effects on these individuals would be negligible.

Small herbivorous mammals in leafy vegetables

The prime habitat of voles is grassland but the species can also be found in cultivated areas. Stable populations can be developed in multi-annual crops, particularly clover/alfalfa fields which perfectly

[7] Jokic G, Vuksa P & Vuksa M (2010), Comparative efficacy of conventional and new rodenticides against Microtus arvalis (Pallas, 1778) in wheat and alfalfa crops. Crop Protection 29: 487-491. BCS Edition no: M-427179-01-1

[8] Lauenstein G (1979), Zur Problematik der Bekämpfung von Feldmäusen (Microtus arvalis Pall.)) auf Grünland. Zeitschrift für angewandte Zoologie 66: 35-59. BCS Edition no M-419441-01-1

[9] Jacob J (2003), Short-term effects of farming practices on populations of common voles. Agriculture, Ecosystems and Environment 95: 321-325. BCS Edition no. M-415511-01-1

match the species ecological needs. Whilst autumn sown fields might serve as overwintering habitat, spring sown fields would only be colonized later in the season (summer) and only during periods of high population density, then representing rather a "sink" than a "source" habitat for the local populations). "

Response RMS: The first tier risk assessment based on the ecologically relevant endpoint of 34 mg/kg bw/d was acceptable in the original DAR, so the public literature referred to was not evaluated. The monitoring trial in hop fields was however evaluated above (Study IIIA, 10.3.3/01).
The live trapping of mammals showed that they preferred the natural habitats instead of the hop yard. The wood mouse was the most abundant species inside the hop yard and was the only species tracked regularly inside the hop yard. Five common voles were radiotracked; one of those spent all its time in the hop yard. This individual, a fertile male, used the habitat hop yard and spent 100% of the 24 h telemetry in it. This individual seemed to be a so-called disperser trapped on study site 4, it was the only common vole trapped on this site. In late July the undersown crops Raphanus sativa, Galinsoga spec, and Chenopodiaceae were already high and therefore the predation risk inside the hop yard decreased. The population densities of common voles in the surroundings in late July was high and the need for new territories increased. These factors indicate that this individual was migrating and used the hop yard not as a sustainable habitat. However, this finding means that common voles can use hop fields as feeding habitat, at least occasionally. Therefore, RMS does not agree with the notifier that the study shows that the common vole is not a relevant species in hop fields.
It should also be noted that the results from this study (for both mammals and birds) are based on hop yards close to nature areas. It is not clear if the same focal species can be used for hop yards in an intensively cultivated area, if present at all. Mammal trapping was only conducted in the hop yards close to nature, therefore it is not possible to compare species abundance between plots in intensively cultivated area and plots close to nature areas.
If the study is used in a refined risk assessment, these remarks should be taken into account.

Furthermore, in case of higher tier, EFSA (2009) requires an uncertainty analysis of the refinements (see 6.8 and 6.9).This is however not available in the dossier. Should a refined risk assessment become necessary (e.g. by change of the endpoint after peer review), an uncertainty analysis should be provided, in the tabular form recommended by EFSA.

Based on the new long-term endpoint for mammals, the TER for the small herbivorous mammal "vole" from the use of the a.s. in lettuce is 4.3, which is below the trigger of 5. The notifier questions the relevance of the "vole" scenario in general. This issue should be considered by a dedicated EFSA working group or the PPR panel. As long as the guidance document includes the vole scenario, it has to be taken into account. Non-relevance of a species (group) can be shown with field monitoring data,

but these are not available for mammals in lettuce fields. Population effects can be investigated with field effect data and/or population modelling, but these are not available either. Based on the available data, a risk to the "vole" cannot be excluded. It is noted that the TER is not far below the trigger, that the only refinement included is one on residue dissipation, that the TER is calculated with the lowest available long-term endpoint which is based on <10% lower bodyweight at the next level, and that the diet composition use in the calculations is worst-case.

Metabolites

The notifier provided the following information: " The toxicological properties of two metabolites of BYI 02960 were investigated in a series of studies and evaluated in more detail in section KIIA 5.8 of this submission. Toxicological studies performed on two other metabolites which are also common metabolites to other agrochemicals are also reported in this section. Only a brief summary of the results of these studies is presented in this section. Overall, the results indicate that none of the metabolites is more toxic to mammals than the parent, so that the risk assessment for wild mammals can be based on the toxicity and exposure ratios calculated for BYI 02960 a.i.

The metabolite difluoroacetic acid (DFA) is a major soil, water and plant metabolite and is found in the rat ADME study at an amount of around 6%. It is considered a non-relevant metabolite in terms of the EU Guidance document Sanco/221/2000-rev.10 (25 February, 2003). This metabolite was devoid of genotoxic potential; the acute oral LD50 was between 300 and 2000 mg/kg. When DFA was administered via the diet to Wistar rats for at least 14 days at concentrations of 500, 2000 and 8000 ppm (equating approximately to 48, 187 and 745 mg/kg body weight/day, respectively in males and 51, 201 and 800 mg/kg body weight/day, respectively in females), slight body weight decrease was observed in both sexes at 8000 ppm. In a 90-day rat study, DFA was administered in the diet administration to Wistar rats at concentrations of 200, 1000 and 6000 ppm (equating approximately to 12.7, 66.2, 380 mg/kg body weight/day, respectively in males and 15.6, 78.7, 472 mg/kg body weight/day, respectively in females). Mean body weight and food consumption were reduced at 6000 and 1000 ppm, respectively in both sexes but not at 200 ppm. When the NOAEL of 200 ppm DFA is expressed in BYI 02960 equivalents, it equates to 38 and 47 mg/kg/day in males and females, respectively. Therefore, DFA was not more toxic than BYI 02960 after subchronic administration to the rat.

BYI 02960-difluoroethyl-amino-furanone (DFEAF) is a minor plant metabolite. The acute oral LD50 in rats was higher than 2000 mg/kg, indicating that DFEAF is not more toxic than BYI 02960.

BYI 02960-CHMP ((6-chloro-3-pyridyl)methanol) is a plant metabolite. The acute oral rat LD50 was 1842 mg/kg in males and 1483 mg/kg in females. In a 90-day rat study, BYI 02960-CHMP was administered in the diet to Sprague Dawley rats (10/sex/group) at concentrations of 160, 800, 4000 and 20 000 ppm, (corresponding to 9.9, 48.9, 250.1 and 1246.6 mg/kg/day, respectively for males and 11.1, 55.9, 275.9 and 1173.7 mg/kg/day, respectively for females). Mean body weights and mean food consumption were decreased at 20000 ppm in both sexes. Based on eosinophilic intranuclear

inclusions in the proximal tubular epithelium of kidneys at 20000 ppm and 4000 ppm, the no observed effects level (NOEL) was 800 ppm (48.9 mg/kg/day) in males, and 4000 ppm (275.9 mg/kg/day) in females, respectively. When the NOEL is expressed in BYI 02960 equivalents, it equates to 97.8 and 551.8 mg/kg/day, respectively. Therefore, BYI 02960-CHMP was less toxic than BYI 02960 after subchronic administration to the rat.
6-CNA (6-chloronicotinic acid) is a major soil and plant metabolite. An acute oral rat toxicity study and an Ames test were performed on this metabolite for the registration of acetamiprid. 6-CNA was not genotoxic and not acutely toxic."
RMS agrees that no separate risk assessment is required for the metabolites.

Exposure via drinking water

An assessment of the risk potentially posed by consumption of contaminated drinking water is required according to the EFSA Guidance Document for Birds and Mammals (2009).
Two scenarios were identified as relevant for assessing the risk of pesticides via drinking water to birds and mammals:

- Leaf scenario, only relevant for birds possibly drinking water from puddles in leaf whorls after application of a pesticide to a crop and subsequent rainfall or irrigation; not relevant for mammals.
- Puddle scenario. Birds and mammals taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil. This scenario is relevant for all types of applications that may cause contamination of soil and only for long-term exposure. A risk assessment is performed below.

<u>Risk assessment for the puddle scenario (field use in hop and lettuce):</u>

An "escape clause" recommended in the EFSA Guidance Document for Birds and Mammals (2009) allows for screening the need for a quantitative risk assessment by a comparison between the application rate and the toxicity of the respective substance. This escape clause specifies that "*due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals …, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (= application rate x MAF) (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≥ 500 L/kg).*" [10].

Table 2.6.1.2-12 Evaluation of potential concern for exposure of mammals drinking water from puddles (escape clause)

[10] EFSA (2009): Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA, p. 66

Compound	Koc [mL/g]	Application rate * MAF [g a.i./ha]	LD50 or NO(A)EL [mg a.i./ kg bw/d]	Ratio (Application rate * MAF) / NO(A)EL	"Escape clause" No concern if ratio	Conclusion
BYI 02960	98.4 [1)]	150 [2)] x 1	1607	0.09	≤ 50	No concern
			~~34~~ 6.4	~~4.4~~ 23	≤ 50	No concern

1) Arithmetic mean of ten K_{OC} values (see B.5)

2) Worst-case application rate in hops

This evaluation confirms that the risk for mammals from drinking from puddles that may contain residues from the use of product is acceptable.

Indirect exposure via contaminated worms or fish

Substances with a high bioaccumulation potential could theoretically bear a risk of secondary poisoning for mammals if feeding on contaminated prey like fish or earthworms. For organic chemicals, a log POW > 3 is used to trigger an in-depth evaluation of the potential for bioaccumulation. As shown, neither the active ingredient nor any of its metabolites has a log Pow above 3 and the potential of bioaccumulation is considered to be very low. Hence, there is no need to evaluate potential effects of secondary poisoning of mammals.

2.6.2 Effects on aquatic species

Summary of aquatic toxicity data

Active substance and formulation

The active ingredient of BYI 02960 SL200, BYI 02960, has been tested on all laboratory indicator species for aquatic organisms representing all trophic levels of the aquatic environment. Acute data on fish toxicity are available for four different species, amongst them the sensitive cold water fish trout, and the salt water fish *Cyprinodon variegatus*. The results indicate that BYI 02960 is practically non-toxic to fish. As such, the LC50 values reported are greater than the highest concentration within the test. Using the formulated product BYI 02960 SL 200G the LC50 as related to the active substance was >100 mg a.i./L.

An early life-stage test for BYI 02960 confirms the low toxicity to fish also on chronic scale. Exposed continuously over the full developmental life-phase of fathead minnow a slightly reduced fry survival at the highest test concentration of 10 mg a.i./L was recorded. As compared to the untreated control survival was reduced by 7% resulting in an overall fry survival of 87.5% at a mean measured

concentration of 8.4 mg a.i./L. Time to hatch and hatching success was not influenced, neither was any effect observed on growth as determined on length and weight.

A very selective non-target activity of BYI 02960 is further confirmed by low toxicity to representatives of three other taxonomic groups, namely amphibians, algae and aquatic plants. There were no adverse effects noted in the 48h acute frog test, and the influence of the active substance on *Pseudokirchneriella subcapitata* and *Lemna gibba* was also negligible. In a test with the formulation no effects on algal development were recorded at the highest test concentration used 250 mg formulation/L corresponding to 42.5 mg a.i./L. For Lemna, an onset of effects was determined at a mean measured concentration of 67.7 mg a.i./L ; therefore aquatic plants are also considered to be insensitive to the active substance.

BYI 02960 is an insecticide against sucking insects. Hence, a side activity against aquatic invertebrates can be anticipated. Data were generated on all laboratory standard indicator species of aquatic arthropods: the cladoceran *Daphnia magna*, the midge *Chironomus riparius* and, the marine species *Americamysis bahia* and the mollusc *Crassostrea virginica*. The most sensitive organism for BYI 02960 was found to be *Chironomus riparius*. While acute toxicity to daphnids was negligible with a NOEC for the active substance at or greater than 77.6 mg a.i./L, the 50% effect concentration (EC50) to the midge was determined to be at a concentration of 0.062 mg a.i./L. For the saltwater species, the LC50 of the mysid shrimp was at 0.26 mg a.i./L and indicates as such a lower sensitivity than the insect. BYI 02960 was non-toxic to the marine oyster.

Chronic tests confirm the high toxicity of the active substance to *Chironomus riparius*. The lowest-observed-effect concentration (LOEC) impacting the emergence of the developed Chironomus larvae was at ~~0.02~~ 0.015 mg a.i./L, only a factor of ~~3~~ 4 below the acute EC50 values. The acute-to-chronic ratio for the most sensitive ecotoxicological endpoint, survival of Chironomus, for BYI 02960 is thus low. The immediate activity observed in the acute test appears to be reflected in the chronic test.

The 28 day chronic mysid shrimp test resulted in a LOEC of 0.0236 mg a.i./L based upon a statistically significant influence on reproductive output. The notifier considers that from the first-tier laboratory test species indicative for a potential effect of an insecticide, Chironomus is considered to be of higher relevance for the risk assessment than the marine shrimp as it reflects a real taxonomic group present in the freshwater biocoenosis. The influence of the formulation BYI 02960 SL 200 G was therefore examined on Chironomus as the most sensitive invertebrate. The lowest-observed-effect concentration was at ~~0.024~~ 0.020 mg a.i./L, the NOEC was established at ~~0.012~~ 0.00989 mg a.i./L.

However, RMS does not accept to disregard the mysid shrimp data. Saltwater species are always included in the risk assessment unless a clear difference in sensitivity is observed between saltwater and freshwater species and it can be proven that exposure of saltwater environments is not relevant. In this case, the mysid shrimp NOEC is 0.0132 mg a.i./L and therefore close to the Chironomus endpoint. Hence, it is not considered acceptable to disregard the data available for the mysid shrimp. However, the Chironomus endpoint is driving the risk assessment since it is slightly lower.

In general, there was no relevant influence of the formulation on the toxicity in comparison to the toxicity with the active substance.

Accepted data on the acute toxicity of BYI 02960 (flupyradifurone) and BYI 02960 200 SL (Sivanto) are summarised in Table 2.6.2-01. In the tables, it is indicated whether endpoints are based on nominal or actual concentrations. Data indicated in bold are the lowest effect concentrations for that species group.

Table 2.6.2-01. The acute toxicity of BYI 02960 and BYI 02960 SL 200 to aquatic life. Bold data are the lowest effect concentrations for that specific group.

Group	Test substance	Time-scale (Test type)	End point	Toxicity[1] (mg/L)
Fish				
Rainbow trout (*Oncorhynchus mykiss)*	a.s.	96 hr (static)	Mortality, LC_{50}	>74.2 ($_{mm}$)
Fathead minnow (*Pimephales promelas*)	a.s.	96 hr (static)	Mortality, LC_{50}	**>70.5 ($_{mm}$)**
Common carp (*Cyprinus carpio)*	a.s.	96 hr (static)	Mortality, LC_{50}	>100 ($_{nom}$)
Sheepshead minnow (*Cyprinodon variegatus)* (saltwater species)	a.s.	96 hr (static)	Mortality, LC_{50}	>83.9 ($_{mm}$)
Rainbow trout (*Oncorhynchus mykiss)*	BYI 02960 SL 200[2]	96 hr (static)	Mortality, LC_{50}	>585 (form, $_{nom}$) >100 (a.s.,$_{nom}$)
Common carp (*Cyprinus carpio)*	BYI 02960 SL 200[2]	96 hr (static)	Mortality, LC_{50}	>585 (form, $_{nom}$) >100 (a.s.,$_{nom}$)
Amphibians				
Frog (*Xenopus laevis)*	a.s.	96 hr (static)	Mortality, LC_{50}	>80 ($_{nom}$)
Aquatic invertebrates				
Daphnia magna	a.s.	48 h (static)	Mortality, EC_{50}	>77.6 ($_{mm}$)
Chironomus riparius	a.s.	48 h (static)	Mortality, EC_{50}	**0.0617** ($_{nom}$)
Eastern oyster (*Crassostrea virginica)* (saltwater species)	a.s.	96 h (static)	Inhibition of shell growth, EC_{50}	>29 ($_{mm}$)
Mysid (*Americamysis bahia)* (saltwater species), juveniles*	a.s.	96 h (static)	Mortality, EC_{50}	0.26 ($_{mm}$)
Daphnia magna	BYI 02960 SL 200[2]	48 h (static)	Mortality, EC_{50}	684 (form, $_{nom}$) 117 (a.s., $_{nom}$)
Algae				
Pseudokirchneriella subcapitata	a.s.	72 h (static)	Biomass: E_bC_{50}, Growth rate: E_rC_{50} & Yield: E_yC_{50}	>80 ($_{nom}$)
Pseudokirchneriella subcapitata	BYI 02960 SL 200[2]	72 h (static)	Biomass: E_bC_{50} & Growth rate: E_rC_{50}	>250 (form, $_{nom}$) **>42.8** (a.s., $_{nom}$)

Group	Test substance	Time-scale (Test type)	End point	Toxicity[1] (mg/L)
Aquatic higher plants				
Lemna gibba	a.s.	7 d (static)	Biomass: E_bC_{50}, Growth rate: E_rC_{50} and Yield: E_yC_{50}	**>67.7** ($_{mm}$)

Metabolites

According to the LoEP from fate and behaviour, the environmental occurring metabolites requiring further assessment by other disciplines (toxicology and ecotoxicology) are:

Surface Water: 6-CNA, DFA, BYI 02960-succinamide, BYI 02960-azabicyclosuccinmaide

Sediment: 6-CNA, DFA

Accepted acute data on the acute toxicity of the relevant metabolites are summarized in Table 2.6.2-02.

Table 2.6.2-02 The acute toxicity of metabolites of BYI 02960 to aquatic life

Group	Test substance	Time-scale (Test type)	End point	Toxicity[1] (mg/L)
Fish				
Rainbow trout (*Oncorhynchus mykiss)*	BYI 02960-succinamide	96 hr (static)	Mortality, EC_{50}	>100 ($_{nom}$)
Rainbow trout (*Oncorhynchus mykiss)*	DFA (BCS-AB60481 (tech.) = sodium difluoroacetate)	96 hr (static)	Mortality, EC_{50}	>10 ($_{nom}$)
Aquatic invertebrates				
Daphnia magna	DFA (BCS-AB60481 (tech.) = sodium difluoroacetate)	48 hr (static)	Mortality, EC_{50}	>10 ($_{nom}$)
Daphnia magna	6-CNA (IC-0)	48 h (static)	Mortality, EC_{50}	>95.1 ($_{mm}$)
Chironomus riparius	BYI 02960-succinamide	48 h (static)	Mortality, EC_{50}	>100 ($_{nom}$)
Chironomus riparius	BYI 02960-azabicyclosuccinamide, sodium salt	48 h (static)	Mortality, EC_{50}	>100 ($_{nom}$)
Chironomus tentans	6-CNA	48 h (static)	Mortality, EC_{50}	>1 ($_{nom}$)

Group	Test substance	Time-scale (Test type)	End point	Toxicity[1] (mg/L)
Algae				
Pseudokirchneriella subcapitata	DFA (BCS-AB60481 (tech.) = sodium difluoroacetate)	72 hr (static)	Growth rate: E_rC_{50} and Yield: E_yC_{50}	>10 ($_{nom}$)
Pseudokirchneriella subcapitata	BYI 02960-succinamide	72 hr (static)	Growth rate: E_rC_{50} and Yield: E_yC_{50}	>10 ($_{nom}$)
Pseudokirchneriella subcapitata	6-CNA (IC-0)	72 hr (static)	Growth rate: E_rC_{50} Yield: E_yC_{50}	>100 ($_{nom}$) 85 ($_{nom}$)

Chronic toxicity data

Active substance and formulation

Accepted data on the chronic toxicity of BYI 02960 (flupyradifurone) and BYI 02960 200 SL (Sivanto) are summarised in Table 2.6.2-03. In the tables, it is indicated whether endpoints are based on nominal or actual concentrations. Data indicated in bold are the lowest effect concentrations for that species group.

Table 2.6.2-03 The chronic toxicity of BYI 02960 and BYI 02960 SL 200 to aquatic life. Bold data are the lowest effect concentrations for that specific group.

Group	Test substance	Time-scale (Test type)	End point	Toxicity[1] (mg/L)
Laboratory tests ‡				
Fish				
Fathead minnow (*Pimephales promelas*)	a.s.	ELS, 35 d (flow-through)	Survival NOEC	**4.41** ($_{mm}$)
Aquatic invertebrate				
Daphnia magna	a.s.	21 d (semi-static)	Reproduction, NOEC	3.2 ($_{nom}$)
Mysid (*Americamysis bahia)* (saltwater species), juveniles	a.s.	28 d (semi-static)	life-cycle toxicity, NOEC	0.0132 ($_{mm}$)
Sediment dwelling organisms				
Chironomus riparius	a.s.	28 d (static; water-spiked)	NOEC	**~~0.010~~ 0.00681** (~~$_{nom}$~~ $_{mm}$)

Group	Test substance	Time-scale (Test type)	End point	Toxicity[1] (mg/L)
Chironomus riparius	BYI 02960 SL 200[2]	28 d (static; water-spiked)	NOEC	~~0.012~~ 0.00989 mg a.s./L (~~nom~~ mm)

Chronic toxicity data for the metabolites are summarised in Table 2.6.2-04.

Table 2.6.2-04 The chronic toxicity of metabolites of BYI 02960 to aquatic life

Group	Test substance	Time-scale (Test type)	End point	Toxicity[1] (mg/L)
Aquatic invertebrates				
Daphnia magna	BYI 02960-succinamide	21 d (semi-static)	Reproduction, NOEC	43.3 ($_{nom}$)
Sediment dwelling organisms				
Chironomus riparius	DFA (BCS-AB60481 (tech.) = sodium difluoroacetate)	28 d (static; water-spiked)	NOEC	100 ($_{nom}$)
Chironomus riparius	6-CNA (IC-0)	28 d (static; water-spiked)	NOEC	100 ($_{nom}$)

Risk assessment

The risk assessment is based on Guidance Document on Aquatic Ecotoxicology, SANCO/3268/2001, rev 4 final, 17 October 2002. Toxicity exposure ratios (TER values) are calculated based on the most sensitive species and worst-case PEC_{SW} values.

The TER-values have been calculated based on the following equations:

TER_A = LC50 or EC50 / maximum PEC_{SW}

TER_{LT} = ErC50 / maximum PEC_{SW}

TER_{LT} = chronic NOEC /maximum PEC_{SW}

The risk is considered acceptable if the TER_A values are ≥ 100, and the TER_{LT} values ≥ 10.

Acute and chronic risk assessment was carried out in tier 1 using the initial PEC_{SW} calculated with FOCUS Surface Water, Step 2, and the lowest available toxicity endpoints for algae, *Daphnia* and other invertebrates, fish, amphibians, and *Lemna*. The resulting TER-values are presented for the

different uses of BYI 02960 SL 200 for the a.s. and the metabolites. TERs that do not meet the relevant trigger are indicated in bold.

Tier 1 risk assessment for fish (and other aquatic vertebrates)

The acute risk assessment for the a.s. is based on the lowest LC50 for fish of >70.5 mg a.s./L; this also covers amphibians since the LC50 for *Xenopus laevis* is higher (>80 mg a.s./L).

Table 2.6.2-05 TER_A calculations for fish based on FOCUS Step 2 for application in hops

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Crop: Hops						
BYI 02960	*P. promelas*	LC_{50}	**>** 70 500	17.36	> 4 061	100
BYI 02960 – succinamide	*O. mykiss*	LC_{50}	**>** 100 000	4.065	> 24 600	
DFA*	*O. mykiss*	LC_{50}	> 10 000	1.268 (1.421*)	>7 886 (> 7 037*)	

*) In a worst-case approach, exposure concentrations for DFA are based upon groundwater calculations; however, dilution will occur during transport to surface waters.

Table 2.6.2-06 TER_A calculations for fish based on FOCUS Step 2 for application in lettuce – biennial (covers annual use)

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Crop: Lettuce						
BYI 02960	*P. promelas*	LC_{50}	**>** 70 500	11.88	> 5 934	100
BYI 02960 – succinamide	*O. mykiss*	LC_{50}	**>** 100 000	0.484	> 206 612	
DFA*	*O. mykiss*	LC_{50}	> 10 000	1.339 (5.599*)	> 7 468 (> 1 786*)	

*) In a worst-case approach, exposure concentrations for DFA are based upon groundwater calculations; however, dilution will occur during transport to surface waters.

The TERA values meet the required trigger for both uses, indicating an acceptable acute risk to fish for application of the product.

Table 2.6.2-07 TER_{LT} calculations for fish based on FOCUS Step 2 for application in hops

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_{LT}	Trigger
Crop: Hops						
BYI 02960	*P. promelas*	NOEC	4410	17.36	254	10

Table 2.6.2-08 TER_{LT} calculations for fish based on FOCUS Step 2 for application in lettuce – biennial (covers annual use)

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_{LT}	Trigger
Crop: Lettuce						
BYI 02960	*P. promelas*	NOEC	4410	11.88	371	10

The TER_{LT} values meet the required trigger for both uses, indicating an acceptable long-term risk to fish for application of the product.

Tier 1 risk assessment for aquatic invertebrates (including sediment-dwelling organisms)

The acute risk assessment for the a.s. is based on the lowest LC50 for aquatic invertebrates of 0.0617 mg a.s./L, for *Chironomus*. Sediment-spiked tests with *Chironomus* are not available, therefore the risk assessment is performed with PECsw only (so PECsed is not used).

Table 2.6.2-09 TER_A calculations for aquatic invertebrates based on FOCUS Step 2 for application in hops

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Crop: Hops						
BYI 02960	*C. riparius*	EC_{50}	61.7	17.36	**3.53**	100
DFA*	*D. magna*	EC_{50}	**>** 10 000	1.268 (1.421*)	> 7 886 (> 7 037*)	
6-CNA	*D. magna*	EC_{50}	> 95 100	0.463	> 205 400	

*) In a worst-case approach, exposure concentrations for DFA are based upon groundwater calculations; however, dilution will occur during transport to surface waters.

Table 2.6.2-10 TER_A calculations for aquatic invertebrates based on FOCUS Step 2 for application in lettuce – biennial (covers annual use)

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Crop: Hops						
BYI 02960	*C. riparius*	EC_{50}	61.7	17.36	**3.53**	100
DFA*	*D. magna*	EC_{50}	> 10 000	1.268 (1.421*)	> 7 886 (> 7 037*)	
6-CNA	*C. tentans*	EC_{50}	> 1 000	0.463	> 2 160	
BYI 02960-succinamide	*C. riparius*	EC_{50}	> 100 000	4.065	> 24 600	
BYI 02960-azabicyclosuccinamide, sodium salt	*C. riparius*	EC_{50}	> 100 000	2.499	>40 016	

*) In a worst-case approach, exposure concentrations for DFA are based upon groundwater calculations; however, dilution will occur during transport to surface waters.

The TERA values meet the required trigger for both uses for the metabolites, indicating an acceptable acute risk to aquatic invertebrates. However, for the acute risk of the a.s., refinement is required.

The long-term risk assessment for the a.s. is based on the lowest NOEC for aquatic invertebrates of ~~0.0102~~ 0.00681 mg a.s./L, for *C.riparius*.

Table 2.6.2-11 TER_{LT} calculations for aquatic invertebrates based on FOCUS Step 2 for application in hops

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_{LT}	Trigger
Crop: Hops						
BYI 02960	*C. riparius*	NOEC	~~10~~ 6.81	17.36	~~**0.58**~~ **0.39**	10
BYI 02960 – succinamide	*D. magna*	NOEC	43 300	4.065	10 652	
DFA*	*C. riparius*	NOEC	100 000	1.268 (1.421*)	78 864 (70 373*)	
6-CNA	*C. riparius*	NOEC	100 000	0.463	215 983	

*) In a worst-case approach, exposure concentrations for DFA are based upon groundwater calculations; however, dilution will occur during transport to surface waters.

Table 2.6.2-012 TER_{LT} calculations for aquatic invertebrates based on FOCUS Step 2 for application in lettuce - biennial (covers annual use)

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_{LT}	Trigger
Crop: Lettuce						
BYI 02960	*C. riparius*	NOEC	~~10~~ 6.81	11.88	~~**0.84**~~ **0.57**	10
BYI 02960 – succinamide	*D. magna*	NOEC	43 300	0.484	89 463	
DFA*	*C. riparius*	NOEC	100 000	1.339 (5.599*)	74 683 (17 860*)	
6-CNA	*C. riparius*	NOEC	100 000	0.579	172 712	

*) In a worst-case approach, exposure concentrations for DFA are based upon groundwater calculations; however, dilution will occur during transport to surface waters.

The TERLT values meet the required trigger for both uses for the metabolite, indicating an acceptable long-term risk to aquatic invertebrates. However, for the long-term risk of the a.s., refinement is required.

Tier 1 risk assessment for algae

The risk assessment for the a.s. is based on the lowest EC50 for algae of >42.8 mg a.s./L, based on the study with the formulated product.

Table 2.6.2-13 TER_A calculations for algae based on FOCUS Step 2 for application in hops

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Crop: Hops						
BYI 02960	*P. subcapitata*	EC50	>42 800	17.36	>2 465	10
DFA	*P. subcapitata*	EC50	>10 000	1.268	>7 886	10
BYI 02960 – succinamide	*P. subcapitata*	EC50	>10 000	4.065	>2 460	10
6-CNA	*P. subcapitata*	EC50	85 000	0.463	183 585	10

Table 2.6.2-14 TER_A calculations for algae based on FOCUS Step 2 for application in lettuce – biennial (covers annual use)

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Crop: Lettuce						
BYI 02960	*P. subcapitata*	EC50	>42 800	11.88	>3 603	10
DFA	*P. subcapitata*	EC50	>10 000	1.339	>7 468	10
BYI 02960 – succinamide	*P. subcapitata*	EC50	>10 000	0.484	>20 661	10
6-CNA	*P. subcapitata*	EC50	85 000	0.579	146 805	10

The TERA values meet the required trigger for both uses for the a.s. and the metabolites, indicating an acceptable risk to algae.

Tier 1 risk assessment for aquatic macrophytes

Table 2.6.2-15 TER_A calculations for lemna based on FOCUS Step 2 for application in hops

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Crop: Hops						
BYI 02960	*L. gibba*	EC50	>67 700	17.36	>3 890	10

Table 2.6.2-16 TER_A calculations for lemna based on FOCUS Step 2 for application in lettuce –biennial (covers annual use)

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Crop: Lettuce						
BYI 02960	*L. gibba*	EC50	>67 700	11.88	>5 699	10

The TERA values meet the required trigger for both uses for the a.s., indicating an acceptable risk to algae.

Refined risk assessment for aquatic invertebrates

Based on the tier 1 risk assessments shown above, acceptable risk is found for the a.s. for fish and other aquatic vertebrates, algae and aquatic macrophytes.
For the relevant metabolites, sufficient toxicity data are available. Acceptable risk is found for all groups.
However, refinement is necessary for the a.s. for the acute and long-term risk to aquatic invertebrates. The risk is refined on the exposure side, with Step 3 and Step 4 FOCUSsw calculations.

For lettuce, FOCUS Step 4 PECsw values are available both for the annual (once per 12 months) and the biennial (once per 24 months) use. The annual use is proposed at BBCH 41-49, the biennial at BBCH 12-49. Because a higher interception percentage can be taken into account at later crop stages, the PEC values from FOCUS Step 2 are higher for the biennial use. In Step 3 and 4, however, the PEC values are sometimes higher for the annual and sometimes for the biennial use, depending on the scenario. This was not presented clearly in the original DAR, but in this revised version TER values are presented both for the biennial and the annual use.

Refined acute risk assessment for aquatic invertebrates

Table 2.6.2-17 TER_A calculations for *C. riparius* based on FOCUS Step 3 for application in hops

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	Scenario	TER_A	Trigger
Crop: Hops							
BYI 02960	*C. riparius*	EC_{50}	61.7	0.394	R1, pond	157	100
				5.531	R1, stream	**11**	

Table 2.6.2-18 TER_A calculations for *C. riparius* based on FOCUS Step 3 for application in lettuce – biennial use

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	Scenario	TER_A	Trigger
Crop: Lettuce							

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Scenario	TER_A	Trigger
BYI 02960	*C. riparius*	EC_{50} 61.7	0.830	D3 (ditch, 1st)	**74.3**	100
			0.840	D3 (ditch, 2nd)	**73.5**	
			1.035	D4 (pond, 1st)	**59.6**	
			0.794	D4 (stream, 1st)	**77.7**	
			1.268	D6 (ditch, 1st)	**48.7**	
			0.060	R1 (pond, 1st)	1028	
			0.858	R1 (stream, 1st)	**71.9**	
			0.097	R1 (pond, 2nd)	636	
			1.186	R1 (stream, 2nd)	**52.0**	
			1.586	R2 (stream, 1st)	**38.9**	
			0.940	R2 (stream, 2nd)	**65.6**	
			2.226	R3 (stream, 1st)	**27.7**	
			3.570	R3 (stream, 2nd)	**17.3**	
			0.522	R4 (stream, 1st)	118.2	
			4.808	R4 (stream, 2nd)	**12.8**	

Table 2.6.2-19 TER_A calculations for *C. riparius* based on FOCUS Step 3 for application in lettuce – annual use

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Scenario	TER_A	Trigger
Crop: Lettuce						
BYI 02960	*C. riparius*	EC_{50} 61.7	0.813	D3 (ditch, 1st)	**75.9**	100
			0.811	D3 (ditch, 2nd)	**76.1**	
			0.983	D4 (pond, 1st)	**62.8**	
			0.969	D4 (stream, 1st)	**63.7**	
			1.206	D6 (ditch, 1st)	**51.2**	
			0.337	R1 (pond, 1st)	183	
			2.827	R1 (stream, 1st)	**21.8**	
			0.139	R1 (pond, 2nd)	444	
			2.189	R1 (stream, 2nd)	**28.2**	
			1.215	R2 (stream, 1st)	**50.8**	

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Scenario	TER_A	Trigger
			1.044	R2 (stream, 2nd)	**59.1**	
			3.698	R3 (stream, 1st)	**16.7**	
			3.297	R3 (stream, 2nd)	**18.7**	
			0.83	R4 (stream, 1st)	**74.3**	
			2.954	R4 (stream, 2nd)	**20.9**	

For use in hops and both uses in lettuce a safe use is shown at Step 3 for at least one subscenario (TER > 100). Potential mitigation measures are investigated considering Step 4 calculations.

Table 2.6.2-19 Refined TER_A calculations for *C. riparius* based on FOCUS Step 4 for application in hops (90 % drift reduction)

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Scenario	TER_A	Trigger
Crop: Hops; 90 % drift reduction (no buffer zone)						
BYI 02960	*C. riparius*	EC_{50} 61.7	0.553	R1, stream	112	100
Crop: Hops; 75 % drift reduction and 10 m buffer zone						
BYI 02960	*C. riparius*	EC_{50} 61.7	0.589	R1, stream	105	100

The above table shows that for hops, acceptable risk can be reached with different combinations of buffer zones and spray drift reduction.

Table 2.6.2-20 Refined TER_A calculations for *C. riparius* based on FOCUS Step 4 for application in lettuce (drift reducing nozzles) – biennial use

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Drift reduction [%]	Scenario	TER_A	Trigger
Crop: Lettuce; 50% drift reduction (No buffer)							
BYI 02960	*C. riparius*	EC_{50} 61.7	**0.434**	50	D3 (ditch, 1st)	143	100
			0.446	50	D3 (ditch, 2nd)	139	
			1.033	90	D4 (pond, 1st)	**59.7**	
			0.721	90	D4 (stream, 1st)	**85.6**	
			1.268	90	D6 (ditch, 1st)	**48.7**	

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Drift reduction [%]	Scenario	TER_A	Trigger
			0.858	90	R1 (stream, 1st)	**71.9**	
			1.186	90	R1 (stream, 2nd)	**52.0**	
			1.586	90	R2 (stream, 1st)	**38.9**	
			0.940	90	R2 (stream, 2nd)	**65.6**	
			2.226	90	R3 (stream, 1st)	**27.7**	
			3.570	90	R3 (stream, 2nd)	**17.3**	
			4.808	90	R4 (stream, 2nd)	**12.8**	

Table 2.6.2-20 shows that for the biennial use in lettuce, most scenario's do not meet the trigger considering drift reducton only. For the annual use in lettuce, PECsw values are not available considering drift reduction only. Therefore, further refinement is necessary for both lettuce uses. FOCUS Step 4 PECsw values are available based on buffer zones (with drift and/or run-off reduction.

Table 2.6.2-21a Refined TER_A calculations for *C. riparius* based on FOCUS Step 4 for application in lettuce (5 m distance) – biennial use

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	Scenario	TER_A	Trigger
Crop: Lettuce; 5 m drift buffer (no drift reduction)							
BYI 02960	*C. riparius*	EC_{50}	61.7	0.252	D3 (ditch, 1st)	246	100
				0.265	D3 (ditch, 2nd)	234	
				1.035	D4 (pond, 1st)	**60**	
				0.721	D4 (stream, 1st)	**86**	
				1.268	D6 (ditch, 1st)	**49**	
				0.858	R1 (stream, 1st)	**72**	
				1.186	R1 (stream, 2nd)	**52**	
				1.586	R2 (stream, 1st)	**39**	
				0.940	R2 (stream, 2nd)	**66**	
				2.226	R3 (stream, 1st)	**28**	
				3.570	R3 (stream, 2nd)	**17**	
				4.808	R4 (stream, 2nd)	**13**	

Table 2.6.2-21b Refined TER_A calculations for *C. riparius* based on FOCUS Step 4 for application in lettuce (5 m distance) – annual use

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	Scenario	TER_A	Trigger
Crop: Lettuce; 5 m drift buffer (no drift reduction)							
BYI 02960	*C. riparius*	EC_{50}	61.7	0.234	D4 (pond, 1st)	264	100
				0.234	D4 (stream, 1st)	264	
				0.982	D4 (pond, 1st)	**59.7**	
				0.969	D4 (stream, 1st)	**85.6**	
				1.206	D6 (ditch, 1st)	**48.7**	
				2.827	R1 (stream, 1st)	**71.9**	
				2.189	R1 (stream, 2nd)	**52.0**	
				1.215	R2 (stream, 1st)	**38.9**	
				1.044	R2 (stream, 2nd)	**65.6**	
				3.698	R3 (stream, 1st)	**27.7**	
				3.297	R3 (stream, 2nd)	**17.3**	
				0.830	R4 (stream, 1st)	**74.34**	
				2.954	R4 (stream, 2nd)	**12.8**	

Table 2.6.2-22a Refined TER_A calculations for *C. riparius* based on FOCUS Step 4 for application in lettuce (10 m distance) – biennial use

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Buffer type	Scenario	TER_A	Trigger
Crop: Lettuce; 10 m distance (spray drift mitigation only (S) or spray drift mitigation + VFS* (R))							
BYI 02960	*C. riparius*	EC_{50} 61.7	1.034	S	D4 (pond, 1st)	**59.7**	100
			0.721	S	D4 (stream, 1st)	**85.6**	
			1.268	S	D6 (ditch, 1st)	**48.7**	
			0.389	R	R1 (stream, 1st)	159	
			0.540	R	R1 (stream, 2nd)	114	
			0.716	R	R2 (stream, 1st)	**86.2**	
			0.422	R	R2 (stream, 2nd)	146	
			1.009	R	R3 (stream, 1st)	**61.2**	
			1.630	R	R3 (stream, 2nd)	**37.9**	
			2.184	R	R4 (stream, 2nd)	**28.3**	

*VFS: vegetated filter strip

Table 2.6.2-22b Refined TER_A calculations for *C. riparius* based on FOCUS Step 4 for application in lettuce (10 m distance) – annual use

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Buffer type	Scenario	TER_A	Trigger
Crop: Lettuce; 10 m distance (spray drift mitigation only (S) or spray drift mitigation + VFS* (R))							
BYI 02960	*C. riparius*	EC_{50} 61.7	0.975	S	D4 (pond, 1st)	**63.3**	100
			0.969	S	D4 (stream, 1st)	**63.7**	
			1.206	S	D6 (ditch, 1st)	**51.2**	
			1.270	R	R1 (stream, 1st)	**48.6**	
			0.954	R	R1 (stream, 2nd)	**64.7**	
			0.553	R	R2 (stream, 1st)	112	
			0.469	R	R2 (stream, 2nd)	132	
			1.683	R	R3 (stream, 1st)	**36.7**	

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Buffer type	Scenario	TER_A	Trigger
			1.506	R	R3 (stream, 2nd)	**41.0**	
			1.345	R	R4 (stream, 1st)	**45.9**	
			0.705	R	R4 (stream, 2nd)	**87.5**	

*VFS: vegetated filter strip

Table 2.6.2-22c Refined TER_A calculations for *C. riparius* based on FOCUS Step 4 for application in lettuce (15 m distance) – biennial use

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Buffer type	Scenario	TER_A	Trigger
Crop: Lettuce; 15 m distance (spray drift mitigation only (S) or spray drift mitigation + VFS* (R))							
BYI 02960	*C. riparius*	EC_{50} 61.7	1.034	S	D4 (pond, 1st)	**59.7**	100
			0.721	S	D4 (stream, 1st)	**85.6**	
			1.268	S	D6 (ditch, 1st)	**48.7**	
			0.375	R	R2 (stream, 1st)	165	
			0.528	R	R3 (stream, 1st)	117	
			0.856	R	R3 (stream, 2nd)	**72.1**	
			1.144	R	R4 (stream, 2nd)	**53.9**	

*VFS: vegetated filter strip

Table 2.6.2-22c Refined TER_A calculations for *C. riparius* based on FOCUS Step 4 for application in lettuce (15 m distance) – annual use

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Buffer type	Scenario	TER_A	Trigger
Crop: Lettuce; 15 m distance (spray drift mitigation only (S) or spray drift mitigation + VFS* (R))							
BYI 02960	*C. riparius*	EC_{50} 61.7	0.975	S	D4 (pond, 1st)	**63.3**	100
			0.969	S	D4 (stream, 1st)	**63.7**	
			1.206	S	D6 (ditch, 1st)	**51.2**	
			1.270	R	R1 (stream, 1st)	**48.6**	
			0.954	R	R1 (stream, 2nd)	**64.7**	
			1.683	R	R3 (stream, 1st)	**36.7**	

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Buffer type	Scenario	TER_A	Trigger
			1.506	R	R3 (stream, 2nd)	**41.0**	
			1.345	R	R4 (stream, 1st)	163	
			0.705	R	R4 (stream, 2nd)	**45.9**	

*VFS: vegetated filter strip

Table 2.6.2-23a Refined TER_A calculations for *C. riparius* based on FOCUS Step 4 for application in lettuce (20 m distance) – biennial use

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Buffer type	Scenario	TER_A	Trigger
Crop: Lettuce; 20 m distance (spray drift mitigation only (S) or spray drift mitigation + VFS* (R))							
BYI 02960	*C. riparius*	EC_{50} 62	1.033	S	D4 (pond, 1st)	**59.7**	100
			0.721	S	D4 (stream, 1st)	**85.6**	
			1.268	S	D6 (ditch, 1st)	**48.7**	
			0.856	R	R3 (stream, 2nd)	**72.1**	
			1.144	R	R4 (stream, 2nd)	**53.9**	

*VFS: vegetated filter strip

Table 2.6.2-23b Refined TER_A calculations for *C. riparius* based on FOCUS Step 4 for application in lettuce (20 m distance) – annual use

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Buffer type	Scenario	TER_A	Trigger
Crop: Lettuce; 20 m distance (spray drift mitigation only (S) or spray drift mitigation + VFS* (R))							
BYI 02960	*C. riparius*	EC_{50} 61.7	0.975	S	D4 (pond, 1st)	**63.3**	100
			0.969	S	D4 (stream, 1st)	**63.7**	
			1.206	S	D6 (ditch, 1st)	**51.2**	
			0.662	R	R1 (stream, 1st)	**93.2**	
			0.500	R	R1 (stream, 2nd)	123	
			0.882	R	R3 (stream, 1st)	**70.0**	
			0.790	R	R3 (stream, 2nd)	**78.1**	
			0.705	R	R4 (stream, 2nd)	**87.5**	

*VFS: vegetated filter strip

Conclusion acute risk aquatic invertebrates:
A safe use has been shown for the subscenario R1 (pond) in both hops and lettuce with no risk mitigation.

For hops, acceptable risk can be reached in the one relevant complete scenario with different combinations of buffer zones and spray drift reduction.

For the biennial use in lettuce, by applying mitigation measures an acceptable use can be shown for for ten of the fifteen FOCUS subscenarios considering different mitigation options (up to 20m VFS). ~~scenario D3 with no buffer zone and drift reducing nozzles, and for some of the subscenarios R1 stream, R2 stream and R3 stream considering different buffer zones (vegetated filter strips) and drift reducing nozzles~~. Of the seven complete scenario's, three meet the trigger.

For the annual use in lettuce, by applying mitigation measures an acceptable use can be shown for for eight of the fifteen FOCUS subscenarios considering different mitigation options (up to 20m VFS). Of the seven complete scenario's, two meet the trigger.

Refined long-term risk assessment for aquatic invertebrates

Table 2.6.2-24 TER_{LT} calculations for *C. riparius* based on FOCUS Step 3 for application in hops

Compound	Species	Endpoint [µg a.i./L]	$PEC_{sw,max}$ [µg/L]	Scenario	TER_{LT}	Trigger
Crop: Hops						
BYI 02960	*C. riparius*	NOEC ~~10~~ 6.81	0.394	R1, pond	~~25~~ 17.3	10
			5.531	R1, stream	~~1.81~~ **1.23**	

Table 2.6.2-25a **TER_{LT} calculations for *C.riparius* based on FOCUS Step 3 for application in lettuce – biennial use**

Compound	Species	Endpoint [µg a.i./L]	$PEC_{sw,max}$ [µg/L]	Scenario	TER_{LT}	Trigger
Crop: Lettuce						
BYI 02960	*C. riparius*	NOEC ~~10~~ 6.81	0.830	D3 (ditch, 1st)	~~12.0~~ **8.20**	10
			0.840	D3 (ditch, 2nd)	~~11.9~~ **8.11**	
			1.035	D4 (pond, 1st)	~~9.7~~ **6.58**	
			0.794	D4 (stream, 1st)	~~12.6~~ **8.58**	
			1.268	D6 (ditch, 1st)	~~7.9~~ **5.37**	
			0.060	R1 (pond, 1st)	~~167~~ 114	
			0.858	R1 (stream, 1st)	~~11.7~~ **7.94**	
			0.097	R1 (pond, 2nd)	~~103~~ 70.2	
			1.186	R1 (stream, 2nd)	~~8.4~~ **5.74**	
			1.586	R2 (stream, 1st)	~~6.3~~ **4.29**	
			0.940	R2 (stream, 2nd)	~~10.6~~ **7.24**	
			2.226	R3 (stream, 1st)	~~4.5~~ **3.06**	
			3.570	R3 (stream, 2nd)	~~2.8~~ **1.91**	
			0.522	R4 (stream, 1st)	~~19.2~~ 13.1	
			4.808	R4 (stream, 2nd)	~~2.1~~ **1.42**	

Table 2.6.2-26b **TER_{LT} calculations for *C.riparius* based on FOCUS Step 3 for application in lettuce – annual use**

Compound	Species	Endpoint [µg a.i./L]	$PEC_{sw,max}$ [µg/L]	Scenario	TER_{LT}	Trigger
Crop: Lettuce						
BYI 02960	*C. riparius*	NOEC ~~10~~ 6.81	0.830	D3 (ditch, 1st)	**8.38**	10
			0.811	D3 (ditch, 2nd)	**8.40**	
			0.983	D4 (pond, 1st)	**6.93**	
			0.969	D4 (stream, 1st)	**7.03**	
			1.206	D6 (ditch, 1st)	**5.65**	
			0.337	R1 (pond, 1st)	20.2	
			2.827	R1 (stream, 1st)	**2.41**	
			0.139	R1 (pond, 2nd)	49.0	

Compound	Species	Endpoint [µg a.i./L]	$PEC_{sw,max}$ [µg/L]	Scenario	TER_{LT}	Trigger
			2.189	R1 (stream, 2nd)	**3.11**	
			1.215	R2 (stream, 1st)	**5.60**	
			1.044	R2 (stream, 2nd)	**6.52**	
			3.698	R3 (stream, 1st)	**1.84**	
			3.297	R3 (stream, 2nd)	**2.07**	
			0.830	R4 (stream, 1st)	**8.20**	
			2.954	R4 (stream, 2nd)	**2.31**	

The TER_{LT} values for aquatic invertebrates do not meet the required trigger based upon Step 3 PECsw values in one of the two subscenario's in hops, in twelve of the fifteen subscenarios in the biennial use in lettuce and in thirteen of the fifteen subscenarios (all of the seven complete scenarios) in the annual use in lettuce. A refined risk assessment with FOCUS Step 4 values, considering mitigation options is required.

Table 2.6.2-26 TER_{LT} calculations for *C. riparius* based on FOCUS Step 4 for application in hops with exposure mitigation measures

Compound	Species	Endpoint [µg a.i./L]	$PEC_{sw,max}$ [µg/L]	Scenario	TER_{LT}	Trigger
Crop: Hops; no buffer zone 90% drift reduction						
BYI 02960	*C.riparius*	NOEC ~~10~~ 6.81	0.553	R1, stream	~~18.1~~ 12.3	10
5 m distance (spray drift buffer) and 90 % drift reduction						
BYI 02960	*C.riparius*	NOEC ~~10~~ 6.81	0.452	R1, stream	~~22.1~~ 15.1	10
10 m distance (spray drift buffer & VFS*) and 90 % drift reduction						
BYI 02960	*C.riparius*	NOEC ~~10~~ 6.81	0.235	R1, stream	~~42.6~~ 29.0	10
15 m distance (spray drift buffer & VFS*) and 75 % drift reduction						
BYI 02960	*C.riparius*	NOEC ~~10~~ 6.81	0.388	R1, stream	~~25.8~~ 17.6	10

*VFS: vegetated filter strip

The above table shows that for hops, acceptable risk can be reached with different combinations of buffer zones and spray drift reduction.

Table 2.6.2-27 TER_{LT} calculations for *C. riparius* based on FOCUS Step 4 for biannial application in lettuce (drift reducing nozzles)

Compound	Species	Endpoint [µg a.i./L]	$PEC_{sw,max}$ [µg/L]	Drift reduction [%]	Scenario	TER_{LT}	Trigger
Crop: Lettuce; drift reduction (no buffer)							
BYI 02960	*C.riparius*	NOEC ~~10~~ 6.81	0.434	50%	D3 (ditch, 1st)	15.7	10
			0.446	50%	D3 (ditch, 2nd)	15.3	
			1.033	90%	D4 (pond, 1st)	~~9.68~~ **6.59**	
			0.721	90%	D4 (stream, 1st)	~~13.9~~ **9.45**	
			1.268	90%	D6 (ditch, 1st)	~~7.89~~ **5.37**	
			0.858	90%	R1 (stream, 1st)	~~11.7~~ **7.94**	
			1.186	90%	R1 (stream, 2nd)	~~8.43~~ **5.74**	
			1.586	90%	R2 (stream, 1st)	~~6.31~~ **4.29**	
			0.940	90%	R2 (stream, 2nd)	~~10.6~~ **7.24**	
			2.226	90%	R3 (stream, 1st)	~~4.49~~ **3.06**	
			3.570	90%	R3 (stream, 2nd)	~~2.80~~ **1.91**	
			4.808	90%	R4 (stream, 2nd)	~~2.08~~ **1.42**	

Table 2.6.2-27shows that for the biennial use in lettuce, most scenario's do not meet the trigger considering drift reducton only. For the annual use in lettuce, PECsw values are not available considering drift reduction only. Therefore, further refinement is necessary for both lettuce uses. FOCUS Step 4 PECsw values are available based on buffer zones, with drift and/or run-off reduction). Since the acute endpoint for C.chironomus is driving the risk assessment, TERLT values are not presented for all possible mitigation measures, but only for the minimum required mitigation that meets the trigger, ór for the strictest mitigation measure in case this does not meet the trigger yet.

Table 2.6.2-28a TER_{LT} calculations for *C.riparius* based on FOCUS Step 4 for biennial application in lettuce (per subscenario, the minimum required mitigation measure is shown to

reach a TER < trigger. If this cannot be reached, the TER is shown at the strictest mitigation measure)

Compound	Species	Endpoint [µg a.i./L]	$PEC_{sw,max}$ [µg/L]	% drift reduction	buffer zone	Scenario	TER_{LT}	Trigger
Crop: Lettuce; D scenarios: buffer (spray drift only) + drift reduction (nozzles); R scenarios: buffer (spray drift + VFS*) + drift reduction)								
BYI 02960	*C.riparius*	NOEC ~~10~~ 6.81	1.033	90%	20	D4 (pond, 1st)	**6.59**	10
			0.721	90%	20	D4 (stream, 1st)	**9.45**	
			1.268	90%	20	D6 (ditch, 1st)	**5.37**	
			0.389	0%	20	R1 (stream, 1st)	17.5	
			0.540	0%	10	R1 (stream, 2nd)	12.6	
			0.375	0%	10	R2 (stream, 1st)	18.2	
			0.422	0%	10	R2 (stream, 2nd)	16.1	
			0.528	90%	15	R3 (stream, 1st)	12.9	
			0.856	90%	20	R3 (stream, 2nd)	**7.96**	
			1.144	90%	20	R4 (stream, 2nd)	**5.95**	

*VFS: vegetated filter strip

Table 2.6.2-28b TER_{LT} calculations for *C.riparius* based on FOCUS Step 4 for annual application in lettuce (per subscenario, the minimum required mitigation measure is shown to reach a TER < trigger. If this cannot be reached, the TER is shown at the strictest mitigation measure)

Compound	Species	Endpoint [µg a.i./L]	$PEC_{sw,max}$ [µg/L]	% drift reduction	buffer zone	Scenario	TER_{LT}	Trigger
Crop: Lettuce; D scenarios: buffer (spray drift only) + drift reduction (nozzles); R scenarios: buffer (spray drift + VFS*) + drift reduction)								
BYI 02960	*C.riparius*	NOEC ~~10~~ 6.81	0.234	0%	5	D3 (ditch, 1st)	29.1	10

Compound	Species	Endpoint [µg a.i./L]	$PEC_{sw,max}$ [µg/L]	% drift reduction	buffer zone	Scenario	TER_{LT}	Trigger
			0.234	0%	5	D3 (ditch, 2nd)	29.1	
			0.975	90%	20	D4 (pond, 1st)	**6.98**	
			0.969	90%	20	D4 (stream, 1st)	**7.03**	
			1.206	90%	20	D6 (ditch, 1st)	**5.65**	
			0.662	0%	20	R1 (stream, 1st)	10.29	
			0.500	0%	20	R1 (stream, 2nd)	13.62	
			0.553	0%	10	R2 (stream, 1st)	12.31	
			0.469	0%	10	R2 (stream, 2nd)	14.52	
			0.882	90%	20	R3 (stream, 1st)	7.72	
			0.790	90%	20	R3 (stream, 2nd)	**8.62**	
			0.379	0%	10	R4 (stream, 1st)	17.97	
			0.705	90%	20	R4 (stream, 2nd)	**9.66**	

*VFS: vegetated filter strip

Conclusion:

Based on the calculations presented above a safe use of the formulation in hops can be shown for all FOCUS scenarios considering different mitigation options (up to 90% drift reducing nozzles).

Based on the calculations presented above a safe biennial use of the formulation in lettuce can be shown for ten of the ~~thir~~fifteen FOCUS subscenarios considering different mitigation options (up to 20m VFS). Of the seven complete scenario's, three meet the trigger.

A safe annual use of the formulation in lettuce can be shown for nine of the fifteen FOCUS subscenarios considering different mitigation options (up to 20m VFS). Of the seven complete scenario's, three meet the trigger.

Overall conclusion risk aquatic invertebrates:
For hops, acceptable risk can be reached in the one relevant complete scenario with different combinations of buffer zones and spray drift reduction.

For the biennial use in lettuce, an acceptable use can be shown for three of the seven complete FOCUS scenarios considering different mitigation options (up to 20m VFS).

For the annual use in lettuce, an acceptable use can be shown for two of the seven complete FOCUS scenarios considering different mitigation options (up to 20m VFS).

Assessment of bioconcentration potential

BYI 02960 and its relevant metabolites have a log Pow of <3, therefore no study on bioaccumulation is required and there is no concern for bioconcentration.

Table 2.6.2-29 Log Pow values

Substance	Maximum Log Pow	Reference
BYI 02960	1.2	Vol.3 B.2.1.13
BYI 02960-succinamide	0.6 at pH 5	Vol.3 B.2 Table B.2.1-2 IIA 7.13b
BYI 02960-azabicyclosuccinamide NA-salt	-1.3 at pH 5	Vol.3 B.2 Table B.2.1-2 IIA 7.13b
DFA	- 3.1 at pH5	Vol.3 B.2 Table B.2.1-2 IIA 7.13b
6-CNA	1.52 at pH 1.98	Vol.3 B.2 Table B.2.1-2 IIA 7.13b

2.6.3 Effects on bees and other arthropod species

2.6.3.1 Bees

Procedures for risk assessment were in agreement with the recommendations in the Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (Working Document Sanco/10329/2002 rev 2 final, 17 October 2002, referring to EPPO (EPPO 170 and EPPO 2002). EPPO 2002 was updated with EPPO 2010[11], an update focussing especially on systemic substances.

[11] EPPO 2010: Environmental risk assessment scheme for plant protection products. Chapter 10: Honeybees. OEPP/EPPO, Bulletin OEPP/EPPO Bulletin 40, 323–331

Exposure to honeybees is not relevant for the glasshouse use in lettuce (lettuce for food production needs no pollination therefore honeybees are not expected to be introduced in or be attracted to lettuce production in glasshouse).

Exposure is relevant for the field uses in hops and lettuce. The crops themselves are not attractive to honeybees for nectar and/or pollen collection. However, honey bees may be present in the field to forage on flowering weeds; bees foraging off-field may be exposed via spray drift. Furthermore, potential exposure routes are via honeydew, succeeding crops and guttation and other drinking water sources.

Acute toxicity data and first-tier acute risk assessment

Below the results of laboratory testing with honeybees are summarised.

Table 2.6.3.1-01 Acute toxicity of technical BYI 02960 to honey bees in the laboratory

Test substance	Ecotoxicological Endpoint: LD_{50}		Reference
BYI 02960 (tech.)	oral 24 h oral 48 h contact 24 h contact 48 h contact 72 h contact 96 h	1.3 µg a.i./bee **1.2 µg a.i./bee** >200 µg a.i./bee >200 µg a.i./bee 158.4 µg a.i./bee **122.8 µg a.i./bee**	Schmitzer & Sekine (2008) KIIA 8.7.1/01
	Ecotoxicological Endpoint: NOED		
	oral contact	0.68 µg a.i./bee* 25 µg a.i./bee	

Bold values: Endpoints considered relevant for risk assessment

*The NOED for mortality is 0.68 µg a.i./bee. However, discoordinated movements and aparthy occurred at this and all higher dose rates. The $NOED_{oral}$ for sublethal effects is therefore set at 0.34 µg a.i./bee. The $NOED_{contact}$ is 25 µg a.i./bee also for sublethal effects.

Table 2.6.3.1-02 Acute toxicity data of the formulated product BYI 02960 SL 200 G to honey bees in the laboratory

Test substance	Ecotoxicological Endpoint: LD_{50}		Reference
BYI 02960 SL 200 G	oral 24 h oral 48 h contact 24 h contact 48 h contact 72 h	3.2 µg a.i./bee **3.2 µg a.i./bee** 21.5 µg a.i./bee 17.1 µg a.i./bee **15.7 µg a.i../bee**	Schmitzer (2009) KIIIA1 10.4.2.1/01
	Ecotoxicological Endpoint: NOED		
	oral contact	2.6 µg a.i./bee 6.3 µg a.i./bee	

Bold values: Endpoints considered relevant for risk assessment

The acute contact toxicity of the formulaton to bumble bees was also tested, see the table below.

Table 2.6.3.1-03 Acute toxicity of of the formulated product BYI 02960 SL 200 G to bumble bees in the laboratory

Test substance	Ecotoxicological Endpoint: LD_{50}		Reference
Flupyradifurone SL 200 G	contact 48 h	>100 µg a.i./bee	Vergé (2012) KIIA 8.7.1/07
	Ecotoxicological Endpoint: NOED		
	contact	≥100 µg a.i./bee	

In the plant metabolism studies, a range of metabolites have been measured in the reproductive plant organs (flowers). The observed metabolites were tested in acute and chronic toxicity studies for potential effects on honey bees. Table 2.6.3.1-04 provides an overview of the investigated metabolites, including the parent compound. Results of the acute toxicity studies are provided in Table 2.6.3.1-05.

Table 2.6.3.1-04 Overview table on names and synonyms of compounds addressed within the bee chapter

Abbreviation / Chemical code	Alternative name
BYI 02960	Flupyradifurone
BYI 02960-DFEAF	BYI 02960-difluoroethyl-amino-furanone
BYI 02960-OH	BYI 02960-hydroxy
DFA	Difluoroacetic acid
6-CNA	6-chloronicotinic acid
BYI 02960 -CHMP	6-chloropicolyl alcohol, 6-CPA

Table 2.6.3.1-05 Acute toxicity of BYI 02960 metabolites to honey bees in the laboratory

Test substance	Ecotoxicological Endpoint	Value	Reference
BYI 02960-DFEAF	LD_{50} - oral 48 h LD_{50} - contact 48 h NOED - oral 48 h NOED - contact 48 h	**>81.5 µg/bee** **>100 µg/bee** ≥81.5 µg/bee ≥100 µg/bee	Schmitzer, 2010 KIIA 8.7.1/02
BYI 02960-OH	LD_{50} - oral 48 h LD_{50} - contact 48 h NOED - oral 48 h NOED - contact 48 h	**>105.3 µg/bee** **>100 µg/bee** ≥105.3 µg/bee ≥100 µg/bee	Schmitzer, 2011 KIIA 8.7.1/03
DFA	LD_{50} - oral 48 h LD_{50} - contact 48 h NOED - oral 48 h NOED - contact 48 h	**>107.9 µg/bee** **>100 µg/bee** ≥107.9 µg/bee ≥100 µg/bee	Schmitzer, 2010 KIIA 8.7.1/04

6-CNA	LD_{50} - oral 48 h LD_{50} - contact 48 h NOED - oral 48 h NOED - contact 48 h	**>107.1 µg/bee** **>100 µg/bee** ≥107.1 µg/bee ≥100 µg/bee	Schmitzer, 2010 KIIA 8.7.1/05
BYI 02960 -CHMP	LD_{50} - oral 48 h LD_{50} - contact 48 h NOED - oral 48 h NOED - contact 48 h	**>106.7 µg/bee** **>100 µg/bee** ≥106.7 µg/bee ≥100 µg/bee	Schmitzer, 2010 KIIA 8.7.1/06

Bold values: Endpoints considered relevant for risk assessment

An indication of hazard (Hazard Quotient or HQ) can be derived according to the EPPO risk assessment scheme, by calculating the ratio between the single application rate (expressed in g/ha) and the lowest laboratory contact and oral LD50 (expressed in µg/bee).

HQ_O and HQ_C resp. = Application rate [g/ha] / LD50 oral or LD50 contact [µg/bee]

HQ values are calculated using data from the studies performed with technical material and the corresponding formulated product. HQ values higher than 50 indicate the need of higher tiered tests to clarify the actual risk to honey bees.

Table 2.6.3.1-06 Hazard quotients for bees – oral exposure

Crop	Exposure route	LD_{50} [µg a.i./bee]	Application rate [g/ha]	Hazard quotient HQ_O	Trigger	Refined risk assessment
BYI 02960 SL 200						
Hops	oral	3.2	150	47	50	No
Lettuce	oral	3.2	125	39	50	No
off-field						
Hops	oral	3.2	150*0.1933	9.1	50	No
Lettuce	oral	3.2	125*0.0277	1.1	50	No
BYI 02960 (technical)						
Hops	oral	1.2	150	**125**	50	**Yes**
Lettuce	oral	1.2	125	**104**	50	**Yes**
off-field						
Hops	oral	1.2	150*0.1933	24	50	No
Lettuce	oral	1.2	125*0.0277	3.5	50	No

The hazard quotients for oral exposure calculated for BYI 02960 technical exceed the empirical trigger value for higher tier testing (HQ_O > 50) for both uses. Therefore, a refined risk assessment will be conducted, see below (higher tier semi-field and field studies).
Because a risk is indicated based on the full in-field dose, the off-field risk is also calculated. The default drift rates of the next section (non-target arthropods other than bees) are used to calculate the

exposure that may occur on the off-field area. Based on these default drift rates and the trigger of 50, an off-field acute risk is not indicated.

Table 2.6.3.1-07 Hazard quotients for bees – contact exposure

Crop	Exposure route	LD_{50} [µg a.i./bee]	Application rate [g/ha]	Hazard quotient HQ_O	Trigger	Refined risk assessment
BYI 02960 SL 200						
Hops	contact	15.7	150	9.6	50	No
Lettuce	contact	15.7	125	8.0	50	No
BYI 02960 (technical)						
Hops	contact	122.8	150	1.22	50	No
Lettuce	contact	122.8	125	1.02	50	No

The hazard quotient for contact exposure is below the empirical trigger value for higher tier testing (HQ_C <50) for both envisaged uses. Therefore, no unacceptable acute risk to bees is to be expected via the contact route of exposure.

Table 2.6.3.1-05 shows that all relevant plant metabolites have a very low acute toxicity to honeybees (all LD50 values >100 µg a.i./bee). Therefore, the acute risk of these metabolites is low.
The bumble bee contact toxicity test does not indicate concern about higher sensitivity of bumble bees than honey bees.

Chronic toxicity data

There are currently no harmonised and ring tested test guidelines available to assess the chronic risk to adult honey bees. Nonetheless, there is some experience within the European honey bee testing community on conducting chronic studies in adult honey bees, by exposing honey bees orally to a treated 50% (w/v) sucrose solution as an exclusive food source for a period of 10 consecutive days by continuous and ad libitum feeding. Since the originally submitted chronic study with the a.s. was deemed unacceptable die to high control mortality and uncertainty about sublethal effects, the notifier performed a new chronic study with the a.s. Table 2.6.3.1-08 provides an overview of the results obtained with parent BYI 02960 and its metabolites.

Table 2.6.3.1-08 Chronic toxicity to adult honey bees in the laboratory

Test substance	Ecotoxicological Endpoint: NOEC / NOED		Reference
BYI 02960	NOEC (nominal) NOED (nominal)	26000 µg a.i./L 0.79 µg a.i./bee/day	Gladbach, Theis et al., 2013 KIIA 8.16.1/09

BYI 02960-DFEAF	NOEC (nominal) NOED (nominal)	≥ 10000 µg a.i./L ≥ 0.435 µg a.i./bee/day	Kling, 2012, KIIA 8.16.1/02
BYI 02960-OH	NOEC (nominal) NOED (nominal)	≥ 10000 µg a.i./L ≥ 0.420 µg a.i./bee/day	Kling, 2012, KIIA 8.16.1/03
DFA	NOEC (nominal) NOED (nominal)	≥ 10000 µg a.i./L ≥ 0.379 µg a.i./bee/day	Kling, 2012, KIIA 8.16.1/04
6-CNA	NOEC (nominal) NOED (nominal)	≥ 10000 µg a.i./L ≥ 0.418 µg a.i./bee/day	Kling, 2012, KIIA 8.16.1/05
BYI 02960-CHMP	NOEC (nominal) NOED (nominal)	≥ 10000 µg a.i./L ≥ 0.413 µg a.i./bee/day	Kling, 2012, KIIA 8.16.1/06

The table shows that all relevant plant metabolites show very low chronic toxicity to honeybees.

The a.s. has chronic mortality endpoints very close to the acute mortality endpoints, see Table 2.6.3.1-09. Thus, the toxicity of the a.s. is not increased when the substance is taken in for several consecutive days; there is no concern about chronic/delayed toxicity with regard to mortality.

Table 2.6.3.1-09 Comparison of acute and chronic endpoints for mortality in adult honey bees

	NOEDoral	$LD(D)_{50}$
Studies with technical grade active substance	[µg a.s./bee(/day)]	[µg a.s./bee(/day)]
Acute adult toxicitv studv (48 hours)	0.68	1.2
Chronic adult toxicitv studv (10 davs)	0.79	1.83

As explained above, the foliage residue toxicity study KIIIA 10.4.3/01 was not evaluated by RMS because guidance on how to perform, evaluate and use this study in the risk assessment is not available in EU.

Chronic toxicity of BYI 02960 to honey bee larvae

There are currently no harmonised and ring tested test guidelines available to assess the chronic risk to honey bee larvae. Nonetheless, there is experience in Europe within some laboratories on

conducting chronic studies in honey bee larvae. A study has been performed with the active substance.

Table 2.6.3.1-10 Chronic toxicity of BYI 02960 honey bee larvae in the laboratory

Test substance	Ecotoxicological Endpoint: NOEC / NOED		Reference
BYI 02960	NOEC (nominal) NOED (nominal)	≥ 10000 µg a.i./kg larval diet ≥ 0.44 µg a.i./bee larvae/day	Nikolakis *et al.*, 2010 KIIIA1 10.4.6.1/01

The chronic toxicity studies with the metabolites show no effects to adult honeybees at the highest tested dose of 10 mg/L diet. The chronic toxicity study with the a.s. shows no effects to honeybee larvae at the highest tested dose of 10 mg/kg diet. Only in the chronic toxicity study on adult honeybees with the a.s., which was performed at higher doses than the other studies, effects were seen. From this study, a NOEC of 26 mg a.i./L/ 0.79 µg a.i./bee/day could be derived. These endpoints could be used in risk calculations based on daily intake values for different castes of honeybees. This is discussed below.

Residue measurements in nectar and pollen and other matrices

Residue measurements in *Phacelia*

An overview of the measured residues of the a.s. and metabolite DFEAF in the semi-field trials on *Phacelia* in the different matrices was compiled by RMS in Tables 2.6.3.1-11-16. In these studies, the first foliar spray applications was done just before flowering and the second foliar spray application was done during full flowering.

Table 2.6.3.1-11 Residues of BYI02960 and metabolite in flowers

Study	Application rate	DALA	Residue BYI 02960 (µg/kg) mean (individual samples)	Residue BYI 02960-DFEAF (µg/kg) mean (individual samples)
KIIIA 10.4.7/03	2x200 g a.s./ha (foliar spray)	0	12729 (Subsample A: 7534-16308-13098; Subsample B: 9925-17310-12199)	11 (Subsample A: 7 – 12 - 11; Subsample B: 9-16-13)
		7	993 (Subsample A: 327-3141-729; Subsample B: 238-1381-143)	25 (Subsample A: 8-44-22; Subsample B: 12-52-13)
KIIIA 10.4.7/04	1x 300 (soil appl.) and 2x 200 g a.s./ha (foliar spray)	0	37592	78
		7	199	9
KIIIA 10.4.7/05	1x 300 (soil appl.) and 2x 200 g a.s./ha (foliar spray)	0	14907 (20083-9533-15110)	60 (37-18-126)
		7	2356 (701-3590-2778)	23 (9-35-26)

Table 2.6.3.1-12 Residues of BYI02960 and metabolite in pollen from forager bees

Study	Application rate	DALA	Residue BYI 02960 (µg/kg) mean (individual samples)	Residue BYI 02960-DFEAF (µg/kg) mean (individual samples)
IIA 8.16.2/08	2x 200 g a.s./ha (foliar spray)	-1	148 (138-146-162)	2.2 (2.2-2.0-2.5)
		0 (+1h)	64743 (59696-69911-64622)	76 (68-86-74)
		0 (+2.5h)	63271 (53137-70667-66009)	101 (92-110-102)
		0 (+4h)	36126 (34628-31623-42126)	120 (116-135-110)
		1	10354 (6469-8481-16113)	57 (36-43-92)
		3	570 (779-410-522)	8.2 (9.4-6.7-8.4)
		4	773 (855-662-803)	9.7 (7.9-9.3-12)
		5	877 (941-714-976)	9.1 (7.8-7.4-12)
		6	925 (605-1041-1128)	7.7 (5.3-8.0-9.7)
		7	766 (410-795-1092)	6.0 (3.2-6.0-8.9)
		8	688 (572-728-763)	6.0 (5.1-6.3-6.7)
		9	368 (174-460-469)	3.0 (1.3-3.3-4.5)
		10	553 (636-377-647)	6.2 (11-2.8-4.9)

Table 2.6.3.1-13 Residues of BYI02960 and metabolite in pollen from combs

Study	Application rate	DALA	Residue BYI 02960 (µg/kg) mean (individual samples)	Residue BYI 02960-DFEAF (µg/kg) mean (individual samples)
KIIIA 10.4.7/04	1x 300 (soil appl.) and 2x 200 g a.s./ha (foliar spray)	7	5759 (3025-6109-8144)	13 (7-13-20)
KIIIA 10.4.7/06	2x 200 g a.s./ha (foliar spray)	27	3.5 (3-4-4-2-4-4)	<1 (6 samples)
KIIA 8.16.2/08	2x 200 g a.s./ha (foliar spray)	-1	194 (240-175-166)	2.1(2.9-1.9-1.7)
		0	5910 (6691-1545-9495)	4.7 (4.8-3.1-6.1)
		1	6433 (11260-5021-3019)	7.3 (12-6.1-3.9)
		2	4836 (4356-7009-3142)	15 (13-20-13)

		3	7912 (13783-4451-5503)	19 (26-16-14)
		4	10171 (7381-22529-603)	29 (22-46-19)
		5	4117 (6194-5171-985)	18.6 (20-28-7.7)
		6	3042 (1807-6017-1301)	15 (13-20-12)
		7	3277 (2943-5249-1640)	14 (12-18-11)
		8	2477 (1915-4491-1026)	14 (14-18-11)
		9	3565 (3634-4495-2566)	14 (14-16-11)
		10	1805 (1580-2115-1719)	11 (12-10-10)
		12	3591 (5791-4843-139)	9.3 (14-12-1.9)
		18	2893 (2671-3115)	7.7 (6.8-8.6)
		24	1709 (2273-1145)	5.6 (9.1-2.1)

Table 2.6.3.1-14 Residues of BYI02960 and metabolite in nectar from forager bees

Study	Application rate	DALA	Residue BYI 02960 (µg/kg) mean (individual samples)	Residue BYI 02960-DFEAF (µg/kg) mean (individual samples)
IIA 8.16.2/08	2x 200 g a.s./ha (foliar spray)	-1	38 (7-59-48)	<LOQ (3 samples)
		0 (+1h)	3048 (2777-3304-3064)	65 (62-65-69)
		0 (+2.5h)	2265 (2038-2031-2726)	89 (87-85-96)
		0 (+4h)	1435 (1235-1709-1360)	104 (92-122-98)
		1	752 (699-871-687)	77 (58-79-95)
		2	337 (301-331-379)	13 (11.2-17-12)
		3	158 (150-148-175)	9.4 (8.6-9.7-10)
		4	223 (191-223-254)	12 (9.7-12-13)
		5	118 (102-119-132)	8.1 (6.9-8.2-9.3)
		6	57 (54-57-60)	4.4 (4.1-4.5-4.5)
		7	41 (37-39-46)	2.9 (2.8-2.7-3.2)
		8	23 (20-21-29)	2.0 (1.8-1.9-2.2)
		9	15 (12-16-17)	1.3 (1.1-1.4-1.4)
		10	36 (83-10-15)	<1 (<LOQ-<LOQ-1.1)

Table 2.6.3.1-15 Residues of BYI02960 in nectar from combs

Study	Application rate	DALA	Residue BYI 02960 (µg/kg) mean (individual samples)	Residue BYI 02960-DFEAF (µg/kg) mean (individual samples)
KIIIA 10.4.7/04	1x 300 (soil appl.) and 2x 200 g a.s./ha (foliar spray)	7	21 (15-18-31)	<1 µg/kg (3 samples)
KIIIA 10.4.7/05	1x 300 (soil appl.) and 2x 200 g a.s./ha (foliar spray)	7	<1.3 (2-<0.1-<1)	<0.4 (<1 - <0.1 - <0.1)
KIIIA 10.4.7/06	2x 200 g a.s./ha (foliar spray)	27	<6.9 (17-8-6-2-<1-6-6-10-6)	<1 (1 sample of 1 and 8 samples of <1)
IIA 8.16.2/08	2x 200 g a.s./ha (foliar spray)	-1	<LOQ (3 samples)	<LOD (3 samples)
		0	<1 (1.2-<LOQ-1.2)	<LOD (3 samples)
		1	127 (186-68)	5.6 (9.4-1.7)
		2	44 (33-84-16)	<1.8 (<LOQ-3.3-<LOQ)
		3	111 (80-108-144)	5.5 (5.0-4.6-6.8)
		4	35 (12-48-46)	<1.7 (<LOQ-1.8-2.2)
		5	93 (69-117)	5.1 (4.8-5.4)
		6	82 (56-108)	4.5 (3.4-5.5)
		7	107 (107)	5.2 (5.2)
		8	95 (95)	4.8 (4.8)
		9	50 (28-31-90)	2.3 (1.2-1.4-4.4)
		10	57.5 (38-77)	3.2 (2.6-3.8)
		12	45 (40-49)	2.6 (2.6-2.5)
		18	38 (30-45)	1.7 (1.7-1.7)
		24	32 (19-44)	1.3 (1.1-1.4)

Table 2.6.3.1-16 Residues of BYI02960 in bee wax from combs

Study	Application rate	DALA	Residue BYI 02960 (µg/kg) mean (individual samples)	Residue BYI 02960-DFEAF (µg/kg) mean (individual samples)
KIIIA 10.4.7/05	1x 300 (soil appl.) and 2x 200 g a.s./ha	7	3.7 (7-3-1)	<1 (3 samples)
KIIIA 10.4.7/06	2x 200 g a.s./ha	27	15 (2-1-21-7-3-1-62-24-10)	<1 (9 samples)

Residue measurements in oilseed rape

An overview of the measured residues of the a.s. and metabolite DFEAF in the honey bee field trials on oil seed rape in the different matrices was compiled by RMS in Tables 2.6.3.1-17-24. In these studies, the first foliar spray application was done just before flowering and the second foliar spray application was done during full flowering.

Table 2.6.3.1-17 Residues of BYI02960 and metabolite in flowers

Study	Application rate	DALA	Residue BYI 02960 (µg/kg)	Residue BYI 02960-DFEAF (µg/kg)
KIIIA 10.4.5/01	10 g a.s./kg OSR seeds (seed treatment), 1x 300 g a.s./ha (soil application). 2x205 g a.s./ha (foliar spray)	-6	14155	9
		-5	9289	15
		-4	6939	20
		-3	5244	16
		-2	2887	10
		-1	2577	10
		0	30379	28
		1	22071	28
		2	14071	45
		3	18308	50
		4	16068	67

		5	12251	48
		6	11230	44
		7	15786	80
		8	12972	61
		9	4294	20
		10	3306	15
		11	1917	10
KIIIA 10.4.5/02	10.1 g a.s./kg OSR seeds (seed treatment), 1x 300 g a.s./ha (soil application). 2x205 g a.s./ha (foliar spray)	-9	7700	5
		-8	7900	20
		-7	5900	14
		-6	5500	17
		-5	3400	13
		-4	3900	14
		-3	4100	18
		-2	2200	9
		-1	2100	8
		0	36000	35
		1	24000	47
		2	17000	40
		3	3400	20
		4	3000	7
		5	3400	9
		6	2100	5
		7	1600	4
		8	1800	7
		9	1500	6
		10	1300	6
		11	660	3
		12	830	4
		13	440	3
		14	440	4
		15	290	3
		16	170	2
		17	150	2
		18	100	1

		19	96	<1
		20	84	<1
		21	89	<1

Table 2.6.3.1-18 Residues of BYI02960 and metabolite in pollen from forager bees

Study	Application rate	DALA	Residue BYI 02960 (µg/kg)	Residue BYI 02960-DFEAF (µg/kg)
KIIIA 10.4.5/01	10 g a.s./kg OSR seeds (seed treatment), 1x 300 g a.s./ha (soil application). 2x205 g a.s./ha (foliar spray)	-4	1523	10
		-3	246	2
		-2	706	4
		-1	760	5
		0	nd	nd
		1	14259	21
		2	6147	19
		3	3252	26
		4	2941	22
		5	2721	20
		6	1814	12
		7	1475	8
		8	2034	10
		9	2318	12
		10	2001	9
		11	1788	7
KIIIA 10.4.5/02	10.1 g a.s./kg OSR seeds (seed treatment), 1x 300 g a.s./ha (soil application). 2x205 g a.s./ha (foliar spray)	-9	3200	6
		-8	nd	nd
		-7	1200	6
		-6	810	5

		-5	1300	12
		-4	1200	12
		-3	1100	8
		-2	1100	6
		-1	1000	5
		0	21000	21
		1	4300	16
		2	1800	9
		3	1600	9
		5	1500	6
		6	1400	5
		7	1400	6
		8	1200	6
		9	950	4
		10	900	4
		11	800	3
		12	670	3
		13	590	2
		14	630	3
		15	990	6
		16	220	1
		17	380	2
		18	nd	nd
		19	220	1
		20	170	1

Table 2.6.3.1-19 Residues of BYI02960 in pollen from combs

Study	Application rate	DALA	Residue BYI 02960 (µg/kg) mean (individual samples)	Residue BYI 02960-DFEAF (µg/kg) mean (individual samples)
KIIIA 10.4.5/01	10 g a.s./kg OSR seeds (seed treatment), 1x 300 g a.s./ha (soil application). 2x205 g a.s./ha (foliar spray)	10	1347 (305- 966- 950-743-1694-594-3905-1617)	7 (3-7-6-6-9-4-12-9)
KIIIA 10.4.5/02	10.1 g a.s./kg OSR seeds (seed	23	349 (23-460-1200-140-340-130-150)	<1.9 (6-2-1-<1-1-<1-<1)

	treatment), 1x 300 g a.s./ha (soil application). 2x205 g a.s./ha (foliar spray)			

Table 2.6.3.1-20 Residues of BYI02960 in beebread

Study	Application rate	DALA	Residue BYI 02960 (µg/kg)	Residue BYI 02960-DFEAF (µg/kg)
KIIIA 10.4.5/01	10 g a.s./kg OSR seeds (seed treatment), 1x 300 g a.s./ha (soil application). 2x205 g a.s./ha (foliar spray)	14	1958	7
		35	6	<0.1
		56	113	2
		77	81	1
		99	38	<1
		18	4	<0.1
		140	11	<0.1
		155	7	<0.1
KIIIA 10.4.5/02	10.1 g a.s./kg OSR seeds (seed treatment), 1x 300 g a.s./ha (soil application). 2x205 g a.s./ha (foliar spray)	44	25	<1
		64	8	<1
		94	9	<1
		116	13	<1
		136	5	<0.1
		157	4	<0.1
		180	4	<0.1

Table 2.6.3.1-21 Residues of BYI02960 in nectar from forager bees

Study	Application rate	DALA	Residue BYI 02960	Residue BYI 02960-

			(µg/kg)	DFEAF (µg/kg)
KIIIA 10.4.5/01	10 g a.s./kg OSR seeds (seed treatment), 1x 300 g a.s./ha (soil application). 2x205 g a.s./ha (foliar spray)	-6	747	<1
		-5	nd	nd
		-4	2365	15
		-3	363	4
		-2	272	3
		-1	269	3
		0	2356	4
		1	2908	10
		2	nd	nd
		3	4108	37
		4	2729	38
		5	1467	16
		6	304	4
		7	341	3
		8	2823	3
		9	nd	nd
		10	2851	4
		11	3617	4
KIIIA 10.4.5/02	10.1 g a.s./kg OSR seeds (seed treatment), 1x 300 g a.s./ha (soil application). 2x205 g a.s./ha (foliar spray)	-8	4300	26
		-7	1500	11
		-6	990	6
		-5	990	15
		-4	730	16
		-3	670	9
		-2	450	6
		-1	490	4
		0	3000	21

		1	3500	20
		2	1600	11
		3	1000	6
		4	590	4
		5	470	3
		6	370	3
		7	370	4
		8	190	3
		9	180	2
		10	150	2
		11	100	1
		12	110	1
		13	50	<1
		14	58	<1
		15	3	<0.1
		16	nd	nd
		17	nd	nd
		18	nd	nd
		19	<0.1	<0.1

Table 2.6.3.1-22 Residues of BYI02960 in nectar from combs

Study	Application rate	DALA	Residue BYI 02960 (µg/kg) mean (individual samples)	Residue BYI 02960-DFEAF (µg/kg) mean (individual samples)
KIIIA 10.4.5/01	10 g a.s./kg OSR seeds (seed treatment), 1x 300 g a.s./ha (soil application). 2x205 g a.s./ha (foliar spray)	10	898 (658-870-991-428-996-758-1347-1137)	9 (7-8-11-5-9-8-13-12)
KIIIA 10.4.5/02	10.1 g a.s./kg OSR seeds (seed treatment), 1x 300 g a.s./ha (soil application). 2x205 g a.s./ha (foliar spray)	23	227 (140-78-490-12-140-110-620)	<1.6 (<1-<1-3-<0.1-1-<1-4)

Table 2.6.3.1-23 Residues of BYI02960 in nectar/honey from the hive

Study	Application rate	DALA	Residue BYI 02960 (µg/kg)	Residue BYI 02960-DFEAF (µg/kg)
KIIIA 10.4.5/01	10 g a.s./kg OSR seeds (seed treatment), 1x 300 g a.s./ha (soil application). 2x205 g a.s./ha (foliar spray)	14	1409	30
		35	45	<1
		56	575	4
		77	223	<1
		99	144	1
		18	380	5
		140	61	<0.1
		155	317	5
KIIIA 10.4.5/02	10.1 g a.s./kg OSR seeds (seed treatment), 1x 300 g a.s./ha (soil application). 2x205 g a.s./ha (foliar spray)	44	920	5
		64	260	1
		94	500	2
		116	170	<1
		136	300	<1
		157	840	2
		180	670	2

Table 2.6.3.1-24 Residues of BYI02960 in beewax from combs

Study	Application rate	DALA	Residue BYI 02960 (µg/kg) mean (individual samples)	Residue BYI 02960-DFEAF (µg/kg) mean (individual samples)
KIIIA 10.4.5/01	10 g a.s./kg OSR seeds (seed treatment), 1x 300	10	43 (6-14-25-6-148-23-94-31)	<0.3 (I sample of 2 and 7 samples of <0.1)

	g a.s./ha (soil application). 2x205 g a.s./ha (foliar spray)			
KIIIA 10.4.5/02	10.1 g a.s./kg OSR seeds (seed treatment), 1x 300 g a.s./ha (soil application). 2x205 g a.s./ha (foliar spray)	23	3.6 (6-2-7-2-2-2-4)	<0.1 (7 samples <0.1)
		64	32	<0.1
		94	11	<0.1
		116	39	<0.1
		136	<1	<0.1
		157	<1	<0.1
		180	6	<0.1

Residue measurements in nectar and pollen of other crops

Four supervised residue trials under semi-field conditions (studies KIIA 8.16.2/04 to /07) were submitted in which the residue levels in melon and tomato flowers, nectar (from combs or honey stomachs) and pollen (from combs or forager honey or bumble bees) were studied. One trial in melon was performed in North-Carolina and studied either a drench or a double foliar application. Two trials in watermelon and one in tomato were performed in Spain and all studied a drench application. The location (for the trial in North Carolina) or the application method (for the drench trials) are less relevant for the GAP under evaluation. Therefore, the residue levels are not summarised in this section nor used in the risk assessment, but all results can be found in Tables 2.6.3.1-16 and -17.

Residue measurements in guttation water

The flupyradifurone residue situation in guttation water was investigated in two field studies in winter oil seed rape (W-OSR) (KIIA 8.16.2/09 and /10, Rexer 2012c and d). The treated seeds in these studies were treated at a rate of nominally 10 g flupyradifurone a.s./kg and were sown at actual rates of 59 and 70 g flupyradifurone a.s./ha. The seeds were sown on field plots which were treated at the day of W-OSR-planting with nominally 300 g flupyradifurone a.s./ha (actually 310 and 311 g flupyradifurone a.s./ha), which was immediately incorporated down to a depth of 15 - 20 cm. Guttation fluid from the W-OSR seedlings were collected from the very first day onwards, at which the first guttation droplets occurred; this started at BBCH 9 - 11, which means at a crop stage with minimal dilution of residues due to plant growth and plant biomass.

The maximum residue level in guttation fluid in the two studies was measured at the very first days where guttation droplets occurred after emergence (actually 12 and 13 days after seed planting); thereafter, the concentrations of flupyradifurone decreased continuously.
In autumn, residues of BYI 02960 above the limit of quantitation (LOQ; 0.010 mg/L) were found in 20 out of 20 analysed samples in autumn 2010 (quantifiable residues ranging from 11 mg/L to 0.039 mg/L in one study and from 21 mg/L to 0.087 mg/L in the other study). Generally, residues of BYI 02960 declined by about two orders of magnitude during the approximately 5 weeks lasting autumn sampling period. In the following spring, residues of BYI 02960 above the LOQ were found in all or the majority of the analysed samples (quantifiable residues ranging from 0.21 mg/L to 0.014 mg/L or 0.15 mg/L to 0.062 mg/L).

In autumn, residues of the metabolite BYI 02960-DFEAF were < LOQ (i.e. < 0.010 mg/L) except for 2 out of 10 or 3 out of 8 analysed samples (collected in the first, (second) and third day of sampling; maximum residue 0.023 mg/L. In spring, all residues of the DFEAF-metabolite were < LOQ.

Residue measurements in soil

Residue measurements in soil were performed in some of the semi-field and field studies, to confirm the artificially incorporated background concentration in soil.

Table 2.6.3.1-25 Residues of BYI02960 in soil

Study	Application rate	DAA1	Residue BYI 02960 (µg/kg) mean (individual samples)	Residue BYI 02960-DFEAF (µg/kg) mean (individual samples)
KIIIA 10.4.7/04	1x 300 (soil appl.) and 2x 200 g a.s./ha (foliar spray)	0	51 (36-51-67)	<1 µg/kg (3 samples)
KIIIA 10.4.7/05	1x 300 (soil appl.) and 2x 200 g a.s./ha (foliar spray)	0	49 (40-56-50)	not done
KIIIA 10.4.5/01	1x 300 g a.s./ha (soil application). 10 g a.s./kg OSR seeds (seed treatment), 2x205 g a.s./ha (foliar	0	75 (75)	not done

	spray)			
KIIIA 10.4.5/02	1x 300 g a.s./ha (soil application). 10.1 g a.s./kg OSR seeds (seed treatment), 2x205 g a.s./ha (foliar spray)	0	61 (61)	not done

Risk calculations for exposure via nectar and pollen

The envisaged use of BYI 02960 in hops is an application rate of 150 g a.i./ha, also during the flowering period of the crop. However, hop is a dioecious plant, with male and female flowers developing on separate plants. The harvested commodity in commercial hop cultivation is the female flower cluster. Male hop plants, which could theoretically offer honey bees pollen, are not cultivated in hop plantations – on the contrary: as any pollination of the female flowers (by male pollen) would significantly reduce hop quality, would significantly reduce the harvesting window and would significantly hamper the processing of the hop in breweries, hop plantations are entirely cultivated with female plants. Female flowers, however, do not offer honey bees nectar and pollen. As such, the commercial hop plantations themselves are not attractive to honey bees at any growth stage.

The envisaged use of BYI 02960 in lettuce accounts for an application rate of 125 g a.i./ha, during the development of harvestable, vegetative plant parts. Lettuce does not flower in commercial cultivation (except in seed production, which is not proposed here) and as such, honey bees are not attracted by the crop. Moreover, spray applications of BYI 02960 during flowering are excluded by the envisaged application window.

In conclusion, the crops themselves are not attractive to honeybees for nectar and/or pollen collection. However, weeds may be present in the field and these may flower. In general, the occurrence of weeds and especially flowering weeds in crop fields is not beneficial for growers, a.o. because these weeds will compete with the crop plants for resources. However, weed occurrence will depend on the crop and the individual farmer and may be MS specific. The presence of flowering weeds can therefore not be excluded a priori. For hops especially, undergrowth vegetation is expected to be present. Therefore, the risk via nectar and pollen of flowering weeds should be addressed for the in-field situation.

A large dataset is available with residue measurements.
One semi-field study in Phacelia studied the residue levels after a twofold spray application of 200 g a.s./ha, one just before and one during flowering. Residue levels on pollen and nectar taken from foraging bees directly after the second spray application are high, but decrease quickly:
Phacelia pollen from forager bees one hour after spraying: ca. 65 mg/kg, decreasing within the first day to ca. 36 mg/kg (four hours after spraying). One day after spraying, a level of ca. 10 mg/kg is reached and this decreases within ten days to ca. 0.5 mg/kg.
Phacelia nectar from forager bees one hour after spraying: ca. 3 mg/kg, decreasing within the first day to ca. 1.4 mg/kg (four hours after spraying). One day after spraying, a level of ca. 0.8 mg/kg is reached and this decreases within ten days to ca. 0.04 mg/kg.
The residue levels in pollen and nectar in the hive (maximum ca. 10 mg/kg and 0.1 mg/kg, respectively) are much lower than the residue levels in pollen and nectar collected by forager bees,

but they remain present for a longer time (24 days after spraying, the residue levels in pollen and nectar in the hive are ca. 2 mg/kg and 0.03 mg/kg, respectively). In two other semi-field trials, in which next to two foliar applications, also a soil application was used during sowing, residue levels were only measured on one timepoint but results are more or less in accordance with the results of the study with only foliar applications.

Two field trials in oilseed rape also studied residue levels in pollen and nectar taken from foraging bees. These study included two foliar applications and a seed treatment and furthermore had a background soil concentration.
The highest residue level in oilseed rape pollen from forager bees was found on the day of spraying: 21 mg/kg. The level decreased less rapidly than in the above Phacelia study (without seed treatment), reaching a level of ca. 1-2 mg/kg within ten days.
The highest residue level in oilseed rape nectar from forager bees was found three days after spraying: ca. 4 mg/kg. The level decreased to ca. 2 mg/kg within ten days.
The residue levels in pollen and nectar in the hive (maximum values of individual samples ca. 4 mg/kg and 1 mg/kg, respectively) are lower than the residue levels in pollen and nectar collected by forager bees. Hive residues were only measured on day 10 or day 23 after the last application and not earlier, so a direct comparison is difficult. Hive residues remain present for a longer time (23 days after spraying, the residue levels in pollen and nectar in the hive are maximum 1 mg/kg and 0.6 mg/kg, respectively; after 180 days, still 0.004 mg/kg is found in beebread).

In all the above studies, residue levels of metabolite DFEAF are much lower than those of the a.s. and decrease in a similar way.

It is difficult to determine a generic RUD value from these residue measurements, as the trials were performed with different application regimes and each trial had multiple applications. However, a general picture can be derived: directly after spraying, high levels of the a.s. can be found in nectar and pollen; residues in pollen are higher than those in nectar; residue levels in pollen and nectar in the hive seem to be lower than the residue levels in pollen and nectar collected by forager bees, but they remain present for a longer time; after six months, still traces of the a.s. can be found in the beebread; the residue levels of metabolite DFEAF are much lower than those of the a.s.

These residue levels could be used in exposure calculations, using daily intake values for nectar and pollen for different castes of bees. The exposure values could then be compared to the toxicity values derived above. If such an exercise is undertaken, it should be noted that the residue levels were determined in studies which used at least two foliar sprays with 200 g a.s./ha each, while the proposed uses in hops and lettuce have only a single application, at 150 and 125 g a.s./ha, respectively, and furthermore are not attractive to bees themselves. However, at the moment there is no harmonised

method available to calculate these exposure levels and especially the corresponding risk. Furthermore, it is not necessary to perform these lower tier calculations, as a large number of higher tier effect studies is available. Please see the section below (higher tier toxicity data and risk assessment).

Higher tier toxicity data and risk assessment for exposure via nectar and pollen

Six independent semi-field (gauze tunnel) studies were submitted, where BYI 02960 formulations were applied to the highly bee attractive surrogate crop Phacelia tanacetifolia with honey bees actively foraging on the crop (i.e. during bee flight). The studies are summarised in Table 2.6.3.1-26. One of these studies was not accepted (except for residue analysis) because of an unequal colony set-up.

The studies were all performed by two different laboratories. Differences between these laboratories were reflected in the report for the use of statistics and preparation of tables or figures. RMS has prepared stacked figures in those cases that figures were missing. In that way the interpretation of the results was more convenient as comparisons between studies are made easier and more transparent.

Table 2.6.3.1-26 Overview of semi-field studies

Specifications and endpoint of the studies	**Number of the semi-field study (grey shading means unacceptable for bee endpoints)**					
	10.4.7/01	**10.4.7/02**	**10.4.7/03**	**10.4.7/04**	**10.4.7/05**	**10.4.7/06**
Authors	Schnorbach, 2012a	Schnorbach, 20102b	Rentschler, 2012a	Pröbsting, 2012a	Pröbsting, 2012b	Rentschler, 2012b
Guideline	OEPP/EPPO guideline No. 170 (3), 2001	OEPP/EPPO guideline No. 170 (3), 2001	OEPP/EPPO guideline No. 170 (3), 2001	OEPP/EPPO guideline No. 170 (3), 2001	OEPP/EPPO guideline No. 170 (4), 2010	OEPP/EPPO guideline No. 75 (2007)
Test facility	Bayer CropScience AG, Monheim	Bayer CropScience AG, Monheim	Eurofins Agroscience Services, Niefern-Öschelbronn	Eurofins Agroscience Services, Niefern-Öschelbronn	Eurofins Agroscience Services, Niefern-Öschelbronn	Eurofins Agroscience Services, Niefern-Öschelbronn
Nr of applications	1	1	2	3	3	2
Type applications	1x foliar spray	1x foliar spray	2x foliar spray (10 d apart)	1x soil application, 2x foliar spray (7 d apart)	1x soil application, 2x foliar spray (18 d apart)	2x foliar spray (10 d apart)
Dose rate	75 and 150 g a.s./ha	150 g a.s./ha, including surfactant	2x 200 g a.s./ha	1 x 300 and 2 x 200 g a.s./ha	1 x 300 and 2 x 200 g a.s./ha	2x 200 g a.s./ha
Number of hives in control	3 / 3 / 3	2 / 2 / 2	3 / 3 / 3	3 / 3 / 3	3 / 3 / 3	3 / 3 / 3

/ treatment / reference						
Hive size (initial nrs of bees)	2500	2500-3000	4000-7000 bees	1000-9000 bees	7000-13800 bees	5313-8570 bees
Tunnel size	50 m2	50 m2	72 m2	60 m2	60 m2	80 m2
Timing application	during flowering and active foraging of bees	during flowering and active foraging of bees	1s foliar application 6 d before hive set-up, 2nd foliar application 10 d after 1st application during flowering and active foraging of bees	1st foliar application 4 d before hive set-up 2nd foliar application 7 d after 1st foliar application (3 d after hive set-up) during flowering and active foraging of bees	1st foliar application 14 d before hive set-up, 2nd foliar application 4 d after hive set-up during flowering and active foraging of bees	1st foliar application 5 d before hive set-up and 2nd foliar application 5 d after set-up during flowering and active foraging of bees
Exposure duration	7 d	12 d	16 d	10 d	12 d	12 d
Observation duration	29 d	29 d	29 d	32 d	33 d	35 d
Ranking worst case exposure	6	5	3	1 – however unequal colony setup	4	2
Flight intensity	Similar to control	Similar to control	Reduction on DALA0-3, significant at DALA0 and DALA2	Cannot be evaluated	Strong reduction on DALA0-7, significant on DALA0aa and DALA5	Strong reduction on DALA0-7, significant on DALA0aa, DALA3 and DALA6
Bee mortality in bee traps linen sheets	Similar to control	Similar to control	No apparent increase of mortality	cannot be evaluated	No apparent increase of mortality	No apparent increase of mortality
Number of bees	Similar to control, Decrease caused by limited forage	Similar to control, Decrease caused by limited forage	More or less equal to control	cannot be evaluated	Small decrease explained limited foraging area. Further comparable to the control	Small decrease explained limited foraging area. Further comparable to the control
Development of brood	Similar to control, same level during	Similar to control, same level during	Decreases during confinement	cannot be evaluated	Decreases during confinement	Decreases during confinement

	confinement, increase afterwards	confinement, increase afterwards	and increases afterwards, similar to control		and increases afterwards, similar to control	and increases afterwards, similar to control
Development of food storage aria	Increase pollen and nectar stores similar to control	Increase pollen and nectar stores similar to control	Decreases during confinement similar to the control, increases after confinement	cannot be evaluated	Decreases during confinement and increases afterwards, similar to control	Decreases during confinement and increases afterwards, similar to control
Presentation condition of colonies	Figures for nectar, pollen etc. individually	Figures for nectar, pollen etc. individually	Tables Stack figures prepared by RMS	Tables Stack figures prepared by RMS	Tables Stack figures prepared by RMS	Stacked figures
Behaviour	Not reported	Not reported	normal	Clustering but further normal	Repellence effect on DAA0aa and DAA1.	Intoxication symptoms, coordination problems and repellence effect on DAA0aa
remark	Non-GLP	Non-GLP	No obvious higher mortality outside the hive can be related to decrease of flight intensity	Unequal colony set-up, therefore most endpoints cannot be evaluated.	No obvious higher mortality outside the hive can be related to decrease of flight intensity	No obvious higher mortality outside the hive can be related to decrease of flight intensity
Statistics for mortality and flight intensity	no	no	yes	yes	yes	yes
Statistics for brood and food storage	no	no	no	no	no	no

According to the notifier, " the results of all of the six semi-field (tunnel) studies under forced and as such worst-case exposure conditions consistently did not show any adverse effects on mortality, foraging activity, behaviour, brood-, food- and population development or on overall colony vitality". They state that "overall, the laboratory database shows that BYI 02960 does not exhibit delayed or chronic effects, either in adult bees or in honey bee larvae. BYI 02960 metabolites are virtually non-toxic to honey bees and there is no indication that BYI 02960 metabolites are of any higher toxicity regarding potentially delayed or chronic effects than the parent compound. These laboratory findings

have been consistently confirmed by in total six independent semi-field tunnel studies in the highly bee attractive surrogate crop Phacelia tanacetifolia. As such, it can be concluded that BYI 02960 can be applied at foliar application rates of up to and including 200 g a.i./ha, even to bee-attractive, full-flowering crops during honey bees actively foraging, without adverse effects on honey bees, honey bee brood and honey bee colonies."

RMS however notes the following:

- Study 01 and /02 were not performed under GLP and are therefore less reliable. However, they represent the two lowest exposure situations in the dataset and are therefore less important for the overall conclusion.

- Endpoints in this type of semi-field study are interrelated. For instance mortality of bees outside the hive will depend on flight intensity. When flight intensity is reduced, mortality in the tunnel might be reduced as well as fewer bees are foraging. This was indeed the case in the studies where reduction of flight intensity was observed. Flight intensity was significantly reduced in studies 10.4.7/03-06; reduction was observed for three to seven days.

- Significant effects on flight intensity are present in studies 10.4.6/3 to 10.4.7/6. In studies 10.4.6/1 and /2 however, flight intensity was not evaluated statistically. Differences between studies may therefore be biased by the performing laboratory. However, based on visual inspection of the data RMS agrees that studies 10.4.6/1 and/2 do not suggest an effect on foraging activity.

- Repellence of flupyradifurone leads to decreased flight intensity. In that way bees will minimize exposure of the hive to flupyradifurone. In the studies in which reduced flight intensity was seen, effects on other endpoints were not clearly visible.

- Of course, a short-term reduction of flight intensity can be an effective way to reduce exposure and possibly related risk for honey bee colonies. It can only be seen as an acceptable effect if it does not lead to effects on bee colonies in the longer term from a shortage of food. This was not apparent in the semi-field studies (brood development, food storage area and colony strength comparable to control in all studies) but effects on these parameters can potentially be masked by the small colony size and confined conditions..

- Exposure in the tunnel was on average 12 days. Longer exposure is not feasible as the foraging opportunities within a tunnel are too limited. It was evident in most studies that there was a reduction of the colony strength during confinement in the tunnel. After placement of the hives in the open field, colonies started to grow again. Any effects of the active substance can therefore be masked by the stronger effect of confinement to the tunnel. Moreover, due to

natural variability between the hives it is extremely difficult to evaluate differences between treatment and control.

- Reduction of flight intensity and effects on behaviour were observed in those studies that were worst case for number of applications and timing of the applications (those ranked by RMS as 1, 2, 3 and 4).

- So far guidance has not been developed for the interpretation of changes of endpoints on brood development. Since the endpoints are interrelated, possible effects are difficult to disentangle. Evaluation of these endpoints is difficult without a clear perspective on normal brood development.

- It is not yet clear if and how results can be assessed statistically. Indeed, in these 6 studies, statistical assessment has not been performed on endpoints of brood development.

- Confined conditions may result in possible stress on bees which may result in a natural reduction of brood activity when bees are kept inside tunnels for longer periods. In these studies reductions of brood activity are indeed often observed during confinement to the tunnels.

- According to the EFSA opinion on bees, it is not possible to assess pollen and nectar storage and hive weight development due to the small size of the colony; therefore, it is difficult to assess potential effects on honey production (i.e. a potential protection goal) when adverse effects are observed on other parameters. As smaller sizes of colonies are used in semi-field studies, the interpretation of impacts on full size colonies will be difficult and the possibility to translate adverse effects on a small colony to a full size colony seems limited. Due to confined conditions, the small size of the colony and the high turnover rate of nectar and pollen in the tunnels, it is not possible to assess hive weight development and direct potential effects on honey production.

Based on the above, the semi-field studies alone are not considered sufficient proof that the risk to bees is acceptable. However, also two field studies and one long-term colony feeding study were submitted. These are summarised in Table 2.6.3.1-27.

Table 2.6.3.1-27 Overview of field and long-term feeding studies

Specifications and endpoint of the studies			
	10.4.5/01	10.4.5/02	8.16.2/03
Authors	Rexer 2012a	Rexer 2012b	Nikolakis, Krieg et al. 2012
Guideline	OEPP/EPPO guideline No. 170	OEPP/EPPO guideline No. 170	OEPP/EPPO Guideline No.

	(4), 2010 with modifications recommendations of the IVA Guideline (Beutel et al., 1992).	(4), 2010 with modifications recommendations of the IVA Guideline (Beutel et al., 1992).	170 (4) (2010) with modifications, the EU Directive 91/414/EEC (1997), and in part, the recommendations of the IVA Guideline (Beutel et al., 1992)
Test facility	Eurofins Agroscience Services EcoChem, Niefern-Öschelbronn	Eurofins Agroscience Services EcoChem, Niefern-Öschelbronn	Bayer CropScience AG, Monheim
Nr of applications	4	4	Not applicable, exposure by feeding
Type applications	1x bare soil 1 seed treatment 2x foliar spray	1x bare soil 1 seed treatment 2x foliar spray	Fortified diet in tunnels
Treatment rate	9.9 g flupyradifurone/kg seed (analysed). 300 g as/ha (soil appl), 205 and 205 g as/ha (2 foliage applications)	10.1 g flupyradifurone/ kg seed (analysed). 300 g as/ha (soil appl), 205 and 205 g as/ha (2 foliage applications)	600 – 2500 - 10000 µg as/kg diet
Crop	Oil seed rape	Oil seed rape	Not applicable, exposure by feeding
Number of hives in control / treatment / reference	8 / 8 / n.a.	8 / 8 / n.a.	5 (3 remaining) / 5 / n.a.
Hive size (mean initial nrs of bees)	9,000	12,000	6000
Timing application	first foliar application immediately pre/early flowering; second foliar application 6 d later during full flowering and active foraging of bees	first foliar application immediately pre/early flowering; second foliar application 9 d later during full flowering and active foraging of bees	No flowering crop in the tunnel, forced diet feeding
Exposure duration	24 d	30 d	42 d
Observation duration	346 d	351 d	326 d
Flight intensity	Similar to control, except slightly lower for 2 d after 2nd foliar application	Similar to control, except clearly lower 2 and 3 d after 2nd foliar application	Similar to control
Bee mortality in bee traps, linen sheets	Similar to control	Similar to control	Similar to control

Number of bees	Similar to control	Similar to control	Similar to control
Weight of colony	Similar to control	Similar to control	Similar to control
Development of brood	Similar to control	Similar to control	Similar to control
Development of food storage aria	Increase pollen and nectar stores similar to control	Increase pollen and nectar stores similar to control	Increase pollen and nectar stores similar to control
Behaviour	intensive cleaning behaviour and locomotion problems in small fraction of bees of treated colonies on day after 2nd foliar application	no treatment-related effects	
Remark			The low number of replicates (especially in the control, with only three remaining replicates) and the apparent variation within the treatment groups in mortality of bees/drones/immature stages make it difficult to draw conclusions on the effect of the treatment. What can be concluded is that, under the specific circumstances of this study, the different treatments of the test substance do not lead to an adverse effect in one of the observed parameters (or the combination of adverse effects of the different parameters) to such an extent, that it led to the collapse of one or more colonies.
Statistics for mortality and flight intensity	no	no	yes
Statistics for brood and food storage	no	no	yes

Thus, these studies confirm the results of the semi-field studies.

In the two field studies on oil seed rape, no adverse effects on honey bees were seen during the exposure period nor during the observation period which included overwintering. The only treatment-related effects were a short-term (two days) reduction on flight intensity in both studies and some behavioural anomalies in a small fraction of bees for one day in one of the two studies. These effects

apparently did not lead to direct or delayed effects on survival or wellbeing of the colonies as compared to the control colonies. Exposure in the field studies was confirmed with residue measurements in nectar and pollen both from forager bees and from hive matrices.

Also, a long-term feeding study was submitted in which colonies were forced to feed on up to 10 mg/kg diet for seven weeks. This is clearly a worst-case exposure for the GAPs of the semi-field and field studies (2x 200 g a.s./ha foliar spray and in some cases also background soil concentration included). In these trials, the initial concentration in pollen and nectar taken from forager bees could be above 10 mg/kg on the first days, but this quickly decreased to below 10 mg/kg. In the hives, the concentration in pollen or nectar never exceeded a maximum value of 10 mg/kg and also decreased to much below that level soon. This study had some shortcomings but it can be concluded that a 7-week exposure to 10mg flupyradifurone/kg diet does not lead to collapse of a colony, including overwintering.

The overall data package shows that the spraying of 2x 200 g a.s./ha on a bee-attractive crop during full flowering and active foraging of honey bees does not result in adverse acute or long-term (including overwintering) effects on honey bee colonies.

This data package covers the proposed uses in the non-attractive crops lettuce and hops with application rates of 1x125 and 1x150 g a.s./ha, respectively. For these crops, the main concern is via flowering weeds especially in the hop plantations. The above studies in highly attractive crops indicate that a low risk is expected from these uses via nectar and pollen of flowering weeds.

Expert discussion in Pesticide Peer Review meeting 124 (December 2014):

The RMS concluded that the data sets indicate that overall no adverse acute or long-term effects (including over-wintering evaluation) were indicated for a spray of 2 x 200 g a.s./ha on bee attractive crops during full flowering and active foraging (1 application prior to flowering and 1 application during flowering). The experts at the meeting agreed with the conclusion of the RMS noting that some slight transient treatment related effects may have been observed.

Risk assessment for other exposure routes

Exposure via honeydew

Aphids are expected to be more sensitive to flupyradifurone than bees because they are target pest species. If they are indeed more sensitive, buildup of honeydew at a level that can harm honeybees is not expected. However, this needs to be confirmed with data. The notifier has submitted dose-response studies with aphids (KIIIA1 6.1.1) . However, actual LD50 values were not found in the dose-

response studies and the doses are expressed in g a.s./ha; therefore they are not useful for a comparison with the toxicity to honeybees. The notifier also submitted an LD50-study with the green peach aphid. The raw data of the LD50-study with the green peach aphid need to be submitted before conclusions can be drawn about the usefulness of the study. However, based on the conclusion above that the risk via direct overspray and via nectar and pollen of highly attractive flowering crops is acceptable, a high risk via honeydew (directly oversprayed or produced (much) later by aphids) is not expected either.

Expert discussion in Pesticide Peer Review meeting 124 (December 2014):

The RMS included a consideration of the risk via insect honey dew in the DAR. The RMS considers that the higher tier studies were sufficient to cover the risk to honey bees via this route of exposure. The experts agreed with the conclusions of the RMS that this route of exposure was addressed.

Risk assessment for other exposure routes

Exposure via honeydew

Aphids are expected to be more sensitive to flupyradifurone than bees because they are target pest species. If they are indeed more sensitive, buildup of honeydew at a level that can harm honeybees is not expected. However, this needs to be confirmed with data. The notifier has submitted dose-response studies with aphids (KIIIA1 6.1.1) . However, actual LD50 values were not found in the dose-response studies and the doses are expressed in g a.s./ha; therefore they are not useful for a comparison with the toxicity to honeybees. The notifier also submitted an LD50-study with the green peach aphid. The raw data of the LD50-study with the green peach aphid need to be submitted before conclusions can be drawn about the usefulness of the study. However, based on the conclusion above that the risk via direct overspray and via nectar and pollen of highly attractive flowering crops is acceptable, a high risk via honeydew (directly oversprayed or produced (much) later by aphids) is not expected either.

Exposure via guttation and other water sources

The relevance of exposure via guttation droplets and other water sources for honey bee risk assessment is not yet clear. The risk via guttation droplets is generally estimated to be highest from droplets on young seedlings grown from treated seeds. The applicant submitted two studies in which the residue level in young oil seed rape seedlings, sown in soil with a background concentration covering the PECplateau of the proposed uses, was measured. A highest level of 21 mg/L was found (initial level, quickly declining). This is comparable to the NOEC-value from the chronic honey bee laboratory study with technical flupyradifurone (10-d feeding of 26 mg/kg in highly bee attractive sugar solution). Therefore, a high risk via guttation is not indicated. Based on the conclusion above (see section higher tier toxicity data and risk assessment) that the risk via direct overspray and via nectar

and pollen of highly attractive flowering crops is acceptable, a high risk via other water sources (e.g. puddles, surface water) is not expected either.

Exposure via succeeding crops

In order to account for potential side-effects on honey bees from a potential carry-over of soil-borne flupyradifurone residues into potentially bee-attractive, succeeding flowering cops, a worst-case soil-plateau concentration was established in two out of four semi-field studies in Phacelia tanacetifolia as well as in both long term honey bee health field studies in W-OSR. In all of these four studies which evaluated potential side-effects on honey bees, nominally 300 g flupyradifurone a.s./ha was applied onto the soil at the day of crop planting, to be immediately incorporated down to depth of 20 cm to establish a worst-case, non-aged flupyradifurone soil plateau concentration within the root zone of the planted, bee-attractive flowering crop. The analytically determined soil concentration in these four studies was in the range of 36 - 76 µg flupyradifurone a.s./kg soil.

These analytically determined soil concentrations can be compared to the soil plateau concentration that has been calculated in the fate section. The PECplateau for lettuce of 0.016 mg/kg (over 20 cm) is clearly covered. For hops, the reported PECplateau is 0.080 mg/kg, but this is calculated over 5 cm for perennial crops without tillage. In case of succeeding crops other than hops, tillage will take place and it can be assumed that the soil concentration will be spread over 20 cm, leading to a soil concentration of 0.020 mg/kg. Thus, risk via succeeding crops of the use in hops is also covered with the effect semi-field and field studies.

2.6.3.2 Other arthropod species

Procedures for risk assessment were in agreement with the recommendations in the Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (Working Document Sanco/10329/2002 rev 2 final, 17 October 2002, referring to ESCORT 2). The recommendations of ESCORT 3, a follow-up workshop, are taken into account for this DAR for issues where guidance is lacking.

In glasshouse crops for which Integrated Pest Management systems are used, introduced natural enemies may be exposed to flupyradifurone and, considering the results of in-field risk calculations for the field uses (see below), significantly affected This issue is not assessed in the framework of the DAR but can be taken account at Member State level.

Toxicity tests on non-target arthropods have been performed with BYI 02960 SL 200 G on the species *Typhlodromus pyri*, *Aphidius rhopalosiphi*, *Coccinella septempunctata*, *Aleochara bilineata* and *Orius*

laevigatus. Furthermore, two full-fauna off-crop field studies were conducted. Table 2.6.3.2-01 below summarises the results of laboratory testing with non-target arthropod species.

Table 2.6.3.2- 01 Ecotoxicological endpoints for arthropods other than bees (BYI 02960 SL 200)

Test species, Dossier-File-No. Reference	Tested Formulation, Study type, Exposure	Ecotoxicological Endpoint
Aphidius rhopalosiphi Jans, 2010 KIIA 8.8.1.1/01 KIIIA1 10.5.1/01	BYI 02960 SL 200 (g/L) Laboratory, glass plates 0.5 g a.i./ha 1.1 g a.i./ha 2.2 g a.i./ha 4.7 g a.i./ha 10 g a.i./ha 10 g a.i./ha 20 g a.i./ha 40 g a.i./ha 80 g a.i./ha 160 g a.i./ha	LR_{50} < 0.5 g a.i./ha Corr. Mortality [%] 85 100 100 100 100 100 100 100 100 100
Typhlodromus pyri Jans, 2010 KIIA 8.8.1.2/01 KIIIA 10.5.1/02	BYI 02960 SL 200 (g/L) Laboratory, glass plates 2 g a.i./ha 4 g a.i./ha 9 g a.i./ha 19 g a.i./ha 40 g a.i./ha	LR_{50} 17 g a.i./ha Corr. Mortality [%] 6.3 6.3 25.0 50.0 89.6
Aphidius rhopalosiphi Jans, 2010 KIIIA1 10.5.2/01	BYI 02960 SL 200 (g/L) Extended lab., exposure on potted barley seedlings 0.5 g a.i./ha 0.89 g a.i./ha 1.58 g a.i./ha 2.81 g a.i./ha 5.0 g a.i./ha	LR_{50} 2.02 g a.i./ha, ER_{50} >0.89 <1.58 g a.i./ha Corr. Mortality [%] / Effect on Reproduction [%] / Repellency rel. to control [%] -3.4 [A] / -6.4 [B] / -42.0 [C] sign. 10.3 / -2.9 [B] / -45.8 [C] n.sign. 37.9 / 57.7 / -26.3 [C] n.sign. 69.0 / n.a. / -67.5 [C] sign. 89.7 / n.a. / -43.7 [C] sign.
Typhlodromus pyri Jans, 2010 KIIIA1 10.5.2/02	BYI 02960 SL 200 (g/L) Extended lab., exposure on detached broad bean leaf discs 15 g a.i./ha 32 g a.i./ha 67 g a.i./ha 142 g a.i./ha 300 g a.i./ha	LR_{50} 177 g a.i./ha, ER_{50} >142 g a.i./ha Corr. Mortality [%] / Effect on Reproduction [%] -1.1 [A] / 6.0 -4.6 [A] / 0.6 6.9 / 5.1 37.9 / -6.8 [B] 75.9 / n.a.

Test species, Dossier-File-No. Reference	Tested Formulation, Study type, Exposure	Ecotoxicological Endpoint		
Coccinella septempunctata Jans, 2010 KIIIA1 10.5.2/03	BYI 02960 SL 200 (g/L) Extended lab., exposure on detached broad bean leaves	LR_{50} 273.9 g a.i./ha		
		Corr. Mortality [%]	Eggs/Female/Day	Hatching [%]
	Control	-	10.4	92.0
	8 g a.i./ha	12.9	15.5	91.4
	17 g a.i./ha	4.1	17.6	95.7
	35 g a.i./ha	-9.7 [A]	9.5	87.3
	72 g a.i./ha	3.2	15.5	93.5
	150 g a.i./ha	32.3	21.6	95.4
	Control	-	13.9	91.9
	100 g a.i./ha	9.7	16.1	92.0
	160 g a.i./ha	19.4	21.0	92.3
	250 g a.i./ha	45.2	25.6	91.3
	380 g a.i./ha	71.0	n.a.	n.a.
	600 g a.i./ha	96.8	n.a.	n.a.
Aleochara bilineata Jans, 2010 KIIIA1 10.5.2/04	BYI 02960 SL 200 (g/L) Extended lab., spray deposits on soil (LUFA 2.1)	ER_{50} > 300 g a.i./ha		
		Effect on Reproduction [%]		
	10 g a.i./ha	7.6		18.2
	21 g a.i./ha			
	45 g a.i./ha	-0.4 [B]		
	95 g a.i./ha			13.2
	200 g a.i./ha			
	300 g a.i./ha	16.5		
		13.0		
Aphidius rhopalosiphi Jans, 2010 KIIIA1 10.5.3/01	BYI 02960 SL 200 (g/L) Aged residue spray deposits on maize plants, 2 appl. of 250 g a.i./ha (spray interval of 10 d)	Corr. Mortality [%]	Effect on Reproduction [%]	Repellency rel. to control [%]
	Residues aged for 0 d:	100	n.a.	68.1 sign.
	Residues aged for 14 d:	90.0	n.a.	47.5 sign.
	Residues aged for 28 d:	76.7	n.a.	30.6 sign.
	Residues aged for 42 d:	29.6	89.9	37.3 n.sign.
	Residues aged for 49 d:	20.0	37.6	-18.8 [C] n.sign.
	Residues aged for 56 d:	6.7	0.6	-6.0 [C] n.sign.
Orius laevigatus Schwarz, 2010 KIIIA1 10.5.3/02	BYI 02960 SL 200 (g/L) Aged residue spray deposits on apple trees, 2 appl. of 250 g a.i./ha (spray interval of 10 d)	Corr. Mortality [%]	Effect on Fecundity [%]	Effect on Fertility [%]
	Residues aged for 0 d:	100	n.a.	n.a.
	Residues aged for 14 d:	75.6	n.a.	n.a.
	Residues aged for 28 d:	24.5	23.0	-6.5 [D]
	Residues aged for 42 d:	9.8	34.7	12.9

A: A negative value indicates a lower mortality in the treatment than in the control.
B: A negative value indicates a higher reproduction rate in the treatment than in the control.
C: A negative value indicates a higher percentage of wasps found on plants in the treatment than in the control.

D: A negative value indicates a higher nymphal hatching rate in the treatment than in the control.
n.a.: Not assessed.
sign.: statistically significant at 5%-level (only indicated for repellency).
n.sign.: not statistically significant (only indicated for repellency).

The tier 1 glass plate studies indicate that insects, as represented by *Aphidius rhopalosiphi* (LR50 < 0.5 g a.i./ha), are more sensitive to the exposure of BYI 02960 SL 200 G than mites like *Typhlodromus pyri* (LR50 17.3 g a.i./ha). This has been confirmed by extended laboratory studies for the same two species. A comparison of the extended laboratory results for *Aphidius rhopalosiphi* (LR50 2.02 g a.i./ha, ER50 >0.89 and <1.58 g a.i./ha) with the results from the additionally tested species *Coccinella septempunctata* (LR50 273.9 g a.i./ha and no effects on reproduction below the LR50) and *Aleochara bilineata* (ER50 >300 g a.i./ha) gives clear evidence, that *Aphidius rhopalosiphi* is by far the most sensitive species.
Hence, aged residue studies were conducted with *Aphidius rhopalosiphi* and - in addition - with the predatory bug *Orius laevigatus*. The results showed that *Orius laevigatus* is also susceptible to the exposure of BYI 02960 SL 200 G (effects <50% after 28 days of aging) but clearly less sensitive than *Aphidius rhopalosiphi* (effects <50% after 49 days of aging). For this reason, the risk assessment of *Orius laevigatus* is considered to be covered by the risk assessment for *Aphidius rhopalosiphi*.

Data have been submitted on *Typhlodromus pyri* and *Aphidius rhopalosiphi* (i.e. two indicator species) as well as three crop-relevant species (*Coccinella septempunctata, Aleochara bilineata* and *Orius laevigatus*). From these studies a risk assessment is performed according to the recommendations of ESCORT 2.

Tier 1 risk assessment

In the 1st tier, the LR50 values of laboratory glassplate tests with the two indicator species are taken into account. The hazard quotient (HQ) is calculated as follows:

In field-HQ = max. single application rate * MAF / LR_{50}
Off-field HQ = max. single application rate * MAF * (drift factor/VDF)*correction factor / LR_{50}

- Max. single application rate = 125 g a.i./ha (lettuce), 150 g a.i./ha (hops)
- MAF (multiple application factor) = 1 (only 1 application)
- Drift factor = 19.33% (hop, 3 m distance, 1 application; ESCORT2) 2.77% (lettuce (field crops), 1m distance, 1 application; ESCORT2)

- VDF (vegetation distribution factor) = 10 (default value as recommended by the Terrestrial Guidance Document, to take into account the 3-dimensional structure of the off-field vegetation)
- Correction factor = 10 (uncertainty factor for the extrapolation from indicator species to all off-field non-target arthropods; default value for tier 1 risk assessment according to the Terrestrial Guidance Document)

The risk is considered acceptable if the calculated HQ is < 2. See the Tables below.

Table 2.6.3.2-02 HQ for terrestrial non-target arthropods for the in-field scenario

Crop (field uses)	Species	Appl. rate [g a.i./ha]	MAF	LR_{50} [g a.i./ha]	HQ	Trigger
Hops	*A. rhopalosiphi*	150	1	<0.5	**>300**	2
Hops	*T. pyri*	150	1	17	**8.8**	2
Lettuce	*A. rhopalosiphi*	125	1	<0.5	**>250**	2
Lettuce	*T. pyri*	125	1	17	**7.4**	2

Conclusion: For both standard species, the in-field HQ values are above the trigger of concern, indicating a need for refinement.

Table 2.6.3.2-03 HQ for terrestrial non-target arthropods for the off-field scenario

Crop	Species	Appl. rate [g a.i./ha]	MAF	Drift [%]	VDF	Corr. factor	LR_{50} [g a.i./ha]	HQ	Trigger
Hops	*A. rhopalosiphi*	150	1	19.33	10	10	<0.5	**>58**	2
Hops	*T. pyri*	150	1	19.33	10	10	17	1.7	2
Lettuce	*A. rhopalosiphi*	125	1	2.77	10	10	<0.5	**>6.9**	2
Lettuce	*T. pyri*	125	1	2.77	10	10	17	0.2	2

Conclusion: The HQ values for *Typhlodromus pyri* are below the trigger of concern for both uses. However, both HQ values for *Aphidius* are above the trigger of 2, indicating a need for refinement.

<u>Tier 2 risk assessment</u>

In the 2nd tier, the results of extended laboratory tests are taken into account (LR50, ER50 and/or effect percentages). These are available for four species.

The exposure is calculated as:

In-field: Application rate * MAF
In-field (high crops) Application rate * MAF * 0.5
Off-field: Application rate * MAF * (drift factor / VDF) *correction factor

- Max. single application rate = 125 g a.i./ha (lettuce), 150 g a.i./ha (hops)
- MAF (multiple application factor) = 1 (only 1 application)

- Factor 0.5 for high crops: The correction factor of 0.5 for the in-field exposure assessment in three-dimensional crops according to ESCORT2 (see footnote "a" in legend on page 19 of the ESCORT2 document) can be applied according to the notifier. However, application in hops can start from a stage in which the plants are still small, BBCH 31. The BBCH scale describes the hop plants between BBCH 31 and 38 as follows:

  Principal growth stage 3: Elongation of bines
  31 Bines have reached 10% of top wire height
  32 Bines have reached 20% of top wire height
  33 Bines have reached 30% of top wire height
  3 . Stages continuous till . . .
  38 Plants have reached the top wire

  At the beginning of the application period (BBCH 31-37), the plants are still thin and small (ca. 40cm based on a top wire size of 4m) and it is not justified to use the factor of 0.5. Furthermore, it is unclear whether the correction factor can really also be used for hops. Since no justification/validation for the use of the factor of 0.5 for other crops than orchards and vineyards, the crops mentioned in ESCORT 2, is available, the risk is calculated without correction factor.
- Drift factor = 19.33% (hop, 3 m distance, 1 application; ESCORT2; considered relevant for all grwoth stages since the same application machinery will be used throughout the season); 2.77% (lettuce (field crops), 1m distance, 1 application; ESCORT2)
- VDF (vegetation distribution factor) = 10 (default value as recommended by the Terrestrial Guidance Document, to take into account the 3-dimensional structure of the off-field vegetation; it can only be applied in the context of 2D test systems)
- Correction factor = 5 (uncertainty factor for the extrapolation from indicator species to all off-field non-target arthropods; default value for tier 2 risk assessment)

Table 2.6.3.2-04 Exposure calculation for in-field assessment

Crop / no. of applications	Appl. rate [g a.i./ha]	MAF	factor to account for high crops	in-field PEC_{max}. [g/ha]
Hops BBCH 31-38 / 1	150	1	n.a.	150
Lettuce / 1	125	1	n.a.	125

Table 2.6.3.2-05 Exposure calculation for off-field risk assessment

Appl. rate [g a.i./ha]	MAF	Drift [%]	Veg. distr. factor	Correction factor	off-field $PEC_{max.}$ [g/ha]	Remark
150	1	19.33	-	5	145	in case of 3-D study design
150	1	19.33	10	5	14.5	in case of 2-D study design
125	1	2.77	-	5	17.3	in case of 3-D study design
125	1	2.77	10	5	1.73	in case of 2-D study design

Tier 2 in-field risk assessment

The in-field exposure PEC values are compared to the results of the extended laboratory studies.

Table 2.6.3.2-06 In-field risk assessment based on study results from extended laboratory studies

Test Species	in-field $PEC_{max.}$, [g a.i./ha]	LR_{50}/ ER_{50} [g a.i./ha]	Trigger	Refined assessment

				required?
Use in hops, 1 x 150 g a.i./ha				
Typhlodromus pyri	150	177 / >142	Effects are < 50%	no*
Aphidius rhopalosiphi	150	2.02 / >0.89	Effects are < 50%	**yes**
Coccinella septempunctata	150	273.9 / no effects up to 250	Effects are < 50%	no
Aleochara bilineata	150	>300	Effects are < 50%	no
Use in lettuce, 1 x 125 g a.i./ha				
Typhlodromus pyri	125	177 / >142	Effects are < 50%	no
Aphidius rhopalosiphi	125	2.02 / >0.89	Effects are < 50%	**yes**
Coccinella septempunctata	125	273.9 / no effects up to 250	Effects are < 50%	no
Aleochara bilineata	125	>300	Effects are < 50%	no

* No adverse effects on reproduction were seen up to 142 g a.s./ha. The LR50 is 177 g a.s./ha. It is likely that at 150 g a.s./ha no adverse effects on *Typhlodromus* will occur.

The higher tier in-field risk assessment for *Typhlodromus, Coccinella* and *Aleochara* indicates that no unacceptable adverse effects are to be expected in the in-field area for arthropod species with a similar sensitivity as these species. However, the in-field risk assessment for *Aphidius rhopalosiphi* indicates that initial effects in the in-field area cannot be excluded. Therefore, further refinement is needed.

Refined in-field risk assessment

The results of the tier 2 risk assessment indicate that initial effects on species with a similar sensitivity as *Aphidius rhopalosiphi* cannot be excluded. As a consequence, an aged residue study was performed to demonstrate the potential for recovery for *Aphidius rhopalosiphi*, the most sensitive tested species, and in addition for the predatory bug *Orius laevigatus*.

An extended laboratory aged residue study has been performed on *Aphidius rhopalosiphi* (Jans, 2010; KIIIA1 10.5.3/01). In this study, BYI 02960 SL 200 was applied 2 times at a rate of 250 g a.i./ha with a 10 days interval on potted maize plants. Spray residues were aged under semi-field conditions. Bioassays with freshly dried residues, residues aged for 14 days and for 28 days, respectively, resulted in a corrected morality of 100, 90 and 76.7%, respectively. After an ageing time of 42, 49 and 56 days, corrected mortalities of 29.6, 20.0 and 6.7% were recorded, respectively. Exposure to residues aged for 42 days resulted in a 89.9% reduction in reproduction relative to the control. No effects on reproduction >50% were observed after an ageing time of 49 and 56 days, respectively. Statistically significant repellency was recorded in the first, second and third bioassay (aged 0, 14 and

28 days), while in the fourth, fifth and sixth bioassay (aged 42, 49 and 56 days) no repellent effects were observed.

A second extended laboratory aged residue study has been performed on *Orius laevigatus* (Schwarz, 2010; KIIIA1 10.5.3/02). BYI 02960 SL 200 was applied 2 times at a rate of 250 g a.i./ha with an application interval of 10 days onto potted apple plants. *Orius laevigatus* showed a corrected mortality of 100 and 75.6%, when exposed to fresh residues of the test item and residues aged for 14 days, respectively. After an ageing time of 28 and 42 days, a corrected mortality of 24.5 and 9.8% was observed, respectively. No adverse effects on reproduction > 50% were observed after an ageing time of 28 and 42 days, respectively. The results indicated that *Orius laevigatus* is as well susceptible to the exposure of BYI 02960 SL 200 G (effects <50% after 28 days of ageing) but clearly less sensitive as *Aphidius rhopalosiphi* (effects <50% after 49 days of ageing).

These aged residue studies indicate that the potential for recovery is given even after 2 applications at a rate of 250 g a.i./ha within 7 weeks for the most sensitive species, *Aphidius rhopalosiphi*. For *Orius laevigatus*, residues aged for only 4 weeks already had no adverse effect on mortality and reproduction. Since the intended use pattern includes only single applications at a rate of up to 150 g a.i./ha in the field it can be concluded that the potential for recovery is given within a few weeks after the application and no unacceptable in-field risk for non-target arthropods has to be expected from the use of BYI 02960 SL 200 according to the proposed use pattern.
It is noted that the ageing in the studies was done under semi-field conditions and on plants which could potentially cause more growth dilution than other plant species, and therefore the aged residue studies do not represent all possible field conditions. Thus, a generic ‘potential for recovery’ period cannot be quantified. However, RMS considers that a potential for recovery within one year (the required period for in-field risk assessment according to ESCORT 2) has been shown.

Tier 2 off-field risk assessment
The off-field exposure PEC values are compared to the results of the extended laboratory studies.

Table 2.6.3.2-07 Off-field risk assessment based on study results from extended laboratory studies

Test Species	off-field $PEC_{max.}$, [g a.i./ha]	LR_{50}/ ER_{50} [g a.i./ha]	Trigger	Refined assessment required?
Use in hops, 1 x 150 g a.i./ha				
Typhlodromus pyri	14.5	177 / >142	Effects are < 50%	no
Aphidius rhopalosiphi	145	2.02 / >0.89	Effects are < 50%	**yes**
Coccinella septempunctata	14.5	273.9 / no	Effects are <	no

		effects up to 250	50%	
Aleochara bilineata	14.5	>300	Effects are < 50%	no
Use in lettuce, 1 x 125 g a.i./ha				
Typhlodromus pyri	1.73	177 / >142	Effects are < 50%	no
Aphidius rhopalosiphi	17.3	2.02 / >0.89	Effects are < 50%	**yes**
Coccinella septempunctata	1.73	273.9 / no effects up to 250	Effects are < 50%	no
Aleochara bilineata	1.73	>300	Effects are < 50%	no

The maximum PEC off-field for the use in hop (worst-case) is calculated to be 14.5 g/ha for 2D-test systems and 144.9 g/ha for 3D-test systems. For *Typhlodromus*, *Coccinella* and *Aleochara* no effects > 50% neither on mortality nor on reproduction were observed in extended laboratory studies on natural substrate at a rate of 142 (*Typhlodromus*), 250 (*Coccinella*, 2nd trial) and 300 g a.i./ha (*Aleochara*), respectively (see Table 2.6.3.2-07). However, for *Aphidius*, initial effects cannot be excluded, indicating a need for further refinement.

Refined off-field risk assessment

To assess off-field effects of BYI 02960 SL 200 on naturally occurring arthropod communities under more realistic conditions, two full-fauna field studies were conducted on grassland as surrogate for off-field habitats in the Netherlands and in Southwestern France.

BYI 02960 SL 200 was applied in a dose-response design at drift rates (0.51, 1.7, 5.1 and 21 g a.i./ha) to grassland habitats with little agricultural input in the Netherlands and Southwestern France. These sites held a diverse and representative off-crop non-target arthropod community. Four replicate plots of 24 x 24 m or 22 x 22 m each were used per treatment (4 application rates, control, reference treatment = 24 plots in total). Arthropods were sampled comprehensively using three different sampling methods (pitfall, suction and weed/Berlese sampling) shortly before the application and 1, 2, 4 and 8 weeks after the application. Overall community changes relative to the control were analyzed using multivariate statistics and depicted by Principal Response Curves (PRC).
In addition, effects on individual arthropod populations were analyzed with univariate statistics for taxa that were sufficiently abundant. In both studies, the toxic reference item treatment caused clear responses both at the arthropod community level and at the population level, demonstrating that the test system was sufficiently sensitive to detect toxic effects.
Both field tests contained sufficient numbers for analysis of effects on the Hymenopteran family Braconidae, to which the most sensitive first tier species *Aphidius rhopalosiphi* belongs.

<u>Results of the trial in the Netherlands:</u>

At community level, no statistically significant effects on arthropod communities were found at any of the rates tested up to and including 21 g a.i./ha. Therefore, 21 g a.i./ha is the community NOER (No Observed Effect Rate) of BYI 02960 SL 200. In addition, 72 taxa were sufficiently abundant for a univariate statistical evaluation at population level. In the three lowest test rates, none of the taxa showed a consistent treatment-related response. Predatory mites of the family *Cunaxidae* and hymenopteran parasitoids of the family *Braconidae* showed transient adverse effects only at one sampling moment shortly after application of the highest test rate (21 g a.i./ha). Both taxa recovered within one week. In conclusion, the population NOEAER (No Observed Ecologically Adverse Effect Rate) of BYI 02960 SL 200 is 21 g a.i./ha and the population NOER (No Observed Effect Rate) is 5.1 g a.i./ha.

Results of the trial in France:

At community level, no statistically significant effects on arthropod communities were found at any of the rates tested up to and including 21 g a.i./ha. Therefore, 21 g a.i./ha is the community NOER (No Observed Effect Rate) of BYI 02960 SL 200.

At the population level, 79 taxa were sufficiently abundant for population level evaluations. Only three phytophagous taxa were adversely affected.

At the test rate of 5.1 g a.i./ha, significant adverse effects on the chrysomelid beetles *Alticinae* and on juvenile leafhoppers (*Cicadellidae*) occurred at a single sampling occasion. At the rate of 21 g a.i./ha, *Alticinae* and *Cicadellidae* showed statistically significant reductions at two sampling moments after application, but recovered already 4 weeks after the application. Aphids (*Aphidoidea*) were reduced at several sampling moments after the application of 21 g a.i./ha; however, the reduction was only statistically significant at the penultimate sampling moment. The observed effects on *Aphidoidea* were to be expected since aphids are a target pest species for the proposed uses of BYI 02960 SL 200 in lettuce and hop. However, aphids can be expected to recover quickly from population declines due to their high asexual reproductive potential. No significant reduction in *Aphidoidea* abundance was observed at the last sampling moment two months after application of the test item. The highest rate tested in this study - BYI 02960 SL 200 applied at 21 g a.i./ha - is therefore classified as the population NOEAER (No Observed Ecologically Adverse Effect Rate) with effect class 3a; the second highest dose rate – 5.1 g a.i./ha is the NOEAER with effect class 2.

In the Terrestrial ecotoxicology guidance document and in ESCORT 2, there is no clear guidance on what endpoints should be used from field studies for risk assessment. The report from the ESCORT 3 workshop contains some relevant recommendations: (chapter 1.8.1.2, p.9) ‘ Class 3 effects may be appropiate for the off-crop in-field margin, and Class 1 and 2 are appropiate for off-field area’; (chapter 5.3, p.40) ‘ Considering field studies for off-crop risk assessment, no effect or only transient effects (e.g. de Jong et al. 2010) are considered acceptable’; and (chapter 7.1, p.49): ‘At the level of field

studies, the NOER community and the NOEAER population should be used for the off-field risk assessment.'.

The drift values used to calculate the off-field exposure were determined at distances from the *crop*. In the current risk assessment, differentiation between ' off-crop in-field' and ' off-field' is not done. Depending on MS, crop type, farmer, etc., there may or may not be an in-field off-crop buffer zone. To ensure that the off-field is adequately protected, in this risk assessment the situation without off-crop in-field zone is assumed.

In both field studies, the community NOER is 21 g a.s./ha.
As NOEAER, three values were set:

Study in NL: 21 g a.s./ha is the population level NOEAER with effect class 2

Study in SW-FR: 5.1 g a.s./ha is the population level NOEAER with effect class 2; 21 g a.s./ha is the population level NOEAER with effect class 3a

Based on the recommendations of ESCORT 3, the community NOER and the community NOEAER values with effect class 2 are considered and the lowest of these is taken. Thus, the relevant endpoint for the risk assessment would be 5.1 g a.s./ha based on the population NOEAER effect class 2 from the study in France. Because two field studies are available, the RMS does not consider an additional safety factor on this value necessary.

The relevant endpoint for the risk assessment from these studies was discussed in Pesticide Peer Review meeting 124 of December 2014. The studies had a checkerboard design, meaning that there were areas of low dose and control which could serve as source of recolonisation for the populations. The experts questioned whether there was more possibility for recolonisation in these studies than there would be in a normal agricultural off-field situation. It was therefore decided in the meeting that the NOER values could be used from these studies as these do not (less likely) account for recovery/ recolonisation.

In both field studies, the community NOER is 21 g a.s./ha.
The NOER population is:

Study in NL: 5.1 g a.s./ha

Study in SW-FR: 1.7 g a.s./ha

The lowest from these values can be used in the risk assessment, i.e. 1.7 g a.s./ha.

It was extensively discussed in the pesticide peer review meeting whether a safety factor should be applied to this value to account from uncertainties in extrapolating the results of field studies to the realistic worst case field situation. Overall, the majority of the experts agreed that no additional safety

factor is required in this case given that the endpoint is a NOER and that two studies (accounting for the representativeness of the taxa) are available.

Refined potential exposure:

In the original DAR, the calculation of the off-field PEC was confusing, as reference was made to the term 'correction factor' while this is only appropiate for extended laboratory studies and not for field studies. This term has therefore been removed from the table below. The possible need for a safety factor, accounting for uncertainty when using field studies in the risk assessment, has already been discussed above. The vegetation distribution factor is not applicable because of the 3-D study design. The refined off-field exposure is calculated in the table below. For the use in hops, a risk is identified using the default drift value; therefore the exposure at increasing distances from the crop (and therefore decreasing drift values) is also shown.

Table 2.6.3.2-08 Refined exposure calculation for off-field risk assessment

Crop / no. of applications	Appl. rate [g a.i./ha]	MAF	Distance from crop	Drift [%]	off-field PECmax. [g/ha]	Endpoint based two full-fauna field studies [g/ha]
Hops / 1	150	1	3*	19.33	29	1.7
	150	1	5	11.57	17	1.7
	150	1	10	5.77	8.7	1.7
	150	1	15	3.84	5.8	1.7
	150	1	20	1.79	2.7	1.7
	150	1	30	0.56	0.84	1.7
Lettuce / 1	125	1	1*	2.77	3.5	1.7
	125	1	5	0.57	0.71	1.7

*Default

Table 2.6.3.2-08 shows that ~~the off-crop exposure is below the relevant off-field endpoint of 5.1 g a.s./ha for the use in lettuce. Therefore, the off-field risk from the use in lettuce is acceptable.~~

~~t~~he expected exposure (29 g a.s./ha) is above the relevant endpoint (1.7 ~~5.1~~ g a.s./ha) for the use in hops at the default distance from the crop. The table shows that a buffer zone of at least 30~~20~~ m would be required for the exposure value to fall below the required level. This means that acceptable use can be achieved based on the European guidance. The off-field risk from the use in hops is acceptable provided that a buffer zone of 30~~20~~ m is used.

For lettuce, the expected exposure (3.5 g a.s./ha) is above the relevant endpoint (1.7 ~~5.1~~ g a.s./ha) at the default distance from the crop. The table shows that a buffer zone of at least 5 m would be required for the exposure value to fall below the required level.

Note that other options for risk mitigation may be possible (e.g. drift reducing nozzles or the presence of off-crop in-field buffer zones which would give the option to use a higher endpoint). Risk mitigation measures are set at Member State level.

2.6.4 Effects on earthworms and other soil macro-organisms

2.6.4.1 Effects on earthworms

Procedures for risk assessment were in agreement with the recommendations in the Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC (Working Document Sanco/10329/2002 rev 2 final, 17 October 2002). Two soil metabolites require further consideration according to the fate section: DFA and 6-CNA.

Acute risk to earthworms

The table below presents a summary of the acute toxicity studies on earthworms.

Table 2.6.4.1-01 Acute effects of BYI 02960 and relevant metabolites on earthworms

Test species	Test design	Ecotoxicological endpoint			Reference
BYI 02960					
Eisenia fetida	acute, 14 d (10% peat in test soil)	LC_{50}	185.6	mg a.i./kg dws	Leicher (2010) KIIA 8.9.1/01
BYI 02960 SL 200 G					
Eisenia fetida	acute, 14 d (5% peat in test soil)	LC_{50}	121	mg a.i./kg dws	Leicher (2010) KIIIA 10.6.2/01
DFA					
Eisenia fetida	acute, 14 d (10% peat in test soil)	LC_{50}	> 958	mg p.m./kg dws	Leicher (2007) KIIA 8.9.1/02
6-CAN					
Eisenia fetida	acute, 14 d (10% peat in test soil)	LC_{50}	> 1000	mg p.m./kg dws	Wetton (1999) KIIA 8.9.1/03

dws = dry weight soil
a.i. = active ingredient; p.m. = pure metabolite

For lipophilic substances (log POW > 2) the Terrestrial Guidance Document recommends to apply an additional assessment factor of 2 for the ecotoxicological endpoints (LC50, NOEC), if the study was conducted in artificial soil with a high content of organic matter (i.e. 10 % peat), to consider the possible sorption of these compounds to the organic matter. However, BYI 02960 and its metabolites have a log POW < 2 and no additional assessment factor has to be applied.

The toxicity values are compared with the PECsoil values from section B.8.3 (maximum concentration after multiple year use: $PEC_{initial}$ after one application + $PEC_{accumulation}$). The $PEC_{initial}$ is expressed over 5 cm. For hops, the $PEC_{accumulation}$ is expressed over 5 cm (perennial crop without tillage). For lettuce, the $PEC_{accumulation}$ is expressed over 20 cm (annual crop with tillage). The acute TER values are presented in Table 2.6.4.1-02.

Table 2.6.4.1-02 TER_{acute} calculations for earthworms

Compound test design	End point	[mg/kg soil]	$PEC_{max,accu}$ [mg/kg soil]	TER_{acute}	Trigger	Refined risk assessment?
Hops						
BYI 02960	LC_{50}	185.6	0.160	2.3E+3	10	
BYI 02960 SL 200	LC_{50}	121	0.160	7.6E+2	10	No
DFA	LC_{50}	>958	0.009	> 11E+4	10	
6-CNA	LC_{50}	>1000	0.007	> 14E+4	10	
Lettuce biennial						
BYI 02960	LC50	185.6	0.141	1.3E+3	10	
BYI 02960 SL 200	LC50	121	0.141	8.6E+2	10	No
DFA	LC50	>958	0.014	> 68E+3	10	
6-CNA	LC50	>1000	0.012	≥ 83E+3	10	
Lettuce annual						
BYI 02960	LC50	185.6	0.063	2.9E+3	10	
BYI 02960 SL 200	LC50	121	0.063	1.9E+3	10	No
DFA	LC50	>958	0.006	> 16E+4	10	
6-CNA	LC50	>1000	0.005	> 20E+4	10	

The TER values in Table 2.6.4.1-02 are far above the Annex VI 91/414 EEC trigger of 10. Hence the acute risk to earthworms is considered to be acceptable.

Chronic risk to earthworms

The table below presents a summary of the chronic toxicity studies on earthworms.

Table 2.6.4.1-03 Chronic effects of BYI 02960 and relevant metabolites on earthworms

Test species	Test design	Ecotoxicological endpoint	Reference
BYI 02960 SL 200 G			
Eisenia fetida	reproduction, 56 d (10% peat in test soil)	NOEC 1.5 mg a.i./kg dws	Leicher (2010) KIIIA1 10.6.3/01
DFA			
Eisenia fetida	reproduction, 56 d (10% peat in test soil) mixing	NOEC 59 mg p.m./kg dws	Leicher (2010) KIIA 8.9.2/02
6-CNA			
Eisenia fetida	reproduction, 56 d (10% peat in test soil) mixing	NOEC 95 mg p.m./kg dws	Leicher (2011) KIIA 8.9.2/03

dws = dry weight soil
a.i. = active ingredient; p.m. = pure metabolite

The toxicity values are compared with the PECsoil values from section B.8.3 (maximum concentration after multiple year use: $PEC_{initial}$ after one application + $PEC_{accumulation}$). The $PEC_{initial}$ is expressed over

5 cm. For hops, the $PEC_{accumulation}$ is expressed over 5 cm (perennial crop without tillage). For lettuce, the $PEC_{accumulation}$ is expressed over 20 cm (annual crop with tillage). The long-term TER values are presented in Table 2.6.4.1-04.

Table 2.6.4.1-04 $TER_{long-term}$ calculations for earthworms

Compound test design	End point	[mg/kg soil]	$PEC_{max,accu}$ [mg/kg soil]	TER_{lt}	Trigger	Refined risk assessment?
Hops						
BYI 02960 SL 200	NOEC	1.5	0.160	9.4	5	No
DFA	NOEC	59	0.009	6.6E+3	5	
6-CNA	NOEC	95	0.007	14E+3	5	
Lettuce biennial						
BYI 02960 SL 200	NOEC	1.5	0.141	11	5	No
DFA	NOEC	59	0.014	4.2E+3	5	
6-CNA	NOEC	95	0.012	7.9E+3	5	
Lettuce annual						
BYI 02960 SL 200	NOEC	1.5	0.063	24	5	No
DFA	NOEC	59	0.006	9.8E+3	5	
6-CNA	NOEC	95	0.005	19E+3	5	

The TER values in Table 2.6.4.1-04 are above the Annex VI 91/414 EEC trigger of 5. Hence the chronic risk to earthworms is considered to be acceptable.

Field studies with earthworms

A field study with earthworms was performed nevertheless. In this one year earthworm field study, BYI 02960 SL 200 G was sprayed on grassland at 300, 600 and 1500 g a.i./ha corresponding to measured concentrations of 271, 472 and 1234 µg/kg soil (mean of four plots). Reduction of abundance (-33%) and biomass (-38%) at 1500 g a.i./ha and biomass at 600 g a.i./ha (-36%) after 1 month were seen, but there was full recovery of earthworm population after 11 months. The measured concentrations in soil at the start of the study (up to 1.234 mg/kg soil measured over 10 cm, see remarks to the field study above) cover the expected concentrations at the proposed uses (up to 0.160 mg/kg soil). ~~The study confirms the expected acceptable risk to earthworms.~~ However, the study was considered invalid by the experts of the pesticide peer review meeting of December 2014. Therefore, this study cannot be used for the risk assessment. This has no influence on the outcome of the risk assessment, since this was already acceptable based on laboratory studies.

2.6.4.2 Effects on soil non-target macro-organisms (IIIA 10.6.6)

Summary and risk assessment

The Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (Sanco 10329/2002, rev 2 final) states that a 'higher degree of scrutiny' for the soil organism risk assessment is required if the field DT90 values in soil are >100 days. The field DT90 values for BYI 02960 and the laboratory DT90 (no field studies available) for metabolites DFA and 6-CNA are >100 days. Therefore, studies with *Folsomia candida* and *Hypoaspis aculeifer* were performed with the formulated product and the two metabolites. See Table 2.6.4.1.

Table 2.6.4.1 Effects on other soil non-target macro-organisms

Test species	Test design	Ecotoxicological endpoint			Reference
BYI 02960 SL 200 G					
Folsomia candida	chronic, 28 d (5% peat in test soil)	NOEC	1.44	mg a.s./kg dws	Frommholz (2009) KIIA 8.14/01, KIIIA1 10.6.6/01
Hypoaspis aculeifer	chronic, 14 d (5% peat in test soil)	NOEC	≥ 170	mg a.s./kg dws	Kratz (2010) KIIA 8.14/04, KIIIA1 10.6.6/02
DFA					
Folsomia candida	chronic, 28 d (5% peat in test soil)	NOEC	≥ 95.8	mg p.m./kg dws	Frommholz (2010) KIIA 8.14/02
Hypoaspis aculeifer	chronic, 14 d (5% peat in test soil)	NOEC	≥ 958	mg p.m./kg dws	Kratz (2010) KIIA 8.14/05
6-CNA					
Folsomia candida	chronic, 28 d (5% peat in test soil)	NOEC	90	mg p.m./kg dws	Frommholz (2010) KIIA 8.14/03
Hypoaspis aculeifer	chronic, 14 d (5% peat in test soil)	NOEC	≥ 100	mg p.m./kg dws	Kratz, A. (2011) KIIA 8.14/06

dws = dry weight soil
p.m. = pure metabolite

The toxicity values are compared with the PECsoil values from section B.8.3 (maximum concentration after multiple year use: $PEC_{initial}$ after one application + $PEC_{accumulation}$). The $PEC_{initial}$ is expressed over 5 cm. For hops, the $PEC_{accumulation}$ is expressed over 5 cm (perennial crop without tillage). For lettuce, the $PEC_{accumulation}$ is expressed over 20 cm (annual crop with tillage). The long-term TER values are presented in Table 2.6.4.2-08 and -09.

Table 2.6.4.2--08 $TER_{long-term}$ calculations for *Folsomia candida*

Compound test design	End point	[mg/kg soil]	$PEC_{max,accu}$ [mg/kg soil]	TER_{lt}	Trigger	Refined risk assessment?
Hops						
BYI 02960 SL 200	NOEC	1.44	0.160	9.0	5	No
DFA	NOEC	≥ 95.8	0.009	11E+3	5	
6-CNA	NOEC	90	0.007	13E+3	5	
Lettuce biennial						
BYI 02960 SL 200	NOEC	1.44	0.141	10	5	No
DFA	NOEC	≥ 95.8	0.014	6.8E+3	5	
6-CNA	NOEC	90	0.012	7.5E+3	5	
Lettuce annual						
BYI 02960 SL 200	NOEC	1.44	0.063	23	5	No
DFA	NOEC	≥ 95.8	0.006	16E+3	5	
6-CNA	NOEC	90	0.005	18E+3	5	

Table 2.6.4.2--09 $TER_{long-term}$ calculations for *Hypoaspis aculeifer*

Compound test design	End point	[mg/kg soil]	$PEC_{max,accu}$ [mg/kg soil]	TER_{lt}	Trigger	Refined risk assessment?
Hops						
BYI 02960 SL 200	NOEC	≥ 170	0.160	≥ 1.1E+3	5	No
DFA	NOEC	≥ 958	0.009	≥ 11E+4	5	
6-CNA	NOEC	≥ 100	0.007	≥ 14E+3	5	
Lettuce biennial						
BYI 02960 SL 200	NOEC	≥ 170	0.141	≥ 1.2E+3	5	No
DFA	NOEC	≥ 958	0.014	≥ 68E+3	5	
6-CNA	NOEC	≥ 100	0.012	≥ 8.3E+3	5	
Lettuce annual						
BYI 02960 SL 200	NOEC	≥ 170	0.063	≥ 2.7E+3	5	No
DFA	NOEC	≥ 958	0.006	≥ 16E+4	5	
6-CNA	NOEC	≥ 100	0.005	≥ 20E+3	5	

The TER values in Table 2.6.4.2-08 and -09 are above the Annex VI 91/414 EEC trigger of 5. Hence the chronic risk to soil macro-organisms other than earthworms is considered to be acceptable.

Effects on organic matter breakdown

Two litterbag studies, one similating spray treatment and one seed treatment, both performed in Germany, show no adverse effects on organic matter breakdown after exposure to ca. 0.24 mg BYI 02960/kg soil dw. These tested concentrations are higher than the calculated maximum concentrations of at most 0.160 mg/kg a.s. (measured over 10 cm) for the proposed uses in hops and lettuce ($PEC_{initial}$ expressed over 5 cm + $PEC_{accumulation}$ expressed over 5 (hops) or 20 (lettuce) cm). Therefore the risk to organic matter breakdown is considered to be low.

2.6.5 Effects on soil micro-organisms

Laboratory studies on microbial turnover are available for the active substance BYI 02960, the formulation BYI 02960 SL 200 G, and the metabolite 6-CNA (N-turnover only).
The results indicate that BYI 02960 and BYI 02960 SL 200 G have no adverse impact on microbial C- and N-turnover in soil. NOECs were clearly above the expected exposure in soil.
Overall, it can be concluded that the functioning of soil micro-organisms is not at risk if BYI 02960 SL 200 G is applied according to the recommended use pattern.

The toxicity of BYI 02960 SL 200 G on soil non-target micro-organisms is summarised in the table below.

Table 2.6.5-01 Effects on soil non-target micro-organisms

Test species	Test design	Ecotoxicological endpoint		Reference
BYI 02960 SL 200 G				
C-cycle	28 d	no influence	16.59 µL prod/kg dws (≡3.335 mg a.s./kg dws)	Frommholz (2010) KIIIA1 10.7.1/01
N-cycle	28 d	no influence	16.59 µL prod/kg dws (≡3.335 mg a.s./kg dws)	Frommholz (2010) KIIIA1 10.7.1/02
BYI 02960				
N-cycle	28 d	no influence	3.8 mg a.i./kg dws	Frommholz (2009) KIIA 8.10.1/01
C-cycle	28 d	no influence	3.8 mg a.i./kg dws	Schulz (2011) KIIA 8.10.2/01
6-CNA				
N-cycle	28 d	no influence	1.33 mg p.m./kg dws	Frommholz (2011) KIIA 8.10.1/02

Risk assessment

According to current regulatory requirements the risk is acceptable, if the effect of the recommended application rate of a compound / product on nitrogen or carbon mineralisation is < 25% after 100 days. In the studies, deviations from the control did not exceed 25% after 28 days, at tested concentrations which are higher than the calculated maximum concentrations of at most 0.160 mg/kg a.s. and 0.012 mg 6-CNA for the proposed uses.

The metabolite 6-CNA was shown to be of general low toxicity for soil organisms (earthworm LC50 >1000 mg/kg, NOEC 95 mg/kg; Hypoaspis NOEC 100 mg/kg; Folsomia NOEC 90 mg/kg). The NOEC of the metabolite 6-CNA for microbial N-turnover was determined to be 111- fold higher than the worst case PEC in soil for 6-CNA. The high margin of safety indicates that microbial C-turnover is not at risk due to formation of 6-chloronicotinic acid in soil.
Similar to 6-chloronicotinic acid the metabolite DFA acid was shown to be of general low toxicity for soil organisms (earthworm LC50 >958 mg/kg, NOEC 59 mg/kg). Assuming a 10-fold higher toxicity of DFA in comparison with to 6-CNA with regard to microbial C- and N-turnover, the risk could still be considered as acceptable.
Furthermore, the metabolites are likely to have been formed in the litterbag studies, in which no risk was identified to organic matter breakdown.
Based on the above, no further studies are required. The risk to soil micro-organisms is considered to be low.

2.6.6 Effects on other non-target organisms (flora and fauna)

The risk assessment is based on the “Guidance Document on Terrestrial Ecotoxicology”, (SANCO/10329/2002 rev2 final, 2002). It is restricted to off-field situations, as non-target plants are non-crop plants located outside the treated area. Spray drift from the treated areas may lead to residues of a product in off-crop areas. Exposure is not relevant for glasshouse uses.

In the case of a non-herbicide, screening results and/or Tier 1 studies give first information about the likelihood for terrestrial plant effects. The risk can be considered acceptable if there are no data indicating more than 50% phytotoxic effect at the maximum application rate.

Seedling emergence (Gosch (2010), KIIIA1 10.8.1.3/01) and vegetative vigour studies Gosch (2010), KIIIA1 10.8.1.2/01) have been conducted with BYI 02960 SL 200 G following OECD testing guidelines 208 and 227, respectively (see Annex Points IIIA1 10.8.1.3 and 10.8.1.2). Each study included 11 species which were tested at the maximum application rate of 410 g a.i./ha.
In the case of BYI 02960 SL 200 G neither the tier 1 seedling emergence nor the vegetative vigour studies showed phytotoxic effects >50% at the maximum application rate of 410 g a.i./ha. Therefore, the ER50 values are set at >410 g a.s./ha.

For the proposed field uses in hops and lettuce, the following scenario is used to describe the exposure to non-target plants:

off-field exposure= Application rate * MAF * drift factor

For the MAF and drift factor, similar assumptions are made as for the off-field risk assessment for non-target arthropods.

As the proposed uses of BYI 02960 SL 200 formulation involves one application only, the multiple application factor (MAF) is 1. Drift values are 19.33% for hops (3 m distance) and 2.77% for lettuce (field crops, 1m distance).

The calculated exposure for the respective uses and the TER values are given in Table 2.6.6-01.

Table 2.6.6-01 Exposure values and TERs for non-target plants

Crop	Max. single dose [g a.s./ha]	number of applications	MAF	Drift [%]	Exposure [g a.s./ha]	ER_{50} [g a.s./ha]	TER	Trigger
hops	150	1	1	19.33	30	>410	>14	5
lettuce	125	1	1	2.77	3.5	>410	>118	5

1: drift in vegetables, > 50 cm.

For both uses, the TERs are above the trigger of 5. A low risk for non-target plants is expected.

2.6.7 Effects on biological methods of sewage treatment

Emission of flupyradifurone to sewage treatment plants may occur following the proposed use of Sivanto in glasshouses. The expected concentration in the STP is not calculated because EU harmonised guidance is lacking. However, based on the results of the activated sludge respiration test, which obtained an EC_{50} > 962 mg a.s./L, no effect on biological methods of sewage treatment are expected.

2.7 Efficacy

The data presented by the applicant supports two representative uses for Flupyradifurone in the EU: Control of *Nasonovia ribisnigri* in lettuce (field and greenhouse) and Phorodon humuli in hops.

Mode of action

Flupyradifurone interacts with insect nicotinic acetylcholine receptors, a class of neurotransmitter-gated cation channels which are involved in excitatory neurotransmission, a target also known for neonicotinoid insecticides.

After uptake into the plant, Flupyradifurone is translocated acropetally in the xylem, in direction of the transpiration stream and is translaminarily distributed into adjacent plant cells.

2.7.1 Dose justification and efficacy

For approval of a new active substance efficacy data is required on one or more representative uses on a widely grown crop in each zone of at least one plant protection product containing the active substance, showing adequate efficacy. The principal objective of the efficacy evaluation of an active substance is to confirm that the dose rates are realistic. The dossier contains trials for the Maritime and north-eastern zone for hop, and data for the Mediterranean and maritime zone for lettuce. The submitted gap is adequate, in that it concerns two very different crops that likely represent worst case situations for the evaluation of other aspects.

No trials were presented from the south-eastern zone. It is not expected that trials from this zone would have presented a worst case situation for efficacy or phytotoxicity when compared to the submitted trials.

Hop

Dose justification trials are available from the maritime zone (Germany and the Czech Republic) and the north-east zone (Poland). The claimed dose rate is 0.75 L/ha (0,150 g a.s/ha). Only trials with sufficient pest pressure were taken into consideration.

In hop high efficacy was found at all tested dose rates, some effect of the dose rate was seen as the lower dose rates than the requested dose rate in general showed equal to or lower efficacy than the reference products while the proposed dose rate showed higher than or equal efficacy.

For hop cultivation it is necessary to reach an efficacy against damson hop aphid close to 100% (to reach acceptable cones quality at harvest), the rate of 0.75 L/ha Flupyradifurone SL200 can be considered as the optimum dose rate, and provided good efficacy.

Lettuce

Nasonovia ribisnigri is a difficult aphid to control, as in lettuce, it is often found concealed between the leaves, it thus provides an adequate test for the product.

11 Minimum Effective Dose trials were presented by the applicant: 7 carried out in the Maritime zone, 4 in the Mediterranean zone. All of them were carried out under field conditions. The intended dose rate is125 g a.s/ha

In general, in the field, little effect of the dose rate was found at the first assessments in the maritime zone trials, but higher dose rates performed better at later assessment dates. In the mediteranean zones a dose response was found at dose rates from 75 to 150 g a.s./ha, the increase in efficacy between 125 and 150 g a.s./ha was only marginal. When compared to reference products based on thyacloprid, imidacloprid or pymetrozine, the dose rate of 125 g a.s. in general performed equal or better.

The reference product based on spirotetramat in general provided longer lasting control than Flupyradifurone 200 SL and the other reference product, but initial efficacy was often lower.

The proposed dose rate of 125 g a.s/ha provided good control (initial and lasting efficacy) and when compared to the other tested dose rates, should be considered as the optimum dose rate for control of *Nasonovia ribisnigri* in lettuce in the field.

All submitted trials for lettuce were performed under field conditions, as such the greenhouse use can not be evaluated based on the submitted data. The aim of the efficacy evaluation for approval of the active substance is to establish if the GAP is realistic. While no large differences in dose rate are expected between the indoor and outdoor use in lettuce, the dose rate could be different because of differences in pest pressure, or higher sensitivity to phytotoxicity because of different growing conditions.

For approval of a new active substance efficacy data is required on one or more representative uses on a widely grown crop in each zone of at least one plant protection product containing the active substance, showing adequate efficacy. Without the greenhouse use for lettuce, the submitted data for approval of the active substance can still be considered sufficient for efficacy.

2.7.2 Possible occurrence of the development of resistance

No data was submitted by the applicant. Flupyradifurone has a mode of action similar to the neonicotinoids. It does not yet have a IRAC classification, but will likely be classified in one of the subgroups of group 4.

Resistance risk would be expected to be similar to neonicotinoid insecticides.

Resistance risk and resistance management strategies will be evaluated in more detail during product registration.

2.7.3 Effects on quality of plants or plant products

No data was submitted by the applicant. No phytotoxic effects were found in the dose justification trials, an effect on quality of plants or plant products is therefore not expected.
Effects on quality of plants or plant products will be evaluated in more detail during product registration.

2.7.4 Effects on transformation processes

No data was submitted by the applicant.
Effects on transformation processes will be evaluated in more detail during product registration.

2.7.5 Effects on the yield of treated plants or plant products

No data was submitted by the applicant. No phytotoxic effects were found in the dose justification trials. Effects on yield will be evaluated in more detail during product registration.

2.7.6 Phytotoxicity to target plants or target plant products

Crop safety in lettuce and hops was considered in all effectiveness and dose justification trials. Eight varieties of lettuce and 7 varieties of hop were included in the trials.

Lettuce

In lettuce, all trials were performed under field conditions, whereas greenhouse conditions would present a more worst case situation for the assessment of phytotoxicity. The main goal of evaluation of efficacy for registration of an active substance is to check if the requested dose rates are realistic, this is important for determination of the risk envelope for the other aspects. Phytotoxic effects could place an upper limit on the dose rates if severe enough. This may have an effect on efficacy, but will result in less worse case situations for the other aspects.
For the purpose of creating a realistic gap for registration of the active substance the submitted phytoxicity data can be considered sufficient. For registration of the product under greenhouse conditions additional trials would be required.

Hops:

In 4 trials in hop in the Czech Republic, a 2N dose rate was included in the trials. In none of the efficacy trials, including those with the 2N dose rate, phytotoxic effects were seen.

2.7.7 Impact on succeeding crops, adjacent crops and on treated plants or plant products used for propagation

Based on the evaluation of data submitted by the applicant, the effects on adjacent crops and effects on treated plants and plant products used for propagation were found to be acceptable.

APPENDIX 1

FLUPYRADIFURONE

PART 1: Standard terms and abbreviations

PART 2: Organisations and publications

PART 3: Preparation (formulation) types and codes

Part 1	**Standard terms and abbreviations**
A	ampere
ACh	acetylcholine
AChE	acetylcholinesterase
ADI	acceptable daily intake
ADP	adenosine diphosphate
AE	acid equivalent
AFID	alkali flame-ionisation detector or detection
A/G	albumin/globulin ratio
ai	active ingredient
ALD_{50}	approximate median lethal dose, 50%
ALT	alanine aminotransferase (SGPT)
AOEL	acceptable operator exposure level
AMD	automatic multiple development
ANOVA	analysis of variance
AP	alkaline phosphatase
approx	approximate
ARC	anticipated residue contribution
ARfD	acute reference dose
as	active substance
AST	aspartate aminotransferase (SGOT)
ASV	air saturation value
ATP	adenosine triphosphate
BCF	bioconcentration factor
bfa	body fluid assay
BOD	biological oxygen demand
bp	boiling point
BSAF	biota-sediment accumulation factor
BSE	bovine spongiform encephalopathie
BSP	bromosulfophthalein
Bt	bacillus thuringiensis
Bti	bacillus thuringiensis israelensis
Btk	bacillus thuringiensis kurstaki
Btt	bacillus thuringiensis tenebrionis
BUN	blood urea nitrogen
bw	body weight
c	centi- (x 10^{-2})
°C	degree Celsius (centigrade)
CA	controlled atmosphere
CAD	computer aided design
CADDY	computer aided dossier and data supply (an electronic dossier interchange and archiving format)
cd	candela
CDA	controlled drop(let) application
cDNA	complementary DNA
CEC	cation exchange capacity
cf	confer, compare to
CFU	colony forming units

ChE	cholinesterase
CI	confidence interval
CL	confidence limits
cm	centimetre
CNS	central nervous system
COD	chemical oxygen demand
CPK	creatinine phosphatase
cv	coefficient of variation
Cv	ceiling value
CXL	Codex Maximum Residue Limit (Codex MRL)
d	day
DAA	days after application
DALA	days after last application
DAT	days after treatment
DES	diethylstilboestrol
DFR	dislodgeable foliar residue
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic Acid
dna	designated national authority
DO	dissolved oxygen
DOC	dissolved organic carbon
dpi	days post inoculation
DRES	dietary risk evaluation system
DT_{50}	period required for 50 percent dissipation (define method of estimation)
DT_{90}	period required for 90 percent dissipation (define method of estimation)
dw	dry weight
DWQG	drinking water quality guidelines
ε	decadic molar extinction coefficient
EC_{50}	median effective concentration
ECD	electron capture detector
ECU	European currency unit
ED_{50}	median effective dose
EDI	estimated daily intake
ELISA	enzyme linked immunosorbent assay
e-mail	electronic mail
EMDI	estimated maximum daily intake
EPMA	electron probe micro analysis
ERC	environmentally relevant concentration
ERL	extraneous residue limit
F	field
F_0	parental generation
F_1	filial generation, first
F_2	filial generation, second
FIA	fluorescence immunoassay
FID	flame ionisation detector
FOB	functional observation battery
fp	freezing point
FPD	flame photometric detector
FPLC	fast protein liquid chromatography

g	gram
G	glasshouse
GAP	good agricultural practice
GC	gas chromatography
GC-EC	gas chromatography with electron capture detector
GC-FID	gas chromatography with flame ionisation detector
GC-MS	gas chromatography-mass spectrometry
GC-MSD	gas chromatography with mass-selective detection
GEP	good experimental practice
GFP	good field practice
GGT	gamma glutamyl transferase
GI	gastro-intestinal
GIT	gastro-intestinal tract
GL	guideline level
GLC	gas liquid chromatography
GLP	good laboratory practice
GM	geometric mean
GMO	genetically modified organism
GMM	genetically modified micro-organism
GPC	gel-permeation chromatography
GPPP	good plant protection practice
GPS	global positioning system
GSH	glutathion
GV	granulosevirus
h	hour(s)
H	Henry's Law constant (calculated as a unitless value) (see also K)
ha	hectare
Hb	haemoglobin
HCG	human chorionic gonadotropin
Hct	haematocrit
HDT	highest dose tested
hL	hectolitre
HEED	high-energy electron diffraction
HID	helium ionisation detector
HPAEC	high performance anion exchange chromatography
HPLC	high pressure liquid chromatography or high performance liquid chromatography
HPLC-MS	high pressure liquid chromatography - mass spectrometry
HPPLC	high pressure planar liquid chromatography
HPTLC	high performance thin layer chromatography
HR	Highest Residue
HRGC	high resolution gas chromatography
H_S	Shannon-Weaver index
Ht	haematocrit
I	indoor
I_{50}	inhibitory dose, 50%
IC_{50}	median immobilisation concentration or median inhibitory concentration
ICM	integrated crop management
ID	ionisation detector

IEDI	international estimated daily intake
IESTI	international estimated short term intake
IGR	insect growth regulator
im	intramuscular
inh	inhalation
ip	intraperitoneal
IPM	integrated pest management
IR	infrared
ISBN	international standard book number
ISSN	international standard serial number
iv	intravenous
IVF	in vitro fertilisation
k	kilo
K	Kelvin or Henry's Law constant (in atmospheres per cubic meter per mole, see also H)
K_{ads}	adsorption constant
K_{des}	apparent desorption coefficient
K_{oc}	organic carbon adsorption coefficient
K_{om}	organic matter adsorption coefficient
kg	kilogram
L	litre
LAN	local area network
LASER	light amplification by stimulated emission of radiation
LBC	loosely bound capacity
LC	liquid chromatography
LC-MS	liquid chromatography- mass spectrometry
LC_{50}	lethal concentration, median
LCA	life cycle analysis
LC_{Lo}	lethal concentration low
LC-MS-MS	liquid chromatography with tandem mass spectrometry
LD_{50}	lethal dose, median; dosis letalis media
LD_{Lo}	lethal dose low
LDH	lactate dehydrogenase
LOAEC	lowest observable adverse effect concentration
LOAEL	lowest observable adverse effect level
LOD	limit of detection
LOEC	lowest observable effect concentration
LOEL	lowest observable effect level
LOQ	limit of quantification (determination)
LPLC	low pressure liquid chromatography
LSC	liquid scintillation counting or counter
LSD	least squared denominator multiple range test
LSS	liquid scintillation spectrometry
LT	lethal threshold
m	metre
M	molar
µm	micrometer (micron)
MC	moisture content
MCH	mean corpuscular haemoglobin

MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
MDL	method detection limit
MFO	mixed function oxidase
µg	microgram
mg	milligram
MHC	moisture holding capacity
min	minute(s)
mL	millilitre
MLT	median lethal time
MLD	minimum lethal dose
mm	millimetre
mo	month(s)
mol	Mole(s)
MOS	margin of safety
mp	melting point
MRE	maximum residue expected
MRL	maximum residue level or limit
mRNA	messenger ribonucleic acid
MS	mass spectrometry
MSDS	material safety data sheet
MTD	maximum tolerated dose
n	normal (defining isomeric configuration) or number of observations
NAEL	no adverse effect level
nd	not detected
NEDI	national estimated daily intake
NESTI	national estimated short term intake
NEL	no effect level
NERL	no effect residue level
ng	nanogram
nm	nanometer
NMR	nuclear magnetic resonance
no	number
NOAEC	no observed adverse effect concentration
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NOED	no observed effect dose
NOEL	no observed effect level
NOIS	notice of intent to suspend
NPD	nitrogen-phosphorus detector or detection
NPV	nuclear polyhedrosis virus
NR	not reported
NTE	neurotoxic target esterase
OC	organic carbon content
OCR	optical character recognition
ODP	ozone-depleting potential
ODS	ozone-depleting substances
OM	organic matter content
op	organophosphorous pesticide

Pa	Pascal
PAD	pulsed amperometric detection
2-PAM	2-pralidoxime
PBI	plant back interval
pc	paper chromatography
PC	personal computer
PCV	haematocrit (packed corpuscular volume)
PEC	predicted environmental concentration
PEC_A	predicted environmental concentration in air
PEC_S	predicted environmental concentration in soil
PEC_{SW}	predicted environmental concentration in surface water
PEC_{GW}	predicted environmental concentration in ground water
PED	plasma-emissions-detector
pH	pH-value
PHED	pesticide handler's exposure data
PHI	pre-harvest interval
PIC	prior informed consent
pic	phage inhibitory capacity
PIXE	proton induced X-ray emission
pKa	negative logarithm (to the base 10) of the dissociation constant)
PNEC	predicted no effect concentration
po	by mouth
P_{OW}	partition coefficient between n-octanol and water
POP	persistent organic pollutants
ppb	parts per billion (10^{-9})
PPE	personal protective equipment
ppm	parts per million (10^{-6})
ppp	plant protection product
ppq	parts per quadrillion (10^{-24})
ppt	parts per trillion (10^{-12})
PSP	phenolsulfophthalein
PrT	prothrombin time
PRL	practical residue limit
PT	prothrombin time
PTDI	provisional tolerable daily intake
PTT	partial thromboplastin time
QSAR	quantitative structure-activity relationship
r	correlation coefficient
r^2	coefficient of determination
RBC	red blood cell
REI	restricted entry interval
Rf	retardation factor
RfD	reference dose
RH	relative humidity
RL_{50}	median residual lifetime
RNA	ribonucleic acid
RP	reversed phase
rpm	rotations per minute
rRNA	ribosomal ribonucleic acid
RRT	relative retention time

RSD	relative standard deviation
s	second
SAC	strong adsorption capacity
SAP	serum alkaline phosphatase
SAR	structure/activity relationship
SBLC	shallow bed liquid chromatography
sc	subcutaneous
sce	sister chromatid exchange
SD	standard deviation
se	standard error
SEM	standard error of the mean
SEP	standard evaluation procedure
SF	safety factor
SFC	supercritical fluid chromatography
SFE	supercritical fluid extraction
SIMS	secondary ion mass spectroscopy
SOP	standard operating procedures
sp	species (only after a generic name)
SPE	solid phase extraction
SPF	specific pathogen free
spp	subspecies
sq	square
SSD	sulphur specific detector
SSMS	spark source mass spectrometry
STEL	short-term exposure limit
STMR	supervised trials median residue
t	tonne (metric ton)
$t_{½}$	half-life (define method of estimation)
T_3	tri-iodothyroxine
T_4	thyroxin
TADI	temporary acceptable daily intake
TBC	tightly bound capacity
TCD	thermal conductivity detector
TC_{Lo}	toxic concentration, low
TID	thermionic detector, alkali flame detector
TD_{Lo}	toxic dose low
TDR	time domain reflectrometry
TER	toxicity exposure ration
TER_I	toxicity exposure ration for initial exposure
TER_{ST}	toxicity exposure ration following repeated exposure
TER_{LT}	toxicity exposure ration following chronic exposure
tert	tertiary (in a chemical name)
TEP	typical end-use product
TGGE	temperature gradient gel electrophoresis
TIFF	tag image file format
TLC	thin layer chromatography
Tlm	median tolerance limit
TLV	threshold limit value
TMDI	theoretical maximum daily intake
TMRC	theoretical maximum residue contribution

TMRL	temporary maximum residue limit
TOC	total organic carbon
Tremcard	Transport emergency card
tRNA	transfer ribonucleic acid
TSH	thyroid stimulating hormone (thyrotropin)
TWA	time weighted average
UDS	unscheduled DNA synthesis
UF	uncertainty factor (safety factor)
ULV	ultra low volume
UV	ultraviolet
v/v	volume ratio (volume per volume)
WBC	white blood cell
wk	week
wt	weight
w/v	weight per volume
ww	wet weight
w/w	weight per weight
XRFA	X-ray fluorescence analysis
yr	year
<	less than
=	less than or equal to
>	greater than
=	greater than or equal to

Part 2 Organisations and Publications

ACPA	American Crop Protection Association
ASTM	American Society for Testing and Materials
BA	Biological Abstracts (Philadelphia)
BART	Beneficial Arthropod Registration Testing Group
CA	Chemical Abstracts
CAB	Centre for Agriculture and Biosciences International
CAC	Codex Alimentarius Commission
CAS	Chemical Abstracts Service
CCFAC	Codex Committee on Food Additives and Contaminants
CCGP	Codex Committee on General Principles
CCPR	Codex Committee on Pesticide Residues
CCRVDF	Codex Committee on Residues of Veterinary Drugs in Food
CE	Council of Europe
CIPAC	Collaborative International Pesticides Analytical Council Ltd
COREPER	Comite des Representants Permanents
EC	European Commission
ECB	European Chemical Bureau
ECCA	European Crop Care Association
ECDIN	Environmental Chemicals Data and Information Network of the European Communities
ECDIS	European Environmental Chemicals Data and Information System
ECE	Economic Commission for Europe
ECETOC	European Chemical Industry Ecology and Toxicology Centre
ECLO	Emergency Centre for Locust Operations
ECMWF	European Centre for Medium Range Weather Forecasting
ECPA	European Crop Protection Association
EDEXIM	European Database on Export and Import of Dangerous Chemicals
EHC (number)	Environmental Health Criteria (number)
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EMIC	Environmental Mutagens Information Centre
EPA	Environmental Protection Agency
EPO	European Patent Office
EPPO	European and Mediterranean Plant Protection Organization
ESCORT	European Standard Characteristics of Beneficials Regulatory Testing
EU	European Union
EUPHIDS	European Pesticide Hazard Information and Decision Support System
EUROPOEM	European Predictive Operator Exposure Model
FAO	Food and Agriculture Organization of the UN
FOCUS	Forum for the Co-ordination of Pesticide Fate Models and their Use
FRAC	Fungicide Resistance Action Committee
GATT	General Agreement on Tariffs and Trade
GAW	Global Atmosphere Watch
GIFAP	Groupement International des Associations Nationales de Fabricants de Produits Agrochimiques (now known as GCPF)

GCOS	Global Climate Observing System
GCPF	Global Crop Protection Federation (formerly known as GIFAP)
GEDD	Global Environmental Data Directory
GEMS	Global Environmental Monitoring System
GIEWS	Global Information and Early Warning System for Food and Agriculture
GRIN	Germplasm Resources Information Network
HRAC	Herbicide Resistance Action Committee
IARC	International Agency for Research on Cancer
IATS	International Academy of Toxicological Science
IBT	Industrial Bio-Test Laboratories
ICBB	International Commission of Bee Botany
ICBP	International Council for Bird Preservation
ICES	International Council for the Exploration of the Seas
ICPBR	International Commission for Plant-Bee Relationships
ILO	International Labour Organization
IMO	International Maritime Organisation
IOBC	International Organisation for Biological Control of Noxious Animals and Plants
IPCS	International Programme on Chemical Safety
IRAC	Insecticide Resistance Action Committee
IRC	International Rice Commission
ISCO	International Soil Conservation Organization
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
JECFA	FAO/WHO Joint Expert Committee on Food Additives
JFCMP	Joint FAO/WHO Food and Animal Feed Contamination Monitoring Programme
JMP	Joint Meeting on Pesticides (WHO/FAO)
JMPR	Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues (Joint Meeting on Pesticide Residues)
NATO	North Atlantic Treaty Organisation
NAFTA	North American Free Trade Agreement
NCI	National Cancer Institute (USA)
NCTR	National Centre for Toxicological Research (USA)
NGO	non-governmental organisation
NTP	National Toxicology Programme (USA)
OECD	Organisation for Economic Co-operation and Development
OLIS	On-line Information Service of OECD
PAN	Pesticide Action Network
RNN	Re-registration Notification Network
RTECS	Registry of Toxic Effects of Chemical Substances (USA)
SCPH	Standing Committee on Plant Health
SETAC	Society of Environmental Toxicology and Chemistry
SI	Systeme International d'Unites
SITC	Standard International Trade Classification

TOXLINE	Toxicology Information On-line
UN	United Nations
UNEP	United Nations Environment Programme
WCDP	World Climate Data Programme
WCP	World Climate Programme
WCRP	World Climate Research Programme
WFP	World Food Programme
WHO	World Health Organization
WTO	World Trade Organization
WWF	World Wildlife Fund

Part 3 Preparation (formulation) types and codes

Code	*Description*	*Definition*
AB	Grain bait	Special forms of bait.
AE	Aerosol dispenser	A container-held preparation which is dispersed generally by a propellant as fine droplets/particles upon actuation of a valve.
AL	Other liquids to be applied undiluted	Self defining.
BB	Block blits	Special forms of bait.
BR	Briquette	Solid block designed for controlled release of active ingredient into water.
CB	Bait concentrate	A solid or liquid intended for dilution before use as a bait.
CG	Encapsulated granule	A granule with a protective or release controlling coating.
CS	Capsule suspension	A stable suspension of capsules in a fluid normally intended for dilution with water before use.
DC	Dispersible concentrate	A liquid homogeneous preparation to be applied as a solid dispersion after dilution in water.
DP	Dustable powder	A free-flowing powder suitable for dusting.
DS	Powder for dry seed treatment	A powder for application in the dry state directly to seed.
EC	Emulsifiable concentrate	A liquid, homogenous preparation to be applied as an emulsion after dilution in water.
ED	Electrochargeable liquid	Special liquid preparation for electrostatic (electrodynamic)spraying
EO	Emulsion. water in oil	A fluid. heterogeneous preparation consisting of a dispersion of fine globules of pesticide in water in a continuous organic liquid phase.
ES	Emulsion for seed treatment	A stable emulsion for application to the seed either directly or after dilution.

Code	***Description***	***Definition***
EW	Emulsion. Oil in water	A fluid, heterogeneous preparation consisting of a dispersion of fine globules of pesticide in an organic liquid in a continuous water phase.
FD	Smoke tin	Special form of smoke generator.
FG	Fine granule	A granule in the particle size range from 300 to 2500 :.
FK	Smoke candle	A smoke generator in the form of a candle.
FP	Smoke cartridge	Special form of smoke generator.
FR	Smoke rodlet	Special form of smoke generator.
FS	Flowable concentrate for seed treatment	A stable suspension for application to the seed either directly or seed treatment after dilution.
FT	Smoke tablet	Special form of smoke generator.
FU	Smoke generator	A combustible preparation generally solid, which upon ignition releases the active substances in the form of a smoke.
FW	Smoke pellet	Special form of smoke generator.
GA	Gas	A gas packed in pressure bottle or pressure tank.
GB	Granular bait	Special forms of bait.
GE	Gas generating product	A preparation which generates a gas by chemical reaction.
GG	Macrogranule	A granule in the particle size range from 2000 to 6000 :.
GP	Flo-dust	Very fine dustable powder for pneumatic application in glasshouses.
GR	Hot fogging concentrate	A free-flowing solid preparation of a defined granule size range ready for use.
GS	Cold fogging concentrate	Very viscous preparation based on oil or fat.
HN	Hot fogging concentrate	A preparation suitable for application by fogging equipment either directly or after dilution.
KN	Cold fogging concentrate	A preparation suitable for application by cold fogging equipment, either directly or after dilution.

Code	***Description***	***Definition***
LA	Lacquer	A solvent based film-forming preparation.
LS	Solution for seed treatment	A solution for application to the seed either directly or after dilution.
MG	Microgranule	A granule in the particle ske range from 100 to 600
OF	Oil miscible flowable (=oil active substances in a miscible suspension)	A stable suspension of concentrate fluid intended for dilution in an organic liquid before use.
OL	Oil miscible liquid	A liquid, homogenous preparation to be applied as a homogenous liquid after dilution in an organic liquid.
OP	Oil dispersible powder	A powder preparation to be applied as a suspension after dispersion in an organic liquid.
PA	Paste	A water based film forming preparation.
PB	Plate bait	Special forms of bait.
PC	Gel or paste concentrate	A solid preparation to be applied as a gel or a paste after dilution with water.
PR	Plant rodlet	A small rodlet, usually a few centimetres in length and a few millimetres in diameter containing active substance.
PS	Seed coated with a pesticide	Self defining.
RB	Bait (ready for use)	A preparation designed to attract and be eaten by the target species.
SB	Scrap bait	Special forms of bait.
SC	Suspension (= flowable concentrate)	A stable suspension of active substance(s) in a fluid intended for dilution with water before use
SE	Suspo-emulsion	A fluid. heterogeneous preparation consisting of a stable dispersion of active substance(s) in the form of solid particles.
SG	Water soluble granules	A preparation consisting of granules to be applied as a true solution of active substance after dissolution in water but many contain insoluble inert ingredients.

Code	***Description***	***Definition***
SL	Soluble concentrate	A liquid homogenous preparation to be applied as a true solution of active substance after dissolution in water but many contain insoluble inert ingredients.
SO	Spreading oil	A preparation designed to form a surface layer on application to water.
SP	Water soluble powder	A powder preparation to be applied as a true solution of the active substance after solution in water but which may contain insoluble inert ingredients.
SS	Water soluble powder for seed treatment	A powder to be dissolved in water before application to the seed.
SU	Ultra low volume (ULV) suspension	A suspension ready for use through ULV equipment.
TB	Tablet	Solid preparation in the form of small flat plates for dissolution in water.
TP	tracking powder	A rodenticidal contact preparation in powder form.
UL	Ultra low volume (ULV) liquid	A homogenous liquid ready for use through ULV equipment.
VP	Vapour releasing product	A preparation containing one or more volatile ingredients, the vapours of which are released into the air. Evaporation rate normally is controlled by using suitable preparations and/or dispensers.
WG	Water dispersible	A preparation granule consisting of granules to be applied after disintegration and dispersion in water.
WP	Wettable powder	A powder preparation to be applied as a suspension after dispersion in water.
WS	Water dispersible powder for slurry seed treatment	A powder to be dispersed at high concentration in water before application as a slurry to the seed.
XX	Others	

APPENDIX 2

FLUPYRADIFURONE

SPECIFIC TERMS AND ABBREVIATIONS

a	absolute organ weight
AAP	Algal Assay Procedure medium
aerob	aerobic test conditions
a-GT	alpha-glutamyl-transferase
ALAT	alanine aminotransferase
ALP	alkaline phosphatase
amu	atomic mass units
anaer	anaerobic test conditions
AR	applied radioactivity
ASAT	aspartate aminotransferase
ASTM	American Society for Testing and Materials
B	bacteria
biodeg	biodegradation
Chr. ab.	chromosome aberrations
CMC	carboxymethylcellulose
CoE	Council of Europe
crit.	criterion
d	decreased, but not statistically significantly
dc	statistically significantly decreased
DFI	Daily Food Intake
DMF	dimethylformamide
DO	Dissolved Oxygen
dr	dose-related
DWI	Daily Water Intake
E	total effect of mortality and fecundity/parasitic capacity, used in arthropod toxicity tests
E. coli	*Escherichia coli*
equal	used when the values given by the notifier are expressed in mg/kg bw/day.
equivalent	used when values given by the notifier are only expressed in mg/kg food, not in mg/kg bw/day, as species-dependent factor is used to translate these data to mg/kg bw/day.
ETE	Estimated Theoretical Exposure
GCP	good clinical practice
GIDH	glutamic-acid dehydrogenase

GOT	glutamic-oxalacetic transaminase
GPT	glutamic-pyruvic transaminase
HDL	high density lipoproteins
HPRT	hypoxanthine- guanine phosphoribosyl transferase
i	increased, but not statistically significantly
ic	statistically significantly increased
MC	moisture content in soil (v/v)
Mc	mammalian cells
MWHC	maximum water holding capacity (soils)
n/a	not applicable
n.d.	not detected
n.r.	not reported
ns	not significant
o.m.	organic matter
PEC	Predicted Environmental Concentration
PEG	polyethylene glycol
pF	moisture tension (soil) in [log $cm_{water\ column}$]
PIEC	Predicted Initial Environmental Concentration
pointmut.	pointmutations
r	relative organ weight
r.a.	radioactivity
res.	result
Ri	Reliability Index, referring to the intrinsic reliability of a test with respect to the quality of the study
S. typh.	*Salmonella typhimurium*
SPE	Solid Phase Extraction
Sub.	Substance
T	temperature
TWA	time weighted average

TWAEC	time weighted average environmental concentration
wat/sed	water/sediment systeem
w/w	weight per weight
-	negative
+	positive
-act.	without activation
+act.	with activation
%v/v	the percentage expressed by volume
%w/w	the percentage expressed by weight

APPENDIX 3

Flupyradifurone

LIST OF END-POINTS

Chapter 2.1 Identity, Physical and Chemical Properties, Details of Uses, Further Information

Active substance (ISO Common Name)	Flupyradifurone
Function (*e.g.* fungicide)	Insecticide

Rapporteur Member State	The Netherlands

Identity (Annex IIA, point 1)

Chemical name (IUPAC)	4-[(6-chloro-3-pyridylmethyl)(2,2-difluoroethyl)amino]furan-2(5H)-one
Chemical name (CA)	2(5H)-furanone, 4-[[(6-chloro-3-pyridinyl)methyl](2,2-difluoroethyl)amino]-
CIPAC No	not allocated
CAS No	951659-40-8
EEC No (EINECS or ELINCS)	not assigned
FAO Specification (including year of publication)	not applicable
Minimum purity of the active substance as manufactured (g/kg)	Min. 960 g/kg Flupyradifurone
Identity of relevant impurities (of toxicological, environmental and/or other significance) in the active substance as manufactured (g/kg)	Based on environmental and (eco)toxicological information, there are no relevant impurities.
Molecular formula	$C_{12}H_{11}ClF_2N_2O_2$
Molecular mass	288.68 g/mol
Structural formula	

Physical-chemical properties (Annex IIA, point 2)

Melting point (state purity)	69.0 °C (99.4%)
Boiling point (state purity)	No boiling point; decomposition occurred at 270°C (99.4%)
Temperature of decomposition	Decomposition occurred at the temperature range 270 – 355 °C (99.4%)
Appearance (state purity)	white powder with weak, not characteristic odour (99.4)
Relative density (state purity)	D_4^{20} = 1.43 (99.4%)
Surface tension	69.1 mN/m at 20°C (97.6%)

Vapour pressure (in Pa, state temperature)	9.1×10^{-7} Pa for 20 °C 1.7×10^{-6} Pa for 25 °C 2.6×10^{-5} Pa for 50 °C (extrapolated, 99.9%)
Henry's law constant (Pa m^3 mol^{-1})	8.2×10^{-8} Pa x m^3 x mol^{-1} at 20 °C
Solubility in water (g/L or mg/L, state pH and temperature)	pH 4 (buffer) 3.2 g/L at 20°C pH 9 (buffer) 3.0 g/L at 20°C In distilled water: pH 7 3.2 g/L at 20°C (99.4)
Solubility in organic solvents (in g/L or mg/L, state temperature)	[g/L at 20 °C] (99.4%) methanol > 250 n-heptane 0.0005 toluene 3.7 dichloromethane > 250 acetone > 250 ethylacetate > 250 dimethyl sulfoxide > 250
Partition coefficient (log P_{OW}, state pH and temperature)	At 25 °C, pH7 (99,4%) Pow log Pow 16 1.2
Hydrolytic stability (DT_{50}, state pH and temperature)	hydrolytically stable at ambient temperature in the range of pH 4 to pH9.
Dissociation constant	No dissociation occurs in aqueous solutions in the pH-range 1< pH <12
UV / VIS absorption (max.) (if absorption >290 nm state ε at wavelength)	UV/Vis-spectrum: MeOH

pH	λmax	ε
7.0	213	9615.06
	259	25800.49
2.0	214	9388.74

	<table><tr><td></td><td>259</td><td>26576.75</td></tr><tr><td>10.0</td><td>213</td><td>9996.41</td></tr><tr><td></td><td>259</td><td>25954.64</td></tr></table> ε: molar absorption coefficient, L/(molxcm) (99.4%)
Photostability (DT_{50}, aqueous, sunlight, state pH)	1st order half-life for photolytic degradation in sterile phosphate buffer (pH 7) was 13.8 experimental hrs.(99.3)
Quantum yield of direct phototransformation in water at Σ >290 nm	D = 0.000138 (99.4%)
Flammability	not highly flammable
Explosive properties	not explosive

Classification and proposed labelling (Annex IIA, point 10)

with regard to physical and chemical data	No classification is proposed

List of representative uses evaluated

Crop and/ or situation (a)	Country	Product Name	F G or I (b)	Pests or Group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)	Remarks: (m)
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	kg as/hL min max	water L/ha min max	kg as/ha min max		
Hops	N-EU (residue zone)	Sivanto	F	Aphids, *Phorodon humuli,*	SL	200	spray	BBCH 31-75 May July.	1	n.a.	0,0045-0,0075	2000-3300	0.150	21	~~Biannual~~ biennial application proposed
Lettuce	N/S-EU (residue zone)	Sivanto	F	Aphids, *Nasonovia ribisnigri*	SL	200	spray	BBCH 12-49 May--Oct	1	n.a.	0,0125-0,0250	500-1000	0.125	10	Max 1 application per **24** months
Lettuce	N/S-EU (residue zone)	Sivanto	F	Aphids, *Nasonovia ribisnigr*	SL	200	spray	BBCH 41-49 May--Oct	1	n.a.	0,0125-0,0250	500-1000	0.125	3	Max 1 application per **12** months
Lettuce (soil bound)	N-EU S-EU (residue zone)	Sivanto	G	Aphids, *Nasonovia ribisnigri,*	SL	200	spray	BBCH 12-49	2	10	0,0125-0,0250	500-1000	0.125	3	Max 2 applications per 12 months

Remarks:
n) in case of group of crops the Codex classification should be used
o) Outdoor of field use (F), or glasshouse application (G)
p) e.g. biting and sucking in insects, soil born insects, foliar fungi
q) e.g. wettable powder (WP), emulsifiable concentration (EC), granule (GR)
r) Use CIPAC/FAO Codes where appropriate
s) All abbreviations used must be explained
t) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench
u) Kind, e.g. overall, broadcast aerial spraying, row, individual plant, between the plants
v) g/l or g/L
w) Growth stage at last treatment
x) The minimum and maximum number of application possible under practical conditions of use must be provided
y) PHI - Pre Harvest Interval
z) Remarks may include: Extent of use/ economic importance/restrictions

Chapter 2.2 – Methods of Analysis

Analytical methods for the active substance (Annex IIA, point 4.1)

Technical as (principle of method)	HPLC-UV at 225 nm
Impurities in technical as (principle of method)	HPLC-UV at 210/256 nm
Plant protection product (principle of method)	HPLC-UV at 280 nm

Analytical methods for residues (Annex IIA, point 4.2)

Residue definitions for monitoring purposes

Food of plant origin		Flupyradifurone DFA, expressed as DFA ~~, sum of both expressed as flupyradifurone equivalents~~ separately
Food of animal origin		Flupyradifurone DFA, expressed as DFA ~~, sum of both expressed as flupyradifurone equivalents~~ separately
Soil		Flupyradifurone
Water	surface	Flupyradifurone, ~~DFA, 6-CNA~~
	drinking/ground	Flupyradifurone, ~~DFA, 6-CNA~~
Air		Flupyradifurone

Monitoring/ Enforcement methods

Analytical methods for residues (Annex IIA, point 4.2)

Food/feed of plant origin (principle of method and LOQ for methods for monitoring purposes)	HPLC-MS/MS method 01330 with acetonitrile/water (4/1, v/v) with 2.2 mL/L formic acid extraction. LOQ 0.01 mg/kg for flupyradifurone for lettuce, wheat, orange, rape seed potato, orange and wheat. LOQ 0.05 mg/kg for hop. LOQ 0.02 ~~1~~ mg/kg for DFA for lettuce, wheat, orange, rape seed potato, orange and wheat. LOQ 0.10 mg/kg for hop.
Food/feed of animal origin (principle of method and LOQ for methods for monitoring purposes)	HPLC-MS/MS acetonitrile/water (4/1, v/v), with the addition of n-heptane in the cases of fat and milk. LOQ 0.01 ~~2~~ mg/kg for flupyradifurone and LOQ 0.02 mg/kg for DFA in fat, liver, kindey, muscle, egg and milk.
Soil (principle of method and LOQ)	HPLC-MS/MS after extraction with acetonitrile. LOQ of 5µg/kg for BYI 02960
Water (principle of method and LOQ)	HPLC-MS/MS LOQ for BYI 02960 is 0.05 µg/L in drinking and surface water.
Air (principle of method and LOQ)	HPLC/MS-MS after extraction with acetonitrile.l LOQ for BYI 029607 µg /m^3
Body fluids and tissues (principle of method and LOQ)	No methods required.

Chapter 2.3 Impact on Human and Animal Health

Absorption, distribution, excretion and metabolism (toxicokinetics) (Annex IIA, point 5.1)

Rate and extent of oral absorption	The gastrointestinal absorption of radioactivity was high and fast, almost completely absorbed within 48h (75-90 % of administered radioactivity was detected in urine after oral administration and 76% was detected after i.v. administration). ~~It accounted for >80 % of the dose independent of the labelling position used.~~
Distribution	Maximum plasma concentration was reached approx. 1 h after administration of a single low dose (2 mg/kg bw) and 2-4 h after a single high dosage (200 mg/kg bw). Flupyradifurone is widely distributed with highest concentrations in liver and kidney. A fast decline was observed for all organs and tissues (<LOQ seven days after administration).
Potential for accumulation	No evidence for accumulation, supported by the low Pow of 1.2.
Rate and extent of excretion	Excretion was very fast, mainly renal and almost completed after 24 h (approx. 77-97% within 24h and 82-100% within 48h); 75-90% was extreted via

	urine within 48h and 7-26% was excreted via faeces.
Metabolism in animals	Moderately metabolised in the rat. The parent compound represented the predominant part of the radioactivity in urine. Main metabolited detected in urine (accounting for >10% of the dose administered) were BYI 02960-OH and BYI 02960-hippuric acid (only high dose male). Metabolites 6-CAN and DFA were detected in urine at levels >5% of the dose administered. Metabolic reactions: • hydroxylation followed by conjugation with glucuronic acid or sulfate, • cleavage of the difluoroethyl group forming BYI 02960-des-difluoroethyl, and difluoroacetic acid (DFA), • cleavage of the molecule at the pyridinylmethylene bridge forming 6-CNA, which was further conjugated with glycine to BYI 02960-hippuric acid and BYI 02960-difluoroethyl-amino-furanone. 6-CNA is considered a major metabolite in male rat but not in females.
Toxicologically relevant compounds (animals and plants)	Parent compound, DFA
Toxicologically relevant compounds (environment)	Parent compound, DFA

Acute toxicity (Annex IIA, point 5.2)

Rat LD_{50} oral	LD_{50} >300-2000 mg/kg ~~cut-off = 2000 mg/kg~~	H302
Rat LD_{50} dermal	LD_{50} > 2 000 mg/kg	
Rat LC_{50} inhalation	LC50 at 4 hours >4.7 mg/L (nose only) ~~> 4671 mg/m3~~	
Skin irritation	Non irritating	
Eye irritation	Slight redness of the conjunctivae, reversed within 48 hours	
Skin sensitisation	Not sensitising (LLNA)	

Short term toxicity (Annex IIA, point 5.3)

Target / critical effect	Liver effects in rodents and dogs, thyroid effects in rats, kidney effects in mice and dogs and degeneration of myofibers in skeletal muscles in dog	
Relevant oral NOAEL	6.0 ~~30.2~~ mg/kg bw/day (90-d rat study) 81 mg/kg bw/day (90-d mouse study) 12 mg/kg bw/day (90-d dog study)	

	7.8 mg/kg bw/day (1-y dog study)	
Relevant dermal NOAEL	500 mg/kg bw/day (28-d rat study)	
Relevant inhalation NOAEL	No data – not required	

Genotoxicity (Annex IIA, point 5.4)

	Flupyradifurone has no genotoxic potential	

Long term toxicity and carcinogenicity (Annex IIA, point 5.5)

Target/critical effect	Liver in both species, thyroid and lung in rats and kidney in mice	
Relevant NOAEL	15.8 mg/kg bw/day (2-year rat carcinogenicity) 43 mg/kg bw/day (2-year mouse carcinogenicity)	
Carcinogenicity	Not carcinogenic in both rats (> 80 mg/kg/day) and mice (>224 mg/kg/day)	

Reproductive toxicity (Annex IIA, point 5.6)

Reproduction toxicity

Reproduction target / critical effect	Parental: decreased body weight gain, increased liver and thyroid weight, centrilobular hypertrophy, decreased bw (gain) Reproductive: decreased estrous cycle number, litter size, number of implants. Offspring: meternal effects leading to effects on pup weight, physical development	
Relevant parental NOAEL	6.4 ~~7.7~~ ~~32~~ mg/kg bw/day	
Relevant reproductive NOAEL	34 mg/kg bw/day	
Relevant offspring NOAEL	34 mg/kg bw/day	

Developmental toxicity

Developmental target / critical effect	Maternal: decreased bw; increased liver weight. Developmental: short costal cartilage (rat only)	
Relevant maternal NOAEL	Rat: 30 mg/kg bw/day Rabbit: 40 mg/kg bw/day	
Relevant developmental NOAEL	Rat: 50 mg/kg bw/day Rabbit: 15 ~~40~~ mg/kg bw/day	

Neurotoxicity (Annex IIA, point 5.7)

Acute neurotoxicity	Piloerection and dilated pupils NOAEL: 35 mg/kg, (m/f)	

Repeated neurotoxicity	90d rat study: Systemic toxicity: body weight and food consumption effect, NOAEL: 29.4 mg/kg bw/d Neurotoxicity: No adverse effects, NO(A)EL: 143/173 mg/kg (m)	
Delayed neurotoxicity	No data available – not required	
Developmental neurotoxicity	Maternal: decreased body weight gain NOAEL: 42.4 mg/kg bw/day Offspring: decreased body weight gain NOAEL: 42.4 mg/kg bw/day Developmental neurotoxicity: slightly increased motor/locomotor activity and startle habituation. NOAEL: 42.4 mg/kg bw/day	

Other toxicological studies (Annex IIA, point 5.8)

Mechanism studies	**Biokinetic study in the plasma of rats following 7 day exposure through the diet:** Plasma concentrations were considered similar between the three times of blood collection for both sexes.
	28-day immunotoxicity study: Systemic NOAEL of 50 mg/kg bw/d Immunotoxic NOAEL of >230 mg/kg bw/d

Studies performed on metabolites or impurities	**Difluoroacetic acid** (DFA, BCS-AA56716, M44) - Ames test: negative - In vitro HPRG LGMA: negative - In vitro CA: Negative - Acute oral: 300 mg/kg <LD_{50} < 2000 mg/kg - 14-day dietary rat: NOAEL = 51 mg/kg bw/d - 90-day dietary rat: NOAEL = 12.7 mg/kg bw/d (m) **BYI 02960-difluoroethyl-amino-furanone** (BCS-CC98193, BYI 02960-DFEAF, M34) - Ames tes: negative - In vitro CA: positive in absence of metabolic activation - In vitro HPRT LGMA: negative - In vivo MNT: negative - In vivo UDS: negative - Acute oral: ≥ 2 000 mg/kg/day - 14-d dietary rat : NOAEL = 135 mg/d bw/d - 28-day dietary rat: NOAEL = 243 mg/kg bw/d **BYI 02960-CHMP** (6-chloropyridin-3-ylmethanol, IM-O, M21) - Ames test: negative - Acute oral: LD_{50} in females = 1483 mg/kg/day - 90-day dietary rat: NOAEL = 48.9 mg/kg bw/d **BYI 02960-6-CNA** (6-chloronicotinic acid, IC-0, M27) - Ames test: negative - Acute oral: LD_{50} ≥5000 mg/kg/day

Medical data (Annex IIA, point 5.9)

Limited as this is a new active ingredient

Summary (Annex IIA, point 5.10)	Value	Study	Safety factor
ADI	0.064 ~~0.08~~ ~~0.12~~ mg/kg bw/day	2-generation rat ~~90-day and 1-y dog study combined~~	100
AOEL	0.064 ~~0.08~~ ~~0.12~~ mg/kg bw/day	2-generation rat ~~90-day and 1-y dog study combined~~	100 *
ARfD	0.15 ~~0.35~~ mg/kg bw	Rabbit developmental ~~acute neurotox study~~	100

*oral absorption is >80%, correction for oral absorption not necessary.

Dermal absorption (Annex IIIA, point 7.3)

BYI 02960 200 SL*	Based on human in vitro absorption values corrected for the large variation: 0.4% at 200 g/L 3 % at 0.625 g/L 8 % at 0.1 g/L 17% at 0.045 g/L (calculated *pro rata*)

~~* A *pro rata* corrected dermal absorption of 17% for the spray dilution is used in the exposure scenarios based on a dilution of 0.045 g/L~~.

Exposure scenarios (Annex IIIA, point 7.2)

		without PPE	with PPE
Operator – use on hop ((% AOEL) Mechanical upward spraying, field	UK-75th	42.9	29.0
	DE-GM	53.1	49.7
Operator – use on lettuce (% AOEL) Mechanical downward spraying, field	UK-75th	50.5	7.7
	DE-GM	20.0	15.9
Operator – use on lettuce (% AOEL) Manual downward spraying, field	UK-75th	119.3	21.8
Operator – use on lettuce (% AOEL) Manual downward spraying, (indoor)	Dutch greenhouse model	97.7	12.3
Workers	exposure up to 46.1 % of the AOEL, without PPE (EUROPOEM II)		
Bystanders	exposure up to 5.8 % of the AOEL (EUROPOEM II)		
Resident	Exposure up to 0.58 % of the AEL (German model for the child)		

Classification and proposed labelling with regard to toxicological data (Annex IIA, point 10)

	RMS/peer review proposal
Substance classified (BYI 02960)	-

Chapter 2.4 Residues

Metabolism in plants (Annex IIA, point 6.1 and 6.7, Annex IIIA, point 8.1 and 8.6)

Plant groups covered	Foliar treatment: fruit (apple), pulses/oilseeds (cotton), cereals (rice) Soil treatment: fruit (tomato), root and tuber vegetables (potato), seed treatment: root and tuber vegetables (potato), cereals (rice)
Rotational crops	Turnips, wheat, Swiss chard
Metabolism in rotational crops similar to metabolism in primary crops?	Yes
Processed commodities	Hydrolysis (pasteurisation, baking, brewing, boiling, sterilisation)
Residue pattern in processed commodities similar to residue pattern in raw commodities?	Parent flupyradifurone is stable, no information on major metabolites DFA and DFEAF
Plant residue definition for monitoring	Flupyradifurone DFA, expressed as DFA ~~, sum of both expressed as flupyradifurone equivalents~~ separately
Plant residue definition for risk assessment	The sum of parent compound flupyradifurone and its metabolites DFA ~~and difluoroethyl-amino-furanone (DFEAF)~~, expressed in flupyradifurone equivalents.
Conversion factor (monitoring to risk assessment)	1

Metabolism in livestock (Annex IIA, point 6.2 and 6.7, Annex IIIA, point 8.1 and 8.6)

Animals covered	Laying hen, lactating goat
Time needed to reach a plateau concentration in milk and eggs	Eggs: 6 days, confirmed by feeding study Milk: 9 days, confirmed by cattle feeding study
Animal residue definition for monitoring	Flupyradifurone DFA, expressed as DFA ~~, sum of both expressed as flupyradifurone equivalents~~ separately
Animal residue definition for risk assessment	The sum of flupyradifurone and metabolite DFA, expressed in flupyradifurone equivalents.
Conversion factor (monitoring to risk assessment)	Not applicable
Metabolism in rat and ruminant similar (yes/no)	yes
Fat soluble residue: (yes/no)	no

Residues in succeeding crops (Annex IIA, point 6.6, Annex IIIA, point 8.5)

Residue trials on succeeding cereals, root crops and leafy crops were conducted in NEU and SEU at a application rate of 200 g as/ha (on soil or lettuce) in three rotations with plant back intervals (PBI) of: 25-33d (1st rotation), 61-145d (2nd rotation) and 266-329d 93rd rotation). For root crops and leafy crops, samples were taken at early harvest (eh) and normal harvest (nh) growth stages. Quantifiable residues were measured in all rotations, median values and range is reported. Residues in first rotation crops were always highest.

1st rotation:

carrot/turnip root (eh): 0.06 mg/kg (0.05-0.14)

carrot/turnip root (nh): 0.08 mg/kg (0.05-0.12)

Lettuce (eh): 0.08 mg/kg (0.06-0.21)

Lettuce (nh): 0.07 mg/kg (<0.04-0.16)

Barley green forage: 0.10 mg/kg (0.05-0.41)

Barley straw: 0.12 mg/kg (<0.07-0.39)

Barley grain: 0.35 mg/kg (0.11-0.65)

2nd rotation:

carrot/turnip root (eh): 0.04 mg/kg (<0.04-0.07)

carrot/turnip root (nh): 0.05 mg/kg (<0.04-0.06)

Lettuce (eh): 0.04 mg/kg (<0.04-0.12)

Lettuce (nh): 0.04 mg/kg (<0.04-0.11)

Barley green forage: 0.05 mg/kg (0.05-0.13)

Barley straw: <0.07 mg/kg (<0.07-0.12)

Barley grain: 0.12 mg/kg (0.06-0.28)

3rd rotation:

carrot/turnip root (eh): 0.05 mg/kg (<0.04-0.06)

carrot/turnip root (nh): 0.05 mg/kg (<0.04-0.06)

Lettuce (eh): <0.04 mg/kg (<0.04-0.10)

Lettuce (nh): <0.04 mg/kg (<0.04-0.07)

Barley green forage: 0.04 mg/kg (<0.04-0.19)

Barley straw: <0.07 mg/kg (<0.07-0.19)

Barley grain: 0.12 mg/kg (0.08-0.39)

Field rotational crop trials at 2x 125 g/ha in various crops after short plant back intervals (30d):

Potatoes: 0.13 mg/kg (0.05-0.27)

Leek: 0.09 mg/kg (0.04-0.25)

Cucumber: 0.32 mg/kg (0.07-0.43)

Onion: 0.09 mg/kg (<0.04-0.18)
Bean (legume): 0.50 mg/kg (0.28-1.1)
Field peas (pulses): 1.6 mg/kg (0.67-2.3)
winter rape: 0.10 mg/kg (0.07-0.17)
Cereals: 0.35 mg/kg (0.11-0.65)

Stability of residues (Annex IIA, point 6 introduction, Annex IIIA, point 8 Introduction)

Flupyradifurone DFEAF, and DFA are stable in orange fruit (high acid content), spinach leaves and tomato fruit (high water content), wheat grain (high starch content), bean seed (high protein content), coffee bean (specific matrix), soybean seed (high oil content), and sugar cane (high water content) for at least 18 months when stored frozen (≤-20°C).
DFA is stable in animal fat, kidney, liver, and muscle for at least 43 days, when stored frozen (≤-20°C).

Residues from livestock feeding studies (Annex IIA, point 6.4, Annex IIIA, point 8.3)

	Ruminant:	Poultry:	Pig:
	Conditions of requirement of feeding studies		
Expected intakes by livestock ≥ 0.1 mg/kg diet (dry weight basis) (yes/no - If yes, specify the level)	Yes	Yes	Yes
Potential for accumulation (yes/no):	No	No	See ruminant
Metabolism studies indicate potential level of residues ≥ 0.01 mg/kg in edible tissues (yes/no)	Yes	Yes	See ruminant
	Feeding studies (Specify the feeding rate in cattle and poultry studies considered as relevant) Dairy cattle: 4.8 mg flupyradifurone/kg dry feed (1.6 ~~1.8N~~ ~~2N~~) Laying hens: 1.5 mg flupyradifurone /kg dry feed (1.2N) Residue levels in matrices : Mean (max) mg/kg*		
Muscle	BYI 02960: 0.04 (0.05) DFA: <0.02 (<0.02) ~~0.063 (0.064)~~	BYI 02960: <0.01 (<0.01) DFA: 0.08 (0.10) ~~0.093 (0.106)~~	-
Liver	BYI 02960: 0.15 (0.17) DFA: <0.02 (<0.02) ~~0.165 (0.188)~~	BYI 02960: <0.01 (<0.01) DFA: 0.10 (0.11) ~~0.114 (0.122)~~	-
Kidney	BYI 02960: 0.16 (0.22)	No data	-

	DFA: 0.022 (<0.02) ~~0.176 (0.281)~~		
Fat	BYI 02960: DFA: <<0.02 (<0.02) ~~0.041 (<0.050)~~	BYI 02960: <0.01 (<0.01) DFA: 0.03 (0.04) ~~0.039 (0.045)~~	-
Milk	BYI 02960: <0.02 DFA: <0.02(<0.02) ~~0.043 (<0.050)~~		
Eggs		BYI 02960: <0.01 (<0.01) DFA: 0.05 (0.05) ~~0.061 (0.063)~~	

* total of DFA and flupyradifurone

Summary of residues data according to the representative uses on raw agricultural commodities and feedingstuffs (Annex IIA, point 6.3, Annex IIIA, point 8.2)

Commodity	Residue region	Outdoor/ indoor	Enforcement		Risk assessment			MRL proposal (mg/kg)		Median CF	Comments
			BYI 02960	DFA	(Sum of BYI 02960 (parent compound) and DFA, expressed as BYI 02960)	STMR (mg/kg)	HR residue (mg/kg)	BYI 02960	DFA		
Lettuce	NEU	I	0.73; 1.4; 1.8; 2.0; 2.1; 2.5; 2.6; 3.5; 6.0	0.009; 0.01; 0.013; 0.018; <0.02; <0.02; <0.02; 0.023; 0.043;	0.79; 1.42; 1.82; 2.02; 2.12; 2.53; 2.64; 3.52; 6.04;	**2.12**	**6.04**	**9**	0.06	1	Agricultural use (2 x 0.125 g a.s./ha, interval = 10 days, PHI = 3 days Unrounded MRL BYI 02960: 8.597 DFA: 0.060 Greenhouse use identified as the worst case residue situation
	NEU	O	0.08; 0.11; 0.13; 0.19 0.20; 0.25; 0.37; 0.57; 0.80;	0.009; 0.016; <0.02; <0.02; <0.02; <0.02; <0.02; <0.02; <0.02; <0.02;	0.10; 0.13; 0.15; 0.21; 0.22; 0.28; 0.39; 0.62; 0.82	0.22	0.82	1.5	0.04	1	Agricultural use (1 x 0.125 g a.s./ha, PHI = 10 days Unrounded MRL BYI 02960: 1.265 DFA: 0.036 No trials compliant with the cGAP with PHI 3d (1x125 g as/ha, int. 10d, PHI 3d). Identified as less critical than Greenhouse use
	NEU	O	0.11; 0.37; 0.43; 0.58; 0.68; 0.83; 1.0; 1.5; 2.9	0.009; 0.009; 0.010; <0.02; <0.02; <0.02; <0.02; <0.02; 0.066	0.13; 0.39; 0.46; 0.60; 0.70; 0.83; 1.02; 1.52; 3.00	0.60	3.00	5.0	**0.1**	1	Home and garden use (2 x 0.125 g a.s./ha, interval = 10 days, PHI = 3 days) Unrounded MRL BYI 02960: 4.430

Commodity	Residue region	Outdoor/ indoor	Enforcement		Risk assessment			MRL proposal (mg/kg)		Median CF	Comments
			BYI 02960	DFA	(Sum of BYI 02960 (parent compound) and DFA, expressed as BYI 02960)	STMR (mg/kg)	HR residue (mg/kg)	BYI 02960	DFA		
											DFA: 0.091
	SEU	O	0.35; 0.40; 0.48; 0.72; 1.1; 1.5; 2.1; 2.7; 3.1	0.007; 0.010; 0.013; 0.016; 0.017; 0.017; <0.02; 0.043; 0.05;	0.38; 0.42; 0.53; 0.76; 1.12; 1.58; 2.15; 2.73; 3.18	1.12	3.18	6	0.08	1	Home and garden use (2 x 0.125 g a.s./ha, interval = 10 days, PHI = 3 days) Unrounded MRL BYI 02960: 5.526 DFA: 0.081
	SEU	O	0.04; 0.04; 0.05; 0.11; 0.29; 0.38; 0.43; 0.55; 0.80	<0.02; <0.02; <0.02; 0.02; 0.029; 0.03; 0.03; 0.046; 0.051	0.06; 0.06; 0.08; 0.14; 0.31; 0.41; 0.48; 0.60; 0.82	0.31	0.82	1.5	0.08	1	Agricultural use (1 x 0.125 g a.s./ha, PHI = 10 days Unrounded MRL BYI 02960: 1.366 DFA: 0.076 No trials compliant with the cGAP with PHI 3d (1x125 g as/ha, int. 10d, PHI 3d). Identified as less critical than Greenhouse use
Hops, dry cones	NEU	O	0.14, 0.31, 0.43, 0.48, 0.77, 0.90, 1.1, 1.8, 2.0	0.09, 0.09, 0.13, 0.17, <0.2, <0.2, <0.2, <0.2, <0.2, <0.2	0.34, 0.51, 0.63, 0.68, 1.05, 1.11, 1.5, 2.27, 2.3	**1.05**	**2.3**	**4**	**0.4**	1	8 trials on hops compliant with the cGAP (1x120 g as/ha, PHI 21d), cone, kiln-dried Unrounded MRL BYI 02960: 3.488 DFA: 0.357

(a) Numbers of trials in which particular residue levels were reported *e.g.* 3 x <0.01, 1 x 0.01, 6 x 0.02, 1 x 0.04, 1 x 0.08, 2 x 0.1, 2 x 0.15, 1 x 0.17

(b) Supervised Trials Median Residue *i.e.* the median residue level estimated on the basis of supervised trials relating to the representative use

(c) Highest residue

Consumer risk assessment (Annex IIA, point 6.9, Annex IIIA, point 8.8)

ADI	0.064 ~~0.08~~ ~~0.12~~ mg/kg bw/d
TMDI (% ADI) according to WHO European diet	15.1 ~~47.3~~ ~~75.6~~% WHO Cluster diet B
TMDI (% ADI) according to national diet	12.1% UK toddler ~~31.6% Danish child~~ ~~60.1% German child~~
IEDI (WHO European Diet) (% ADI)	Not required
NEDI (specify diet) (% ADI)	Not required
Factors included in IEDI and NEDI	Not applicable
ARfD	0.15 ~~0.35~~ mg/kg bw
IESTI (% ARfD)	82.3% ~~161.4%~~ Lettuce, German child ~~524.6% Dutch child (celery, import tolerance, MRL application)~~
NESTI (% ARfD) according to national (to be specified) large portion consumption data	See IESTI ~~81.8 69.2% German child (lettuce)~~ ~~83.4% UK infant (oranges, import tolerance, MRL application)~~
Factors included in IESTI and NESTI	Proposed MRLs, HR and processing factors for lettuce inner heads, beer (hops) and melon peel/pulp distribution

Processing factors (Annex IIA, point 6.5, Annex IIIA, point 8.4)

Crop/ process/ processed product	Number of studies (valid studies)	Processing factors Transfer factor (mean)	Amount transferred (%) (Optional)
Lettuce outer leaves	4	1.8	
Lettuce inner head parts	4	0.72 ~~0.73~~	
Lettuce inner leaves	4	0.87 ~~0.76~~	
Lettuce washed inner leaves	4	0.65 ~~0.67~~	
Lettuce washing water	4	0.02 ~~0.07~~	
Hops draff	2	<0.11	
Brewer's yeast	2	<0.11	
Beer	2	0.02 ~~0.01~~	

Processing factors for DFA were all <0.2.

Proposed MRLs (Annex IIA, point 6.7, Annex IIIA, point 8.6)

Crop	MRL proposal (mg/kg)	
	Flupyradifurone	**DFA, expressed as DFA**
Lettuce	9	0.1
Hops	4	0.4
Rotational root and tuber vegetables	0.01	0.09
Rotational stem vegetables	0.01	0.08
Rotational bulb vegetables	0.01	0.06
Rotational fruiting vegetables	0.01	0.15
Rotational legume vegetables	0.01	0.4
Rotational leafy vegetables	0.08	0.04
Rotational cereals and sweet corn	0.01	0.3
Rotational pulses	0.01	0.8
Rotational oilseeds	0.01	0.05
eggs	0.01*	0.03
poultry meat (muscle)	0.01*	0.05
poultry fat	0.01*	0.03
poultry liver/offal	0.01*	0.1
milk	0.01*	0.03
Ruminant, pig and horse meat (muscle)	0.01*	0.1
Ruminant, pig and horse fat	0.01*	0.1
Ruminant, pig and horse liver	0.01*	0.1
Ruminant, pig and horse kidney	0.01*	0.15
other ruminant, horse and pig offal	0.01*	0.15

Parallel to the peer review of flupyradifurone under Reg (EC) 1107/2009, an application for the setting of MRLs from additional uses in the EU ~~and import tolerances~~ is ongoing.

Chapter 2.5 Fate and behaviour

Route of degradation (aerobic) in soil (Annex IIA, point 7.1.1.1.1)

Mineralization after 100 days *	29.4-58.6% (n=4, PYM-^{14}C BYI 02960) 20.2-57.4% (n=3, PYR-^{14}C BYI 02960) 12.3-38.9% (n=6, FUR-^{14}C BYI 02960) 25.9-42.3% (n=3, ETH-^{14}C BYI 02960)
Non-extractable residues after 100 days *	12.5-16.8% (n=4, PYM-^{14}C BYI 02960) 11.3-25.5% (n=3, PYR-^{14}C BYI 02960) 16.4-34.1% (n=6, FUR-^{14}C BYI 02960) 14.3-17.9% (n=3, ETH-^{14}C BYI 02960)
Metabolites requiring further consideration - name and/or code, % of applied (range and maximum)	difluoroacetic acid (DFA, max. 33.9%) 6-chloronicotinic acid (6-CNA, max. 17.1%)

PYM (= pyrindinyl-methyl-^{14}C-label), PYR (= pyridine-2,6-^{14}C-label), FUR (= furanone-4-^{14}C-label) and ETH (= ethyl-1-^{14}C-label)
* at the end of the experiment at 120 days; n = number of soils tested

Route of degradation in soil - Supplemental studies (Annex IIA, point 7.1.1.1.2)

Anaerobic degradation

Mineralization after 100 days*	6.0-26.6% (n=3, PYR-^{14}C BYI 02960) 15.9% (n=1, FUR-^{14}C BYI 02960) 6.9% (n=1, ETH-^{14}C BYI 02960) Formed at the aerobic phase
Non-extractable residues after 100 days*	12.9-17.2% (n=3, PYR-^{14}C BYI 02960) 30.1% (n=1, FUR-^{14}C BYI 02960) 15.2% (n=1, ETH-^{14}C BYI 02960)
Metabolites that may require further consideration for risk assessment - name and/or code, % of applied (range and maximum)	DFA (max. 26.2%; mainly during aerobic phase) 6-CNA (max. 14.2%; mainly during aerobic phase)

* duration experiment 120 days, value at the end of the experiment; n = number of soils

Soil photolysis

Metabolites that may require further consideration for risk assessment - name and/or code, % of applied (range and maximum)	no major metabolites	
	mineralization 0.1-2.2%	8 d cont. irradiation
	bound residues0.8-0.9%	8 d cont. irradiation
	DT_{50} (SFO) 99.6-109.3 d	8 d cont. irradiation
	419.2->1000 d	8 d dark control

Rate of degradation in soil (Annex IIA, point 7.1.1.2, Annex IIIA, point 9.1.1)

Laboratory studies

Parent	**Aerobic conditions**							
Soil type[1] (Origin)	**BYI 02960 label**	**pH ($CaCl_2$)**	**t. °C / % MWHC**	**DT_{50} (d)**	**DT_{90} (d)**	**DT_{50} (d) 20˚C pF 2**	**χ2**	**Method of calculation**
Sandy loam (AX)	PYM	6.4	20 / 55	63.4	443.3	169.1 *[a]	1.2	DFOP
Sandy loam (AX)	FUR	6.1	20 / 55	62.2	390.6	141.5 *[b]	1.6	DFOP
Loamy sand (AX)	ETH	6.2	20 / 55	62.0	538.1	210.3 *[c]	1.6	DFOP
Ax geometric mean				**62.5**	**453.3**	**171.3**		
Silt loam (HF)	PYM	6.5	20 / 55	52.4	209.3	54.4 **	0.6	DFOP
Silt loam (HF)	FUR	6.5	20 / 55	33.2	229.5	40.5 **	1.7	DFOP
Silt loam (HF)	ETH	6.5	20 / 55	34.1	329.8	43.0 **	2.3	DFOP
Silt loam (HF)	PYR	6.5	20 / 55	33.0	221.3	90.0 **	2.0	DFOP
HF geometric mean				**37.4**	**243.3**	**54.0**		
Loam (HN)	PYM	5.4	20 / 55	120.0	489.7	157.5 *[d]	1.2	DFOP
Silt loam (HN)	FUR	4.8	20 / 55	98.3	462.5	157.5 *[e]	2.0	DFOP
HN geometric mean				**108.6**	**475.9**	**157.5**		
Clay loam (DD)	PYM	7.4	20 / 55	56.4	265.1	60.1 **	1.7	DFOP
Silty clay (DD)	FUR	7.1	20 / 55	49.3	303.1	55.1 **	2.3	DFOP
Clay loam (DD)	ETH	7.1	20 / 55	33.9	649.6	38.6 **	1.9	DFOP
DD geometric mean				**45.5**	**373.7**	**50.4**		
Silt loam (SF)	FUR	6.5	20 / pF2.0-2.5	228	757	179.7 **	1.3	SFO
Silt loam (SF)	PYR	6.5	20 / pF2.0-2.5	242	898	166.4 **	0.7	DFOP
SF geometric mean				**234.9**	**824.5**	**172.9**		
Sandy loam (S)	FUR	7.0	20 / pF2.0-2.5	58.3	273	58.8 **	1.1	DFOP
Sandy loam (S)	PYR	7.0	20 / pF2.0-2.5	56.3	324	55.5 **	1.8	FOMC
Sanger (S) geometric mean				**56.8**	**522.5**	**57.1**		
Overall geometric mean				**73.2**	**405.0**	**94.8**		
Overall median				**59.7**	**413.5**	**107.3**		

[1] USDA classification; * DFOP slow fase DT50 ; ** SFO for modelling DT50
Ax = Laacher Hof, HF = Hofchen an Hohenseh, HN = , DD = Dollendorf II SF = Springfield, S = Sanger soil
PYM (= pyrindinyl-methyl-14C-label), PYR (= pyridine-2,6-14C-label), FUR (= furanone-4-14C-label) and ETH (= ethyl-1-14C-label); a: k_1 0.0438, k_2: 0.0041, g 0.3822; b: k_1 0.0650, k_2: 0.0049, g 0.3312; c: k_1 0.0546, k_2: 0.0033, g 0.4023; d: k_1 0.1031 , k_2: 0.0044, g 0.1571; e: k_1 0.1079, k_2: 0.0044, g 0.2280

6-CNA	Aerobic conditions							
Soil type[1]	**pH ($CaCl_2$)**	**t. °C / % MWHC**	**DT_{50} (d)**	**DT_{90} (d)**	**DT_{50} (d) 20°C pF2**	**f.f.**	**χ2**	**Method of calculation**
Sandy loam	5.8	20 / 45	2.9	9.7	2.9	-	8.5	SFO
Clay	7.4	20 / 45	2.2	7.4	2.2	-	6.9	SFO
Loam	7.0	20 / 45	5.3	17.5	5.3	-	8.5	SFO
Silt loam	6.5	20 / 55	3.1 3.0*	10.4 9.9*	- 3.0	- 0.266	17.0 15.4	FOMC-SFO DFOP-SFO
Sandy loam	7.0	20 / pF2.0-2.5	36.6 24.8*	121 82.4*	- 22.4#	- 0.694	13.8 15.1	DFOP-SFO SFO-SFO
Geometric mean			**5.2**	**17.4**	**4.7**			
Arithmetic mean						**0.480**		

[1] USDA classification; f.f. = formation fraction; # normalised to pF2 ; * not included in the geometric mean

DFA	Aerobic conditions							
Soil type[1]	**pH ($CaCl_2$)**	**t. °C / % MWHC**	**DT_{50} (d)**	**DT_{90} (d)**	**DT_{50} (d) 20°C pF2**	**f.f.**	**χ2**	**Method of calculation**
Clay loam	7.1	20 / 55	44.9 32.0*	149.0 106.2*	- 32.0	- 0.909	5.4 5.2	DFOP-SFO SFO-SFO
Loamy sand	6.2	20 / 55	73.6	244.5	73.6	0.590	8.4	SFO-SFO
Silt loam	6.5	20 / 55	67.4 37.8*	223.9 125.7*	- 37.8	- 1.00	7.4 4.0	FOMC-SFO SFO-SFO
Geometric mean			**60.6**	**201.3**	**44.7**			
Arithmetic mean						**0.833**		

[1] USDA classification; f.f. = formation fraction; # normalised to pF2 ; * not included in the geometric mean

Field studies

BYI 0960	Aerobic conditions									
Soil type (bare soil)	**Location (country or USA state)**	**pH[1]**	**Depth (cm)**	**DT_{50} (d) actual**	**DT_{90} (d) actual**	**χ2**	**Method of calculation**	**DT_{50} (d) Norm.**	**χ2**	**Method of calculation**
Sandy loam	Germany	6.3	0-30	41.0	749	7.5	DFOP	n.c.	n.r.	n.r.
Clay laom	UK	5.8	0-30	251	>1000	7.5	DFOP	n.c.	n.r.	n.r.
Silt loam	Germany	6.3	0-30	42.8	484	6.3	DFOP	n.c.	n.r.	n.r.
Clay loam	Italy	7.4	0-30	8.3	279	7.1	DFOP	n.c.	n.r.	n.r.
Loam	Spain	5.9	0-30	22.6	215	6.6	DFOP	n.c.	n.r.	n.r.

Loam	Germany	5.5	0-30	39.0	579	11.3	DFOP	n.c.	n.r.	n.r.
							Geometric mean	**n.c.**		

[1] pH $CaCl_2$; n.c. not calculated; n.r. not relevant

pH dependence (yes / no) (if yes type of dependence)	no
Soil accumulation and plateau concentration	0.080 mg/kg $PEC_{plateau}$ and 0.160 mg/kg $PEC_{plateau}$ + PIEC for hops over 5 cm for perennial crops without tillage 0.016 mg/kg $PEC_{plateau}$ and 0.141 mg/kg $PEC_{plateau}$ + PIEC for lettuce over 20 cm

Laboratory studies

Parent	**Anaerobic conditions**							
Soil type	**BYI 02960 label**	**pH (CaCl2)**	**t. °C / % MWHC**	**DT_{50}**	**DT_{90} (d)**	**DT_{50} (d) 20°C pF2/10kPa**	**$\chi 2$**	**Method of calculation**
Silt loam	PYR	6.4	20 / 55 *	581.8	>1000	n.c.	1.4	SFO
Silt loam	FUR	6.4	20 / 55 *	693.2	>1000	n.c.	1.3	SFO
Silt loam	ETH	6.4	20 / 55 *	631.0	>1000	n.c.	0.9	SFO
Silt loam geometric mean				**633.7**	**>1000**	**n.c.**		
Loamy sand	PYR	6.7	20 / 55 *	152	506	n.c.	11.9	SFO
Sandy clay loam	PYR	6.5	20 / 55 *	>1000**	>1000**	n.c.	n.r.	n.r.
Geometric mean				**458.4**	**796.8**	**n.c.**		

PYR (= pyridine-2,6-^{14}C-label), FUR (= furanone-4-^{14}C-label) and ETH (= ethyl-1-^{14}C-label); n.c. not calculated; not relevant
* %MWHC before flooding at aerobic stage; ** no degradation observed DT_{50} and DT_{90} not calculated

Soil adsorption/desorption (Annex IIA, point 7.1.2)

Parent BYI 02960					
Soil Type[1]	**OC %**	**Soil pH ($CaCl_2$)**	**Kf (mL/g)**	**Kfoc (mL/g)**	**1/n**
Sandy loam *	2.1	6.2	2.077	98.9	0.8445
Loam *	2.4	6.6	2.213	92.2	0.8682
Loam *	2.2	5.3	2.354	107.0	0.8643
Loam *	5.1	7.2	3.822	74.9	0.8648
Sandy loam	0.7	6.8	0.597	85.2	0.9021
Silt loam	1.9	6.5	2.512	132.2	0.8505
Arithmetic mean				**98.4**	**0.8657**
pH dependence, Yes or No			No		

[1] USDA classification; * soils used for determination of 1/n in the time dependent sorption

~~Time dependent sorption (TDS)~~	~~R_{TDS} values at t = 0 1.46, 1.91, 3.35 and 4.66 mL/g~~ ~~R_{TDS} values increased by a factor of 4.4, 2.9, 3.2 and 2.6 (mean of all four soils = 3.3) at t =120 days~~ ~~Arithmetic mean Koc of 80.2 L/kg;~~ ~~Arithmetic mean 1/n = 0.8605 (of four soils)~~ ~~Geometric mean DT_{50} 58.0 days~~

Metabolite 6-CNA					
Soil Type[1]	**OC %**	**Soil pH ($CaCl_2$)**	**Kf (mL/g)**	**Kfoc (mL/g)**	**1/n**
Loamy Sand	2.54	6.2	1.027	70	1.0069
Silt loam	0.76	6.6	0.569	129	0.9706
Clay	2.05	7.5	0.833	70	0.8941
Clay loam	1.41	8.3	0.690	84	0.9262
			Arithmetic mean	**88.3**	**0.9495**
pH dependence (yes or no)			No		

[1] USDA classification

Metabolite DFA					
Soil Type[1]	**OC %**	**Soil pH ($CaCl_2$)**	**Kf (mL/g)**	**Kfoc (mL/g)**	**1/n**
Silt loam	2.4	6.5	1.74	9.5	0.9053
Loam	2.9	5.8	1.07	7.8	0.8013
Clay loam	4.5	7.4	1.78	8.2	0.9579
Sandy Loam	0.5	6.0	0.594	6.7	0.6902
Silty clay loam	1.7	6.5	0.274	1.7	0.8194
			Arithmetic mean	**6.8**	**0.8348**
pH dependence (yes or no)			No		

[1] USDA classification

Mobility in soil (Annex IIA, point 7.1.3, Annex IIIA, point 9.1.2)

Column leaching	Performed for Brazilian registration, not required for EU registration
Aged residues leaching ‡	Not performed, not required Leaching assessment based on batch adsorption values
Lysimeter/ field leaching studies ‡	Not performed, not required For parent substance and metabolites leaching based on batch adsorption values

PEC (soil) (Annex IIIA, point 9.1.3)

Parent Method of calculation	DT_{50} (d): Kinetics: Field or Lab:	0.02 (fast) and 462 (slow) DFOP (k_1=34.66; k_2=0.0015; g=0.27) Field
Application data	Crop: Depth of soil layer: Soil bulk density: % plant interception: Number of appl.: Interval (d): Application rate(s):	hops lettuce (biennial application) lettuce (annual application) 5 cm 1500 kg/m3 60 for hops (BBCH stage 31-75) 25 for lettuce (BBCH stage 12-49) (biennial) 70 for lettuce (BBCH stage 41-49) (annual) 1 n.a. 150 g as/ha for hops 125 g as/ha for lettuce (field application)

PEC_{soil} of BYI 02960 after application

		Hops		Lettuce (biennial)		Lettuce (annual)	
	Time* **[d]**	**$PEC_{soil,act}$** **[mg kg^{-1}]**	**$PEC_{soil,twa}$** **[mg kg^{-1}]**	**$PEC_{soil,act}$** **[mg kg^{-1}]**	**$PEC_{soil,twa}$** **[mg kg^{-1}]**	**$PEC_{soil,act}$** **[mg kg^{-1}]**	**$PEC_{soil,twa}$** **[mg kg^{-1}]**
Initial	0	0.080	-	0.125	-	0.050	-
Short-term	1	0.058	0.069	0.091	0.108	0.036	0.043
	2	0.058	0.066	0.091	0.102	0.036	0.041
	4	0.058	0.063	0.091	0.098	0.036	0.039
Long-term	7	0.058	0.061	0.090	0.095	0.036	0.038
	14	0.057	0.059	0.089	0.093	0.036	0.037
	21	0.057	0.058	0.088	0.091	0.035	0.037
	28	0.056	0.058	0.087	0.091	0.035	0.036
	50	0.054	0.057	0.085	0.089	0.034	0.035
	100	0.050	0.054	0.079	0.085	0.031	0.034

*Time: days after maximum concentration ($PEC_{soil,act}$) or time interval ($PEC_{soil,twa}$)

Long term PEC_{soil} of BYI 02960

	Residues distributed over … [cm]	Seasonal $PEC_{s, max}$, max. soil residue in 1st year [mg/kg]	Long-term plateau / background conc. C_{min} [mg/kg]	Long-term maximum conc. C_{max} [mg/kg]	Background C_{min} + max. of 1 year in 5 cm [mg/kg]
Hops 1 x 150 g/ha	**5**	0.080	0.080	**0.160**	-
	10	0.040	0.040	0.080	0.120
	20	0.020	0.020	0.040	0.100
Lettuce 1 [1)] 1 x 125 g/ha	5	0.125	0.046	0.171	-
	10	0.063	0.023	0.085	0.148
	20	0.031	0.011	0.043	**0.136**
Lettuce 2 [2)] 1 x 125 g/ha	5	0.050	0.050	0.100	-
	10	0.025	0.025	0.050	0.075
	20	0.013	0.013	0.025	**0.063**

[1)] Max 1 application per 24 months (biennial)
[2)] Max 1 application per 12 months (annual)
In bold: Generally, for long-term assessments the substance distribution in soil for annual crops with tillage should be assumed over a depth of 20 cm (lettuce) and for perennial crops without tillage over a depth of 5 – 10 cm (e.g. hops).

Metabolites Method of calculation	**6-CNA** Molecular weight: 157.6 g/mol Molecular weight relative to the parent: 0.546 DT_{50} (d): 36.6 Kinetics: SFO Field or Lab: Lab **DFA** Molecular weight: 96.0 g/mol Molecular weight relative to the parent: 0.333 DT_{50} (d): 73.6 Kinetics: SFO Field or Lab: Lab
Application data	**6-CNA** Maximum occurrence: 17.1% **DFA** Maximum occurrence: 33.9%

Initial PEC_{soil} of BYI 02960 metabolites after application (5 cm soil)

		Hops	Lettuce (biennial)	Lettuce (annual)
	Time* [d]	$PEC_{soil,act}$ [mg kg^{-1}]	$PEC_{soil,act}$ [mg kg^{-1}]	$PEC_{soil,act}$ [mg kg^{-1}]
6-CNA	0	0.007	0.012	0.005
DFA	0	0.009	0.014	0.006

Long term PECsoil of BYI 02960 metabolite 6-CNA

	Residues distributed over … [cm]	Seasonal $PEC_{s,max}$, max. soil residue in 1st year [mg/kg]	Long-term plateau / background conc. C_{min} [mg/kg]	Long-term maximum conc. C_{max} [mg/kg]
Hops 1 x 150 g/ha	**5**	0.015	<0.001	0.015
Lettuce 1 [1)] 1 x 125 g/ha	**20**	0.004	<0.001	0.004
Lettuce 2 [2)] 1 x 125 g/ha	**20**	0.002	<0.001	0.002

[1)] Max 1 application per 24 months (biennial); [2)] Max 1 application per 12 months (annual)

Long term PECsoil of BYI 02960 metabolite DFA

	Residues distributed over … [cm]	Seasonal $PEC_{s,max}$, max. soil residue in 1st year [mg/kg]	Long-term plateau / background conc. C_{min} [mg/kg]	Long-term maximum conc. C_{max} [mg/kg]
Hops 1 x 150 g/ha	**5**	0.018	0.001	0.019
Lettuce 1 [1)] 1 x 125 g/ha	**20**	0.005	<0.001	0.005
Lettuce 2 [2)] 1 x 125 g/ha	**20**	0.003	<0.001	0.003

[1)] Max 1 application per 24 months (biennial); [2)] Max 1 application per 12 months (annual)

Route and rate of degradation in water (Annex IIA, point 7.2.1)

Hydrolytic degradation of the active substance and metabolites above 10 %	pH 4, 7 and 9, 50 ºC: stable no metabolites >10%
Photolytic degradation of active substance and metabolites above 10 %	Tested BYI 02960 furanone-4-^{14}C-label Parent DT_{50} : 13.8 hours (under study conditions) Estimated DT_{50} at 33.3°N: 1.75 days Estimated DT_{50} at 38.03°N: 2.7 days Estimated DT_{50} at 51.32°N: 3.4 days Reaction products >10%: BYI 02960-succinamide and BYI 02960-azabicyclosuccinamide
Quantum yield of direct photo transformation in water at Σ > 290 nm	1.38 x 10^{-4} mol · Einstein $^{-1}$
Readily biodegradable (yes/no)	Test not performed, active-substance classified as not ready biodegradable.

Degradation in water / sediment

Parent BYI 02960	Distribution in water decreased to 11.4% (Sandy loam), 22.3% (Loamy sand) 14.1-14.3% (Loam) and 35.6-36.8% (Sand) at termination of the studies. Maximum sediment 59.4% (Sandy loam) at 59 d, 49.5% (Loamy sand) at termination, 58.3-58.7% (Loam) after 45-60 d and 37.6-38.9 % (Sand) at termination.								
Water / sediment system	**pH water phase**	**pH sed.**	**t. °C**	**$D_{eg}T_{50}$ / $D_{eg}T_{90}$ whole system**	**χ2**	**Method of calc.**	**DT_{50} / DT_{90} water**	**χ2**	**Method of calc.**
Sandy loam	7.4	5.2	20±2	193.1 / 641.3	1.2	SFO	8.5 / 174.6	1.6	FOMC
Loamy sand	7.7	6.7	20±2	246.9 / 820.1	1.3	SFO	34.5 / 181.8	4.4	DFOP
Loam	6.5	4.8	20±1	208.2 / 691.6	1.5	SFO	48.5 / 161.0	47.1	DFOP

Sand	6.9	6.8	20±1	246.1 / 817.4	1.3	SFO	123.8 / 411.2	2.9	DFOP
Loam	6.5	4.8	20±1	202.4 / 672.2	1.6	SFO	50.2 / 116.9	4.9	DFOP
Sand	6.9	6.8	20±1	285.0 / 946.9	1.0	SFO	117.5 / 390.3	2.4	DFOP
Geometric mean				**228.1 / 757.7**					

BYI 02960 Mineralization and non extractable residues				
Water / sediment system	**BYI 02960 label**	**Mineralization x % after n d. (end of the study)**	**Non-extractable residues in sed. Max x % after n d**	**Non-extractable residues in sed. x % after n d (end of the study)**
Sandy loam	PYR	6.8	25.0 after 119 d	25.0 after 119 d
Loamy sand	PYR	8.5	13.6 after 119 d	13.6 after 119 d
Loam	FUR	3.9	22.6 after 120 d	22.6 after 120 d
Sand	FUR	5.5	17.9 after 120 d	17.9 after 120 d
Loam	ETH	1.5	26.6 after 120 d	26.6 after 120 d
Sand	ETH	0.9	15.2 after 120 d	15.2 after 120 d

PYR (= pyridine-2,6-^{14}C-label), FUR (= furanone-4-^{14}C-label) and ETH (= ethyl-1-^{14}C-label)

DFA	Distribution in water decrease to 32.3% (Loam) at termination of the study and 72.3% (Loamy sand) at 79 d. Maximum sediment 25.2% (Loam) and 16.5% (Loamy sand) both after 79 d								
Water / sediment system	**pH water phase**	**pH sed.**	**t. °C**	**$D_{eg}T_{50}$ / $D_{eg}T_{90}$ whole system**	**χ2**	**Method of calc.**	**DT_{50} / DT_{90} water**	**χ2**	**Method of calc.**
Loam	6.9	5.2	19.2±0.1	109.0 / 362.2	2.9	SFO	75.3 / 250.3	1.6	DFOP
Loamy sand	7.5	7.0	19.2±0.1	567.2 / >1000	3.0	SFO	371.5 / >1000	4.8	SFO
Geometric mean				**248.6 / 601.8**					

PEC (surface water) and PEC sediment (Annex IIIA, point 9.2.3)

Parent Parameters used in FOCUSsw step 1 and 2, 3 and 4	Version control no. of FOCUS calculator: FOCUS step 1 and 2 version 1.1. Swash verion 3.1 (step 3) and Swan version 1.1.4 (step 4) Molecular weight (g/mol): 288.7 Water solubility (mg/L): 3200 at 20ºC Vapour pressure (Pa): 9.1×10^{-7} K_{OC} (L/kg): 98.4 Freudlich exponent (1/n): 0.8657 Geometric mean DT_{50} soil (d): 94.8 Geometric mean DT_{50} water/sed (d): 228 DT_{50} water (d): 228 DT_{50} sediment (d): 228 (STEP 1 and 2) DT_{50} sediment (d): 1000 (STEP 3 an 4) Crop interception (%): 50 for hops (biennial)

		25 for lettuce (biennial) 50 for lettuce (annual)
Application rate	Crop: Number of applications: Interval (d): Application rate(s): Application window: Crop: Number of applications: Interval (d): Application rate(s): Application window:	hops 1 n.a. 150 g as/ha NE- March-May SE- March-May leafy vegetables (lettuce) 1 (for field application) n.a. 125 g as/ha NE- March-May SE- March-May

FOCUS STEP 1 Scenario	**crop**	**$PEC_{sw,max}$ (µg/L)**	**$PEC_{sed,max}$ (µg/kg)**
		Actual	**Actual**
	Hops	53.86	43.49
	Lettuce (biennial)	53.86	43.49
	Lettuce (annual)	53.86	43.49

FOCUS STEP 2 Scenario	**Crop**	**$PEC_{sw,max}$ (µg/L)**	**$PEC_{sed,max}$ (µg/kg)**
		Actual	**Actual**
	Hops (NE)	13.07	12.49
	Hops (SE)	17.36	16.70
	Lettuce (NE - biennial)	6.41	6.25
	Lettuce (SE - biennial)	11.88	11.51
	Lettuce (NE - annual)	3.19	3.09
	Lettuce (SE - annual)	5.34	5.20

FOCUS STEP 3 Hops Scenario	**Entry route**	**$PEC_{sw, max}$ (µg/L)**	**$PEC_{sed, max}$ (µg/kg)**
R1, pond	Spray drift	0.394	0.795
R1, stream	Spray drift	5.531	0.362

Time dependent PEC values or time-weighted average concentrations are not included in the LoEP, because they were not used in the risk assessment.

FOCUS STEP 3 Lettuce (biennial) Scenario	**Entry route**	**$PEC_{sw, max}$ (µg/L)**	**$PEC_{sed, max}$ (µg/kg)**

D3 (ditch, 1st)	Spray drift	0.830	0.380
D3 (ditch, 2nd)	Spray drift	0.840	0.460
D4 (pond, 1st)	Drainage	1.035	4.545
D4 (stream, 1st)	Spray drift	0.794	1.772
D6 (ditch, 1st)	Drainage	1.268	1.766
R1 (pond, 1st)	Run-off	0.060	0.162
R1 (stream, 1st)	Run-off	0.858	0.211
R1 (pond, 2nd)	Run-off	0.097	0.254
R1 (stream, 2nd)	Run-off	1.186	0.334
R2 (stream, 1st)	Run-off	1.586	0.521
R2 (stream, 2nd)	Run-off	0.940	0.342
R3 (stream, 1st)	Run-off	2.226	0.469
R3 (stream, 2nd)	Run-off	3.570	1.011
R4 (stream, 1st)	Spray drift	0.522	0.054
R4 (stream, 2nd)	Run-off	4.808	1.255

Time dependent PEC values or time-weighted average concentrations are not included in the LoEP, because they were not used in the risk assessment.

FOCUS STEP 4
Hops, 1 x 150 g/ha

Buffer Width & Type	FOCUS Scenario	PEC_{sw} [µg/L] Drift Reduction				PEC_{sed} [µg/kg] Drift Reduction			
		25%	50%	75%	90%	25%	50%	75%	90%
0m (drift)	R1 (pond, 1st)	0.296	0.197	0.099	0.039	0.607	0.415	0.218	0.094
	R1 (stream, 1st)	4.149	2.766	1.383	0.553	0.273	0.184	0.093	0.072
5m (drift)	R1 (pond, 1st)	0.445	0.223	0.111	0.045	0.891	0.465	0.244	0.105
	R1 (stream, 1st)	4.515	2.258	1.129	0.452	0.297	0.151	0.076	0.071
10m (drift & run-off)	R1 (pond, 1st)	0.253	0.126	0.063	0.025	0.522	0.273	0.142	0.061
	R1 (stream, 1st)	2.354	1.177	0.589	0.235*	0.157	0.080	0.04	0.031
15m (drift & run-off)	R1 (pond, 1st)	0.141	0.071	0.035	0.014*	0.302	0.158	0.083	0.036
	R1 (stream, 1st)	1.554	0.777	0.388	0.155*	0.104	0.053	0.032	0.03
20m (drift & run-off)	R1 (pond, 1st)	0.078	0.039	0.02*	0.008*	0.174	0.091	0.048	0.021
	R1 (stream, 1st)	0.708	0.354	0.177*	0.071*	0.048	0.025	0.017	0.016

The mitigation from 10 m onwards includes spray drift and concurrent run-off buffer. However, as can be seen from the linear decrease of PEC_{sw} values with increasing drift reduction, the PEC_{sw} is always drift dominated and the run-off buffer does not drive the PEC_{sw}. * reduction of the overall drift percentage is >95%, compared to STEP3, in case drift leads to PECsw max.

FOCUS STEP 4
Lettuce, 1 x 125 g/ha (biennial)

Buffer Width & Type	FOCUS Scenario	PECsw [µg/L] Drift Reduction				PECsed [µg/kg] Drift Reduction			
		25%	50%	75%	90%	25%	50%	75%	90%

0m (drift)	D3 (ditch, 1st)	0.632	0.434	0.235	0.117	0.335	0.334	0.333	0.332
	D3 (ditch, 2nd)	0.643	0.446	0.249	0.130	0.419	0.399	0.398	0.397
	D4 (pond, 1st)	1.034	1.034	1.033	1.033	4.538	4.531	4.524	4.520
	D4 (stream, 1st)	0.721	0.721	0.721	0.721	1.771	1.771	1.771	1.771
	D6 (ditch, 1st)	1.268	1.268	1.268	1.268	1.766	1.765	1.765	1.764
	R1 (pond, 1st)	0.055	0.050	0.045	0.043	0.148	0.134	0.119	0.111
	R1 (stream, 1st)	0.858	0.858	0.858	0.858	0.210	0.208	0.207	0.206
	R1 (pond, 2nd)	0.092	0.087	0.082	0.079	0.240	0.225	0.210	0.201
	R1 (stream, 2nd)	1.186	1.186	1.186	1.186	0.333	0.332	0.330	0.330
	R2 (stream, 1st)	1.586	1.586	1.586	1.586	0.520	0.520	0.519	0.518
	R2 (stream, 2nd)	0.940	0.940	0.940	0.940	0.342	0.341	0.341	0.340
	R3 (stream, 1st)	2.226	2.226	2.226	2.226	0.466	0.462	0.459	0.456
	R3 (stream, 2nd)	3.570	3.570	3.570	3.570	1.006	1.000	0.995	0.991
	R4 (stream, 1st)	0.392	0.261	0.131	0.074	0.041	0.027	0.025	0.024
	R4 (stream, 2nd)	4.808	4.808	4.808	4.808	1.253	1.251	1.249	1.248

FOCUS STEP 4
Lettuce, 1 x 125 g/ha (biennial)

Buffer Width & Type	**FOCUS Scenario**	**PEC_{sw} [µg/L] Drift Reduction**				**PEC_{sed} [µg/kg] Drift Reduction**			
		0%	**50%**	**75%**	**90%**	**0%**	**50%**	**75%**	**90%**
5m (drift)	D3 (ditch, 1st)	0.252	0.145	0.091	0.059*	0.333	0.332	0.332	0.332
	D3 (ditch, 2nd)	0.265	0.158	0.105	0.073*	0.398	0.397	0.397	0.396
	D4 (pond, 1st)	1.035	1.034	1.033	1.033	4.541	4.529	4.523	4.520
	D4 (stream, 1st)	0.721	0.721	0.721	0.721	1.771	1.771	1.771	1.771
	D6 (ditch, 1st)	1.268	1.268	1.268	1.268	1.765	1.764	1.764	1.764
	R1 (pond, 1st)	0.057	0.049	0.045	0.042	0.154	0.130	0.117	0.11
	R1 (stream, 1st)	0.858	0.858	0.858	0.858	0.208	0.207	0.206	0.206
	R1 (pond, 2nd)	0.094	0.086	0.082	0.079	0.247	0.221	0.208	0.201
	R1 (stream,2nd)	1.186	1.186	1.186	1.186	0.331	0.330	0.33	0.329
	R2 (stream, 1st)	1.586	1.586	1.586	1.586	0.519	0.518	0.518	0.518
	R2 (stream,2nd)	0.940	0.940	0.940	0.940	0.341	0.341	0.34	0.34
	R3 (stream, 1st)	2.226	2.226	2.226	2.226	0.460	0.458	0.456	0.455
	R3 (stream,2nd)	3.570	3.570	3.570	3.570	0.997	0.993	0.991	0.99
	R4 (stream, 1st)	0.191	0.095	0.074	0.074	0.025	0.025	0.024	0.024
	R4 (stream,2nd)	4.808	4.808	4.808	4.808	1.250	1.248	1.247	1.247
10m (drift and run-	D3 (ditch, 1st)	0.151	0.094	0.066*	0.049*	0.332	0.332	0.332	0.332
	D3 (ditch, 2nd)	0.165	0.108	0.080*	0.063*	0.397	0.397	0.396	0.396
	D4 (pond, 1st)	1.034	1.033	1.033	1.033	4.534	4.526	4.522	4.519

FOCUS STEP 4 Lettuce, 1 x 125 g/ha (biennial)									
Buffer Width & Type	**FOCUS Scenario**	**PEC_{sw} [µg/L] Drift Reduction**				**PEC_{sed} [µg/kg] Drift Reduction**			
		0%	**50%**	**75%**	**90%**	**0%**	**50%**	**75%**	**90%**
off)	D4 (stream, 1st)	0.721	0.721	0.721	0.721	1.771	1.771	1.771	1.771
	D6 (ditch, 1st)	1.268	1.268	1.268	1.268	1.764	1.764	1.764	1.764
	R1 (pond, 1st)	0.029	0.022	0.019	0.018	0.083	0.064	0.055	0.049
	R1 (stream, 1st)	0.389	0.389	0.389	0.389	0.097	0.097	0.097	0.096
	R1 (pond, 2nd)	0.043	0.037	0.034	0.032	0.122	0.103	0.094	0.088
	R1 (stream,2nd)	0.540	0.540	0.540	0.540	0.157	0.157	0.157	0.156
	R2 (stream, 1st)	0.716	0.716	0.716	0.716	0.228	0.228	0.228	0.228
	R2 (stream,2nd)	0.422	0.422	0.422	0.422	0.151	0.151	0.151	0.151
	R3 (stream, 1st)	1.009	1.009	1.009	1.009	0.219	0.217	0.216	0.216
	R3 (stream,2nd)	1.630	1.630	1.630	1.630	0.452	0.450	0.449	0.448
	R4 (stream, 1st)	0.101	0.051	0.034	0.034	0.012	0.012	0.012	0.011
	R4 (stream,2nd)	2.184	2.184	2.184	2.184	0.585	0.584	0.584	0.584
15m (drift and run-off)	D3 (ditch, 1st)	0.115	0.076	0.057*	0.045*	0.332	0.332	0.332	0.332
	D3 (ditch, 2nd)	0.129	0.090	0.071*	0.059*	0.397	0.396	0.396	0.396
	D4 (pond, 1st)	1.034	1.033	1.033	1.033	4.531	4.524	4.521	4.519
	D4 (stream, 1st)	0.721	0.721	0.721	0.721	1.771	1.771	1.771	1.771
	D6 (ditch, 1st)	1.268	1.268	1.268	1.268	1.764	1.764	1.764	1.764
	R1 (pond, 1st)	0.018	0.013	0.011	0.009	0.055	0.039	0.032	0.027
	R1 (stream, 1st)	0.204	0.204	0.204	0.204	0.053	0.052	0.052	0.052
	R1 (pond, 2nd)	0.025	0.021	0.018	0.017	0.076	0.060	0.052	0.047
	R1 (stream,2nd)	0.283	0.283	0.283	0.283	0.086	0.085	0.085	0.085
	R2 (stream, 1st)	0.375	0.375	0.375	0.375	0.122	0.122	0.122	0.122
	R2 (stream,2nd)	0.220	0.220	0.220	0.220	0.081	0.081	0.081	0.081
	R3 (stream, 1st)	0.528	0.528	0.528	0.528	0.119	0.118	0.117	0.117
	R3 (stream,2nd)	0.856	0.856	0.856	0.856	0.245	0.243	0.242	0.242
	R4 (stream, 1st)	0.069	0.035	0.018	0.018	0.008	0.006	0.006	0.006
	R4 (stream,2nd)	1.144	1.144	1.144	1.144	0.318	0.317	0.317	0.316
20m (drift and run-off)	D3 (ditch, 1st)	0.097	0.067*	0.052*	0.045*	0.332	0.332	0.332	0.332
	D3 (ditch, 2nd)	0.110	0.081*	0.066*	0.057*	0.397	0.396	0.396	0.396
	D4 (pond, 1st)	1.033	1.033	1.033	1.033	4.529	4.523	4.520	4.518
	D4 (stream, 1st)	0.721	0.721	0.721	0.721	1.771	1.771	1.771	1.771

FOCUS STEP 4 Lettuce, 1 x 125 g/ha (biennial)									
Buffer Width & Type	**FOCUS Scenario**	**PEC_{sw} [µg/L] Drift Reduction**				**PEC_{sed} [µg/kg] Drift Reduction**			
		0%	**50%**	**75%**	**90%**	**0%**	**50%**	**75%**	**90%**
	D6 (ditch, 1st)	1.268	1.268	1.268	1.268	1.764	1.764	1.764	1.764
	R1 (pond, 1st)	0.016	0.012	0.010	0.009	0.050	0.037	0.03	0.026
	R1 (stream, 1st)	0.204	0.204	0.204	0.204	0.052	0.052	0.052	0.052
	R1 (pond, 2nd)	0.024	0.020	0.018	0.016	0.071	0.057	0.05	0.046
	R1 (stream,2nd)	0.283	0.283	0.283	0.283	0.086	0.085	0.085	0.085
	R2 (stream, 1st)	0.375	0.375	0.375	0.375	0.122	0.122	0.122	0.122
	R2 (stream,2nd)	0.220	0.220	0.220	0.220	0.081	0.081	0.081	0.081
	R3 (stream, 1st)	0.528	0.528	0.528	0.528	0.118	0.117	0.117	0.117
	R3 (stream,2nd)	0.856	0.856	0.856	0.856	0.244	0.243	0.242	0.242
	R4 (stream, 1st)	0.053	0.026	0.018	0.018	0.007	0.006	0.006	0.006
	R4 (stream,2nd)	1.144	1.144	1.144	1.144	0.317	0.317	0.317	0.316

* reduction of the overall drift percentage is >95%, compared to STEP3, in case drift leads to PECsw max.

FOCUS STEP 4 Lettuce, 1 x 125 g/ha (annual)									
Buffer Width & Type	**FOCUS Scenario**	**PEC_{sw} [µg/L] Drift Reduction**				**PEC_{sed} [µg/kg] Drift Reduction**			
		0%	**50%**	**75%**	**90%**	**0%**	**50%**	**75%**	**90%**
5m (drift)	D3 (ditch, 1st)	0.234	0.127	0.073	0.040*	0.188	0.187	0.187	0.186
	D3 (ditch, 2nd)	0.234	0.127	0.073	0.041*	0.177	0.173	0.172	0.172
	D4 (pond, 1st)	0.982	0.978	0.976	0.975	3.750	3.732	3.723	3.718
	D4 (stream, 1st)	0.969	0.969	0.969	0.969	1.395	1.394	1.394	1.394
	D6 (ditch, 1st)	1.206	1.206	1.206	1.206	1.958	1.958	1.958	1.958
	R1 (pond, 1st)	0.334	0.325	0.320	0.317	0.744	0.723	0.712	0.706
	R1 (stream, 1st)	2.827	2.827	2.827	2.827	0.694	0.693	0.692	0.692
	R1 (pond, 2nd)	0.137	0.130	0.127	0.125	0.404	0.379	0.367	0.359
	R1 (stream,2nd)	2.189	2.189	2.189	2.189	0.411	0.410	0.409	0.409
	R2 (stream, 1st)	1.215	1.215	1.215	1.215	0.510	0.509	0.508	0.508
	R2 (stream,2nd)	1.044	1.044	1.044	1.044	0.243	0.242	0.242	0.241
	R3 (stream, 1st)	3.698	3.698	3.698	3.698	0.780	0.775	0.772	0.771
	R3 (stream,2nd)	3.297	3.297	3.297	3.297	0.949	0.945	0.942	0.941

FOCUS STEP 4 Lettuce, 1 x 125 g/ha (annual)									
Buffer Width & Type	**FOCUS Scenario**	**PEC_{sw} [µg/L] Drift Reduction**				**PEC_{sed} [µg/kg] Drift Reduction**			
		0%	**50%**	**75%**	**90%**	**0%**	**50%**	**75%**	**90%**
	R4 (stream, 1st)	0.830	0.830	0.830	0.830	0.239	0.238	0.238	0.238
	R4 (stream,2nd)	2.954	2.954	2.954	2.954	0.797	0.796	0.795	0.794
10m (drift and run-off)	D3 (ditch, 1st)	0.133	0.076	0.048*	0.031*	0.187	0.187	0.186	0.186
	D3 (ditch, 2nd)	0.133	0.077	0.048*	0.031*	0.173	0.172	0.172	0.172
	D4 (pond, 1st)	0.980	0.977	0.976	0.975	3.740	3.727	3.720	3.717
	D4 (stream, 1st)	0.969	0.969	0.969	0.969	1.394	1.394	1.394	1.394
	D6 (ditch, 1st)	1.206	1.206	1.206	1.206	1.958	1.958	1.958	1.958
	R1 (pond, 1st)	0.143	0.135	0.132	0.130	0.339	0.323	0.314	0.309
	R1 (stream, 1st)	1.270	1.270	1.270	1.270	0.294	0.293	0.293	0.292
	R1 (pond, 2nd)	0.060	0.055	0.052	0.051	0.196	0.177	0.168	0.162
	R1 (stream,2nd)	0.954	0.954	0.954	0.954	0.193	0.193	0.192	0.192
	R2 (stream, 1st)	0.553	0.553	0.553	0.553	0.207	0.206	0.206	0.206
	R2 (stream,2nd)	0.469	0.469	0.469	0.469	0.108	0.108	0.108	0.108
	R3 (stream, 1st)	1.683	1.683	1.683	1.683	0.367	0.365	0.363	0.362
	R3 (stream,2nd)	1.506	1.506	1.506	1.506	0.403	0.400	0.399	0.398
	R4 (stream, 1st)	0.379	0.379	0.379	0.379	0.112	0.112	0.111	0.111
	R4 (stream,2nd)	1.345	1.345	1.345	1.345	0.373	0.372	0.372	0.372
15m (drift and run-off)	D3 (ditch, 1st)	0.097	0.058*	0.038*	0.027*	0.187	0.186	0.186	0.186
	D3 (ditch, 2nd)	0.098	0.059*	0.039*	0.028*	0.173	0.172	0.172	0.172
	D4 (pond, 1st)	0.979	0.977	0.976	0.975	3.734	3.724	3.719	3.716
	D4 (stream, 1st)	0.969	0.969	0.969	0.969	1.394	1.394	1.394	1.394
	D6 (ditch, 1st)	1.206	1.206	1.206	1.206	1.958	1.958	1.958	1.958
	R1 (pond, 1st)	0.140	0.134	0.131	0.129	0.332	0.319	0.313	0.309
	R1 (stream, 1st)	1.270	1.270	1.270	1.270	0.293	0.293	0.293	0.292
	R1 (pond, 2nd)	0.058	0.054	0.052	0.051	0.188	0.173	0.166	0.161
	R1 (stream,2nd)	0.954	0.954	0.954	0.954	0.193	0.193	0.192	0.192
	R2 (stream, 1st)	0.553	0.553	0.553	0.553	0.207	0.206	0.206	0.206
	R2 (stream,2nd)	0.469	0.469	0.469	0.469	0.108	0.108	0.108	0.107
	R3 (stream, 1st)	1.683	1.683	1.683	1.683	0.366	0.364	0.363	0.362
	R3 (stream,2nd)	1.506	1.506	1.506	1.506	0.401	0.399	0.398	0.398
	R4 (stream, 1st)	0.379	0.379	0.379	0.379	0.112	0.111	0.111	0.111

FOCUS STEP 4 Lettuce, 1 x 125 g/ha (annual)									
Buffer Width & Type	**FOCUS Scenario**	**PEC_{sw} [µg/L] Drift Reduction**				**PEC_{sed} [µg/kg] Drift Reduction**			
		0%	**50%**	**75%**	**90%**	**0%**	**50%**	**75%**	**90%**
	R4 (stream,2nd)	1.345	1.345	1.345	1.345	0.373	0.372	0.372	0.372
20m (drift and run-off)	D3 (ditch, 1st)	0.078	0.049*	0.034*	0.025*	0.187	0.186	0.186	0.186
	D3 (ditch, 2nd)	0.079	0.049*	0.035*	0.026*	0.172	0.172	0.172	0.172
	D4 (pond, 1st)	0.978	0.976	0.976	0.975	3.731	3.723	3.718	3.716
	D4 (stream, 1st)	0.969	0.969	0.969	0.969	1.394	1.394	1.394	1.394
	D6 (ditch, 1st)	1.206	1.206	1.206	1.206	1.958	1.958	1.958	1.958
	R1 (pond, 1st)	0.074	0.069	0.067	0.066	0.185	0.173	0.167	0.164
	R1 (stream, 1st)	0.662	0.662	0.662	0.662	0.156	0.155	0.155	0.155
	R1 (pond, 2nd)	0.032	0.028	0.027	0.026	0.111	0.097	0.091	0.087
	R1 (stream,2nd)	0.500	0.500	0.500	0.500	0.104	0.104	0.104	0.104
	R2 (stream, 1st)	0.290	0.290	0.290	0.290	0.109	0.108	0.108	0.108
	R2 (stream,2nd)	0.244	0.244	0.244	0.244	0.058	0.057	0.057	0.057
	R3 (stream, 1st)	0.882	0.882	0.882	0.882	0.199	0.198	0.197	0.196
	R3 (stream,2nd)	0.790	0.790	0.790	0.790	0.214	0.212	0.212	0.211
	R4 (stream, 1st)	0.199	0.199	0.199	0.199	0.060	0.060	0.060	0.060
	R4 (stream,2nd)	0.705	0.705	0.705	0.705	0.202	0.202	0.202	0.202

* reduction of the overall drift percentage is >95%, compared to STEP3, in case drift leads to PECsw max.

Parameters used in FOCUSsw step 1 and 2 **Metabolite 6-CNA**	Molecular weight (g mol^{-1}): 157.6 Water solubility (mg/L): 1430 at 20ºC Soil or water metabolite: soil K_{oc} (L/kg): 88 Geometric mean DT_{50} soil (d): 4.7 at 20ºC/pF_2 Geometric mean DT_{50} water/sed (d): 1000 DT_{50} water (d): 1000 (system value) DT_{50} sediment (d): 1000 (system value) Crop interception (%): see parent Maximum occurrence observed (% molar basis with respect to the parent) Water/sediment: 0 Soil: 17.1

Parameters used in FOCUSsw step 1 and 2 **Metabolite DFA**	Molecular weight (g mol^{-1}): 96.0 Water solubility (mg/L): 500000 at 20ºC Soil or water metabolite: soil and water K_{oc} (L/kg): 6.8 Geometric mean DT_{50} soil (d): 44.7 at 20ºC/pF_2 Geometric mean DT_{50} water/sed (d): 249 DT_{50} water (d): 249 (system value) DT_{50} sediment (d): 249 (system value) Crop interception (%): see parent Maximum occurrence observed (% molar basis with respect to the parent) Water/sediment: 6.9 Soil: 33.9
Parameters used in FOCUSsw step 1 and 2 **Metabolite BYI 02960-succinamide**	Molecular weight (g mol^{-1}): 306.7 Water solubility (mg/L): 120000 at 20ºC Soil or water metabolite: water K_{oc} (L/kg): 0 DT_{50} soil (d): 0.1 DT_{50} water/sed (d): 1000 DT_{50} water (d): 1000 DT_{50} sediment (d): 1000 Crop interception (%): see parent Maximum occurrence observed (% molar basis with respect to the parent) Water/sediment: 39.6 (photolysis) Soil: 0
Parameters used in FOCUSsw step 1 and 2 **Metabolite BYI 02960-azabicyclo succinamide**	Molecular weight (g mol^{-1}): 288.3 Water solubility (mg/L): 180000 at 20ºC Soil or water metabolite: water K_{oc} (L/kg): 0 DT_{50} soil (d): 0.1 DT_{50} water/sed (d): 1000 DT_{50} water (d): 1000 DT_{50} sediment (d): 1000 Crop interception (%): see parent Maximum occurrence observed (% molar basis with respect to the parent) Water/sediment: 25.9 (photolysis) Soil: 0
Application rate	See parent
Main routes of entry	Drift of parent

Crop	**FOCUS STEP**	**Difluoroacetic acid**	**6-Chloronicotinic acid**	**BYI 02960-succinamide**	**BYI 02960-azabicyclo-succinamide**

		PEC_{sw} (µg/L)	PEC_{sed} (µg/kg)	PEC_{sw} (µg/L)	PEC_{sed} (µg/kg)	PEC_{sw} (µg/L)	PEC_{sed} (µg/kg)	PEC_{sw} (µg/L)	PEC_{sed} (µg/kg)
Hops 1 x 150 g/ha	1	5.810	0.380	4.177	3.675	4.065	<0.001	2.499	<0.001
	2 N-EU	0.743	0.050	0.232	0.204	4.065	<0.001	2.499	<0.001
	2 S-EU	1.268	0.086	0.463	0.408	4.065	<0.001	2.499	<0.001
Lettuce (biennial) 1 x 125 g/ha	1	4.683	0.317	3.481	3.063	0.484	<0.001	0.297	<0.001
	2 N-EU	0.682	0.046	0.289	0.255	0.484	<0.001	0.297	<0.001
	2 S-EU	1.339	0.091	0.579	0.509	0.484	<0.001	0.297	<0.001
Lettuce (annual) 1 x 125 g/ha	1	4.683	0.317	3.481	3.063	0.484	<0.001	0.297	<0.001
	2 N-EU	0.551	0.037	0.193	0.170	0.484	<0.001	0.297	<0.001
	2 S-EU	1.076	0.073	0.386	0.340	0.484	<0.001	0.297	<0.001

PEC (ground water) (Annex IIIA, point 9.2.1)

Method of calculation and type of study (*e.g.* modelling, field leaching, lysimeter)	For FOCUS gw modeling, values used – Modeling using FOCUS model(s), with appropriate FOCUSgw scenarios, according to FOCUS guidance. Models used: FOCUS-PEARL 4.4.4 PELMO 4.4.3 Scenarios (list of names): seven for hops all nine for lettuce Crop: hops leafy vegetables for lettuce
Flupyradifurone (BYI 02960)	Molecular weight (g/mol): 288.7 Water solubility (mg/L): 3200 at 20ºC Vapour pressure (Pa): 9.1×10^{-7} Geometric mean DT_{50} (d): 94.8 (normalisation to 10kPa or pF2, 20°C with Q_{10} of 2.58; Tier 1) DT_{50} (d): 33.4 (fast; Tier 2a (DFOP)) 94.8 (slow; Tier 2a (DFOP)) g 0.43 (Tier 2a (DFOP)) Arithmetic mean K_{om} (L/kg): 57.1 (Tier 1 and 2a (DFOP)) Arithmetic mean K_{oc} (L/kg): 98.4 (Tier 1 and 2a (DFOP)) Freundlich exponent (1/n): 0.8657 (Tier 1 and 2a(DFOP)) Plant uptake factor: 0.5 (systemic active substance) Q_{10}: 2.58 Molar activation energy (kJ/mol): 65.4

Metabolite 6-CNA	Molecular weight (g/mol): 157.6 * Water solubility (mg/L): 1430 Vapour pressure (Pa): 1.0×10^{-10} Geometric mean DT_{50} (d): 4.7 (normalisation to 10kPa or pF_2, 20°C with Q_{10} of 2.58) Arithmetic mean K_{om} (L/kg): 51 Arithmetic mean K_{oc} (L/kg): 88 Freundlich exponent (1/n): 0.949 Kinetic formation fraction: 0.478 from parent ** Plant uptake factor: 0.0 ~~0.5 ****~~ Q_{10}: 2.58 Molar activation energy (kJ/mol): 65.4
Metabolite DFA	Molecular weight (g/mol): 96.0 Water solubility (mg/L): 500000 Vapour pressure (Pa): 1.0×10^{-10} Geometric mean DT_{50} (d): 44.7 (normalisation to 10kPa or pF_2, 20°C with Q_{10} of 2.58) Arithmetic mean K_{om} (L/kg): 3.9 Arithmetic mean K_{oc} (L/kg): 6.8 Freundlich exponent (1/n): 0.835 Kinetic formation fraction: 0.833 from parent ** Plant uptake factor: 0.0 ~~0.5 ****~~ Q_{10}: 2.58 Molar activation energy (kJ/mol): 65.4
Application rate	Application rate (g/ha): 150 (hops, annual and biennial) 125 (lettuce, field annual and biennial) 125 (lettuce, glasshouse)~~***~~ Number of applications: 1 (hops annual and biennial) 1 (lettuce, field annual and biennial) 2 (lettuce, glasshouse) Interval (d): n.a. (hops and lettuce field) 10 (lettuce, glass) Time of application: BBCH 31-75 (hops annual and biennial) BBCH 41-49 (lettuce annual) BBCH 12-49 (lettuce biennual) (month or season) BBCH 12-49 (lettuce, glasshouse) Plant interception (%): 60 (hops annual and biennial) 70 (lettuce annual) 25 (lettuce biennual)) 25 (lettuce, glasshouse)

* The sum of formation fractions of both metabolites is > 1. In order to run the modeling with PELMO, the molar mass of 6-CNA was set to 451 g/mol, resulting from $M_{auxiliary}$ (6-CNA) = M (6-CNA) × 0.478 / (1-0.833) = 451 g/mol
** Kinetic formation fraction used for PEARL, values used for PELMO see DAR;
Additional higher Tier PECgw calculations with time dependent sorption are presented in the DAR.
~~*** Glass house application worst case for groundwater, no field applications included in LoEP~~
~~**** Data provided to justify that the plant uptake factor for the active substance and metabolites is 0.5~~

PEC(gw) - FOCUS modelling results (80th percentile annual average concentration at 1m)

HOPS annual and biennial

Scenario	PEC_{gw} BYI02960 (µg/L) annual			
	Tier I		Tier 2a (DFOP)	
	Hops PEARL	Hops PELMO	Hops PEARL	Hops PELMO
Châteaudun	0.457	0.409	0.275	0.247
Hamburg	0.567	0.591	0.338	0.358
Kremsmünster	0.409	0.461	0.246	0.277
Piacenza	0.377	0.458	0.224	0.275
Porto	0.214	0.275	0.130	0.166
Sevilla	0.219	*0.060*	0.134	*0.037*
Thiva	0.178	0.154	0.108	*0.094*

In italics: values pass the trigger of 0.1 µg/L

Scenario	PEC_{gw} BYI02960 (µg/L) biennial			
	Tier I		Tier 2a (DFOP)	
	Hops PEARL	Hops PELMO	Hops PEARL	Hops PELMO
Châteaudun	0.197	0.169	0.119	0.103
Hamburg	0.271	0.230	0.164	0.140
Kremsmünster	0.169	0.189	0.102	0.114
Piacenza	0.162	0.210	*0.097*	0.126
Porto	*0.091*	0.115	*0.055*	*0.069*
Sevilla	*0.090*	*0.022*	*0.055*	*0.014*
Thiva	*0.073*	*0.058*	*0.045*	*0.036*

In italics: values pass the trigger of 0.1 µg/L

Scenario	PEC_{gw} 6-CNA (µg/L)			
	Tier I annual		Tier I biennial	
	Hops PEARL	Hops PELMO	Hops PEARL	Hops PELMO
Châteaudun	*0.010*	*0.009*	*0.005*	*0.004*
Hamburg	*0.011*	*0.012*	*0.006*	*0.005*
Kremsmünster	*0.009*	*0.010*	*0.004*	*0.004*
Piacenza	*0.008*	*0.009*	*0.004*	*0.005*
Porto	*0.006*	*0.007*	*0.002*	*0.003*
Sevilla	*0.005*	*0.002*	*0.002*	*0.001*
Thiva	*0.004*	*0.004*	*0.002*	*0.002*

In italics: values pass the trigger of 0.1 µg/L

Scenario	PEC_{gw} DFA (µg/L) annual			
	Tier I		Tier 2a (DFOP parent)	
	Hops PEARL	Hops PELMO	Hops PEARL	Hops PELMO
Châteaudun	1.554	1.377	1.459	1.299
Hamburg	1.699	1.830	1.631	1.771
Kremsmünster	1.064	1.228	1.020	1.189
Piacenza	1.059	0.957	0.953	0.904
Porto	**0.696**	0.758	**0.608**	**0.691**
Sevilla	0.976	0.776	0.916	**0.715**
Thiva	0.912	0.916	0.817	0.838

In bold: values pass the trigger of 0.75 µg/L

Scenario	PEC_{gw} DFA (µg/L) biennial			
	Tier I		Tier 2a (DFOP parent)	
	Hops PEARL	Hops PELMO	Hops PEARL	Hops PELMO
Châteaudun	0.754	**0.697**	**0.722**	**0.681**
Hamburg	0.793	0.852	0.772	0.836
Kremsmünster	**0.525**	**0.596**	**0.515**	**0.592**
Piacenza	**0.511**	**0.448**	**0.473**	**0.439**
Porto	**0.318**	**0.350**	**0.277**	**0.315**
Sevilla	**0.479**	**0.428**	**0.459**	**0.424**
Thiva	**0.450**	**0.426**	**0.415**	**0.395**

In bold: values pass the trigger of 0.75 µg/L

Lettuce field annual

Scenario	PEC_{gw} BYI02960 (µg/L) annual (1st cropping)			
	Tier I		Tier 2a (DFOP)	
	Lettuce PEARL	Lettuce PELMO	Lettuce PEARL	Lettuce PELMO
Châteaudun	0.106	*0.065*	*0.065*	*0.040*
Hamburg	0.259	0.212	0.156	0.129
Jokionen	*0.095*	*0.080*	*0.059*	*0.050*
Kremsmünster	0.175	0.149	0.106	*0.090*
Porto	0.106	0.130	*0.064*	*0.078*
Sevilla	*0.002*	*0.001*	*0.002*	*0.001*
Thiva	*0.043*	*0.031*	0.027	*0.020*

In italics: values pass the trigger of 0.1µg/L
In bold: values pass the trigger of 0.75 µg/L

Scenario	PECgw BYI02960 (µg/L) annual (2nd cropping)			
	Tier I		Tier 2a (DFOP)	
	Lettuce PEARL	Lettuce PELMO	Lettuce PEARL	Lettuce PELMO
Châteaudun	0.135	*0.089*	*0.083*	0.056
Hamburg	0.319	0.286	0.193	0.175
Kremsmünster	0.216	0.185	0.130	0.113
Porto	0.211	0.253	0.128	0.155
Sevilla	*0.004*	*0.001*	*0.003*	*0.001*

In italics: values pass the trigger of 0.1µg/L
In bold: values pass the trigger of 0.75 µg/L

Scenario	PEC_{gw} 6-CNA (µg/L) annual			
	Tier I (1st cropping)		Tier I (2nd cropping)	
	Lettuce PEARL	Lettuce PELMO	Lettuce PEARL	Lettuce PELMO
Châteaudun	*0.003*	*0.002*	*0.003*	*0.003*
Hamburg	*0.005*	*0.005*	*0.007*	*0.006*
Jokionen	*0.003*	*0.002*	-	-
Kremsmünster	*0.004*	*0.004*	*0.005*	*0.004*
Porto	*0.003*	*0.004*	*0.005*	*0.007*
Sevilla	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Thiva	*0.001*	*0.002*	-	-

In italics: values pass the trigger of 0.1µg/L
In bold: values pass the trigger of 0.75 µg/L

Scenario	PEC_{gw} DFA (µg/L) annual (1st cropping)			
	Tier I		Tier 2a (DFOP parent)	
	Lettuce PEARL	Lettuce PELMO	Lettuce PEARL	Lettuce PELMO
Châteaudun	**0.691**	**0.554**	**0.657**	**0.543**
Hamburg	1.165	0.990	1.141	0.987
Jokionen	1.064	0.961	1.037	0.974
Kremsmünster	**0.670**	**0.613**	**0.650**	**0.618**
Porto	**0.412**	**0.393**	**0.373**	**0.374**
Sevilla	**0.237**	**0.179**	**0.211**	**0.164**
Thiva	**0.280**	**0.261**	**0.252**	**0.238**

In italics: values pass the trigger of 0.1µg/L
In bold: values pass the trigger of 0.75 µg/L

Scenario	PEC_{gw} DFA (µg/L) annual (2st cropping)			
	Tier I		Tier 2a (DFOP parent)	
	Lettuce PEARL	Lettuce PELMO	Lettuce PEARL	Lettuce PELMO
Châteaudun	**0.691**	**0.567**	**0.654**	**0.558**
Hamburg	1.179	1.082	1.153	1.088
Kremsmünster	**0.671**	**0.632**	**0.651**	**0.638**
Porto	**0.456**	**0.435**	**0.479**	**0.472**
Sevilla	**0.362**	**0.272**	**0.376**	**0.291**

In italics: values pass the trigger of 0.1µg/L
In bold: values pass the trigger of 0.75 µg/L

Lettuce field biennial

Scenario	PEC_{gw} BYI02960 (µg/L) biennial (1st cropping)			
	Tier I		Tier 2a (DFOP)	
	Lettuce PEARL	Lettuce PELMO	Lettuce PEARL	Lettuce PELMO
Châteaudun	**0.154**	**0.106**	*0.065*	*0.040*
Hamburg	**0.379**	**0.288**	**0.156**	0.129
Jokionen	**0.110**	*0.084*	*0.059*	*0.050*
Kremsmünster	**0.242**	**0.203**	**0.106**	*0.090*
Porto	**0.138**	**0.169**	*0.064*	*0.078*
Sevilla	*0.006*	*0.002*	*0.002*	*0.001*
Thiva	**0.111**	*0.065*	*0.027*	*0.020*

In italics: values pass the trigger of 0.1µg/L
In bold: values pass the trigger of 0.75 µg/L

Scenario	PECgw BYI02960 (µg/L) biennial (2nd cropping)			
	Tier I		Tier 2a (DFOP)	
	Lettuce PEARL	Lettuce PELMO	Lettuce PEARL	Lettuce PELMO
Châteaudun	**0.207**	**0.137**	**0.126**	*0.083*
Hamburg	**0.443**	**0.398**	**0.270**	**0.237**
Kremsmünster	**0.291**	**0.244**	**0.177**	**0.149**
Porto	**0.253**	**0.302**	**0.153**	**0.178**
Sevilla	*0.009*	*0.002*	*0.006*	*0.002*

In italics: values pass the trigger of 0.1µg/L
In bold: values pass the trigger of 0.75 µg/L

Scenario	PEC_{gw} 6-CNA (µg/L) biennual			
	Tier I (1st cropping)		Tier I (2nd cropping)	
	Lettuce PEARL	Lettuce PELMO	Lettuce PEARL	Lettuce PELMO
Châteaudun	*0.004*	*0.003*	*0.005*	*0.003*
Hamburg	*0.008*	*0.006*	*0.009*	*0.008*
Jokionen	*0.003*	*0.003*	-	-
Kremsmünster	*0.005*	*0.005*	*0.006*	*0.006*
Porto	*0.004*	*0.004*	*0.006*	*0.007*
Sevilla	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Thiva	*0.003*	*0.002*	-	-

In italics: values pass the trigger of 0.1µg/L
In bold: values pass the trigger of 0.75 µg/L

Scenario	PEC_{gw} DFA (µg/L) biennual (1st cropping)			
	Tier I		Tier 2a (DFOP parent)	
	Lettuce PEARL	Lettuce PELMO	Lettuce PEARL	Lettuce PELMO
Châteaudun	0.847	**0.668**	0.801	**0.651**
Hamburg	1.393	1.169	1.357	1.168
Jokionen	1.393	1.197	1.372	1.207
Kremsmünster	0.852	**0.778**	0.823	0.769
Porto	**0.461**	**0.394**	**0.397**	**0.365**
Sevilla	**0.295**	**0.236**	**0.258**	**0.219**
Thiva	**0.626**	**0.519**	**0.668**	**0.561**

In italics: values pass the trigger of 0.1µg/L
In bold: values pass the trigger of 0.75 µg/L

Scenario	PEC_{gw} DFA (µg/L) biennual (2st cropping)			
	Tier I		Tier 2a (DFOP parent)	
	Lettuce PEARL	Lettuce PELMO	Lettuce PEARL	Lettuce PELMO
Châteaudun	0.946	**0.748**	0.955	0.783
Hamburg	1.517	1.411	1.546	1.462
Kremsmünster	0.892	**0.880**	0.906	0.906
Porto	**0.660**	**0.626**	**0.688**	**0.653**
Sevilla	**0.379**	**0.290**	**0.369**	**0.293**

In italics: values pass the trigger of 0.1µg/L
In bold: values pass the trigger of 0.75 µg/L

Lettuce glasshouse

Scenario	PEC_{gw} BYI02960 (µg/L) Glasshouse (1st cropping)			
	Tier I		Tier 2a (DFOP)	
	Lettuce PEARL	Lettuce PELMO	Lettuce PEARL	Lettuce PELMO
Châteaudun	1.132	0.786	**0.682**	**0.474**
Hamburg	2.179	1.879	1.310	1.125
Jokionen	0.985	0.769	**0.598**	**0.473**
Kremsmünster	1.503	1.330	0.898	0.797
Porto	0.877	0.980	**0.524**	**0.576**
Sevilla	*0.078*	*0.018*	*0.050*	*0.012*
Thiva	0.997	0.717	**0.606**	**0.438**

In italics: values pass the trigger of 0.1 µg/L
In bold: values pass the trigger of 0.75 µg/L

Scenario	PECgw BYI02960 (µg/L) Glasshouse (2nd cropping)			
	Tier I		Tier 2a (DFOP)	
	Lettuce PEARL	Lettuce PELMO	Lettuce PEARL	Lettuce PELMO
Châteaudun	1.543	1.073	0.927	**0.684**
Hamburg	2.843	2.540	1.706	1.534
Kremsmünster	1.819	1.654	1.089	0.991
Porto	1.691	1.787	1.008	1.070
Sevilla	**0.116**	*0.024*	*0.076*	*0.016*

In italics: values pass the trigger of 0.1 µg/L
In bold: values pass the trigger of 0.75 µg/L

Scenario	PEC_{gw} 6-CNA (µg/L) Glasshouse			
	Tier I (1st cropping)		Tier I (2nd cropping)	
	Lettuce PEARL	Lettuce PELMO	Lettuce PEARL	Lettuce PELMO
Châteaudun	*0.022*	*0.016*	*0.029*	*0.021*
Hamburg	*0.038*	*0.036*	*0.050*	*0.048*
Jokionen	*0.021*	*0.018*	-	-
Kremsmünster	*0.028*	*0.026*	*0.034*	*0.031*
Porto	*0.020*	*0.022*	*0.035*	*0.038*
Sevilla	*0.002*	*0.001*	*0.003*	*0.001*
Thiva	*0.019*	*0.015*	-	-

In italics: values pass the trigger of 0.1µg/L
In bold: values pass the trigger of 0.75 µg/L

Scenario	PEC_{gw} DFA (µg/L) Glasshouse (1st cropping)			
	Tier I		Tier 2a (DFOP parent)	
	Lettuce PEARL	Lettuce PELMO	Lettuce PEARL	Lettuce PELMO
Châteaudun	3.511	2.857	3.605	2.894
Hamburg	6.305	5.095	6.353	5.297
Jokionen	6.390	5.751	6.230	5.829
Kremsmünster	3.573	3.331	3.685	3.402
Porto	1.863	1.703	2.059	1.833
Sevilla	1.098	0.827	1.185	0.917
Thiva	3.020	2.649	2.727	2.416

In italics: values pass the trigger of 0.1µg/L
In bold: values pass the trigger of 0.75 µg/L

Scenario	PEC_{gw} DFA (µg/L) Glasshouse (2st cropping)			
	Tier I		Tier 2a (DFOP parent)	
	Lettuce PEARL	Lettuce PELMO	Lettuce PEARL	Lettuce PELMO
Châteaudun	4.267	3.627	4.127	3.474
Hamburg	7.008	6.341	6.836	6.244
Kremsmünster	3.970	3.761	3.948	3.647
Porto	2.959	2.773	2.760	2.650
Sevilla	1.669	1.283	1.589	1.280

In italics: values pass the trigger of 0.1µg/L
In bold: values pass the trigger of 0.75 µg/L

Fate and behaviour in air (Annex IIA, point 7.2.2, Annex III, point 9.3)

Direct photolysis in air	Not studied - no data requested
Quantum yield of direct phototransformation	1.38 x 10^{-4} mol/Einstein in water
Photochemical oxidative degradation in air	DT_{50} of 4.4 hours (0.18 d) derived by the Atkinson model (AOPWIN version 1.92a). OH (12 h) concentration assumed = 1.5 x 10^{-6} OH/cm^3
Volatilisation	From plant surfaces (BBA guideline): no data Vapour pressure: 9.1 x 10^{-7} Pa at 20°C
	From soil surfaces (BBA guideline): no data
Metabolites	No data

PEC (air)

Method of calculation	Expert judgement, based on Vapour pressure 9.1 x 10^{-7} Pa at 20°C,

	Henry's Law Constant 4.13 x 10^{-7} Pa m^3 mol^{-1} Atmospheric half life 4.4 – 13.1 hours residues in air are expected to be negligible.

$PEC_{(a)}$

Maximum concentration	No data provided - none requested

Residues requiring further assessment

Environmental occurring metabolite requiring further assessment by other disciplines (toxicology and ecotoxicology).	Soil:	BYI 02960, 6-CNA, DFA
	Surface Water:	BYI 02960, 6-CNA, DFA, BYI 02960-succinamide, BYI 02960-azabicyclosuccinmaide
	Sediment:	BYI 02960, 6-CNA, DFA
	Ground water:	BYI 02960, 6-CNA, DFA
	Air:	BYI 02960

Monitoring data, if available (Annex IIA, point 7.4)

Soil (indicate location and type of study)	New active substance, not applicable
Surface water (indicate location and type of study)	New active substance, not applicable
Ground water (indicate location and type of study)	New active substance, not applicable
Air (indicate location and type of study)	New active substance, not applicable

Points pertinent to the classification and proposed labelling with regard to fate and behaviour data

Not readily biodegradable; candidate for R53

Chapter 2.6 Ecotoxicology

Effects on terrestrial vertebrates (Annex IIA, point 8.1, Annex IIIA, points 10.1 and 10.3)

Species	Test substance	Time scale	End point (mg/kg bw/day)	End point (mg/kg feed)
Birds ‡				
Bobwhite quail *(Colinus virginianus)*	a.s.	Acute	LD_{50} 232	
Canary *(Serinus canaria)*	a.s.	Acute	LD_{50} 330	
Bobwhite quail *(Colinus virginianus)*	BYI 02960 SL 200[1]	Acute	LD_{50} 431 mg a.s. /kg bw	
Hen *(Gallus domesticus)*	BYI 02960 SL 200[1]	Acute	LD_{50} >342 mg a.s. /kg bw	
Mallard duck (*Anas platyrhynchos*)	a.s.	Short-term	LC_{50} >825	>4741
Bobwhite quail (*Colinus virginianus*)	a.s.	Short-term	LC_{50} >470	>4876
Mallard duck (*Anas platyrhynchos*)	a.s.	Long-term	NOAEL 83 (measured)	845
Bobwhite quail *(Colinus virginianus)*	a.s.	Long-term	NOAEL 14 (nominal)	111
Mammals ‡				
Rat	a.s.	Acute	LD_{50} 2000 (cut-off)	
Rat	a.s.	Acute neurotoxicity	LD_{50} >800	
		Combined acute endpoint :	LD_{50} 1607	
Rat	BYI 02960 SL 200 g/L[1]	Acute	LD_{50} > 2000 mg form/kg bw (>5000 cut-off)	
Rat	a.s.	Long-term	NOAEL ~~34~~ 6.4	~~500~~ 100
Rat	DFA, DFEAF, BYI 02960-CHMP, 6-CNA	see LoE mamtox		
Additional higher tier studies ‡				
-				

[1] BYI 02960 SL 200 G = BYI 02960 SL 200 g/L = Flupyradifurone SL 200 G = Sivanto

Toxicity/exposure ratios for terrestrial vertebrates (Annex IIIA, points 10.1 and 10.3)

Crop (BBCH stage) / Generic focal species	Time scale	DDD	TER[1]	Annex VI Trigger[3]
Tier 1 – uptake via diet (Birds)				
Hops (BBCH 31-75) – 1x 0.150 g a.s./ha				
Hops (≥ 20)– Small insectivorous bird “finch”				
	Acute	3.80	61	10
	Long-term	0.84	17	5
Hops (20-39)– Small granivorous bird “finch”				
	Acute	1.85	126	10
	Long-term	0.45	32	5
Lettuce (BBCH 12-49) – 1x 0.125 g a.s./ha				
Lettuce (10-19)– Medium herbivorous/granivorous bird “pigeon”				
	Acute	6.94	33	10
	Long-term	1.50	9.3	5
Lettuce (10-49)– Small granivorous bird “finch”				
	Acute	3.43	68	10
	Long-term	0.83	17	5
Lettuce (10-49)– Small omnivorous bird “lark”				
	Acute	3.0	77	10
	Long-term	0.72	19	5
Lettuce (10-19)– Small insectivorous bird “wagtail”				
	Acute	3.35	69	10
	Long-term	0.75	19	5

Crop (BBCH stage) / Generic focal species	Time scale	DDD	TER[1]	Annex VI Trigger[3]
Tier 1 – uptake via diet (mammals)				
Hops (BBCH 31-75) – 1x 0.150 g a.s./ha				
Hops (≥ 20)– Small insectivorous mammal “shrew”				
	Acute	0.81	1984	10
	Long-term	0.151	~~225~~ 42	5
Hops (≥ 40)– Small herbivorous mammal "vole"				
	Acute	6.14	262	10
	Long-term	1.73	~~20~~ **3.7**	5

Crop (BBCH stage) / Generic focal species	Time scale	DDD	TER[1]	Annex VI Trigger[3]
Hops (20-39)– Small omnivorous mammal “mouse”				
	Acute	1.29	1246	10
	Long-term	0.31	~~110~~ 21	5
Lettuce (BBCH 12-49) – 1x 0.125 g a.s./ha				
Lettuce (≥ 20) - Small insectivorous mammal “shrew”				
	Acute	0.68	2381	10
	Long-term	0.13	~~270~~ 51	5
Lettuce (40-49)– Small herbivorous mammal "vole"				
	Acute	17.05	94	10
	Long-term	4.79	~~7.1~~ **1.3**	5
Lettuce (all season)– Large herbivorous mammal “lagomorph”				
	Acute	4.39	366	10
	Long-term	0.95	~~36~~ 6.8	5
Lettuce (10-49)– Small omnivorous mammal “mouse”				
	Acute	2.15	747	10
	Long-term	0.52	~~66~~ 12.4	5
Tier 2 – uptake via diet (mammals). Refined ftwa of 0.164 can be used for all weed types and lettuce crop plants for the representative uses.				
Hops (≥ 40)– Small herbivorous mammal "vole"				
	Long-term	0.53	12	5
Lettuce (40-49)– Small herbivorous mammal "vole"				
	Long-term	1.48	**4.3**	5

Tier 1– uptake via drinking water (Birds and mammals)				
	Time scale	DDD	TER[1]	Annex VI Trigger[3]
Leaf scenario (use in lettuce, 1x0.125 kg a.s./ha) (birds only)	Acute	23	10.1	10
Puddle scenario (birds and mammals)	Not relevant based on escape clause			
Tier 1 – secondary poisoning (Birds and mammals): not relevant, logPow <3				

Toxicity data for aquatic species (most sensitive species of each group) (Annex IIA, point 8.2, Annex IIIA, point 10.2)

Group	Test substance	Time-scale (Test type)	End point	Toxicity[1] (mg/L)

Group	Test substance	Time-scale (Test type)	End point	Toxicity[1] (mg/L)
Laboratory tests ‡				
Fish				
Rainbow trout (*Oncorhynchus mykiss)*	a.s.	96 h (static)	Mortality, LC_{50}	>74.2 ($_{mm}$)
Fathead minnow (*Pimephales promelas*)	a.s.	96 h (static)	Mortality, LC_{50}	>70.5 ($_{mm}$)
Common carp (*Cyprinus carpio)*	a.s.	96 h (static)	Mortality, LC_{50}	>100 ($_{nom}$)
Sheepshead minnow (*Cyprinodon variegatus)* (saltwater species)	a.s.	96 h (static)	Mortality, LC_{50}	>83.9 ($_{mm}$)
Fathead minnow (*Pimephales promelas*)	a.s.	ELS, 35 d (flow-through)	Survival NOEC	4.41 ($_{mm}$)
Rainbow trout (*Oncorhynchus mykiss)*	BYI 02960 SL 200 G[2]	96 h (static)	Mortality, LC_{50}	>585 (form, $_{nom}$) >100 (a.s.,$_{nom}$)
Common carp (*Cyprinus carpio)*	BYI 02960 SL 200 G[2]	96 h (static)	Mortality, LC_{50}	>585 (form, $_{nom}$) >100 (a.s.,$_{nom}$)
Rainbow trout (*Oncorhynchus mykiss)*	BYI 02960-succinamide	96 h (static)	Mortality, EC_{50}	>100 ($_{nom}$)
Rainbow trout (*Oncorhynchus mykiss)*	DFA (BCS-AB60481 (tech.) = sodium difluoroacetate)	96 h (static)	Mortality, EC_{50}	>10 ($_{nom}$)
Amphibians				
Frog (*Xenopus laevis)*	a.s.	96 h (static)	Mortality, LC_{50}	>80 ($_{nom}$)
Aquatic invertebrates				
Daphnia magna	a.s.	48 h (static)	Mortality, EC_{50}	>77.6 ($_{mm}$)
Eastern oyster (*Crassostrea virginica)* (saltwater species)	a.s.	96 h (static)	Inhibition of shell growth, EC_{50}	>29 ($_{mm}$)
Mysid (*Americamysis bahia)* (saltwater species), juveniles[3]	a.s.	96 h (static)	Mortality, EC_{50}	0.26 ($_{mm}$)
Daphnia magna	a.s.	21 d (semi-static)	Reproduction, NOEC	3.2 ($_{nom}$)

Group	Test substance	Time-scale (Test type)	End point	Toxicity[1] (mg/L)
Mysid (*Americamysis bahia)* (saltwater species), juveniles	a.s.	28 d (semi-static)	life-cycle toxicity, NOEC	0.0132 [4] (mm)
Daphnia magna	BYI 02960 SL 200 G[2]	48 h (static)	Mortality, EC_{50}	684 (form, nom) 117 (a.s., nom)
Daphnia magna	DFA (BCS-AB60481 (tech.) = sodium difluoroacetate)	48 h (static)	Mortality, EC_{50}	>10 (nom)
Daphnia magna	6-CNA (IC-0)	48 h (static)	Mortality, EC_{50}	>95.1 (mm)
Daphnia magna	BYI 02960-succinamide	21 d (semi-static)	Reproduction, NOEC	43.3 (nom)
Sediment dwelling organisms				
Chironomus riparius	a.s.	48 h (static; water-spiked)	Mortality, EC_{50}	0.0617 (nom)
Chironomus riparius	a.s.	28 d (static; water-spiked)	NOEC	~~0.010~~ 0.00681 (~~nom~~ mm)
Chironomus riparius	BYI 02960-succinamide	48 h (static; water-spiked)	Mortality, EC_{50}	>100 (nom)
Chironomus riparius	BYI 02960-azabicyclosuccinamide, sodium salt	48 h (static; water-spiked)	Mortality, EC_{50}	>100 (nom)
Chironomus tentans	6-CNA	96 h (static; water-spiked)	Mortality, EC_{50}	>1 (nom)
Chironomus riparius	DFA (BCS-AB60481 (tech.) = sodium difluoroacetate)	28 d (static; water-spiked)	NOEC	100 (nom)
Chironomus riparius	6-CNA (IC-0)	28 d (static; water-spiked)	NOEC	100 (nom)
Chironomus riparius	BYI 02960 SL 200 G[2]	28 d (static; water-spiked)	NOEC	~~0.012~~ 0.00989 mg a.s./L (~~nom~~ mm)

Group	Test substance	Time-scale (Test type)	End point	Toxicity[1] (mg/L)
Algae				
Pseudokirchneriella subcapitata	a.s.	72 h (static)	Biomass: E_bC_{50}, Growth rate: E_rC_{50} & Yield: E_yC_{50}	>80 ($_{nom}$)
Pseudokirchneriella subcapitata	BYI 02960 SL 200 G[2]	72 h (static)	Biomass: E_bC_{50} & Growth rate: E_rC_{50}	>250 (form, $_{nom}$) >42.8 (a.s., $_{nom}$)
Pseudokirchneriella subcapitata	DFA (BCS-AB60481 (tech.) = sodium difluoroacetate)	72 h (static)	Growth rate: E_rC_{50} and Yield: E_yC_{50}	>10 ($_{nom}$)
Pseudokirchneriella subcapitata	BYI 02960-succinamide	72 h (static)	Growth rate: E_rC_{50} and Yield: E_yC_{50}	>10 ($_{nom}$)
Pseudokirchneriella subcapitata	6-CNA (IC-0)	72 h (static)	Growth rate: E_rC_{50} Yield: E_yC_{50}	>100 ($_{nom}$) 85 ($_{nom}$)
Aquatic higher plants				
Lemna gibba	a.s.	7 d (static)	Biomass: E_bC_{50}, Growth rate: E_rC_{50} and Yield: E_yC_{50}	>67.7 ($_{mm}$)
Microcosm or mesocosm tests				
Not required				

[1] indicate whether based on nominal ($_{nom}$) or mean measured concentrations ($_{mm}$). In the case of preparations indicate whether end points are presented as units of preparation or a.s.

[2] BYI 02960 SL 200 G = BYI 02960 SL 200 g/L = Flupyradifurone SL 200 G = Sivanto = 200 g/L flupyradifurone (nominal), 201 g/L flupyradifurone (analysed) ;

[3] Unknown if juveniles are the most sensitive life stage, see B.9

[4] ~30% difference on average from the control, but this is considered not biologically relevant - see detailed discussion in DAR

Maximum PEC_{sw} values and TER values – application to hops at 1 × 0.150 g as/ha

TER_A calculations for fish based on FOCUS Step 2 for application in hops

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Crop: Hops					
BYI 02960	*P. promelas*	LC_{50} > 70 500	17.36	> 4 061	100
BYI 02960 – succinamide	*O. mykiss*	LC_{50} > 100 000	4.065	> 24 600	
DFA*	*O. mykiss*	LC_{50} > 10 000	1.268 (1.421*)	>7 886 (> 7 037*)	

*) In a worst-case approach, exposure concentrations for DFA are based upon groundwater calculations; however, dilution will occur during transport to surface waters.

TER_{LT} calculations for fish based on FOCUS Step 2 for application in hops

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_{LT}	Trigger
Crop: Hops						
BYI 02960	*P. promelas*	NOEC	4410	17.36	254	10

TER_A calculations for aquatic invertebrates based on FOCUS Step 2 for application in hops

Compound	**Species**	**Endpoint [µg/L]**		**$PEC_{sw,max}$ [µg/L]**	**TER_A**	**Trigger**
Crop: Hops						
BYI 02960	*C. riparius*	EC_{50}	61.7	17.36	**3.53**	100
DFA*	*D. magna*	EC_{50}	> 10 000	1.268 (1.421*)	> 7 886 (> 7 037*)	
6-CNA	*C. tentans*	EC_{50}	> 1 000	0.463	> 2 160	
BYI 02960-succinamide	*C. riparius*	EC_{50}	> 100 000	4.065	> 24 600	
BYI 02960-azabicyclosuccinamide, sodium salt	*C. riparius*	EC_{50}	> 100 000	2.499	>40 016	

*) In a worst-case approach, exposure concentrations for DFA are based upon groundwater calculations; however, dilution will occur during transport to surface waters.

TER_{LT} calculations for aquatic invertebrates based on FOCUS Step 2 for application in hops

Compound	**Species**	**Endpoint [µg/L]**		**$PEC_{sw,max}$ [µg/L]**	**TER_{LT}**	**Trigger**
Crop: Hops						
BYI 02960	*C. riparius*	NOEC	~~10~~ 6.81	17.36	**~~0.58~~ 0.39**	10
BYI 02960 – succinamide	*D. magna*	NOEC	43 300	4.065	10 652	
DFA*	*C. riparius*	NOEC	100 000	1.268 (1.421*)	78 864 (70 373*)	
6-CNA	*C. riparius*	NOEC	100 000	0.463	215 983	

*) In a worst-case approach, exposure concentrations for DFA are based upon groundwater calculations; however, dilution will occur during transport to surface waters.

TER_A calculations for *C. riparius* based on FOCUS Step 3 for application in hops

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	Scenario	TER_A	Trigger
Crop: Hops							
BYI 02960	*C. riparius*	EC_{50}	61.7	0.394	R1, pond	157	100
				5.531	R1, stream	**11**	

TER_{LT} calculations for *C. riparius* based on FOCUS Step 3 for application in hops

Compound	**Species**	**Endpoint [µg a.i./L]**	**$PEC_{sw,max}$ [µg/L]**	**Scenario**	**TER_{LT}**	**Trigger**
Crop: Hops						
BYI 02960	*C. riparius*	NOEC 10	0.394	R1, pond	25	10
			5.531	R1, stream	**1.81**	

Refined TER_A calculations for *C. riparius* based on FOCUS Step 4 for application in hops

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Scenario	TER_A	Trigger
Crop: Hops; 90 % drift reduction (no buffer zone)						
BYI 02960	*C. riparius*	EC_{50} 61.7	0.553	R1, stream	112	100
Crop: Hops; 75 % drift reduction and 10 m buffer zone						
BYI 02960	*C. riparius*	EC_{50} 61.7	0.589	R1, stream	105	100

TER_{LT} calculations for *C. riparius* based on FOCUS Step 4 for application in hops with exposure mitigation measures

Compound	**Species**	**Endpoint [µg a.i./L]**	**$PEC_{sw,max}$ [µg/L]**	**Scenario**	**TER_{LT}**	**Trigger**
Crop: Hops; no buffer zone 90% drift reduction						
BYI 02960	*C.riparius*	NOEC 10	0.553	R1, stream	18.1	10
5 m distance (spray drift buffer) and 90 % drift reduction						
BYI 02960	*C.riparius*	NOEC 10	0.452	R1, stream	22.1	10
10 m distance (spray drift buffer & VFS*) and 90 % drift reduction						
BYI 02960	*C.riparius*	NOEC 10	0.235	R1, stream	42.6	10
15 m distance (spray drift buffer & VFS*) and 75 % drift reduction						
BYI 02960	*C.riparius*	NOEC 10	0.388	R1, stream	25.8	10

*VFS: vegetated filter strip

TER_A calculations for algae based on FOCUS Step 2 for application in hops

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Crop: Hops					
BYI 02960	*P. subcapitata*	EC50 >42 800	17.36	>2 465	10
DFA	*P. subcapitata*	EC50 >10 000	1.268	>7 886	10
BYI 02960 – succinamide	*P. subcapitata*	EC50 >10 000	4.065	>2 460	10

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
6-CNA	*P. subcapitata*	EC50	85 000	0.463	183 585	10

TER_A calculations for lemna based on FOCUS Step 2 for application in hops

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Crop: Hops						
BYI 02960	*L. gibba*	EC50	>67 700	17.36	>3 890	10

Maximum PEC_{sw} values and TER values – application to lettuce at 1 × 0.125 g as/ha- biennial use (once every two years, at BBCH 12-49) and annual use (use once per year, at BBCH 41-49)

TER_A calculations for fish based on FOCUS Step 2 for application in lettuce – biennial (covers annual use)

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Crop: Lettuce						
BYI 02960	*P. promelas*	LC_{50}	**>** 70 500	11.88	> 5 934	100
BYI 02960 – succinamide	*O. mykiss*	LC_{50}	**>** 100 000	0.484	> 206 612	
DFA*	*O. mykiss*	LC_{50}	> 10 000	1.339 (5.599*)	> 7 468 (> 1 786*)	

*) In a worst-case approach, exposure concentrations for DFA are based upon groundwater calculations; however, dilution will occur during transport to surface waters.

TER_{LT} calculations for fish based on FOCUS Step 2 for application in lettuce – biennial (covers annual use)

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_{LT}	Trigger
Crop: Lettuce						
BYI 02960	*P. promelas*	NOEC	4410	11.88	371	10

TER_A calculations for aquatic invertebrates based on FOCUS Step 2 for application in lettuce – biennial (covers annual use)

Compound	**Species**	**Endpoint [µg/L]**		**$PEC_{sw,max}$ [µg/L]**	**TER_A**	**Trigger**
Crop: Lettuce						
BYI 02960	*C. riparius*	EC_{50}	61.7	11.88	**5.20**	100
DFA*	*D. magna*	EC_{50}	**>** 10 000	1.339 (5.599*)	> 7 468 (> 1 786*)	
6-CNA	*C. tentans*	EC_{50}	> 1 000	0.579	> 1 727	

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
BYI 02960-succinamide	*C. riparius*	EC_{50}	> 100 000	0.484	> 206 612	
BYI 02960-azabicyclosuccinamide, sodium salt	*C. riparius*	EC_{50}	> 100 000	0.297	> 336 700	

*) In a worst-case approach, exposure concentrations for DFA are based upon groundwater calculations; however, dilution will occur during transport to surface waters.

TER_{LT} calculations for aquatic invertebrates based on FOCUS Step 2 for application in lettuce– biennial (covers annual use)

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_{LT}	Trigger
Crop: Lettuce						
BYI 02960	*C. riparius*	NOEC	~~10~~ 6.81	11.88	~~**0.84**~~ **0.57**	10
BYI 02960 – succinamide	*D. magna*	NOEC	43 300	0.484	89 463	
DFA*	*C. riparius*	NOEC	100 000	1.339 (5.599*)	74 683 (17 860*)	
6-CNA	*C. riparius*	NOEC	100 000	0.579	172 712	

*) In a worst-case approach, exposure concentrations for DFA are based upon groundwater calculations; however, dilution will occur during transport to surface waters.

TER_A calculations for *C. riparius* based on FOCUS Step 3 for application in lettuce – biennial use

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	Scenario	TER_A	Trigger
Crop: Lettuce							
BYI 02960	*C. riparius*	EC_{50}	61.7	0.830	D3 (ditch, 1st)	**74**	100
				0.840	D3 (ditch, 2nd)	**74**	
				1.035	D4 (pond, 1st)	**60**	
				0.794	D4 (stream, 1st)	**78**	
				1.268	D6 (ditch, 1st)	**49**	
				0.060	R1 (pond, 1st)	1028	
				0.858	R1 (stream, 1st)	**72**	
				0.097	R1 (pond, 2nd)	636	
				1.186	R1 (stream, 2nd)	**52**	
				1.586	R2 (stream, 1st)	**39**	
				0.940	R2 (stream, 2nd)	**66**	
				2.226	R3 (stream, 1st)	**28**	

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Scenario	TER_A	Trigger
			3.570	R3 (stream, 2nd)	**17**	
			0.522	R4 (stream, 1st)	118	
			4.808	R4 (stream, 2nd)	**13**	

TER_A calculations for *C. riparius* based on FOCUS Step 3 for application in lettuce – annual use

Compound	**Species**	**Endpoint [µg/L]**	**$PEC_{sw,max}$ [µg/L]**	**Scenario**	**TER_A**	**Trigger**
Crop: Lettuce						
BYI 02960	*C. riparius*	EC_{50} 61.7	0.813	D3 (ditch, 1st)	**76**	100
			0.811	D3 (ditch, 2nd)	**76**	
			0.983	D4 (pond, 1st)	**63**	
			0.969	D4 (stream, 1st)	**64**	
			1.206	D6 (ditch, 1st)	**51**	
			0.337	R1 (pond, 1st)	183	
			2.827	R1 (stream, 1st)	**22**	
			0.139	R1 (pond, 2nd)	444	
			2.189	R1 (stream, 2nd)	**28**	
			1.215	R2 (stream, 1st)	**51**	
			1.044	R2 (stream, 2nd)	**59**	
			3.698	R3 (stream, 1st)	**17**	
			3.297	R3 (stream, 2nd)	**18**	
			0.83	R4 (stream, 1st)	**74**	
			2.954	R4 (stream, 2nd)	**21**	

TER_{LT} calculations for *C.riparius* based on FOCUS Step 3 for application in lettuce – biennial use

Compound	**Species**	**Endpoint [µg a.i./L]**	**$PEC_{sw,max}$ [µg/L]**	**Scenario**	**TER_{LT}**	**Trigger**
Crop: Lettuce						
BYI 02960	*C. riparius*	NOEC ~~10~~ 6.81	0.830	D3 (ditch, 1st)	~~12.0~~ **8.20**	10

Compound	Species	Endpoint [µg a.i./L]	$PEC_{sw,max}$ [µg/L]	Scenario	TER_{LT}	Trigger
			0.840	D3 (ditch, 2nd)	~~11.9~~ **8.11**	
			1.035	D4 (pond, 1st)	~~9.7~~ **6.58**	
			0.794	D4 (stream, 1st)	~~12.6~~ **8.58**	
			1.268	D6 (ditch, 1st)	~~7.9~~ **5.37**	
			0.060	R1 (pond, 1st)	~~167~~ 114	
			0.858	R1 (stream, 1st)	~~11.7~~ **7.94**	
			0.097	R1 (pond, 2nd)	~~103~~ 70.2	
			1.186	R1 (stream, 2nd)	~~8.4~~ **5.74**	
			1.586	R2 (stream, 1st)	~~6.3~~ **4.29**	
			0.940	R2 (stream, 2nd)	~~10.6~~ **7.24**	
			2.226	R3 (stream, 1st)	~~4.5~~ **3.06**	
			3.570	R3 (stream, 2nd)	~~2.8~~ **1.91**	
			0.522	R4 (stream, 1st)	~~19.2~~ 13.1	
			4.808	R4 (stream, 2nd)	~~2.1~~ **1.42**	

TER_{LT} calculations for *C.riparius* based on FOCUS Step 3 for application in lettuce – annual use

Compound	Species	Endpoint [µg a.i./L]	$PEC_{sw,max}$ [µg/L]	Scenario	TER_{LT}	Trigger
Crop: Lettuce						
BYI 02960	*C. riparius*	NOEC ~~10~~ 6.81	0.830	D3 (ditch, 1st)	**8.38**	
			0.811	D3 (ditch, 2nd)	**8.40**	
			0.983	D4 (pond, 1st)	**6.93**	
			0.969	D4 (stream, 1st)	**7.03**	
			1.206	D6 (ditch, 1st)	**5.65**	
			0.337	R1 (pond, 1st)	20.2	
			2.827	R1 (stream, 1st)	**2.41**	10
			0.139	R1 (pond, 2nd)	49.0	
			2.189	R1 (stream, 2nd)	**3.11**	
			1.215	R2 (stream, 1st)	**5.60**	
			1.044	R2 (stream, 2nd)	**6.52**	

Compound	Species	Endpoint [µg a.i./L]	$PEC_{sw,max}$ [µg/L]	Scenario	TER_{LT}	Trigger
			3.698	R3 (stream, 1st)	**1.84**	
			3.297	R3 (stream, 2nd)	**2.07**	
			0.830	R4 (stream, 1st)	**8.20**	
			2.954	R4 (stream, 2nd)	**2.31**	

Refined TER_A calculations for *C. riparius* based on FOCUS Step 4 for application in lettuce – biennial use* (drift reducing nozzles)

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Drift reduction [%]	Scenario	TER_A	Trigger
Crop: Lettuce; 50% drift reduction (No buffer)							
BYI 02960	*C. riparius*	EC_{50} 61.7	0.434	50	D3 (ditch, 1st)	143	100
			0.446	50	D3 (ditch, 2nd)	139	
			1.033	90	D4 (pond, 1st)	**60**	
			0.721	90	D4 (stream, 1st)	**86**	
			1.268	90	D6 (ditch, 1st)	**49**	
			0.858	90	R1 (stream, 1st)	**72**	
			1.186	90	R1 (stream, 2nd)	**52**	
			1.586	90	R2 (stream, 1st)	**39**	
			0.940	90	R2 (stream, 2nd)	**66**	
			2.226	90	R3 (stream, 1st)	**28**	
			3.570	90	R3 (stream, 2nd)	**17**	
			4.808	90	R4 (stream, 2nd)	**13**	

*The PECsw calculated with only drift reducing nozzles are not available for the annual use in lettuce

Refined TER_A calculations for *C. riparius* based on FOCUS Step 4 for application in lettuce (5 m distance) – biennial use

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Scenario	TER_A	Trigger
Crop: Lettuce; 5 m drift buffer (no drift reduction)						
BYI 02960	*C. riparius*	EC_{50} 61.7	1.035	D4 (pond, 1st)	**59.7**	100
			0.721	D4 (stream, 1st)	**85.6**	
			1.268	D6 (ditch, 1st)	**48.7**	
			0.858	R1 (stream, 1st)	**71.9**	
			1.186	R1 (stream, 2nd)	**52.0**	
			1.586	R2 (stream, 1st)	**38.9**	
			0.940	R2 (stream, 2nd)	**65.6**	
			2.226	R3 (stream, 1st)	**27.7**	
			3.570	R3 (stream, 2nd)	**17.3**	
			4.808	R4 (stream, 2nd)	**12.8**	

Refined TER_A calculations for *C. riparius* based on FOCUS Step 4 for application in lettuce (5 m distance) – annual use

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Scenario	TER_A	Trigger
Crop: Lettuce; 5 m drift buffer (no drift reduction)						
BYI 02960	*C. riparius*	EC_{50} 61.7	0.234	D4 (pond, 1st)	264	100
			0.234	D4 (stream, 1st)	264	
			0.982	D4 (pond, 1st)	**59.7**	
			0.969	D4 (stream, 1st)	**85.6**	
			1.206	D6 (ditch, 1st)	**48.7**	
			2.827	R1 (stream, 1st)	**71.9**	
			2.189	R1 (stream, 2nd)	**52.0**	
			1.215	R2 (stream, 1st)	**38.9**	
			1.044	R2 (stream, 2nd)	**65.6**	
			3.698	R3 (stream, 1st)	**27.7**	
			3.297	R3 (stream, 2nd)	**17.3**	
			0.830	R4 (stream, 1st)	**74.34**	
			2.954	R4 (stream, 2nd)	**12.8**	

Refined TER_A calculations for *C. riparius* based on FOCUS Step 4 for application in lettuce (10 m distance) – biennial use

Compound	Species	Endpoint [µg/L]	PEC$_{sw,max}$ [µg/L]	Buffer type	Scenario	TER$_A$	Trigger
Crop: Lettuce; 10 m distance (spray drift mitigation only (S) or spray drift mitigation + VFS* (R))							
BYI 02960	*C. riparius*	EC$_{50}$ 61.7	1.034	S	D4 (pond, 1st)	**59.7**	100
			0.721	S	D4 (stream, 1st)	**85.6**	
			1.268	S	D6 (ditch, 1st)	**48.7**	
			0.389	R	R1 (stream, 1st)	159	
			0.540	R	R1 (stream, 2nd)	114	
			0.716	R	R2 (stream, 1st)	**86.2**	
			0.422	R	R2 (stream, 2nd)	146	
			1.009	R	R3 (stream, 1st)	**61.2**	
			1.630	R	R3 (stream, 2nd)	**37.9**	
			2.184	R	R4 (stream, 2nd)	**28.3**	

*VFS: vegetated filter strip

Refined TER$_A$ calculations for *C. riparius* based on FOCUS Step 4 for application in lettuce (10 m distance) – annual use

Compound	Species	Endpoint [µg/L]	PEC$_{sw,max}$ [µg/L]	Buffer type	Scenario	TER$_A$	Trigger
Crop: Lettuce; 10 m distance (spray drift mitigation only (S) or spray drift mitigation + VFS* (R))							
BYI 02960	*C. riparius*	EC$_{50}$ 61.7	0.975	S	D4 (pond, 1st)	**63.3**	100
			0.969	S	D4 (stream, 1st)	**63.7**	
			1.206	S	D6 (ditch, 1st)	**51.2**	
			1.270	R	R1 (stream, 1st)	**48.6**	
			0.954	R	R1 (stream, 2nd)	**64.7**	
			0.553	R	R2 (stream, 1st)	112	
			0.469	R	R2 (stream, 2nd)	132	
			1.683	R	R3 (stream, 1st)	**36.7**	
			1.506	R	R3 (stream, 2nd)	**41.0**	
			1.345	R	R4 (stream, 1st)	**45.9**	

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Buffer type	Scenario	TER_A	Trigger
			0.705	R	R4 (stream, 2nd)	**87.5**	

*VFS: vegetated filter strip

Refined TER_A calculations for *C. riparius* based on FOCUS Step 4 for application in lettuce (15 m distance) – biennial use

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Buffer type	Scenario	TER_A	Trigger
Crop: Lettuce; 15 m distance (spray drift mitigation only (S) or spray drift mitigation + VFS* (R))							
BYI 02960	*C. riparius*	EC_{50} 61.7	1.034	S	D4 (pond, 1st)	**59.7**	100
			0.721	S	D4 (stream, 1st)	**85.6**	
			1.268	S	D6 (ditch, 1st)	**48.7**	
			0.375	R	R2 (stream, 1st)	165	
			0.528	R	R3 (stream, 1st)	117	
			0.856	R	R3 (stream, 2nd)	**72.1**	
			1.144	R	R4 (stream, 2nd)	**53.9**	

*VFS: vegetated filter strip

Refined TER_A calculations for *C. riparius* based on FOCUS Step 4 for application in lettuce (15 m distance) – annual use

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Buffer type	Scenario	TER_A	Trigger
Crop: Lettuce; 15 m distance (spray drift mitigation only (S) or spray drift mitigation + VFS* (R))							
BYI 02960	*C. riparius*	EC_{50} 61.7	0.975	S	D4 (pond, 1st)	**63.3**	100
			0.969	S	D4 (stream, 1st)	**63.7**	
			1.206	S	D6 (ditch, 1st)	**51.2**	
			1.270	R	R1 (stream, 1st)	**48.6**	
			0.954	R	R1 (stream, 2nd)	**64.7**	
			1.683	R	R3 (stream, 1st)	**36.7**	
			1.506	R	R3 (stream, 2nd)	**41.0**	
			1.345	R	R4 (stream, 1st)	163	

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Buffer type	Scenario	TER_A	Trigger
			0.705	R	R4 (stream, 2nd)	**45.9**	

*VFS: vegetated filter strip

Refined TER_A calculations for *C. riparius* based on FOCUS Step 4 for application in lettuce (20 m distance) – biennial use

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Buffer type	Scenario	TER_A	Trigger
Crop: Lettuce; 20 m distance (spray drift mitigation only (S) or spray drift mitigation + VFS* (R))							
BYI 02960	*C. riparius*	EC_{50} 62	1.033	S	D4 (pond, 1st)	**59.7**	100
			0.721	S	D4 (stream, 1st)	**85.6**	
			1.268	S	D6 (ditch, 1st)	**48.7**	
			0.856	R	R3 (stream, 2nd)	**72.1**	
			1.144	R	R4 (stream, 2nd)	**53.9**	

*VFS: vegetated filter strip

Refined TER_A calculations for *C. riparius* based on FOCUS Step 4 for application in lettuce (20 m distance) – annual use

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Buffer type	Scenario	TER_A	Trigger
Crop: Lettuce; 20 m distance (spray drift mitigation only (S) or spray drift mitigation + VFS* (R))							
BYI 02960	*C. riparius*	EC_{50} 61.7	0.975	S	D4 (pond, 1st)	**63.3**	100
			0.969	S	D4 (stream, 1st)	**63.7**	
			1.206	S	D6 (ditch, 1st)	**51.2**	
			0.662	R	R1 (stream, 1st)	**93.2**	
			0.500	R	R1 (stream, 2nd)	123	
			0.882	R	R3 (stream, 1st)	**70.0**	
			0.790	R	R3 (stream, 2nd)	**78.1**	
			0.705	R	R4 (stream, 2nd)	**87.5**	

*VFS: vegetated filter strip

Table B.9.2.5.5-11 TER_{LT} calculations for *C. riparius* based on FOCUS Step 4 for biennial application in lettuce (drift reducing nozzles)

Compound	Species	Endpoint [µg a.i./L]	$PEC_{sw,max}$ [µg/L]	Drift reduction [%]	Scenario	TER_{LT}	Trigger
Crop: Lettuce; drift reduction (no buffer)							
BYI 02960	*C.riparius*	NOEC ~~10~~ 6.81	0.434	50%	D3 (ditch, 1st)	15.7	10
			0.446	50%	D3 (ditch, 2nd)	15.3	
			1.033	90%	D4 (pond, 1st)	~~9.68~~ **6.59**	
			0.721	90%	D4 (stream, 1st)	~~13.9~~ **9.45**	
			1.268	90%	D6 (ditch, 1st)	~~7.89~~ **5.37**	
			0.858	90%	R1 (stream, 1st)	~~11.7~~ **7.94**	
			1.186	90%	R1 (stream, 2nd)	~~8.43~~ **5.74**	
			1.586	90%	R2 (stream, 1st)	~~6.31~~ **4.29**	
			0.940	90%	R2 (stream, 2nd)	~~10.6~~ **7.24**	
			2.226	90%	R3 (stream, 1st)	~~4.49~~ **3.06**	
			3.570	90%	R3 (stream, 2nd)	~~2.80~~ **1.91**	
			4.808	90%	R4 (stream, 2nd)	~~2.08~~ **1.42**	

TER_{LT} calculations for *C.riparius* based on FOCUS Step 4 for biennial application in lettuce**

Compound	Species	Endpoint [µg a.i./L]	$PEC_{sw,max}$ [µg/L]	% drift reduction	buffer zone	Scenario	TER_{LT}	Trigger
Crop: Lettuce; D scenarios: buffer (spray drift only) + drift reduction (nozzles); R scenarios: buffer (spray drift + VFS*) + drift reduction)								
BYI 02960	*C.riparius*	NOEC ~~10~~ 6.81	1.033	90%	20	D4 (pond, 1st)	**6.59**	10
			0.721	90%	20	D4 (stream, 1st)	**9.45**	

Compound	Species	Endpoint [µg a.i./L]	$PEC_{sw,max}$ [µg/L]	% drift reduction	buffer zone	Scenario	TER_{LT}	Trigger
			1.268	90%	20	D6 (ditch, 1st)	**5.37**	
			0.389	0%	20	R1 (stream, 1st)	17.5	
			0.540	0%	10	R1 (stream, 2nd)	12.6	
			0.375	0%	10	R2 (stream, 1st)	18.2	
			0.422	0%	10	R2 (stream, 2nd)	16.1	
			0.528	90%	15	R3 (stream, 1st)	12.9	
			0.856	90%	20	R3 (stream, 2nd)	**7.96**	
			1.144	90%	20	R4 (stream, 2nd)	**5.95**	

*VFS: vegetated filter strip
** per subscenario, the minimum required mitigation measure is shown to reach a TER < trigger. If this cannot be reached, the TER is shown at the strictest mitigation measure

TER_{LT} calculations for *C.riparius* based on FOCUS Step 4 for annual application in lettuce**

Compound	Species	Endpoint [µg a.i./L]	$PEC_{sw,max}$ [µg/L]	% drift reduction	buffer zone	Scenario	TER_{LT}	Trigger
Crop: Lettuce; D scenarios: buffer (spray drift only) + drift reduction (nozzles); R scenarios: buffer (spray drift + VFS*) + drift reduction)								
BYI 02960	*C.riparius*	NOEC ~~10~~ 6.81	0.234	0%	5	D3 (ditch, 1st)	29.1	
			0.234	0%	5	D3 (ditch, 2nd)	29.1	
			0.975	90%	20	D4 (pond, 1st)	**6.98**	10
			0.969	90%	20	D4 (stream, 1st)	**7.03**	
			1.206	90%	20	D6 (ditch, 1st)	**5.65**	

Compound	Species	Endpoint [µg a.i./L]	$PEC_{sw,max}$ [µg/L]	% drift reduction	buffer zone	Scenario	TER_{LT}	Trigger
			0.662	0%	20	R1 (stream, 1st)	10.29	
			0.500	0%	20	R1 (stream, 2nd)	13.62	
			0.553	0%	10	R2 (stream, 1st)	12.31	
			0.469	0%	10	R2 (stream, 2nd)	14.52	
			0.882	90%	20	R3 (stream, 1st)	7.72	
			0.790	90%	20	R3 (stream, 2nd)	**8.62**	
			0.379	0%	10	R4 (stream, 1st)	17.97	
			0.705	90%	20	R4 (stream, 2nd)	**9.66**	

*VFS: vegetated filter strip
** per subscenario, the minimum required mitigation measure is shown to reach a TER < trigger. If this cannot be reached, the TER is shown at the strictest mitigation measure

TER_A calculations for algae based on FOCUS Step 2 for application in lettuce

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Crop: Hops						
BYI 02960	*P. subcapitata*	EC50	>42 800	11.88	>3 603	10
DFA	*P. subcapitata*	EC50	>10 000	1.339	>7 468	10
BYI 02960 – succinamide	*P. subcapitata*	EC50	>10 000	0.484	>20 661	10
6-CNA	*P. subcapitata*	EC50	85 000	0.579	146 805	10

TER_A calculations for lemna based on FOCUS Step 2 for application in lettuce

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Crop: Hops					
BYI 02960	*L. gibba*	EC50 >67 700	11.88	>5 699	10

Bioconcentration						
	Active substance	BYI 02960-succinamide	BYI 02960-azabicyclo-succinamide	DFA	BYI 02960-DFEAF	6-CNA
$logP_{O/W}$	1.2	-1.3	-2.7	-3.1	-0.2	1.52
Bioconcentration factor (BCF)[1] ‡	not required					
Annex VI Trigger for the bioconcentration factor	100 (a.s. not readily biodegradable)					
Clearance time (days) (CT_{50})	-					
(CT_{90})	-					
Level and nature of residues (%) in organisms after the 14 day depuration phase	-					

[1] only required if log $P_{O/W}$ >3.

Effects on honeybees (Annex IIA, point 8.7, Annex IIIA, point 10.4)

Acute toxicity studies on adult honeybees		
Test substance	Acute oral toxicity (LD_{50} µg/bee)	Acute contact toxicity (LD_{50} µg/bee)
a.s.	1.2	122.8
Flupyradifurone SL 200 G[1]	3.2 (a.s.)	15.7 (a.s.)
BYI02960-difluoroethyl-amino-furanone (BYI02960-DFEAF)	>81.5	>100
BYI02960 - hydroxy	>105.3	>100
Difluoroacetic acid	>107.9	>100
6-chloronicotinic acid (6-CNA = IC-0);	>107.1	>100
6-chloro-picolylalcohol	>106.7	>100

[1] BYI 02960 SL 200 G = BYI 02960 SL 200 g/L = Flupyradifurone SL 200 G = Sivanto

Chronic toxicity studies on adult honeybees*		
Test substance	LC_{50} µg/L	LC_{50} µg/bee/d
a.s.	61100 µg/kg	1.83
BYI02960-difluoroethyl-amino-furanone (BYI02960-DFEAF)	>10000	>0.435
BYI02960 - hydroxy	>10000	>0.420
Difluoroacetic acid	>10000	>0.379
6-chloronicotinic acid (6-CNA = IC-0);	>10000	>0.418
6-chloro-picolylalcohol	>10000	>0.413

* These studies were performed in 2012/2013. Some uncertainties were noted during peer review arising from the lack of toxic standard, the lack of information on the history of the used bees, the lack of experience of conducting these kind of tests and the lack of experience for the interpretation of these tests. However, it is noted that currently no harmonised guideline is available, nor an agreed reference performance for any potential toxic standard. The studies were used in the risk assessment as additional information only .

Chronic toxicity studies on honeybee larvae*		
Test substance	NOEC µg/diet	NOEC µg/larvae
a.s.	≥ 10000 (nominal)	1.32 µg a.s./larvae (nominal) (accumulated exposure on day +4, +5 and +6 after grafting of the larvae)

* This study was performed in 2010. Some uncertainties were noted during peer review arising the lack of experience of conducting these kind of tests and the lack of experience for the interpretation of these tests. The study was used in the risk assessment as additional information only .

Acute toxicity studies on adult bumblebees	
Test substance	Acute contact toxicity (LD_{50} µg/bee)
Flupyradifurone SL 200 G	>100 (a.s.)

[1] BYI 02960 SL 200 G = BYI 02960 SL 200 g/L = Flupyradifurone SL 200 G = Sivanto

Field or semi-field tests
Five acceptable semi-field (gauze tunnel) studies are available, where BYI 02960 formulations were applied to Phacelia tanacetifolia with honey bees actively foraging on the crop (i.e. during bee flight) with different application regimes (ranging from 1x 75 g a.s. foliar spray to 1x 300 g a.s./ha soil application + 2x 200 g a.s./ha. Furthermore, two field studies are available in oil seed rape where BYI 02960 formulations were applied with honey bees actively foraging on the crop (i.e. during bee flight) at 2x 200 g a.s./ha with

preceding 1x 300 g a.s./ha soil application and 10 g a.s./kg seed seed treatment.

In the semi-field and field studies, the only clearly treatment-related effects seen were short-term reduced flight intensity (one to at most seven days after application) and behaviour anomalies in some bees (one day after application) after the foliar application during full flowering. Furthermore some slight, transient, potential effects were observed on other parameters. However this did not lead to reduced colony performance or survival.

Also, a large residue package is available with a.o. residues measured in pollen and nectar taken both from foragers and from hives, from studies in Phacelia and oil seed rape.

Lastly, a long-term feeding study was submitted in which colonies were forced to feed on up to 10 mg/kg diet for seven weeks. This did not lead to collapse of the colonies, including overwintering.

The overall data package shows that the spraying of 2x 200 g a.s./ha on a bee-attractive crop during full flowering and active foraging of honey bees may have caused some slight transient treatment related effects, but does not result in adverse acute or long-term (including overwintering) effects on honey bee colonies. Therefore, the risk to honey bees from application on the non-bee attractive crops hops and lettuce is clearly covered.

Hazard quotients for honey bees (Annex IIIA, point 10.4)

Application rate: 0.150 kg a.s./ha (hops, worst case for lettuce)

Test substance	Route	Hazard quotient	Annex VI Trigger
a.s.	Contact	1.22	50
a.s.	oral	**125**	50
Flupyradifurone SL 200 G	Contact	9.6	50
Flupyradifurone SL 200 G	oral	47	50

Effects on other arthropod species (Annex IIA, point 8.3.2, Annex IIIA, point 10.5)

Laboratory tests with standard sensitive species

Species	Test Substance	End point	Effect (LR_{50} g a.s./ha)
Typhlodromus pyri ‡	BYI 02960 SL200 G [1]	Mortality	17
Aphidius rhopalosiphi ‡	BYI 02960 SL200 G [1]	Mortality	<0.5

[1] BYI 02960 SL 200 G = BYI 02960 SL 200 g/L = Flupyradifurone SL 200 G = Sivanto

Hops, 1 x 0.150 g a.s./ha

Test substance	Species	Effect (LR_{50} g a.s./ha)	HQ in-field	HQ off-field[1]	Trigger
BYI 02960 SL200 G	*Typhlodromus pyri*	17	**8.8**	1.7	2
	Aphidius rhopalosiphi	<0.5	**>300**	**>58**	2

[1] at distance of 3 m and drift rate of 19.33%

Lettuce, 1 x 0.125 g a.s./ha

Test substance	Species	Effect (LR_{50} g a.s./ha)	HQ in-field	HQ off-field[1]	Trigger
BYI 02960 SL200 G	*Typhlodromus pyri*	17	**7.4**	0.2	2
	Aphidius rhopalosiphi	<0.5	**>250**	**>6.9**	2

[1] at distance of 1 m and drift rate of 2.77% for vegetables < 50 cm.

Further laboratory and extended laboratory studies ‡

Species	Life stage	Test substance, substrate and duration	Dose (g a.s./ha)	End point	% effect[1]	Trigger value
Extended laboratory test						
Aphidius rhopalosiphi	adults, < 48 h	BYI 02960 SL200 [3] Initial residues on barley seedlings	0.5, 0.89, 1.58, 2.81, 5.0	Mortality Reproduction	LR50: 2.02 g a.s./ha ER50: >0.89 and <1.58 g a.s./ha	50 %
Typhlodromus pyri	protonymphs	BYI 02960 SL200 [3] Initial residues on bean leaf discs	15, 32, 67, 142, 300	Mortality Reproduction	LR50: 177 g a.s./ha ER50: >142 g a.s./ha	50%
Coccinella semtempunctata	larvae 4 d	BYI 02960 SL200 [3] Initial residues on bean leaves	8, 17, 35, 72, 100, 150, 160, 250, 380, 600	Mortality Reproduction	LR50: 273.9 g a.s./ha No effects up to 250 g a.s./ha	50%
Aleochara bilineata	adults	BYI 02960 SL200 [3] Initial residues sprayed on soil	10, 21, 45, 95, 200, 300	Reproduction	ER50: >300 g a.s./ha	50%
Aphidius rhopalosiphi	adults, < 48 h	BYI 02960 SL200 G [3] Semi-field aged residues on maize seedlings	2x 250 with 10d interval; ageing up to 56 d	Mort. / Repr. 0 DAA 14 DAA 28 DAA 42 DAA 49 DAA 56 DAA	 100* / n.a. 90* / n.a. 76.7* / n.a. 29.6* / 89.9* 20.0* / 37.6 6.7 / 0.6	50 %

Species	Life stage	Test substance, substrate and duration	Dose (g a.s./ha)	End point	% effect[1]	Trigger value
Orius laevigatus	nymphs, 4-5 d	BYI 02960 SL200 G [3] Semi-field aged residues on apple trees	2x 250 with 10d interval; ageing up to 42 d	Mort. / Repr. 0 DAA 14 DAA 28 DAA 42 DAA	 100* / n.a. 76* / n.a. 25* / -6.5. 9.8 / 13	50 %

[1] Corrected mortality / reduction of reproduction relative to control. Positive percentages relate to adverse effects (e.g. a negative value for reproduction means an increase compared to the control).
* statistically significant; DAA = day after last application; n.a. = not assessed.
[3] BYI 02960 SL 200 G = BYI 02960 SL 200 g/L = Flupyradifurone SL 200 G = Sivanto

Field or semi-field tests
Two NTA field studies were performed with BYI 02960 SL 200 (200 g flupyradifurone/L) on grassland to similate an off-crop community. Soil-surface and plant-dwelling arthropod communities were sampled just before application and 1, 2, 4 and 8 weeks after application. Netherlands: NTA full fauna off-crop field study. Spray application rates: 1x 0.51, 1.7, 5.1, 21 g a.i./ha Community level NOER = 21 g a.i./ha Population level NOER = 5.1 g a.i./ha Population level NOEAER = 21 g a.i./ha, effect class 2 (two taxa adversely affected on one sampling moment shortly after application in the highest treatment rate, but both populations recovered withtin the next sampling moment one or two weeks later). SW France: NTA full fauna off-crop field study. Spray application rates: 1x 0.51, 1.7, 5.1, 21 g a.i./ha Community level NOER = 21 g a.i./ha Population level NOER = 1.7 g a.i./ha Population level NOEAER = 5.1 g a.i./ha effect class 2 (two taxa adversely affected on one sampling moment, recovery at next sampling moment). Population level NOEAER = 21 g a.i./ha effect class 3a (three taxa adversely affected, of which one on two sampling moments; effects no longer statistically significant on the last two sampling dates) NOER: No Observed Effect Rate NOEAER: No Observed Ecologically Adverse Effect Rate The underlined value is used in the off-field risk assessment (without SF)

Effects on earthworms, other soil macro-organisms and soil micro-organisms (Annex IIA points 8.4 and 8.5. Annex IIIA, points, 10.6 and 10.7)

Test organism	Test substance	Time scale	End point[1]
Earthworms			
	a.s.	Acute 14 days	LC_{50} 185.6 mg a.s./kg d.w.soil (10% organic matter)
	BYI 02960 SL 200 G [3]	Acute 14 days	LC_{50} 709 mg form/kg d.w.soil = 121 mg a.s./kg d.w.soil (5% organic matter)
	BYI 02960 SL 200 G [3]	Chronic 56 days	NOEC 8.9 mg form/kg d.w.soil = 1.5 mg a.s./kg d.w.soil (10% organic matter)
	BYI 02960-difluoroacetic acid	Acute 14 days	LC_{50} > 958 mg /kg d.w.soil (10% organic matter)
	BYI 02960-difluoroacetic acid	Chronic 56 days	NOEC 59 mg /kg d.w.soil (10% organic matter)
	IC-0 = 6-CAN	Acute 14 days	LC_{50} > 1000 mg /kg d.w.soil (10% organic matter)
	IC-0 = 6-CAN	Chronic 56 days	NOEC 95 mg /kg d.w.soil (5% organic matter)
Other soil macro-organisms			
Hypoaspis aculeifer	BYI 02960 SL 200 G [3]	Chronic 28 days	NOEC 1000 mg form/kg d.w.soil = 170 mg a.s./kg d.w.soil (5% organic matter)
	BYI 02960-difluoroacetic acid	Chronic 14 days	NOEC 958 mg /kg d.w.soil (5% organic matter)
	IC-0 = 6-CAN	Chronic 14 days	NOEC 100 mg /kg d.w.soil (5% organic matter)
Collembola			
Folsomia candida	BYI 02960 SL 200 G [3]	Chronic 28 days	NOEC 8.47 mg form/kg d.w.soil = 1.44 mg a.s./kg d.w.soil (5% organic matter)
	BYI 02960-difluoroacetic acid	Chronic 28 days	NOEC 95.8 mg /kg d.w.soil (5% organic matter)
	IC-0 = 6-CAN	Chronic 28 days	NOEC 90 mg /kg d.w.soil (5% organic matter)
Soil micro-organisms			
Nitrogen mineralisation	a.s.		< 25% effect at day 28 at 3.8 mg a.s/kg soil
	BYI 02960 SL 200 G [3]		< 25% effect at day 28 at 16.59 µL form/kg d.w.soil (3.335 mg a.s/kg soil)

Test organism	Test substance	Time scale	End point[1]
	IC-0 = 6-CAN		< 25% effect at day 28 at 1.33 mg /kg d.w.soil
Carbon mineralisation	a.s.		< 25% effect at day 28 at 3.8 mg a.s/kg soil
	BYI 02960 SL 200 G [3]		< 25% effect at day 28 at 16.59 µL form/kg d.w.soil (3.335 mg a.s/kg soil)
Field studies[2]			
Litter bag study (overspray): Soil treated with 150 g a.i./ha for plateau concentration (incorporated) + the annual rate of 300 g a.i./ha (oversprayed), both with product BYI 02960 SL 200 G [3]. At a measured initial concentration of 237 µg as/kg dry soil no statistically significant effects were found on soil litter degradation 217 d after treatment. Litter bag study (seed treatment): Soil treated with 150 g a.i./ha for plateau concentration (incorporated, product BYI 02960 SL 200 G [3]) + the annual rate of 265 g a.i./ha (by sowing of treated wheat seed, product BYI 02960 FS 480 G). At a measured initial concentration of 243 µg as/kg dry soil no statistically significant effects were found on soil litter degradation 217 d after treatment.			

[1] Correction of endpoints not necessary due to log Pow <2.0

[2] litter bag, field arthropod studies not included at 8.3.2/10.5 above, and earthworm field studies (a previously included earthworm field study was removed because it was considered invalid in the pesticide peer review meeting)

[3] BYI 02960 SL 200 G = BYI 02960 SL 200 g/L = Flupyradifurone SL 200 G = Sivanto

Toxicity/exposure ratios for soil organisms

Hops, 1 x 0.150 kg a.s./ha (annual use, covers biennial)

Test organism	Test substance	Time scale	Soil PEC[1]	TER	Trigger
Earthworms					
	a.s.	Acute	0.160	2.3E+3	10
	BYI 02960 SL 200 G	Acute	0.160	7.6E+2	10
	DFA	Acute	0.009	> 11E+4	5
	6-CNA	Acute	0.007	> 14E+4	10
	BYI 02960 SL 200 G	Chronic	0.160	9.4	10
	DFA	Chronic	0.009	6.6E+3	5
	6-CNA	Chronic	0.007	14E+3	10

Test organism	Test substance	Time scale	Soil PEC[1]	TER	Trigger
Other soil macro-organisms					
Soil mite	BYI 02960 SL 200 G	Chronic	0.160	≥ 1.1E+3	5
	DFA	Chronic	0.009	≥ 11E+4	5
	6-CNA	Chronic	0.007	≥ 14E+3	5
Collembola	BYI 02960 SL 200 G	Chronic	0.160	9.0	5
	DFA	Chronic	0.009	11E+3	5
	6-CNA	Chronic	0.007	13E+3	5

[1] For the a.s., the $PEC_{initial}$ + $PEC_{accumulation}$ is used.

Lettuce, 1 x 0.125 kg a.s./ha (biennual use, covers annual use)

Test organism	Test substance	Time scale	Soil PEC[1]	TER	Trigger
Earthworms					
	a.s.	Acute	0.141	1.3E+3	10
	BYI 02960 SL 200 G	Acute	0.141	8.6E+2	10
	DFA	Acute	0.014	> 68E+3	5
	6-CNA	Acute	0.012	≥ 83E+3	10
	BYI 02960 SL 200 G	Chronic	0.141	11	10
	DFA	Chronic	0.014	4.2E+3	5
	6-CNA	Chronic	0.012	7.9E+3	10
Other soil macro-organisms					
Soil mite	BYI 02960 SL 200 G	Chronic	0.141	≥ 1.2E+3	5
	DFA	Chronic	0.014	≥ 68E+3	5
	6-CNA	Chronic	0.012	≥ 8.3E+3	5
Collembola	BYI 02960 SL 200 G	Chronic	0.141	10	5
	DFA	Chronic	0.014	6.8E+3	5
	6-CNA	Chronic	0.012	7.5E+3	5

1 For the a.s., the PECinitial + PECaccumulation is used.

Effects on non target plants (Annex IIA, point 8.12, Annex IIIA, point 10.8)

Preliminary screening data

Not required as ER_{50} tests are provided

Laboratory dose response tests

Most sensitive species	Test substance	ER_{50} (g a.s./ha) vegetative vigour	ER_{50} (g a.s./ha) seedling emergence	Exposure[1] (g a.s./ha)	TER	Trigger
11 species: Cucumber (*Cucumis sativa*), buckwheat (*Fagopyrum esculentum*), oilseed rape (*Brassica napus*), sugar beet (*Beta vulgaris*), soybean (*Glycine max*), lettuce (*Lactuca sativa*), tomato (*Lycopersicon esculentum*), onion (*Allium cepa*), oat (*Avena sativa*), ryegrass (*Lolium perenne*), corn (*Zea mays*)	BYI 02960 SL 200 g/L[2]	>410 (g a.s./ha)	>410 (g a.s./ha)	30 g a.s./ha	>14	5

[1] for the worst case use in hops, 150 g a.s./ha, at distance of 3 m and drift rate of 19.77%
[2] BYI 02960 SL 200 G = BYI 02960 SL 200 g/L = Flupyradifurone SL 200 G = Sivanto

Additional studies (e.g. semi-field or field studies)

Not required

Effects on biological methods for sewage treatment (Annex IIA 8.15)

Test type/organism	end point
Activated sludge	>962 mg a.s./L

Ecotoxicologically relevant compounds

Active substance (in terms of compound under consideration, e.g. a variant such as an ester) must always be included in the soil, water and groundwater compartment. Ecotoxicological relevance of metabolites should be based on risk assessment.

Compartment	
soil	Parent
water	Parent
sediment	Parent

groundwater	Parent

Classification and proposed labelling with regard to ecotoxicological data (Annex IIA, point 10 and Annex IIIA, point 12.3)

	RMS/peer review proposal
Active substance	1999/45/EC: R50/53
Active substance	EC 12725/2008: Acute 1 and Chronic 1; GHS09; H400 and H410

	RMS/peer review proposal
BYI 02960 SL 200 G	1999/45/EC: R50/53
	EC 12725/2008: Acute 1 and Chronic 1; GHS09; H400 and H410

LEVEL 3

Flupyradifurone

PROPOSAL FOR THE DECISION

3 Proposed decision with respect to the application for approval of the active substance in Annex I

3.1 Background to the proposed decision

The representative formulation for flupyradifurone is Sivanto SL200. Sivanto SL200 is a soluble concentrate (SL) containing 200 grams per litre (g/L) of flupyradifurone and is proposed as an insecticide in agriculture to control mainly sucking insects after foliar spray application on various crops.

The submitted gap is adequate, in that it concerns two very different crops that likely represent worst case situations for the evaluation of other aspects. The aim of the efficacy evaluation for approval of the active substance is to establish if the GAP is realistic.
The claimed dose rates for hops, 0.75 L/ha (0.150 g a.s./ha) and for lettuce grown in the field, 0.125 g a.s./ha were found to be the minimum effective dose rates. No trials were submitted for lettuce grown in the greenhouse, and therefore the dose rate for this use has not been proven to be the correct dose rate.
While no large differences in dose rate are expected between the indoor and outdoor use in lettuce, the dose rate could be different because of differences in pest pressure, or higher sensitivity to phytotoxicity because of different growing conditions.

For approval of a new active substance data on efficacy is required on one or more representative uses on a widely grown crop in each zone of at least one plant protection product containing the active substance, showing adequate efficacy. Without the greenhouse use for lettuce, the submitted data for approval of the active substance can still be considered sufficient for efficacy.

The representative formulation caused no unacceptable phytotoxic effects.

The physical and chemical properties of both the active substance Flupyradifurone and the representative formulation Sivanto SL200 are sufficiently described.
The provided analytical methods for the determination of the active substance and/or impurities in technical material and in the plant protection product are acceptable. The provided analytical methods for residue analysis in plants and plant products, foodstuffs and feeding stuffs, soil, water and air are acceptable.

For flupyradifurone, an Acceptable Daily Intake (ADI) of 0.08 ~~0.12~~ mg/kg bw/day, an ARfD of 0.15 ~~0.35~~ mg/kg bw and and AOEL of 0.08 ~~0.12~~ mgkg bw/d can be established.
Safe uses were identified for operators without PPE using the DE-GM and UK-POEM model (outdoors), manual spraying using UK-POEM model (outdoors) and the Dutch-90th greenhouse model

(indoors). Safe uses were also identified for workers (without PPE) and bystanders using the EUROPOEM II 2002 model (90th percentile) and residents (Martin *et al*.).

Residue levels and metabolic pathway in crops treated with flupyradifurone according to the intended use and the intended use in the MRL dossier are sufficiently described. Residues are expected in rotational crops and livestock. MRLs are proposed for rotational crops and products of animal origin. Based on the evaluation of the intended use for approval and intended use in the MRL dossier, the use of flupyradifurone is not considered to result in an unacceptable risk for the consumer due to exposure to residues in food products.

The fate and behaviour of the active substance flupyradifurone ~~is being~~ has been assessed for soil, groundwater, suface water and sediment and air. The active substance is not persistent in soil: geometric mean non-normalised laboratory DT_{50} trigger value of 73.2 days ~~and~~. Two major metabolites 6-CNA and DFA are formed in laboratory soil degradation studies (max 17.1% and 33.9% respectively). ~~The obtained PEC_{soils} are used in the ecotoxicological assessment.~~ The active substance flupyradifurone has the potential to leach to the groundwater. The groundwater triggers were passed with higher tier approaches, such as kinetic sorption and DFOP approaches. The leaching of the active substance flupyradifurone is for some scenarios critical and ~~in many scenarios~~ shows exceedings of the threshlod values in many scenarios. DFA, one of the major metabolites, shows a potential for leaching to the groundwater. DFA is declared a non relevant metabolite for groundwater assessment. The other metabolite 6-CNA does not show a potential ~~for leach~~ to leach to the groundwater. The active substance flupyradifurone is persistent in water sediment systems (geometric mean DT_{50} value of 228 days). ~~The obtained PECsw/sed are used in the ecotoxicolgical assessment.~~ Flupyradifurone is readily degraded in air.

For the proposed uses in hops (1-150 g a.s./ha) and lettuce (1x125 g a.s./ha) acceptable risk is expected for birds, ~~wild mammals,~~ bees, earthworms, other soil macro-organisms, organic matter breakdown, soil micro-organisms, terrestrial non-target plants and activated sludge. For mammals, the acute risk for both uses and the long-term risk for the use in hops are acceptable, but a long-term risk for small herbivorous mammals cannot be excluded for the use in lettuce.
Acceptable risk to aquatic organisms is expected provided that mitigation options are used ~~(for the use in hops up to 90% drift reducing nozzles; for the use in lettuce up to 20m VFS and 90% drift reducing nozzles)~~. For hops, acceptable risk can be reached in the one relevant complete scenario with different combinations of buffer zones and spray drift reduction. For the biennial use in lettuce, an acceptable use can be shown for three of the seven complete FOCUS scenarios considering different mitigation options (up to 20m VFS). For the annual use in lettuce, an acceptable use can be shown for two of the seven complete FOCUS scenarios considering different mitigation options (up to 20m VFS).

Acceptable risk to non-target arthropods is expected provided that for both uses , risk mitigation is used (in hops e.g. a buffer zone of ~~2~~30 m, in lettuce e.g. a buffer zone of 5 m). ~~Risk mitigation for non-target arthropods is not necessary for the use in lettuce.~~

The active substance does not comply with the criteria for POP, PBT, vPvB.

3.2 Proposed decision concerning the approval of the active substance according to Regulation (EC) No 1107/2009

It is proposed to approve flupyradifurone according to Regulation (EC) No 1107/2009.

3.3 Rational for the conditions and restrictions to be associated with a proposed approval of the active substance according to Regulation (EC) No 1107/2009

No conditions or restrictions are to be associated with the proposed approval of flupyradifurone.

LEVEL 4

Flupyradifurone

DEMAND FOR FURTHER INFORMATION

4 Further information to permit a decision to be made, or to support a review of the conditions and restrictions associated with the proposed inclusion in Annex I

4.1 Identity of the active substance

Please refer to the confidential annex C (volume 4).

4.2 Physical and chemical properties of the active substance

No additional data required.

4.3 Data on application and further information

No additional data required.

4.4 Classification, packaging and labelling

No additional data required.

4.5 Methods of analysis

No additional data required.

4.6 Toxicology and metabolism

No additional data required.

4.7 Residues

~~No additional data required.~~ Additional data required to address the expected residue levels of DFA in products of animal origin resulting from exposure to DFA in feed and additional data to address the magnitude of residue in rotational crops conducted at realistic-worst-case plant back intervals with regard to the following crop selected, and providing in addition soil analyses.

4.8 Fate and behaviour

No additional data required.

4.9 Ecotoxicology

~~No additional data required.~~ Additional data required to address the long-term risk to small herbivorous mammals from the use in lettuce.

4.10 Efficacy

No additional data required.

European Commission

Flupyradifurone

VOLUME 2

ANNEX A

Rapporteur Member State: The Netherlands

January 2014/November 2014

Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of flupyradifurone in Regulation (EU) 1107/2009.

Contents

A List of the tests and studies submitted and of information available

(Annex IIA and Annex IIIA) (Dossier Documents J, I, K-II, L-II, K-III and L-III and other information available to or brought to the attention of the Rapporteur Member State)

A.1	Identity (Annex IIA 1, 3.1 to 3.4; Annex IIIA 1, 3.1 to 3.7, 3.9 and 12.1)	1
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Lists of Annex IIA and Annex IIIA submitted data and studies are based on the reference lists provided by the notifier. In addition to the references given below, the notifier submitted Documents A-I, J, L, M, N and O.

Section A.1 Identity (Annex IIA 1, 3.1 to 3.4; Annex IIIA 1, 3.1 to 3.7, 3.9 and 12.1)

References on identity are listed in Volume 4.

Section A.2 Physical chemical properties (Annex IIA 2, Annex IIIA 2)

References for the active substance

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Bogdoll, B.; Eyrich, U.	KIIA 2.3.2 /01	2011	Flupyradifurone (BYI 02960): Calculation of the Henry´s law constant Bayer CropScience, Report No.: AF09/042, Edition Number: M-414341-01-2 EPA MRID No.: 48843653 Date: 2011-08-15 GLP/GEP: no, unpublished ...also filed: KIIA 7.4.9 /02	Y	BCS
Bogdoll, B.; Strunk, B.	KIIA 2.2 /01	2011	Flupyradifurone (BYI 02960), pure substance: Relative density Bayer CropScience, Report No.: PA09/006, Edition Number: M-412635-01-2 EPA MRID No.: 48843632 Date: 2011-08-18 GLP/GEP: Y, unpublished	Y	BCS
Bogdoll, B.; Strunk, B.	KIIA 2.4.1 /01	2011	Flupyradifurone (BYI 02960), pure substance - Physical characteristics colour, physical state and odour Bayer CropScience, Report No.: PA09/008, Edition Number: M-412655-01-2 EPA MRID No.: 48843616 Date: 2011-08-18 GLP/GEP: Y, unpublished ...also filed: KIIA 2.4.2 /01	Y	BCS
Bogdoll, B.; Strunk, B.	KIIA 2.4.2 /01	2011	Flupyradifurone (BYI 02960), pure substance - Physical characteristics colour, physical state and odour Bayer CropScience, Report No.: PA09/008, Edition Number: M-412655-01-2 EPA MRID No.: 48843616 Date: 2011-08-18 GLP/GEP: Y, unpublished ...also filed: KIIA 2.4.1 /01	Y	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Bogdoll, B.; Strunk, B.	KIIA 2.8.1 /01	2011	Flupyradifurone (BYI 02960), pure substance: Partition coefficient 1-octanol / water at pH 4, pH 7 and pH 9 (HPLC-method) Bayer CropScience, Report No.: PA09/004, Edition Number: M-414485-01-2 EPA MRID No.: 48843639 Date: 2011-09-26 GLP/GEP: Y, unpublished ...also filed: KIIA 2.8.2 /01	Y	BCS
Bogdoll, B.; Strunk, B.	KIIA 2.8.2 /01	2011	Flupyradifurone (BYI 02960), pure substance: Partition coefficient 1-octanol / water at pH 4, pH 7 and pH 9 (HPLC-method) Bayer CropScience, Report No.: PA09/004, Edition Number: M-414485-01-2 EPA MRID No.: 48843639 Date: 2011-09-26 GLP/GEP: Y, unpublished ...also filed: KIIA 2.8.1 /01	Y	BCS
Eyrich, U.; Bogdoll, B.	KIIA 2.2 /02	2011	Flupyradifurone (BYI 02960), technical substance : Relative density Bayer CropScience, Report No.: PA11/063, Edition Number: M-414075-01-2 EPA MRID No.: 48843633 Date: 2011-09-16 GLP/GEP: Y, unpublished	Y	BCS
Eyrich, U.; Bogdoll, B.	KIIA 2.4.1 /02	2011	Flupyradifurone (BYI 02960), technical substance: Physical characteristics colour, physical state and odour Bayer CropScience, Report No.: PA11/062, Edition Number: M-414072-01-2 EPA MRID No.: 48843617 Date: 2011-09-16 GLP/GEP: Y, unpublished ...also filed: KIIA 2.4.2 /02	Y	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Eyrich, U.; Bogdoll, B.	KIIA 2.4.2 /02	2011	Flupyradifurone (BYI 02960), technical substance: Physical characteristics colour, physical state and odour Bayer CropScience, Report No.: PA11/062, Edition Number: M-414072-01-2 EPA MRID No.: 48843617 Date: 2011-09-16 GLP/GEP: Y, unpublished ...also filed: KIIA 2.4.1 /02	Y	BCS
Eyrich, U.; Bogdoll, B.	KIIA 2.7 /01	2011	Flupyradifurone (BYI 02960): Solubility in organic solvents Bayer CropScience, Report No.: PA09/005, Edition Number: M-414064-01-2 EPA MRID No.: 48843656 Date: 2011-09-16 GLP/GEP: Y, unpublished	Y	BCS
Eyrich, U.; Bogdoll, B.	KIIA 2.14 /01	2011	Flupyradifurone (BYI 02960), technical substance : Determination of the surface tension Bayer CropScience, Report No.: PA11/065, Edition Number: M-414086-01-2 EPA MRID No.: 48843659 Date: 2011-09-16 GLP/GEP: Y, unpublished	Y	BCS
Eyrich, U.; Bogdoll, B.	KIIA 2.16 /01	2011	Flupyradifurone (BYI 02960), pure substance: Determination of the pH-value in distilled water Bayer CropScience, Report No.: PA09/007, Edition Number: M-412128-01-2 EPA MRID No.: 48843626 Date: 2011-08-09 GLP/GEP: Y, unpublished	Y	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Eyrich, U.; Bogdoll, B.	KIIA 2.16 /02	2011	Flupyradifurone (BYI 02960), technical substance : Determination of the pH-value in distilled water Bayer CropScience, Report No.: PA11/064, Edition Number: M-414084-01-2 EPA MRID No.: 48843627 Date: 2011-09-16 GLP/GEP: Y, unpublished	Y	BCS
Eyrich, U.; Bogdoll, B.	KIIA 2.18 /01	2011	Flupyradifurone (BYI 02960), technical substance : The oxidation or reduction properties Bayer CropScience, Report No.: PA11/066, Edition Number: M-414080-01-2 EPA MRID No.: 48843620 Date: 2011-09-16 GLP/GEP: Y, unpublished	Y	BCS
Hall, L. R.	KIIA 2.9.2 /01	2011	Phototransformation of [14C]BYI 02960 in aqueous pH 7 buffer - amended report Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: MERVP042-1, Edition Number: M-418426-02-2 EPA MRID No.: 48843669 Date: 2011-11-28 ...Amended: 2012-03-05 GLP/GEP: Y, unpublished ...also filed: KIIA 2.9.4 /01 ...also filed: KIIA 7.6 /01	Y	BCS
Hall, L. R.	KIIA 2.9.4 /01	2011	Phototransformation of [14C]BYI 02960 in aqueous pH 7 buffer - amended report Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: MERVP042-1, Edition Number: M-418426-02-2 EPA MRID No.: 48843669 Date: 2011-11-28 ...Amended: 2012-03-05 GLP/GEP: Y, unpublished ...also filed: KIIA 2.9.2 /01 ...also filed: KIIA 7.6 /01	Y	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Heinemann, O.	KIIA 2.9.3 /01	2011	BYI 02960: Determination of the quantum yield and assessment of the environmental half-life of the direct photo-degradation in water Bayer CropScience, Report No.: MEF-11/554, Edition Number: M-414756-01-2 EPA MRID No.: 48843668 Date: 2011-09-26 GLP/GEP: Y, unpublished ...also filed: KIIA 2.9.4 /02 ...also filed: KIIA 7.6 /02	Y	BCS
Heinemann, O.	KIIA 2.9.4 /02	2011	BYI 02960: Determination of the quantum yield and assessment of the environmental half-life of the direct photo-degradation in water Bayer CropScience, Report No.: MEF-11/554, Edition Number: M-414756-01-2 EPA MRID No.: 48843668 Date: 2011-09-26 GLP/GEP: Y, unpublished ...also filed: KIIA 2.9.3 /01 ...also filed: KIIA 7.6 /02	Y	BCS
Hellpointner; E.	KIIA 2.10 /01	2010	BYI 02960: Calculation of the chemical half-life in the troposphere Bayer CropScience, Report No.: MEF-10/896, Edition Number: M-398741-01-2 EPA MRID No.: 48843671 Date: 2010-12-27 GLP/GEP: no, unpublished ...also filed: KIIA 7.10 /01	Y	BCS
Mislankar, S.; Woodard, D.	KIIA 2.9.1 /01	2011	BYI-02960: Hydrolytic degradation Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: MERVP019, Edition Number: M-398952-01-2 EPA MRID No.: 48843667 Date: 2011-01-07 GLP/GEP: Y, unpublished ...also filed: KIIA 7.5 /01	Y	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Peters; S.	KIIA 2.5.1.1 /01	2009	Spectral data set of BYI 02960 a.i. - Reference material Bayer CropScience, Report No.: 15-600-2439, Edition Number: M-345761-01-2 EPA MRID No.: 48843628 Date: 2009-04-07 GLP/GEP: Y, unpublished ...also filed: KIIA 2.5.1.2 /01 ...also filed: KIIA 2.5.1.3 /01 ...also filed: KIIA 2.5.1.4 /01 ...also filed: KIIA 2.5.1.5 /01	Y	BCS
Peters; S.	KIIA 2.5.1.2 /01	2009	Spectral data set of BYI 02960 a.i. - Reference material Bayer CropScience, Report No.: 15-600-2439, Edition Number: M-345761-01-2 EPA MRID No.: 48843628 Date: 2009-04-07 GLP/GEP: Y, unpublished ...also filed: KIIA 2.5.1.1 /01 ...also filed: KIIA 2.5.1.3 /01 ...also filed: KIIA 2.5.1.4 /01 ...also filed: KIIA 2.5.1.5 /01	Y	BCS
Peters; S.	KIIA 2.5.1.3 /01	2009	Spectral data set of BYI 02960 a.i. - Reference material Bayer CropScience, Report No.: 15-600-2439, Edition Number: M-345761-01-2 EPA MRID No.: 48843628 Date: 2009-04-07 GLP/GEP: Y, unpublished ...also filed: KIIA 2.5.1.1 /01 ...also filed: KIIA 2.5.1.2 /01 ...also filed: KIIA 2.5.1.4 /01 ...also filed: KIIA 2.5.1.5 /01	Y	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Peters; S.	KIIA 2.5.1.4 /01	2009	Spectral data set of BYI 02960 a.i. - Reference material Bayer CropScience, Report No.: 15-600-2439, Edition Number: M-345761-01-2 EPA MRID No.: 48843628 Date: 2009-04-07 GLP/GEP: Y, unpublished ...also filed: KIIA 2.5.1.1 /01 ...also filed: KIIA 2.5.1.2 /01 ...also filed: KIIA 2.5.1.3 /01 ...also filed: KIIA 2.5.1.5 /01	Y	BCS
Peters; S.	KIIA 2.5.1.5 /01	2009	Spectral data set of BYI 02960 a.i. - Reference material Bayer CropScience, Report No.: 15-600-2439, Edition Number: M-345761-01-2 EPA MRID No.: 48843628 Date: 2009-04-07 GLP/GEP: Y, unpublished ...also filed: KIIA 2.5.1.1 /01 ...also filed: KIIA 2.5.1.2 /01 ...also filed: KIIA 2.5.1.3 /01 ...also filed: KIIA 2.5.1.4 /01	Y	BCS
Petrovic, P.	KIIA 2.18 /02	2011	Flupyradifurone (BYI 02960), technical substance: Complex formation ability in water Allessa Chemie GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: B 015/2011, Edition Number: M-414563-01-2 EPA MRID No.: 48843660 Date: 2011-09-21 GLP/GEP: Y, unpublished	Y	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Schloesser, G.; Mans, H. C.	KIIA 2.18 /07	2011	BYI 02960 - Determination of metallic impurities Currenta GmbH & Co. OHG, Leverkusen, Germany Bayer CropScience, Report No.: 2011/0073/01, Edition Number: M-412025-01-2 EPA MRID No.: 48843661 Date: 2011-08-10 GLP/GEP: Y, unpublished confidential	Y	BCS
Smeykal, H.	KIIA 2.1.1 /01	2010	BYI 02960, pure substance: Melting point, boiling point, thermal stability Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20090051.01, Edition Number: M-367370-01-2 EPA MRID No.: 48843630 Date: 2010-03-25 GLP/GEP: yes, unpublished ...also filed: KIIA 2.1.2 /01 ...also filed: KIIA 2.1.3 /01	Y	BCS
Smeykal, H.	KIIA 2.1.1 /02	2011	Flupyradifurone (BYI 02960), technical substance: Melting point, boiling point, thermal stability Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20110197.01, Edition Number: M-414242-01-2 EPA MRID No.: 48843631 Date: 2011-09-16 GLP/GEP: Y, unpublished ...also filed: KIIA 2.1.2 /02 ...also filed: KIIA 2.1.3 /02	Y	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Smeykal, H.	KIIA 2.1.2 /01	2010	BYI 02960, pure substance: Melting point, boiling point, thermal stability Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20090051.01, Edition Number: M-367370-01-2 EPA MRID No.: 48843630 Date: 2010-03-25 GLP/GEP: Y, unpublished ...also filed: KIIA 2.1.1 /01 ...also filed: KIIA 2.1.3 /01	Y	BCS
Smeykal, H.	KIIA 2.1.2 /02	2011	Flupyradifurone (BYI 02960), technical substance: Melting point, boiling point, thermal stability Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20110197.01, Edition Number: M-414242-01-2 EPA MRID No.: 48843631 Date: 2011-09-16 GLP/GEP: Y, unpublished ...also filed: KIIA 2.1.1 /02 ...also filed: KIIA 2.1.3 /02	Y	BCS
Smeykal, H.	KIIA 2.1.3 /01	2010	BYI 02960, pure substance: Melting point, boiling point, thermal stability Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20090051.01, Edition Number: M-367370-01-2 EPA MRID No.: 48843630 Date: 2010-03-25 GLP/GEP: Y, unpublished ...also filed: KIIA 2.1.1 /01 ...also filed: KIIA 2.1.2 /01	Y	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Smeykal, H.	KIIA 2.1.3 /02	2011	Flupyradifurone (BYI 02960), technical substance: Melting point, boiling point, thermal stability Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20110197.01, Edition Number: M-414242-01-2 EPA MRID No.: 48843631 Date: 2011-09-16 GLP/GEP: Y, unpublished ...also filed: KIIA 2.1.1 /02 ...also filed: KIIA 2.1.2 /02	Y	BCS
Smeykal, H.	KIIA 2.3.1 /01	2008	BYI 02960, pure substance: Vapour pressure - Final report Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20080615.01, Edition Number: M-309853-01-3 EPA MRID No.: 48843650 Date: 2008-10-10 GLP/GEP: Y, unpublished ...also filed: KIIA 7.4.9 /01	Y	BCS
Smeykal, H.	KIIA 2.11.1 /01	2011	Flupyradifurone (BYI 02960), technical substance: flammability (solids) Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20110197.03, Edition Number: M-414249-01-2 EPA MRID No.: 48843621 Date: 2011-09-16 GLP/GEP: Y, unpublished	Y	BCS
Smeykal, H.	KIIA 2.11.2 /01	2011	Flupyradifurone (BYI 02960), technical substance: auto-flammability Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20110197.05, Edition Number: M-414252-01-2 EPA MRID No.: 48843657 Date: 2011-09-16 GLP/GEP: Y, unpublished	Y	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Smeykal, H.	KIIA 2.11.2 /02	2011	Flupyradifurone (BYI 02960), technical substance: autoflammability (UN Bowes Cameron cage test) Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20110197.07, Edition Number: M-414257-01-2 EPA MRID No.: 48843658 Date: 2011-09-16 GLP/GEP: Y, unpublished	Y	BCS
Smeykal, H.	KIIA 2.13 /01	2011	Flupyradifurone (BYI 02960), technical substance: explosive properties Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20110197.04, Edition Number: M-414250-01-2 EPA MRID No.: 48843622 Date: 2011-09-16 GLP/GEP: Y, unpublished	Y	BCS
Smeykal, H.	KIIA 2.15 /01	2011	Flupyradifurone (BYI 02960), technical substance: oxidizing properties Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20110197.06, Edition Number: M-414253-01-2 EPA MRID No.: 48843619 Date: 2011-09-16 GLP/GEP: Y, unpublished	Y	BCS
Smeykal, H.	KIIA 2.18 /03	2011	Flupyradifurone (BYI 02960), technical substance: Particle size distribution Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20110197.02, Edition Number: M-414246-01-2 EPA MRID No.: 48843638 Date: 2011-09-16 GLP/GEP: Y, unpublished	Y	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Wagner, S.	KIIA 2.17.1 /01	2011	Chemical storage stability of BYI 02960 - Flupyradifurone (BYI 02960) Bayer CropScience, Report No.: 15-155-2438, Edition Number: M-411305-01-2 EPA MRID No.: 48843623 Date: 2011-07-19 GLP/GEP: Y, unpublished	Y	BCS
Wagner, S.	KIIA 2.17.2 /01	2011	Stability to normal and elevated temperature, metals, and metal ions and corrosion characteristics to plastic containers of flupyradifurone (BYI 02960) according to OPPTS 830.6313 and 830.6320 Bayer CropScience, Report No.: 15-160-2527, Edition Number: M-413798-01-2 EPA MRID No.: 48843618 Date: 2011-09-12 GLP/GEP: Y, unpublished	Y	BCS
Wiche, A.; Bogdoll, B.	KIIA 2.6 /01	2011	BYI 02960, pure substance: Solubility in distilled water (pH 7), at pH 4 and pH 9 (flask method) Bayer CropScience, Report No.: PA09/003, Edition Number: M-409513-01-2 EPA MRID No.: 48843644 Date: 2011-06-17 GLP/GEP: Y, unpublished	Y	BCS
Wiche, A.; Bogdoll, B.	KIIA 2.9.5 /01	2011	Flupyradifurone (BYI 02960), pure substance : Dissociation constant in water Bayer CropScience, Report No.: PA10/048, Edition Number: M-414102-01-2 EPA MRID No.: 48843634 Date: 2011-09-16 GLP/GEP: Y, unpublished	Y	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Ziemer, F.	KIIA 2.18 /04	2011	Flupyradifurone (BYI 02960), technical substance: Statement on the viscosity according to OPPTS 830.7100 Bayer CropScience, Report No.: AF11/027, Edition Number: M-417799-01-2 EPA MRID No.: 48843629 Date: 2011-11-17 GLP/GEP: no, unpublished	Y	BCS
Ziemer, F.	KIIA 2.18 /05	2011	Flupyradifurone (BYI 02960), technical substance: Statement on the miscibility according to OPPTS 830.6319 Bayer CropScience, Report No.: AF11/026, Edition Number: M-417793-01-2 EPA MRID No.: 48843624 Date: 2011-11-17 GLP/GEP: no, unpublished	Y	BCS
Ziemer, F.	KIIA 2.18 /06	2011	Flupyradifurone (BYI 02960), technical substance: Statement on the dielectric breakdown voltage according to OPPTS 830.6321 Bayer CropScience, Report No.: AF11/028, Edition Number: M-417802-01-2 EPA MRID No.: 48843625 Date: 2011-11-17 GLP/GEP: no, unpublished	Y	BCS

References for the formulation

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Hennig-Gizewski, S.; Hoppe, M.	KIIIA1 2.1 /01	2011	Physical, chemical and technical properties of BYI 02960 SL 200 (200 g/L) - BYI 02960 SL 200 G - amendment no.: 1 Bayer CropScience, Report No.: FM0076(PC00)G01, Edition Number: M-402943-02-1 Date: 2011-02-14 ...Amended: 2011-09-19 GLP/GEP: Y, unpublished ...also filed: KIIIA1 2.4.2 /01 ...also filed: KIIIA1 2.5.1 /01 ...also filed: KIIIA1 2.5.2 /01 ...also filed: KIIIA1 2.5.3 /01 ...also filed: KIIIA1 2.6.1 /01 ...also filed: KIIIA1 2.8.2 /01 ...also filed: KIIIA1 2.8.4 /01	Y	BCS
Hennig-Gizewski, S.; Hoppe, M.	KIIIA1 2.4.2 /01	2011	Physical, chemical and technical properties of BYI 02960 SL 200 (200 g/L) - BYI 02960 SL 200 G - amendment no.: 1 Bayer CropScience, Report No.: FM0076(PC00)G01, Edition Number: M-402943-02-1 Date: 2011-02-14 ...Amended: 2011-09-19 GLP/GEP: Y, unpublished ...also filed: KIIIA1 2.1 /01 ...also filed: KIIIA1 2.5.1 /01 ...also filed: KIIIA1 2.5.2 /01 ...also filed: KIIIA1 2.5.3 /01 ...also filed: KIIIA1 2.6.1 /01 ...also filed: KIIIA1 2.8.2 /01 ...also filed: KIIIA1 2.8.4 /01	Y	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Hennig-Gizewski, S.; Hoppe, M.	KIIIA1 2.5.1 /01	2011	Physical, chemical and technical properties of BYI 02960 SL 200 (200 g/L) - BYI 02960 SL 200 G - amendment no.: 1 Bayer CropScience, Report No.: FM0076(PC00)G01, Edition Number: M-402943-02-1 Date: 2011-02-14 ...Amended: 2011-09-19 GLP/GEP: Y, unpublished ...also filed: KIIIA1 2.1 /01 ...also filed: KIIIA1 2.4.2 /01 ...also filed: KIIIA1 2.5.2 /01 ...also filed: KIIIA1 2.5.3 /01 ...also filed: KIIIA1 2.6.1 /01 ...also filed: KIIIA1 2.8.2 /01 ...also filed: KIIIA1 2.8.4 /01	Y	BCS
Hennig-Gizewski, S.; Hoppe, M.	KIIIA1 2.5.2 /01	2011	Physical, chemical and technical properties of BYI 02960 SL 200 (200 g/L) - BYI 02960 SL 200 G - amendment no.: 1 Bayer CropScience, Report No.: FM0076(PC00)G01, Edition Number: M-402943-02-1 Date: 2011-02-14 ...Amended: 2011-09-19 GLP/GEP: Y, unpublished ...also filed: KIIIA1 2.1 /01 ...also filed: KIIIA1 2.4.2 /01 ...also filed: KIIIA1 2.5.1 /01 ...also filed: KIIIA1 2.5.3 /01 ...also filed: KIIIA1 2.6.1 /01 ...also filed: KIIIA1 2.8.2 /01 ...also filed: KIIIA1 2.8.4 /01	Y	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Hennig-Gizewski, S.; Hoppe, M.	KIIIA1 2.5.3 /01	2011	Physical, chemical and technical properties of BYI 02960 SL 200 (200 g/L) - BYI 02960 SL 200 G - amendment no.: 1 Bayer CropScience, Report No.: FM0076(PC00)G01, Edition Number: M-402943-02-1 Date: 2011-02-14 ...Amended: 2011-09-19 GLP/GEP: Y, unpublished ...also filed: KIIIA1 2.1 /01 ...also filed: KIIIA1 2.4.2 /01 ...also filed: KIIIA1 2.5.1 /01 ...also filed: KIIIA1 2.5.2 /01 ...also filed: KIIIA1 2.6.1 /01 ...also filed: KIIIA1 2.8.2 /01 ...also filed: KIIIA1 2.8.4 /01	Y	BCS
Hennig-Gizewski, S.; Hoppe, M.	KIIIA1 2.6.1 /01	2011	Physical, chemical and technical properties of BYI 02960 SL 200 (200 g/L) - BYI 02960 SL 200 G - amendment no.: 1 Bayer CropScience, Report No.: FM0076(PC00)G01, Edition Number: M-402943-02-1 Date: 2011-02-14 ...Amended: 2011-09-19 GLP/GEP: Y, unpublished ...also filed: KIIIA1 2.1 /01 ...also filed: KIIIA1 2.4.2 /01 ...also filed: KIIIA1 2.5.1 /01 ...also filed: KIIIA1 2.5.2 /01 ...also filed: KIIIA1 2.5.3 /01 ...also filed: KIIIA1 2.8.2 /01 ...also filed: KIIIA1 2.8.4 /01	Y	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Hennig-Gizewski, S.; Hoppe, M.	KIIIA1 2.7.1 /01	2011	Storage stability and shelf life of BYI 02960 SL 200 (200 g/L) - [Packaging material:HDPE] - Final report (2 years) Bayer CropScience, Report No.: FM0076(SL01)N01, Edition Number: M-402996-02-1 Date: 2011-02-14 ...Amended: 2012-07-25 GLP/GEP: no, unpublished ...also filed: KIIIA1 2.7.3 /01 ...also filed: KIIIA1 2.7.4 /01 ...also filed: KIIIA1 2.7.5 /01 ...also filed: KIIIA1 4.1.3 /01	Y	BCS
Hennig-Gizewski, S.; Hoppe, M.	KIIIA1 2.7.2 /01	2012	Storage stability data of BYI 02960 SL 200 (200 g/L) - [Packaging material:HDPE] Bayer CropScience, Report No.: FM0076(EP01)G01, Edition Number: M-432583-01-1 EPA MRID No.: 48844507 Date: 2012-06-18 GLP/GEP: no, unpublished	Y	BCS
Hennig-Gizewski, S.; Hoppe, M.	KIIIA1 2.7.3 /01	2011	Storage stability and shelf life of BYI 02960 SL 200 (200 g/L) - [Packaging material:HDPE] - Final report (2 years) Bayer CropScience, Report No.: FM0076(SL01)N01, Edition Number: M-402996-02-1 Date: 2011-02-14 ...Amended: 2012-07-25 GLP/GEP: no, unpublished ...also filed: KIIIA1 2.7.1 /01 ...also filed: KIIIA1 2.7.4 /01 ...also filed: KIIIA1 2.7.5 /01 ...also filed: KIIIA1 4.1.3 /01	Y	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Hennig-Gizewski, S.; Hoppe, M.	KIIIA1 2.7.4 /01	2011	Storage stability and shelf life of BYI 02960 SL 200 (200 g/L) - [Packaging material:HDPE] - Final report (2 years) Bayer CropScience, Report No.: FM0076(SL01)N01, Edition Number: M-402996-02-1 Date: 2011-02-14 ...Amended: 2012-07-25 GLP/GEP: no, unpublished ...also filed: KIIIA1 2.7.1 /01 ...also filed: KIIIA1 2.7.3 /01 ...also filed: KIIIA1 2.7.5 /01 ...also filed: KIIIA1 4.1.3 /01	Y	BCS
Hennig-Gizewski, S.; Hoppe, M.	KIIIA1 2.7.5 /01	2011	Storage stability and shelf life of BYI 02960 SL 200 (200 g/L) - [Packaging material:HDPE] - Final report (2 years) Bayer CropScience, Report No.: FM0076(SL01)N01, Edition Number: M-402996-02-1 Date: 2011-02-14 ...Amended: 2012-07-25 GLP/GEP: no, unpublished ...also filed: KIIIA1 2.7.1 /01 ...also filed: KIIIA1 2.7.3 /01 ...also filed: KIIIA1 2.7.4 /01 ...also filed: KIIIA1 4.1.3 /01	Y	BCS
Hennig-Gizewski, S.; Hoppe, M.	KIIIA1 2.8.2 /01	2011	Physical, chemical and technical properties of BYI 02960 SL 200 (200 g/L) - BYI 02960 SL 200 G - amendment no.: 1 Bayer CropScience, Report No.: FM0076(PC00)G01, Edition Number: M-402943-02-1 Date: 2011-02-14 ...Amended: 2011-09-19 GLP/GEP: Y, unpublished ...also filed: KIIIA1 2.1 /01 ...also filed: KIIIA1 2.4.2 /01 ...also filed: KIIIA1 2.5.1 /01 ...also filed: KIIIA1 2.5.2 /01 ...also filed: KIIIA1 2.5.3 /01 ...also filed: KIIIA1 2.6.1 /01 ...also filed: KIIIA1 2.8.4 /01	Y	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Hennig-Gizewski, S.; Hoppe, M.	KIIIA1 2.8.4 /01	2011	Physical, chemical and technical properties of BYI 02960 SL 200 (200 g/L) - BYI 02960 SL 200 G - amendment no.: 1 Bayer CropScience, Report No.: FM0076(PC00)G01, Edition Number: M-402943-02-1 Date: 2011-02-14 ...Amended: 2011-09-19 GLP/GEP: Y, unpublished ...also filed: KIIIA1 2.1 /01 ...also filed: KIIIA1 2.4.2 /01 ...also filed: KIIIA1 2.5.1 /01 ...also filed: KIIIA1 2.5.2 /01 ...also filed: KIIIA1 2.5.3 /01 ...also filed: KIIIA1 2.6.1 /01 ...also filed: KIIIA1 2.8.2 /01	Y	BCS
Rexer, K.; Schumacher, R.	KIIIA1 2.2.1 /01	2011	Safety relevant technical properties of BYI 02960 SL 200 (200 g/L) - Final report - Bayer CropScience, Report No.: FF0091(SA00)G01, Edition Number: M-401926-01-1 Date: 2011-02-14 GLP/GEP: Y, unpublished ...also filed: KIIIA1 2.2.2 /01 ...also filed: KIIIA1 2.3.1 /01 ...also filed: KIIIA1 2.3.3 /01	Y	BCS
Rexer, K.; Schumacher, R.	KIIIA1 2.2.2 /01	2011	Safety relevant technical properties of BYI 02960 SL 200 (200 g/L) - Final report - Bayer CropScience, Report No.: FF0091(SA00)G01, Edition Number: M-401926-01-1 Date: 2011-02-14 GLP/GEP: Y, unpublished ...also filed: KIIIA1 2.2.1 /01 ...also filed: KIIIA1 2.3.1 /01 ...also filed: KIIIA1 2.3.3 /01	Y	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Rexer, K.; Schumacher, R.	KIIIA1 2.3.1 /01	2011	Safety relevant technical properties of BYI 02960 SL 200 (200 g/L) - Final report - Bayer CropScience, Report No.: FF0091(SA00)G01, Edition Number: M-401926-01-1 Date: 2011-02-14 GLP/GEP: Y, unpublished ...also filed: KIIIA1 2.2.1 /01 ...also filed: KIIIA1 2.2.2 /01 ...also filed: KIIIA1 2.3.3 /01	Y	BCS
Rexer, K.; Schumacher, R.	KIIIA1 2.3.3 /01	2011	Safety relevant technical properties of BYI 02960 SL 200 (200 g/L) - Final report - Bayer CropScience, Report No.: FF0091(SA00)G01, Edition Number: M-401926-01-1 Date: 2011-02-14 GLP/GEP: Y, unpublished ...also filed: KIIIA1 2.2.1 /01 ...also filed: KIIIA1 2.2.2 /01 ...also filed: KIIIA1 2.3.1 /01	Y	BCS

Section A.3 Further information (Annex IIA 3, Annex IIIA 3 and 4)

References for the active substance

Author(s)	Annex point / reference number	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. claimed	Owner
Anon.	KIIA 3.7 /01	2012	Material safety data sheet for flupyradifurone TC Bayer CropScience, Report No.: M-424153-02-1, Edition Number: M-424153-02-1 EPA MRID No.: 48844202 Date: 2012-07-26 GLP/GEP: n.a., unpublished ...also filed: KIIA 9 /01	Y	BCS
Nauen, R.	KIIA 3.6 /01	2012	Statement: Possible development of resistance or cross-resistance flupyradifurone (BYI 2960) Bayer CropScience, Report No.: M-428792-01-2, Edition Number: M-428792-01-2 EPA MRID No.: 48844201 Date: 2012-04-04 GLP/GEP: n.a., unpublished	Y	BCS
Nuesslein, F.	KIIA 3.8.1 /01	2012	Flupyradifurone - Incineration as a safe means of disposal and pyrolytic behaviour under controlled conditions Bayer CropScience, Report No.: M-422993-01-2, Edition Number: M-422993-01-2 EPA MRID No.: 48844203 Date: 2012-01-23 GLP/GEP: no, unpublished	Y	BCS
Rauen, H. W.	KIIA 3.4.1 /01	2012	Document D1 - Safe uses to support the EU Dossier for Annex I inclusion for which data have been provided for flupyradifurone TC Bayer CropScience Bayer CropScience, Report No.: M-434984-01-1, Edition Number: M-434984-01-1 Date: 2012-08-06 GLP/GEP: n.a., unpublished **...also filed: D 1 /01**	Y	BCS

Author(s)	Annex point / reference number	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. claimed	Owner
Rauen, H. W.; Huang, J.; Lidstone, R.; Ledesma, A.; Perkins, G.; Paranhos, M.	KIIA 3.4.1 /02	2012	Document D3 - Intended uses supported in Australia, Brazil, Canada, Mexico and USA (Partners in the global joint review) for which data will be provided for flupyradifurone TC and its formulants Sivanto 200 SL and BYI 02960 480 FS Bayer CropScience Bayer CropScience, Report No.: M-434988-01-1, Edition Number: M-434988-01-1 Date: 2012-08-06 GLP/GEP: n.a., unpublished **...also filed: D 3 /01**	Y	BCS

References for the plant protection product

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. Claimed	Owner
Anon.	KIIIA1 4.1 /01	2012	Butenolide (BYI 02960) SL200 8X2L CAS CA Bayer CropScience, Report No.: M-423030-03-1, Edition Number: M-423030-03-1 GLP/GEP: n.a., unpublished ...also filed: KIIIA1 11.3 /01 ...also filed: KIIIA1 11.4 /01	Y	BCS
Hennig-Gizewski, S.; Hoppe, M.	KIIIA1 4.1.3 /01	2011	Storage stability and shelf life of BYI 02960 SL 200 (200 g/L) - [Packaging material:HDPE] - Final report (2 years) Bayer CropScience, Report No.: FM0076(SL01)N01, Edition Number: M-402996-02-1 Date: 2011-02-14 ...Amended: 2012-07-25 GLP/GEP: no, unpublished ...also filed: KIIIA1 2.7.1 /01 ...also filed: KIIIA1 2.7.3 /01 ...also filed: KIIIA1 2.7.4 /01 ...also filed: KIIIA1 2.7.5 /01	Y	BCS
Nuesslein, F.	KIIIA1 4.7 /01	2012	Flupyradifurone - Incineration as a safe means of disposal and pyrolytic behaviour under controlled conditions Bayer CropScience, Report No.: M-422993-01-1, Edition Number: M-422993-01-1 Date: 2012-01-23 GLP/GEP: no, unpublished	Y	BCS

Section A.4 Proposals for classification and labelling (Annex IIA 10; Annex IIIA 12.3 and 12.4)

The proposed classification and labelling for the active substance Flupyradifurone and the formulations is based on the physico-chemical, toxicological and ecotoxicological evaluation as presented in section B.2, B.6 and B.9 respectively.

Section A.5 Methods of analysis (Annex IIA 4; Annex IIIA 5)

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Ballard, T. D.	KIIA 4.3 /09	2012	FDA PAM multiresidue method (MRM) testing for BYI 02960 and Four Metabolites EN-CAS Analytical Laboratories, Winston-Salem, NC, USA BCS, Report No.: RARVP039, Edition Number: M-432818-01-1 EPA MRID No.: 48843839 Date: 2012-06-19 GLP/GEP: yes, unpublished	Y	Bayer CropScience
Brumhard, B.: Reineke, A.	KIIA 4.4 /01	2009	Analytical method 01074 for the determination of BYI 02960 in soil using LC/MS/MS Bayer CropScience, Report No.: 01074, Edition Number: M-337752-01-2 Method Report No.: MR-07/337 EPA MRID No.: 48843829 Date: 2009-02-24 GLP/GEP: yes, unpublished	Y	BCS
Fargeix, G.; Rosati, D.	KIIA 4.5 /01	2012	Analytical method no 01213 for the determination of residues of BYI 02960 in drinking and surface water by HPLC-MS/MS Bayer S.A.S., Bayer CropScience, Lyon, France Bayer CropScience, Report No.: 01213, Edition Number: M-428019-01-2 Method Report No.: MR-12/022 EPA MRID No.: 48843834 Date: 2012-03-29 GLP/GEP: yes, unpublished	Y	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Fenn, L.	KIIA 4.5 /04	2012	Independent laboratory validation of Bayer method - RV-005-W12-01 Analytical method for the determination of residues of BYI 02960 and its metabolites difluoroacetic acid, BYI 02960-succinamide, BYI 02960-azabicycclosuccinamide in water using LC/MS/MS JRF America, Audubon, PA, USA Bayer CropScience, Report No.: RARVP048, Edition Number: M-433352-01-1 EPA MRID No.: 48843837 Date: 2012-06-28 GLP/GEP: yes, unpublished	Y	BCS
Heinz, N.	KIIA 4.7 /01	2011	BYI 02960: Analytical method for determination in air PTRL Europe GmbH, Ulm, Germany Bayer CropScience, Report No.: P 2419 G, Edition Number: M-420657-01-1 EPA MRID No.: 48843838 Date: 2011-12-14 GLP/GEP: yes, unpublished	Y	BCS
Justus, K.	KIIA 4.3 /06	2011	Extraction efficiency testing of the residue analytical method RV-001-P10-02 for the determination of BYI 02960, 6-chloronicotinic acid, difluoroacetic acid and difluoroethyl-amino-furanone in plant matrices using aged radioactive residues Bayer CropScience, Report No.: MEF-11/793, Edition Number: M-419323-01-1 EPA MRID No.: 48843821 Date: 2011-12-01 GLP/GEP: yes, unpublished	Y	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Konrad, S.	KIIA 4.3 /02	2012	Independent lab validation of BCS method 01330 for the determination of residues of BYI 02960 and its metabolite difluoroacetic acid in/on plant matrices by HPLC-MS/MS Currenta GmbH & Co. OHG, Leverkusen, Germany Bayer CropScience, Report No.: 2011/0134/01, Edition Number: M-427133-01-1 Method Report No.: 2011/0134/01 EPA MRID No.: 48843818 Date: 2012-02-28 GLP/GEP: yes, unpublished	Y	BCS
Konrad, S.	KIIA 4.3 /11	2012	Independent lab validation of BCS method 01214 for the determination of residues of BYI 02960 and its metabolite difluoroacetic acid in/on animal matrices by HPLC-MS/MS Currenta GmbH & Co. OHG, Leverkusen, Germany BCS, Report No.: 2011/0164/01, Edition Number: M-427160-01-1 Method Report No.: 2011/0164/01 EPA MRID No.: 48843826 Date: 2012-02-28 GLP/GEP: yes, unpublished	Y	Bayer CropScience
Kraemer, F.; Ruengeler, W.	KIIA 4.2.3 /05	2011	Flupyradifurone - Determination of diisopropylethylamine, butyronitrile, 1-butanol and tert.-butylmethylether in flupyradifurone (BYI 02960) GLC - external standard (Headspace) Bayer CropScience, Report No.: AM015011MP1, Edition Number: M-411557-01-2 EPA MRID No.: 48843612 Date: 2011-05-05 GLP/GEP: no, unpublished confidential	Y	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Kraemer, F.; Ruengeler, W.	KIIA 4.2.3 /06	2011	Validation of GLC-method AM015011MP1 - Determination of tert.-butylmethylether and methylcyclohexane in flupyradifurone - GLC - external standard (Headspace) Bayer CropScience, Report No.: VB1-AM015011MP1, Edition Number: M-411560-01-2 EPA MRID No.: 48843613 Date: 2011-05-05 GLP/GEP: yes, unpublished confidential	Y	BCS
Li, Y.	KIIA 4.3 /04	2012	An analytical method for the determination of residues of BYI 02960, 6-chloronicotinic acid, difluoroacetic acid, and difluoroethyl-amino-furanone in plant matrices using LC/MS/MS Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: RV-001-P10-03, Edition Number: M-433355-01-1 Method Report No US: RV-001-P10-03 EPA MRID No.: 48843823 Date: 2012-06-27 GLP/GEP: no, unpublished	Y	BCS
Li, Y.; Moore, S.	KIIA 4.3 /12	2012	An analytical method for the determination of residues of BYI 02960 and its metabolites BYI02960-hydroxy, BYI 02960-acetyl-AMCP, and difluoroacetic acid in animal matrices and biota using LC/MS/MS BCS LP, Stilwell, KS, USA BCS, Report No.: RV-004-A11-05, Edition Number: M-429871-01-1 Method Report No.: RV-004-A11-05 EPA MRID No.: 48843827 Date: 2012-04-24 GLP/GEP: no, unpublished	Y	Bayer CropScience

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Li, Y.; Schoening, R.	KIIA 4.3 /05	2011	Amendment No. 1 - Validation of Bayer CropScience method RV-001-P10-02 - An analytical method for the determination of residues of BYI 02960, 6-chloronicotinic acid, difluoroacetic acid, and difluoroethyl-amino-furanone in plant matrices using LC/MS/MS BCS LP, Stilwell, KS, USA Bayer CropScience, Report No.: RARVP013, Edition Number: M-415504-02-2 Method Report No US: RARVP013 EPA MRID No.: 48843820 Date: 2011-10-12 **...Amended: 2012-01-11** GLP/GEP: yes, unpublished	Y	BCS
Moore, S. M.; Harbin, A. M.	KIIA 4.3 /13	2012	BYI 02960 - Magnitude of the residue in dairy cows - Amended report BCS LP, Stilwell, KS, USA Bayer CropScience, Report No.: RARVP050-1, Edition Number: M-428416-02-1 EPA MRID No.: 48843842 Date: 2012-04-03 **...Amended: 2012-05-31** GLP/GEP: yes, unpublished ...also filed: KIIA 6.1.1 /02 ...also filed: KIIA 6.4.2 /01	Y	BCS
Netzband, D.	KIIA 4.4 /02	2010	An analytical method for the determination of residues of BYI 02960, 6-chloronicotinic acid (6-CNA) and difluoroacetic acid (DFA) in soil and sediment using LC/MS/MS Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: RV-002-S10-01, Edition Number: M-401212-01-1 EPA MRID No.: 48843830 Date: 2010-10-21 GLP/GEP: no, unpublished	Y	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Netzband, D.	KIIA 4.4 /03	2011	Bayer method RV-002-S10-02 - An analytical method for the determination of residues of BYI 02960, 6-chloronicotinic acid (6-CNA) and difluoroacetic acid (DFA) in soil and sediment using LC/MS/MS Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: RV-002-S10-02, Edition Number: M-428409-01-1 EPA MRID No.: 48843831 Date: 2011-12-06 GLP/GEP: no, unpublished	Y	BCS
Netzband, D.	KIIA 4.5 /02	2012	Bayer method RV-005-W12-01 - Analytical method for the determination of residues of BYI 02960 and its metabolites Difluoroacetic acid, BYI 2960-Succinamide and BYI 2960-Azabicyclosuccinamide in water using LC/MS/MS Bayer CropScience, Stilwell, KS, USA Bayer CropScience, Report No.: RV-005-W12-01, Edition Number: M-435207-01-1 EPA MRID No.: 48843835 Date: 2012-05-04 GLP/GEP: no, unpublished	Y	BCS
Netzband, D. J.	KIIA 4.5 /03	2012	In house laboratory validation of an analytical method for the determination of residues of BYI 02960 and its metabolites difluoroacetic acid, BYI 02960-succinamide and BYI 02960-azabicyclosuccinamide in water using LC/MS/MS Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: RARVP010, Edition Number: M-433268-01-1 Method Report No US: RARVP010 EPA MRID No.: 48843836 Date: 2012-06-25 GLP/GEP: yes, unpublished	Y	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Netzband, D. J.; Stoughton, S. M.	KIIA 4.4 /04	2012	In house laboratory validation of an analytical method for the determination of residues of BYI 02960 and its metabolites 6-chloronicotinic acid (6-CNA) and difluoroacetic acid (DFA) in soil and sediment using LC/MS/MS Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: RARVP008, Edition Number: M-428405-01-1 EPA MRID No.: 48843832 Date: 2012-04-03 GLP/GEP: yes, unpublished	Y	BCS
Pennell, M. F.	KIIA 4.4 /05	2012	Independent laboratory validation of Bayer method RV-002-S10-02 for the determination of BYI 02960 and its two acid metabolites in soil and sediment en-cas Analytical Laboratories, Winston-Salem, NC, USA Bayer CropScience, Report No.: RARVP049, Edition Number: M-431282-01-1 EPA MRID No.: 48843833 Date: 2012-05-16 GLP/GEP: yes, unpublished	Y	BCS
Rosati, D.	KIIA 4.3 /08	2012	Analytical method no 01212 for the determination of residues of BYI 02960 and its metabolites BCS-AA56716 (DFA), AE F161089 (6CNA) and BCS-CC98193 (furanone) in/on plant material by HPLC-MS/MS Bayer S.A.S., Bayer CropScience, Lyon, France BCS, Report No.: 01212, Edition Number: M-428017-01-2 Method Report No.: MR-10/174 EPA MRID No.: 48843824 Date: 2012-03-27 GLP/GEP: yes, unpublished	Y	Bayer CropScience

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Schierhoff, R.	KIIA 4.3 /07	2012	Independent laboratory validation - Bayer method RV-001-P10-02 for BYI 02960, 6-chloronicotinic acid, difluoroacetic acid, and difluoroethyl-amino-furanone in plant matrices using LC/MS/MS ABC Laboratories, Inc., Columbia, MO, USA Bayer CropScience, Report No.: ABC 67567, Edition Number: M-432661-01-2 EPA MRID No.: 48843822 Date: 2012-05-21 GLP/GEP: yes, unpublished	Y	BCS
Schulte, G.; Bauer, J.	KIIA 4.3 /01	2012	Analytical method 01330 for the determination of residues of BYI 02960 and its metabolite difluoroacetic acid in/on plant matrix by HPLC-MS/MS - Enforcement method plant Bayer CropScience, Report No.: 01330, Edition Number: M-425848-01-2 Method Report No.: 01330 EPA MRID No.: 48843817 Date: 2012-02-22 GLP/GEP: yes, unpublished	Y	BCS
Schulte, G.; Bauer, J.	KIIA 4.3 /10	2012	Analytical method 01214 for the determination of residues of BYI 02960 and its metabolite difluoroacetic acid in/on animal matrices by HPLC-MS/MS - Enforcement method animal BCS, Report No.: 01214, Edition Number: M-425837-01-1 Method Report No.: 01214 EPA MRID No.: 48843825 Date: 2012-02-22 GLP/GEP: yes, unpublished	Y	Bayer CropScience

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Wade, J. M.; Netzband, D. J.	KIIA 4.3 /14	2012	BYI 02960 - Magnitude of the residue in laying hens Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: RARVP041, Edition Number: M-428933-02-1 EPA MRID No.: 48843843 Date: 2012-04-05 **...Amended: 2012-06-25** GLP/GEP: yes, unpublished ...also filed: KIIA 6.4.1 /01	Y	BCS
Wagner, N.; Wessa, S.	KIIA 4.2.3 /07	2010	Flupyradifurone (BYI 02960) - Determination of sulphated ashes in flupyradifurone (BYI 02960) Bayer CropScience, Report No.: AM016411MP1, Edition Number: M-414525-01-2 EPA MRID No.: 48843614 Date: 2010-02-22 GLP/GEP: no, unpublished	Y	BCS
Wagner, S.	KIIA 4.2.1 /02	2011	Validation of AM008809MP1 - Flupyradifurone (BYI 02960) - Determination of technical grade active substance HPLC - ISTD Bayer CropScience, Report No.: VB1-AM008809MP1, Edition Number: M-409002-01-2 EPA MRID No.: 48843607 Date: 2011-06-06 GLP/GEP: no, unpublished	Y	BCS
Wagner, S.	KIIA 4.2.3 /03	2011	Validation of AM013011MP2 - Flupyradifurone (BYI 02960) - Impurities in technical grade active substance - HPLC-ESTD Bayer CropScience, Report No.: VB1-AM013011MP2, Edition Number: M-417931-01-2 EPA MRID No.: 48843609 Date: 2011-11-18 GLP/GEP: no, unpublished confidential	Y	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Wagner, S.	KIIA 4.2.3 /04	2011	Validation of AM014011MP2 - Flupyradifurone (BYI 02960) polar impurities in technical grade active substance HPLC-ESTD Bayer CropScience, Report No.: VB1-AM014011MP2, Edition Number: M-417933-01-2 EPA MRID No.: 48843611 Date: 2011-11-21 GLP/GEP: no, unpublished confidential	Y	BCS
Wagner, S.	KIIA 4.2.3 /08	2011	Validation of AM016411MP1 - Determination of sulphated ashes in flupyradifurone (BYI 02960) Bayer CropScience, Report No.: VB1-AM016411MP1, Edition Number: M-415243-01-2 EPA MRID No.: 48843615 Date: 2011-10-07 GLP/GEP: yes, unpublished confidential	Y	BCS
Willoh, J. M.	KIIA 4.3 /15	2012	Independent laboratory validation of the residue analytical method: an analytical method for the determination of residues of BYI 02960 and its metabolites BYI 02960-hydroxy, BYI 02960-acetyl-AMCP, and difluoroacetic acid in animal matrices and biota using LC/MS/MS, Bayer Method No. RV-004-A11-04 Morse Laboratories, Inc., Sacramento, CA, USA Bayer CropScience, Report No.: RARVP042, Edition Number: M-432185-02-1 EPA MRID No.: 48843828 Date: 2012-05-30 **...Amended: 2012-06-20** GLP/GEP: yes, unpublished	Y	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Ying Li	KIIA 4.3 /03	2010	Bayer Method RV-001-P10-02 - An analytical method for the determination of residues of BYI 02960, 6-chloronicotinic acid, difluoroacetic acid, and difluoroethyl-amino-furanone in plant matrices using LC/MS/MS Bayer CropScience LP, Stilwell, KS, USA BCS, Report No.: RV-001-P10-02, Edition Number: M-401023-01-2 EPA MRID No.: 48843819 Date: 2010-11-05 GLP/GEP: yes, unpublished	Y	BCS
zur Muehlen, U.; Wagner, S.	KIIA 4.2.1 /01	2011	Flupyradifurone (BYI 02960) - Determination of technical grade active substance HPLC - ISTD Bayer CropScience, Report No.: AM008809MP1, Edition Number: M-407218-01-2 EPA MRID No.: 48843606 Date: 2011-05-09 GLP/GEP: no, unpublished	Y	BCS
zur Muehlen, U.; Wagner, S.	KIIA 4.2.3 /01	2011	Flupyradifurone (BYI 02960) - Impurities in technical grade active substance - HPLC - external standard Bayer CropScience, Report No.: AM013011MP2, Edition Number: M-407220-02-2 EPA MRID No.: 48843608 Date: 2011-05-09 **...Amended: 2011-09-27** GLP/GEP: no, unpublished confidential	Y	BCS
zur Muehlen, U.; Wagner, S.	KIIA 4.2.3 /02	2011	Analytical method - Flupyradifurone (BYI 02960) polar impurities in technical grade active substance HPLC - external standard Bayer CropScience, Report No.: AM014011MP2, Edition Number: M-406294-02-2 EPA MRID No.: 48843610 Date: 2011-09-27 **...Amended: 2011-09-29** GLP/GEP: no, unpublished confidential	Y	BCS

Section A.6 Toxicology and metabolism (Annex IIA 5; Annex IIIA 7)

References for the active

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
[redacted].	KIIA 5.3.1 /02	2008	BYI 02960 - Exploratory 28-day toxicity study in the rat by dietary administration [redacted] Bayer CropScience, Report No.: SA 07047, Edition Number: M-297120-01-2 EPA MRID No.: 48844150 Date: 2008-02-01 GLP/GEP: no, unpublished	Yes	BCS
[redacted]	KIIA 5.3.1 /03	2007	BYI 02960 : Preliminary 28-day toxicity study in the mouse by dietary administration [redacted] Bayer CropScience, Report No.: SA 07013, Edition Number: M-294820-01-2 EPA MRID No.: 48844151 Date: 2007-11-23 GLP/GEP: no, unpublished	Yes	BCS
[redacted]	KIIA 5.3.4 /01	2012	A chronic toxicity feeding study in the Beagle dog with technical grade BYI 02960 [redacted] Bayer CropScience, Report No.: 09-C76-RZ, Edition Number: M-425272-01-2 EPA MRID No.: 48844121 Date: 2012-02-17 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 5.3.7 /01	2012	A subacute dermal toxicity study in rats with BYI 02960 [redacted] Bayer CropScience, Report No.: 11-S22-US, Edition Number: M-432336-01-1 EPA MRID No.: 48844115 Date: 2012-06-05 GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
[redacted]	KIIA 5.3.1 /01	2007	BYI 02960 - Exploratory 28-day toxicity study in the rat by gavage [redacted] Bayer CropScience, Report No.: SA 06075, Edition Number: M-283421-02-2 EPA MRID No.: 48844149 Date: 2007-02-02 **...Amended: 2009-02-24** GLP/GEP: no, unpublished	Yes	BCS
[redacted]	KIIA 5.3.3 /01	2010	A 90-day toxicity feeding study in the beagle dog with technical grade BYi 02960 [redacted] Bayer CropScience, Report No.: 09-S76-QQ, Edition Number: M-369978-01-2 EPA MRID No.: 48844114 Date: 2010-04-22 GLP/GEP: yes, unpublished	Yes	BCS
Entian, G.	KIIA 5.4.3 /01	2009	BYI 02960 (tested as BYI 02960 technical) (project: BYI 02960) - V79/HPRT test in vitro for the detection of induced forward mutations Bayer Schering Pharma AG, Wuppertal, Germany Bayer CropScience, Report No.: AT05625, Edition Number: M-359743-01-2 EPA MRID No.: 48844128 Date: 2009-10-29 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 5.2.3 /01	2010	BYI 02960 - Activity ID TXRVP033 - Acute inhalation toxicity in rats [redacted] Report No.: AT05727, Edition Number: M-362791-01-2 EPA MRID No.: 48844105 Date: 2010-01-07 GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
[redacted]	KIIA 5.5.2 /01	2012	BYI 02960 - Chronic toxicity and carcinogenicity study in the Wistar rat by dietary administration [redacted] Bayer CropScience, Report No.: SA 08337, Edition Number: M-428257-01-1 EPA MRID No.: 48844123 Date: 2012-03-05 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 5.7.1 /01	2011	BYI 02960 An acute neurotoxicity study in the rat by oral administration [redacted] Bayer CropScience, Report No.: SA 10096, Edition Number: M-415408-01-2 EPA MRID No.: 48844138 Date: 2011-09-30 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 5.7.4 /01	2011	BYI 02960 - A 90-day neurotoxicity study in the rat by dietary administration [redacted] Report No.: SA 09283, Edition Number: M-410022-01-2 EPA MRID No.: 48844139 Date: 2011-06-28 GLP/GEP: yes, unpublished	Yes	BCS
[redacted].	KIIA 5.8 /04	2010	BCS-AA56716 - Acute oral toxicity in rats - Acute toxic class method [redacted] Bayer CropScience, Report No.: 37066 TAR, Edition Number: M-393372-01-2 EPA MRID No.: 48844102 Date: 2010-10-22 GLP/GEP: no, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
[redacted].	KIIA 5.2.1 /01	2009	BYI 02960 - Acute toxicity in the rat after oral administration [redacted] Bayer CropScience, Report No.: AT05287, Edition Number: M-349992-01-2 EPA MRID No.: 48844101 Date: 2009-06-08 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 5.2.2 /01	2009	BYI 02960 - Acute toxicity in the rat after dermal administration [redacted] Bayer CropScience, Report No.: AT05288, Edition Number: M-349995-01-2 EPA MRID No.: 48844104 Date: 2009-06-08 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 5.7.5 /01	2012	A developmental neurotoxicity study with technical grade BYI 02960 in Wistar rats [redacted] Bayer CropScience, Report No.: 11-D72-UW, Edition Number: M-434203-01-1 EPA MRID No.: 48844140 Date: 2012-07-09 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 5.2.4 /01	2009	BYI 02960 - Acute skin irritation/corrosion on rabbits [redacted] Bayer CropScience, Report No.: AT05342, Edition Number: M-353761-01-2 EPA MRID No.: 48844107 Date: 2009-07-08 GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
[redacted]	KIIA 5.2.5 /01	2009	BYI 02960 - Acute eye irritation on rabbits [redacted] Bayer CropScience, Report No.: AT05341 A, Edition Number: M-361319-02-2 EPA MRID No.: 48844106 Date: 2009-07-08 **...Amended: 2009-10-29** GLP/GEP: yes, unpublished	Yes	BCS
Hall, C.	KIIA 5.8 /02	2010	BCS-AA56716 (metabolite of BYI 02960) - In vitro chromosome aberration test in Chinese hamster V79 cells Harlan CCR, Rossdorf, Germany Bayer CropScience, Report No.: 1351103, Edition Number: M-409726-01-2 EPA MRID No.: 48844132 Date: 2010-12-15 GLP/GEP: no, unpublished	Yes	BCS
Hall, C.	KIIA 5.8 /03	2010	BCS-AA56716 (metabolite of BYI 02960) - Gene mutation assay in Chinese hamster v79 cells in vitro (V79 / HPRT) Harlan CCR, Rossdorf, Germany Bayer CropScience, Report No.: 1351102, Edition Number: M-409727-01-2 EPA MRID No.: 48844129 Date: 2010-12-20 GLP/GEP: no, unpublished	Yes	BCS
Hall, C.	KIIA 5.8 /08	2010	BYI 02960-difluoroethyl-amino-furanone (metabolite of BYI 02960) - In vitro chromosome aberration test in Chinese hamster V79 cells Harlan CCR, Rossdorf, Germany Bayer CropScience, Report No.: 1399703, Edition Number: M-420108-01-2 EPA MRID No.: 48844133 Date: 2010-10-07 GLP/GEP: no, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Hall, C.	KIIA 5.8 /09	2010	BYI 0960-difluoroethyl-amino-furanone (metabolite of BYI 02960) - Gene mutation assay in Chinese hamster V79 cells in vitro (V79 / HPRT) Harlan CCR, Rossdorf, Germany Bayer CropScience, Report No.: 1399702, Edition Number: M-420095-01-2 EPA MRID No.: 48844130 Date: 2010-12-20 GLP/GEP: yes, unpublished	Yes	BCS
Hall, C.	KIIA 5.8 /10	2011	Micronucleus assay in bone marrow cells of the mouse with BYI 02960-difluoroethyl-aminofuranone (metabolite of BYI 02960) Harlan Cytotest Cell Research GmbH, Rossdorf, Germany Bayer CropScience, Report No.: M-420540-01-2, Edition Number: M-420540-01-2 EPA MRID No.: 48844136 Date: 2011-11-28 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 5.8 /11	2011	In vivo unscheduled DNA synthesis in rat hepatocytes with BYI 02960-difluoroethyl-amino-furanone (metabolite of BYI 02960) [redacted] Bayer CropScience, Report No.: 1421402, Edition Number: M-420111-01-2 EPA MRID No.: 48844137 Date: 2011-10-26 GLP/GEP: no, unpublished	Yes	BCS
Herbold, B.	KIIA 5.4.1 /01	2009	BYI 02960 (tested as BYI 02960 technical) (project: BYI 02960) - Salmonella/microsome test plate incorporation and preincubation method Bayer Schering Pharma AG, Wuppertal, Germany Bayer CropScience, Report No.: AT05387, Edition Number: M-354173-01-2 EPA MRID No.: 48844124 Date: 2009-07-24 GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
[redacted]	KIIA 5.4.4 /01	2009	BYI 02960 - Micronucleus-test on the male mouse [redacted] Bayer CropScience, Report No.: AT05350, Edition Number: M-353785-01-2 EPA MRID No.: 48844134 Date: 2009-07-09 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 5.5.3 /01	2012	BYI 02960 - Carcinogenicity study in the C57BL/6J mouse by dietary administration [redacted] Bayer CropScience, Report No.: SA 08338, Edition Number: M-425975-01-1 EPA MRID No.: 48844122 Date: 2012-02-24 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 5.6.11 /01	2012	BYI 02960 - Developmental toxicity study in the rabbit by gavage [redacted] Bayer CropScience, Report No.: SA 10314, Edition Number: M-423559-01-1 EPA MRID No.: 48844117 Date: 2012-01-26 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 5.8 /05	2011	BCS-AA56716 (difluoroacetic acid): Preliminary 14-day toxicity study in the rat by dietary administration [redacted] Bayer CropScience, Report No.: SA 10323, Edition Number: M-414152-01-2 EPA MRID No.: 48844153 Date: 2011-09-19 GLP/GEP: no, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
[redacted]	KIIA 5.8 /06	2012	BCS-AA56716 (Difluoroacetic acid) - 90-day toxicity study in the rat by dietary administration [redacted] Bayer CropScience, Report No.: SA 10324, Edition Number: M-424611-01-2 EPA MRID No.: 48844113 Date: 2012-02-02 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 5.1.1 /01	2012	[Pyridinylmethyl-14C]BYI 02960 - Absorption, distribution, excretion, and metabolism in the rat [redacted] Report No.: MEF-11/747, Edition Number: M-422210-01-1 EPA MRID No.: 48844141 Date: 2012-01-12 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 5.1.2 /02	2011	Quantitative whole body autoradiography of [furanone-4-14C]BYI 02960 in male and female rats: Distribution of total radioactivity and elimination from blood, organs and tissues after single oral administration including determination of radioactivity in the excreta and exhaled 14CO2 [redacted] Report No.: MEF-11/275, Edition Number: M-409859-01-2 EPA MRID No.: 48844144 Date: 2011-05-30 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 5.1.2 /03	2011	[Furanone-4-14C]BYI 02960 - Metabolism in organs and tissues of male and female rats [redacted] Report No.: MEF-11/271, Edition Number: M-414034-02-2 EPA MRID No.: 48844145 Date: 2011-09-12 **...Amended: 2012-02-02** GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
[redacted]	KIIA 5.1.3 /02	2011	[Ethyl-1-14C]BYI 02960 - Metabolism in organs and tissues of male and female rats (3 time-points) [redacted] Report No.: MEF-11/270, Edition Number: M-415416-02-1 EPA MRID No.: 48844147 Date: 2011-09-29 **...Amended: 2012-02-02** GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 5.1.1 /02	2011	Quantitative whole body autoradiography of [pyridinylmethyl-14C]BYI 02960 in male and female rats: Distribution of total radioactivity and elimination from blood, organs and tissues after single oral administration including determination of radioactivity in the excreta and exhaled 14CO2 [redacted] Report No.: MEF-11/276, Edition Number: M-409993-01-2 EPA MRID No.: 48844142 Date: 2011-05-30 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 5.8 /13	2012	BYI 02960-difluoroethyl aminofuranone: A 14-day dose range finding toxicity/palatability study in rats [redacted] Report No.: 11/116-100PE, Edition Number: M-426158-01-2 EPA MRID No.: 48844109 Date: 2012-02-24 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 5.8 /14	2012	BYI 02960-difluoroethyl aminofuranone: A 28-day dietary toxicity study in wistar rats [redacted] Bayer CropScience, Report No.: 11/116-100P, Edition Number: M-426136-01-2 EPA MRID No.: 48844110 Date: 2012-02-29 GLP/GEP: yes, unpublished	Yes	BCS

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[redacted]	KIIA 5.6.10 /01	2010	BYI 02960: Developmental toxicity study in the rat by gavage [redacted] Bayer CropScience, Report No.: SA 08347, Edition Number: M-363938-01-2 EPA MRID No.: 48844116 Date: 2010-02-22 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 5.6.10 /02	2012	BYI 02960 - Complementary maternal tolerability study in the pregnant Sprague-Dawley rat by gavage [redacted] Bayer CropScience, Report No.: SA 11140, Edition Number: M-425810-01-2 EPA MRID No.: 48844118 Date: 2012-02-21 GLP/GEP: yes, unpublished	Yes	BCS
Lasserre, D.	KIIA 5.10 /03	2012	BYI 02960 and its major metabolite DFA (difluoroacetic acid) - Toxicological profile comparison Bayer S.A.S., Bayer CropScience, Sophia Antipolis, France Bayer CropScience, Report No.: M-428487-01-2, Edition Number: M-428487-01-2 EPA MRID No.: 48844155 Date: 2012-05-31 GLP/GEP: n.a., unpublished	Yes	BCS
[redacted]	KIIA 5.6.1 /01	2010	Technical grade BYI 02960: A dose range-finding reproductive toxicity study in the Wistar rat [redacted] Bayer CropScience, Report No.: 09-P72-RB, Edition Number: M-394208-01-2 EPA MRID No.: 48844120 Date: 2010-11-01 GLP/GEP: yes, unpublished	Yes	BCS

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[redacted]	KIIA 5.6.1 /02	2009	Technical grade BYF 02960: A two-generation reproductive toxicity study in the Wistar rat [redacted] Report No.: 09-R72-SA, Edition Number: M-417665-01-2 EPA MRID No.: 48844119 Date: 2009-10-05 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 5.8 /16	1997	Acute oral toxicity study in rats IM-0 [redacted] Report No.: G-0887, Report includes Trial Nos.: 3662 Edition Number: M-195899-01-2 EPA MRID No.: 44988421 Date: 1997-09-30 GLP/GEP: yes, unpublished	Yes	Nippon Soda Co., Ltd., JPN
[redacted]	KIIA 5.8 /19	1997	Acute oral toxicity study in rats IC-0 [redacted] Report No.: G-0941, Report includes Trial Nos.: 3686 Edition Number: M-195930-01-2 EPA MRID No.: 44988420 Date: 1997-09-30 GLP/GEP: yes, unpublished	Yes	Nippon Soda Co., Ltd., JPN
Nobuo, M.; Yukihiro, K.	KIIA 5.8 /15	1997	Reverse mutation study on bacteria IM-0 Nippon Soda Co., Ltd., Odawara Reseach Center, Japan Nippon Soda Co., Ltd., JPN, Report No.: G-949, Report includes Trial Nos.: 9862 Edition Number: M-195904-01-2 EPA MRID No.: 44988432 Date: 1997-09-30 GLP/GEP: yes, unpublished	Yes	Nippon Soda Co., Ltd., JPN

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Nobuo, M.; Yukihiro, K.	KIIA 5.8 /18	1997	Reverse mutation study on bacteria IC-0 Nippon Soda Co., Ltd., Odawara Reseach Center, Japan Nippon Soda Co., Ltd., JPN, Report No.: G-942, Report includes Trial Nos.: 9854 Edition Number: M-195932-01-2 EPA MRID No.: 44988502 Date: 1997-09-30 GLP/GEP: yes, unpublished	Yes	Nippon Soda Co., Ltd., JPN
[redacted].	KIIA 5.3.1 /04	2008	Preliminary 28-day toxicity study in the dog by dietary administration [redacted] Bayer CropScience, Report No.: SA07290, Edition Number: M-312461-01-3 EPA MRID No.: 48844152 Date: 2008-12-09 GLP/GEP: no, unpublished	Yes	BCS
[redacted]	KIIA 5.3.2 /01	2009	BYI 02960 - 90-day toxicity study in the rat by dietary administration - Amendment no.2 [redacted] Bayer CropScience, Report No.: SA 07294, Edition Number: M-329048-03-2 EPA MRID No.: 48844111 Date: 2009-02-10 **...Amended: 2012-03-21** GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 5.3.2 /02	2009	BYI 02960 - 90-day toxicity study in the mouse by dietary administration - Amendment no.2 [redacted] Report No.: SA 07295, Edition Number: M-328668-03-2 EPA MRID No.: 48844112 Date: 2009-02-06 **...Amended: 2012-03-22** GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
[redacted]	KIIA 5.10 /01	2010	BYI 02960 - Biokinetic in the plasma of rats following 7 days exposure through the diet [redacted] Bayer CropScience, Report No.: SA 09334, Edition Number: M-385777-01-2 EPA MRID No.: 48844154 Date: 2010-07-08 GLP/GEP: no, unpublished	Yes	BCS
[redacted]	KIIA 5.10 /02	2011	BYI 02960: 28-day immunotoxicity study in the female wistar rat by dietary administration [redacted] Bayer CropScience, Report No.: SA 10353, Edition Number: M-414754-01-2 EPA MRID No.: 48844148 Date: 2011-09-22 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 5.8 /12	2011	BYI-02960-difluoroethyl-amino-furanone acute oral toxicity in rats acute toxic class method [redacted] Bayer CropScience, Report No.: 37503 TAR, Edition Number: M-409674-01-2 EPA MRID No.: 48844103 Date: 2011-05-19 GLP/GEP: yes, unpublished	Yes	BCS
Sokolowski, A.	KIIA 5.4.1 /02	2011	1st amendment to report Salmonella typhimurium reverse mutation assay with BYI 02960 Harlan Cytotest Cell Research GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 1425802, Edition Number: M-420539-02-2 EPA MRID No.: 48844125 Date: 2011-09-23 **...Amended: 2011-10-17** GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Sokolowski, A.	KIIA 5.8 /01	2010	Salmonella typhimurium - Reverse mutation assay with BCS-AA56716 (metabolite of BYI 02960) Harlan CCR, Rossdorf, Germany Bayer CropScience, Report No.: 1351101, Edition Number: M-409724-01-2 EPA MRID No.: 48844126 Date: 2010-09-30 GLP/GEP: no, unpublished	Yes	BCS
Sokolowski, A.	KIIA 5.8 /07	2011	Salmonella typhimurium reverse mutation assay with BYI 02960-difluoroethyl-amino-furanone (metabolite of byi-02960) Harlan CCR, Rossdorf, Germany Bayer CropScience, Report No.: 1399701, Edition Number: M-409728-01-2 EPA MRID No.: 48844127 Date: 2011-05-24 GLP/GEP: no, unpublished	Yes	BCS
Spiegel, K.	KIIA 5.10 /04	2012	Dietary assessment - Toxicological coverage of BYI 02960 plant and livestock metabolites Bayer CropScience, Report No.: M-428863-01-2, Edition Number: M-428863-01-2 EPA MRID No.: 48844157 Date: 2012-05-31 GLP/GEP: n.a., unpublished	Yes	BCS
[redacted]	KIIA 5.8 /17	1997	Thirteen-week dietary subchronic toxicity study in rats IM-0 [redacted] Report No.: G-0889, Report includes Trial Nos.: 0259 Edition Number: M-195901-01-2 EPA MRID No.: 44988427 Date: 1997-11-28 GLP/GEP: yes, unpublished	Yes	Nippon Soda Co., Ltd., JPN

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Thum, M.	KIIA 5.4.2 /01	2009	BYI 02960 (tested as BYI 02960 technical) - In vitro chromosome aberration test with chinese hamster V79 cells Bayer Schering Pharma AG, Wuppertal, Germany Bayer CropScience, Report No.: AT05626, Edition Number: M-359746-01-2 EPA MRID No.: 48844131 Date: 2009-11-11 GLP/GEP: yes, unpublished	Yes	BCS
Vohr, H. W.	KIIA 5.2.6 /01	2009	BYI 02960 - Local lymph node assay in mice (LLNA/IMDS) Bayer Schering Pharma AG, Wuppertal, Germany Bayer CropScience, Report No.: AT05334, Edition Number: M-353715-01-2 EPA MRID No.: 48844108 Date: 2009-06-29 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 5.1.2 /01	2011	[Furanone-4-14C]BYI 02960 - Absorption, distribution, excretion, and metabolism in the rat [redacted] Report No.: MEF-11/556, Edition Number: M-421499-01-1 EPA MRID No.: 48844143 Date: 2011-12-22 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 5.1.3 /01	2011	[Ethyl-1-14C]BYI 02960 - Absorption, distribution, excretion, and metabolism in male rats [redacted] Report No.: MEF-11/555, Edition Number: M-415647-01-1 EPA MRID No.: 48844146 Date: 2011-10-10 GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Wieland, J.	KIIA 5.4.4 /02	2011	Micronucleus assay in bone marrow cells of the mouse with BYI 02960-a.i. Harlan Cytotest Cell Research GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 1425801, Edition Number: M-420536-01-2 EPA MRID No.: 48844135 Date: 2011-11-10 GLP/GEP: yes, unpublished	Yes	BCS

References for the product

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Anon.	KIIIA1 7.3.1 /01	2010	Southern European greenhouse model overview European Crop Protection Association ECPA, Report No.: M-400719-01-1, Edition Number: M-400719-01-1 Date: 2010-10-31 GLP/GEP: n.a., unpublished	Yes	ECPA
[redacted]	KIIIA1 7.1.3 /01	2010	BYI 02960 SL 200 g/L - Acute inhalation toxicity in rats [redacted] Bayer CropScience, Report No.: AT06016, Edition Number: M-392826-01-1 Date: 2010-10-18 GLP/GEP: yes, unpublished	Yes	BCS
Freitag, T.; Stuke, S.	KIIIA1 7.7.1 /01	2011	Determination of the dislodgeable foliar residues (DFR) of BYI 02960 after spraying of BYI 02960 SL 200 on lettuce in the field in the Netherlands Bayer CropScience, Report No.: 10-2916, Report includes Trial Nos.: 10-2916-01 Edition Number: M-420640-01-1 Date: 2011-12-12 GLP/GEP: yes, unpublished	Yes	BCS
Freitag, T.; Stuke, S.	KIIIA1 7.7.1 /02	2011	Determination of the dislodgeable foliar residues (DFR) of BYI 02960 after spraying of BYI 02960 SL 200 on lettuce in the field in Portugal Bayer CropScience, Report No.: 10-2917, Report includes Trial Nos.: 10-2917-01 Edition Number: M-420656-01-1 Date: 2011-12-15 GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Freitag, T.; Stuke, S.	KIIIA1 7.7.1 /03	2011	Determination of the dislodgeable foliar residues (DFR) of BYI 02960 after spraying of BYI 02960 SL 200 on lettuce in the greenhouse in the Netherlands Bayer CropScience, Report No.: 10-2918, Report includes Trial Nos.: 10-2918-01 Edition Number: M-420641-01-1 Date: 2011-12-13 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIIA1 7.1.1 /01	2010	BYI 02960 SL 200 g/L - Acute toxicity in the rat after oral administration [redacted] Bayer CropScience, Report No.: AT05943, Edition Number: M-385422-01-1 Date: 2010-06-17 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIIA1 7.1.2 /01	2010	BYI 02960 SL 200 g/L - Acute toxicity in the rat after dermal application [redacted] Bayer CropScience, Report No.: AT05944, Edition Number: M-385421-01-1 Date: 2010-06-17 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIIA1 7.1.4 /01	2010	BYI 02960 SL 200 g/L - Acute skin irritation/corrosion on rabbits [redacted] Bayer CropScience, Report No.: AT05908, Edition Number: M-370381-01-1 Date: 2010-05-06 GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
[redacted]	KIIIA1 7.1.5 /01	2010	BYI 02960 SL 200 g/L - Acute eye irritation on rabbits [redacted] Bayer CropScience, Report No.: AT05812, Edition Number: M-364511-01-1 Date: 2010-02-25 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIIA1 7.6.1 /01	2010	BYI 02960 (SL200) - In vivo dermal absorption study in the male rat [redacted] Bayer CropScience, Report No.: SA 09255, Edition Number: M-396841-01-1 Date: 2010-11-30 GLP/GEP: yes, unpublished	Yes	BCS
Odin-Feurtet, M.	KIIIA1 7.6.2 /01	2010	BYI 02960 (SL200) - Comparative in vitro dermal absorption study using human and rat skin Bayer S.A.S., Bayer CropScience, Sophia Antipolis, France Bayer CropScience, Report No.: SA 09254, Edition Number: M-391215-01-1 Date: 2010-09-28 GLP/GEP: yes, unpublished	Yes	BCS
Repetto, M.	KIIIA1 7.1.6 /01	2010	BYI 02960 SL 200 g/L - Evaluation of potential skin sensitization in the local lymph node assay in the mouse Bayer S.A.S., Bayer CropScience, Sophia Antipolis, France Bayer CropScience, Report No.: SA 10101, Edition Number: M-368808-01-1 Date: 2010-05-07 GLP/GEP: yes, unpublished	Yes	BCS

Section A.7 Residue data (Annex IIA 6; Annex IIIA 8 and 12.2)

Author(s)	**Annex point / reference number**	**Year**	**Title** **Source** (where different from company) **Company name, Report No., Date, GLP/GEP status** (where relevant)**, published or not**	**Data protect. claimed**	**Owner**
A. Klempner	KIIA 6.6.2 /01	2011	Metabolism of [furanone-4-^{14}C]BYI 02960 in confined rotational crops Bayer CropScience, Report No.: MEF-11/365, Edition Number: M-421861-01-2 Date: 2011-12-16 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 6.2.2 /01	2012a	[Pyridinylmethyl-^{14}C]BYI 02960: Metabolism in the laying hen [redacted] Report No.: MEF-11/199, Edition Number: M-422162-01-2 Date: 2012-01-10 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 6.2.2 /02	2012b	[Furanone-4-^{14}C]BYI 02960: Metabolism in the laying hen [redacted] Report No.: MEF-11/200, Edition Number: M-422263-01-2 Date: 2012-01-10 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 6.2.3 /01	2011a	[Pyridinylmethyl-14C]BYI 02960: Metabolism in the lactating goat [redacted] Report No.: MEF-11/269, Edition Number: M-419701-01-2 Date: 2011-12-07 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 6.2.3 /02	2011b	[Furanone-4-^{14}C]BYI 02960: Metabolism in the lactating goat [redacted], Report No.: MEF11/268, Edition Number: M-421995-01-2 Date: 2011-12-16 GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title **Source** (where different from company) **Company name, Report No., Date, GLP/GEP status** (where relevant)**, published or not**	Data protect. claimed	Owner
Breuer-Rehm, M.	KIIA 6.6.2 /02	2011	Metabolism of [pyridinylmethyl-^{14}C]BYI 02960 in confined rotational crops Bayer CropScience, Report No.: MEF-10/892, Edition Number: M-419853-02-1 Date: 2011-12-02 ...Amended: 2012-02-22 GLP/GEP: yes, unpublished	Yes	BCS
Justus, K.	KIIA 6.2.1 /01	2011a	Metabolism of [furanone-4-^{14}C]BYI 02960 in tomatoes Bayer CropScience, Report No.: MEF-11/016, Edition Number: M-411352-01-2 Date: 2011-07-25 GLP/GEP: yes, unpublished	Yes	BCS
Justus, K.	KIIA 6.2.1 /02	2011b	Metabolism of [pyridinylmethyl-^{14}C]BYI 02960 in tomatoes Bayer CropScience, Report No.: MEF-11/182, Edition Number: M-411500-01-2 Date: 2011-07-26 GLP/GEP: yes, unpublished	Yes	BCS
Justus, K.	KIIA 6.2.1 /04	2011c	Metabolism of [furanone-4-^{14}C]BYI 02960 in potatoes Bayer CropScience, Report No.: MEF-10/769, Edition Number: M-415234-01-2 Date: 2011-09-14 GLP/GEP: yes, unpublished	Yes	BCS
Justus, K.	KIIA 6.2.1 /05	2011d	Metabolism of [pyridinylmethyl-^{14}C]BYI 02960 in potatoes Bayer CropScience, Report No.: MEF-10/710, Edition Number: M-415078-01-2 Date: 2011-09-14 GLP/GEP: yes, unpublished	Yes	BCS
Justus, K.	KIIA 6.2.1 /06	2011e	Metabolism of [furanone-4-^{14}C]BYI 02960 in apples Bayer CropScience, Report No.: MEF-11/499, Edition Number: M-422562-01-1 Date: 2011-12-29 GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title **Source** (where different from company) **Company name, Report No., Date, GLP/GEP status** (where relevant)**, published or not**	Data protect. claimed	Owner
Justus, K.	KIIA 6.2.1 /07	2011f	Metabolism of [pyridinylmethyl-^{14}C]BYI 02960 in apples Bayer CropScience, Report No.: MEF-11/198, Edition Number: M-414678-01-2 Date: 2011-09-09 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 6.1.1 /02	2012a	BYI 02960 - Magnitude of the residue in dairy cows [redacted] Bayer CropScience, Report No.: RARVP050, Edition Number: M-428416-01-1 Date: 2012-04-03 GLP/GEP: yes, unpublished **...also filed: KIIA 4.3 /08** **...also filed: KIIA 6.4.2 /01**	Yes	BCS
[redacted]	KIIA 6.4.2 /01	2012b	BYI 02960 - Magnitude of the residue in dairy cows [redacted] Bayer CropScience, Report No.: RARVP050, Edition Number: M-428416-01-1 Date: 2012-04-03 GLP/GEP: yes, unpublished **...also filed: KIIA 4.3 /08** **...also filed: KIIA 6.1.1 /02**	Yes	BCS
Netzband, D.; Timberlake, B. C.; Harbin, A. M.	KIIA 6.1.1 /01	2012	Storage stability of BYI 02960, difluoroacetic acid, and difluoroethyl-amino-furanone in plant matrices (18-month data) Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: RARVP046-1, Edition Number: M-428412-02-1 Date: 2012-04-03 ...Amended: 2012-11-01 GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title **Source** (where different from company) **Company name, Report No., Date, GLP/GEP status** (where relevant)**, published or not**	Data protect. claimed	Owner
Noss, G.; Ballmann, C.	KIIA 6.3.1.2 /01	2012a	Determination of the residues of BYI 02960 in/on hop after spraying of BYI 02960 SL 200 in the field in Germany Bayer CropScience, Report No.: 10-2225, Report includes Trial Nos.: 10-2225-01 10-2225-02 10-2225-03 10-2225-04 Edition Number: M-425351-01-1 Date: 2012-02-13 GLP/GEP: yes, unpublished	Yes	BCS
Noss, G.; Ballmann, C.	KIIA 6.3.1.2 /02	2012b	Determination of the residues of BYI 02960 in/on hop after spray application of BYI 02960 SL 200 in Germany Bayer CropScience, Report No.: 11-2076, Report includes Trial Nos.: 11-2076-01 11-2076-02 11-2076-03 11-2076-04 Edition Number: M-425339-01-1 Date: 2012-02-13 GLP/GEP: yes, unpublished	Yes	BCS
Noss, G.; Bauer, J.	KIIA 6.3.1.1 /01	2012	Determination of the residues of BYI 02960 in/on lettuce after spraying of BYI 02960 SL 200 in the field in the Netherlands, Belgium, France (North) and Germany Bayer CropScience, Report No.: 10-2223, Report includes Trial Nos.: 10-2223-01 10-2223-02 10-2223-03 10-2223-04 10-2223-05 Edition Number: M-424742-01-1 Date: 2012-02-08 GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title **Source** (where different from company) **Company name, Report No., Date, GLP/GEP status** (where relevant)**, published or not**	Data protect. claimed	Owner
Schmeling, S.; Weber, E.	KIIA 6.2.1 /08	2011a	Metabolism of [furanone-4-14C]BYI 02960 in cotton after spray application Bayer CropScience, Report No.: MEF-11/392, Edition Number: M-421625-01-2 Date: 2011-12-12 GLP/GEP: yes, unpublished	Yes	BCS
Schmeling, S.; Weber, E.	KIIA 6.2.1 /09	2011b	Metabolism of [pyridinylmethyl-^{14}C]BYI 02960 in cotton after spray application Bayer CropScience, Report No.: MEF-11/393, Edition Number: M-421691-01-2 Date: 2011-12-12 GLP/GEP: yes, unpublished	Yes	BCS
Schmeling, S.; Weber, E.	KIIA 6.2.1 /10	2011c	Metabolism of [furanone-4-^{14}C]BYI 02960 in paddy rice Bayer CropScience, Report No.: MEF-11/058, Edition Number: M-414219-01-2 Date: 2011-09-09 GLP/GEP: yes, unpublished	Yes	BCS
Schmeling, S.; Weber, E.	KIIA 6.2.1 /11	2011d	Metabolism of [pyridinylmethyl-^{14}C]BYI 02960 in paddy rice Bayer CropScience, Report No.: MEF-11/059, Edition Number: M-414328-01-2 Date: 2011-09-09 GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title **Source** (where different from company) **Company name, Report No., Date, GLP/GEP status** (where relevant)**, published or not**	Data protect. claimed	Owner
Schoening, R.; Bauer, J.	KIIA 6.3.1.1 /03	2011	Determination of the residues of BYI 02960 in/on lettuce, head after spray application of BYI 02960 SL 200 in the field in France (South), Spain and Italy - Amendment no. 0001 to report no. 10-2213 Bayer CropScience, Report No.: 10-2213, Report includes Trial Nos.: 10-2213-01 10-2213-02 10-2213-03 10-2213-04 10-2213-05 Edition Number: M-425913-02-1 Date: 2011-09-14 ...Amended: 2012-02-27 GLP/GEP: yes, unpublished	Yes	BCS
Schoening, R.; Bauer, J.	KIIA 6.6.3 /01	2012	Determination of the residues of BYI 02960 in/on the field rotational crops barley, carrot, lettuce and turnip after spray application of BYI 02960 SL 200 on lettuce and soil in the field in Germany, the Netherlands, France (South) and Spain Bayer CropScience, Report No.: 10-2503, Report includes Trial Nos.: 10-2503-01 10-2503-02 10-2503-03 10-2503-04 Edition Number: M-429091-01-1 Date: 2012-04-11 GLP/GEP: yes, unpublished	Yes	BCS
Schoening, R.; Ruhl, S.	KIIA 6.2.1 /12	2012	Determination of residues of difluoroacetic acid in extracts of samples from plant metabolism and confined rotational crops studies after application of BYI 02960 Bayer CropScience, Report No.: MR-11/050, Edition Number: M-422550-01-1 Date: 2012-01-17 GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title **Source** (where different from company) **Company name, Report No., Date, GLP/GEP status** (where relevant)**, published or not**	Data protect. claimed	Owner
Schulte, G.	KIIA 6.3.1.1 /05	2012a	Determination of the residues of BYI 02960 in/on lettuce after spraying of BYI 02960 SL 200 in the greenhouse in France (North), Germany, the Netherlands and Italy Bayer CropScience, Report No.: 10-2212, Report includes Trial Nos.: 10-2212-01 10-2212-02 10-2212-03 10-2212-04 10-2212-05 Edition Number: M-425829-01-1 Date: 2012-02-22 GLP/GEP: yes, unpublished	Yes	BCS
Schulte, G.	KIIA 6.6.3.1.2 /01	2012b	Determination of the residues of BYI 02960 in/on the field rotational crop potato after spray application of BYI 02960 SL 200 to bare soil in the field in northern France, the Netherlands, Spain and Italy - Limited rotational crop study Bayer CropScience, Report No.: 11-2550, Report includes Trial Nos.: 11-2550-01 11-2550-02 11-2550-03 11-2550-04 Edition Number: M-438341-01-1 Date: 2012-09-10 GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title **Source** (where different from company) **Company name, Report No., Date, GLP/GEP status** (where relevant)**, published or not**	Data protect. claimed	Owner
Schulte, G.	KIIA 6.6.3.1.3 /01	2012c	Determination of the residues of BYI 02960 in/on the field rotational crop leek after spray application of BYI 02960 SL 200 to bare soil in the field in northern France, Germany, Spain and Italy - Limited rotational crop study Bayer CropScience, Report No.: 11-2551, Report includes Trial Nos.: 11-2551-01 11-2551-02 11-2551-03 11-2551-04 Edition Number: M-438384-01-1 Date: 2012-09-13 GLP/GEP: yes, unpublished	Yes	BCS
Schulte, G.	KIIA 6.6.3.1.4 /01	2012d	Determination of the residues of BYI 02960 in/on the field rotational crop cucumber after spray application of BYI 02960 SL 200 to bare soil in the field in northern France, Germany, Spain and Italy - Limited rotational crop study Report No.: 11-2552, Report includes Trial Nos.: 11-2552-01 11-2552-02 11-2552-03 11-2552-04 Edition Number: M-438343-01-1 Date: 2012-09-13 GLP/GEP: yes, unpublished	Yes	BCS
Schulte, G.	KIIA 6.6.3.1.5 /01	2012fe	Determination of the residues of BYI 02960 in/on the field rotational crop onion after spray application of BYI 02960 SL 200 on bare soil in the field in France (North), Germany, Italy and Spain - Limited rotational crop study Bayer CropScience, Report No.: 11-2553, Report includes Trial Nos.: 11-2553-01 11-2553-02 11-2553-03 11-2553-04 Edition Number: M-438397-01-1 Date: 2012-09-14 GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title **Source** (where different from company) **Company name, Report No., Date, GLP/GEP status** (where relevant)**, published or not**	Data protect. claimed	Owner
Schulte, G.	KIIA 6.6.3.1.6 /01	2012f	Amendment no. 1 to report no: 11.2555 - Determination of the residues of BYI 02960 in/on the field rotational crop french bean after spray application of BYI 02960 SL 200 to bare soil in the field in northern France, Germany, Italy and Spain - Limited rotational crop study Bayer CropScience, Report No.: 11-2555, Report includes Trial Nos.: 11-2555-01 11-2555-02 11-2555-03 11-2555-04 Edition Number: M-438394-02-1 Date: 2012-09-14 ...Amended: 2012-09-27 GLP/GEP: yes, unpublished	Yes	BCS
Schulte, G.	KIIA 6.6.3.1.7 /01	2012g	Determination of the residues of BYI 02960 in/on the field rotational crop pea after spray application of BYI 02960 SL 200 on bare soil in the field in France (North), Germany, Italy and Spain - Limited rotational crop study Bayer CropScience, Report No.: 11-2556, Report includes Trial Nos.: 11-2556-01 11-2556-02 11-2556-03 11-2556-04 Edition Number: M-438582-01-1 Date: 2012-09-17 GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title **Source** (where different from company) **Company name, Report No., Date, GLP/GEP status** (where relevant)**, published or not**	Data protect. claimed	Owner
Schulte, G.	KIIA 6.6.3.1.8 /01	2012h	Determination of the residues of BYI 02960 in/on the field rotational crop winter rape after spray application of BYI 02960 SL 200 on bare soil in the field in northern France, Germany, Italy and Spain - Limited rotational crop study Bayer CropScience, Report No.: 11-2554, Report includes Trial Nos.: 11-2554-01 11-2554-02 11-2554-03 11-2554-04 Edition Number: M-443092-01-l Date: 2012-12-07 GLP/GEP: yes, unpublished	Yes	BCS
Schulte, G.; Bauer, J.	KIIA 6.5.4 /01	2012a	Determination of the residues of BYI 02960 in/on lettuce and head lettuce and the processed fractions (head, inner parts; leaf, outer; leaf, inner; leaf, inner, washed and washings) after spraying of BYI 02960 SL 200 in the field in the Netherlands, Belgium and Germany Bayer CropScience, Report No.: 10-3223, Report includes Trial Nos.: 10-3223-01 10-3223-02 10-3223-04 10-3223-05 Edition Number: M-426982-01-1 Date: 2012-03-07 GLP/GEP: no, unpublished	Yes	BCS
Schulte, G.; Bauer, J.	KIIA 6.5.4 /02	2012b	Determination of the residues of BYI 02960 in/on hop (cone, green and cone, kiln-dried) and the processed fractions (hops draff, brewer's yeast and beer) after spraying of BYI 02960 SL 200 in the field in Germany Bayer CropScience, Report No.: 10-3407, Report includes Trial Nos.: 10-3407-01 10-3407-02 Edition Number: M-425311-01-1 Date: 2012-02-13 GLP/GEP: no, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title **Source** (where different from company) **Company name, Report No., Date, GLP/GEP status** (where relevant)**, published or not**	Data protect. claimed	Owner
Thielert, W.; Schoening, R.	KIIA 6.10 /01	2012	Systemicity of flupyradifurone and specific metabolites in tomato (foliar/soil application), melon (soil application) and cucumber (foliar application) under greenhouse conditions - Flupyradifurone SL 200 (200 g/L) Bayer CropScience Bayer CropScience, Report No.: MR-12/052, Edition Number: M-434311-01-1 Date: 2012-07-11 GLP/GEP: n.a., unpublished	Yes	BCS
Uceda, L.	KIIA 6.3.1.1 /02	2012a	Amendment No. 1 - Determination of the residues of BYI 02960 in/on lettuce after spray application of BYI 02960 SL 200 in the field in Germany, northern France and Belgium Bayer S.A.S., Bayer CropScience, Lyon, France Bayer CropScience, Report No.: 11-2082, Report includes Trial Nos.: 11-2082-01 11-2082-02 11-2082-03 11-2082-04 Edition Number: M-425941-02-1 Date: 2012-02-23 ...Amended: 2012-03-05 GLP/GEP: yes, unpublished	Yes	BCS
Uceda, L.	KIIA 6.3.1.1 /04	2012b	Amendment No.1 - Determination of the residues of BYI 02960 in/on lettuce after spray application of BYI 02960 SL 200 in the field in Spain, Italy, Southern France and Portugal Bayer CropScience, Lyon, France Bayer CropScience, Report No.: 11-2071, Report includes Trial Nos.: 11-2071-01 11-2071-02 11-2071-03 11-2071-04 Edition Number: M-425784-02-1 Date: 2012-02-23 GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title **Source** (where different from company) **Company name, Report No., Date, GLP/GEP status** (where relevant)**, published or not**	Data protect. claimed	Owner
Uceda, L.	KIIA 6.3.1.1 /06	2012c	Determination of the residues of BYI 02960 in/on lettuce after spray application of BYI 02960 SL 200 in the greenhouse in northern France, Italy, Spain and Germany Bayer CropScience, Lyon, France Bayer CropScience, Report No.: 11-2070, Report includes Trial Nos.: 11-2070-01 11-2070-02 11-2070-03 11-2070-04 Edition Number: M-425786-01-1 Date: 2012-02-23 GLP/GEP: yes, unpublished	Yes	BCS
Unold, M.; Justus, K	KIIA 6.2.1 /03	2011	Metabolism of [ethyl-1-^{14}C]BYI 02960 in tomatoes Bayer CropScience, Report No.: MEF-11/498, Edition Number: M-413996-01-2 Date: 2011-09-05 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 6.4.1 /01	2012	BYI 02960 - Magnitude of the residue in laying hens [redacted] Bayer CropScience, Report No.: RARVP041, Edition Number: M-428933-01-1 Date: 2012-04-05 GLP/GEP: yes, unpublished **...also filed: KIIA 4.3 /09**	Yes	BCS
Weber, E.	KIIA 6.5.1 /01	2011	Nature of the residues of [pyridinylmethyl-14C]BYI02960 in processed commodities - High temperature hydrolysis Bayer CropScience, Report No.: MEF-10/856, Edition Number: M-402311-01-2 EPA MRID No.: 48843966 Date: 2011-02-09 GLP/GEP: yes, unpublished	Yes	BCS

Section A.8 Environmental fate and behaviour (Annex IIA 7; Annex IIIA 9)

References for the active substance

Author(s)	Annex point/ reference number	Year	Title, Source, Company, Report No, GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner

Author(s)	Annex point/ reference number	Year	Title, Source, Company, Report No, GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
Bogdoll, B.; Eyrich, U.	IIA 7.4.9/02 IIA 2.3.2/01	2011	Flupyradifurone (BYI 02960): Calculation of the Henry´s law constant Bayer CropScience, Report No.: AF09/042, Edition Number: M-414341-01-1 Date: 2011-08-15 GLP/GEP: no, unpublished	Y	BCS
Bruns, E.	IIA 7.8.3/04	2012	Fate of BYI 02960 (tech.) in outdoor microcosm ponds simulating actual exposure conditions in agricultural use Bayer CropScience, Report No.: EBRVP109, Edition Number: M-427167-01-1 Date: 2012-03-19 GLP/GEP: yes, unpublished	Y	BCS
de Souza, T. J. T.	IIA 7.4.3/01	2012	BYI 02960 - Mobility of [pyridine-2,6-14C]-BYI 02960 in Brazilian soils - Soil columns leaching method Bioensaios Análises e Consultoria Ambiental, Viamão, RS, Brazil Bayer CropScience, Report No.: 2301-LIX-344-11, Edition Number: M-424966-01-2 Date: 2012-02-08 GLP/GEP: yes, unpublished	Y	BCS
Hall, L. R.	IIA 7.6/01 IIA 2.9.2/01 IIA 2.9.4/01	2011	Phototransformation of [14C] BYI 02960 in aqueous pH 7 buffer - amended report Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: MERVP042-1, Edition Number: M-418426-02-1 Date: 2011-11-28 ...Amended: 2012-03-05 GLP/GEP: yes, unpublished	Y	BCS
Hall, L. R.	IIA 7.6/03	2011	Phototransformation of [14C] BYI 02960 in natural water Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: MERVP020, Edition Number: M-415368-01-1 Date: 2011-08-16 GLP/GEP: yes, unpublished	Y	BCS

Author(s)	Annex point/ reference number	Year	Title, Source, Company, Report No, GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
Heinemann, O.	IIA 7.3.1/01	2011	Determination of the residues of BYI 02960 in/on soil after spraying of BYI 02960 SL 200 in the field in Germany, Italy, Spain and the United Kingdom Bayer CropScience, Report No.: 09-2702, Report includes Trial Nos.: 09-2702-01 09-2702-02 09-2702-03 09-2702-05 09-2702-06 09-2702-07 Edition Number: M-414245-01-1 Date: 2011-09-13 GLP/GEP: yes, unpublished	Y	BCS
Heinemann, O.	IIA 7.6/02	2011	BYI 02960: Determination of the quantum yield and assessment of the environmental half-life of the direct photo-degradation in water Bayer CropScience, Report No.: MEF-11/554, Edition Number: M-414756-01-2 Date: 2011-09-26 GLP/GEP: yes, unpublished	Y	BCS
Hellpointner, E.	IIA 7.1.2/01	2012	[Furanone-4-14C] and [Ethyl-1-14C] and [Pyridine-2,6-14C] BYI 02960: Anaerobic Soil Metabolism Bayer CropScience, Report No.: MEF-11/514, Edition Number: M-421504-01-2 Date: 2012-01-03 GLP/GEP: yes, unpublished	Y	BCS
Hellpointner, E.; Unold, M.	IIA 7.8.3/01	2012	[Pyridine-2,6-14C] BYI 02960: Aerobic aquatic metabolism Bayer CropScience, Report No.: MEF-11/907, Edition Number: M-422359-01-1 Date: 2012-01-12 GLP/GEP: yes, unpublished	Y	BCS
Hellpointner, E.; Unold, M.	IIA 7.8.3/03	2012	[1-14C] BYI 02960-DFA (BCS-AB60481): Aerobic aquatic degradation Bayer CropScience, Report No.: M-422371-01-1, Edition Number: M-422371-01-1 Date: 2012-01-12 GLP/GEP: yes, unpublished	Y	BCS

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Hellpointner; E.	IIA 7.10/01	2010	BYI 02960: Calculation of the chemical half-life in the troposphere Bayer CropScience, Report No.: MEF-10/896, Edition Number: M-398741-01-2 Date: 2010-12-27 GLP/GEP: no, unpublished	Y	BCS
Liu, A. C.	IIA 7.4.2/01	1997	Soil adsorption/desorption study 6-chloronicotinic acid (Acetamiprid metabolite) Rhone-Poulenc Ag Company, RTP, NC, USA Bayer CropScience, Report No.: C007666, Edition Number: M-196394-01-1 Date: 1997-09-15 GLP/GEP: yes, unpublished	N	NISSO
Lowden, P.; Oddy, A. M.; Jones, M. K.	IIA 7.2.3/01	1997	Rate of degradation of the acid metabolite, (14C)-IC-O in three soils NI-25 Rhone-Poulenc Agriculture Ltd., Ongar, Essex, United Kingdom Bayer CropScience, Report No.: C007660, Edition Number: M-196378-01-1 Date: 1997-08-14 GLP/GEP: yes, unpublished	N	NISSO
Menke, U.	IIA 7.1.1/01, IIA 7.2.1/01, IIA 7.4.1/03	2011	[Pyridinylmethyl-14C] BYI 02960: Aerobic soil metabolism/degradation and time-dependent sorption in soils Bayer CropScience, Report No.: MEF-07/334, Edition Number: M-414615-01-2 Date: 2011-08-05 GLP/GEP: yes, unpublished	Y	BCS
Menke, U.	IIA 7.2.1 /01 IIA 7.1.1/01 IIA 7.4.1/03	2011	[Pyridinylmethyl-14C] BYI 02960: Aerobic soil metabolism/degradation and time-dependent sorption in soils Bayer CropScience, Report No.: MEF-07/334, Edition Number: M-414615-01-2 Date: 2011-08-05 GLP/GEP: yes, unpublished	Y	BCS

Author(s)	Annex point/ reference number	Year	Title, Source, Company, Report No, GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
Menke, U.	IIA 7.4.1/03 IIA 7.1.1/01 IIA 7.1.2/01	2011	[Pyridinylmethyl-14C] BYI 02960: Aerobic soil metabolism/degradation and time-dependent sorption in soils Bayer CropScience, Report No.: MEF-07/334, Edition Number: M-414615-01-2 Date: 2011-08-05 GLP/GEP: yes, unpublished	Y	BCS
Menke, U.; Telscher, M.	IIA 7.4.1/01	2008	[Pyridinylmethyl-14C] BYI 02960: Adsorption to and desorption from soils Bayer CropScience, Report No.: MEF-08/261, Edition Number: M-327492-01-2 Date: 2008-12-17 GLP/GEP: yes, unpublished	Y	BCS
Menke, U.; Unold, M.	IIA 7.1.1/02, IIA 7.2.1/02	2011	[Furanone-4-14C] BYI 02960: Aerobic soil metabolism/degradation Bayer CropScience, Report No.: MEF-10/804, Edition Number: M-411625-01-2 Date: 2011-07-28 GLP/GEP: yes, unpublished	Y	BCS
Menke, U.; Unold, M.	IIA 7.1.1/04, IIA 7.2.1/04	2011	[Ethyl-1-14C] BYI 02960: Aerobic soil metabolism Bayer CropScience, Report No.: MEF-10/858, Edition Number: M-414981-01-1 Date: 2011-09-08 GLP/GEP: yes, unpublished	Y	BCS
Menke, U.; Unold, M.	IIA 7.1.1/05, IIA 7.2.1/05,	2011	[Pyridine-2,6-14C] BYI 02960: Aerobic soil metabolism Bayer CropScience, Report No.: MEF-10/880, Edition Number: M-411693-01-2 Date: 2011-07-28 GLP/GEP: yes, unpublished	Y	BCS
Menke, U.; Unold, M.	IIA 7.1.3/01	2011	[Pyridinylmethyl-14C] BYI 02960 and [furanone-4-14C] BYI 02960: Phototransformation on soil Bayer CropScience, Report No.: MEF-10/351, Edition Number: M-405776-01-2 Date: 2011-03-24 GLP/GEP: yes, unpublished	Y	BCS

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Menke, U.; Unold, M.	IIA 7.2.1/02 IIA 7.1.1/02	2011	[Furanone-4-14C] BYI 02960: Aerobic soil metabolism/degradation Bayer CropScience, Report No.: MEF-10/804, Edition Number: M-411625-01-2 Date: 2011-07-28 GLP/GEP: yes, unpublished	Y	BCS
Menke, U.; Unold, M.	IIA 7.2.1/04 IIA 7.1.1/04	2011	[Ethyl-1-14C] BYI 02960: Aerobic soil metabolism Bayer CropScience, Report No.: MEF-10/858, Edition Number: M-414981-01-1 Date: 2011-09-08 GLP/GEP: yes, unpublished	Y	BCS
Menke, U.; Unold, M.	IIA 7.2.1/05 IIA 7.1.1/05	2011	[Pyridine-2,6-14C] BYI 02960: Aerobic soil metabolism Bayer CropScience, Report No.: MEF-10/880, Edition Number: M-411693-01-2 Date: 2011-07-28 GLP/GEP: yes, unpublished	Y	BCS
Menke, U.; Unold, M.	IIA 7.4.2/02	2011	[1-14C] BYI 02960-DFA (BCS-AB60481): Adsorption to and desorption from five soils Bayer CropScience, Report No.: MEF-10/538, Edition Number: M-413836-01-2 Date: 2011-08-26 GLP/GEP: yes, unpublished	Y	BCS
Mislankar, S. G.; Woodard, D.	IIA 7.1.2/02	2012	[Pyridine-2,614C] BYI 02960: Anaerobic soil metabolism Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: MERVP094, Edition Number: M-421993-01-1 Date: 2012-01-10 GLP/GEP: yes, unpublished	Y	BCS
Mislankar, S.; Woodard, D.	IIA 7.5/01 IIA 2.9.1/01	2011	BYI-02960: Hydrolytic degradation Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: MERVP019, Edition Number: M-398952-01-1 Date: 2011-01-07 GLP/GEP: yes, unpublished	Y	BCS

Author(s)	Annex point/ reference number	Year	Title, Source, Company, Report No, GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
Ripperger, R. J.	IIA 7.1.1/03, IIA 7.2.1/03	2011	[Furanone-4-14C] BYI 02960: Aerobic soil metabolism in two US soils Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: MERVP037-2, Edition Number: M-405497-03-1 Date: 2011-01-14 ...Amended: 2012-01-05 GLP/GEP: yes, unpublished	Y	BCS
Ripperger, R. J.	IIA 7.2.1/03 IIA 7.1.1/03	2011	[Furanone-4-14C] BYI 02960: Aerobic soil metabolism in two US soils Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: MERVP037-2, Edition Number: M-405497-03-1 Date: 2011-01-14 ...Amended: 2012-01-05 GLP/GEP: yes, unpublished	Y	BCS
IIA 7.4.1/05	Scherr, F.	2014	Evaluation of the kinetic sorption parameters for flupyradifurone and their dependence on starting values Bayer CropScience, Report No.: EnSa-14-0804v2, Edition Number: M-491714-02-1 Date: 2014-07-10 **...Amended: 2014-07-30** GLP/GEP: no, unpublished	Y	BCS
Shepherd, J. J.	IA 7.1.1/06, IIA 7.2.1/06, IIA 7.2.3/04	2011	[Pyridine-2,6-14C] BYI 02960: Aerobic soil metabolism in two US soils Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: MERVP038-1, Edition Number: M-413425-02-1 Date: 2011-09-06 ...Amended: 2012-01-05 GLP/GEP: yes, unpublished	Y	BCS
Shepherd, J. J.	IIA 7.2.1/06 IIA 7.1.1/06 IIA 7.2.3/04	2011	[Pyridine-2,6-14C] BYI 02960: Aerobic soil metabolism in two US soils Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: MERVP038-1, Edition Number: M-413425-02-1 Date: 2011-09-06 ...Amended: 2012-01-05 GLP/GEP: yes, unpublished	Y	BCS

Author(s)	Annex point/ reference number	Year	Title, Source, Company, Report No, GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
Shepherd, J. J.	IIA 7.2.3/04 IIA 7.1.1/06 IIA 7.2.1/06	2011	[Pyridine-2,6-14C] BYI 02960: Aerobic soil metabolism in two US soils Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: MERVP038-1, Edition Number: M-413425-02-1 Date: 2011-09-06 ...Amended: 2012-01-05 GLP/GEP: yes, unpublished	Y	BCS
Smeykal, H.	IIA 7.4.9/01 IIA 2.3.1/01	2008	BYI 02960, pure substance: Vapour pressure - Final report Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20080615.01, Edition Number: M-309853-01-1 Date: 2008-10-10 GLP/GEP: yes, unpublished	Y	BCS
Stroech, K.	IIA 7.4.1/02	2010	[Pyridinylmethyl-14C] BYI 02960: Adsorption/desorption on two soils Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: MERVP017, Edition Number: M-363541-01-1 Date: 2010-01-29 GLP/GEP: yes, unpublished	Y	BCS
Sur, R.; Dorn, S.	IIA 7.2.1/07	2012	Kinetic evaluation of the aerobic metabolism of BYI 02960 in four soils for the determination of modelling endpoints Bayer CropScience, Report No.: MEF-11/619, Edition Number: M-423020-01-1 Date: 2012-01-19 GLP/GEP: no, unpublished	Y	BCS
Sur, R.; Dorn, S.	IIA 7.2.1/08	2012	Kinetic evaluation of the aerobic metabolism of BYI 02960 in four soils for the determination of modelling endpoints Bayer CropScience, Report No.: MEF-11/620, Edition Number: M-423347-01-1 Date: 2012-01-19 GLP/GEP: no, unpublished	Y	BCS

Author(s)	Annex point/ reference number	Year	Title, Source, Company, Report No, GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
Sur, R.; Dorn, S.	IIA 7.2.1/09 IIA 7.2.3/05	2012	Kinetic evaluation of the degradation rate of [Ethyl-1-14C] BYI 02960 and its metabolite difluoroacetic acid (BYI 02960-DFA) for the determination of trigger and modelling endpoints Bayer CropScience, Report No.: MEF-11/855, Edition Number: M-422874-01-1 Date: 2012-01-18 GLP/GEP: no, unpublished	Y	BCS
Sur, R.; Dorn, S.	IIA 7.2.1/10 IIA 7.2.3/03	2012	Kinetic evaluation of the degradation rate of (pyridine-2,6-14C] BYI 02960 and its metabolite 6-chloronicotinic accid (6-CNA) for the determination of trigger and modelling endpoints Bayer CropScience, Report No.: MEF-11/838, Edition Number: M-422853-01-1 Date: 2012-01-18 GLP/GEP: no, unpublished	Y	BCS
Sur, R.; Dorn, S.	IIA 7.2.3/02	2012	Kinetic evaluation of the degradation rate of 6-chloronicotinic acid (6-CNA) for the determination of trigger and modelling endpoints Bayer CropScience, Report No.: MEF-11/837, Edition Number: M-422843-01-1 Date: 2012-01-18 GLP/GEP: no, unpublished	Y	BCS
Sur, R.; Dorn, S.	IIA 7.2.3/03 IIA 7.2.1/10	2012	Kinetic evaluation of the degradation rate of (pyridine-2,6-14C] BYI 02960 and its metabolite 6-chloronicotinic accid (6-CNA) for the determination of trigger and modelling endpoints Bayer CropScience, Report No.: MEF-11/838, Edition Number: M-422853-01-1 Date: 2012-01-18 GLP/GEP: no, unpublished	Y	BCS
Sur, R.; Dorn, S.	IIA 7.2.3/05 IIA 7.2.1/09	2012	Kinetic evaluation of the degradation rate of [Ethyl-1-14C] BYI 02960 and its metabolite difluoroacetic acid (BYI 02960-DFA) for the determination of trigger and modelling endpoints Bayer CropScience, Report No.: MEF-11/855, Edition Number: M-422874-01-1 Date: 2012-01-18 GLP/GEP: no, unpublished	Y	BCS

Author(s)	Annex point/ reference number	Year	Title, Source, Company, Report No, GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
Sur, R.; Scherr, F.	IIA 7.4.1/04	2012	Evaluation of the time-dependent sorption of flupyradifurone based on laboratory time-dependent sorption experiments in four soils Bayer CropScience, Report No.: MEF-11/723, Edition Number: M-422824-01-1 Date: 2012-01-18 GLP/GEP: no, unpublished	Y	BCS
Unold, M.; Menke, U.	IIA 7.8.3/02	2012	[Furanone-4-14C] and [ethyl-1-14C] BYI 02960: Aerobic aquatic metabolism Bayer CropScience, Report No.: MEF-10/730, Edition Number: M-426504-01-1 Date: 2012-02-16 GLP/GEP: yes, unpublished	Y	BCS
Woodard, D.	IIA 7.1.2/03	2012	[Pyridine-2,614C] BYI 02960: Anaerobic soil metabolism in Springfield, Nebraska (USA) soil Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: MERVL006, Edition Number: M-424987-01-1 Date: 2012-02-14 GLP/GEP: yes, unpublished	Y	BCS
Xu, T.	IIA 7.8.2/01	2012	[Pyridine-2,6-14C] BYI 02960: Anaerobic aquatic metabolism in two water/sediment systems Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: MERVP027, Edition Number: M-422616-01-1 Date: 2012-01-17 GLP/GEP: yes, unpublished	Y	BCS

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Schaefer, D.; Stroech, K.; Garside, C. M.	IIIA 9.2.2/01	2014	Concentrations of flupyradifurone and its metabolites at drinking water abstraction points Bayer CropScience Bayer CropScience, Report No.: M-495332-01-1, Edition Number: M-495332-01-1 Date: 2014-09-02 GLP/GEP: n.a., unpublished	Y	BCS
Scherr, F.	IIIA1 9.6.1/07 IIIA1 9.6.2/07	2013	Flupyradifurone (FPF) and metabolites: PECgw FOCUS PEARL, PELMO EUR - Use in lettuce (glasshouse) in Europe Bayer CropScience, Report No.: EnSa-13-0468, Edition Number: M-461318-01-1 Date: 2013-07-25 GLP/GEP: no, unpublished	Y	BCS
Scherr, F; Ellerich, C.	IIIA1 9.4/03 IIIA1 9.5/03	2014	FPF PECsoil FOCUS EUR: Predicted environmental concentrations in soil (PECsoil) of flupyradifurone and its metabolites- Use in hops and lettuce in Europe Bayer CropScience, Report No.: EnSa-14-0903, Edition Number: M-495501-01-1 Date: 2014-08-01 GLP/GEP: no, unpublished	Y	BCS
Scherr, F.; Ellerich, C.	IIIA1 9.6.1/08 IIIA1 9.6.2/08	2013	Flupyradifurone (FPF) and metabolites: PECgw FOCUS PEARL, PELMO EUR - Use in lettuce and hops in Europe Bayer CropScience, Report No.: EnSa-13-0426, Edition Number: M-461289-01-1 Date: 2013-07-25 GLP/GEP: no, unpublished	Y	BCS

Author(s)	Annex point/ reference number	Year	Title, Source, Company, Report No, GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
Scherr, F.; Ellerich, C.	IIIA1 9.6.1/09 IIIA1 9.6.2/09	2014	Flupyradifurone (FPF) and metabolites: PECgw FOCUS PEARL, PELMO EUR - Use in lettuce and hops in Europe Bayer CropScience, Report No.: EnSa-14-0869, Edition Number: M-495503-01-1 Date: 2014-07-15 GLP/GEP: no, unpublished	Y	BCS
Scherr, F.; Ellerich, C.	IIIA1 9.7/02 IIIA1 9.8/02	2013	Flupyradifurone (FPF) and metabolites: PECsw,sed FOCUS EUR - Use in lettuce and hops in Europe Bayer CropScience, Report No.: EnSa-13-0431, Edition Number: M-461364-01-1 Date: 2013-07-22 GLP/GEP: no, unpublished	Y	BCS
Schriever, C.; Gottesbüren, B.; Ebert, D.; Sur, R.; Schmitt, W.	IIIA1 9.6.1/10 IIIA1 9.6.2/10	2013	ECPA background paper Plant uptake of compounds via root systems – A hydroponic test system to determine plant uptake as input parameter for leaching models EPCA Date: January 2013 GLP/GEP: no unplublished	N	ECPA
Sur, R.; Ellerich, C.	IIIA1 9.4/01 IIIA1 9.5/01	2012	FPF PECsoil FOCUS EUR: Predicted environmental concentrations in soil (PECsoil) of flupyradifurone and its metabolites- Use in hops and lettuce in Europe Bayer CropScience, Report No.: EnSa-12-0117, Edition Number: M-428042-01-1 Date: 2012-03-21 GLP/GEP: no, unpublished	Y	BCS
Sur, R.; Ellerich, C.	IIIA1 9.4/02 IIIA1 9.5/02	2013	FPF PECsoil FOCUS EUR: Predicted environmental concentrations in soil (PECsoil) of flupyradifurone and its metabolites- Use in hops and lettuce in Europe Bayer CropScience, Report No.: EnSa-13-0432, Edition Number: M-461359-01-1 Date: 2013-07-09 GLP/GEP: no, unpublished	Y	BCS

Author(s)	Annex point/ reference number	Year	Title, Source, Company, Report No, GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
Sur, R.; Ellerich, C.	IIIA1 9.6.1/01 IIIA1 9.6.2/01	2012	FPF PECgw FOCUS EUR: Predicted environmental concentrations in groundwater recharge based on models FOCUS PEARL and FOCUS PELMO - Use in hops in Europe Bayer CropScience, Report No.: EnSa-12-0089, Edition Number: M-427737-01-1 Date: 2012-03-16 GLP/GEP: no, unpublished	Y	BCS
Sur, R.; Ellerich, C.	IIIA1 9.6.1/02 IIIA1 9.6.2/02	2012	Tier 2a (DFOP) FPF PECgw EU: Predicted environmental concentrations in groundwater recharge based on models Focus Pearl and Focus Pelmo - Use in hops in Europe - Flupyradifurone (BYI 02960) - Difluoroacetic acid (DFA) - 6-Chloronicotinic acid (6-CNA) Bayer CropScience, Report No.: EnSa-12-0090, Edition Number: M-427991-01-1 Date: 2012-03-21 GLP/GEP: no, unpublished	Y	BCS
Sur, R.; Ellerich, C.	IIIA1 9.6.1/03 IIIA1 9.6.2/03	2012	FPF PECgw FOCUS EUR (TDS): Predicted environmental concentrations in groundwater recharge based on models FOCUS PEARL and FOCUS PELMO - Use in hops in Europe Bayer CropScience, Report No.: EnSa-12-0091, Edition Number: M-427980-01-1 Date: 2012-03-21 GLP/GEP: no, unpublished	Y	BCS
Sur, R.; Ellerich, C.	IIIA1 9.6.1/04 IIIA1 9.6.2/04	2012	FPF PECgw FOCUS EUR: Predicted environmental concentrations in groundwater recharge based on models FOCUS PEARL and FOCUS PELMO - Use in lettuce in Europe Bayer CropScience, Report No.: EnSa-12-0097, Edition Number: M-427736-01-1 Date: 2012-03-16 GLP/GEP: no, unpublished	Y	BCS

Author(s)	Annex point/ reference number	Year	Title, Source, Company, Report No, GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
Sur, R.; Ellerich, C.	IIIA1 9.6.1/05 IIIA1 9.6.2/05	2012	Tier 2a (DFOP) FPF PECgw EU: Predicted environmental concentrations in groundwater recharge based on models Focus Pearl and Focus Pelmo - Use in lettuce in Europe - Flupyradifurone (BYI 02960) - Difluoroacetic acid (DFA) - 6-Chloronicotinic acid (6-CNA) Bayer CropScience, Report No.: EnSa-12-0098, Edition Number: M-427987-01-1 Date: 2012-03-21 GLP/GEP: no, unpublished	Y	BCS
Sur, R.; Ellerich, C.	IIIA1 9.6.1/06 IIIA1 9.6.2/06	2012	FPF PECgw FOCUS EUR (TDS): Predicted environmental concentrations in groundwater recharge based on models FOCUS PEARL and FOCUS PELMO - Use in lettuce in Europe Bayer CropScience, Report No.: EnSa-12-0099, Edition Number: M-427981-01-1 Date: 2012-03-21 GLP/GEP: no, unpublished	Y	BCS
Sur, R.; Ellerich, C.	IIIA1 9.7/01 IIIA1 9.8/01	2012	FPF PECsw FOCUS EUR: Predicted environmental concentrations in surface water and sediment - Use in hops and lettuce in Europe Bayer CropScience, Report No.: EnSa-12-0071, Edition Number: M-427646-01-1 Date: 2012-03-16 GLP/GEP: no, unpublished	Y	BCS

Section A.9 Ecotoxicology

References for the active substance

Author(s)	Annex point / reference number	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. Claimed Y/N	Owner
[redacted]	KIIA 8.1.4/01a	2013	Mallard repro, BYI 2960, revised data [redacted] Bayer CropScience Edition no. M-450547-01-1 (supplement to edition M-412917-02-1) Date: 2013-03-22 GLP/GEP: yes , unpublished	Yes	BCS
[redacted]	KIIA 8.6 /01	2010	Toxicity of BYI 02960 technical to duckweed (Lemna gibba G3) under static-renewal conditions [redacted] Bayer CropScience, Report No.: EBRVP043, Edition Number: M-398376-01-1 Date: 2010-12-21 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 8.2.1.1 /02	2011	Acute toxicity of BYI 02960 to Xenopus laevis under flow-through conditions [redacted] Bayer CropScience, Report No.: EBRVP187, Edition Number: M-417822-01-1 Date: 2011-11-18 GLP/GEP: yes, unpublished	Yes	BCS
Banman, C. S.; Lam, C. V.	KIIA 8.3.1.1 /01	2009	Acute toxicity of BYI 02960 to Daphnia magna under static conditions Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: EBRVP032, Edition Number: M-357476-01-1 Date: 2009-10-14 GLP/GEP: yes, unpublished	Yes	BCS
Banman, C. S.; Lam, C. V.	KIIA 8.4 /01	2010	Toxicity of BYI 02960 technical to the green alga Pseudokirchneriella subcapitata Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: EBRVP030, Edition Number: M-397552-01-1 Date: 2010-12-10 GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. Claimed Y/N	Owner
[redacted]	KIIA 8.11.1 /01	2009	Acute toxicity of BYI 02960 technical to the sheepshead minnow (Cyprinodon variegatus) under static conditions [redacted] Bayer CropScience, Report No.: EBRVP034, Edition Number: M-357479-01-1 Date: 2009-10-14 GLP/GEP: yes, unpublished	Yes	BCS
Bocksch, S.	KIIA 8.16.2/05	2012a	Determination of residues of BYI 02960 applied via drench application in watermelon in the semi-field; Eurofins Agroscience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany; Report No.: S09-01391; Document No.: M-424666-01-2; February 09, 2012; Pages: 173	Yes	BCS
Bocksch, S.	KIIA 8.16.2/06	2012b	Determination of residues of BYI 02960 applied via drench application in tomato in the semi-field; Eurofins Agroscience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany; Report No.: S10-03818; Document No.: M-424683-01-2; February 10, 2012; Pages: 108	Yes	BCS
Bocksch, S.	KIIA 8.16.2/07	2012c	Determination of residues of BYI 02960 applied via drench application in watermelon in the semi-field; Eurofins Agroscience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany; Report No.: S10-03825; Document No.: M-424675-01-2; February 08, 2012; Pages: 117	Yes	BCS
Bowers, L. M.; Lam, C. V.	KIIA 8.3.1.2 /04	1998	Acute toxicity of 6-chloronicotinic acid (a metabolite of imidacloprid) to Chironomus tentans under static renewal conditions Bayer Corporation, Stilwell, KS, USA Bayer CropScience, Report No.: 108127, Edition Number: M-048448-01-1 Date: 1998-04-23 GLP/GEP: yes, unpublished	No	NISSO

Author(s)	Annex point / reference number	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. Claimed Y/N	Owner
[redacted]	KIIA 8.2.1.2 /02	2011	Acute toxicity of BYI 02960 (tech.) to fish (Cyprinus carpio) under static conditions (limit test) [redacted] Report No.: EBRVP186, Edition Number: M-420407-01-2 Date: 2011-12-19 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 8.2.1.3 /01	2011	Acute toxicity of BYI 02960 succinamide (tech.) to fish (Oncorhynchus mykiss) under static conditions (limit test) [redacted] Report No.: EBRVP203, Edition Number: M-414293-01-2 Date: 2011-09-21 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 8.2.1.3 /02	2011	Acute toxicity of sodium difluoro acetate (BCS AB60481, tech.) to fish (Oncorhynchus mykiss) under static conditions (limit test) [redacted] Report No.: EBRVP080, Edition Number: M-413889-01-2 Date: 2011-09-12 GLP/GEP: yes, unpublished	Yes	BCS
Bruns, E.	KIIA 8.3.1.1 /02	2011	Acute toxicity of BCS-AB60481 to the waterflea Daphnia magna in a static laboratory test system - limit test- Bayer CropScience, Report No.: EBRVP079, Edition Number: M-409326-01-2 Date: 2011-06-10 GLP/GEP: yes, unpublished	Yes	BCS
Bruns, E.	KIIA 8.3.1.2 /01	2011	Acute toxicity of BYI 02960 (tech.) to larvae of Chironomus riparius in a 48 h static laboratory test system Bayer CropScience, Report No.: EBRVP026, Edition Number: M-414739-01-2 Date: 2011-09-26 GLP/GEP: yes, unpublished ...also filed: KIIA 8.5.1 /01	Yes	BCS

Author(s)	Annex point / reference number	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. Claimed Y/N	Owner
Bruns, E.	KIIA 8.3.1.2 /02	2011	Acute toxicity of BYI 02960-succinamide to larvae of Chironomus riparius in a 48 h static laboratory test system Bayer CropScience, Report No.: EBRVP202, Edition Number: M-417386-01-2 Date: 2011-11-17 GLP/GEP: yes, unpublished	Yes	BCS
Bruns, E.	KIIA 8.3.1.2 /03	2012	Acute toxicity of BYI 02960-azabicyclosuccinamide (BCS-CS64875) to larvae of Chironomus riparius in a 48 h static laboratory test system Bayer CropScience, Report No.: EBRVP207, Edition Number: M-424404-01-1 Date: 2012-02-02 GLP/GEP: yes, unpublished	Yes	BCS
Bruns, E.	KIIA 8.3.2.2 /01	2011	Chironomus riparius 28-day chronic toxicity test with BYI 02960 (tech.) in a water-sediment system using spiked water Bayer CropScience, Report No.: EBRVP025, Edition Number: M-401792-01-2 Date: 2011-02-14 GLP/GEP: yes, unpublished ...also filed: KIIA 8.5.2 /01	Yes	BCS
Bruns, E.	KIIA 8.3.2.2 /02	2011	Chironomus riparius 28-day chronic toxicity test with Sodium difluoroacetate in a water-sediment system using spiked water - limit test Bayer CropScience, Report No.: EBRVP181, Edition Number: M-415913-01-2 Date: 2011-10-06 GLP/GEP: yes, unpublished	Yes	BCS
Bruns, E.	KIIA 8.3.2.2 /03	2011	Chironomus riparius 28-day chronic toxicity test with 6-Chloronicotinic acid in a water-sediment system using spiked water - limit test Bayer CropScience, Report No.: EBRVP183, Edition Number: M-416604-02-2 Date: 2011-10-18 ...Amended: 2011-12-20 GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. Claimed Y/N	Owner
Bruns, E.	KIIA 8.4 /02	2011	Pseudokirchneriella subcapitata growth inhibition test with BCS-AB60481 - limit test Bayer CropScience, Report No.: EBRVP077, Edition Number: M-409118-01-2 Date: 2011-06-10 GLP/GEP: yes, unpublished	Yes	BCS
Bruns, E.	KIIA 8.4 /04	2012	Pseudokirchneriella subcapitata growth inhibition test with 6 - chloronicotinic acid Bayer CropScience, Report No.: EBRVP242, Edition Number: M-424145-01-2 Date: 2012-02-01 GLP/GEP: yes, unpublished	Yes	BCS
Bruns, E.	KIIA 8.5.1 /01	2011	Acute toxicity of BYI 02960 (tech.) to larvae of Chironomus riparius in a 48 h static laboratory test system Bayer CropScience, Report No.: EBRVP026, Edition Number: M-414739-01-2 Date: 2011-09-26 GLP/GEP: yes, unpublished ...also filed: KIIA 8.3.1.2 /01	Yes	BCS
Bruns, E.	KIIA 8.5.2 /01	2011	Chironomus riparius 28-day chronic toxicity test with BYI 02960 (tech.) in a water-sediment system using spiked water Bayer CropScience, Report No.: EBRVP025, Edition Number: M-401792-01-2 Date: 2011-02-14 GLP/GEP: yes, unpublished ...also filed: KIIA 8.3.2.2 /01	Yes	BCS
Bruns, E.; Hoffmann, K.; Hager, J.	KIIA 8.11.1 /01a	2013	Flupyradifurone (BYI 02960) - BCS opinion on the reproduction endpoint of the chronic study with Americamysis bahia Bayer CropScience AG, Monheim, Germany Edition Number: M-452374-01-1 Edition Date: 2013-04-26 GLP/GEP: No	Yes	BCS

Author(s)	Annex point / reference number	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. Claimed Y/N	Owner
Caspers, N.	KIIA 8.15 /01	2010	Activated sludge, respiration inhibition test with BYI 02960 (tech.) Currenta GmbH & Co. OHG, Leverkusen, Germany Bayer CropScience, Report No.: 2010/0089/01, Edition Number: M-377311-01-1 Date: 2010-06-21 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 8.1.4/01b	2013	SynTech response for the mallard reproduction study exposed to BYI 02960 [redacted] Bayer CropScience Edition Number: M-450549-01-1 Date: 2013-03-26 GLP/GEP: no , unpublished	Yes	BCS
[redacted]	KIIA 8.1.4 /02a	2013	SynTech response for the quail reproduction study exposed to BYI 02960 [redacted] Bayer CropScience Edition Number: M-451967-01-1 Date: 2013-04-18 GLP/GEP: no , unpublished	Yes	BCS
[redacted] [redacted]	KIIA 8.1.1 /02	2011	Toxicity of BYI 02960 technical during an acute oral LD50 with the canary (Serinus canaria) [redacted] Bayer CropScience, Report No.: EBRVP036, Edition Number: M-408514-01-1 Date: 2011-05-25 GLP/GEP: yes, unpublished	Yes	BCS
Claude, M. B.; Kendall, T. Z.; Krueger, H. O.	KIIA 8.11.1 /04	2011	BYI 02960: A flow-through life-cycle toxicity test with the saltwater mysid (Americanysis bahia) Wildlife International Ltd., Easton, MD, USA Bayer CropScience, Report No.: EBRVP038, Edition Number: M-420783-01-1 Date: 2011-09-08 GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. Claimed Y/N	Owner
[redacted]	KIIA 8.1.2 /01	2010	Toxicity of BYI 02960 technical during an acute dietary LC50 with the mallard duck (Anas platyrhynchos) [redacted] Bayer CropScience, Report No.: EBRVP020, Edition Number: M-388718-01-1 Date: 2010-08-26 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 8.1.1 /01	2010	Toxicity of BYI 02960 technical during an acute oral LD50 with the northern bobwhite quail (Colinus virginianus) [redacted] Bayer CropScience, Report No.: EBRVP022, Edition Number: M-386036-01-1 Date: 2010-07-14 GLP/GEP: yes, unpublished	Yes	BCS
Frommholz, U.	KIIA 8.10.1 /01	2009	BYI 02960 a.s.: Determination of effects on nitrogen transformation in soil Bayer CropScience, Report No.: FRM-N-130/09, Edition Number: M-359803-01-2 Date: 2009-12-03 GLP/GEP: yes, unpublished	Yes	BCS
Frommholz, U.	KIIA 8.10.1 /02	2011	6-chloronicotinic acid (AE F161089): Determination of effects on nitrogen transformation in soil Bayer CropScience, Report No.: FRM-N-156/11, Edition Number: M-408028-01-2 Date: 2011-05-20 GLP/GEP: yes, unpublished	Yes	BCS
Frommholz, U.	KIIA 8.14 /01	2009	BYI 02960 SL 200 G: Influence on the reproduction of the collembola species Folsomia candida tested in artificial soil with 5 % peat Bayer CropScience, Report No.: FRM-COLL-75/09, Edition Number: M-359728-01-2 Date: 2009-12-02 GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. Claimed Y/N	Owner
Frommholz, U.	KIIA 8.14 /02	2010	Metabolite BYI 02960-difluoroacetic acid: Influence on the reproduction of the collembolan species Folsomia candida tested in artificial soil Bayer CropScience, Report No.: FRM-COLL-85/10, Edition Number: M-368675-01-2 Date: 2010-05-12 GLP/GEP: yes, unpublished	Yes	BCS
Frommholz, U.	KIIA 8.14 /03	2011	6-chloronicotinic acid (AE F161089): Influence on the reproduction of the collembolan species Folsomia candida tested in artificial soil Bayer CropScience, Report No.: FRM-COLL-111/11, Edition Number: M-407861-01-2 Date: 2011-05-18 GLP/GEP: yes, unpublished	Yes	BCS
Gallagher, S. P.; Kendall, T. Z.; Krueger, H. O.	KIIA 8.11.1 /02	2009	BYI 02960: A 96-hour shell deposition test with the eastern oyster (Crassostrea virginica) Wildlife International Ltd., Easton, MD, USA Bayer CropScience, Report No.: EBRVP023, Edition Number: M-361668-01-1 Date: 2009-12-01 GLP/GEP: yes, unpublished	Yes	BCS
Gallagher, S. P.; Kendall, T. Z.; Krueger, H.O.	KIIA 8.11.1 /03	2009	BYI 02960: A 96-hour static acute toxicity test with the saltwater mysid (Americamysis bahia) Wildlife International Ltd., Easton, MD, USA Bayer CropScience, Report No.: 149A-236, Edition Number: M-364620-01-1 Date: 2009-12-08 GLP/GEP: yes, unpublished	Yes	BCS
Gladbach, D.; Theis, M.; Przygoda, D.; Nikolakis, A.	KIIA 8.16.1 /09	2013	Assessment of chronic effects of BYI 02960 tech. to the honey bee, Apis mellifera L., in a 10 days continuous laboratory feeding test; Bayer CropScience AG, Monheim, Germany; Report No.: E 318 4561-8; Document No.: M-462475-01-1; August 26, 2013; Pages: 96	Yes	BCS

Author(s)	Annex point / reference number	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. Claimed Y/N	Owner
Gosch, H.	KIIA 8.12 /01	2010	BYI 02960 SL 200 g/L - Effects on the vegetative vigour of eleven species of non-target terrestrial plants (Tier 1) Bayer CropScience, Report No.: VV 10/002, Edition Number: M-397734-01-2 Date: 2010-12-14 GLP/GEP: yes, unpublished	Yes	BCS
Gosch, H.	KIIA 8.12 /02	2010	BYI 02960 SL 200 g/L - Effects on the seedling emergence and growth of eleven species of non-target terrestrial plants (Tier 1) Bayer CropScience, Report No.: SE10/001, Edition Number: M-397727-01-2 Date: 2010-12-14 GLP/GEP: yes, unpublished	Yes	BCS
Gould, T. J.; Lawrence, J.; Harbin, A. M.	KIIA 8.16.2/04	2012	Determination of residues of BYI 02960 in blossoms, nectar, and pollen when applied via soil drench and Foliar Spray to melon under semi-field Conditions in North Carolina; Eurofins Agroscience Services, Mebane, NC, USA; Report No.: RARVP019; Document No.: M-435037-01-1; July 24, 2012; Pages: 209	Yes	BCS
Hager, J.	Q 14 /01 (relevant for KIIA 8.3.2.2)	2014	Flupyradifurone (BYI 02960): BCS opinion on the need for additional data on mayflies (Ephemeroptera) Bayer CropScience, Report No.: M-496218-01-1, Edition Number: M-496218-01-1 Date: 2014-08-29 GLP/GEP: n.a., unpublished	Yes	BCS
Jans, D.	KIIA 8.8.1.1 /01	2010	Toxicity to the parasitoid wasp Aphidius rhopalosiphi (DESTEPHANI-PEREZ) (Hymenoptera: Braconidae) using a laboratory test; BYI 02960 SL 200 g/L Bayer CropScience, Report No.: CW09/079, Edition Number: M-366965-01-2 Date: 2010-04-15 GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. Claimed Y/N	Owner
Jans, D.	KIIA 8.8.1.2 /01	2010	Toxicity to the predatory mite Typhlodromus pyri SCHEUTEN (Acari, Phytoseiidae) using a laboratory test; BYI 02960 SL 200 g/L Bayer CropScience, Report No.: CW09/073, Edition Number: M-366957-01-2 Date: 2010-04-15 GLP/GEP: yes, unpublished	Yes	BCS
Kling, A.	KIIA 8.16.1 /02	2012	BYI 02960-difluoroethyl-amino-furanone (BYI 02960-DFEAF) - Assessment of chronic effects to the honey bee, Apis mellifera L., in a 10 days continuous laboratory feeding limit test Eurofins Agroscience Services EcoChem GmbH, Niefern-Öschelbronn, Germany Bayer CropScience, Report No.: S11-01959, Report includes Trial Nos.: S11-01959-L1_BLCF Edition Number: M-425174-01-2 Date: 2012-02-14 GLP/GEP: yes, unpublished	Yes	BCS
Kling, A.	KIIA 8.16.1 /03	2012	BYI 02960-hydroxy - Assessment of chronic effects to the honey bee, Apis mellifera L., in a 10 days continuous laboratory feeding limit test Eurofins Agroscience Services EcoChem GmbH, Niefern-Öschelbronn, Germany Bayer CropScience, Report No.: S11-01960, Report includes Trial Nos.: S11-01960-L1_BLCF Edition Number: M-425212-01-2 Date: 2012-02-15 GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. Claimed Y/N	Owner
Kling, A.	KIIA 8.16.1 /04	2012	Difluoroacetic acid - Assessment of chronic effects to the honey bee, Apis mellifera L., in a 10 days continuous laboratory feeding limit test Eurofins Agroscience Services EcoChem GmbH, Niefern-Öschelbronn, Germany Bayer CropScience, Report No.: S11-01939, Report includes Trial Nos.: S11-01939-L1_BLCF Edition Number: M-425105-01-1 Date: 2012-02-13 GLP/GEP: yes, unpublished	Yes	BCS
Kling, A.	KIIA 8.16.1 /05	2012	6-chloronicotinic acid - Assessment of chronic effects to the honey bee, Apis mellifera L., in a 10 days continuous laboratory feeding limit test Eurofins Agroscience Services EcoChem GmbH, Niefern-Öschelbronn, Germany Bayer CropScience, Report No.: S11-01957, Report includes Trial Nos.: S11-01957-L1_BLCF Edition Number: M-425155-01-2 Date: 2012-02-13 GLP/GEP: yes, unpublished	Yes	BCS
Kling, A.	KIIA 8.16.1 /06	2012	6-chloropicolyl alcohol - Assessment of chronic effects to the honey bee, Apis mellifera L., in a 10 days continuous laboratory feeding limit test Eurofins Agroscience Services EcoChem GmbH, Niefern-Öschelbronn, Germany Bayer CropScience, Report No.: S11-01958, Report includes Trial Nos.: S11-01958-L1_BLCF Edition Number: M-425159-01-2 Date: 2012-02-13 GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. Claimed Y/N	Owner
Kratz, M.-A.	KIIA 8.14 /04	2009	BYI 02960 SL 200 G: Influence on mortality and reproduction on the soil mite species Hypoaspis aculeifer tested in artificial soil with 5 % peat Bayer CropScience, Report No.: KRA-HR-19/09, Edition Number: M-358752-01-2 Date: 2009-11-10 GLP/GEP: yes, unpublished	Yes	BCS
Kratz, M.-A.	KIIA 8.14 /05	2010	BYI 02960-DFA (BCS-AA56716): Influence on mortality and reproduction on the soil mite species Hypoaspis aculeifer tested in artificial soil with 5 % peat Bayer CropScience, Report No.: KRA-HR-27/10, Edition Number: M-390091-01-2 Date: 2010-09-15 GLP/GEP: yes, unpublished	Yes	BCS
Kratz, M.-A.	KIIA 8.14 /06	2011	6-chloronicotinic acid (AE F161089): Influence on mortality and reproduction on the soil mite species Hypoaspis aculeifer tested in artificial soil Bayer CropScience, Report No.: KRA-HR-44/11, Edition Number: M-404434-01-2 Date: 2011-03-24 GLP/GEP: yes, unpublished	Yes	BCS
Leicher, T.	KIIA 8.9.1 /01	2010	BYI 02960 (tech.): Acute toxicity to earthworms (Eisenia fetida) tested in artificial soil Bayer CropScience, Report No.: LRT/RG-A-131/09, Edition Number: M-363742-01-2 Date: 2010-02-18 GLP/GEP: yes, unpublished	Yes	BCS
Leicher, T.	KIIA 8.9.1 /02	2010	BYI 02960-difluoroacetic acid: acute toxicity to earthworms (Eisenia fetida) tested in artificial soil Bayer CropScience, Report No.: LRT/RG-A-135/10, Edition Number: M-368835-01-2 Date: 2010-04-12 GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. Claimed Y/N	Owner
Leicher, T.	KIIA 8.9.2 /01	2010	BYI 02960 SL 200 G: Effects on survival, growth and reproduction on the earthworm Eisenia fetida tested in artificial soil Bayer CropScience, Report No.: LRT-RG-R-76/09, Edition Number: M-392964-01-2 Date: 2010-10-21 GLP/GEP: yes, unpublished	Yes	BCS
Leicher, T.	KIIA 8.9.2 /02	2010	BYI 02960 Difluoroacetic acid: Effects onsurvival, growth and reproduction on the earthworm Eisenia fetida tested in artifical soil with 10 % peat Bayer CropScience, Report No.: LRT-RG-R-81/10, Edition Number: M-398061-01-2 Date: 2010-12-16 GLP/GEP: yes, unpublished	Yes	BCS
Leicher, T.	KIIA 8.9.2 /03	2011	6-chloronicotinic acid (AE F161089): Effects on survival, growth and reproduction on the earthworm Eisenia fetida tested in artificial soil with 5 % peat Bayer CropScience, Report No.: LRT-RG-R-101/11, Edition Number: M-413562-02-2 Date: 2011-09-05 ...Amended: 2012-02-23 GLP/GEP: yes, unpublished	Yes	BCS
Leicher, T.	KIIA 8.16.2 /01	2011	BYI 02960: Effects on soil litter degradation after spray application Bayer CropScience, Report No.: LRT-SLD-45/11, Edition Number: M-413408-01-2 Date: 2011-09-06 GLP/GEP: yes, unpublished	Yes	BCS
Leicher, T.	KIIA 8.16.2 /02	2011	BYI 02960: Effects on soil litter degradation if applied as seed treatment Bayer CropScience, Report No.: LRT-SLD-46/11, Edition Number: M-413416-01-2 Date: 2011-09-06 GLP/GEP: yes, unpublished	Yes	BCS

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[redacted]	KIIA 8.2.1.1 /01	2010	Acute toxicity of BYI 02960 technical to the rainbow trout (Oncorhynchus mykiss) under static conditions [redacted] Bayer CropScience, Report No.: EBRVP041, Edition Number: M-390611-01-1 Date: 2010-09-27 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 8.2.1.2 /01	2010	Acute toxicity of BYI 02960 technical to the fathead minnow (Pimephales promelas) under static conditions [redacted] Bayer CropScience, Report No.: EBRVP035, Edition Number: M-392560-01-1 Date: 2010-10-21 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 8.2.4 /01	2011	Early life stage toxicity of BYI 02960 technical to the Fathead minnow (Pimephales promelas) under flow-through conditions [redacted] Bayer CropScience, Report No.: EBRVP033, Edition Number: M-409339-01-1 Date: 2011-06-14 GLP/GEP: yes, unpublished	Yes	BCS
McElligott, A.	KIIA 8.3.1.1 /03	1997	Acute toxicity (48 hours) to Daphnids (Daphnia magna) under semi-static conditions IC-0 Rhone-Poulenc Agro, Sophia Antipolis, France Bayer CropScience, Report No.: C007748, Edition Number: M-196569-01-1 Date: 1997-05-20 GLP/GEP: yes, unpublished	No	NISSO
Nauen, R.	KIIA 8.16.1 /08	2013	Determination of the oral LD50-value for flupyradifurone against the green peach aphis, Myzus persicae Sulzer (Homoptera: Aphididae); Bayer CropScience AG, Monheim, Germany; Report No.: M-461449-01-1; Document No.: M-461449-01-1; August 01, 2013; Pages: 6	Yes	BCS

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Nikolakis, A.; Krieg, V.; Aumeier, P.; Gladbach, D.	KIIA 8.16.2/03	2012	Honey bee colony feeding study, evaluating the effects of BYI 02960-fortified sugar- and pollen diet on the development of honey bee colonies under confined semi-field conditions, followed by a post-exposure field observation period Bayer CropScience, Report No.: E 319 4111-0, Edition Number: M-438748-01-1 Date: 2012-09-24 GLP/GEP: yes, unpublished	Yes	BCS
Nikolakis, A.; Theis, M.; Przygoda, D.	KIIA 8.16.1 /07	2011	BYI 02960 tech.: Effects of exposure to spiked diet on honeybee larvae (Apis mellifera carnica) in an in vitro laboratory testing design Bayer CropScience, Report No.: E 318 3897-9, Edition Number: M-406645-01-2 Date: 2011-05-02 GLP/GEP: yes, unpublished	Yes	BCS
Rexer, H. U.	KIIA 8.16.2/08	2013	Determination of residues of BYI 02960 after application of BYI 02960 SL 200 G once before and once during flowering in a semi-field honeybee (Apis mellifera L.) study in Phacelia tanacetifolia in 2012; Eurofins-GAB GmbH, Niefern-Oeschelbronn, Germany; Report No.: S12-00038; Document No.: M-457246-01-1; May 31, 2013; Pages: 192	Yes	BCS
Rexer, H. U.	KIIA 8.16.2/09	2012c	A field study to determine residues of BYI 02960 in guttation liquid from winter oil-seed rape (OSR) plants in Northern Germany in 2010/2011; Eurofins Agroscience Services GmbH, Niefern-Oeschelbronn, Germany; Report No.: S10-03312; Document No.: M-438826-01-1; September 27, 2012; Pages: 107	Yes	BCS
Rexer, H. U.	KIIA 8.16.2/10	2012d	A field study to determine residues of BYI 02960 in guttation liquid from winter oil-seed rape (OSR) plants in France in 2010/2011; Eurofins Agroscience Services GmbH, Niefern-Oeschelbronn, Germany; Report No.: S10-03313; Document No.: M-438829-01-1; September 27, 2012; Pages: 106	Yes	BCS

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Riebschlaeger, T.	KIIA 8.3.2.1 /01	2011	Effects of BYI 02960 (techn.) on development and reproductive output of the waterflea Daphnia magna in a static-renewal laboratory test system Bayer CropScience, Report No.: EBRVP209, Edition Number: M-414066-01-2 Date: 2011-09-15 GLP/GEP: yes, unpublished	Yes	BCS
Riebschlaeger, T.	KIIA 8.3.2.1 /02	2012	Influence of BYI02960-succinamide (tech.) on development and reproductive output of the waterflea Daphnia magna in a static-renewal laboratory test system Bayer CropScience, Report No.: EBRVP185, Edition Number: M-424700-01-2 Date: 2012-02-10 GLP/GEP: yes, unpublished	Yes	BCS
Schmitzer, S.	KIIA 8.7.1 /01	2012	Revised final report no.: 1 - Effects of BYI 02960 (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 41121035, Edition Number: M-308904-02-1 Date: 2012-01-14 ...Amended: 2012-03-22 GLP/GEP: yes, unpublished	Yes	BCS
Schmitzer, S.	KIIA 8.7.1 /02	2010	Effects of BYI02960 - difluoroethyl - amino - furanone (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 60291035, Edition Number: M-398557-01-2 Date: 2010-12-21 GLP/GEP: yes, unpublished	Yes	BCS

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Schmitzer, S.	KIIA 8.7.1 /03	2011	Effects of BYI 02960 - hydroxy (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 63901035, Edition Number: M-409606-01-2 Date: 2011-06-15 GLP/GEP: yes, unpublished	Yes	BCS
Schmitzer, S.	KIIA 8.7.1 /04	2010	Effects of difluoroacetic acid (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 56331035, Edition Number: M-367915-01-2 Date: 2010-04-29 GLP/GEP: yes, unpublished	Yes	BCS
Schmitzer, S.	KIIA 8.7.1 /05	2010	Effects of 6-chloronicotinic acid (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 60281035, Edition Number: M-395279-01-2 Date: 2010-11-19 GLP/GEP: yes, unpublished	Yes	BCS
Schmitzer, S.	KIIA 8.7.1 /06	2010	Effects of 6-chloro-picolylalcohol (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 50911035, Edition Number: M-361234-01-2 Date: 2010-01-11 GLP/GEP: yes, unpublished	Yes	BCS
Schulz, L.	KIIA 8.10.2 /01	2011	BYI 02960 a.s.: Effects on the activity of soil microflora (carbon transformation test) BioChem Agrar GmbH, Gerichshain, Germany Bayer CropScience, Report No.: 11 10 48 058 C, Edition Number: M-417194-01-2 Date: 2011-11-11 GLP/GEP: yes, unpublished	Yes	BCS

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Sobczyk, H.	KIIA 8.4 /03	2011	Pseudokirchneriella subcapitata growth inhibition test with BYI 02960 - succinamide - limit test Bayer CropScience, Report No.: EBRVP184, Edition Number: M-414090-01-2 Date: 2011-09-23 GLP/GEP: yes, unpublished	Yes	BCS
Springer, T	KIIA 8.11.1 /01b	2013	Effect size, variation, and dependability of conclusions from NOEC and ECx calculations for for project 149A-242B, BYI 02960: A flow-through life-cycle toxicity test Wildlife International Edition Number: M-452382-01-1 Edition Date: 2013-04-25 GLP/GEP: No	Yes	BCS
[redacted]	KIIA 8.1.4 /01	2011	Toxicity of BYI 02960 technical on reproduction to the mallard duck (Anas platyrhynchos) [redacted] Bayer CropScience, Report No.: EBRVP018-1, Edition Number: M-412917-02-1 Date: 2011-08-25 ...Amended: 2012-03-19 GLP/GEP: yes, unpublished	Yes	BCS
[redacted].	KIIA 8.1.4 /02	2012	Toxicity of BYI 02960 technical on reproduction to the northern bobwhite quail (Colinus virginianus) [redacted] Bayer CropScience, Report No.: EBRVP019, Edition Number: M-424704-01-1 Date: 2012-02-09 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIA 8.1.2 /02	2010	Toxicity of BYI 02960 technical during an acute dietary LC50 with the northern bobwhite quail (Colinus virginianus [redacted] Bayer CropScience, Report No.: EBRVP021, Edition Number: M-394535-01-1 Date: 2010-11-10 GLP/GEP: yes, unpublished	Yes	BCS

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Vergé, E	KIIA 8.7.1 /07	2012	Flupyradifurone SL 200 G: Acute contact toxicity to the bumblebee Bombus terrestris L.(Hymenoptera, Apidae) under laboratory conditions (multi doses test) Source: Eurofins-GAB GmbH, Niefern-Oeschelbronn, Germany Edition Number: M-443696-01-1 Study Completion Date: 2012-12-13 GLP: yes, unpublished	Yes	BCS
Voerste, A.; Malsam, O.	KIIA 8.14.1 /01	2010	Determination of the insecticidal efficacy of the metabolite difluoroacetic acid (BCS-AA56716) compared to the parent compound BYI02960 (BCS-BZ89914) Bayer CropScience, Report No.: VAR 2010-01, Edition Number: M-386333-01-1 Date: 2010-06-10 GLP/GEP: no, unpublished	Yes	BCS
Wetton, P. M.	KIIA 8.9.1 /03	1999	Acute toxicity to earthworms (Eisenia foetida) IC-0 Safepharm Lab. Ltd., Derby, United Kingdom Bayer CropScience, Report No.: C007758, Edition Number: M-196591-01-1 Date: 1999-08-25 GLP/GEP: yes, unpublished	No	NISSO

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Author(s)	Annex point / reference number	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. claimed	Owner
Aldershof, S.; Bakker, F.	KIIIA1 10.5.4 /01	2012	A field study to assess the effects of BYI 02960 SL 200 g/L on the non-target, surface- and plantdwelling, arthropod fauna of a grassland habitat (off-crop) in The Netherlands during summer MITOX Consultants, Amsterdam, The Netherlands Bayer CropScience, Report No.: B154FFN, Edition Number: M-425092-01-2 Date: 2012-02-17 GLP/GEP: yes, unpublished	Yes	BCS
Aldershof, S.; Bakker, F.	KIIIA1 10.5.4 /02	2012	A field study to assess the effects of BYI 02960 (SL 200 g/L) on the non-target, surface- and plant-dwelling arthropod fauna of a grassland habitat (off-crop) in SW France during summer MITOX Consultants, Amsterdam, The Netherlands Bayer CropScience, Report No.: B153FFN, Edition Number: M-425080-01-2 Date: 2012-02-17 GLP/GEP: yes, unpublished	Yes	BCS
Barfknecht, R.; Wilkens, S.	KIIIA1 10.1.6 /02	2012	Acute oral toxicity of chicken (Gallus gallus domesticus) with BYI 02960 SL 200, according to OECD 223 - limit test- Bayer CropScience, Report No.: BAR/LD 142, Edition Number: M-423043-01-2 Date: 2012-01-06 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIIA1 10.2.2.1 /01	2010	Acute toxicity of BYI 02960 SL 200 G to fish (Oncorhynchus mykiss) under static conditions-limit test [redacted] Report No.: EBRVP098, Edition Number: M-398721-02-2 Date: 2010-12-20 ...Amended: 2012-01-19 GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. claimed	Owner
[redacted]	KIIIA1 10.2.2.1 /02	2011	Acute toxicity of BYI 02960 SL 200 G to fish (Cyprinus carpio) under static conditions (limit test) [redacted] Report No.: EBRVP199, Edition Number: M-420485-01-2 Date: 2011-12-19 GLP/GEP: yes, unpublished	Yes	BCS
Bruns, E.	KIIIA1 10.2.2.3 /01	2010	Pseudokirchneriella subcapitata growth inhibition test with BYI 02960 SL 200 G Bayer CropScience, Report No.: EBRVP095, Edition Number: M-397244-01-2 Date: 2010-12-20 GLP/GEP: yes, unpublished	Yes	BCS
Frommholz, U.	KIIIA1 10.6.6 /01	2009	BYI 02960 SL 200 G: Influence on the reproduction of the collembola species Folsomia candida tested in artificial soil with 5 % peat Bayer CropScience, Report No.: FRM-COLL-75/09, Edition Number: M-359728-01-2 Date: 2009-12-02 GLP/GEP: yes, unpublished	Yes	BCS
Frommholz, U.	KIIIA1 10.7.1 /01	2010	BYI 02960 SL 200 G : Determination of effects on carbon transformation in soil Bayer CropScience, Report No.: FRM-C-116/10, Edition Number: M-395469-01-2 Date: 2010-11-23 GLP/GEP: yes, unpublished	Yes	BCS
Frommholz, U.	KIIIA1 10.7.1 /02	2010	BYI 02960 SL 200 G: Determination of effects on nitrogen transformation in soil Bayer CropScience, Report No.: FRM-N-148/10, Edition Number: M-396112-01-2 Date: 2010-11-29 GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. claimed	Owner
Gosch, H.	KIIIA1 10.8.1.2 /01	2010	BYI 02960 SL 200 g/L - Effects on the vegetative vigour of eleven species of non-target terrestrial plants (Tier 1) Bayer CropScience, Report No.: VV 10/002, Edition Number: M-397734-01-2 Date: 2010-12-14 GLP/GEP: yes, unpublished	Yes	BCS
Gosch, H.	KIII A, 10.8.1.3 /01	2010	BYI 02960 SL 200 g/L - Effects on the seedling emergence and growth of eleven species of non-target terrestrial plants (Tier 1) Bayer CropScience, Report No.: SE10/001, Edition Number: M-397727-01-2 Date: 2010-12-14 GLP/GEP: yes, unpublished	Yes	BCS
Jans, D.	KIIIA1 10.5.1 /01	2010	Toxicity to the parasitoid wasp Aphidius rhopalosiphi (DESTEPHANI-PEREZ) (Hymenoptera: Braconidae) using a laboratory test; BYI 02960 SL 200 g/L Bayer CropScience, Report No.: CW09/079, Edition Number: M-366965-01-2 Date: 2010-04-15 GLP/GEP: yes, unpublished	Yes	BCS
Jans, D.	KIIIA1 10.5.1 /02	2010	Toxicity to the predatory mite Typhlodromus pyri SCHEUTEN (Acari, Phytoseiidae) using a laboratory test; BYI 02960 SL 200 g/L Bayer CropScience, Report No.: CW09/073, Edition Number: M-366957-01-2 Date: 2010-04-15 GLP/GEP: yes, unpublished	Yes	BCS
Jans, D.	KIIIA1 10.5.2 /01	2010	Toxicity to the parasitoid wasp Aphidius rhopalosiphi (DESTEPHANI-PEREZ) (Hymenoptera: Braconidae) using an extended laboratory test on barley BYI 02960 SL 200 (g/L) Bayer CropScience, Report No.: CW09/083, Edition Number: M-366970-01-2 Date: 2010-04-13 GLP/GEP: yes, unpublished	Yes	BCS

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Jans, D.	KIIIA1 10.5.2 /02	2010	Toxicity to the predatory mite Typhlodromus pyri SCHEUTEN (Acari, Phytoseiidae) using an extended laboratory test on Phaseolus vulgaris BYI 02960 SL 200 (g/L) Bayer CropScience, Report No.: CW09/076, Edition Number: M-366968-01-2 Date: 2010-04-13 GLP/GEP: yes, unpublished	Yes	BCS
Jans, D.	KIIIA1 10.5.2 /03	2010	Toxicity to the ladybird beetle Coccinella septempunctata L. (Coleoptera, Coccinellidae) using an extended laboratory test on Phaseolus vulgaris; BYI 02960 SL 200 (g/L) Bayer CropScience, Report No.: CW09/074, Edition Number: M-384754-01-2 Date: 2010-06-30 GLP/GEP: yes, unpublished	Yes	BCS
Jans, D.	KIIIA1 10.5.2 /04	2010	Chronic toxicity (ER50) of BYI 02960 SL 200 (g/L) to the rove beetle Aleochara bilineata Gyll. (Coleoptera: Staphylinidae) under extended laboratory conditions Bayer CropScience, Report No.: CW09/072, Edition Number: M-384433-01-2 Date: 2010-06-30 GLP/GEP: yes, unpublished	Yes	BCS
Jans, D.	KIIIA1 10.5.3 /01	2010	Toxicity to the parasitoid wasp Aphidius rhopalosiphi (DESTEPHANI-PEREZ) (Hymenoptera: Braconidae) in an extended laboratory test (under semi-field conditions aged residues on Zea mays) BYI 02960 SL 200 (g/L) Bayer CropScience, Report No.: CW10/018, Edition Number: M-396372-01-2 Date: 2010-11-30 GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. claimed	Owner
Kratz, M.-A.	KIIIA1 10.6.6 /02	2009	BYI 02960 SL 200 G: Influence on mortality and reproduction on the soil mite species Hypoaspis aculeifer tested in artificial soil with 5 % peat Bayer CropScience, Report No.: KRA-HR-19/09, Edition Number: M-358752-01-2 Date: 2009-11-10 GLP/GEP: yes, unpublished	Yes	BCS
Leicher, T.	KIIIA1 10.6.2 /01	2010	BYI 02960 SL 200 G: acute toxicity to earthworms (Eisenia fetida) tested in artificial soil with 5 percent peat Bayer CropScience, Report No.: LRT/RG-A-143/10, Edition Number: M-397720-01-2 Date: 2010-12-14 GLP/GEP: yes, unpublished	Yes	BCS
Leicher, T.	KIIIA1 10.6.3 /01	2010	BYI 02960 SL 200 G: Effects on survival, growth and reproduction on the earthworm Eisenia fetida tested in artificial soil Bayer CropScience, Report No.: LRT-RG-R-76/09, Edition Number: M-392964-01-2 Date: 2010-10-21 GLP/GEP: yes, unpublished	Yes	BCS
Menke, U.	KIIIA1 10.6.4 /01	2012	BYI 02960 SL 200 G: Effects on the earthworm fauna of a grassland area within one year Bayer CropScience, Report No.: MNU/RG-F-8/12, Edition Number: M-426607-01-1 Date: 2012-03-07 GLP/GEP: yes, unpublished	Yes	BCS
Neumann, P.	Q 15 /01 (relevant for KIIIA 10.5.4)	2014	Extended summaries of the non-target arthropod off-crop field studies conducted for flupyradifurone SL 200 (including the evaluation of the EU RMS) Bayer CropScience, Report No.: M-496127-01-1, Edition Number: M-496127-01-1 Date: 2014-09-09 GLP/GEP: n.a., unpublished	Yes	BCS

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Nikolakis, A.; Theis, M.; Przygoda, D.	KIIIA1 10.4.6.1 /01	2011	BYI 02960 tech.: Effects of exposure to spiked diet on honeybee larvae (Apis mellifera carnica) in an in vitro laboratory testing design Bayer CropScience, Report No.: E 318 3897-9, Edition Number: M-406645-01-2 Date: 2011-05-02 GLP/GEP: yes, unpublished	Yes	BCS
Porch, J. R.; Krueger, H. O.	KIIIA1 10.4.3 /01	2011	BYI 02960 200 SL: A foliage residue toxicity study with the honeybee Wildlife International Ltd., Easton, MD, USA Bayer CropScience, Report No.: EBRVP044, Report includes Trial Nos.: 149-220 Edition Number: M-413084-01-1 Date: 2011-07-13 GLP/GEP: yes, unpublished	Yes	BCS
Proebsting, A.	KIIIA1 10.4.7 /04	2012a	BYI 02960 SL 200 G: A semi-field study in Denmark 2010 to evaluate effects of spray applications in Phacelia tanacetifolia on the honeybee Apis mellifera L. (Hymenoptera, Apidae) Eurofins Agroscience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Bayer CropScience, Report No.: S10-01954, Report includes Trial Nos.: S10-01954-01_BZEU Edition Number: M-423156-01-2 Date: 2012-01-19 GLP/GEP: yes, unpublished	Yes	BCS

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Proebsting, A.	KIIIA1 10.4.7 /05	2012b	BYI 02960 SL 200 G: A semi-field study in Italy 2011 to evaluate effects of spray applications in Phacelia tanacetifolia on the honeybee Apis mellifera L. (Hymenoptera, Apidae) Eurofins Agroscience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Bayer CropScience, Report No.: S10-01955, Report includes Trial Nos.: S10-01955-01-BZEU Edition Number: M-423172-01-2 Date: 2012-01-18 GLP/GEP: yes, unpublished	Yes	BCS
Rentschler, S.	KIIIA1 10.4.7 /03	2012a	BYI 02960 SL 200 G: A semi-field study in Germany 2009 to evaluate effects of spray applications in Phacelia tanacetifolia on the honeybee Apis mellifera L. (Hymenoptera, Apidea) Eurofins Agroscience Services EcoChem GmbH, Niefern-Öschelbronn, Germany Bayer CropScience, Report No.: S09-00854, Report includes Trial Nos.: S09-00854-01_BZEU Edition Number: M-425576-01-2 Date: 2012-02-17 GLP/GEP: yes, unpublished	Yes	BCS
Rentschler, S.	KIIIA1 10.4.7 /06	2012b	Determination of side-effects of BYI 02960 SL 200 G on honey bee (Apis mellifera L.) brood under semi-field conditions Eurofins Agroscience Services, EcoChem GmbH, Niefern-Oeschelbronn, Germany Bayer CropScience, Report No.: S10-03819, Report includes Trial Nos.: S10-03819-01 Edition Number: M-427438-01-1 Date: 2012-03-19 GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. claimed	Owner
Rexer, H. U.	KIIIA 10.4.5/01	2012a	Assessment of side effects on the honeybee (Apis mellifera L.), exposed to winter oil-seed rape, grown from seeds treated with BYI 02960 FS 480 G and sequentially sprayed with BYI 02960 SL 200 G during immediate pre- and full flowering in a long-term field study in Northern Germany Source: Eurofins Agroscience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Edition Number: M-438818-01-1 Date: 2012-09-27 GLP: yes, unpublished	Yes	BCS
Rexer, H. U.	KIIIA 10.4.5/02	2012b	Assessment of side effects on the honeybee (Apis mellifera L.), exposed to winter oil-seed rape, grown from seeds treated with BYI 02960 FS 480 G and sequentially sprayed with BYI 02960 SL 200 G during immediate pre- and full flowering in a long-term field study in France Source: Eurofins Agroscience Services GmbH, Niefern-Oeschelbronn, Germany Edition Number: M-438819-01-1 Date: 2012-07-24 GLP: yes, unpublished	Yes	BCS
Riebschlaeger, T.	KIIIA1 10.2.2.2 /01	2010	Acute toxicity of BYI 02960 SL 200 G to the waterflea Daphnia magna in a static laboratory test system Bayer CropScience, Report No.: EBRVP097, Edition Number: M-393738-01-2 Date: 2010-10-28 GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. claimed	Owner
Scherr, F.; Ellerich, C.	KIIIA1 10.3.3 /03	2013	Scherr, F.; Ellerich, C.; Statement on residue dissipation of flupyradifurone in treated foliage of lettuce: kinetic evaluation; Bayer CropScience AG, Monheim, Germany; Report No.: EnSa-12-0154; Document No.: M-428040-02-1; August 09, 2013; Pages: 127	Yes	BCS
Schmitzer, S.	KIIIA1 10.4.2.1 /01	2009	Revised final report no.: 1 - Effects of BYI 02960 SL 200 G (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 52331035, Edition Number: M-359920-02-1 Date: 2009-12-03 ...Amended: 2012-03-22 GLP/GEP: yes, unpublished	Yes	BCS
Schnorbach, H. J.	KIIIA1 10.4.7 /01	2012a	Evaluation of the effects of BYI 02960 SL 100 on honey bees (Apis mellifera) in a semifield tunnel test in full-flowering Phacelia tanacetifolia Bayer CropScience, Report No.: IA08DVG048G001, Edition Number: M-427040-01-1 Date: 2012-03-15 GLP/GEP: no, unpublished	Yes	BCS
Schnorbach, H. J.	KIIIA1 10.4.7 /02	2012b	Evaluation of the effects of BYI 02960 SL 200 on honey bees (Apis mellifera) in a semifield tunnel test in full-flowering Phacelia tanacetifolia Bayer CropScience, Report No.: IA09DVG051K619, Edition Number: M-427046-01-1 Date: 2012-03-15 GLP/GEP: no, unpublished	Yes	BCS
Schwarz, A.	KIIIA1 10.5.3 /02	2010	Effects of BYI 02960 SL 200 (g/L) on the predatory bug Orius laevigatus extended laboratory study - aged residue test IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 58691053, Edition Number: M-394033-01-2 Date: 2010-10-28 GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. claimed	Owner
Silke, G.	KIIIA1 10.2.6.2 /01	2011	Chironomus riparius 28-day chronic toxicity test with BYI 02960 SL 200 G in a water-sediment system using spiked water Bayer CropScience, Report No.: EBRVP182, Edition Number: M-416145-01-2 Date: 2011-10-17 GLP/GEP: yes, unpublished	Yes	BCS
[redacted]	KIIIA1 10.1.6 /01	2012	Toxicity of BYI 02960 SL 200 during an acute oral LD50 with the Northern bobwhite quail (Colinus virginianus) [redacted] Bayer CropScience, Report No.: EBRVP093, Edition Number: M-424312-01-1 Date: 2012-02-03 GLP/GEP: yes, unpublished	Yes	BCS
Sur, R.; Ellerich, C.	KIIIA1 10.3.3 /04	2012	Statement on residue dissipation of flupyradifurone in treated foliage of monocotyledonous plants: kinetic evaluation Bayer CropScience, Report No.: EnSa-12-0155, Edition Number: M-428041-01-1 Date: 2012-03-23 GLP/GEP: no, unpublished	Yes	BCS
Uceda, L.	KIIIA1 10.3.3 /02	2012	Determination of the residues of BYI 02960 in/on barley and wheat after spray application of BYI 02960 SL 200 in the field in Germany, Southern France and Italy Bayer S.A.S., Bayer CropScience, Lyon, France Bayer CropScience, Report No.: 11-2958, Report includes Trial Nos.: 11-2958-01 11-2958-02 11-2958-03 11-2958-04 Edition Number: M-427494-01-1 Date: 2012-03-22 GLP/GEP: yes, unpublished	Yes	BCS

Author(s)	Annex point / reference number	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. claimed	Owner
Wolf, C.	KIIIA1 10.3.3 /01	2004	Generic field monitoring of birds and mammals in hop-cultivation in Southern Germany Bayer CropScience, Report No.: WFC/FS 009, Edition Number: M-123479-01-1 Date: 2004-11-12 GLP/GEP: yes, unpublished	Yes	BCS

Section A.10 Efficacy

Author(s)	References relied on Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Dorcier, C.	KIIIA1 6.1.1 /02	2010	Comparative uptake, distribution and growth effects of radiolabelled BYI 02960, imidacloprid and thiamethoxam in tomato plants after soil drench under different soil water conditions Bayer CropScience, Report No.: M-428103-01-1, Edition Number: M-428103-01-1 Date: 2010-06-21 GLP/GEP: n.a., unpublished	Yes	BCS
Kiatkowski, P.; Rauen, H.W.; Spiegel, K.; Garside, C.M. Bayer cropscience, 2012, pp 92-94		2012	Document N - Tier 3 Summary of the Active Substance for BYI 02960 (Flupyradifurone) and its formulation Sivanto (Flupyradifurone BYI 02960) SL 200.Chapter 7 efficacy data and information	Yes	BCS
Reckmann, U.	KIIIA1 6.1.1 /03	2007	Formulation check of the translaminar aphicidal efficacy of a SL 200 and a WG 50 formulation of BCS-BZ 89914 = VRF 2960 Bayer CropScience, Report No.: b01008, Edition Number: M-428088-01-1 Date: 2007-11-13 GLP/GEP: no, unpublished	Yes	BCS
Reckmann, U.	KIIIA1 6.1.1 /04	2009	Formulation check: translaminar aphicidal efficacy of BCS-BZ 89914 (= VRF 2960) SL200 Bayer CropScience, Report No.: b00909, Edition Number: M-428099-01-1 Date: 2009-02-02 GLP/GEP: no, unpublished	Yes	BCS
Reckmann, U.	KIIIA1 6.1.1 /05	2008	Formulation check of the aphidical activity of different EC formulations of the sulfoximines BCS-CL 88386 (= JES 6515) EC010 in comparison to BCS-BZ 89914 (= VRF 2960) and imidacloprid Bayer CropScience, Report No.: b12208, Edition Number: M-428097-01-1 Date: 2008-11-07 GLP/GEP: no, unpublished	Yes	BCS

Author(s)	References relied on Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Reckmann, U.	KIIIA1 6.1.1 /06	2008	BCS-BZ 89914=VRF 2960 Speed of action - 3 trials Bayer CropScience, Report No.: b06308, Edition Number: M-428090-01-1 Date: 2008-06-16 GLP/GEP: no, unpublished	Yes	BCS
Reckmann, U.	KIIIA1 6.1.1 /07	2006	Selection of a BYI 02960 GR 2 against Aphis gossypii and Myzus persicae after soil mixing Bayer CropScience, Report No.: b05406, Edition Number: M-428084-01-1 Date: 2006-08-17 GLP/GEP: no, unpublished	Yes	BCS
Reckmann, U.	KIIIA1 6.1.1 /08	2007	Influence of soil moisture on the aphicidal activity of BYI 02290 SL100 and BYI 02960 SL100 after drench application Bayer CropScience, Report No.: b10107, Edition Number: M-428085-01-1 Date: 2007-11-14 GLP/GEP: no, unpublished	Yes	BCS
Reckmann, U.	KIIIA1 6.1.1 /09	2007	Influence of the soil type on the aphicidal activity of BYI 02290 SL100 and BYI 02960 SL100 after drench application Bayer CropScience, Report No.: b10207, Edition Number: M-428080-01-1 Date: 2007-11-14 GLP/GEP: no, unpublished	Yes	BCS
Reckmann, U.	KIIIA1 6.1.1 /10	2008	Aphicidal speed of action of BCS-AA 10233 and BYI 02960 after drench application: comparison to Movento and imidacloprid Bayer CropScience, Report No.: M-428096-01-1, Edition Number: M-428096-01-1 Date: 2008-11-07 GLP/GEP: no, unpublished	Yes	BCS

Author(s)	References relied on Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Reckmann, U.	KIIIA1 6.1.1 /11	2007	Formulation check of the translaminar efficacy against Bemisia tabaci of BYI 02960 SL 100 (contains 40 percent KS1371B) and SL 300 (without adjuvant) Bayer CropScience, Report No.: b10907, Edition Number: M-428086-01-1 Date: 2007-11-13 GLP/GEP: no, unpublished	Yes	Bayer CropScience BCS
Reckmann, U.	KIIIA1 6.1.1 /12	2008	Efficacy of BCS-BZ89914 (= VRF 2960) SL200 and SL300 against Bemisia tabaci on cotton: initial activity on adults and population development Bayer CropScience, Report No.: b11708, Edition Number: M-428092-01-1 Date: 2008-11-05 GLP/GEP: no, unpublished	Yes	BCS
Thielert, W.	KIIIA1 6.1.1 /01	2012	Study report for flupyradifurone SL 200 Bayer CropScience Bayer CropScience, Report No.: M-428101-01-1, Edition Number: M-428101-01-1 Date: 2012-03-29 GLP/GEP: n.a., unpublished	Yes	BCS
van Waetermeulen, X.	KIIIA1 6.1.2 /01	2012	Compilation of trial report for flupyradifurone SL200 flupyradifurone SL200 (200 g/L) - Minimum effective dose tests lettuce Bayer CropScience Bayer CropScience, Report No.: M-427719-01-1, Edition Number: M-427719-01-1 Date: 2012-03-27 GLP/GEP: yes, unpublished	Yes	BCS
Van Watermeulen, X.; Voslajer, Z.; Thielert, W.; Haas, M.	MIIIA1 Section 7 Efficacy data	2012	Document M, Annex IIIA Section 7, Point 6: Tier 2 Summary of the Efficacy Data and Information on the Plant Protection Product for Flupyradifurone (BYI 02960) SL 200 (Sivanto 200 SL) (specification number 102000021884),	Yes	BCS

Author(s)	References relied on Annex point / reference number	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
Voslajer, Z.	KIIIA1 6.1.2 /02	2012	Compilation of trial report for flupyradifurone SL200 flupyradifurone SL200 (200 g/L) - Minimum effective dose tests - hop Bayer CropScience Bayer CropScience, Report No.: M-427830-01-1, Edition Number: M-427830-01-1 Date: 2012-03-28 GLP/GEP: yes, unpublished	Yes	BCS

European Commission

Flupyradifurone

VOLUME 3

ANNEX B

B.1 Identity
B.2 Physical and chemical properties
B.3 Data on application and further information
B.4 Proposals for classification and labelling
B.5 Methods of analysis

Rapporteur Member State: The Netherlands

November 2014
~~January 2014~~

Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible approval of flupyradifurone under Regulation (EC) 1107/2009

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B.1 Identity

B.1.1 Identity of active substance (Annex IIA 1 and 3.1)

All points of Annex IIA, Section I are addressed in Volume 1, Level 1.

B.1.2 Identity of the plant protection product (Annex IIIA 1)

All points of Annex IIIA, Section 1 are addressed in Volume 1, Level 1.

B.1.3 References relied on

No specific references.

B.2 Physical and chemical properties

B.2.1 Physical and chemical properties of the active substance (Annex IIA 2)

See Table B.2.1-1.

Furthermore, some physical and chemical properties are presented for metabolites: BCS-CC98193 (BYI 02960-DFEAF), BCS-CR74729 (BYI 02960-succinamide), BCS-CU93236, BCS-AA56716 (Difluoroacetic acid), IC-0 (6-CNA).

B.2.2 Physical, chemical and technical properties of the plant protection product (Annex IIIA 2)

See Table B.2.2.

Table B.2.1-1 **Summary of the physical and chemical properties of the active substance** (studies were conducted under GLP and completed to an acceptable standard and results were considered to be valid unless specified otherwise).

section (Annex point)	study	purity	method	results	comment	reference
B.2.1.1 (IIA 2.1.1)	Melting point	99.4% 97.6%	EC A.1, OECD 102 GLP:Y	The melting point of Flupyradifurone, pure at atmospheric pressure is 69.0 °C. The melting point of Flupyradifurone, technical at atmospheric pressure is 67.1 °C.	Acceptable	Smeykal, H.; 2010 Smeykal, H.; 2011
B.2.1.2 (IIA 2.1.2)	Boiling point	99.4% 97.6%	EC A.2, OECD 103 GLP:Y	The test item had no boiling point at atmospheric conditions; the test item decomposed first at a temperature of 270 °C The test item had no boiling point; the test item decomposes first starting at a temperature of 245 °C	Acceptable	Smeykal, H.; 2010 Smeykal, H.; 2011
B.2.1.3 (IIA 2.1.3)	Thermal stability	99.4% 97.6%	EC A.2, OECD 103 GLP:Y	The test item showed an exothermal decomposition in the temperature range 270 – 355 °C with a mean decomposition energy of 895 J/g. The test item Flupyradifurone (BYI 02960), technical substance, showed an exothermal effect in the temperature range of 245 – 400 °C (245 – 355 °C respectively) with an energy of 836 to 938 J/g.	Acceptable. Decomposition temperature by DSC; visual observations (capillary method) showed change of colour to brown at 240°C. Test performed under helium atmosphere.	Smeykal, H.; 2010 Smeykal, H.; 2011

section (Annex point)	study	purity	method	results	comment	reference
B.2.1.4 (IIA 2.2)	Relative density	99.4% 97.6%	EC A.3, OECD 109, OPPTS 830.7300	Active substance, pure: D_4^{20} = 1.43 Active substance, technical: D_4^{20} = 1.52	Acceptable	Bogdoll, B.; Strunk, B.; 2011 Eyrich, U.; Bogdoll, B.; 2011
B.2.1.5 (IIA 2.3.1)	Vapour pressure	99.9%	EC A.4, OECD 104	Extrapolated: 9.1 x 10^{-7} Pa for 20 °C 1.7 x 10^{-6} Pa for 25 °C 2.6 x 10^{-5} Pa for 50 °C	Acceptable	Smeykal, H.; 2008
B.2.1.6 (IIA 2.3.2)	Volatility, Henry's law constant		Calculation.	Henry's law constant at 20 °C in distilled water (pH: 7.0) 8.2 x 10^{-8} Pa.m^3.mol^{-1} Henry's law constants at 20 °C at different pH values: at pH 4: 8.2 x 10^{-8} Pa.m^3.mol^{-1} at pH 9: 8.8 x 10^{-8} Pa.m^3.mol^{-1} A vapour pressure of 9.1 x 10^{-7} Pa (20 °C) and the follwing water solubility values at • pH 4: 3.2 g/L pH 9: 3.0 g/L were used to calculate the Henry's law constant.	Acceptable	Bogdoll, B.; Eyrich, U.; 2011

section (Annex point)	study	purity	method	results	comment	reference
B.2.1.7 (IIA 2.4.1)	Appearance: physical state	99.4% 97.6%	Visual	Active substance, pure (at 21°C): white powder Active substance, technical (at 23°C): beige powder	Acceptable	Bogdoll, B.; Strunk, B.; 2011 Eyrich, U., Bogdoll, B.; 2011
B.2.1.8 (IIA 2.4.1)	Appearance: colour	99.4% 97.6%	visual	Active substance, pure (at 21°C): white powder Active substance, technical (at 23°C): beige powder	Acceptable	
B.2.1.9 (IIA 2.4.2)	Appearance: odour	99.4% 97.6%	OPPTS 830.6304	active substance, pure (at 21°C): weak, not characteristic Active substance, technical (at 23°C): distinct, solvent-like odour		Bogdoll, B.; Strunk, B.; 2011; Eyrich, U., Bogdoll, B.; 2011;

section (Annex point)	study	purity	method	results	comment	reference
B.2.1.10 (IIA 2.5.1)	Spectra (as)	99.4%	OECD 101, UV/Vis spectrum. GLP:Y	UV (methanol) Recorded range of wavelengths: 200 – 800 nm Peak maxima molar absorptivity [nm] [1000 cm²/mol] 213 9615.06 259 25800.49 no peak maxima above 290 nm Buffer solution pH 2 Peak maxima molar absorptivity [nm] [1000 cm²/mol] 214 9388.74 259 26576.75 Buffer solution pH 10 Peak maxima molar absorptivity [nm] [1000 cm²/mol] 213 9996.41 259 25954.64	In the dissociation constant determination (see B.2.1.18) it was concluded that at least in the neutral pH range no repeatable UV spectra could be determined.	Peters, S.; 2009;
		99.4%	OECD 101	FT-IR Assignment of wave number to functional groups, s. report	Acceptable	Peters, S.; 2009;
		99.4%	OECD 101 NMR spectrum.	1H-NMR (600.13 MHz, d3-acetonitrile) 13C-NMR (150.90 MHz, d3-acetonitrile)	Acceptable	Peters, S.; 2009;
		99.4%	OECD 101 mass spectrum.	Mass spectra (LC-MS/ESI$^+$-spectrum) m/z-value assignment 289.1 [M+H]+ 577.3 [2M+H]+	Acceptable	Peters, S.; 2009;

section (Annex point)	study	purity	method	results	comment	reference
B.2.1.10 (IIA 2.5.2)	Spectra (impurities)			None of the impurities present in the active substance as manufactured is of toxicological or environmental significance	Acceptable	
B.2.1.11 (IIA 2.6)	Solubility in water	99.4%	EC A.6, OECD 105 GLP:Y	pH 4 (buffer) 3.2 g/L at 20°C pH 9 (buffer) 3.0 g/L at 20°C In distilled water: pH 7 3.2 g/L at 20°C	Acceptable	Wiche, A., Bogdoll, B.; 2011
B.2.1.12 (IIA 2.7)	Solubility in organic solvents	99.4%	EC A.6, OECD 105	[g/L at 20 °C] methanol > 250 n-heptane 0.0005 toluene 3.7 dichloromethane > 250 acetone > 250 ethylacetate > 250 dimethyl sulfoxide > 250	Acceptable	Eyrich, U., Bogdoll, B.; 2011;
B.2.1.13 (IIA 2.8)	Partition co-efficient	99.4%	ECA.8 OECD 117; High Performance Liquid Chromatography (HPLC) Method.	Determination of the partition coefficient of Flupyradifurone in 1-octanol / water (25 °C) Pow log Pow pH 7 16 1.2	Acceptable	Bogdoll, B.; Stunk, B.; 2011;
B.2.1.15 (IIA 2.9.1)	Hydrolysis rate	Radio chemical purity 99%	Furanone-4-^{14}C] BYI 02960, pure Vial No. C-1116	BYI 02960 is hydrolytically stable at ambient temperature in the range of pH 4 to pH9. 3 minor components were observed during 5 days at 50°C which accounted for a total of 4.9%, none were more than 2.7% of the applied activity in any of the pH	See fate section	Mislankar, S.; Woodard, D., 2011

section (Annex point)	study	purity	method	results	comment	reference
B.2.1.16 (IIA 2.9.2)	Photochemical degradation	Radio chemical purity 99.3%	[Furanone-4-^{14}C] BYI 02960, pure Vial No. C-1116A	1st order half-life for photolytic degradation of BYI 02960 in sterile phosphate buffer (pH 7) was 13.8 experimental hrs.	See fate section	Hall, L.R., 2011
B.2.1.17 (IIA 2.9.3)	Quantum yield	purity 99.4 % w/w	BYI 02960, pure NLL 7780-47-4	A mean quantum yield of **<D** = 0.000138 was calculated on the basis of UV absorption data and the degradation kinetics determined from 2 degradation experiments.	Environmental half-lives of sunlight exposed top surface water layers containing BYI 02960 can be estimated using respective models (GC SOLAR or Kloepffer Model) dependent on season and location of exposure.	Heinemann, O. 2011
B.2.1.18 (IIA 2.9.5)	Dissociation constant (pKa)	purity 99.4 % w/w	OECD 112	No dissociation occurs in aqueous solutions in the pH-range 1< pH <12		Wiche, A., Bogdoll, B.; 2011
B.2.1.19 (IIA 2.10)	Stability in air, photochemical oxidative degradation		Atkinson calculation by AOPWIN™ version 1.92a	The half-life time ($t_{1/2}$) was estimated within a range of 4.4 hours (short-term scenario) to 13.1 hours (long-term scenario), depending on the mean concentration of hydroxyl radicals present in the troposphere. In addition, BYI 02960 is susceptible to reactions with ozone, however, that attack and its resulting chemical half-life is considered to be more slowly by a factor of 2 to 10. As a consequence of the short half life in air, no long-range transport BYI 02960 in the atmosphere is likely to occur nor an accumulation of BYI02960 in the environmental compartment air.	See fate section	Hellpointner, E.;

section (Annex point)	study	purity	method	results	comment	reference
B.2.1.20 (IIA 2.11.1 and 2.11.2)	Flammability and auto-flammability (technical active substance)	Tech. a.s. 97.6%	EEC A.10 GLP:Y	Technical substance is not a highly flammable solid in the sense of the EC guideline (EC) No. 440/2008 Method AIO	Acceptable	Smeykal, H, 2011
B.2.1.21 (IIA 2.12)	Flash point (technical active substance)		Statement	Not applicable. The active substance as manufactured is a solid; its melting point is > 40 °C.		
B.2.1.22 (IIA 2.13)	Explosive properties (technical active substance)	Tech. a.s. 97.6%	EC A.14 OECD 113	Not explosive in the sense of EC guideline A.14	Acceptable	Smeykal, H.; 2011;
B.2.1.23 (IIA 2.14)	Surface tension (technical active substance)	Tech. a.s. 97.6%	EC A.5, OECD 115	Technical substance: 69.1 mN/m at 20°C	Acceptable	Eyrich, U., Bogdoll, B.; 2011;
(IIA 2.15)	Oxidizing properties of the active substance as manufactured	BYI 02960, technical PFV107N 004 purity 97.6 % w/w	EC A.17	Flupyradifurone (BYI 2960) has no oxidizing properties in the sense of EC guideline A.17.	Acceptable	Smeykal, H.; 2011;

section (Annex point)	study	purity	method	results	comment	reference
(IIA 2.17.1)	Storage stability (technical active substance)	BYI 02960, technical 2009-000239 purity 96.6% PFV087N 002 purity 98.7% PFV087N 007 purity 98.6%	OPPTS 830.6317	The results from 24 months stability study at ambient temperature (22.1°C) and 30°C showed Flupyradifurone to be stable in polypropylene and polyethylene containers. Even at elevated temperature no decomposition of the test substance and the storage vessels could be established.	Not applicable	Wagner, S.; 2011

Table B.2.1-2 Summary of the physical and chemical properties of the following metabolites BCS-CC98193 (BYI 02960-DFEAF), BCS-CR74729 (BYI 02960-succinamide), BCS-CU93236, BCS-AA56716 (Difluoroacetic acid), IC-0 (6-CNA) are outlined here.

Studies were conducted under GLP and completed to an acceptable standard and results were considered to be acceptable unless specified otherwise.

section (Annex point)	study	purity	method	results	comment	reference
IIA 7.13a	Solubility in water	BCS-CC98193 (BYI 02960-DFEAF) 98.5%	EC A.6 OECD 105 Flask method GLP: y	The solubility C_s at 20 ^oC was determined to be: pH 5: 35.5 g/L pH 7: 36.7 g/L pH 9: 36.4 g/L	Acceptable	Bogdoll B; Strunk B, 2011 [PA11/018]
		BCS-CR74729 (BYI 02960-succinamide) 97.8%	EC A.6 OECD 105 Flask method GLP: y	The solubility C_s at 20 ^oC was determined to be : pH 5 : 5.3 g/L pH 7 : >120 g/L (138.1 g/L) pH 9 : >120 g/L (122.9 g/L)	Buffers were not adequate: pH was adjusted by adding small amounts of aqueous sodium hydroxide and hydrochloric acid solutions to the solution of the test item. Deviation from EC A.6: two samples were taken at identical equilibration times. Considering the measured solubility, the followed procedure is adequate. Acceptable	Wiche A; Ziemer F, 2011 [PA11/078]

section (Annex point)	study	purity	method	results	comment	reference
		BCS-CU93236 97.3%	EC A.6 OECD 105 Flask method GLP: y	The solubility C_s at 20 °C was determined to be : pH 5 : > 175 g/L (179 g/L) pH 7 : > 180 g/L (181 g/L) pH 9 : > 180 g/L (181 g/L)	Deviation from EC A.6: two samples were taken at identical equilibration times. Considering the measured solubility, the followed procedure is adequate. Acceptable	Ziemer F; Strunk B, 2011 [PA11/094]
		BCS-AA56716 (Difluoroacetic acid) 96.7%	EC A.6 OECD 105 Flask method GLP: y	Difluoroacetic acid was miscible with distilled water in any ratio at 20 °C. The solubility at 20 °C was determined to be > 500 g/L in a pH range of 1.6-13.0.	Due to the extremely high water solubility at all pH values a visual evaluation was made. Acceptable	Bogdoll B; Strunk B, 2011 [PA10/042]
		IC-0 (6-CNA) 99.7%	EC A.6 OECD 105 Flask method GLP: y	The solubility at 20 °C was determined to be: Distilled water: 1.43 g/L (pH 2.1 after incubation) pH 4 buffer: 4.62 g/L (pH 3.2 after incubation) pH 10 buffer: 18.6 g/L (pH 4.4 after incubation)	Acceptable	Miya K, 2001 [NCAS 01-129]
IIA 7.13b	Partition co-efficient	BCS-CR74729 (BYI 02960-succinamide) 97.8%	EC A.8 OECD 107 Shake flask method GLP:Y	Partition coefficient at 23 °C pH 5: log P_{OW} = 0.6 pH 7: log P_{OW} = -1.3 pH 9: log P_{OW} = -2.5	Acceptable	Eyrich U; Ziemer F, 2011 [PA11/079]

section (Annex point)	study	purity	method	results	comment	reference
		BCS-CU93236 97.3%	EC A.8 OECD 107 Shake flask method GLP:Y	Partition coefficient at 23 °C pH 5: log P_{OW} = -1.3 pH 7: log P_{OW} = -2.7 pH 9: log P_{OW} = -3.5	Acceptable	Eyrich U; Ziemer F, 2011 [PA11/093]
		BCS-AA56716 (Difluoroacetic acid) 96.7%?	EC A.8 OECD 107 Shake flask method GLP:Y	Partition coefficient at 23 °C pH 5: log P_{OW} = -3.1 pH 7: log P_{OW} = -3.1 pH 9: log P_{OW} = -3.5	Acceptable	Eyrich U; Ziemer F, 2011 [PA10/043]
		IC-0 (6-CNA) 99.7%	EC A.8 OECD 107 Shake flask method GLP:Y	Log P_{OW} = 1.52 at 25 °C (pH 1.98 buffer solution used)	Acceptable	Higashida S, 2001 [NCAS 01-127]
IIA 7.13c	Dissociation constant (pKa)	BCS-CC98193 (BYI 02960-DFEAF) 98.5%	OECD 112 Spectrophotometric method GLP:Y	No dissociation constant pKa was found in aqueous solution in the pH-range of 2<pH<12. BCS-CC98193 shows no acidic or basic properties in the range of approximately 2<pHa<12.	Acceptable	Wiche A; Bogdoll B, 2011 [PA11/021]
		BCS-AA56716 (Difluoroacetic acid) 96.7%	OECD 112 Titration method GLP:Y	pKa = 1.57	Acceptable	Winkler S, 2011 [20100366.02]

section (Annex point)	study	purity	method	results	comment	reference
		IC-0 (6-CNA) 99.7%	OECD 112 Titration method GLP:Y	pKa = 3.28	Acceptable	Miya K, 2001 [NCAS 01-140]
IIA7.13d	Vapour pressure	BCS-CC98193 (BYI 02960-DFEAF) 98.5%	EC A.4 OECD 104 Vapour pressure balance GLP:Y	2.1×10^{-5} Pa (20 ^{o}C) 4.7×10^{-5} Pa (25 ^{o}C) 2.0×10^{-3} Pa (50 ^{o}C) Vapour pressure could be measured above 55 ^{o}C. Vapour pressures at lower temperatures have been extrapolated.	According to EC A.4, the vapour pressure balance method is recommended for the range of 10^{-3} - 1 Pa. For the vapour pressures determined at 20 ^{o}C and 25 ^{o}C, the gas saturation method would have been more appropriate. Acceptable	Dornhagen J, 2011 [20110091.01]
		BCS-AA56716 (Difluoroacetic acid) 99.6%	EC A.4 OECD 104 Dynamic method GLP:Y	Determined by extrapolation 2.8×10^{2} Pa (20 ^{o}C) 4.0×10^{2} Pa (25 ^{o}C) 2.0×10^{3} Pa (50 ^{o}C)	Acceptable	Smeykal H, 2011 [20100366.01]

section (Annex point)	study	purity	method	results	comment	reference
IIA7.13e	Henry's law constant	BCS-CC9819 3 (BYI 02960-DFEAF)	Calculation GLP: not applicable	Henry's law constant of BCS-CC9819 at 20°C: pH 5.0: 9.6 x 10^{-8} $Pa.m^3.mol^{-1}$ pH 7.0: 9.3 x 10^{-8} $Pa.m^3.mol^{-1}$ pH 9.0: 9.4 x 10^{-8} $Pa.m^3.mol^{-1}$ Based on a vapour pressure of 2.1 x 10^{-5} Pa at 20 ^oC. water solubility (20 ^oC) pH 5: 35.5 g/L pH 7: 36.7 g/L pH 9: 36.4 g/L molecular weight of 163.12 g/mol	Acceptable	Ziemer F; Eyrich U, 2011 [AF11/029]
		BCS-AA5671 6 (Difluoro acetic acid)	Calculation GLP: not applicable	Henry's law constant of difluoroacetic acid at 20 ^oC in a pH range of 1.6 – 13.0 was calculated to be <0.054 $Pa.m^3.mol^{-1}$ Based on a vapour pressure of 2.8 x 10^2 Pa at 20 ^oC water solubility >500 g/L at 20 ^oC molecular weight of 96.03 g/mol	Acceptable	Ziemer F; Eyrich U, 2011 [AF11/017]

Table B.2.2 Summary of the physical and chemical properties of the plant protection product
Product name: Flupyradifurone (BYI 2960)

(studies were conducted under GLP and completed to an acceptable standard and results were considered to be valid unless specified otherwise).

section (Annex point)	study	method	results	comment	reference
B.2.2.1 (IIIA 2.1.1)	Appearance: physical state	Visual assessment 17.1% w/w BYI 2960 GLP:N	soluble concentrate, liquid	Acceptable	Hennig-Gizewski, S. Hoppe, M. M-402943-02-1
B.2.2.2 (IIIA 2.1.1)	Appearance: colour	Visual assessment 17.1% w/w BYI 2960 GLP:N	Clear brown	Acceptable	Hennig-Gizewski, S. Hoppe, M. M-402943-02-1
B.2.2.3 (IIIA 2.1.2)	Appearance: odour	Organoleptic assessment 17.1% w/w BYI 2960 GLP:N	weak characteristic	Acceptable	M-402943-02-1 Hennig-Gizewski, S. Hoppe, M.
B.2.2.4 (IIIA 2.2.1)	Explosive properties	EC A.14 GLP:Y	The test item causes no danger of explosion	Acceptable	Rexer, K.; Schumacher, R. M-401926-01-1
B.2.2.5 (IIIA 2.2.2)	Oxidising properties	EC A.21 GLP:N	The test item is a non oxidising material	Acceptable	Rexer, K.; Schumacher, R. M-401926-01-1
B.2.2.6 (IIIA 2.3.2)	Flammability		Not applicable	Acceptable	Rexer, K.; Schumacher, R. M-401926-01-1

section (Annex point)	study	method	results	comment	reference
B.2.2.7 (IIIA 2.3.3)	Auto-flammability	EEC A.15	Auto-ignition temperature:420 °C	Acceptable	Rexer, K.; Schumacher, R. M-401926-01-1
B.2.2.8 (IIIA 2.3.1)	Flash point	EEC A.9 GLP:N	> 100°C	Acceptable	Rexer, K.; Schumacher, R. M-401926-01-1
B.2.2.9 (IIIA 2.4.1)	Acidity/alkalinity		Not required since the preparation is not acidic nor alkaline.	Acceptable pH of the preparation (1%) is 5.4	
B.2.2.10 (IIIA 2.4.2)	pH	CIPAC MT 75.3 GLP:Y	pH of the neat formulation is 5.4	Acceptable	Hennig-Gizewski, S. Hoppe, M. M-402943-02-1
B.2.2.11 (IIIA 2.5.3)	Surface tension	OECD 115 GLP:Y	34 mN/m (undiluted at 25 °C) 41 mN/m (1 g/L Milli-Q water at 20 °C	Acceptable	Hennig-Gizewski, S. Hoppe, M. M-402943-02-1

section (Annex point)	study	method	results	comment	reference
B.2.2.12 (IIIA 2.5.2)	Viscosity	CIPAC MT 192 GLP:Y	Kinematic viscosity at 20°C: **v**= 1.13E-04 m^2/s (shear rate of 20 s^1) **v**= 1.13E-04 m^2/s (shear rate of 100 s^1) at 40°C: **v**= 4.76E-05 m^2/s (shear rate of 20 s^1) **v**= 4.75E-05 m^2/s (shear rate of 100 s^1) Dynamic viscosity: ***r*l**= 0.1323 Pa*s (shear rate of 20 s^{-1}) ***r*l**= 0.1323 Pa *s (shear rate of 100 s^{-1}) at 40°C: η= 0.0550 Pa * s (shear rate of 20 s^{-1}) η= 0.0550 Pa* s (shear rate of 100 s^{-1})	Acceptable	Hennig-Gizewski, S. Hoppe, M. M-402943-02-1
B.2.2.13 (IIIA 2.6.1)	Relative density	OECD 109 GLP:Y	Mean value: D_4^{20}: 1.174	Acceptable	Hennig-Gizewski, S. Hoppe, M. M-402943-02-1
B.2.2.14 (IIIA 2.6.2)	Bulk (tap) density		Not applicable		
B.2.2.14 (IIIA 2.7.1)	Storage stability	CIPAC MT 46.3	Stable for 2 weeks at 54 °C in HDPE. The active substance determined after 2 weeks storage at 54°C was decreased by 1.18%, which is considered acceptable. See summary table below **Table B 2.2-1 Summary Storage stability and shelf life** for details regarding the phys chem. properties storage.	Acceptable	Hennig-Gizewski, S. Hoppe, M. M-402996-02-1

section (Annex point)	study	method	results	comment	reference
B.2.2.14 (IIIA 2.7.4)	Storage stability, low temperature	CIPAC MT 39.3	No separated (precipitated) material found		Hennig-Gizewski, S. Hoppe, M. M-402996-02-1
B.2.2.15 (IIIA 2.7.5)	Shelf life	According to GIFAP Monograph No. 17, 6.1	Stable for 2 years at 20 °C in HDPE. Properties determined before and after storage: A.i. content, appearance, packaging stability, pH, relative density, persistent foaming, dilution stability. See summary table below **Table B 2.2-1 Summary Storage stability and shelf life** for details regarding the phys chem. properties after shelf life.	Acceptable	Hennig-Gizewski, S. Hoppe, M., 2012 M-402996-02-1
B.2.2.16 (IIIA 2.8.1)	Wettability		Not applicable		
B.2.2.17 (IIIA 2.8.2)	Persistent foaming	CIPAC MT 47.2 GLP:N	0.05% of the preparation in CIPAC D water: 19 mL after 10 sec 0 mL after 1 min 0 mL after 3 min 0 mL after 12 min 0.75% of the preparation in CIPAC D water: 29 mL after 10 sec 0 mL after 1 min 0 mL after 3 min 0 mL after 12 min	Acceptable	Hennig-Gizewski, S. Hoppe, M. M-402943-02-1
B.2.2.18 (IIIA 2.8.3.1)	Suspensibility		Not applicable		

section (Annex point)	study	method	results	comment	reference
B.2.2.19 (IIIA 2.8.3.2)	Suspension stability Spontaneity		Not applicable		
B.2.2.20 (IIIA 2.8.4)	Dilution stability		0.05% of the preparation in CIPAC D water: completely solved 0.75% of the preparation in CIPAC D water: completely solved		Hennig-Gizewski, S. Hoppe, M. M-402943-02-1
B.2.2.21 (IIIA 2.8.5.1)	Dry sieve test		Not applicable		
B.2.2.22 (IIIA 2.8.5.2)	Wet sieve test		Not applicable		
B.2.2.23 (IIIA 2.8.6.1)	Particle size distribution		Not applicable		
B.2.2.24 (IIIA 2.8.6.3)	Content of dust/fines		Not applicable		
B.2.2.25 (IIIA 2.8.6.5)	Attrition and friability		Not applicable		
B.2.2.26 (IIIA 2.8.7.1 - 3)	Emulsifiability, re-emulsifiability and emulsion stability		Not applicable		
B.2.2.27 (IIIA 2.8.7.4)	Stability of dilute emulsion		Not applicable		
B.2.2.28 (IIIA 2.8.8.1)	Flowability		Not applicable		

section (Annex point)	study	method	results	comment	reference
B.2.2.29 (IIIA 2.8.8.2)	Pourability (rinsibility)		Not applicable		
B.2.2.30 (IIIA 2.8.8.3)	Dustability		Not applicable		
B.2.2.31 (IIIA 2.10)	Adherence and distribution to seeds		Not applicable		
(IIIA 2.9.1)	Physical compatibility with other products		Not applicable		
(IIIA 2.9.2)	Chemical compatibility with other products		Not applicable		
(IIIA 2.13)	Corrosion characteristics		Not applicable		

Table B 2.2-1 Summary Storage stability and shelf life

	Initial	2 weeks 54 °C	2 years at ambient temp.
A.S. Content			
flupyradifurone	16.9 % 198 g/L	16.7 % 196 g/L	16.9 % 198 g/L
Appearance colour, condition (visual)	soluble concentrate clear brown liquid	soluble concentrate clear brown liquid	soluble concentrate clear brown liquid
odour (olfactory)	weak characteristic	weak characteristic	weak characteristic
Acidity/Alkalinity *, method: CIPAC MT 31	not determined	not determined	not determined
pH-Value, method: CIPAC MT 75.3 (1% in de-ionized water)	5.4	6.0	6.4
Relative density, method: OECD 109 D_4^{20}	1.174	1.174	1.168
Persistent foaming method: CIPAC MT 47.2, **0.05 %** preparation in CIPAC D water			
after 10 s	19 mL	17 mL	23 mL
after 1 min	0 mL	0 mL	2 mL
after 3 min	0 mL	0 mL	0 mL
after 12 min	0 mL	0 mL	0 mL
Persistent foaming method: CIPAC MT 47.2, **0.75 %** preparation in CIPAC D water			
after 10 s	29 mL	30 mL	33 mL
after 1 min	0 mL	2 mL	0 mL
after 3 min	0 mL	0 mL	0 mL
after 12 min	0 mL	0 mL	2 mL
Dilution stability method: CIPAC MT 41 **0.05 %** prep. In CIPAC D water			
after 18 h	completely solved	completely solved	completely solved
Dilution stability method: CIPAC MT 41 **0.75 %** prep. in CIPAC D water			
initial dispersion	completely solved	completely solved	completely solved

B.2.3 Summary of physical and chemical properties

B.2.3.1 Active substance

Physical and chemical properties of the active substance

Flupyradifurone is an insecticide. Its vapour pressure and volatility are very low. The solubility in water is about 3.2 g/L at a range of pH between 4 and 9. With a log Pow of 1.2 it has no bioaccumulative potential in lipophilic matrices. Flupyradifurone is hydrolytically stable under acidic, neutral and under alkaline conditions at ambient temperature. The hydrolytic degradation is negligible under acidic, neutral and alkaline conditions. Flupyradifurone is rather susceptible for reactions with hydroxyl radicals with photochemical degradation process to contribute significantly to the overall degradation of the substance in case of its occurrence in the atmosphere. Its flammability, explosive and oxidizing properties are not critical

B.2.3.2 Plant protection product

Flupryadifurone (BYI 2960)SL 200 is a brown liquid with a weak characteristic odour.. The product has a flash point > 100°C, an auto-ignition temperature of 420°C and has neither explosive nor oxidizing properties. The pH of a 1% dilution is 5.4. When diluted, the recommended end-use concentration is not surface active. An accelerated storage test of 14 days at 54°C, has been performed. A shelf life study after 2 years at ambient temperature in HDPE has indicated the physico-chemical stability of the product.

B.2.4 References relied on

References for the active substance

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner *

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner *
KIIA 2.1.1 /01	Smeykal, H.	2010	BYI 02960, pure substance: Melting point, boiling point, thermal stability Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20090051.01, Edition Number: M-367370-01-1 Date: 2010-03-25 GLP/GEP: Y, unpublished	Y	BCS
KIIA 2.1.1 /02	Smeykal, H.	2011	Flupyradifurone (BYI 02960), technical substance: Melting point, boiling point, thermal stability Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20110197.01, Edition Number: M-414242-01-1 Date: 2011-09-16 GLP/GEP: Y, unpublished	Y	BCS
KIIA 2.1.2 /01	Smeykal, H.	2010	BYI 02960, pure substance: Melting point, boiling point, thermal stability Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20090051.01, Edition Number: M-367370-01-1 Date: 2010-03-25 GLP/GEP: Y, unpublished	Y	BCS
KIIA 2.1.2 /02	Smeykal, H.	2011	Flupyradifurone (BYI 02960), technical substance: Melting point, boiling point, thermal stability Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20110197.01, Edition Number: M-414242-01-1 Date: 2011-09-16 GLP/GEP: Y, unpublished	Y	BCS
KIIA 2.1.3 /01	Smeykal, H.	2010	BYI 02960, pure substance: Melting point, boiling point, thermal stability Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20090051.01, Edition Number: M-367370-01-1 Date: 2010-03-25 GLP/GEP: Y, unpublished	Y	BCS
KIIA 2.1.3 /02	Smeykal, H.	2011	Flupyradifurone (BYI 02960), technical substance: Melting point, boiling point, thermal stability Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20110197.01, Edition Number: M-414242-01-1 Date: 2011-09-16 GLP/GEP: Y, unpublished	Y	BCS

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner *
KIIA 2.2 /01	Bogdoll, B.; Strunk, B.	2011	Flupyradifurone (BYI 02960), pure substance: Relative density Bayer CropScience, Report No.: PA09/006, Edition Number: M-412635-01-1 Date: 2011-08-18 GLP/GEP: Y, unpublished	Y	BCS
KIIA 2.2 /02	Eyrich, U.; Bogdoll, B.	2011	Flupyradifurone (BYI 02960), technical substance : Relative density Bayer CropScience, Report No.: PA11/063, Edition Number: M-414075-01-1 Date: 2011-09-16 GLP/GEP: Y, unpublished	Y	BCS
KIIA 2.3.1 /01	Smeykal, H.	2008	BYI 02960, pure substance: Vapour pressure - Final report Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20080615.01, Edition Number: M-309853-01-1 Date: 2008-10-10 GLP/GEP: Y, unpublished	Y	BCS
KIIA 2.3.2 /01	Bogdoll, B.; Eyrich, U.	2011	Flupyradifurone (BYI 02960): Calculation of the Henry´s law constant Bayer CropScience, Report No.: AF09/042, Edition Number: M-414341-01-1 Date: 2011-08-15 GLP/GEP: no, unpublished	Y	BCS
KIIA 2.4.1 /01	Bogdoll, B.; Strunk, B.	2011	Flupyradifurone (BYI 02960), pure substance - Physical characteristics colour, physical state and odour Bayer CropScience, Report No.: PA09/008, Edition Number: M-412655-01-1 Date: 2011-08-18 GLP/GEP: Y, unpublished	Y	BCS
KIIA 2.4.1 /02	Eyrich, U.; Bogdoll, B.	2011	Flupyradifurone (BYI 02960), technical substance: Physical characteristics colour, physical state and odour Bayer CropScience, Report No.: PA11/062, Edition Number: M-414072-01-1 Date: 2011-09-16 GLP/GEP: Y, unpublished	Y	BCS

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner *
KIIA 2.4.2 /01	Bogdoll, B.; Strunk, B.	2011	Flupyradifurone (BYI 02960), pure substance - Physical characteristics colour, physical state and odour Bayer CropScience, Report No.: PA09/008, Edition Number: M-412655-01-1 Date: 2011-08-18 GLP/GEP: Y, unpublished	Y	BCS
KIIA 2.4.2 /02	Eyrich, U.; Bogdoll, B.	2011	Flupyradifurone (BYI 02960), technical substance: Physical characteristics colour, physical state and odour Bayer CropScience, Report No.: PA11/062, Edition Number: M-414072-01-1 Date: 2011-09-16 GLP/GEP: Y, unpublished	Y	BCS
KIIA 2.5.1.1 /01	Peters; S.	2009	Spectral data set of BYI 02960 a.i. - Reference material Bayer CropScience, Report No.: 15-600-2439, Edition Number: M-345761-01-1 Date: 2009-04-07 GLP/GEP: Y, unpublished	Y	BCS
KIIA 2.5.1.2 /01	Peters; S.	2009	Spectral data set of BYI 02960 a.i. - Reference material Bayer CropScience, Report No.: 15-600-2439, Edition Number: M-345761-01-1 Date: 2009-04-07 GLP/GEP: Y, unpublished	Y	BCS
KIIA 2.5.1.3 /01	Peters; S.	2009	Spectral data set of BYI 02960 a.i. - Reference material Bayer CropScience, Report No.: 15-600-2439, Edition Number: M-345761-01-1 Date: 2009-04-07 GLP/GEP: Y, unpublished	Y	BCS
KIIA 2.5.1.4 /01	Peters; S.	2009	Spectral data set of BYI 02960 a.i. - Reference material Bayer CropScience, Report No.: 15-600-2439, Edition Number: M-345761-01-1 Date: 2009-04-07 GLP/GEP: Y, unpublished	Y	BCS
KIIA 2.5.1.5 /01	Peters; S.	2009	Spectral data set of BYI 02960 a.i. - Reference material Bayer CropScience, Report No.: 15-600-2439, Edition Number: M-345761-01-1 Date: 2009-04-07 GLP/GEP: Y, unpublished	Y	BCS

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner *
KIIA 2.6 /01	Wiche, A.; Bogdoll, B.	2011	BYI 02960, pure substance: Solubility in distilled water (pH 7), at pH 4 and pH 9 (flask method) Bayer CropScience, Report No.: PA09/003, Edition Number: M-409513-01-1 Date: 2011-06-17 GLP/GEP: Y, unpublished	Y	BCS
KIIA 2.7 /01	Eyrich, U.; Bogdoll, B.	2011	Flupyradifurone (BYI 02960): Solubility in organic solvents Bayer CropScience, Report No.: PA09/005, Edition Number: M-414064-01-1 Date: 2011-09-16 GLP/GEP: Y, unpublished	Y	BCS
KIIA 2.8.1 /01	Bogdoll, B.; Strunk, B.	2011	Flupyradifurone (BYI 02960), pure substance: Partition coefficient 1-octanol / water at pH 4, pH 7 and pH 9 (HPLC-method) Bayer CropScience, Report No.: PA09/004, Edition Number: M-414485-01-1 Date: 2011-09-26 GLP/GEP: Y, unpublished	Y	BCS
KIIA 2.8.2 /01	Bogdoll, B.; Strunk, B.	2011	Flupyradifurone (BYI 02960), pure substance: Partition coefficient 1-octanol / water at pH 4, pH 7 and pH 9 (HPLC-method) Bayer CropScience, Report No.: PA09/004, Edition Number: M-414485-01-1 Date: 2011-09-26 GLP/GEP: Y, unpublished	Y	BCS
KIIA 2.9.1 /01	Mislankar, S.; Woodard, D.	2011	BYI-02960: Hydrolytic degradation Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: MERVP019, Edition Number: M-398952-01-1 Date: 2011-01-07 GLP/GEP: Y, unpublished	Y	BCS
KIIA 2.9.2 /01	Hall, L. R.	2011	Phototransformation of [14C]BYI 02960 in aqueous pH 7 buffer - amended report Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: MERVP042-1, Edition Number: M-418426-02-1 Date: 2011-11-28 **...Amended: 2012-03-05** GLP/GEP: Y, unpublished	Y	BCS

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner *
KIIA 2.9.3 /01	Heinemann, O.	2011	BYI 02960: Determination of the quantum yield and assessment of the environmental half-life of the direct photo-degradation in water Bayer CropScience, Report No.: MEF-11/554, Edition Number: M-414756-01-2 Date: 2011-09-26 GLP/GEP: Y, unpublished	Y	BCS
KIIA 2.9.4 /01	Hall, L. R.	2011	Phototransformation of [14C]BYI 02960 in aqueous pH 7 buffer - amended report Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: MERVP042-1, Edition Number: M-418426-02-1 Date: 2011-11-28 **...Amended: 2012-03-05** GLP/GEP: Y, unpublished	Y	BCS
KIIA 2.9.4 /02	Heinemann, O.	2011	BYI 02960: Determination of the quantum yield and assessment of the environmental half-life of the direct photo-degradation in water Bayer CropScience, Report No.: MEF-11/554, Edition Number: M-414756-01-2 Date: 2011-09-26 GLP/GEP: Y, unpublished	Y	BCS
KIIA 2.9.5 /01	Wiche, A.; Bogdoll, B.	2011	Flupyradifurone (BYI 02960), pure substance : Dissociation constant in water Bayer CropScience, Report No.: PA10/048, Edition Number: M-414102-01-1 Date: 2011-09-16 GLP/GEP: Y, unpublished	Y	BCS
KIIA 2.10 /01	Hellpointner; E.	2010	BYI 02960: Calculation of the chemical half-life in the troposphere Bayer CropScience, Report No.: MEF-10/896, Edition Number: M-398741-01-2 Date: 2010-12-27 GLP/GEP: no, unpublished	Y	BCS
KIIA 2.11.1 /01	Smeykal, H.	2011	Flupyradifurone (BYI 02960), technical substance: flammability (solids) Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20110197.03, Edition Number: M-414249-01-1 Date: 2011-09-16 GLP/GEP: Y, unpublished	Y	BCS

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner *
KIIA 2.11.2 /01	Smeykal, H.	2011	Flupyradifurone (BYI 02960), technical substance: auto-flammability Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20110197.05, Edition Number: M-414252-01-1 Date: 2011-09-16 GLP/GEP: Y, unpublished	Y	BCS
KIIA 2.11.2 /02	Smeykal, H.	2011	Flupyradifurone (BYI 02960), technical substance: autoflammability (UN Bowes Cameron Cage Test) Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20110197.07, Edition Number: M-414257-01-1 Date: 2011-09-16 GLP/GEP: Y, unpublished	Y	BCS
KIIA 2.13 /01	Smeykal, H.	2011	Flupyradifurone (BYI 02960), technical substance: explosive properties Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20110197.04, Edition Number: M-414250-01-1 Date: 2011-09-16 GLP/GEP: Y, unpublished	Y	BCS
KIIA 2.14 /01	Eyrich, U.; Bogdoll, B.	2011	Flupyradifurone (BYI 02960), technical substance : Determination of the surface tension Bayer CropScience, Report No.: PA11/065, Edition Number: M-414086-01-1 Date: 2011-09-16 GLP/GEP: Y, unpublished	Y	BCS
KIIA 2.15 /01	Smeykal, H.	2011	Flupyradifurone (BYI 02960), technical substance: oxidizing properties Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20110197.06, Edition Number: M-414253-01-1 Date: 2011-09-16 GLP/GEP: Y, unpublished	Y	BCS
KIIA 2.16 /01	Eyrich, U.; Bogdoll, B.	2011	Flupyradifurone (BYI 02960), pure substance: Determination of the pH-value in distilled water Bayer CropScience, Report No.: PA09/007, Edition Number: M-412128-01-1 Date: 2011-08-09 GLP/GEP: Y, unpublished	Y	BCS

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner *
KIIA 2.16 /02	Eyrich, U.; Bogdoll, B.	2011	Flupyradifurone (BYI 02960), technical substance : Determination of the pH-value in distilled water Bayer CropScience, Report No.: PA11/064, Edition Number: M-414084-01-1 Date: 2011-09-16 GLP/GEP: Y, unpublished	Y	BCS
KIIA 2.17.1 /01	Wagner, S.	2011	Chemical storage stability of BYI 02960 - Flupyradifurone (BYI 02960) Bayer CropScience, Report No.: 15-155-2438, Edition Number: M-411305-01-1 Date: 2011-07-19 GLP/GEP: Y, unpublished	Y	BCS
KIIA 2.17.2 /01	Wagner, S.	2011	Stability to normal and elevated temperature, metals, and metal ions and corrosion characteristics to plastic containers of flupyradifurone (BYI 02960) according to OPPTS 830.6313 and 830.6320 Bayer CropScience, Report No.: 15-160-2527, Edition Number: M-413798-01-1 Date: 2011-09-12 GLP/GEP: Y, unpublished	Y	BCS
KIIA 2.18 /01	Eyrich, U.; Bogdoll, B.	2011	Flupyradifurone (BYI 02960), technical substance : The oxidation or reduction properties Bayer CropScience, Report No.: PA11/066, Edition Number: M-414080-01-1 Date: 2011-09-16 GLP/GEP: Y, unpublished	Y	BCS
KIIA 2.18 /02	Petrovic, P.	2011	Flupyradifurone (BYI 02960), technical substance: Complex formation ability in water Allessa Chemie GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: B 015/2011, Edition Number: M-414563-01-1 Date: 2011-09-21 GLP/GEP: Y, unpublished	Y	BCS
KIIA 2.18 /03	Smeykal, H.	2011	Flupyradifurone (BYI 02960), technical substance: Particle size distribution Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20110197.02, Edition Number: M-414246-01-1 Date: 2011-09-16 GLP/GEP: Y, unpublished	Y	BCS

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner *
KIIA 2.18 /04	Ziemer, F.	2011	Flupyradifurone (BYI 02960), technical substance: Statement on the viscosity according to OPPTS 830.7100 Bayer CropScience, Report No.: AF11/027, Edition Number: M-417799-01-1 Date: 2011-11-17 GLP/GEP: no, unpublished	Y	BCS
KIIA 2.18 /05	Ziemer, F.	2011	Flupyradifurone (BYI 02960), technical substance: Statement on the miscibility according to OPPTS 830.6319 Bayer CropScience, Report No.: AF11/026, Edition Number: M-417793-01-1 Date: 2011-11-17 GLP/GEP: no, unpublished	Y	BCS
KIIA 2.18 /06	Ziemer, F.	2011	Flupyradifurone (BYI 02960), technical substance: Statement on the dielectric breakdown voltage according to OPPTS 830.6321 Bayer CropScience, Report No.: AF11/028, Edition Number: M-417802-01-1 Date: 2011-11-17 GLP/GEP: no, unpublished	Y	BCS
KIIA 2.18 /07	Schloesser, G.; Mans, H. C.	2011	BYI 02960 - Determination of metallic impurities Currenta GmbH & Co. OHG, Leverkusen, Germany Bayer CropScience, Report No.: 2011/0073/01, Edition Number: M-412025-01-1 Date: 2011-08-10 GLP/GEP: Y, unpublished confidential	Y	BCS

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Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner *

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner *
KIIIA1 2.1 /01	Hennig-Gizewski, S.; Hoppe, M.	2011	Physical, chemical and technical properties of BYI 02960 SL 200 (200 g/L) - BYI 02960 SL 200 G - amendment no.: 1 Bayer CropScience, Report No.: FM0076(PC00)G01, Edition Number: M-402943-02-1 Date: 2011-02-14 **...Amended: 2011-09-19** GLP/GEP: Y, unpublished ...also filed: KIIIA1 2.4.2 /01 ...also filed: KIIIA1 2.5.1 /01 ...also filed: KIIIA1 2.5.2 /01 ...also filed: KIIIA1 2.5.3 /01 ...also filed: KIIIA1 2.6.1 /01 ...also filed: KIIIA1 2.8.2 /01 ...also filed: KIIIA1 2.8.4 /01	Y	BCS
KIIIA1 2.2.1 /01	Rexer, K.; Schumacher, R.	2011	Safety relevant technical properties of BYI 02960 SL 200 (200 g/L) - Final report - Bayer CropScience, Report No.: FF0091(SA00)G01, Edition Number: M-401926-01-1 Date: 2011-02-14 GLP/GEP: Y, unpublished ...also filed: KIIIA1 2.2.2 /01 ...also filed: KIIIA1 2.3.1 /01 ...also filed: KIIIA1 2.3.3 /01	Y	BCS
KIIIA1 2.2.2 /01	Rexer, K.; Schumacher, R.	2011	Safety relevant technical properties of BYI 02960 SL 200 (200 g/L) - Final report - Bayer CropScience, Report No.: FF0091(SA00)G01, Edition Number: M-401926-01-1 Date: 2011-02-14 GLP/GEP: Y, unpublished ...also filed: KIIIA1 2.2.1 /01 ...also filed: KIIIA1 2.3.1 /01 ...also filed: KIIIA1 2.3.3 /01	Y	BCS
KIIIA1 2.3.1 /01	Rexer, K.; Schumacher, R.	2011	Safety relevant technical properties of BYI 02960 SL 200 (200 g/L) - Final report - Bayer CropScience, Report No.: FF0091(SA00)G01, Edition Number: M-401926-01-1 Date: 2011-02-14 GLP/GEP: Y, unpublished ...also filed: KIIIA1 2.2.1 /01 ...also filed: KIIIA1 2.2.2 /01 ...also filed: KIIIA1 2.3.3 /01	Y	BCS

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner *
KIIIA1 2.3.3 /01	Rexer, K.; Schumacher, R.	2011	Safety relevant technical properties of BYI 02960 SL 200 (200 g/L) - Final report - Bayer CropScience, Report No.: FF0091(SA00)G01, Edition Number: M-401926-01-1 Date: 2011-02-14 GLP/GEP: Y, unpublished ...also filed: KIIIA1 2.2.1 /01 ...also filed: KIIIA1 2.2.2 /01 ...also filed: KIIIA1 2.3.1 /01	Y	BCS
KIIIA1 2.4.2 /01	Hennig-Gizewski, S.; Hoppe, M.	2011	Physical, chemical and technical properties of BYI 02960 SL 200 (200 g/L) - BYI 02960 SL 200 G - amendment no.: 1 Bayer CropScience, Report No.: FM0076(PC00)G01, Edition Number: M-402943-02-1 Date: 2011-02-14 **...Amended: 2011-09-19** GLP/GEP: Y, unpublished ...also filed: KIIIA1 2.1 /01 ...also filed: KIIIA1 2.5.1 /01 ...also filed: KIIIA1 2.5.2 /01 ...also filed: KIIIA1 2.5.3 /01 ...also filed: KIIIA1 2.6.1 /01 ...also filed: KIIIA1 2.8.2 /01 ...also filed: KIIIA1 2.8.4 /01	Y	BCS
KIIIA1 2.5.1 /01	Hennig-Gizewski, S.; Hoppe, M.	2011	Physical, chemical and technical properties of BYI 02960 SL 200 (200 g/L) - BYI 02960 SL 200 G - amendment no.: 1 Bayer CropScience, Report No.: FM0076(PC00)G01, Edition Number: M-402943-02-1 Date: 2011-02-14 **...Amended: 2011-09-19** GLP/GEP: Y, unpublished ...also filed: KIIIA1 2.1 /01 ...also filed: KIIIA1 2.4.2 /01 ...also filed: KIIIA1 2.5.2 /01 ...also filed: KIIIA1 2.5.3 /01 ...also filed: KIIIA1 2.6.1 /01 ...also filed: KIIIA1 2.8.2 /01 ...also filed: KIIIA1 2.8.4 /01	Y	BCS

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner *
KIIIA1 2.5.2 /01	Hennig-Gizewski, S.; Hoppe, M.	2011	Physical, chemical and technical properties of BYI 02960 SL 200 (200 g/L) - BYI 02960 SL 200 G - amendment no.: 1 Bayer CropScience, Report No.: FM0076(PC00)G01, Edition Number: M-402943-02-1 Date: 2011-02-14 **...Amended: 2011-09-19** GLP/GEP: Y, unpublished ...also filed: KIIIA1 2.1 /01 ...also filed: KIIIA1 2.4.2 /01 ...also filed: KIIIA1 2.5.1 /01 ...also filed: KIIIA1 2.5.3 /01 ...also filed: KIIIA1 2.6.1 /01 ...also filed: KIIIA1 2.8.2 /01 ...also filed: KIIIA1 2.8.4 /01	Y	BCS
KIIIA1 2.5.3 /01	Hennig-Gizewski, S.; Hoppe, M.	2011	Physical, chemical and technical properties of BYI 02960 SL 200 (200 g/L) - BYI 02960 SL 200 G - amendment no.: 1 Bayer CropScience, Report No.: FM0076(PC00)G01, Edition Number: M-402943-02-1 Date: 2011-02-14 **...Amended: 2011-09-19** GLP/GEP: Y, unpublished ...also filed: KIIIA1 2.1 /01 ...also filed: KIIIA1 2.4.2 /01 ...also filed: KIIIA1 2.5.1 /01 ...also filed: KIIIA1 2.5.2 /01 ...also filed: KIIIA1 2.6.1 /01 ...also filed: KIIIA1 2.8.2 /01 ...also filed: KIIIA1 2.8.4 /01	Y	BCS
KIIIA1 2.6.1 /01	Hennig-Gizewski, S.; Hoppe, M.	2011	Physical, chemical and technical properties of BYI 02960 SL 200 (200 g/L) - BYI 02960 SL 200 G - amendment no.: 1 Bayer CropScience, Report No.: FM0076(PC00)G01, Edition Number: M-402943-02-1 Date: 2011-02-14 **...Amended: 2011-09-19** GLP/GEP: Y, unpublished ...also filed: KIIIA1 2.1 /01 ...also filed: KIIIA1 2.4.2 /01 ...also filed: KIIIA1 2.5.1 /01 ...also filed: KIIIA1 2.5.2 /01 ...also filed: KIIIA1 2.5.3 /01 ...also filed: KIIIA1 2.8.2 /01 ...also filed: KIIIA1 2.8.4 /01	Y	BCS

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner *
KIIIA1 2.7.1 /01	Hennig-Gizewski, S.; Hoppe, M.	2011	Storage stability and shelf life of BYI 02960 SL 200 (200 g/L) - [Packaging material:HDPE] - Final report (2 years) Bayer CropScience, Report No.: FM0076(SL01)N01, Edition Number: M-402996-02-1 Date: 2011-02-14 **...Amended: 2012-07-25** GLP/GEP: no, unpublished ...also filed: KIIIA1 2.7.3 /01 ...also filed: KIIIA1 2.7.4 /01 ...also filed: KIIIA1 2.7.5 /01 ...also filed: KIIIA1 4.1.3 /01	Y	BCS
KIIIA1 2.7.2 /01	Hennig-Gizewski, S.; Hoppe, M.	2012	Storage stability data of BYI 02960 SL 200 (200 g/L) - [Packaging material:HDPE] Bayer CropScience, Report No.: FM0076(EP01)G01, Edition Number: M-432583-01-1 EPA MRID No.: 48844507 Date: 2012-06-18 GLP/GEP: no, unpublished	Y	BCS
KIIIA1 2.7.3 /01	Hennig-Gizewski, S.; Hoppe, M.	2011	Storage stability and shelf life of BYI 02960 SL 200 (200 g/L) - [Packaging material:HDPE] - Final report (2 years) Bayer CropScience, Report No.: FM0076(SL01)N01, Edition Number: M-402996-02-1 Date: 2011-02-14 **...Amended: 2012-07-25** GLP/GEP: no, unpublished ...also filed: KIIIA1 2.7.1 /01 ...also filed: KIIIA1 2.7.4 /01 ...also filed: KIIIA1 2.7.5 /01 ...also filed: KIIIA1 4.1.3 /01	Y	BCS
KIIIA1 2.7.4 /01	Hennig-Gizewski, S.; Hoppe, M.	2011	Storage stability and shelf life of BYI 02960 SL 200 (200 g/L) - [Packaging material:HDPE] - Final report (2 years) Bayer CropScience, Report No.: FM0076(SL01)N01, Edition Number: M-402996-02-1 Date: 2011-02-14 **...Amended: 2012-07-25** GLP/GEP: no, unpublished ...also filed: KIIIA1 2.7.1 /01 ...also filed: KIIIA1 2.7.3 /01 ...also filed: KIIIA1 2.7.5 /01 ...also filed: KIIIA1 4.1.3 /01	Y	BCS

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner *
KIIIA1 2.7.5 /01	Hennig-Gizewski, S.; Hoppe, M.	2011	Storage stability and shelf life of BYI 02960 SL 200 (200 g/L) - [Packaging material:HDPE] - Final report (2 years) Bayer CropScience, Report No.: FM0076(SL01)N01, Edition Number: M-402996-02-1 Date: 2011-02-14 **...Amended: 2012-07-25** GLP/GEP: no, unpublished ...also filed: KIIIA1 2.7.1 /01 ...also filed: KIIIA1 2.7.3 /01 ...also filed: KIIIA1 2.7.4 /01 ...also filed: KIIIA1 4.1.3 /01	Y	BCS
KIIIA1 2.8.2 /01	Hennig-Gizewski, S.; Hoppe, M.	2011	Physical, chemical and technical properties of BYI 02960 SL 200 (200 g/L) - BYI 02960 SL 200 G - amendment no.: 1 Bayer CropScience, Report No.: FM0076(PC00)G01, Edition Number: M-402943-02-1 Date: 2011-02-14 **...Amended: 2011-09-19** GLP/GEP: Y, unpublished ...also filed: KIIIA1 2.1 /01 ...also filed: KIIIA1 2.4.2 /01 ...also filed: KIIIA1 2.5.1 /01 ...also filed: KIIIA1 2.5.2 /01 ...also filed: KIIIA1 2.5.3 /01 ...also filed: KIIIA1 2.6.1 /01 ...also filed: KIIIA1 2.8.4 /01	Y	BCS
KIIIA1 2.8.4 /01	Hennig-Gizewski, S.; Hoppe, M.	2011	Physical, chemical and technical properties of BYI 02960 SL 200 (200 g/L) - BYI 02960 SL 200 G - amendment no.: 1 Bayer CropScience, Report No.: FM0076(PC00)G01, Edition Number: M-402943-02-1 Date: 2011-02-14 **...Amended: 2011-09-19** GLP/GEP: Y, unpublished ...also filed: KIIIA1 2.1 /01 ...also filed: KIIIA1 2.4.2 /01 ...also filed: KIIIA1 2.5.1 /01 ...also filed: KIIIA1 2.5.2 /01 ...also filed: KIIIA1 2.5.3 /01 ...also filed: KIIIA1 2.6.1 /01 ...also filed: KIIIA1 2.8.2 /01	Y	BCS

B.3 Data on application and further information

B.3.1 Data on application relevant to the active substance (Annex IIA 3.1 to 3.6)

B.3.1.1 Function (Annex IIA 3.1)

The active substance flupyradifurone (BYI 02960) will be used as an insecticide.

B.3.1.2 Effects on harmful organisms (Annex IIA 3.2.1)

Flupyradifurone interacts with insect nicotinic acetylcholine receptors, a class of neurotransmitter-gated cation channels which are involved in excitatory neurotransmission, a target also known for neonicotinoid insecticides. Blockage of these receptors leads to paralysis and death of the target insects.

B.3.1.3 Translocation in plants (Annex IIA 3.2.2)

Flupyradifurone is a systemic insecticide. After uptake into the plant, Flupyradifurone is translocated acropetally in the xylem, in direction of the transpiration stream and is translaminarily distributed into adjacent plant cells.

B.3.1.4 Fields of use (Annex IIA 3.3)

Intended for agricultural use. Control of aphids in lettuce and hop were chosen as representative uses for the representative formulation, the product Sivanto (flupyradifurone 200SL)

B.3.1.5 Pests controlled and crops protected (Annex IIA 3.4.1, 3.4.2)

The active substance Flupyradifurone according to the applicant, exhibits high activity especially against sucking insects like aphids and whiteflies. Control of aphids in lettuce and hop were chosen as representative uses for the representative formulation, the product Sivanto (flupyradifurone 200SL)

Crops	**Harmful organisms***
Lettuce	Lettuce aphid *Nasonovia ribisnigri*
Hop	Hop Aphid *Phorodon humili*

B.3.1.6 Mode of action (Annex IIA 3.5.1)

Flupyradifurone interacts with insect nicotinic acetylcholine receptors, a class of neurotransmitter-gated cation channels which are involved in excitatory neurotransmission, a target also known for

neonicotinoid insecticides. Blockage of these receptors leads to paralysis and death of the target insects.

After uptake into the plant, Flupyradifurone is translocated acropetally in the xylem, in direction of the transpiration stream and is translaminarily distributed into adjacent plant cells.

B.3.1.8 Information on the possible occurrence of the development of resistance or cross-resistance (Annex IIA 3.6)

Studies on the possible occurrence of the development of resistance or cross resistance are evaluated in Volume 3, Annex B, section B10 efficacy in chapter 10.2

B.3.2 Data on application relevant to the plant protection product (Annex IIIA 3)

Sivanto SL 200 g/L, or flupyradifuone SL200, is a soluble concentrate (SL) containing 200 grams per litre (g/L) of Flupyradifurone for use on lettuce and hop .

B.3.2.1 Field of use (Annex IIIA 3.1)

Intended for agricultural use. Control of aphids in lettuce and hop were chosen as representative uses for this evaluation.

B.3.2.2 Nature of the effects on harmful organisms (Annex IIIA 3.2)

Flupyradifurone interacts with insect nicotinic acetylcholine receptors, a class of neurotransmitter-gated cation channels which are involved in excitatory neurotransmission, a target also known for neonicotinoid insecticides. Blockage of these receptors leads to paralysis and death of the target insects.

B.3.2.3 Pests controlled and crops protected (Annex IIIA 3.3)

Crops	**Harmful organisms***
Lettuce	Lettuce aphid *Nasonovia ribisnigri*
Hop	Hop Aphid *Phorodon humili*

Rate of application (Annex IIIA 3.4)

See the GAP (Table 3.3.1 Summary of intended uses in chapter B.3.3 in this document)

Concentration of active substance in material used (Annex IIIA 3.5)

The representative formulation for flupyradifurone is Sivanto SL 200 g/L, this is a soluble concentrate (SL) containing 200 grams per litre (g/L) of Flupyradifurone.

Description of the method of application, type of equipment used and type and volume of diluent per unit of area or volume (Annex IIIA 3.6)

Flupyradifurone 200SL (Sivanto) is applied with traditional foliar sprayer.

Number and timing of applications and duration of protection afforded (Annex IIIA 3.7)

See the GAP (Table 3.3.1 Summary of intended uses in chapter B.3.3 in this document)

B.3.2.4 Minimum waiting periods or other precautions between last application and sowing or planting of succeeding crops. Limitations on choice of succeeding crops (Annex IIIA 3.8)

See the GAP (Table 3.3.1 Summary of intended uses in chapter B.3.3 in this document)

B.3.3 Summary of data on application

See table 3.3.1: Summary of intended uses.

Table 3.3.1 Summary of intended uses

Crop and/ or situation (a)	Country	Product Name	F G or I (b)	Pests or Group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)	Remarks: (m)
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	kg as/hL min max	water L/ha min max	kg as/ha min max		
Hops	N-EU (residue zone)	Sivanto	F	Aphids, *Phorodon humuli,*	SL	200	spray	BBCH 31-75 May July.	1	n.a.	0,0045-0,0075	2000-3300	0.150	21	Biannual application proposed
Lettuce	N/S-EU (residue zone)	Sivanto	F	Aphids, *Nasonovia ribisnigri*	SL	200	spray	BBCH 12-49 May--Oct	1	n.a.	0,0125-0,0250	500-1000	0.125	10	Max 1 application per **24** months
Lettuce	N/S-EU (residue zone)	Sivanto	F	Aphids, *Nasonovia ribisnigr*	SL	200	spray	BBCH 41-49 May--Oct	1	n.a.	0,0125-0,0250	500-1000	0.125	3	Max 1 application per **12** months
Lettuce (soil bound)	N-EU S-EU (residue zone)	Sivanto	G	Aphids, *Nasonovia ribisnigri,*	SL	200	spray	BBCH 12-49	2	10	0,0125-0,0250	500-1000	0.125	3	Max 2 applications per 12 months

Remarks:
a) in case of group of crops the Codex classification should be used
b) Outdoor of field use (F), or glasshouse application (G)
c) e.g. biting and sucking in insects, soil born insects, foliar fungi
d) e.g. wettable powder (WP), emulsifiable concentration (EC), granule (GR)
e) Use CIPAC/FAO Codes where appropriate
f) All abbreviations used must be explained
g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench
h) Kind, e.g. overall, broadcast aerial spraying, row, individual plant, between the plants
i) g/l or g/L
j) Growth stage at last treatment
k) The minimum and maximum number of application possible under practical conditions of use must be provided
l) PHI - Pre Harvest Interval
m) Remarks may include: Extent of use/ economic importance/restrictions

B.3.4 Further information on the active substance (Annex IIA 3.7 to 3.9)

B.3.4.1 Recommended methods and precautions concerning handling, storage, transport or fire (Annex IIA 3.7)

B.3.4.1.1 Handling

Advice on safe handling

Use only in area provided with appropriate exhaust ventilation.

Hygiene measures

Avoid contact with skin, eyes and clothing.
Keep working clothes separately.
Wash hands immediately after work, if necessary take a shower.
Remove soiled clothing immediately and clean thoroughly before using again.
Garments that cannot be cleaned must be destroyed (burnt).

B.3.4.1.2 Storage

Requirements for storage areas and containers

Store in original container.
Keep containers tightly closed in a dry, cool and well-ventilated place.
Store in a place accessible by authorized persons only.
Protect from frost.
Keep away from direct sunlight.

Advice on common storage

Keep away from food, drink and animal feedingstuffs.

Suitable materials

HDPE (high density polyethylene)

B.3.4.1.3 Transport

ADR/RID/ADN

UN number:	**3082**
Proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, LIQUID, N.O.S., (FLUPYRADIFURONE SOLUTION)
Transport hazard class(es):	9
Packing group:	III

Environm. Hazardous Mark: YES
Hazard no.: 90
Tunnel Code: E
This classification is in principle not valid for carriage by tank vessel on inland waterways. Please refer to the manufacturer for further information.

IMDG

UN number: **3082**
Proper shipping name: ENVIRONMENTALLY HAZARDOUS SUBSTANCE, LIQUID,, N.O.S., (FLUPYRADIFURONE SOLUTION)
Transport hazard class(es): 9
Packing group: III
Marine pollutant: YES

IATA

UN number: **3082**
Proper shipping name: ENVIRONMENTALLY HAZARDOUS SUBSTANCE, LIQUID, N.O.S., (FLUPYRADIFURONE SOLUTION)
Transport hazard class(es): 9
Packing group: III
Environm. Hazardous Mark: YES

Transport in bulk according to Annex II of MARPOL 73/78 and the IBC Code

No transport in bulk according to the IBC Code.

Fire

Extinguishing media

Suitable extinguishing media:

Water spray
Carbon dioxide (CO2)
Foam
Sand

Special hazards arising from the substance or mixture:

In the event of fire the following may be released:
Hydrogen chloride (HCl)

Hydrogen cyanide (hydrocyanic acid)
Hydrogen fluoride
Carbon monoxide (CO)
Nitrogen oxides (NOx)

Advice for firefighters

Special protective equipment for fire-fighters

In the event of fire and/or explosion do not breathe fumes.
In the event of fire, wear self-contained breathing apparatus.

Further information

Contain the spread of the fire-fighting media.
Do not allow run-off from fire fighting to enter drains or water course

B.3.4.2 Procedures for destruction or decontamination of the active substance, contaminated packaging and contaminated material

B.3.4.2.1 Controlled incineration

In accordance with current regulations and, if necessary, after consultation with the site operator and/or with the responsible authority, the product may be taken to a waste disposal site or incineration plant. Not completely emptied packagings should be disposed of as hazardous waste.

B.3.4.2.2 Others

Waste key for the unused product: 020108 agrochemical waste containing dangerous substances

B.3.4.3 Emergency measures in the case of an accident

Personal precautions:

In normal use and handling conditions please refer to the label and/or leaflet. In all other cases the following recommendations would apply.

Respiratory protection:

If product is handled while not enclosed, and if contact may occur: Wear respirator with an organic vapours and gas filter mask, (protection factor 10) conforming to EN140 type A or equivalent. Respiratory protection should only be used to control residual risk of short duration activities, when all reasonably practicable steps have been taken to reduce exposure at source e.g. containment and/or local extract ventilation. Always follow respirator manufacturer's instructions regarding wearing and maintenance.

Hand protection:
Wear CE Marked (or equivalent) nitrile rubber gloves (minimum thickness 0,40 mm). Wash when contaminated. Dispose of when contaminated inside, when perforated or when contamination outside cannot be removed. Wash hands always before eating, drinking, smoking or using the toilet.

Eye protection:
Wear goggles conforming to EN166 (Field of Use 5 or equivalent).

Skin and body protection:
Wear standard coverall and type 3 suit. Wear two layers of clothing wherever possible. Polyester/cotton or cotton overalls should be worn under chemical protection suit and should be professionally laundered frequently. If chemical protection suit is splashed, sprayed or significantly contaminated, decontaminate as far as possible, then carefully remove and dispose of as advised by manufacturer.

Protective measures
If product is handled while not enclosed, and if contact may occur: Complete suit protecting against chemicals

Environmental precautions:
Do not allow to get into surface water, drains and ground water
Methods for cleaning up or taking up:
Soak up with inert absorbent material (e.g. sand, silica gel, acid binder, universal binder, sawdust). Clean contaminated floors and objects thoroughly, observing environmental regulations. Keep in suitable, closed containers for disposal.

B.3.4.4 Summary of further information and assessment

No further information.

B.3.5 Further information of the plant protection product (Annex IIIA 4)

Product name: Sivanto SL 200 g/L

B.3.5.1.1 Packaging, compatibility on the preparation with proposed packaging materials (Annex IIIA 4.1)

Description and specification of the packaging

Specific Requirement:	Examples:
■ Material (declare the primary packaging material from **inside to outside**)	Bottle / Canister: HDPE
■ Capacity:	Standard bottle / canister up to 10 liter, packed in outer corrugated fibreboard: • 20 x 0.250 liter • 20 x 0.500 liter • 12 x 1 liter • 4 x 3 liter • 4 x 5 liter • 2 x 10 liter Standard canister / IBC over 10 liter such as: • 20 liter • 25 liter • 50 liter • 200 liter • 640 liter • 1000 liter
■ Type of closure and size of opening	Screw cap 36-50-63 mm (with HF seal or internal wad) - to fit container neck as defined in ECPA One Trip Container Guidelines. Tri – Sure, S 56 x 4 mm, guarantees closure. Screw cap DN 50 guarantees closure and DN 150 guarantees closure.
■ Application devices	None

B.3.5.1.2 Packaging suitability

Statement of compliance:

All packagings comply with ADR regulations and have been tested using the ADR test methods appropriate to the pack type and material and classification of the contents.

B.3.5.1.3 Packaging resistance

The resistance of packaging material to its contents has been tested in accordance with GIFAP Technical Monograph No 17 (May 1993).

B.3.5.2 Procedures for cleaning application equipment (Annex IIIA 4.2)

All application equipment and contaminated protective clothing should be washed/cleaned with water or a dilute detergent solution and thoroughly rinsed. Care should be taken not to spill the contaminated

washings from application equipment into waste water channels. Contaminated cleaning liquids should be disposed safely according to local regulations.

Application equipment

Field spraying equipment should be sufficiently cleaned in order to avoid product left over. Cleaning of field spaying equipment is in general an essential part of the recommendations for use of plant protection products.

Empty the contents of the spraying equipment completely onto the field just sprayed. Remove all filters and nozzles, scrub clean and rinse them with clean water. Put 10 % clean water into the tank to cover the agitator and add about 0.5% liquid detergent. Operate a tank flushing system if fitted. Circulate water through the pump and controls for at least one minute. Drain sprayer, collect washings. Repeat procedure once more. Pump last washing water out through boom feed hoses and pipes. Collect washings. Clean off the outside of the sprayer using minimum water volumes. Collect washings. Replace cleaned nozzles and filters. Collect and put all washings back into the tank and spray out onto the field headland, or otherwise safely dispose of them. Ensure the sprayer systems are completely drained before storage. Plant protection equipment should always be stored in a properly designated storage facility.

Protective clothing

All contaminated clothing should be washed / cleaned with a dilute detergent solution and thoroughly rinsed with clean water.

Impermeable overalls, boots and face shields should be washed clean and dried.

Permeable overalls should be laundered after use.

Disposable overalls and gloves should be washed and disposed of as contaminated waste.

Gloves and boots should be washed clean, if necessary on the insides as well.

B.3.5.3 Re-entry periods, necessary waiting periods or other precautions to protect man, livestock and the environment (Annex IIIA 4.3)

Pre-harvest interval (in days)

Crop	Pre-harvest Interval (PHI)
hop:	21
lettuce, field	10
lettuce, glasshouse	3

Re-entry period (in days) for livestock, to areas to be grazed.

Re-entry period needs not to be established as the representative crops are not grazed.

Re-entry period (in hours or days) for man to crops, buildings or spaces treated

A safe re-entry or handling of treated crops is possible when spray deposit has dried.

Withholding period (in days) for animal foodstuffs

Lettuce:
Due to the time between last treatment and harvest, as defined by the GAPs, it is not necessary to set a withholding period for use of treated plants as animal feeding stuffs. The withholding period is covered by the vegetation period of the crop. However, a poultry feeding study with parent BYI 02960 was conducted at dose rates which reflect maximum possible exposure to livestock based on uses in numerous crops, many of which will be submitted in a separate document. That study covers all aspects of residues in livestock and thus no specific withholding period is required.

Hops:
Not relevant for hops since they are not used as animal feeding stuffs.

Waiting period (in days) between application and handling treated products

Given the toxicological profile of the product and the residue levels at harvest in treated agricultural commodities, there is no need to define a waiting period between application and handling treated products.

Waiting period (in days) between last application and sowing or planting of succeeding crops

A full program of residue trials in rotational crops is presented either in this dossier (the "main", multi-crop, multi-rotation study) or in a separate document (multiple additional crops). The data collected in these studies yield information on the level of residues to be expected in following crops, and are reflected in the dietary risk assessment. Therefore, no waiting period needs to be specified.

Conditions under which the product should not be used

Product should not be used in windy conditions in order to avoid drift into water bodies.

B.3.5.4 Recommended methods and precautions concerning: handling, storage, transport or fire (Annex IIIA 4.4)

For details see Material Safety Data Sheet.

Warehouse storage

Keep out of the reach of children.
Keep away from food, drink and animal feeding stuffs.
When using, do not eat, drink or smoke.
Keep away from source of ignition.
Store in the original container under usual warehouse conditions! Keep containers tightly closed in a cool, well-ventilated place.
Store in a place accessible by authorized persons only! Keep away from direct sunlight and protect again frost.
For more information see:

- Guidelines for the safe handling of pesticides during their formulation, packing, storage and transport (GCPF).
- Guidelines for the safe warehousing of crop protection products (GCPF).

User level storage

Keep out of the reach of children.
Keep away from food, drink and animal feeding stuffs.
When using, do not eat, drink or smoke.
Keep away from source of ignition.

Transport

Risks during transport of product are minimal. Follow the general rules and good practice for transport and the Dangerous Goods regulations. Do not stow the product together with food, feed and consumable items.
Transport classification of the product in Europe is:
Road and rail (ADR and RID): UN3082 (environmentally hazardous substance), Hazard No. 90.

Fire

Extinguish fires with foam or carbon dioxide (CO_2), sand or water spray. Do not allow run-off from fire fighting to enter drains or water courses, e.g. by bundling the area with sand or earth.

Wear a self-contained breathing apparatus. Remove product from areas of fire or otherwise cool containers with water in order to avoid pressure being built up due to heat.
Water should be used as sparingly as possible and if contaminated it must not come in contact with surface water. It must be collected and burned in a commercial incinerator. If burning facilities are inadequate, obtain the manufacturer's advice on decontamination methods.

Protective clothing and equipment – Nature
If product is handled such that there is a risk:

Respiratory protection:	If product is handled while not enclosed, and if skin contact may occur: Full mask Multi-range filter ABEK/P2
Hand protection: Eye protection: Hygiene measures:	PVC or other plastic material gloves Safety glasses with side-shields Avoid contact with skin, eyes and clothing. Keep working clothes separately. Wash hands before breaks and at the end of workday. Wash hands immediately after work, if necessary take a shower. Remove soiled or soaked clothing immediately and clean thoroughly before using again. Garments that cannot be cleaned must be destroyed (burnt).

Protective clothing and equipment – characteristics

Respiratory protection	If product is handled while not enclosed, and if skin contact may occur: Full mask Multi-range filter ABEK/P2
Hand protection	PVC or other plastic material gloves
Eye protection	Safety glasses with side-shields

Suitability and effectiveness of the protective clothing and equipment

Safety glasses with side-shields are recommended to protect eyes from contact with the product. Protective clothing is recommended as routine hygienic measure. Chemical resistant gloves are well known to sufficiently protect hands from contact with the product handled.

Procedures to minimize the generation of waste

Product

To minimise product waste, users are required not to prepare more than they will use for the treatment.

Package

To minimise package waste, empty and rinsed containers should be delivered to a local container collection programme, where available. Otherwise, they should be rendered unusable, e.g. by puncturing, and disposed in accordance with local regulations.

Packaging that cannot be cleaned should be disposed of as product waste. It must be incinerated in an authorised special waste incineration plant. Comply with local legislation.

To minimise generation of leftovers, users are recommended to store not more than will be consumed within the shelf life period of the product.

Information on likely combustion products in the event of fire

The following can be released in case of fire:

Carbon dioxide (CO_2) Carbon monoxide (CO) Nitrous gases (NOx) Hydrogen cyanide (hydrocyanic acid)

Detailed procedures in the event of an accident during transport

Containment of spillages

Keep people and animals away.

Do not discharge into drains / surface water / groundwater. Cover any spilled liquid with absorbent material such as sand, earth or a proprietary absorbent material. After the liquid has been absorbed, carefully sweep it up into a disposable container and tightly seal. Dispose by incineration in an authorised special waste incineration plant. Comply with local legislation. For larger quantities, please contact manufacturer.

If spillage enters drains leading to sewage works, inform local company immediately. If spillage enters rivers or watercourses, inform the Environmental Agency.

Decontamination of areas, vehicles and buildings

Take up with adsorbent material (e.g. sand, earth or a proprietary absorbent material).
Transfer collected material to heavy duty plastic drums and keep safe for disposal. Clean contaminated floors and objects thoroughly, observing environmental regulations.

Disposal of damaged packaging, adsorbents and other material

After the liquid has been absorbed (sand, earth or proprietary absorbent material), carefully sweep it up into a disposable container and tightly seal. Damaged packaging and adsorbents should be collected into heavy duty plastic drums.

Containers and drums can be taken to waste disposal site or incineration plant, after consultation with site operator and/or with the responsible authority. Dispose of by incineration in an authorised special waste incineration plant. Comply with local legislation. For larger quantities, contact manufacturer.

Protection of emergency workers and bystanders

Keep people and animals away. Avoid contact with spilled product or contaminated surfaces and wear personal protective equipment (see Section IIIA1 4.4.6).

In the event of fire and/or explosion, do not breathe fumes. Wear self-contained breathing apparatus for fire fighting if necessary. Remove product from area of fire or otherwise cool containers with water in order to avoid pressure being built up due to heat.

When dealing with a spillage, do not eat, drink or smoke. Remove soiled or soaked clothing immediately. Clean hands and face at work intervals and after work.

First aid measures

General advice:	Move out of dangerous area. If the patient is likely to become unconscious, place and transport in stable sideways position. Remove soiled or soaked clothing immediately.
After inhalation:	Move the patient to fresh air and keep at rest. Call a physician immediately.
After eye contact:	In the case of contact with eyes, rinse immediately with plenty of water. Call a physician immediately.
After skin contact:	After contact with skin, wash immediately with soap and plenty of water. Call a physician immediately.
After ingestion:	Do not induce vomiting. Wash out mouth with water. Call a physician Immediately.

B.3.5.6 Procedures for destruction or decontamination of the plant protection product and its packaging (Annex IIIA 4.6)

B.3.5.6.1 Possibility of neutralisation

No neutralization procedure (e.g. reaction with alkali to form less toxic compounds) is necessary for Flupyradifurone SL 200.

B.3.5.6.3 Others

No other methods for disposal of Sivanto SL 200 g/L are available.

B.3.5.7 Summary of further information and assessment

No further information.

B.3.6 References relied on

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source GLP or GEP status Published or not	Data Protection Claimed Y/N	Owner
IIA 3.4.1 /01	Rauen, H. W.	2012	Document D1 - Safe uses to support the EU Dossier for Annex I inclusion for which data have been provided for flupyradifurone (BYI 02960) Bayer CropScience Bayer CropScience, Report No.: M-421862-01-1, Edition Number: M-421862-01-1 Date: 2012-01-06 GLP/GEP: n.a., unpublished	Y	BCS
KIIA 3.4.1 /02	Rauen, H. W.	2012	Document D1 - Safe uses to support the EU Dossier for Annex I inclusion for which data have been provided for flupyradifurone (BYI 02960) Bayer CropScience Bayer CropScience, Report No.: M-421862-01-1, Edition Number: M-421862-01-1 Date: 2012-01-06 GLP/GEP: n.a., unpublished	Y	BCS
KIIA 3.4.1 /03	Rauen, H. W.; Testenière, O.	2012	Document D-3 - Intended uses supported in the EU for which data will be provided for flupyradifurone (BYI 02960) Bayer CropScience Bayer CropScience, Report No.: M-427337-01-1, Edition Number: M-427337-01-1 Date: 2012-02-15 GLP/GEP: n.a., unpublished	Y	BCS
KIIA 3.4.1 /04	Rauen, H. W.; Testenière, O.	2012	Document D-3 - Intended uses supported in the EU for which data will be provided for flupyradifurone (BYI 02960) Bayer CropScience Bayer CropScience, Report No.: M-427337-01-1, Edition Number: M-427337-01-1 Date: 2012-02-15 GLP/GEP: n.a., unpublished	Y	BCS
KIIA 3.6 /01	Nauen, R.	2012	Statement: Possible development of resistance or cross-resistance flupyradifurone (BYI 2960) Bayer CropScience, Report No.: M-428792-01-1, Edition Number: M-428792-01-1 Date: 2012-04-04 GLP/GEP: n.a., unpublished	Y	BCS
KIIA 3.7 /01	Moens, S.	2012	Flupyradufurone TC 1x300kg FBC WW Bayer CropScience, Report No.: M-424153-01-1, Edition Number: M-424153-01-1 Date: 2012-01-30 GLP/GEP: n.a., unpublished	Y	BCS

KIIA 3.7 /02	Moens, S.	2012	Flupyradufurone TC 1x300kg FBC WW Bayer CropScience, Report No.: M-424153-01-1, Edition Number: M-424153-01-1 Date: 2012-01-30 GLP/GEP: n.a., unpublished	Y	BCS
KIIA 3.8.1 /01	Nuesslein, F.	2012	Flupyradifurone - Incineration as a safe means of disposal and pyrolytic behaviour under controlled conditions Bayer CropScience, Report No.: M-422993-01-1, Edition Number: M-422993-01-1 Date: 2012-01-23 GLP/GEP: no, unpublished	Y	BCS
KIIA 3.8.1 /02	Nuesslein, F.	2012	Flupyradifurone - Incineration as a safe means of disposal and pyrolytic behaviour under controlled conditions Bayer CropScience, Report No.: M-422993-01-1, Edition Number: M-422993-01-1 Date: 2012-01-23 GLP/GEP: no, unpublished	Y	BCS

B.4 Proposals for classification and labelling

B.4.1 Proposals for the classification and labelling of the active substance (Annex IIA 10)

Proposed classification based on Regulation EC 1272/2008:

Physical and chemical properties
No classification is required.

Human health effects
Flupyradifurone (BYI 02960) needs to be classified as Acute oral toxic Cat. 4 H302 according to Regulation (EC) 1272/2008. Flupyradifurone does not need to be classified on the basis of its acute dermal, and inhalation toxicology in rats (Category 5/ Unclassified). Flupyradifurone is considered not irritating to skin and eyes (Category 5/ Unclassified). Flupyradifurone does not have sensitising properties in a LLNA (Category 5/ Unclassified).

Ecotoxicological properties

Category:	Acute 1 and Chronic 1
GHS picogram:	GHS09
Signal word:	Warning
Hazard statement:	H400 “Very toxic to aquatic life”
	H410 “Very toxic to aquatic life with long lasting effects “
Precautionary statements:	(the following may be considered):
	P273 “ Avoid release to the environment”,
	P391 “ Collect spillage”
	P 501 “ Dispose of contents/container to … [in accordance with national/international regulations (to be specified)].

Explanation:
The standard species fish, daphnids and algae are not sensitive to flupyradifurone (L/EC50 >70 mg/L). However, aquatic insects are much more sensitive. The classification is based on the lowest acute L/EC_{50} of flupyradifurone, which is <1 mg/L (*C.riparius* EC50 = 0.0617 mg/L). Therefore, flupyradifurone is classified in Category Acute 1.

Flupyradifurone is considered not ready biodegradable (see LoE fate and behaviour). There are adequate chronic toxicity data available. The NOECs for fish and daphnids are >1 mg/L, but as said above, daphnids are not sensitive to flupyradifurone and it is therefore necessary to take into account other, sensitive, species. The lowest NOEC is <0.1 mg/L (0.010 mg/L for *C. riparius*). Therefore, flupyradifurone is classified in Category Chronic 1.

The M-factor for flupyradifurone is 10 based on both acute and chronic toxicity.

The precautionary statements may be chosen according to national regulations.

B.4.2 Proposals for the classification and labelling of preparations (Annex IIIA 11.3 and 11.4)

Proposed classification based on Regulation EC 1272/2008:

Physical and chemical properties
No classification is required.

Human health effects
The formulation BYI 02960 SL 200 g/L does not need to be classified on the basis of its acute oral, dermal toxicology. According to Regulation (EC) 1272/2008 formulation BYI 02960 SL 200 g/L is classified with H332, Harmful if inhaled (R20, Harmful by inhalation according to Directive 67/548/EEC). BYI 02960 SL 200 g/L is considered not irritating to skin and eyes.
BYI 02960 SL 200 g/L has sensitising properties in a LLNA and is classified with H317 (may cause an allergic skin reaction)/R43 (may cause sensitization by skin contact).

Ecotoxicological properties

Category:	Acute 1 and Chronic 1
GHS picogram:	GHS09
Signal word:	Warning
Hazard statement:	H400 “Very toxic to aquatic life”
	H410 “Very toxic to aquatic life with long lasting effects “
Precautionary statements:	(the following may be considered):
	P273 “ Avoid release to the environment”,
	P391 “ Collect spillage”
	P 501 “ Dispose of contents/container to … [in accordance with national/international regulations (to be specified)].

Explanation:
Toxicity studies with the preparation on the standard species fish, daphnids and algae are available and it is clear that these species are not sensitive to Sivanto (BYI 02960 SL 200) (L/EC50 >100 mg formulation/L). However, aquatic insects are much more sensitive to the a.s.. There is only a chronic study available on aquatic insects for the preparation but not an acute one. Therefore, it would underestimate the acute toxicity to base the classification of the preparation on the available studies.

Instead, the acute classification is based on the summation method. Flupyradifurone is classified as H400 based on its lowest acute L/EC_{50}, which is 0.0617 mg/L, and has an M-factor of 10. The concentration of flupyradifure in the preparation (ca.20%) multiplied by the M-factor (10) is greater than 25%. Therefore, the preparation is also classified in Category Acute 1 (H400). Since this is the worst-case acute classification, the other components of the product do not need to be discussed.
The lowest chronic NOEC for the preparation is ca. 0.06 mg preparation/L (for *C. riparius)*. Therefore, the preparation is classified in Category Chronic 1 (with H410).
The precautionary statements may be chosen according to national regulations.

B.4.3 References relied on

The proposed classification and labelling is based on the physical-chemical, toxicological and ecotoxicological evaluation as presented in section B.2, B.6 and B.9, respectively.

B.5 Methods of analysis

B.5.1 Analytical methods for technical active substance (IIA 4.2.1)) and plant protection product (IIIA 5.2.1)

B.5.1.1 Technical active substance (IIA 4.2.1)

STUDY 1

Reference	:	zur Mühlen, U., Wagner S., 2011 (IIIA 4.2.1/01)	GLP statement	:	-
Type of study	:	Determination of technical grade active substance HPLC -ISTD	Guideline	:	SANCO 3030/99 rev 4
Year of execution	:	2011	Acceptability	:	Acceptable
Test substance	:	Flupyradifurone (BYI 2960)	Method reference	:	AM008809MP1

STUDY 2

Reference	:	Wagner S., 2011 (IIIA 4.2.1/02)	GLP statement	:	-
Type of study	:	Validation of AM008809MP1 Flupyradifurone (BYI 02960) Determination of technical grade active substance HPLC - ISTD	Guideline	:	SANCO 3030/99 rev 4
Year of execution	:	2011	Acceptability	:	Acceptable
Test substance	:	Flupyradifurone (BYI 2960)	Method reference	:	VB1-AM008809MP1

Description of the method

The active substance Flupyradifurone is determined in the technical material as manufactured by reversed-phase liquid chromatography (HPLC) using Diethylphthalate as internal standard (ISTD). The quantitative determination is performed with a specific UV detector (DAD detector) at 225 nm.

Results

Linearity	5 concentrations with double measurements; range 84.3 – 120.4% (corresponding to 84.3 mg/100 mL to 120.4 mg/100 mL); correlation coefficient r^2: 0.9999; regression equation and chromatograms are given in the report; the function is linear in the operating range.
Precision Repeatability	5 synthetic samples with double measurements; no outliers are reported, RSD: 0.30 %; acceptable according to the Horwitz
Accuracy	5 synthetic samples with double measurements; mean recovery: 99.87 %; RSD:
Specificity/Interference	Retention times and UV-spectra from reference substance, sample and spiked sample were compared. The UV-spectra show no spectral differences, the corresponding retention times are identical. Chromatograms of reference substances, sample and spiked sample were checked and found to be free of interfering compounds.

Conclusion

The HPLC-method AM008809MP1 for the determination of Flupyradifurone (BYI 02960) in technical grade active substance is found to be valid.

Guidelines & Limitations

None.

B.5.1.2 Plant protection product (IIIA 5.2.1)

STUDY 3

Reference	:	Zitzmann W., 2009 (IIIA 5.2.1/01)	GLP statement	:	-
Type of study	:	Analytical methods for plant protection product	Guideline	:	SANCO 3030/99 rev 4
Year of execution	:	2009	Acceptability	:	Acceptable
Test substance	:	Flupyradifurone (BYI 2960) SL 200, specification number 102000021884	Method reference	:	AM012609MF1

STUDY 4

Reference	:	Kienow A. Seidel E.., 2010 (IIIA 5.2.1/02)	GLP statement	:	-
Type of study	:	Analytical methods for plant protection product Validation of HPLC-method AM012609MF1 Determination of BYI 02960 in formulations BYI 02960 SL 200 (200 g/L)	Guideline	:	SANCO 3030/99 rev 4
Year of execution	:	2010	Acceptability	:	Acceptable
Test substance	:	Flupyradifurone (BYI 2960) SL 200, specification number 102000021884	Method reference	:	VB2-AM012609MF1

Determination of BYI 02960 in formulations Assay HPLC, external standard. The HPLC method AM012609MF1 is applicable for the determination of the content of Flupyradifurone (BYI 2960) in formulations (*e.g*. ‘Flupyradifurone (BYI 2960) SL 200’).

Description of the method

The active substance (BYI 02960) is separated from formulation constituents by reversed phase chromatography using isocratic elution. After UV detection (280 nm), the quantitative evaluation is carried out by comparing the peak areas with those of reference substances, using an external standard of BYI 02960.

Results

Linearity	6 concentrations with single injections; range 50 -150%; correlation co-efficient $r_{K:}$ 0.9999; regression equation: y = 73.3404x + 21.4446 Chromatograms are given; the function is linear in the operating range.
Precision	6 samples from one batch; single injection; no outliers; acceptable according to the Horwitz equation; relative standard deviation RSD: 0.31%.
Accuracy	6 samples of laboratory-prepared synthetic formulation; mean recovery: 100.3%, confidence interval of recovery: 100.28 ± 0.98 the method shows no constant or proportional systematic error.
Specificity	The UV-spectra of active substance and reference substance show no spectral difference; the retention times of active substance and reference substance are identical.
Interference	No interferences are found.

Conclusion

The HPLC method AM012609MF1for the determination of Flupyradifurone (BYI 2960) in the formulation Flupyradifurone (BYI 2960) SL 200, specification number 102000021884 is found to be valid.

Guidelines & Limitations

None.

B.5.2 Analytical methods (residue) for plants, plant products, foodstuffs of plant and animal origin, feedingstuffs (IIA 4.3 and IIIA 5.4, IIIA 5.6 IIIA 5.7,)

Method 01330 (including its modification M001) was developed as an *enforcement method*. It determines BYI 02960 and its metabolite DFA by HPLC-MS/MS. Matrix-matched standards were used for quantification. The LOQs were generally 0.01 mg/kg and 0.02 mg/kg for BYI 02960 and DFA, respectively, except for hop matrices, for which the respective LOQs were 0.05 and 0.10 mg/kg. Results of an ILV demonstrated inter-laboratory reproducibility of the method.

The European multi-residue methods DFG S 19 and QuEChERS are unsuitable for the enforcement of this compound because the determination of the metabolite DFA is not possible – none of the extraction processes described in either of the methods would allow appropriate extraction of this molecule.

Method 01214 was developed as an *enforcement method* for animal matrices. It determines BYI 02960 and its metabolite DFA by HPLC-MS/MS. For quantification, matrix-matched standards were used. The LOQs were 0.01 mg/kg and 0.02 mg/kg for BYI 02960 and DFA, respectively, in all matrices. Results of an ILV demonstrated inter-laboratory reproducibility of the method.

DFA= Difluoroacetic acid

STUDY 5

Reference	:	Schulte, G., & Bauer, J.; 2012 (IIA 4.3/01)	GLP statement	:	Yes
Type of study	:	Analytical method 01330 for the determination of residues of BYI 02960 and its metabolite difluoroacetic acid in/on plant matrix by HPLC-MS/MS - Enforcement method plant	Guidelines	:	SANCO/825/00 rev. 7 SANCO/3029/99 rev. 4
Year of execution	:	2012	Acceptability	:	Acceptable
Test substance	:	Flupyradifurone (BYI 2960)	Method reference	:	01330, report no. MR-011/096

STUDY 6

Reference	:	Konrad, S.; 2012 (IIA 4.3/02)	GLP statement	:	Yes
Type of study	:	Independent lab validation of method 01330 for the determination of residues of BYI 02960 and its metabolite difluoroacetic acid in/on plant matrices by HPLC-MS/MS	Guidelines	:	SANCO/825/00 rev. 8.1 SANCO/3029/99 rev. 4
Year of execution	:	2012	Acceptability	:	Acceptable
Test substance	:	Flupyradifurone (BYI 2960)	Method reference	:	2011/0134/01

Description of the method

Residue analytical method 01330 (Schulte & Bauer, 2012; KIIA 4.3/01) was developed as an *EU enforcement method* for the determination of the residues of BYI 02960 (parent compound), and its metabolite DFA in/on plant materials.

The residues were extracted twice from 5 g of plant material with acetonitrile/water (4/1, v/v) with 2.2 mL/L formic acid. The materials tested included lettuce (head), rape (seed), orange (fruit), wheat (grain), and hop (cone), representing a wide variety of crops/crop types as requested by EU guidance. After dilution, an aliquot of the raw extract was filtered for measurement. The solution was analyzed by HPLC-MS/MS; residues were quantified against matrix-matched standards.

Results

Note:

The residues were extracted twice from 5 g of plant material with acetonitrile/water (4/1, v/v) with 2.2 mL/L formic acid. The materials tested included lettuce (head), rape (seed), orange (fruit), wheat (grain), and hop (cone), representing a wide variety of crops/crop types as requested by EU guidance. After dilution, *each* aliquot of the raw extract was filtered *for separate measurement of BYI 02960 and DFA. The solutions were analyzed by HPLC-MS/MS using either a C18-column (BYI 02960) or a ZIC®-HILIC SeQuant™ or a Hypercarb column (DFA)*; residues were quantified against matrix-matched standards.

Note:

The extraction efficiency of the residue method for the determination of the relevant residues of BYI 02960 in plant matrices, consisting of the parent compound and its metabolite DFA, was assured by choosing the same extraction procedures as used in the plant metabolism studies (cf. chapter 6.2.1 of the Annex II dossier).
In addition, an extraction efficiency study (see KIIA 4.3/04) was conducted using method 01304. As the extraction procedures for method 01330 are the same as for 01304, the results of the study prove satisfactory extraction efficiency with method 01330 in all matrices.The extraction efficiency was conducted in agreement with SANCO/825/00 rev. 8.1.

Recovery results from the method validation of method 01330 (enforcement method) Recoveries and relative standard deviations (RSDs) for BYI 02960.

Sample material	FL* [mg/kg]	Individual values [%]					Mean value	RSD [%]	LOQ [mg/kg]
QUANTIFICATION MRM (289/126)									
lettuce / head	0.01	102	84	94	105	102	97	8.8	0.01
	0.10	92	99	88	97	99	95	5.1	
		Overall recovery (n = 10)					***96***	***6.9***	
rapé / seed	0.01	99	98	97	92	91	95	3.8	0.01
	0.10	96	98	92	92	94	94	2.8	
		Overall recovery (n = 5)					***95***	***3.2***	
orange / fruit	0.01	96	97	97	97	88	95	4.1	0.01
	0.10	100	106	103	99	104	102	2.8	
		Overall recovery (n = 10)					***99***	***5.1***	
wheat / grain	0.01	100	104	106	104	109	105	3.1	0.01
	0.10	109	104	105	105	105	106	1.8	
		Overall recovery (n = 10)					***105***	***2.5***	

hop / cone	0.01	86[1]	89	94	90	91	91	2.4	0.05
	0.10	92	94	94	92	93	93	1.1	
		Overall recovery (n = 10)					***92***	***2.0***	
CONFIRMATORY MRM (289/90)									
lettuce / head	0.01	103	85	95	107	104	99	9.0	0.01
	0.10	91	97	87	96	98	94	5.0	
		Overall recovery (n = 10)					***96***	***7.5***	
rapé / seed	0.01	100	99	97	93	91	96	4.0	0.01
	0.10	94	97	89	91	91	92	3.4	
		Overall recovery (n = 5)					***94***	***4.1***	
orange / fruit	0.01	97	97	97	96	91	96	2.7	0.01
	0.10	98	105	102	98	104	101	3.2	
		Overall recovery (n = 10)					***99***	***4.2***	
wheat / grain	0.01	100	101	107	104	108	104	3.4	0.01
	0.10	105	100	101	102	102	102	1.8	
		Overall recovery (n = 10)					***103***	***2.8***	
hop / cone	0.01	84[1]	88	94	88	88	90	3.4	0.05
	0.10	88	92	91	89	91	90	1.8	
		Overall recovery (n = 10)					***90***	***2.5***	

Sample material	FL* [mg/kg]	Individual values [%]					Mean value [%]	RSD [%]	LOQ [mg/kg]
QUANTIFICATION MRM (289/126)									
rape / seed	0.01	104	106	100	97	99	101	3.7	0.01
	0.10	95	99	91	99	99	97	3.7	
		Overall recovery (n = 10)					*99*	*4.2*	
CONFIRMATORY MRM (289/90)									
rape / seed	0.01	107	105	107	104	103	105	1.7	0.01
	0.10	99	102	95	102	101	100	3.0	
		Overall recovery (n = 10)					*103*	*3.6*	

Recovery results from the method validation of method 01330 (enforcement method).
Recoveries and relative standard deviations (RSDs) for DFA.

Sample material	FL* [mg/kg]	Individual values [%]					Mean value	RSD [%]	LOQ [mg/kg]
HILIC COLUMN (PRIMARY)									
lettuce / head	0.02	100	80	88	99	104	94	10.5	0.02
	0.20	91	100	88	99	101	96	6.1	
		Overall recovery (n = 10)					***95***	***8.1***	
rapé / seed	0.02	68	71	61	64	62	65[1]	6.5	0.02
	0.20	63	74	61	56	57	62[1]	11.6	
		Overall recovery (n = 5)					***64[1]***	***9.1***	
orange / fruit	0.02	101	103	104	94	84	97	8.6	0.02
	0.20	99	97	95	97	99	97	1.7	
		Overall recovery (n = 10)					***97***	***5.8***	
wheat / grain	0.02	104	89	92	83	86	91	8.9	0.02
	0.20	96	87	88	82	87	88	5.7	
		Overall recovery (n = 10)					***89***	***7.3***	
hop / cone	0.10	97	96	105	101	105	101	4.2	0.10
	1.0	105	102	105	97	102	102	3.2	
		Overall recovery (n = 10)					***102***	***3.6***	
HYPERCARB COLUMN (CONFIRMATORY)									
lettuce / head	0.02	100	78	88	94	96	91	9.4	0.02
	0.20	94	101	90	100	100	97	4.9	
		Overall recovery (n = 10)					***94***	***7.7***	
rapé / seed	0.02	73	74	66	68	68	70	5.0	0.02
	0.20	67	71	60	60	61	64[1]	7.8	
		Overall recovery (n = 5)					***671***	***7.7***	
orange / fruit	0.02	101	99	101	97	89	97	5.1	0.02
	0.20	103	100	101	98	103	101	2.1	
		Overall recovery (n = 10)					***99***	***4.1***	
wheat / grain	0.02	86	90	90	84	86	87	3.1	0.02
	0.20	92	92	93	87	92	91	2.6	
		Overall recovery (n = 10)					***89***	***3.6***	
hop / cone	0.10	113	110	113	100	103	108	5.5	0.10
	1.0	97	94	96	101	102	98	3.5	
		Overall recovery (n = 10)					***103***	***6.7***	

* fortified compound DFA determined as DFA

Footnotes:

1: mean recoveries were below 70%; this was accepted due to the RSD of < 20%.

Recovery results from the method validation of method 01330 (enforcement method) Recoveries and relative standard deviations (RSDs) for DFA

Sample material	FL* [mg/kg]	Individual values [%]					Mean value [%]	RSD [%]	LOQ [mg/kg]
HILIC COLUMN (PRIMARY)									
rape / seed	0.02	84	84	80	85	85	84	2.5	0.02
	0.20	71	74	77	71	74	73	3.4	
		Overall recovery (n = 10)					*79*	*7.4*	
HYPERCARB COLUMN (CONFIRMATORY)									
rape / seed	0.02	73	78	80	82	82	79	4.7	0.02
	0.20	94	80	89	81	90	88	8.8	
		Overall recovery (n = 10)					*83*	*8.8*	

Recovery results from independent laboratory validation of method 01330 recoveries and relative standard deviations (RSDs) for BYI 02960

Sample material	FL [mg/kg]	Individual values [%]					Mean value	RSD [%]	LOQ [mg/kg]
QUANTIFICATION MRM (289/126)									
lettuce / head	0.01	89	91	90	92	89	90	1.4	0.01
	0.1	89	91	91	94	92	91	2.0	
		Overall recovery (n = 10)					***91***	***1.8***	
wheat / grain	0.01	77	84	92	89	86	86	6.7	0.01
	0.1	80	92	95	95	92	91	6.8	
		Overall recovery (n = 10)					***88***	***7.0***	
orange / fruit	0.01	89	92	96	93	92	92	3.0	0.01
	0.1	96	101	100	102	98	**99**	2.4	
		Overall recovery (n = 10)					***96***	***4.5***	
CONFIRMATORY MRM (289/90)									
lettuce / head	0.01	94	88	93	93	90	92	2.5	0.01
	0.1	95	95	92	96	94	94	1.5	
		Overall recovery (n = 10)					***93***	***2.5***	
wheat / grain	0.01	67	74	75	81	76	75	7.0	0.01
	0.1	84	95	98	98	93	93	6.3	
		Overall recovery (n = 10)					***84***	***13.4***	
orange / fruit	0.01	94	92	103	100	101	98	4.5	0.01
	0.1	99	104	104	104	102	103	1.9	
		Overall recovery (n = 10)					***100***	***4.0***	

Recovery results from independent laboratory validation of method 01330 recoveries and relative standard deviations (RSDs) for BYI 02960

Sample material	FL [mg/kg]	Individual values [%]					Mean value [%]	RSD [%]	LOQ [mg/kg]
QUANTIFICATION MRM (289/126)									
rape / seed	0.01	76	85	83	83	78	81	4.7	0.01
	0.10	93	94	94	89	93	93	2.4	
		Overall recovery (n = 10)					87	7.7	
CONFIRMATORY MRM (289/90)									
rape / seed	0.01	77	84	85	82	81	82	3.9	0.01
	0.10	94	93	92	88	95	92	2.8	
		Overall recovery (n = 10)					87	7.2	

Recovery results from independent laboratory validation of method 01330 recoveries and relative standard deviations (RSDs) for DFA

Sample material	FL [mg/kg]	Individual values [%]					Mean value	RSD [%]	LOQ [mg/kg]
HILIC COLUMN (PRIMARY)									
lettuce / head	0.01	95	92	93	97	93	94	2.1	0.01
	0.1	100	100	96	97	99	**99**	1.8	
		Overall recovery (n = 10)					***96***	***3.2***	
wheat / grain	0.01	85	86	86	87	89	87	2.1	0.01
	0.1	71	70	72	65	60	68	7.2	
		Overall recovery (n = 10)					***77***	***13.7***	
orange / fruit	0.01	87	88	106	91	94	93	8.3	0.01
	0.1	103	99	103	98	98	100	2.5	
		Overall recovery (n = 10)					***97***	***6.8***	
HYPERCARB COLUMN (CONFIRMATORY)									
lettuce / head	0.01	79	78	81	72	80	78	4.3	0.01
	0.1	88	88	84	91	89	**88**	2.9	
		Overall recovery (n = 10)					***83***	***7.1***	
wheat / grain	0.01	88	84	86	98	93	90	6.2	0.01
	0.1	74	72	71	76	71	73	2.7	
		Overall recovery (n = 10)					***81***	***12.1***	
orange / fruit	0.01	89	96	81	92	87	89	6.4	0.01
	0.1	91	88	86	91	86	**88**	2.8	
		Overall recovery (n = 10)					***89***	***4.7***	

Recovery results from independent laboratory validation of method 01330 recoveries and relative standard deviations (RSDs) for DFA

Sample material	FL [mg/kg]	Individual values [%]					Mean value [%]	RSD [%]	LOQ [mg/kg]
HILIC COLUMN (PRIMARY)									
rape / seed	0.02	96	103	107	108	98	103	5.1	0.01
	0.20	95	102	102	97	100	99	3.0	
		Overall recovery (n = 10)					*101*	*4.3*	
HYPERCARB COLUMN (CONFIRMATORY)									
rape / seed	0.02	90	102	94	110	92	97	8.6	0.01
	0.20	86	92	93	87	92	90	3.3	
		Overall recovery (n = 10)					*94*	*7.6*	

Conclusions

As DFA cannot be determined by DFG multi-residue method S 19 or by the QuEChERS procedure, a specific method, such as 01330, is suitable to determine the proposed enforcement residue definition for BYI 02960.

Method 01330 meets the necessary performance requirements to determine residues of BYI 02960 and its metabolite DFA in plant materials, with an LOQ of 0.01 mg/kg for BYI 02960 (0.05 in hops), and of 0.02 mg/kg for DFA (0.10 in hops). Results of an ILV showed that method 01330 fulfills the reproducibility requirements and is, therefore, applicable as an enforcement method.

Report:	KIIA 4.3/10, Schulte, G., & Teubner, L.; 2012
Title:	Modification M001 of the analytical method 01330 for the determination of residues of BYI 02960 and its metabolite difluoroacetic acid in/on plant matrix by HPLC-MS/MS-Enforcement Method Plant
Report No. & Edition No.	Method no. 01330/M001, report no. MR-012/054 M-438310-01-1, September, 10, 2012
Guidelines:	– EU Regulation (EC) No. 1107/2009 – EU Guidance Document for residue analytical methods SANCO/825/00 rev. 8.1 – EU Guidance Document for residue analytical methods SANCO/3029/99 – OECD Guidance Document on pesticide residue analytical methods ENV/JM/Mono (2007) – US EPA Residue Chemistry Test Guideline OPPTS 860.1340, residue analytical method
GLP:	yes (certified laboratory)

Report:	KIIA 4.3/11, Konrad, S.; 2012
Title:	Independent lab validation of BCS method 01330/M001 for the determination of residues of BYI 02960 and its metabolite difluoroacetic acid in/on plant matrices by HPLC-MS/MS
Report No. & Edition No.	2012/0082/01 M-439855-01-1, , October, 15, 2012
Guidelines:	– EU Regulation (EC) No. 1107/2009 – EU Guidance Document for residue analytical methods SANCO/825/00 rev. 8.1 – EU Guidance Document for residue analytical methods SANCO/3029/99 – OECD Guidance Document on pesticide residue analytical methods ENV/JM/Mono (2007) – US EPA Residue Chemistry Test Guideline OPPTS 860.1340, residue analytical

	methods
GLP:	yes (certified laboratory)

Guidelines & Limitations

None.

Table B.5.2-2

Summary of residue analytical methods for BYI 02960 in plant and animal matrices.

Matrix	Analyte	Method No.	Method principle	LOQ*	Reference
Plant	BYI 02960 DFA	01330t	HPLC-MS/MS	BYI 02960: 0.01 or 0.05 mg/kg DFA: 0.02 or 0.10 mg/kg	IIA 4.3/01 IIA 4.3/02
Plant	BYI 02960 - DFEAF DFA 6-	RARVP 013 (01304)*	HPLC-MS/MS	BYI 02960: 0.01 mg/kg -DFEAF: 0.01 mg/kg DFA: 0.02 or 0.05 mg/kg 6-CNA:	**Data generation** residues
Plant	BYI 02960 - DFEAF DFA 6-	01212*	HPLC-MS/MS	BYI 02960: 0.01 mg/kg -DFEAF: 0.01 mg/kg DFA: 0.02 mg/kg 6-CNA:	IIA 4.3/05 **Data generation**
Animal	BYI 02960 DFA	01214t	HPLC-MS/MS	BYI 02960: 0.01 mg/kg DFA: 0.02 mg/kg	IIA 4.3/06 IIA 4.3/07
Animal	BYI 02960 DFA -acetyl- AMCP -OH	(RV-004-All-04)*	HPLC-MS/MS	BYI 02960: 0.01 mg/kg DFA: 0.01 or 0.02 mg/kg -acetyl-AMCP: 0.01 mg/kg -OH: 0.01	**Data generation** IIA 4.3/08 IIA 4.3/09

*It should be noted that these methods are data generation methods only.

STUDY 7

Reference	:	Schulte, G., & Bauer, J.; 2012 (IIA 4.3/03)	GLP statement	:	Yes
Type of study	:	Analytical method 01214 for the determination of residues of BYI 02960 and its metabolite difluoroacetic acid in/on animal matrices by HPLC-MS/MS - Enforcement method animal	Guidelines	:	SANCO/825/00 rev. 7
Year of execution	:	2012	Acceptability	:	Acceptable
Test substance	:	Flupyradifurone (BYI 2960)	Method reference	:	01214

STUDY 8

Reference	:	Konrad, S.; 2012 (IIA 4.3/04)	GLP statement	:	Yes
Type of study	:	Independent lab validation of BCS method 01214 for the determination of residues of BYI 02960 and its metabolite difluoroacetic acid in/on animal matrices by HPLC-MS/MS	Guidelines	:	SANCO/825/00 rev. 8.1 SANCO/3029/99 rev. 4
Year of execution	:	2012	Acceptability	:	Acceptable
Test substance	:	Flupyradifurone (BYI 2960)	Method reference	:	2011/0164/01

Description of the method

Residue analytical method 01214 (Schulte & Bauer, 2012; KIIA 4.3/06) was developed as an EU *enforcement method* for the determination of the residues of BYI 02960 (parent compound), and its metabolite DFA in animal matrices.

The residues were extracted twice from 5 g of animal-based material with acetonitrile/water (4/1, v/v), with the addition of n-heptane in the cases of fat and milk. The materials tested included bovine muscle, liver, kidney, and milk; and chicken fat and egg, representing the variety of matrix types requested by EU guidance. After the addition of formic acid and dilution, an aliquot of the raw extract was filtered for measurement. After dilution, *each* aliquot of the raw extract was filtered for separate measurement of BYI 02960 and DFA. The solutions were analyzed by HPLC-MS/MS *using either a C18-column (BYI 02960) or a ZIC®-HILIC SeQuantTM or a Hypercarb column (DFA)*; residues were quantified against matrix-matched standards.~~The solution was analyzed by HPLC-MS/MS; residues were quantified against matrix-matched standards.~~

Extraction Efficiency

The extraction efficiency of the residue method for the determination of the relevant residues of BYI 02960 in animal matrices, consisting of the parent compound and its metabolite DFA, was assured by choosing the same extraction procedures as used in the livestock metabolism studies (cf. chapter 6.2.2 and 6.2.3 of the Annex II dossier). In addition an extraction efficiency examination was conducted as part of the poultry (KIIA 4.3/09) and cattle (KIIA 4.3/08) feeding studies. Aged residues in respective samples were analysed using procedures described in method RV-004-A11-04 and, in parallel, using the procedures described in the metabolism studies (cf. chapter 6.1 of Annex II dossier). As the extraction procedures for method RV-004-A11-04 are the same as for method 01214, the

results of the study prove satisfactory extraction efficiency with method 01214. The sample materials were eggs, fat, liver, and muscle in the poultry report, and kidney and milk in the ruminant report.

Blank value of untreated control samples:
From each sample material, two untreated control samples were examined. All residues of BYI 02960 and difluoroacetic acid (expressed as parent equivalents) were well below 30% of the corresponding LOQ level.

Ionisation type and MS/MS transition:
The experiments were performed on a triple-quadrupole mass spectrometer system, fitted with an electrospray interface operated in the positive ion mode for BYI 02960 and in negative mode for difluoroacetic acid under MRM conditions.

BYI 02960: ESI positive	1st MRM: 289 => 126 (quantification)
	2nd MRM: 289 => 90 (confirmatory)
DFA: ESI negative	1st MRM: 95 => 51

Results

Note

The extraction efficiency of the residue method for the determination of the relevant residues of BYI 02960 in animal matrices, consisting of the parent compound and its metabolite DFA, was assured by choosing the same extraction procedures as used in the livestock metabolism studies (cf. chapter 6.2.2 and 6.2.3 of the Annex II dossier).
In addition an extraction efficiency examination was conducted as part of the poultry (KIIA 4.3/09) and cattle (KIIA 4.3/08) feeding studies. Aged residues in respective samples were analysed using procedures described in method RV-004-A11-04 and, in parallel, using the procedures described in the metabolism studies (cf. chapter 6.1 of the Annex II dossier). As the extraction procedures for method RV-004-A11-04 are the same as for 01214, the results of the study prove satisfactory extraction efficiency with method 01214 in all matrices. The sample materials were eggs, fat, liver, and muscle in the poultry report, and kidney and milk in the ruminant report. The extraction efficiency was conducted in agreement with SANCO/825/00 rev. 8.1.

Recovery results from the method validation of method 01214 (enforcement method) Recoveries and relative standard deviations (RSDs) for BYI 02960

Sample material	FL* [mg/kg]	Individual values [%]					Mean value [%]	RSD [%]	LOQ [mg/kg]
PRIMARY TRANSITION (289/126)									
bovine muscle	0.01	111	100	116	119	116	112[1]	6.7	0.01
	0.10	105	105	110	106	112	108	3.0	
		Overall recovery (n = 10)					*110*	*5.5*	
bovine liver	0.01	100	104	107	106	77	99	12.6	0.01
	0.10	99	100	101	100	96	99	1.9	
		Overall recovery (n = 5)					*99*	*8.5*	
bovine kidney	0.01	100	105	104	104	105	104	2.0	0.01
	0.10	83	95	99	102	93	94	7.7	
		Overall recovery (n = 10)					*99*	*7.1*	
chicken fat	0.01	101	106	113	110	106	107	4.2	0.01
	0.10	106	110	114	103	103	107	4.4	
		Overall recovery (n = 10)					*107*	*4.1*	
bovine milk	0.01	103	109	103	103	104	104	2.5	0.01
	0.10	102	106	93	109	104	103	5.9	
		Overall recovery (n = 10)					*104*	*4.3*	
chicken egg	0.01	97	96	97	97	99	97	1.1	0.01
	0.10	92	84	91	102	98	93	7.4	
		Overall recovery (n = 10)					*95*	*5.3*	
CONFIRMATORY TRANSITION (289/90)									
bovine muscle	0.01	112	98	119	114	115	112[1]	7.2	0.01
	0.10	106	105	106	105	112	107	2.8	
		Overall recovery (n = 10)					*109*	*5.7*	
bovine liver	0.01	105	101	108	106	76	99	13.3	0.01
	0.10	101	100	104	97	96	100	3.2	
		Overall recovery (n = 5)					*99*	*9.1*	
bovine kidney	0.01	104	104	105	106	104	105	0.9	0.01
	0.10	82	96	96	100	92	93	7.4	
		Overall recovery (n = 10)					*99*	*7.7*	
chicken fat	0.01	104	105	109	103	105	105	2.2	0.01
	0.10	108	109	111	102	103	107	3.7	
		Overall recovery (n = 10)					*106*	*2.9*	
bovine milk	0.01	102	106	109	103	99	104	3.7	0.01
	0.10	101	108	93	109	104	103	6.3	
		Overall recovery (n = 10)					*103*	*4.9*	
chicken egg	0.01	96	93	95	93	104	96	4.7	0.01
	0.10	93	87	92	102	100	95	6.5	
		Overall recovery (n = 10)					*96*	*5.4*	

Recovery results from the method validation of method 01214 (enforcement method) Recoveries and relative standard deviations (RSDs) for DFA

Sample material	FL* [mg/kg]	Individual values [%]					Mean value	RSD [%]	LOQ [mg/kg]
PRIMARY METHOD (HILIC COLUMN)									
bovine muscle	0.02	106	106	105	107	104	106	1.1	0.02
	0.20	97	101	100	98	105	100	3.1	
		Overall recovery (n = 10)					***103***	***3.5***	
bovine liver	0.02	97	99	105	102	72	95	13.9	0.02
	0.20	105	102	104	100	93	101	4.7	
		Overall recovery (n = 5)					***98***	***10.1***	
bovine kidney	0.02	107	103	106	108	106	106	1.8	0.02
	0.20	94	96	100	101	96	97	3.0	
		Overall recovery (n = 10)					***102***	***5.0***	
chicken fat	0.02	99	106	107	106	106	105	3.1	0.02
	0.20	103	104	111	101	103	104	3.7	
		Overall recovery (n = 10)					***105***	***3.2***	
bovine milk	0.02	110	108	107	101	109	107	3.3	0.02
	0.20	103	108	92	105	102	102	5.9	
		Overall recovery (n = 10)					***105***	***5.1***	
chicken egg	0.02	90	94	90	89	98	92	4.1	0.02
	0.20	90	81	84	94	99	90	8.1	
		Overall recovery (n = 10)					***91***	***6.2***	
CONFIRMATORY METHOD (HYPERCARB COLUMN)									
bovine muscle	0.02	109	105	107	107	112	108	2.1	0.02
	0.20	103	105	105	103	107	105	1.6	
		Overall recovery (n = 10)					***106***	***2.4***	
bovine liver	0.02	98	103	102	99	72	95	13.6	0.02
	0.20	92	90	94	91	88	91	2.5	
		Overall recovery (n = 5)					***93***	***9.6***	
bovine kidney	0.02	100	102	97	104	96	100	3.4	0.02
	0.20	88	90	960	102	96	94	5.9	
		Overall recovery (n = 10)					***97***	***5.3***	
chicken fat	0.02	105	105	109	107	106	106	1.6	0.02
	0.20	100	105	105	99	101	102	2.8	
		Overall recovery (n = 10)					***104***	***3.1***	
bovine milk	0.02	104	105	103	101	104	103	1.5	0.02
	0.20	103	104	93	103	103	101	4.5	
		Overall recovery (n = 10)					***102***	***3.4***	
chicken egg	0.02	100	101	102	98	100	100	1.5	0.02
	0.20	94	86	93	97	98	94	5.0	
		Overall recovery (n = 10)					***97***	***4.9***	

Recovery results from independent laboratory validation of method 01214 recoveries and relative standard deviations (RSDs) for BYI 02960

Sample material	FL* [mg/kg]	Individual values [%]					Mean value	RSD [%]	LOQ [mg/kg]
PRIMARY TRANSITION (289/126)									
chicken egg	0.01	94	109	105	93	91	98	8.3	0.01
	0.1	90	84	84	88	87	87	3.1	
		Overall recovery (n = 10)					***93***	***9.1***	
bovine milk	0.01	99	100	100	101	98	100	1.0	0.01
	0.1	97	91	94	96	95	95	2.2	
		Overall recovery (n = 10)					***97***	***3.2***	
bovine kidney	0.01	92	94	94	93	93	93	0.8	0.01
	0.1	88	89	92	91	92	90	1.9	
		Overall recovery (n = 10)					***92***	***2.2***	
bovine fat	0.01	98	90	96	100	96	96	4.2	0.01
	0.1	93	89	90	95	97	93	3.6	
		Overall recovery (n = 10)					***94***	***4.2***	
bovine muscle	0.01	95	97	97	98	93	96	2.3	0.01
	0.1	90	96	94	96	95	94	2.5	
		Overall recovery (n = 10)					***95***	***2.4***	
bovine liver	0.01	97	98	98	98	100	98	0.9	0.01
	0.1	96	97	99	101	98	98	1.8	
		Overall recovery (n = 10)					***98***	***1.4***	
CONFIRMATORY TRANSITION (289/90)									
chicken egg	0.01	96	111	108	93	90	99	9.4	0.01
	0.1	92	89	89	89	91	90	1.7	
		Overall recovery (n = 10)					***95***	***8.4***	
bovine milk	0.01	101	97	101	102	96	99	2.8	0.01
	0.1	100	94	95	97	97	97	2.3	
		Overall recovery (n = 10)					***98***	***2.8***	
bovine kidney	0.01	93	94	95	96	98	95	2.2	0.01
	0.1	90	94	97	94	94	94	2.5	
		Overall recovery (n = 10)					***94***	***2.4***	
bovine fat	0.01	100	93	98	98	95	97	2.6	0.01

	0.1	97	92	92	97	99	95	3.5	
		Overall recovery (n = 10)					***96***	***3.0***	
bovine muscle	0.01	96	95	95	97	88	94	3.9	0.01
	0.1	92	98	96	98	96	96	2.6	
		Overall recovery (n = 10)					***95***	***3.3***	
bovine liver	0.01	100	101	95	98	97	98	2.5	0.01
	0.1	96	99	98	102	101	99	2.6	
		Overall recovery (n = 10)					***99***	***2.4***	

Recovery results from independent laboratory validation of method 01214 recoveries and relative standard deviations (RSDs) for difluoroacetic acid

Sample material	**FL* [mg/kg]**	**Individual values [%]**					**Mean value**	**RSD [%]**	**LOQ [mg/kg]**
PRIMARY METHOD (HILIC COLUMN)									
chicken egg	0.02	105	96	103	92	95	98	5.7	0.02
	0.2	90	102	93	91	93	94	5.0	
		Overall recovery (n = 10)					***96***	***5.6***	
bovine milk	0.02	100	102	99	107	97	101	3.7	0.02
	0.2	97	96	94	98	96	96	1.6	
		Overall recovery (n = 10)					***99***	***3.8***	
bovine kidney	0.02	93	102	98	106	102	100	4.9	0.02
	0.2	89	94	93	92	92	92	2.2	
		Overall recovery (n = 10)					***96***	***5.8***	
bovine fat	0.02	100	100	90	100	95	97	4.4	0.02
	0.2	91	86	96	92	90	91	4.1	
		Overall recovery (n = 10)					***94***	***5.2***	
bovine muscle	0.02	95	98	100	100	99	98	2.1	0.02
	0.2	83	91	88	86	87	87	3.4	
		Overall recovery (n = 10)					***93***	***6.8***	
bovine liver	0.02	89	79	88	83	105	89	11.1	0.02
	0.2	101	94	96	99	96	97	2.8	
		Overall recovery (n = 10)					***98***	***8.7***	
CONFIRMATORY METHOD (HYPERCARB COLUMN)									
chicken egg	0.02	80	80	83	78	78	80	2.3	0.02
	0.2	81	96	82	84	82	85	7.2	
		Overall recovery (n = 10)					***82***	***6.2***	
bovine milk	0.02	76	79	77	85	75	78	5.0	0.02
	0.2	85	83	83	85	86	84	1.6	
		Overall recovery (n = 10)					***81***	***5.2***	
bovine kidney	0.02	71	72	72	79	75	74	4.4	0.02
	0.2	76	78	78	78	76	77	1.4	
		Overall recovery (n = 10)					***76***	***3.9***	

bovine fat	0.02	73	74	75	75	76	75	1.5	0.02
	0.2	87	88	94	91	85	89	3.6	
		Overall recovery (n = 10)					***82***	***9.7***	
bovine muscle	0.02	73	72	74	72	73	73	1.3	0.02
	0.2	79	83	84	88	85	84	3.8	
		Overall recovery (n = 10)					***78***	***8.0***	
bovine liver	0.02	78	72	73	77	96	79	12.3	0.02
	0.2	89	86	92	92	90	90	2.8	
		Overall recovery (n = 10)					***84***	***10.3***	

Conclusion

As DFA cannot be determined by DFG multi-residue method S 19 or by the QuEChERS procedure, a specific method, such as 01214, is valid for the determination of the proposed enforcement residue definition for BYI 02960.

Method 01214 meets the necessary performance requirements to determine residues of BYI 02960 and its metabolite DFA in animal matrices, with LOQs of 0.01 mg/kg for BYI 02960 and 0.02 mg/kg for DFA. Results of an ILV showed that method 01214 fulfils the reproducibility requirements and is, therefore, applicable as an enforcement method.

Guidelines & Limitations

None.

B.5.3 Analytical methods (residue) soil, water, air (IIA 4.4, IIA 4.5, IIA 4.7 ; IIIA 5.4, IIIA 5.6 to 5.8)

STUDY 9

Reference	:	Brumhard, B., Reineke, A.; 2009 (IIA 4.4/01)	GLP statement	:	Yes
Type of study	:	Analytical Method 01074 for the Determination of BYI 02960 in Soil using LC/MS/MS	Guidelines	:	SANCO/825/00 rev. 7
Year of execution	:	2009	Acceptability	:	Acceptable
Test substance	:	Flupyradifurone (BYI 2960)	Method reference	:	MR-07/337

Description of the method

Soil samples of 20 g are extracted in a microwave extractor with 40 mL of a mixture of acetonitrile (1:4; v/v). Then a subsample is centrifuged to remove fine particles of the soil. An aliquot of the sample solution was injected into the high performance liquid chromatograph and subjected to reversed phase chromatography coupled with tandem mass spectrometry (MS/MS) with electrospray ionisation. The MS/MS instrument was operated in the Multiple Reaction Monitoring mode (MRM). The pseudomolecular ions of the analytes ([M+H]+) were selected by the first quadrupole. These precursor ions were impulsed with nitrogen in the collision cell (second quadrupole) and the resulting fragment ions (product ions) were separated according to their m/z ratio in the third quadrupole. Two of these product ions per analyte were selected: one product ion (MRM-transition) serving for quantitation and the second for confirmation. The first MRM transition of BYI 02960 is the quantification ion with the mass 126 [m/z 126] and the second MRM transition is the confirmatory ion with the mass 99 [m/z 99].
The method was validated using a silt soil (Höfchen) and a sandy loam soil (Laacher Hof). Two different soils were used in order to assess a possible influence of different soil characteristics.

Variations in equipment or sample characteristics and/or deterioration of system performance may require slight modifications in the chromatographic or detector conditions listed in order to obtain adequate chromatographic peak shapes or sensitivity. Instrument parameters and mobile phase may be adjusted to improve separation from unexpected interfering peaks. Therefore, the given LC/MS/MS parameters listed (see following table) may require adaptation.

The detection by MS/MS was performed on a triple-quadrupole tandem mass spectrometer, equipped with a Turbo IonSpray (ESI) interface operated in positive ion mode and multiple reaction monitoring (MRM). Optimal collisionally-activated dissociation (CAD) conditions for fragmentation of the pseudomolecular ions of the analyte was applied with nitrogen as the collision gas.

For calculation of the concentrations, calibration curves were used. These curves were calculated automatically after each sequence run with the Applied Biosystem quantitation software Analyst (Version 1.4.1) using linear regression. Further calculations were performed using the software EXCEL 2003 (Office 2003®).

Results

Selectivity: The high selectivity of the method resulted from the HPLC separation in combination with MS/MS detection. Two MRM transitions were monitored for BYI 02960 (m/z 289 ⟶ 126 for quantitation and m/z 289 ⟶ 99 for confirmation). No signals / peaks interfering with the detection of the analyte were observed in extracts of untreated blank control specimens.

Linearity: For both mass transitions (m/z 289 ⟶ 126 and m/z 289 ⟶ 99) of BYI 02960 the correlation between the injected amount of substance and the detector response was linear for standards in matrix (both soil matrices: Höfchen and Laacher Hof) (l.Ong/L to 100 ng/L) corresponding to a concentration in soil of 2 to 200 p. µg/kg. The correlation coefficients of the 1/x weighted linear regression ranged from 0.9960 to 0.9973.

Untreated Control Samples: Apparent residues in control samples were below 0.3 **x** LOQ (1.5 H µg/kg) for BYI 02960.

Recovery Rates (Accuracy) and Precision (Repeatability): Recovery rates were determined at fortification levels of 5 µg/kg (= LOQ level), and 50 µg/kg. The lowest fortification level experimentally providing a mean recovery between 70 and 110% with a relative standard deviation of ≤ 20% per definition is the Limit of Quantitation (LOQ), provided that the blank values were below 30% of this level. Results are presented in the following tables.
As a measure for the precision of the method, the intra-laboratory repeatability (n = 5) is given as relative standard deviation (% RSD) for all sample materials at fortification levels of 5 and 50 µg/kg. The RSD of the repeatability tests at each recovery set ranged from 1.3 to 3.8 for the quantifier mass transition and from 1.5 to 3.3 % for the confirmatory mass transition.

Recoveries for BYI 02960 Quantifier Mass Transition (m/z 126) RSD: Relative Standard Deviation

Fortification [µg/kg]	Soil	Single values [%]					Mean [%]	RSD [%]
5.0	Höfchen	94	92	92	92	90	92	1.3
5.0	Laacher Hof	102	100	99	97	96	99	2.3
Mean of all 5.0 µg/kg single values							95	4.1
50.0	Höfchen	90	88	85	85	85	86	2.5
50.0	Laacher Hof	100	94	93	94	91	94	3.8
Mean of all 50.0 µg/kg single values							90	5.6
Mean of all Höfchen samples							89	3.8
Mean of all Laacher Hof samples							97	3.7
Overall mean							93	5.5

Recoveries for BYI 02960 Confirmatory Mass Transition (m/z 99) RSD: Relative Standard Deviation

Fortification [µg/kg]	Soil	Single values [%]					Mean [%]	RSD [%]
5.0	Höfchen	93	93	91	94	91	92	1.5
5.0	Laacher Hof	100	99	98	101	94	98	2.8
Mean of all 5.0 µg/kg single values							95	4.0

50.0	Höfchen	91	90	88	87	87	88	1.9
50.0	Laacher Hof	98	95	92	94	90	94	3.3
Mean of all 50.0 µg/kg single values							91	4.0
Mean of all Höfchen samples							90	2.7
Mean of all Laacher Hof samples							96	3.9
Overall mean							93	4.5

Limit of quantification (LOQ): The target limit of quantitation of the method is 5 µg/kg for BYI 02960. The target limit of detection of the method is 1.5 µg/kg for BYI 02960.

Conclusion

The method 01074 (report MR-07/337) was developed for determination of BYI 02960 in soil with target limit of quantitation of 5 µg/kg for BYI 02960. The method validation data are in compliance with the guideline requirements for residue data generation and enforcement. Thus, the method is the recommended enforcement method for monitoring of BYI 02960 residues in soil.

Guidelines & Limitations

None.

STUDY 10

Reference	:	Fargeix, G., Rosati, D.; 2012	GLP statement	:	Yes
Type of study	:	Analytical Method N°01213 for the Determination of Residues of BYI 02960 in Drinking and Surface Water by HPLC-MS/MS, parent and metabolites	Guidelines	:	SANCO/825/00 rev. 8
Year of execution	:	2012	Acceptability	:	Acceptable
Test substance	:	Flupyradifurone (BYI 2960)	Method reference	:	MR-12/022

Description of the method

BYI 02960 is determined by direct injection of filtered aliquot of sample into the high performance liquid chromatograph and subjected to reversed phase chromatography coupled with tandem mass spectrometry (MS/MS) with electrospray ionization, using positive ion mode without further clean-up. Concentrations are quantified using external matrix-matched standard solutions or standards in solvent: it is advised to quantify against matrix matched standards.
The MS/MS instrument is operated in the Multiple Reaction Monitoring mode (MRM). The pseudomolecular ions of the analytes ($[M+H]^+$ or any adducts) were selected by the first quadrupole. These precursor ions were impulsed with nitrogen in the collision cell (second quadrupole), and the resulting fragment (product ions) are separated according to their m/z ratio in the third quadrupole. Two of these product ions per analyte were selected: one product ion (MRM-transition) for quantitation and the second for confirmation. The first MRM transition of BYI 02960 is the quantification ion with the mass 126 [m/z 126] and the second MRM transition is the confirmatory ion with the mass 90 [m/z 90].

Variations in equipment or sample characteristics and/or deterioration of system performance may require slight modifications in the chromatographic or detector conditions listed in order to obtain adequate chromatographic peak shapes or sensitivity. Instrument parameters and mobile phase may be adjusted to improve separation from unexpected interfering peaks. Therefore, the given LC/MS/MS parameters listed (see following table) may require adaptation. Before running a batch of analyses, it is necessary to sufficiently equilibrate the LC column for the analysis of this compound. It is advised to inject 5 calibration points for equilibrium time.

Results

Selectivity/Specificity: The high selectivity of the method resulted from the HPLC separation in combination with MS/MS detection. Two MRM transitions were monitored for BYI 02960 (m/z 289 ➟ 126 for quantitation and m/z 289 ➟ 90 for confirmation). Therefore, the HPLC-MS/MS method is highly specific and an additional confirmatory method is not necessary. No signals / peaks interfering with the detection of the analyte were observed in extracts of untreated control samples. Apparent concentrations in control samples were below 0.3 x LOQ.

Linearity: The correlation between the injected amount of substance and the detector response was linear (1/x weighted) for aqueous Standard solutions ranging from 0.01 to 20 ug/L. The correlation coefficients were > 0.9998 for both MRM transitions.

Limit of Quantification (LOQ) and Limit of Detection (LOD): The LOQ for BYI 02960 is 0.05 ~~H~~ µg/L (rounded value) in surface water, and the LOD is 0.02 ~~u~~ µg/L.

Repeatability (Precision): As a measure for the precision of the method, the intra-laboratory repeatability is given as relative Standard deviation (% RSD) for surface water samples at fortification levels of 0.05 ~~u~~ µg/L and 0.5 ~~u~~ µg/L. The relative Standard deviation for the peak area of the quantification MRM was 5.2% (0.05 ug/L) and 2.5% (0.5 ~~u~~ µg/L).

Recovery Rates (Accuracy): Because of the direct measurement of fortified samples without separate extraction and clean-up steps it is not possible to determine recovery rates in a classical way and therefore, an estimate of the accuracy of the analytical technique was made by an assessment of the linearity of matrix calibration and by determination of the repeatability of sample analysis.

However, for additional demonstration of the reliability of the method, the validation samples were evaluated like recovery rates. Mean recoveries for each fortification level and the overall mean recoveries were within the 70 - 110% range for both MRM transitions. The results are shown in the following tables. All method validation data are in compliance with the guideline requirements for enforcement methods

Matrix Effects: The MS/MS detection of BYI 02960 was slightly affected by the matrix. Even if the results meet all guideline criteria on method validation, the method users are advice to use matrix matched standards because the recovery results are slightly closer to 100%, then.

Recoveries for BYI 02960 Quantifier Mass Transition (m/z 126); RSD = Relative Standard Deviation

Fortification [µg/L]	Matrix (measured by)	Single values [%]					Mean [%]	RSD [%]
0.05	Surface Water (matrix matched standards)	82	88	87	80	79	83	4.7
0.5		84	88	86	84	89	86	5.7
Mean		Mean single values					85	4.1
0.05	Surface Water (solvent standards)	76	82	81	75	74	78	4.7
0.5		81	84	83	73	77	80	5.7
Mean		Mean single values					79	5.1

Recoveries for BYI 02960 Confirmatory Mass Transition (m/z 90); RSD = Relative Standard Deviation

Fortification [µg/L]	Matrix (measured by)	Single values [%]					Mean [%]	RSD [%]
0.05	Surface Water (matrix matched standards)	94	94	94	90	90	92	2.4
0.5		81	82	79	84	84	82	2.6
Mean		Mean single values					87	6.7

Conclusion

All method validation data are in compliance with the guideline requirements for residue data generation and enforcement. Thus, the method is the recommended enforcement method for monitoring of BYI 02960 residues in drinking and surface water at 0.05 ug/L.

Guidelines & Limitations

None.

STUDY 11

Reference	:	Heinz, N., 2011	GLP statement	:	Yes
Type of study	:	Analytical methods for soil, air and water, Analytical Method for Determination in Air	Guidelines	:	SANCO/825/00 rev. 8.1
Year of execution	:	2011	Acceptability	:	Acceptable
Test substance	:	Flupyradifurone (BYI 2960)	Method reference	:	P 2419 G

Description of the method

Air sampling uses adsorption tubes, e.g. flame sealed glass tubes (OD: 8 mm, L: 100 mm) filled with two layers (front layer A: 100 mg, mesh size 20 and rear layer B: 50 mg, mesh size 40) of porous polymer (XAD: ORBO^{TM}-44, Supelco Cat. No. 20260-U), held in place by three glass wool plugs. Particles and aerosols are trapped by filtration or impact onto the adsorbent material. Both ends of sealed cartridges were opened and the fortification solution (10 µL) was dosed directly onto the front layer XAD adsorbent material (layer A, 100 mg adsorption material). The solvent was allowed to evaporate.

The sampling cartridges (including two blank control cartridges) were placed on a SPE station with the fortified layer A at the upper end. A laboratory air pump was attached to the pressure gauge of the station with a tube providing an air flow of 0.83 L/min. The SPE station was placed in a chamber where the air could be heated with a hot air fan. For air sampling under warm and humid conditions, warm air (heated by the fan in the partially closed cabinet) was passed over warm water before diverting the humidified air flow over the sampling cartridges. The water load of the air was calculated from the water loss in the round bottomed flask. The total relative humidity of the air passing through the sampling cartridges was obtained as the sum of the original humidity measured with a humidity sensor at the air inlet, plus the humidity added by evaporation.

After sampling of air (6 hours, i.e. a total air sampling volume of approx. 0.3 m^3), the cartridges were sealed with the provided plastic plugs. If not analyzed immediately, the cartridges may be stored at freezer temperature for up to 5 days. Storage stability was demonstrated as part of this study by storing two fortified cartridges (at LOQ level) for 5 days in a freezer at approx. -20°C. All solutions were stored in a refrigerator (at approximately ≤ 8 °C) when not in use.

The adsorbent is extracted three times with approx. 3 mL of acetonitrile. The extracts are combined and the volume is adjusted to 10 mL. Then a 100-µL or 50-µL aliquot of the raw extract is further diluted into water with 0.1% formic acid resulting in a final volume of 1.0 mL.

The analyte is determined by liquid chromatography with tandem mass spectrometric detection (LC-MS/MS), monitoring two parent-daughter ion transitions. The pseudomolecular ions of the analytes ($[M+H]^+$) were selected by the first quadrupole. These precursor ions were impulsed with nitrogen in the collision cell (second quadrupole) and the resulting fragment ions (product ions) were separated according to their m/z ratio in the third quadrupole. Two of these product ions per analyte were selected: one product ion (MRM-transition) serving for quantitation and the second for confirmation. The first MRM transition of BYI 02960 is the quantification ion with the mass 126 [m/z 126] and the second MRM transition is the confirmatory ion with the mass 99 [m/z 99]. These parameters are

given only as guidance and were established for the specimens of the present study, using the mentioned apparatus and analytical column.

Results

Recovery Results: Extraction Efficiency and Storage Stability

Specimen Type	Fortified BYI 02960 ug	290 m/z ->	126 m/z Mean *	290 m/z ->	99 m/z Mean *
Extraction Efficiency	2.0	101%	105%	99%	103%
		109%		108%	
Stability of Extracts Refrigerated for 6 days at < 8 °C	2.0	105%	105%	105%	104%
		105%		103%	
Storage Stability of Adsorbed BYI 02960 for 5 days at Freezer Temperature: -19 to -23 °C	2.0	100%	101%	100%	101
		103%		102%	

*: each two specimens included in calculation

Recovery Results: Retention Recoveries

Specimen Type	Fortified BYI 02960	Average CAir ug / m³	290 m/z --> Average Recovery	126 m/z Mean* (RSD)	290 m/z --> Average Recovery	99 m/z Mean* (RSD)
Warm Humid Air (temp. 35 °C, relative humidity 92%)	2.0	6.7 (at LOQ)	109%	109% (0.3%)	106%	108% (1%)
			109%		107%	
			110%		109%	
			110%		109%	
			110%		109%	
	20	67 (at 10xLOQ)	107%	107% (2%)	108%	107% (1%)
			105%		105%	
			110%		109%	
			107%		106%	
			104%		106%	

*: each five specimens included in calculation
Average CAir: Average fortified concentration of BYI 02960 in air.

Limit of quantification (LOQ): The method achieves a limit of quantification (LOQ) of 7 µg BYI 02960/m^3 air. Based on the detectability of 2.0 ng BYI 02960/mL and assuming a theoretical final volume of 100 mL and an air sample volume of approx. 0.3 m^3, the limit of detection (LOD) in the final extracts is estimated to be <0.7 µg/m^3.

Conclusion

The LC-MS/MS based analytical method (report no. P 2419 G) was developed for determination of BYI 02960 in air with target limit of quantitation of 7 µg BYI 02960/m³. The method validation data were in compliance with the guideline requirements for residue data generation and enforcement. Thus, the method is the recommended enforcement method for monitoring of BYI 02960 residues in

air.

Guidelines & Limitations

None.

B.5.4 Analytical methods (residue) for body fluids and tissues (IIA 4.8; IIIA 5.8)

A method of analysis for body fluids and tissues is not required, since the active substance is of low acute toxicity (GHS category 4).

B. 5.5 Evaluation and assessment

Analytical methods for analysis of the active substance as manufactured

The HPLC-method AM008809MP1 for the determination of Flupyradifurone (BYI 02960) in technical grade active substance can be used for determination of Flupyradifurone.

Analytical methods for formulation analysis

The HPLC method AM012609MF1for the determination of Flupyradifurone (BYI 2960) can be used for determination of flypyradifurone in the formulation Flupyradifurone (BYI 2960) SL 200.

Analytical methods for residue analysis

The following table gives an overview on the methods that may be used to monitor relevant flupyradifurone residues (comprising parent flupyradifurone and metabolite DFA) in plant and animal matrices.

Table 5.5.-1 Overview of monitoring methods in plant and animal material for flupyradifurone residues

Matrix group / crop group	Residue definition for monitoring	LOQ (mg/kg)	Methods[1]		
			Primary method	Confirmatory method	Independent lab validation
Dry commodities (high protein/high starch content)	BYI 02960, DFA	0.01 0.02	Method 01330 (LC-MS/MS)	yes	yes
Commodities with high water content	BYI 02960, DFA	0.01 0.02	Method 01330 (LC-MS/MS)	yes	yes
Commodities with high oil content	BYI 02960, DFA	0.01 0.02	Method 01330 M001 (LC-MS/MS)	yes	yes
Commodities with high acid content	BYI 02960, DFA	0.01 0.02	Method 01330 (LC-MS/MS)	yes	yes
Commodities which are difficult to analyse (hops)	BYI 02960, DFA	0.05 0.1	Method 01330 (LC-MS/MS)	yes	no
Milk	BYI 02960, DFA	0.01 0.02	Method 01214 (LC-MS/MS)	yes	yes
Eggs	BYI 02960, DFA	0.01 0.02	Method 01214 (LC-MS/MS)	yes	yes
Meat	BYI 02960, DFA	0.01 0.02	Method 01214 (LC-MS/MS)	yes	yes
Fat	BYI 02960, DFA	0.01 0.02	Method 01214 (LC-MS/MS)	yes	yes
Kidney	BYI 02960, DFA	0.01 0.02	Method 01214 (LC-MS/MS)	yes	yes
Liver	BYI 02960, DFA	0.01 0.02	Method 01214 (LC-MS/MS)	yes	yes

[1] BYI 02960: Two MRM transitions were measured (1st MRM for quantification: 289/126; 2nd MRM for confirmation: 289/90; ESI positive; C18 column)

DFA:	One MRM transition was measured using different HPLC conditions (ZIC-HILIC SeQuant column for quantification; Hypercarb column for confirmation; MRM: 95/51, ESI negative)

The ILV (independent lab validation) for hops (matrix difficult to analyze) is under preparation.

The submitted methods are suitable of monitoring in the relevant matrices.

Residues in body fluids and tissues

A method of analysis for body fluids and tissues is not required, since the active substance is of low acute toxicity (GHS category 4).

5.6 References relied on

References for the active substance

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source GLP or GEP status Published or not	Data Protection Claimed Y/N	Owner

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source GLP or GEP status Published or not	Data Protection Claimed Y/N	Owner
KIIA 4.2.1 /01	zur Muehlen, U.; Wagner, S.	2011	Flupyradifurone (BYI 02960) - Determination of technical grade active substance HPLC - ISTD Bayer CropScience, Report No.: AM008809MP1, Edition Number: M-407218-01-1 Date: 2011-05-09 GLP/GEP: no, unpublished	Y	BCS
KIIA 4.2.1 /02	Wagner, S.	2011	Validation of AM008809MP1 - Flupyradifurone (BYI 02960) - Determination of technical grade active substance HPLC - ISTD Bayer CropScience, Report No.: VB1-AM008809MP1, Edition Number: M-409002-01-1 Date: 2011-06-06 GLP/GEP: no, unpublished	Y	BCS
KIIA 4.3 /01	Schulte, G.; Bauer, J.	2012	Analytical method 01330 for the determination of residues of BYI 02960 and its metabolite difluoroacetic acid in/on plant matrix by HPLC-MS/MS - Enforcement method plant Bayer CropScience, Report No.: 01330, Edition Number: M-425848-01-1 Method Report No.: 01330 Date: 2012-02-22 GLP/GEP: Y, unpublished	Y	BCS
KIIA 4.3 /02	Konrad, S.	2012	Independent lab validation of BCS method 01330 for the determination of residues of BYI 02960 and its metabolite difluoroacetic acid in/on plant matrices by HPLC-MS/MS Currenta GmbH & Co. OHG, Leverkusen, Germany Bayer CropScience, Report No.: 2011/0134/01, Edition Number: M-427133-01-1 Method Report No.: 2011/0134/01 Date: 2012-02-28 GLP/GEP: Y, unpublished	Y	BCS
KIIA 4.3 /03	Li, Y.; Schoening, R.	2011	Amendment No. 1 - Validation of Bayer CropScience method RV-001-P10-02 - An analytical method for the determination of residues of BYI 02960, 6-chloronicotinic acid, difluoroacetic acid, and difluoroethyl-amino-furanone in plant matrices using LC/MS/MS Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: RARVP013, Edition Number: M-415504-02-1 Method Report No US: RARVP013 Date: 2011-10-12 **...Amended: 2012-01-11** GLP/GEP: Y, unpublished	Y	BCS

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source GLP or GEP status Published or not	Data Protection Claimed Y/N	Owner
KIIA 4.3 /04	Justus, K.	2011	Extraction efficiency testing of the residue analytical method RV-001-P10-02 for the determination of BYI 02960, 6-chloronicotinic acid, difluoroacetic acid and difluoroethyl-amino-furanone in plant matrices using aged radioactive residues Bayer CropScience, Report No.: MEF-11/793, Edition Number: M-419323-01-1 Date: 2011-12-01 GLP/GEP: Y, unpublished	Y	BCS
KIIA 4.3 /06	Schulte, G.; Bauer, J.	2012	Analytical method 01214 for the determination of residues of BYI 02960 and its metabolite difluoroacetic acid in/on animal matrices by HPLC-MS/MS - Enforcement method animal Bayer CropScience, Report No.: 01214, Edition Number: M-425837-01-1 Method Report No.: 01214 Date: 2012-02-22 GLP/GEP: Y, unpublished	Y	BCS
KIIA 4.3 /07	Konrad, S.	2012	Independent lab validation of BCS method 01214 for the determination of residues of BYI 02960 and its metabolite difluoroacetic acid in/on animal matrices by HPLC-MS/MS Currenta GmbH & Co. OHG, Leverkusen, Germany Bayer CropScience, Report No.: 2011/0164/01, Edition Number: M-427160-01-1 Method Report No.: 2011/0164/01 Date: 2012-02-28 GLP/GEP: Y, unpublished	Y	BCS
KIIA 4.4 /01	Brumhard, B.: Reineke, A.	2009	Analytical method 01074 for the determination of BYI 02960 in soil using LC/MS/MS Bayer CropScience, Report No.: 01074, Edition Number: M-337752-01-1 Method Report No.: MR-07/337 Date: 2009-02-24 GLP/GEP: Y, unpublished	Y	BCS
KIIA 4.5 /01	Fargeix, G.; Rosati, D.	2012	Analytical method no 01213 for the determination of residues of BYI 02960 in drinking and surface water by HPLC-MS/MS Bayer S.A.S., Bayer CropScience, Lyon, France Bayer CropScience, Report No.: 01213, Edition Number: M-428019-01-1 Method Report No.: MR-12/022 Date: 2012-03-29 GLP/GEP: Y, unpublished	Y	BCS

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source GLP or GEP status Published or not	Data Protection Claimed Y/N	Owner
KIIA 4.7 /01	Heinz, N.	2011	BYI 02960: Analytical method for determination in air PTRL Europe GmbH, Ulm, Germany Bayer CropScience, Report No.: P 2419 G, Edition Number: M-420657-01-1 Date: 2011-12-14 GLP/GEP: Y, unpublished	Y	BCS

European Commission

Flupyradifurone

VOLUME 3

ANNEX B

B.6 Toxicology and metabolism

Rapporteur Member State: The Netherlands

January 2014 November 2014 December 2014

Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible approval of flupyradifurone under Regulation (EU) No 1107/2009.

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B.6 TOXICOLOGY AND METABOLISM

B.6.1 ABSORPTION, DISTRIBUTION, EXCRETION AND METABOLISM (TOXICOKINETICS) (ANNEX IIA 5.1)

Data requirement 2.1:

6-CNA cannot be regarded as a major metabolite in the rat studies (the level of 6.3%of the administered dose was obtained only in males treated with 200 mg/kg bw and was indiscriminately found in urine and faeces). Pending on the fate and behaviour section conclusion on 6-CNA calculated levels in groundwater, further toxicological data may be needed for this metabolite; see reporting table 2(1).

The toxicity of 6-CNA is assumed to be covered by the endpoints derived for the parent compound since 6-CNA and its succeeding metabolite BYI 02960-hippuric acid - which can be formed from 6-CNA only – represent a prominent portion in the urine of male rats.

The two metabolites, 6-CNA (0.4% to 6.0%) and BYI 02960-hippuric acid (1.1% to 10.4%) were detected in the urine of male and female rats after administration of [pyridinylmethyl-^{14}C]BYI 02960 at dose levels of 2 and 200 mg/kg bw, as well as in male rats after i.v. administration (female rats were not tested). The two metabolites represented a prominent portion in male rats, however showed lower concentrations in female rats.

	Proportion of dose detected in urine (% of total dose administered)[1]				
Compound	**Male, p.o. 2 mg/kg bw**	**Female, p.o. 2 mg/kg bw**	**Male, p.o. 200 mg/kg bw**	**Female, p.o. 200 mg/kg bw**	**Male, i.v. 2 mg/kg bw**
6-CNA	2.3	0.4	6.0	1.3	2.7
BYI 02960-hippuric acid	7.4	1.1	10.4	2.2	5.1
Sum	**9.7**	**1.5**	**16.4**	**3.5**	**7.8**

[1] Klempner, A.; 2012: [Pyridinylmethyl-^{14}C]BYI 02960 – Absorption, Distribution, Excretion, and Metabolism in the Rat, Report No. MEF 11/747, Document No. M-422210-01-1

In sum the metabolites represented 8% to 16% of the administered dose in male rats. Since BYI 02960-hippuric acid can be formed from 6-CNA only, the proportion of BYI 02960-hippuric acid can be added to the 6-CNA proportion to estimate the proportion of 6-CNA which was systemically available in the rat. When considering the two metabolites together, 6-CNA accounted for ≥ 10% of the dose administered dose in male rats after oral administration. Thus, 6-CNA was present in a sufficient concentration to contribute to the toxicological effects when testing the parent compound. Since the toxicological endpoints derived for parent compound were not dependent on the sex of the animals, it

can be concluded that the different metabolic behaviour of male and female rats had no influence on the toxicological profile.

In the fate section, 6-CNA was identified as a potential groundwater metabolite, however when calculating the worst case PECgw concentrations, no assessment of relevance has been triggered. The worst-case PECgw values were below the trigger concentration of 0.1 µg/L for all intended uses in Europe.

Results from expert consultation 2.1

6-CNA is mentioned as intermediate forming hippuric acid in the rat metabolic pathway. Adding hippuric acid to 6-CNA in the metabolism studies result in 6-CNA being a major rat metabolite in males, but not in females. It was agreed to sum up 6-CNA and hippuric acid for males and females. It was noted that in females both metabolites are not major rat metabolites, even when summing them up (at any dose level in the ADME studies). For 6-CNA, oral LD_{50} > 2000 mg/kg bw (less acutely toxic than the parent), no data are available for hippuric acid. This is not sufficient to conclude on the toxicity of the metabolites for the females, however for males, the experts agreed that the toxicity of the parent would cover the toxicity exerted by the metabolite 6-CNA.

B.6.1.1 Toxicokinetic studies – single dose, oral route in rats

Seven studies are summarised in this chapter; two with the pyridinylmethyl-^{14}C label (study 1 and 2), three with the furanone-^{14}C label (study 3-5), and two with the ethyl-1-^{14}C label (study 6 and 7). All are single dose studies.

Pyridinylmethyl-^{14}C Flupyradifurone

Furanone-4-^{14}C flupyradifurone

Ethyl-1-^{14}C flupyradifurone

Study 1 is an ADME-study in which male and female rats were orally administered with a single low (2 mg/kg/bw) or high dose (200 mg/kg bw) of flupyradifurone. Additionally, male rats were administered intravenously with flupyradifurone (2 mg/kg bw). The excretion via urine and faeces was investigated,

as well as the distribution in the plasma and the radioactivity concentration in organs and tissues after sacrifice. The metabolism was investigated in urine and faeces.

Study 2 is a quantitative whole body autoradiography study following a single oral dose of flupyradifurone (5 mg/kg bw) to male and female rats. At various time points the excretion of radioactivity was determined in urine, faeces and expired air, as well as the distribution in the plasma and the radioactivity concentration in the organs and tissues.

Study 3 is an ADME-study in which male and female rats were orally administered with a single dose of flupyradifurone (2 mg/kg bw). The excretion via urine and faeces was investigated, as well as the distribution in the plasma and the radioactivity concentration in organs and tissues after sacrifice. The metabolism was investigated in urine and faeces.

Study 4 is a quantitative whole body autoradiography study following a single oral dose of flupyradifurone (5 mg/kg bw) to male and female rats. At various time points the excretion of radioactivity was determined in urine, faeces and expired air, as well as the distribution in the plasma and the radioactivity concentration in the organs and tissues.

Study 5 is an organ metabolism study. Male and female rats were orally administered with a single dose of flupyradifurone (3 mg/kg bw) and sacrificed after 6 hours. The metabolism was investigated in urine, plasma, and in extracts of liver, kidney, muscle and fat tissues.

Study 6 is an ADME-study in which male rats were orally administered with a single dose of flupyradifurone (2 mg/kg bw). The excretion via urine and faeces was investigated, as well as the distribution in the plasma and the radioactivity concentration in organs and tissues after sacrifice. The metabolism was investigated in urine and faeces.

Study 7 is an organ metabolism study. Male and female rats were orally administered with a single dose of flupyradifurone (3 mg/kg bw) and sacrificed after 1, 6 or 24 hours. The metabolism was investigated in urine, plasma, and in extracts of liver, kidney, muscle and fat tissues.

As all single dose experiments revealed no indication of a potential for retention, accumulation and/or persistence of the administered radioactivity in organs or tissues. in line with paragraph 26 of the OECD Test Guideline 417, a repeated dose study was not considered necessary. This observation is supported by the low log P_{OW} of flupyradifurone of 1.2.

The different studies and their results are described in more detail below.

Study 1

Characteristics

Reference	:	[redacted]	exposure	:	Single exposure by gavage or iv
type of study	:	Toxicokinetic study (ADME in rat)	dose	:	2 mg/kg or 200 mg/kg bw (gavage) 2 mg/kg bw (iv)
year of execution	:	2009-2011	vehicle	:	0.5% aqueous Tragacanth® suspension (gavage), water (iv)
test substance	:	[pyridinylmethyl-^{14}C]-flupyradifurone, batch no. KATH 6264 (test 1-4) and KATH 6351 (test 5), chemical purity >99%, radiochemical purity >99%, 4.37 MBq/mg	GLP statement	:	yes
Route	:	oral, iv	guideline	:	OECD 417
Species	:	Rat Wistar Hsd/Cpb: WU	acceptability	:	acceptable
group size	:	4/dose/sex (oral), 4 males (iv)			

Study design

The study was performed according to OECD Guideline 417. Male and female rats in groups of four rats/sex were administered orally by gavage a single dose of flupyradifurone suspended in 0.5% aqueous Tragacanth® (2 mL) at target dose levels of 2 mg/kg bw or 200 mg/kg bw. In addition, a group of four male rats was administered intravenously with a single dose of flupyradifurone in water (0.5 mL) at a dose level of 2 mg/kg bw (Table 6.1.1.-01). Flupyradifurone was radiolabelled with ^{14}C in the pyridinylmethyl bridge of the molecule.

* denotes the ^{14}C-label position

Urine and faeces were collected for the time range between dosing and sacrifice and the animals were sacrificed after 72 hours. Plasma levels of radioactivity were followed from each animal for 17 timepoints. Total radioactivity which included the test compound and metabolites was determined in excreta and in organs and tissues collected at sacrifice. Metabolism was investigated in urine and faeces.

Samples were analyzed by radio HPLC, radio TLC, LC-MS and NMR methods. Toxicokinetic parameters were calculated with the software TOPFIT version 2.0 where the dose normalized values were used for better comparison of the low tests among themselves and to the high dose tests.

Table 6.1.1.-01. Experimental groups for each dose level of [pyridinylmethyl-^{14}C]-flupyradifurone

Group no.	No. of animals	Route	Dose	Sacrifice time (hoiurs after last dose)
1	4 M	Oral (gavage)	2 mg/kg bw	72
2	4 F	Oral (gavage)	2 mg/kg bw	72
3	4 M	Oral (gavage)	200 mg/kg bw	72
4	4 F	Oral (gavage)	200 mg/kg bw	72
5	4 M	I.v.	2 mg/kg bw	72

Results

Recovery

The total recovery for the orally administered test was between approximately 97% and 103% of the administered dose in the excreta and the body of male and female rats. The total recovery was approximately 91% of the administered dose in the low dose intravenous administrated male rats.

Table 6.1.1.-02. Recovery of radioactivity in urine, gastrointestinal tract and the body following oral or intravenous dosing of [pyridinylmethyl-^{14}C] flupyradifurone

	% of the administered radioactivity				
	Male 2 mg/kg bw (oral)	Female 2 mg/kg bw (oral)	Male 200 mg/kg bw (oral)	Female 200 mg/kg bw (oral)	Male 2 mg/kg bw (i.v.)
Faeces	23.09	7.49	26.14	10.32	14.64
Urine	75.45	90.07	76.26	85.95	76.24
Sum of excreta	**98.55**	**97.56**	**102.40**	**96.26**	**90.88**
Body without GIT	0.119	0.064	0.128	0.241	0.141
GIT	0.069	0.010	0.086	0.064	0.086
Total in body	**0.188**	**0.074**	**0.214**	**0.306**	**0.227**
Balance	**98.73**	**97.63**	**102.60**	**96.57**	**91.11**

Absorption

The bioavailability factor was determined from plasma kinetics of the oral and i.v. low dose test with male rats (group 1 and 5). A bioavailability factor of F = 0.93 was obtained demonstrating that the orally administered test compound was almost completely bioavailable. This high bioavailability factor was confirmed by the high radioactivity (76% to 90%) detected in the urines and the bodies without

GIT of male and female rats. The absorption started immediately after dosing as could be seen from the quick increase of radioactivity in plasma.

Table 6.1.1.-03.Time course of radioactivity in the plasma following oral or intravenous dosing of [pyridinylmethyl-^{14}C] flupyradifurone

Time [h]	Concentration [mg a.s. equiv. /kg]				
	Male 2 mg/kg bw (oral)	Female 2 mg/kg bw (oral)	Male 200 mg/kg bw (oral)	Female 200 mg/kg bw (oral)	Male 2 mg/kg bw (i.v.)
0.17	0.4465	0.5287	32.5100	30.9100	1.6070
0.33	1.0380	1.2010	66.3400	57.2100	1.6970
0.67	1.5690	1.7240	88.9400	74.9300	1.7670
1	1.7130	1.8540	94.3200	84.4500	1.7060
1.5	1.6740	1.7920	96.0300	87.7900	1.5570
2	1.5500	1.7630	96.8600	92.7700	1.3850
3	1.3410	1.6100	95.6200	98.3200	1.1180
4	1.1990	1.4500	96.3100	99.9900	0.8976
6	0.7990	1.1350	80.6500	91.7700	0.6250
8	0.5281	0.8420	69.2600	81.9800	0.4357
24	0.0200	0.0354	6.4460	22.4000	0.0193
28	0.0134	0.0228	3.3800	17.8300	0.0157
32	0.0100	0.0157	2.1660	10.1800	0.0103
48	---	0.0052	0.6626	1.4800	0.0062
52	---	---	0.6165	1.2700	0.0053
46	---	---	0.5762	0.9593	0.0038
72	---	---	0.3602	0.5697	0.0044

Distribution and plasma kinetics

Flupyradifurone was quickly absorbed and distributed. The C_{max} was reached at approximately 1 hour after administration at the low dose tests (Figure 6.1.1.-01). In the high dose tests C_{max} was reached approximately 2 hours for the males and at approximately 4 hours for the females. The plasma concentrations declined to 50% of C_{max} within 4 - 8 hours and to 1 - 2% of C_{max} within 24 hours in the low dose test with both male and female rats. In the high dose test the plasma concentrations declined to 50% within 8 to 24 hours and to 1-2% of C_{max} after 48 hours. At time of sacrifice, 72 hours after administration, the plasma concentrations were below or around LOQ for the low dose animals and approximately 0.5% of C_{max} for the high dose animals. An overview of all important results of the TOPFIT analysis is presented in table 6.1.1.-04.

Figure 6.1.1.-01.Comparison of dose normalised plasma curves following oral administration of low and high doses of [pyridinylmethyl-^{14}C] flupyradifurone to male and female rats

Table 6.1.1.-04. Pharmacokinetic parameters and calculation of the bioavailability of [pyridinylmethyl-^{14}C] flupyradifurone after oral administration to male and female rats, and after i.v. administration to male rats

	Male 2 mg/kg bw (oral)	Female 2 mg/kg bw (oral)	Male 200 mg/kg bw (oral)	Female 200 mg/kg bw (oral)	Male 2 mg/kg bw (i.v.)
t_{max} measured [h]	1.0	1.0	2.0	4.0	0.67
t_{max} calculated [h]	1.13	1.15	2.23	3.35	0.38
C_{max} measured values	0.878	0.937	0.497	0.578	1.04
C_{max} calculated (fitted) values	0.880	0.929	0.500	0.582	1.0400
$t_{1/2\ abs}$ [h]	0.21	0.17	0.13	0.17	0.06
$t_{1/2\ elim\ 1}$ [h]	3.9	3.0	3.6	8.1	3.8
$AUC_{0-\infty}$ [mg/L·h]	6.10	7.96	6.16	9.73	6.55
MRT_{tot} [h]	6.07	6.69	8.70	13.10	5.71

The biokinetic parameters were comparable in the three low dose tests. Only the AUC value for females of the low dose test was approx. 1.3 times higher than for males, which is related to the slightly broader peak maximum of the plasma curve of female rats. In the high dose tests, the AUC

value for males was proportional to the dose. For females, the AUC value of the high dose test was approx. 1.2 times higher than for the low dose test with females and approx. 1.6 times higher than for the high dose test with males. According to the broader maximum of the plasma curve of female rats in the high dose test, C_{max} was reached later than in males (approx. 4 hours instead of 2 hours). The biokinetic model confirmed a fast absorption phase in all tests with short half-lives of max. 0.2 hours followed by a short elimination phase with half-lives of about 3 – 4 hours. Only females in the high dose test showed a slightly longer elimination phase with a half-life of about 8 hours. The mean residence times in the low dose tests were in the range of approx. 6 – 7 hours. The mean residence times were slightly longer in the high dose tests and amounted to about 9 hours for males and 13 hours for females.

Excretion

Approximately 76% of the administered dose was detected in the urine of male rats, for low or high oral dose and low i.v. dose. For females 90% and 86% was detected in urine in the low and high dose, respectively. The major route of excretion is therefore by urine. Faecal excretion accounted for ca. 7 - 10% of the given dose in females and was slightly higher for males, where between approximately 15 to 26% was excreted with the faeces. Also the major part of faecal residues, approx. 89 - 92% of the radioactivity in faeces in the low dose tests and approximately 47% (females) and 77% (males) in the high dose tests, was excreted within the first day after treatment and was almost complete 48 hours post administration. The cumulative excretion results are summarized in Table 6.1.1-05.

Table 6.1.1.-05. Cumulative excretion of radioactive residues via urine and faeces after oral administration of [pyridinylmethyl-^{14}C] flupyradifurone to male and female rats, and after i.v. administration to male rats

Time [h]	% of dose				
	Male 2 mg/kg bw (oral)	Female 2 mg/kg bw (oral)	Male 200 mg/kg bw (oral)	Female 200 mg/kg bw (oral)	Male 2 mg/kg bw (i.v.)
Faeces					
24	20.55	6.86	20.22	4.90	13.31
48	22.82	7.38	25.84	9.64	14.41
72	23.09	7.49	26.14	10.32	14.64
Urine					
4	17.25	5.34	14.89	9.50	26.12
8	40.99	33.80	31.33	28.56	44.02
12	51.67	---	---	---	---
24	72.61	86.95	68.26	72.15	73.80
48	75.15	89.77	75.56	84.15	75.75
72	75.45	90.07	76.26	85.95	76.24
Total excreted	**98.55**	**97.56**	**102.40**	**96.26**	**90.88**

Radioactive residues in organs and tissues at sacrifice

Approximately 0.1 - 0.3% of the total dose was detected in the body of male and female rats at the time of sacrifice 72 hours after oral and i.v. administration; only a trace amount of 0.01 - 0.09% was found in the GIT. The residual concentrations of radioactivity were low in the low dose tests and ranged from 0.0007 to 0.0175 mg/kg. The highest concentrations were detected in blood cells and the GIT, and in the eyes of female rats. But basically, levels for most organs and tissues were very similar and in the range of approx. 0.001 to 0.007 mg/kg. The residual concentrations of the high dose tests were almost dose proportional and ranged from 0.0859 to 2.345 mg/kg. The highest concentrations were also detected in blood cells and the GIT, as well as in the eyes of female rats. For most organs and tissues the residue concentrations in males were slightly higher as compared to females.
The equivalent concentrations of the residual radioactivity in organs and tissues at sacrifice are presented in Table 6.1.1-06.

Table 6.1.1.-06. Total radioactive residues in organs and tissues at sacrifice after administration of [pyridinylmethyl-^{14}C] flupyradifurone to male and female rats.

Organ or tissue	Concentration [mg a.s. equiv. /kg]				
	Male 2 mg/kg bw (oral)	Female 2 mg/kg bw (oral)	Male 200 mg/kg bw (oral)	Female 200 mg/kg bw (oral)	Male 2 mg/kg bw (i.v.)
Blood cells	0.0175	0.0067	2.3450	1.5770	0.0158
Plasma	0.0020	0.0013	0.2989	0.2963	0.0025
Carcass	0.0021	0.0011	0.1794	0.2377	0.0020
Heart	0.0024	n.c.	0.2643	0.3328	0.0023
Brain	0.0008	n.c.	0.0859	0.0922	0.0008
Kidneys	0.0064	0.0033	0.7975	0.6691	0.0067
Liver	0.0068	0.0034	0.8741	0.7720	0.0063
GIT	0.0141	0.0019	1.7290	1.1450	0.0167
Testes	0.0008	---	0.1020	---	0.0011
Ovaries	---	n.c.	---	0.2880	---
Uterus	---	0.0016	---	0.4599	---
Adrenal gland	0.0048	0.0032	0.4436	0.5606	0.0045
Harderian gland	0.0050	0.0022	0.4101	0.7218	0.0034
Thyroid	n.c.	n.c.	n.c.	n.c.	n.c.
Spleen	0.0030	0.0017	0.3586	0.3398	0.0032
Lung	0.0060	0.0035	0.6648	0.5663	0.0054
Eyes	0.0064	0.0133	0.5996	1.3430	0.0066
Skin	0.0018	0.0014	0.2354	0.9710	0.0024
Bone (femur)	n.c.	0.0023	0.1771	0.2794	0.0024
Fat (perirenal)	0.0018	n.c.	n.c.	0.1245	0.0030
Muscle (leg)	0.0008	0.0007	0.1206	0.2098	0.0012

n.c. : not calculated

Metabolites in urine and faeces

Flupyradifurone was metabolized to numerous metabolites, most of them being minor ones. The parent compound was the predominant part of the radioactivity in urine of male and female rats. In faeces samples of male rats, the metabolite BYI 02960-OH was more prominent than the parent compound. Two metabolites, BYI 02960-6-CNA and BYI 02960-hippuric acid were also prominent in male rats but not in females. All other identified and characterised metabolites represented a minor part of the dose. The metabolic profiles in urine and faeces were very similar for both sexes but male rats showed a higher rate of metabolite formation as compared to female animals. The metabolism results are summarized in the Table 6.1.1-07.

Table 6.1.1.-07. Identified metabolites in urine and faeces after administration of [pyridinylmethyl-^{14}C] flupyradifurone to male and female rats.

Peak no.	Compound	% of the given dose				
		Male 2 mg/kg bw (oral)	Female 2 mg/kg bw (oral)	Male 200 mg/kg bw (oral)	Female 200 mg/kg bw (oral)	Male 2 mg/kg bw (i.v.)
26	Parent compound	40.9	77.7	39.6	65.5	47.3
7	BYI 2960-6-CNA	2.4	0.4	6.3	1.3	2.8
9	Hippuric acid	7.6	1.1	10.5	2.2	5.1
14	BYI 2960-OH-gluA (isomer 1)	1.8	0.4	1.6	0.7	1.6
16	BYI 2960-OH-gluA (isomer 3)	2.4	0.4	2.3	1.1	1.0
18	BYI 2960-des-difluoroethyl	2.2	2.4	1.8	2.7	1.7
24	BYI 2960-OH-SA	0.2	0.3	0.5	0.5	0.2
25	BYI 2960-OH	28.9	10.8	24.0	15.1	22.3
27	BYI 2960-iso-OH	0.4	<0.1	0.4	0.1	0.5
	Total identified	**86.9**	**93.7**	**86.9**	**89.2**	**82.5**
1	unknown 1	0.2	<0.1	0.3	0.1	0.3
2	unknown 2	0.7	0.1	1.1	0.3	0.6
3	unknown 3	0.5	0.1	0.5	0.1	0.4
4	unknown 4	0.3	0.1	0.5	0.3	0.3
5	unknown 5	0.3	0.1	0.2	0.1	0.1
5a	unknown 6	0.2	---	0.1	---	---
6	unknown 7	0.5	0.2	0.2	0.1	0.3
8	unknown 8	0.2	0.2	0.5	0.3	0.3
10	unknown 9	0.4	<0.1	0.3	0.1	0.2
11	unknown 10	0.1	0.1	0.4	0.3	0.2
12	unknown 11	0.9	0.3	0.8	0.6	0.5
13	unknown 12	0.2	0.1	0.2	0.2	0.3
15	unknown 13	0.4	0.3	0.4	0.2	0.3
17	unknown 14	0.2	0.2	0.3	0.2	0.2

19	unknown 15	---	0.1	---	0.2	---
20	unknown 16	0.5	0.1	0.6	0.1	0.5
21	unknown 17	---	---	---	---	0.1
22	unknown 18	---	---	<0.1	---	0.1
23	unknown 19	0.2	0.1	0.2	0.1	0.2
	Total characterized	**5.8**	**2.1**	**6.7**	**3.4**	**4.7**
	Total	**92.7**	**95.9**	**93.7**	**92.7**	**87.2**

Acceptability

The study was considered acceptable.

Conclusions

Flupyradifurone was completely absorbed in all test as demonstrated by the high bioavailability after oral administration and by the high amounts of radioactivity detected in urine and the body without GIT at sacrifice. Excretion of radioactivity was fast and mainly renal. Female rats showed slightly higher renal excretion rates of approx. 86% and 90% of the administered dose than males with approx. 76% of the dose. The major part of the dose (>84%) was excreted within 24 hours after treatment. The parent compound, three major and five minor metabolites were identified in all samples. Identification rates were high and amounted to approx. 83% - 94% of the given dose. The metabolic transformation of flupyradifurone was principally oxidative in nature and took place at least at 3 different structural positions.

<u>Study 2</u>

Characteristics

Reference	:	[redacted]	exposure	:	Single exposure by gavage
type of study	:	Toxicokinetic study (Quantitative whole body autoradiography)	dose	:	5 mg/kg bw
year of execution	:	2009-2011	vehicle	:	0.5% aqueous Tragacanth® suspension
test substance	:	[pyridinylmethyl-^{14}C]-Flupyradifurone, batch no. KATH 6264, chemical purity >99%, radiochemical purity >99%, 4.37 MBq/mg	GLP statement	:	yes
Route	:	oral	guideline	:	OECD 417
Species	:	Rat Wistar Hsd/Cpb: WU	acceptability	:	acceptable
group size	:	8 males + 1 control, 8 females + 1 control			

Study design

The study was performed according to OECD Guideline 417. Male and female rats in groups of eight rats were administered orally by gavage a single dose of [pyridinylmethyl-^{14}C]-flupyradifurone at target

dose levels of 5 mg/kg bw after about 16 hours of fasting. A control animal of each gender was treated with 5 mg/kg bw non-radiolabelled flupyradifurone (Table 6.1.1.-08).

* denotes the ^{14}C-label position

After administration of [pyridinylmethyl-^{14}C]-flupyradifurone, the rats were kept individually, which allowed for separate and quantitative collection of urine, faeces, and expired air. Urine was collected separately for each animal in intervals of 1h, 4 h, 8 h, 24 h, and every 24 h until 168 h. The radioactivity was determined by LSC. The faeces samples were collected every 24 h separately for each animal. The radioactivity was determined by combustion/LSC. Carbon dioxide and other volatiles from expired air were collected from four male and four female animals for the time ranges 0 - 24 h and 24 - 48 h. At sampling, the exact volume was determined, from which an aliquot was taken for the determination of radioactivity by LSC.

One animal of each gender was sacrificed using carbon dioxide at 1, 4, 8, 24, 48, 72, 120, and 168 hours post administration. The control animals were sacrificed 4 hours after dosing. The distribution of total radioactivity to, and elimination from blood, organs and tissues was analyzed qualitatively and quantitatively by whole-body auroradioluminography.

Table 6.1.1.-08. Experimental groups for each dose level of [pyridinylmethyl-^{14}C]-flupyradifurone

Group no.	No. of animals	Route	Dose	Sacrifice time (hoiurs after last dose)
1	8 M	Oral (gavage)	5 mg/kg bw	1, 4, 8, 24, 48, 72, 120, 168
	1 M (control)	Oral (gavage)	5 mg/kg bw	4
2	8 F	Oral (gavage)	5 mg/kg bw	1, 4, 8, 24, 48, 72, 120, 168
	1 F (control)	Oral (gavage)	5 mg/kg bw	4

Results

Distribution

For almost all organs and tissues, maximum concentrations were reached at one hour after administration. At t_{max}, the organ/blood concentration ratios for many organs and tissues were in a range of approx. 0.8 - 1.2, *i.e.* the radioactivity concentration in these organs and tissues was in the range of ± 20% of the concentration in blood. The radioactivity administered with [pyridinylmethyl-^{14}C] flupyradifurone was rapidly cleared from the blood and distributed to the entire body, preferably to

those organs or tissues responsible for metabolism (liver), excretion (kidney) and secretion (*e.g.* adrenal, thyroid, Harderian and salivary glands). The lowest values were measured for perirenal fat, the spinal cord, and brain.

The equivalent concentrations in blood, organs and tissues declined rapidly following approximately first order kinetics in the time range between 1 and 48 hours. At 24 hours after dosage, the concentrations in blood and also in most other tissues were below 5% of the maximum concentration, except nasal mucosa and vitreal body with approx. 7 - 11% of CEQ_{max}. At 48 hours after administration, the concentrations had declined to below 1% of the maximum concentration, except vitreal body and nasal mucosa which exhibited approx. 3 - 8% of $CEQ_{max.}$ After seven days, the equivalent concentrations in almost all organs and tissues had fallen below the limit of quantification. Very low concentrations of radioactivity were still detectable in blood, renal medulla, liver, and lung. For all these organs, the concentration was lower than in the blood. The results are summarized in Table 6.1.1.-09 (male rats) and Table 6.1.1.-10 (female rats) .

Table 6.1.1.-09. Distribution of radioactivity in organs and tissues of male rats after a single oral dose of [pyridinylmethyl-^{14}C] flupyradifurone (5 mg/kg bw)

Organ or tissue	concentration [mg a.s. equiv./kg]							
	Time of sacrifice [hours after administration]							
	1 h	4 h	8 h	24 h	48 h	72 h	120 h	168 h
Blood	3.171	2.376	1.692	0.122	0.026	0.020	0.013	0.009
Liver	5.645	4.326	3.332	0.246	0.027	0.014	0.008	0.005
Renal cortex	4.441	3.267	2.565	0.146	0.016	0.007	0.005	<LOQ
Renal medulla	6.398	4.538	3.680	0.248	0.021	0.013	0.008	0.006
Kidney total	5.420	3.903	3.122	0.197	0.019	0.010	0.006	0.005
Brown fat	3.288	2.479	2.023	0.098	0.007	---	---	---
Perirenal fat	0.446	0.351	0.219	0.013	<LOQ	<LOQ	<LOQ	<LOQ
Skeleton muscle	3.246	2.283	1.608	0.096	0.005	<LOQ	<LOQ	<LOQ
Myocardium	4.426	3.155	2.250	0.142	0.007	<LOQ	<LOQ	<LOQ
Lung	2.450	2.168	1.373	0.082	0.015	0.013	0.005	0.005
Spleen	2.873	2.093	1.557	0.096	0.010	<LOQ	<LOQ	<LOQ
Pancreas	4.089	2.882	2.170	0.129	0.005	<LOQ	<LOQ	<LOQ
Bone marrow	2.770	1.825	1.391	0.083	---	---	---	---
Testis	2.251	2.198	1.466	0.092	<LOQ	<LOQ	<LOQ	<LOQ
Brain	1.214	0.852	0.619	0.036	<LOQ	<LOQ	<LOQ	<LOQ
Spinal cord	1.334	1.004	0.777	0.042	<LOQ	---	---	---
Pituitary gland	3.539	2.435	1.889	0.105	---	---	---	---
Pineal body	3.033	1.488	1.680	0.089	0.005	<LOQ	---	---
Adrenal gland	5.626	4.429	3.338	0.213	0.017	0.007	<LOQ	<LOQ
Thymus	3.173	2.328	1.646	0.096	<LOQ	<LOQ	<LOQ	<LOQ
Thyroid gland	4.252	3.284	2.219	0.143	0.012	<LOQ	---	---
Salivary gland	4.123	3.097	2.195	0.131	0.008	<LOQ	<LOQ	<LOQ

Nasal mucosa	1.409	1.477	1.210	0.250	0.120	0.077	0.043	0.037
Vitreal body	1.575	1.370	1.459	0.273	0.073	0.036	0.011	<LOQ
Harderian gland	4.469	3.083	2.415	0.151	0.014	---	---	---

--- : Organ or tissue was visible in the rat sections but not discernible in the radioluminograms
< LOQ : below limit of quantification

Table 6.1.1.-10. Distribution of radioactivity in organs and tissues of female rats after a single oral dose of 5 [pyridinylmethyl-^{14}C] flupyradifurone (mg/kg bw)

Organ or tissue	concentration [mg a.s. equiv./kg]							
	Time of sacrifice [hours after administration]							
	1 h	4 h	8 h	24 h	48 h	72 h	120 h	168 h
Blood	4.155	3.211	2.005	0.090	0.016	0.012	0.010	0.007
Liver	7.451	5.878	3.778	0.171	0.016	0.008	0.006	<LOQ
Renal cortex	5.460	4.414	2.834	0.124	0.009	0.005	0.005	<LOQ
Renal medulla	8.212	7.083	4.877	0.222	0.013	0.009	0.007	0.008
Kidney total	6.836	5.748	3.856	0.173	0.011	0.007	0.006	0.006
Brown fat	3.229	3.174	1.811	0.095	<LOQ	---	---	---
Perirenal fat	0.413	0.502	0.315	0.019	<LOQ	<LOQ	<LOQ	<LOQ
Skeleton muscle	3.746	3.140	1.926	0.075	<LOQ	<LOQ	<LOQ	<LOQ
Myocardium	5.198	4.474	2.701	0.105	0.006	<LOQ	<LOQ	<LOQ
Lung	3.230	1.142	1.311	0.054	0.008	0.007	0.005	0.005
Spleen	3.742	2.975	1.932	0.077	<LOQ	<LOQ	<LOQ	<LOQ
Pancreas	5.245	3.961	2.626	0.100	<LOQ	---	<LOQ	<LOQ
Bone marrow	2.843	2.344	1.476	0.062	---	---	---	---
Ovary	3.688	3.101	1.915	0.077	0.006	<LOQ	<LOQ	---
Uterus	3.950	3.262	2.052	0.081	<LOQ	<LOQ	<LOQ	<LOQ
Brain	1.279	1.052	0.626	0.026	<LOQ	<LOQ	<LOQ	<LOQ
Spinal cord	1.348	1.074	0.692	0.029	<LOQ	---	---	---
Pituitary gland	4.306	3.793	2.293	0.082	---	---	---	---
Pineal body	3.427	2.797	1.914	0.075	---	---	---	---
Adrenal gland	7.289	5.669	4.029	0.201	0.017	0.008	<LOQ	<LOQ
Thymus	4.090	3.180	1.971	0.075	<LOQ	<LOQ	<LOQ	<LOQ
Thyroid gland	5.548	4.284	2.809	0.102	0.006	---	---	---
Salivary gland	5.411	4.177	2.642	0.102	0.006	<LOQ	<LOQ	<LOQ
Nasal mucosa	1.585	2.381	1.720	0.164	0.102	0.068	0.047	0.037
Vitreal body	2.303	2.065	1.376	0.248	0.062	0.024	0.009	0.008
Harderian gland	5.008	4.048	2.483	0.107	0.019	<LOQ	---	---

--- : Organ or tissue was visible in the rat sections but not discernible in the radioluminograms
< LOQ : below limit of quantification

Excretion

[Pyridinylmethyl-^{14}C] flupyradifurone was excreted rapidly and completely within ca. 48 hours. The major part of the radioactivity (up to 85% in male and up to 93% in female rats) was excreted with urine and the minor part (about 20% for male and ca. 8% for female rats) with the faeces. One day after dosing >90% of the dose was excreted and the excretion was almost complete after two days. The expiration of ^{14}C-carbon dioxide and other ^{14}C-labelled volatiles was tested with male and female animals for a test period of 48 hours. Less than 0.1% of the administered dose was expired during the sampling period. This demonstrates the stability of the pyridinylmethyl-^{14}C label with regard to possible

formation of volatile products. The excretion behavior is summarized in Table 6.1.1.-11 (male rats) and Table 6.1.1.-12 (female rats).

Table 6.1.1.-11. Excretion of radioactivity in urine, faeces and expired air of male rats after a single oral administration of [pyridinylmethyl-^{14}C] flupyradifurone (5 mg/kg bw)

	% of the given dose							
	Time of sacrifice [h after administration]							
	1	**4**	**8**	**24**	**48**	**72**	**120**	**168**
Exhaled air								
24 h					0.06	0.06	0.07	0.06
48 h					0.07	0.08	0.09	0.09
Urine								
1 h	2.38							
4 h		22.39	12.55	14.62	2.71	2.16	2.03	11.86
8 h			42.07	45.19	23.14	30.59	44.03	29.61
24 h				78.07	68.52	79.11	76.13	75.12
48 h					72.34	84.54	79.85	79.67
72 h						85.12	80.35	80.05
96 h							80.53	80.19
120 h							80.70	80.29
144 h								80.38
168 h								80.50
Faeces								
24 h	*	*	*	14.93	23.63	17.37	20.26	17.49
48 h					26.75	20.34	22.49	21.59
72 h						20.54	22.74	21.92
96 h							22.82	21.98
120 h							22.85	22.04
144 h								22.05
168 h								22.12
Sum total	**2.38**	**22.39**	**42.07**	**92.99**	**99.17**	**105.74**	**103.64**	**102.71**

* : faeces not collected

Table 6.1.1.-12. Excretion of radioactivity in urine, faeces and expired air of female rats after a single oral administration of [pyridinylmethyl-^{14}C] flupyradifurone (5 mg/kg bw)

	Percent of radioactive dose administered							
	Time of sacrifice [h after administration]							
	1	**4**	**8**	**24**	**48**	**72**	**120**	**168**
Exhaled air								
24 h					0.02	0.02	0.02	0.02
48 h					0.03	0.03	0.02	0.03
Urine								
1 h	7.19							
4 h		9.79	9.69	12.35	11.53	3.55	25.51	4.35
8 h			32.74	33.40	27.68	49.84	26.53	4.41
24 h				86.47	84.60	86.82	90.42	78.34
48 h					89.90	88.76	92.95	88.13
72 h						88.94	93.16	89.93
96 h							93.23	91.15
120 h							93.28	91.61
144 h								91.95
168 h								92.46
Faeces								
24 h	*	*	*	10.62	8.61	8.75	6.70	4.54
48 h					10.58	9.75	7.82	6.98
72 h						9.78	7.89	7.36
96 h							7.92	7.47
120 h							7.93	7.49
144 h								7.52
168 h								7.53
Sum total	**7.19**	**9.79**	**32.74**	**97.09**	**100.50**	**98.76**	**101.23**	**100.03**

* : faeces not collected

Acceptability

The study was considered acceptable.

Conclusions

[pyridinylmethyl-^{14}C] flupyradifurone was readily absorbed from the gastrointestinal tract of male and female rats after single oral administration of 5 mg/kg bw. The radioactivity was distributed throughout the body immediately after administration but with a clear preference to liver and kidney. The radioactivity was quickly excreted, mainly with the urine (>80%). More than 90% of the administered dose was excreted within 24 hours, and 95% to 100% after 48 hours. Based on the results of this study, any accumulation or significant retention of [pyridinylmethyl-^{14}C] Flupyradifurone in male and female rats can be excluded.

Study 3

Characteristics

Reference	:	[redacted]	exposure	:	Single exposure by gavage
type of study	:	Toxicokinetic study (ADME in rat)	dose	:	2 mg/kg bw
year of execution	:	2009-2011	vehicle	:	0.5% aqueous Tragacanth® suspension
test substance	:	[Furanone-4-^{14}C]-Flupyradifurone, batch no. KATH 6279, chemical purity >99%, radiochemical purity >99%, 3.94 MBq/mg	GLP statement	:	yes
Route	:	oral	guideline	:	OECD 417
Species	:	Rat Wistar Hsd/Cpb: WU	acceptability	:	acceptable
group size	:	4/sex			

Study design

The study was performed according to OECD Guideline 417. Male and female rats in groups of four rats per sex were administered orally by gavage a single dose of flupyradifurone suspended in 0.5% aqueous Tragacanth® (2 mL) at target dose levels of 2 mg/kg bw (Table 6.1.1-13). Flupyradifurone was radiolabelled with ^{14}C in the 4-position of the furanone ring of the molecule.

* denotes the ^{14}C-label position

Urine and faeces were collected for the time range between dosing and sacrifice and the animals were sacrificed after 168 hours. Plasma levels of radioactivity were followed from each animal for 23 timepoints. Total radioactivity which included the test compound and metabolites was determined in excreta and in organs and tissues collected at sacrifice. Metabolism was investigated in urine and faeces.

Samples were analyzed by radio HPLC, radio TLC, LC-MS and NMR methods. Toxicokinetic parameters were calculated with the software TOPFIT version 2.0.

Table 6.1.1.-13 Experimental groups for each dose level of [Furanone-4-^{14}C] flupyradifurone

Group no.	No. of animals	Route	Dose	Sacrifice time (hoiurs after last dose)
1	4 M	Oral (gavage)	2 mg/kg bw	168

2	4 F	Oral (gavage)	2 mg/kg bw	168

Results

Recovery

The total recovery for the orally administered test was approximately between 96% and 102% of the administered dose in the excreta and the body of male and female rats.

Table 6.1.1-14. Recovery of radioactivity in urine, faeces, gastrointestinal tract and the body following a single oral dose of [furanone-4-^{14}C] flupyradifurone (2 mg/kg bw)

	% of administered dose	
	Male	**Female**
Faeces	16.59	10.38
Urine	78.96	91.37
Sum of excreta	**95.56**	**101.0**
Body without GIT	0.48	0.17
GIT	0.02	0.01
Total Body	**0.49**	**0.18**
Balance	**96.05**	**101.90**

Absorption

The total amount of absorbed [furanone-4-^{14}C] flupyradifurone is at least 79% for males and 91% for females since the sum of the dose in urine and body excluding GIT is 79% and 91%, respectively. The absorption started immediately after dosing as could be seen from the quick increase of radioactivity in plasma.

Table 6.1.1.-15. Time course of radioactivity in the plasma following oral dosing of [furanone-4-^{14}C] flupyradifurone

Time [h]	Concentration [mg a.s. equiv./kg]	
	Male, 2mg/kg	**Female, 2mg/kg**
0.17	0.2883	0.4562
0.33	0.7373	1.1720
0.67	1.2720	1.7870
1	1.4180	1.8570
1.5	1.4570	1.9120
2	1.4460	1.8610
3	1.3570	1.7410
4	1.2550	1.5880

6	1.0220	1.2990
8	0.8145	1.0140
24	0.0621	0.0515
28	0.0462	0.0326
32	0.0375	0.0264
48	0.0168	0.0091
52	0.0162	0.0079
56	0.0149	0.0076
72	0.0113	0.0062
96	0.0087	n.d.
120	0.0066	n.d.
144	0.0048	n.d.
152	0.0048	n.d.
168	n.d.	n.d.

Distribution and plasma kinetics

[Furanone-4-^{14}C] flupyradifurone was quickly absorbed and distributed. The C_{max} was reached at approximately 1.5 hour after administration. The plasma concentrations declined to 50% of C_{max} within 8 hours and to 2 - 3% of C_{max} within 24 hours for both male and female rats. At time of sacrifice, 168 hours after administration, the plasma concentrations were below LOQ for both male and female rats. Male rats exhibited about two-fold higher concentrations than females in the time range ≥48 hours. This behavior is probably caused by higher amounts of [furanone-4-^{14}C] flupyradifurone metabolized to C1 and C2 fragments being incorporated into biomolecules or exhaled as $^{14}CO_2$ in males. At the time of sacrifice, 168 hours after administration, the plasma concentration was below LOQ for both male and female animals (Table 6.1.1.-15).

The mean values of total radioactivity were used for a toxicokinetic modeling using the TOPFIT software. A good fit could only be achieved with a three compartment model due to the obvious biphasic nature of the plasma curves. The modeling was performed for the time range between 0 and 152 hours for males and 0 to 72 hours for females. The biokinetic parameters were very similar for both sexes but females exhibited a somewhat shorter mean residence time than males. The area under curve (AUC) was 16.0 mg/kg*h for male and 18.2 mg/kg*h for female rats. The model confirmed a biphasic decline of plasma radioactivity with a short first half life of elimination of approximately 3 hours which is probably mainly attributable to the elimination of the parent compound and a significantly longer second half-life of about 53 hours, probably related to the incorporation of radioactivity into biomolecules. The corresponding concentration-time curves of the modeled and the measured data are shown in Figure 6.1.1.-02 below.

Figure 6.1.1.-2. Timecourse of radioactivity in the plasma following a single oral dose of [furanone-4-^{14}C] flupyradifurone (2 mg/kg bw). (upper curve: male rats; lower curve: female rats)

Excretion

Approximately 79% and 91% of the administered dose was detected in the urine of male and female rats, respectively, and about 75% and 87% was excreted within 24 hours. The major route of excretion is therefore by urine, and is somewhat higher in female rats compared to male rats. Faecal excretion accounted for about 17% of the given dose in male rats, and the major part of faecal residues, approximately 15% of the dose, was excreted within the first 24 hours. In female rats approximately 10% of the dose is found in faeces where about 9% of the dose is excreted in the first 24 hours. The cumulative excretion results are summarized in Table 6.1.1-16.

Table 6.1.1.-16. Cumulative excretion of radioactive residues via urine and faeces after oral administration of [furanone-4-^{14}C] flupyradifurone to male and female rats

Time [h]	% of dose	
Faeces	**Male, 2 mg/kg**	**Female, 2 mg/kg**
24	14.76	9.45
48	16.40	10.29
72	16.50	10.33
96	16.54	10.35
120	16.56	10.36
144	16.57	10.37
168	16.59	10.38
Urine		
4	8.74	6.52
8	33.86	18.32
12	39.55	---
24	75.04	87.31
48	78.40	90.44
72	78.75	90.89
96	78.86	91.14
120	78.91	91.24
144	78.94	91.33
168	78.96	91.37
Total	**95.56**	**101.70**

Radioactive residues in organs and tissues at sacrifice

Approx. 0.5% of the dose was detected in the body of male rats at sacrifice 168 hours after oral administration; only a very minor amount of 0.02% was found in the GIT. Residual concentrations of radioactivity were in the range of 0.0025 to 0.0336 mg/kg. The lowest concentration was detected in the plasma and the highest value in the thyroid. But basically, levels for most organs and tissues were very similar and in the range of approx 0.005 to 0.01 mg/kg. For female rats, approx. 0.2% of the dose was still present in the body at sacrifice and only a negligible proportion of 0.01% in the GIT. Residual concentrations of radioactivity were in the range of 0.0012 to 0.0131 mg/kg. The lowest concentration was detected in the plasma and the highest value in the thyroid. As for males, levels for most organs and tissues were very similar and in the range of approx 0.002 to 0.005 mg/kg. For most organs and tissues the residues in males were 2 - 3 times higher as compared to females. This difference is also very likely caused by the higher rate of incorporation of radioactivity into biomolecules

The equivalent concentrations of the residual radioactivity in organs and tissues at sacrifice are presented in Table 6.1.1.-17.

Table 6.1.1.17. Total radioactive residues in organs and tissues at sacrifice after oral administration of [furanone-4-^{14}C] flupyradifurone to male and female rats.

Organ or tissue	concentration [mg a.s. equiv./kg]	
	Male, 2 mg/kg bw	Female, 2 mg/kg bw
Blood cells	0.0083	0.0038
Plasma	0.0025	0.0012
Carcass	0.0079	0.0031
Heart	0.0065	0.0029
Brain	0.0072	0.0033
Kidneys	0.0104	0.0045
Liver	0.0128	0.0081
Testes	0.0059	---
Ovaries	---	0.0039
Uterus	---	0.0035
Adrenal gland	0.0200	0.0114
Harderian gland	0.0241	0.0091
Thyroid gland	0.0336	0.0131
Spleen	0.0081	0.0032
Lung	0.0075	0.0048
Eye	0.0053	0.0048
Skin	0.0111	0.0047
Bone femur	0.0085	0.0049
Fat perirenal	0.0118	0.0058
Muscle leg	0.0069	0.0023

Metabolites in urine and faeces

Flupyradifurone was metabolized to approximately 20 metabolites. The parent compound represented the predominant part of the radioactivity in urine while in faeces the amounts of the metabolite BYI 02960-OH were more or less similar. All other identified and characterised metabolites represented a minor part of the dose. The metabolic profiles in urines and faeces were very similar for both sexes but male rats exhibited a higher rate of metabolite formation as compared to female animals. A summary of the distribution of the parent compound and metabolites in the excreta is provided in Table 6.1.1-18.

Table 6.1.1.-18. Amounts of metabolites in the excreta of rats after administration of [furanone-4-^{14}C] flupyradifurone

Compound	% of the total dose administered	
	Male: 2 mg/kg	Female: 2 mg/kg
Parent compound	54.68	75.96
difluoroethyl-amino-furanone	3.49	0.96
BYI 02960-OH-gluA (isomer 1)	1.13	0.49

BYI 02960-OH-gluA (isomer 3)	2.17	1.01
BYI 02960-des-difluoroethyl	2.12	3.35
BYI 02960-OH-SA	0.24	0.34
BYI 02960-OH	20.60	13.25
BYI 02960-iso-OH	0.26	0.07
Total identified	**84.70**	**95.42**
unknown 1*	5.30	2.45
unknown 2	0.46	0.19
unknown 3	0.57	0.05
unknown 4	0.03	---
unknown 5	0.40	0.12
unknown 6	0.05	0.16
unknown 7	0.02	0.09
unknown 8	0.03	0.11
unknown 9	0.24	0.44
unknown 10	0.02	0.02
unknown 11	0.25	0.23
unknown 12	---	0.14
unknown 13	0.53	0.02
unknown 14	0.10	0.06
unknown 15	0.02	---
Total characterized	**8.00**	**4.08**
Total	**95.55**	**101.75**

* unknown 1 from urine of male rats was further characterized by TLC analysis of the isolated metabolite fraction.

Acceptability

The study was considered acceptable.

Conclusions

Flupyradifurone was completely absorbed as demonstrated by the high amounts of radioactivity detected in urine and the body without GIT at sacrifice (>79% of the administered dose). Excretion of radioactivity was fast and mainly renal. Female rats showed a slightly higher renal excretion rate (91%) than male rats (78%). The major part of the dose (>89% for males and >96% for females) was excreted within 24 hours after treatment. The parent compound, one major and six minor metabolites were identified in all samples. Identification rates were high and amounted to >90% of the given dose. The metabolic transformation of flupyradifurone was principally oxidative in nature and took place at least at 3 different structural positions. Basically, male rats showed a higher rate of metabolism with only ca. 55% of unchanged parent compound found in excreta whereas 76% of unchanged BYI 02960 was found in the excreta of female rats. But the metabolic patterns were very similar in males and females.

Study 4

Characteristics

Reference	:	[redacted]	exposure	:	Single exposure by gavage
type of study	:	Toxicokinetic study (Quantitative whole body autoradiography)	dose	:	5 mg/kg bw
year of execution	:	2008-2010	vehicle	:	0.5% aqueous Tragacanth® suspension
test substance	:	[furanone-4-^{14}C] flupyradifurone, batch no. KATH 6109 (test 1 and 2, males) and KATH 6252 (test 3, females), chemical purity >99%, radiochemical prurity >99%, 3.94 MBq/mg	GLP statement	:	yes
Route	:	oral	guideline	:	OECD 417
Species	:	Rat Wistar Hsd/Cpb: WU	acceptability	:	acceptable
group size	:	8 males + 1 control, 8 females + 1 control			

Study design

The study was performed according to OECD Guideline 417. Male and female rats in groups of eight rats were administered orally by gavage a single dose of [furanone-4-^{14}C] flupyradifurone at target dose levels of 5 mg/kg bw after ca. 16 hours of fasting. A control animal of each gender was treated with 5 mg/kg bw non-radiolabelled flupyradifurone (Table 6.1.1.-19).

* denotes the ^{14}C-label position

After administration of [furanone-4-^{14}C] flupyradifurone, the rats were kept individually, which allowed for separate and quantitative collection of urine, faeces, and expired air. Urine was collected separately for each animal in intervals of 1, 4, 8, 24 hours, and every 24 hours until 168 hours. The radioactivity was determined by LSC. The faeces samples were collected every 24 hours separately for each animal. The radioactivity was determined by combustion/LSC. Carbon dioxide and other volatiles from expired air were collected from four male and four female animals for the time ranges 0 - 24 h and 24 - 48 h. At sampling, the exact volume was determined, from which an aliquot was taken for the determination of radioactivity by LSC.
One animal of each gender was sacrificed using carbon dioxide at 1, 4, 8, 24, 48, 72, 120, and 168 hours post administration, respectively. The control animals were sacrificed 4 hours after dosing. The distribution of total radioactivity to, and elimination from blood, organs and tissues was analyzed qualitatively and quantitatively by whole-body autoradioluminography.

Table 6.1.1.-19. Experimental groups for each dose level of [furanone-4-^{14}C] flupyradifurone

Group no.	No. of animals	Route	Dose	Sacrifice time (hoiurs after last dose)
1	8 M	Oral (gavage)	5 mg/kg bw	1, 4, 8, 24, 48, 72, 120, 168
	1 M (control)	Oral (gavage)	5 mg/kg bw	4
2	8 F	Oral (gavage)	5 mg/kg bw	1, 4, 8, 24, 48, 72, 120, 168
	1 F (control)	Oral (gavage)	5 mg/kg bw	4

Results

Distribution

The distribution in male and female rats was fast; maximum concentrations were reached at one hour after administration. The maximum concentrations in several organs and organs, *i.e.* liver, kidney, brown fat, myocardium, almost all glandular organs and the olfactory bulb were higher than in blood. The administered dose of [furanone-4-^{14}C] flupyradifurone was rapidly absorbed and distributed to the entire body, preferably to those organs or tissues that are responsible for metabolism. The equivalent concentrations in blood, organs and tissues declined following a biphasic kinetics showing a fast

phase from 1 to 24 hours (females: 1 to 48 hours) and a slower decline from 24 to 168 hours (female: 48 to 168 hours). After 168 hours the radioactivity concentrations in blood and all organs and tissues had declined significantly but were still higher in almost all of the organs than in blood. Slightly lower values were detected in the skeleton muscle, myocardium, lung, pancreas, pineal and vitreal body. Highest values were in perirenal fat (males), uterus and nasal mucosa. For all these organs, the concentration was lower than in the blood. The results are summarized in Table 6.1.1.-20 (male rats) and Table 6.1.1.-21 (female rats) .

Table 6.1.1.-20. Distribution of radioactivity in organs and tissues of male rats after a single oral dose of [furanone-4-^{14}C] flupyradifurone (5 mg/kg bw)

Organ or tissue	Equivalent concentration CEQ [µg a.s. equiv./g]							
	Time of sacrifice [hours after administration]							
	1 h	4 h	8 h	24 h	48 h	72 h	120 h	168 h
Blood	3.896	2.737	1.634	0.086	0.039	0.039	0.024	0.017
Liver	6.860	4.734	2.976	0.303	0.168	0.150	0.080	0.036
Renal cortex	5.743	4.346	2.247	0.146	0.056	0.059	0.035	0.021
Renal medulla	7.529	6.423	3.037	0.205	0.071	0.075	0.042	0.027
Kidney total	6.636	5.384	2.642	0.176	0.063	0.067	0.038	0.024
Brown fat	4.286	2.554	1.665	0.145	0.110	0.085	0.059	0.030
Perirenal fat	0.552	0.418	0.258	0.033	0.020	0.012	0.031	0.018
Skeleton muscle	3.733	2.896	1.426	0.073	0.026	0.029	0.018	0.014
Myocardium	5.274	3.544	2.131	0.097	0.037	0.035	0.026	0.016
Lung	2.794	2.555	1.250	0.077	0.030	0.028	0.017	0.013
Spleen	3.754	2.878	1.464	0.103	0.056	0.057	0.035	0.019
Pancreas	4.960	3.144	2.003	0.117	0.044	0.046	0.026	0.016
Bone marrow	3.320	2.395	1.309	0.182	0.104	0.081	0.041	0.019
Testis	2.493	2.066	1.229	0.080	0.044	0.042	0.030	0.021
Brain	1.372	1.055	0.715	0.081	0.066	0.060	0.052	0.033
Spinal cord	1.624	1.253	0.788	0.108	0.080	0.079	0.067	0.042
Pituitary gland	4.639	2.890	1.766	0.123	0.077	0.073	0.040	0.026
Pineal body	3.763	2.534	1.506	0.105	---	0.048	0.031	0.014
Adrenal gland	7.487	4.793	2.914	0.358	0.143	0.131	0.072	0.039
Thymus	3.869	2.635	1.587	0.140	0.106	0.097	0.045	0.022
Thyroid gland	5.391	3.848	2.280	0.304	0.253	0.174	0.080	0.036
Salivary gland	5.224	3.691	2.237	0.138	0.063	0.059	0.037	0.020
Nasal mucosa	1.943	1.880	1.727	0.592	0.419	0.401	0.179	0.164
Vitreal body	1.785	1.875	1.301	0.197	0.091	0.059	0.049	0.015
Harderian gland	5.384	4.421	---	0.443	0.367	0.313	0.090	0.050
Olfactory bulb	5.823	4.097	2.875	0.547	0.434	0.371	0.128	0.052

--- : Organ or tissue was visible in the rat sections but not discernible in the radioluminograms

Table 6.1.1.-21. Distribution of radioactivity in organs and tissues of female rats after a single oral dose of [furanone-4-^{14}C] flupyradifurone (5 mg/kg bw)

Organ or tissue	Equivalent concentration CEQ [µg a.s. equiv. /g]							
	Time of sacrifice [hours after administration]							
	1 h	4 h	8 h	24 h	48 h	72 h	120 h	168 h
Blood	4.250	2.587	1.341	0.061	0.009	0.007	0.006	0.006
Liver	7.757	4.648	2.705	0.143	0.024	0.018	0.011	0.009
Renal cortex	5.937	3.373	2.027	0.094	0.015	0.010	0.008	0.008
Renal medulla	7.971	5.223	3.525	0.156	0.018	0.015	0.012	0.011
Kidney total	6.954	4.298	2.776	0.125	0.016	0.012	0.010	0.009
Brown fat	3.604	2.221	0.938	0.089	0.018	0.019	0.010	0.008
Perirenal fat	0.654	0.365	0.179	0.014	<LOQ	<LOQ	<LOQ	<LOQ
Skeleton muscle	4.498	2.441	1.398	0.058	0.006	<LOQ	<LOQ	<LOQ
Myocardium	5.606	3.174	1.826	0.075	0.009	0.006	0.006	0.006
Lung	3.714	1.997	1.036	0.050	0.006	0.005	0.004	<LOQ
Spleen	4.238	2.273	1.313	0.068	0.013	0.010	0.007	0.007
Pancreas	5.546	3.081	1.691	0.082	0.010	0.006	0.005	---
Bone marrow	3.187	2.295	1.002	0.075	0.019	0.013	0.008	0.006
Ovary	4.143	2.246	1.447	0.075	0.012	0.011	0.006	<LOQ
Uterus	4.305	2.670	1.414	0.068	0.018	0.013	0.010	0.008
Brain	1.376	0.811	0.462	0.023	0.007	0.007	0.006	0.009
Spinal cord	1.464	0.941	0.495	0.029	0.009	0.009	0.008	0.011
Pituitary gland	4.838	2.950	1.516	0.076	0.012	---	---	---
Pineal body	3.176	2.444	1.244	0.053	---	---	---	---
Adrenal gland	7.927	4.739	2.498	0.172	0.057	0.036	0.021	0.026
Thymus	4.287	2.577	1.319	0.072	0.021	0.019	0.010	0.007
Thyroid gland	6.024	3.585	1.806	0.127	0.050	0.032	0.026	0.026
Salivary gland	5.732	3.450	1.833	0.085	0.013	0.010	0.007	0.006
Nasal mucosa	1.689	1.622	0.751	0.390	0.250	0.203	0.160	0.117
Vitreal body	1.831	1.615	1.110	0.212	0.041	0.032	0.013	0.009
Harderian gland	6.238	3.491	2.034	0.120	0.021	0.011	0.014	---
Olfactory bulb	---	---	2.393	0.194	0.068	0.064	0.021	0.017

--- : **Organ or tissue was visible in the rat sections but not discernible in the radioluminograms**

<LOQ : **below limit of quantitation**

Excretion

[furanone-4-^{14}C] flupyradifurone was excreted rapidly and completely within ca. 48 hours. The major part of the radioactivity (up to 81% in male and up to 88% in female rats) was excreted with urine and the minor part (about 16% for male and about 7% for female rats) with faeces. The exhalation of ^{14}C-carbon dioxide was tested with male and female animals for a period of 48 hours. Between 2.02% and 3.25% (males) and 0.50% and 0.96% (females) of the administered radioactivity was exhaled during this period. This demonstrated that for a small portion of the dose (higher in males than in females) the furanone ring of the molecule obviously underwent biotransformation to C1- and C2-fragments and the terminal product $^{14}CO_2$.

Table 6.1.1.-22: Excretion of radioactivity in urine, faeces and expired air of male rats after a single oral administration of [furanone-4-^{14}C] flupyradifurone (5 mg/kg bw)

	% of the given dose							
	Time of sacrifice [h after administration]							
	1	**4**	**8**	**24*)**	**48**	**72**	**120**	**168**
Exhaled air								
24 h					2.33	2.74	2.54	1.71
48 h					2.83	3.25	3.01	2.02
Urine								
1 h	4.30							
4 h		13.29	4.49	6.27	3.44	3.56	6.76	19.80
8 h			41.15	41.14	27.44	28.12	48.81	50.47
24 h				45.15	73.40	69.05	74.38	77.72
48 h					75.77	71.95	77.50	80.11
72 h						72.26	77.87	80.39
96 h							78.00	80.48
120 h							78.09	80.52
144 h								80.56
168 h								80.59
Faeces								
24 h	*	*	*	41.20	13.54	13.41	10.80	12.76
48 h					15.09	15.48	13.07	13.68
72 h						15.84	13.30	13.76
96 h							13.36	13.79
120 h							13.39	13.80
144 h								13.82
168 h								13.83
Sum total	**4.30**	**13.29**	**41.15**	**86.35**	**93.69**	**91.35**	**94.50**	**96.44**

*) : Because of the untypical excretion behavior (41.20% in faeces after 24 hours), this animal was not considered
* : faeces not collected

Table 6.1.1.-23. Excretion of radioactivity in urine, faeces and expired air of female rats after a single oral administration of [furanone-4-^{14}C] flupyradifurone (5 mg/kg bw)

	Percent of radioactive dose administered							
	Time of sacrifice [h after administration]							
	1	**4**	**8**	**24**	**48 *)**	**72**	**120**	**168**
Exhaled air								
24 h					0.50	0.63	0.66	0.82
48 h					0.58	0.71	0.77	0.96
Urine								
1 h	3.82							
4 h		32.45	35.72	38.42	36.43	42.56	28.90	35.24
8 h			60.08	54.58	53.23	48.11	35.08	54.17
24 h				87.30	54.81	90.78	76.99	84.22
48 h					57.56	92.71	80.60	86.19
72 h						92.89	81.22	86.49
96 h							81.60	86.66
120 h							82.01	87.88
144 h								87.98
168 h								88.04
Faeces								
24 h	*	*	*	6.57	33.30	4.32	4.94	4.69
48 h					34.84	5.18	6.46	5.60
72 h						5.22	6.58	5.75
96 h							6.73	5.79
120 h							6.76	5.81
144 h								5.83
168 h								5.84
Sum total	**3.82**	**32.45**	**60.08**	**93.87**	**92.98**	**98.82**	**89.54**	**94.84**

*) : Because of the untypical excretion behavior (41.20% in faeces after 24 hours), this animal was not considered
* : faeces not collected

Acceptability

The study was considered acceptable.

Conclusions

[furanone-4-^{14}C] flupyradifurone was readily absorbed from the gastrointestinal tract of male and female rats and evenly distributed throughout the rat bodies immediately after the single oral administration of 5 mg/kg bw. [furanone-4-^{14}C] flupyradifurone related radioactivity was rapidly cleared from blood and distributed primarily to those organs or tissues that are responsible for metabolism, excretion and secretion. The excretion of radioactivity *via* urine and faeces was almost completed two days after administration with more than 80% of the dose detected in the urine of both sexes.
The detection of $^{14}CO_2$ in the exhaled air during a sampling period of 48 hours (up to 3.25% in male and 0.96% in female rats) demonstrated that for a small portion of the dose the furanone ring obviously underwent biotransformation to C1- and C2-fragments and the terminal product $^{14}CO_2$. Peak

concentrations of radioactivity for almost all organs and tissues were reached already one hour after dosing. From then onwards, the concentrations declined following a biphasic kinetics. The second slower decline phase, which started after 24 hours in males and after 48 hours in females, is probably due to the formation of small carbon units (C1- or C2-fragments) that entered the carbon pool used for the biosynthesis of endogenous compounds. This is presumably also the reason that at the end of the test (day 7) low radioactive residues were still measured in almost all organs and tissues.
The terminal residues were always by a factor of 1.4 to 4.7 higher in males than in females. A similar ratio of approx. 3 was also found for the formation of $^{14}CO_2$ in males as compared to females. The reason is probably a gender specific quantitative difference in metabolism leading to more C1- and C2-fragments and also a higher incorporation of these components into the endogenous carbon pool. From the results of this study, any significant accumulation or retention of [furanone-4-^{14}C]BYI 02960 in male and female rats can be excluded.

Study 5

Characteristics

Reference	:	[redacted]	exposure	:	Single exposure by gavage
type of study	:	Toxicokinetic study (Metabolism in Organs and Tissues in rat)	dose	:	3 mg/kg bw
year of execution	:	2010-2011	vehicle	:	0.5% aqueous Tragacanth® suspension
test substance	:	[Furanone-4-^{14}C]-Flupyradifurone, batch no. KML 9029, chemical purity >99%, radiochemical purity >99%, 3.94 MBq/mg	GLP statement	:	yes
Route	:	oral	guideline	:	OECD 417
Species	:	Rat Wistar Hsd/Cpb: WU	acceptability	:	acceptable
group size	:	4/sex			

Study design

The study was performed according to OECD Guideline 417. Male and female rats in groups of four rats were administered orally by gavage a single dose of flupyradifurone at target dose levels of 3 mg/kg bw (Table 6.1.1.-24). Flupyradifurone was radiolabelled with ^{14}C in the 4-position of the furanone ring of the molecule.

* denotes the ^{14}C-label position

Urine and faeces were collected for the time range between dosing and sacrifice, the animals were sacrificed after 6 hours. Total radioactivity which included the test compound and metabolites was determined at sacrifice in urine, plasma, liver, kidney, muscle (leg) and fat (perirenal). The metabolism was investigated in urine and plasma samples and in extracts of liver, kidney, muscle and fat. Samples were analyzed by radio HPLC, radio TLC, LC-MS and NMR methods.

Table 6.1.1.-24. Experimental groups for each dose level of [furanone-4-^{14}C] flupyradifurone

Group no.	No. of animals	Route	Dose	Sacrifice time (hoiurs after last dose)
1	4 M	Oral (gavage)	5 mg/kg bw	6
2	4 F	Oral (gavage)	5 mg/kg bw	6

Results

Recovery

The total recovery for the orally administered test was between approximately 98% and 101% of the administered dose in urine, organs and tissues, skin and the combined GIT and faeces samples of male and female rats (Table 6.1.1.-25). In male and female rats, approx. 40% of the dose was detected in organs and tissues, the highest amount was found in the residual carcass (approx. 24 - 27%), followed by skin, liver, muscle, kidney, and fat for which the lowest values were measured.(approx. 0.06 - 0.09%). For the 0 - 6 h collection period, the renal excretion in female rats was by a factor of approx. 1.17 higher than in male rats.

Table 6.1.1.-25: Recovery of radioactivity in urine, plasma and organs 6 h after a single oral dose of [furanone-4-^{14}C] flupyradifurone (3 mg/kg bw)

Percent of given dose (mean values)		
	Male	**Female**
Urine	36.64	42.82
Plasma	0.70	0.55
Carcass	24.00	26.73
Kidneys	0.73	1.04
Liver	3.56	3.61
GIT + faeces	23.63	12.57
Skin	9.18	9.42
Fat (perirenal)	0.06	0.09
Muscle (leg)	2.09	1.26
Balance	**100.60**	**98.09**

Metabolism:

Table 6.1.1.-26 shows the amount of flupyradifurone and its metabolites after six hours in urine of male and female rats as percentage of the given dose. In urine, the parent compound was the dominating radioactive component (approximately 22% of the dose in males and 38% in females). The concentrations of radioactivity in plasma can be found in Table 6.1.1.-27, in liver in Table 6.1.1.-28, in kidney in Table 6.1.1.-29, in muscle in table 6.1.1.-30 and in fat tissue in table 6.1.1.-31. The highest radioactivity concentrations were detected in the liver (approx. 2.9 mg/kg for both sexes) and kidney (approx. 2.7 mg/kg for males and 4.3 mg/kg for females) as the main metabolic and excretory organs. The residue concentrations for plasma and the other tissues were comparable for both sexes and ranged from approx. 0.6 mg/kg for the perirenal fat to approx. 1.5 mg/kg for the leg muscle. Six hours after administration [furanone-4-^{14}C] flupyradifurone was only incompletely metabolized. Metabolic reactions took place at least at 3 different structural positions of the molecule. The majority of the radioactive residues were identified (approximately 89% - 100% in plasma and in extracts of organs and tissues as well as ≥ 88% of radioactivity in urine samples). In all samples of plasma, organs and tissues the parent compound was the by far largest component accounting for more than 72% of the total radioactivity. None of the identified metabolites accounted for more than 12% of the total radioactivity. The metabolism was qualitatively similar in male and female rats, but with quantitative differences. The degradation of the parent compound to the different metabolites was significantly higher in male as compared to female rats.

Table 6.1.1.-26: Quantification of parent compound and metabolites in urine of male and female rats sacrificed 6 h after single oral administration of [furanone-4-^{14}C] flupyradifurone (3 mg/kg bw)

	% of the given dose	
	Male	Female
Sampling period [h]	0 - 6 h	0 - 6 h
Parent compound	22.1	37.6
unknown	1.4	0.4
unknown	1.5	0.4
unknown	0.2	---
unknown	0.4	---
unknown	0.3	---
BYI 02960-difluoroethyl-amino-furanone	1.5	0.2
BYI 02960-OH-gluA (isomer 1)	0.4	---
unknown	0.2	---
BYI 02960-OH-gluA (isomer 3)	0.9	---
BYI 02960-des-difluoroethyl	0.8	0.8
unknown	0.2	---
unknown	0.1	---
BYI 02960-OH	6.9	3.3
Total identified	32.5	42.0
Total characterized *	4.1	0.8

Sum total	**36.6**	**42.8**
Identification rate	**88.8%**	**98.1%**

* : Peaks were characterized based on their retention time in HPLC-analysis

Table 6.1.1.-27. Concentration of parent compound and metabolites in plasma of male and female rats sacrificed 6 h after single oral administration of [furanone-4-^{14}C] flupyradifurone (3 mg/kg bw). Data are presented as mg/kg

	Male	**Female**
Sampling period [h]	**0 - 6 h**	**0 - 6 h**
Parent compound	1.087	1.385
unknown	0.056	0.007
unknown	---	0.010
BYI 02960-difluoroethyl-amino-furanone	0.101	0.012
BYI 02960-des-difluoroethyl	---	0.008
BYI 02960-OH	0.068	0.026
Total identified	1.257	1.369
Total characterized *	0.056	0.016
Sum total	**1.313**	**1.385**

* : Peaks were characterized based on their retention time in HPLC-analysis

Table 6.1.1.-28: Concentration of parent compound and metabolites in the liver of male and female rats sacrificed 6 h after single oral administration of 3 mg/kg [furanone-4-^{14}C] flupyradifurone (3 mg/kg bw)

	Concentration [mg a.s. equiv./kg]	
	Male	**Female**
Sampling [h]	**6 h**	**6 h**
Parent compound	2.110	2.778
unknown	0.150	---
unknown	0.015	---
BYI 02960-difluoroethyl-amino-furanone	0.108	0.015
BYI 02960-OH-gluA (isomer 1)	0.026	---
BYI 02960-OH-gluA (isomer 3)	0.055	0.013
BYI 02960-des-difluoroethyl	0.036	0.024
unknown	0.012	---
unknown	0.007	---
unknown	0.016	---
BYI 02960-OH	0.242	0.077
Total identified	2.577	2.908
Total characterized *	0.200	---
Sum total	**2.928**	**2.937**

* : Peaks were characterized based on their retention time in HPLC-analysis

Table 6.1.1.-29. Concentration of parent compound and metabolites in the kidney of male and female rats sacrificed 6 h after single oral administration of [furanone-4-^{14}C] flupyradifurone (3 mg/kg bw)

	Concentration [mg a.s. equiv./kg]	
	Male	Female
Sampling [h]	6 h	6 h
Parent compound	1.969	4.025
unknown	0.174	0.060
unknown	0.017	---
BYI 02960-difluoroethyl-amino-furanone	0.125	---
BYI 02960-OH-gluA (isomer 1)	0.010	---
BYI 02960-OH-gluA (isomer 3)	0.020	---
BYI 02960-des-difluoroethyl	0.036	0.047
BYI 02960-OH	0.319	0.197
Total identified	2.479	4.269
Total characterized *	0.191	0.060
Sum total	**2.732**	**4.346**

* : Peaks were characterized based on their retention time in HPLC-analysis

Table 6.1.1.-30. Concentration of parent compound and metabolites in muscle of male and female rats sacrificed 6 h after single oral administration of [furanone-4-^{14}C] flupyradifurone (3 mg/kg bw)

	Concentration [mg a.s. equiv./kg]	
	Male	Female
Sampling [h]	6 h	6 h
Parent compound	1.160	1.435
unknown	0.026	---
BYI 02960-difluoroethyl-amino-furanone	0.090	0.009
BYI 02960-des-difluoroethyl	0.013	0.012
BYI 02960-OH	0.083	0.032
Total identified	1.346	1.487
Total characterized *	0.026	---
Sum total	**1.382**	**1.489**

* : Peaks were characterized based on their retention time in HPLC-analysis

Table 6.1.1.-31. Concentration of parent compound and metabolites in the fat of male and female rats sacrificed 6 h after single oral administration of [furanone-4-^{14}C] flupyradifurone (3 mg/kg bw)

	Concentration [mg a.s. equiv./kg]	
	Male	Female
Sampling [h]	6 h	6 h
Parent compound	0.474	0.650
unknown		---
BYI 02960-difluoroethyl-amino-furanone	0.023	---
BYI 02960-OH	0.040	---
Total identified	0.537	0.650

Sum total	**0.558**	**0.651**

Acceptability

The study was considered acceptable.

Conclusions

The distribution of the radioactivity within the organs and tissues (*i.e.* blood, liver, kidney, muscle and fat) showed a distinctive preference for liver and kidney as the main metabolizing and excretory organs. The metabolic transformation of flupyradifurone was principally oxidative in nature and took place at least at 3 different structural positions of the molecule. The metabolic pattern was in good accordance with that obtained from the corresponding ADME rat studies. With regard to the extent of metabolism, a clear sex difference was observed since it was higher in male than in female rats, *i.e.* the metabolic degradation of the parent compound was much less pronounced in females.

Study 6

Characteristics

Reference	:	[redacted]	exposure	:	single dose by gavage
type of study	:	Toxicokinetic study (ADME in male rat)	dose	:	2 mg/kg
year of execution	:	2010-2011	vehicle	:	0.5% aqueous Tragacanth® suspension
test substance	:	[Ethyl-1-^{14}C] flupyradifurone, batch no. KATH 6412, chemical purity >99%, radiochemical purity >99%, 3.93 MBq/mg	GLP statement	:	yes
Route	:	oral	guideline	:	OECD 417
Species	:	Rat Wistar Hsd/Cpb: WU	acceptability	:	acceptable
group size	:	4 male			

Study design

The study was performed according to OECD Guideline for testing chemicals 417. Four male rats were administered orally by gavage a single dose of [Ethyl-1-^{14}C] flupyradifurone suspended in 0.5% aqueous Tragacanth® (2 mL) at target dose level of 2 mg/kg bw. Flupyradifurone was radiolabelled with ^{14}C in the 1-position of the ethyl side chain:

* denotes the position of the ^{14}C-label

Urine and faeces were collected for the time range between dosing and sacrifice and the animals were sacrificed after 72 hours (Table 6.1.1.-32). Plasma levels of radioactivity were followed from each animal for 12 time points. Total radioactivity which included the test compound and metabolites was determined in excreta and in organs and tissues collected at sacrifice. Metabolism was investigated in urine and faeces. Samples were analyzed by radio HPLC, radio TLC, LC-MS and NMR methods. Toxicokinetic parameters were calculated with the software TOPFIT version 2.0.

Table: 6.1.1.-32. Experimental groups for each dose level of [Ethyl-1-^{14}C] flupyradifurone

Group no.	No. of animals	Route	Dose	Sacrifice time (hoiurs after last dose)
1	4 M	Oral (gavage)	2 mg/kg bw	72

Results

Recovery

The results in percent of the given dose in expired air, urine, faeces, organs and tissues at sacrifice are summarized in Table 6.1.1.-33. The total recovery for the orally administred test was approximately 100%.

Table 6.1.1.-33. Recovery of radioactivity in urine, faeces, gastrointestinal tract and the body following a single oral dose of [ethyl-1-^{14}C] flupyradifurone (2 mg/kg bw)

	% of given dose
Expired air	0.20
Faeces	13.51
Urine	82.24
Sum of excreta	**95.95**
Body excluding GIT	3.19
GIT	0.73
Total Body	3.92
Balance	**99.86**

Absorption

[Ethyl-1-^{14}C] flupyradifurone was almost completely absorbed in male rats. The absorption rate was at least 85% because >82% of the dose was detected in urine and >3% in the body without GIT. The absorption commenced immediately after dosing as can be seen from the quick increase of radioactivity in plasma micro samples (Table 6.1.1-34).

Table 6.1.1.-34. Timecourse of radioactivity in the plasma following a single oral dose of [ethyl-1-^{14}C] flupyradifurone (2 mg/kg bw)

Time point	Equivalent concentration (mg/kg)
10 min	0.663
20 min	1.391
40 min	1.878
1 h	2.017
1.5 h	1.963
2 h	1.911
4 h	1.532
8 h	0.995
24 h	0.357
32 h	0.358
48 h	0.230
72 h	0.162

Distribution and plasma kinetics

Flupyradifurone was quickly distributed as can be seen form the analysis of plasma at different time points. The maximum concentration of radioactivity (C_{max}) was already reached one hour after administration. At this time, the radioactivity level in plasma corresponded approximately to the equidistribution concentration. The plasma concentration then declined to approx. 50% of C_{max} within 8 hours and to approx. 10% of the maximum value within 48 hours. At the time of sacrifice, the plasma concentration amounted to ca. 8% of C_{max}.

The mean values of total radioactivity were used for a toxicokinetic modelling using the TOPFIT software. The fitting was conducted assuming a two-compartment model with no data weighting. A good fit was achieved for the entire time range (Figure 6.1.1.-03). The AUC was 45.4 mg/L*h and the half life of elimination was 49.9 hours.

Figure 6.1.1-03. Timecourse of radioactivity in the plasma following a single oral dose of [ethyl-1-^{14}C] flupyradifurone (2 mg/kg bw)

Excretion

Only a very small proportion of the dose (0.2% in total) was detected in the expired air. This result confirms the stability of the radio label in the ethyl group with regard to extensive metabolic transformation. The major route of excretion was renal. In total, approximately 82% of the dose was detected in urine, the majority of which (about 76% of the dose) was excreted within 24 hours. Faecal excretion accounted for ca. 13.5% of the given dose. Also the major part of faecal residues (about 11% of the dose) was excreted within 24 hours after treatment. These results are in good accordance with the excretion behavior detected in all other rat studies with flupyradifurone. The cumulative excretion results in percent of the administered radioactivity are summarized in Table 6.1.1.-34.

Table 6.1.1.-34. Cumulative excretion of radioactive residues via urine, faeces and expired air after a single oral dose of [ethyl-1-^{14}C] flupyradifurone (2 mg/kg bw).

	Time [h]	% of dose
Expired Air	24	0.15
	48	0.19
	72	0.20
Faeces	24	11.08
	48	13.12
	72	13.51
Urine	4	14.95
	8	46.40
	12	60.48
	24	76.12
	48	80.60
	72	82.24
Total		**95.95**

Radioactive residues in organs and tissues at sacrifice

Approximately 4% of the dose was detected in the body at sacrifice 72 hours after oral administration; 0.73% was found in the GIT and 3.19% in the body without GIT. The residual concentration of radioactivity was in the range of 0.025 to 0.158 mg/kg. The lowest concentration was detected in the Harderian gland and the highest value was found in plasma. However, concentrations for most organs and tissues were very similar and in the range of approximately 0.05 to 0.1 mg/kg. The equivalent concentrations of the residual radioactivity in organs and tissues at sacrifice are presented in Table 6.1.1.-35.

Table 6.1.1.-35: Total radioactive residues in organs and tissues at sacrifice after a single oral dose of [ethyl-1-^{14}C] flupyradifurone (2 mg/kg bw)

	Equivalent concentration (mg/kg)
Blood cells	0.104
Plasma	0.158
Carcass	0.064
Heart	0.078
Brain	0.083
Kidneys	0.066
Liver	0.095
GIT	0.129
Testes	0.065
Adrenal gland	0.073
Harderian gland	0.025
Thyroid	0.088
Spleen	0.075
Lung	0.088
Eye	0.138
Skin	0.079
Bone (femur)	0.052
Perirenal fat	0.054
Muscle	0.055

Metabolites in urine and faeces

Flupyradifurone was metabolized into a number of metabolites. The unmetabolized parent compound represented the predominant part of the radioactivity in urine while in faeces the metabolite BYI 02960-OH was more prominent. BYI 02960-DFA was detected in both, urine and faeces. All other identified and characterized metabolites represented a minor part of the dose and were not common to either urine or faeces. A summary of the distribution of the parent compound and metabolites in urine and faeces is provided in Table 6.1.1.-36.

Table 6.1.1.-36. Amounts of metabolites in urine and faeces after a single oral dose of [ethyl-1-^{14}C] flupyradifurone (2 mg/kg bw)

Peak no.	(BYI 02960-)	% of the dose		
		Urine	Faeces	Total
1	DFA	5.28	0.49	5.77
6	difluoroethyl-amino-furanone	3.63	---	3.63
7	OH-gluA (isomer 1)	1.40	---	1.40
8	OH-gluA (isomer 3)	1.79	---	1.79
6	difluoroethyl-amino-furanone	3.63	---	3.63

7	OH-gluA (isomer 1)	1.40	---	1.40
8	OH-gluA (isomer 3)	1.79	---	1.79
11	OH	16.13	7.60	23.73
12	Parent compound	51.96	3.79	55.75
13	iso-OH	---	0.43	0.43
	Identified	80.19	12.31	92.50
2	unknown 1	0.64	---	0.64
3	unknown 2	0.68	---	0.68
4	unknown 3	0.36	---	0.36
5	unknown 4	0.37	---	0.37
9	unknown 5	---	0.10	0.10
10	unknown 6	---	0.17	0.17
	Characterized	2.04	0.27	2.31
	Total in extracts	82.23	12.58	94.81
Total in urine and faeces		**82.23**	**13.51**	**95.74**

Acceptability

The study was considered acceptable.

Conclusions

Flupyradifurone was almost completely absorbed; more than 85% of the administered dose was detected in the urine and the body without GIT at sacrifice The excretion of radioactivity was fast and mainly renal. The major part of the dose (>87%) was excreted within 24 hours after treatment, but continued until sacrifice. At sacrifice, a small proportion of ca. 3% of the dose was detected in the body without GIT. The residue concentration in plasma was 0.158 mg/kg; for most other organs and tissues levels were in the range between 0.05 and 0.1 mg/kg. The parent compound, one major and five minor metabolites were identified in all samples. Identification rates were >95% of the total radioactivity in urine and >85% of the total radioactivity in faeces..The metabolic transformation of flupyradifurone was principally oxidative in nature and took place at 3 different positions of molecule

<u>Study 7</u>

Characteristics

Reference	:	[redacted]	exposure	:	Single dose by gavage
type of study	:	Toxicokinetic study (Metabolism study in organs and tissues of male and female rats (3 time-points).	dose	:	3 mg/kg bw
year of execution	:	2010-2011	vehicle	:	0.5% aqueous Tragacanth® suspension
test substance	:	[Ethyl-1-^{14}C] flupyradifurone, batch no. KML 9028, chemical purity >98%, radiochemical purity	GLP statement	:	yes

		>99% (HPLC) or >98% (TLC), 3.93 MBq/mg			
Route	:	oral	guideline	:	OECD 417
Species	:	Rat Wistar Hsd/Cpb: WU	acceptability	:	acceptable
group size	:	4/time point/sex			

Study design

The study was performed according to OECD Guideline 417. Male and female rats in groups of four rats were administered orally by gavage a single dose of flupyradifurone at target dose levels of 3 mg/kg bw (Table 6.1.1.-24). Flupyradifurone was radiolabelled with ^{14}C in the 1-position of the ethyl side chain:

* denotes the position of the ^{14}C-label

Groups of male and female rats (4/time point/sex) were orally administered with a single dose of 3 mg/kg bw [ethyl-1-^{14}C] flupyradifurone by gavage in 0.5% aqueous Tragacanth®. The animals were sacrificed at 1, 6 or 24 hours after dosing. The total radioactivity was determined over different time periods (0 - 1 hour, 0 - 6 hours, or 0 – 24 hours) in urine, while in plasma, liver, kidney, muscle and fat tissue after sacrifice. The metabolism was investigated in urine, plasma, and in extracts of liver, kidney, muscle, and fat tissue. Samples were analyzed by radio HPLC, radio TLC, LC-MS and NMR methods.

Table 6.1.1.-37. Experimental groups for each dose level of [furanone-4-^{14}C] flupyradifurone

Group no.	No. of animals	Route	Dose	Sacrifice time (hoiurs after last dose)
1	4 M	Oral (gavage)	5 mg/kg bw	1
	4 F	Oral (gavage)	5 mg/kg bw	1
2	4 M	Oral (gavage)	5 mg/kg bw	6
	4 F	Oral (gavage)	5 mg/kg bw	6
3	4 M	Oral (gavage)	5 mg/kg bw	24
	4 F	Oral (gavage)	5 mg/kg bw	24

Results

Recovery

The detailed recovery rates of radioactivity in urine, organs and tissues and the combined GIT and faeces sample are shown in Table 6.1.1-38. The mean recoveries for male rats ranged from approximately 96% to 98% and for female rats from approximately 100% to 104% of the given dose. In male rats, sacrificed at 1 hour after administration, approx. 64.6% of the dose was detected in organs and tissues and approx. 27.2% in the GIT and faeces sample (Table 6.1.1.-38). After 24 hours, the value for organs and tissues declined to approx. 7.5%. These values indicated a fast distribution of the absorbed radioactivity within the body followed by a quick elimination finally leading to a significant increase of the urinary excretion from ca. 6.2% to ca. 71.8%. The situation was slightly different in female rats. After sacrifice at 1 hour h after administration, approx. 81% of the dose was detected in organs and tissues and approx. 13.9% in the GIT and faeces sample. After 24 hours, the value of organs and tissues declined to approx. 5.3%. These values indicated again a fast distribution of the absorbed radioactivity within the body followed by a quick elimination finally leading to a significant increase of the urinary excretion from approx. 8.8% to approx. 85.9%.

Table 6.1.1.-38. Recovery of radioactivity in urine, plasma and organs following a single oral dose of [ethyl-1-^{14}C] flupyradifurone (3 mg/kg bw)

	as % of the given dose					
	Male			Female		
Test period [hour]	1	6	24	1	6	24
Urine	6.22	36.53	71.80	8.76	39.70	85.88
Plasma	1.09	0.62	0.21	1.27	0.61	0.13
Carcass	38.32	24.47	4.25	51.19	29.57	3.08
Kidneys	1.30	0.73	0.08	1.17	0.63	0.06
Liver	6.80	3.44	0.60	7.30	3.62	0.39
GIT + faeces	27.24	21.18	16.61	13.94	14.26	9.44
Skin	14.64	9.19	2.13	17.54	10.48	1.45
Fat	0.10	0.04	0.01	0.15	0.09	0.01
Muscle	2.31	1.14	0.17	2.34	1.35	0.13
Balance	**98.03**	**97.33**	**95.85**	**103.70**	**100.30**	**100.60**

Metabolism

Table 6.1.1.-39 shows the amount of flupyradifurone and its metabolites after 1, 6, and 24 hours in urine of male and female rats as percentage of the given dose. In urine, the parent compound was the dominating radioactive component. The concentrations of radioactivity in plasma can be found in Table 6.1.1.-40, in liver in Table 6.1.1.-41, in kidney in Table 6.1.1.-42, in muscle in table 6.1.1.-43 and in fat tissue in table 6.1.1.-44. Flupyradifurone was intensively metabolized. Metabolic reactions took place at least at 3 different structural positions of the molecule. The majority of the radioctive residues were identified (approx. 97 - 100% in plasma, and in extracts of organs and tissues as well as ≥ 95% of radioactivity in urine). In the 24 hours samples of plasma, and organs and tissues BYI 02960-DFA was by far the dominating metabolite accounting for more than 50% of the radioactivity. All other

identified metabolites contributed to less than 10%. The contribution of the parent compound in these samples ranged from 6 to 38% of the radioactivity. In urine, the parent compound was the dominating radioactive component (approximately 48% of the dose in males and 77% in females).

The metabolism was qualitatively similar in male and female rats, but with quantitative differences. The degradation of the parent compound to the different metabolites in male rats was significantly higher than in female rats.

Table 6.1.1.-39. Quantification of parent compound and metabolites in urine of male and female rats sacrificed 1 h, 6 h, and 24 h after a single oral dose of [ethyl-1-^{14}C] flupyradifurone (3 mg/kg bw)

	% of the given dose					
	Male			Female		
Sampling period	**0 - 1 h**	**0 - 6 h**	**0 - 24 h**	**0 - 1 h**	**0 - 6 h**	**0 - 24 h**
Parent compound	4.96	25.48	47.69	8.19	36.15	76.48
DFA	---	0.22	1.91	---	0.11	1.70
unknown	---	0.38	0.72	---	0.18	0.42
unknown	---	0.15	0.35	---	---	---
unknown	---	0.30	0.67	---	---	---
unknown	---	---	0.43	---	---	---
unknown	---	---	0.27	---	---	---
BYI 02960-difluoroethyl-amino-furanone	0.12	1.55	3.09	0.07	0.33	0.87
BYI 02960-OH-gluA (isomer 1)	0.12	0.66	1.55	---	0.10	---
unknown	---	---	0.46	---	0.14	---
unknown	---	---	---	---	0.38	---
BYI 02960-OH-gluA (isomer 3)	0.13	0.74	1.72	---	0.08	---
unknown	0.08	---	0.34	---	---	---
unknown	---	0.07	0.10	---	---	---
unknown	---	0.15	0.18	---	---	---
BYI 02960-OH	0.82	6.85	12.33	0.51	2.24	6.42
Total identified	6.15	35.49	68.28	8.76	39.00	85.46
Total characterized *	0.08	1.04	3.52	---	0.70	0.42
Sum total	**6.22**	**36.53**	**71.80**	**8.76**	**39.70**	**85.88**
Identification rate	**98.8%**	**97.2%**	**95.1%**	**100.0%**	**98.2%**	**99.5%**

* : Peaks were characterized based on their retention time in HPLC-analysis:

Table 6.1.1.-40. Concentration of parent compound and metabolites in plasma of male and female rats sacrificed 1 h, 6 h, and 24 h after a single oral dose following a single oral dose of [ethyl-1-^{14}C] flupyradifurone (3 mg/kg bw)

	Concentration [mg a.s. equiv./kg]					
	Male			Female		
Sampling time	**1 h**	**6 h**	**24 h**	**1 h**	**6 h**	**24 h**

Parent compound	1.998	0.976	0.031	2.671	1.486	0.063
BYI 02960-DFA	0.058	0.348	0.448	0.057	0.175	0.280
Unknown	---	0.024	---	---	---	---
BYI 02960-difluoroethyl-amino-furanone	0.052	0.084	0.005	---	---	---
Unknown	---	0.018	---	---	---	---
BYI 02960-OH	0.058	0.045	0.007	---	---	---
Total identified	2.166	1.452	0.491	2.728	1.661	0.343
Total characterized *	---	0.042	---	---	---	---
Sum total**	**2.166**	**1.494**	**0.491**	**2.728**	**1.661**	**0.343**

* : Peaks were characterized based on their retention time in HPLC-analysis
** : Sum total = total identified or characterized + solids (unextractable) + samples not analyzed

Table 6.1.1..41. Concentration of parent compound and metabolites in the liver of male and female rats sacrificed 1 h, 6 h, and 24 h after a single oral dose following a single oral dose of [ethyl-1-^{14}C] flupyradifurone (3 mg/kg)

	Concentration [mg a.s. equiv./kg]					
	Male			Female		
Sampling time	**1 h**	**6 h**	**24 h**	**1 h**	**6 h**	**24 h**
Parent compound	3.900	1.903	0.084	5.519	2.977	0.084
BYI 02960-DFA	0.049	0.183	0.218	0.033	0.116	0.136
Unknown	---	0.012	---	0.019	---	---
Unknown	---	0.012	---	---	---	---
BYI 02960-difluoroethyl-amino-furanone	0.070	0.086	---	0.022	0.018	---
BYI 02960-OH-gluA (isomer 1)	---	0.021	---	---	---	---
unknown	---	0.013	---	---	---	---
unknown	---	0.013	---	0.017	0.016	---
unknown	---	---	---	---	---	0.002
unknown	---	---	---	---	---	0.002
unknown	---	---	---	0.014	---	---
unknown	0.042	0.031	---	0.021	0.010	---
BYI 02960-OH	0.187	0.150	0.014	0.106	0.063	0.005
Total identified	4.206	2.343	0.316	5.696	3.190	0.225
Total characterized *	0.042	0.081	---	0.055	0.010	0.004
Sum total **	**4.281**	**2.507**	**0.367**	**5.770**	**3.225**	**0.243**

* : Peaks were characterized based on their retention time in HPLC-analysis
** : Sum total = total identified or characterized + solids (unextractable) + samples not analyzed

Table 6.1.1.-42: Concentration of parent compound and metabolites in the kidney of male and female rats sacrificed 1 h, 6 h, and 24 h after a single oral dose of [ethyl-1-^{14}C] flupyradifurone (3 mg/kg bw)

	Concentration [mg a.s. equiv./kg]					
	Male			Female		
Sampling time	**1 h**	**6 h**	**24 h**	**1 h**	**6 h**	**24 h**

Parent compound	4.268	2.108	0.082	4.695	2.530	0.102
BYI 02960-DFA	0.042	0.176	0.200	0.038	0.133	0.144
unknown	---	0.036	---	---	---	---
unknown	---	0.019	---	---	---	---
BYI 02960-difluoroethyl-amino-furanone	0.084	0.153	---	---	---	---
unknown	---	0.034	---	---	---	---
BYI 02960-OH	0.341	0.334	0.020	0.155	0.115	0.011
Total identified	4.735	2.772	0.303	4.888	2.778	0.257
Total characterized *	---	0.088	---	---	---	---
Sum total **	**4.747**	**2.896**	**0.317**	**4.901**	**2.795**	**0.267**

* : Peaks were characterized based on their retention time in HPLC-analysis
** : Sum total = total identified or characterized + solids (unextractable) + samples not analyzed

Table 6.1.1.-43. Concentration of parent compound and metabolites in muscle of male and female rats sacrificed 1 h, 6 h, and 24 h after a single oral dose of [ethyl-1-^{14}C] flupyradifurone (3 mg/kg bw)

	Concentration [mg a.s. equiv./kg]					
	Male			Female		
Sampling time	**1 h**	**6 h**	**24 h**	**1 h**	**6 h**	**24 h**
Parent compound	2.201	1.097	0.075	2.827	1.568	0.055
BYI 02960-DFA	0.021	0.129	0.124	0.023	0.070	0.108
BYI 02960-difluoroethyl-amino-furanone	0.050	0.070	---	0.016	0.019	---
BYI 02960-OH	0.061	0.049	---	0.035	0.021	0.003
Total identified	2.332	1.346	0.198	2.901	1.678	0.166
Sum total **	**2.336**	**1.352**	**0.208**	**2.902**	**1.682**	**0.167**

* : Peaks were characterized based on their retention time in HPLC-analysis
** : Sum total = total identified or characterized + solids (unextractable) + samples not analyzed

Table 6.1.1.44. Concentration of parent compound and metabolites in fat of male and female rats sacrificed 1 h, 6 h, and 24 h after a single oral dose of [ethyl-1-^{14}C] flupyradifurone (3 mg/kg bw)

	Concentration [mg a.s. equiv./kg]					
	Male			Female		
Sampling time	**1 h**	**6 h**	**24 h**	**1 h**	**6 h**	**24 h**
Parent compound	0.911	0.389	0.030	1.079	0.624	0.013
BYI 02960-DFA	---	0.051	0.074	---	0.031	0.032
unknown	---	0.035	---	---	---	---
BYI 02960-OH	---	0.018	---	---	---	---
Total identified	0.911	0.458	0.104	1.079	0.654	0.045
Total characterized *	---	0.035	---	---	---	---
Sum total **	**0.911**	**0.493**	**0.108**	**1.079**	**0.655**	**0.047**

* Peaks were characterized based on their retention time in HPLC-analysis
** Sum total = Total identified or characterized + solids (unextractable) + samples not analyzed

Acceptability

The study was considered acceptable.

Conclusions

The distribution of the radioactivity within the organs and tissues (*i.e.* blood, liver, kidney, muscle and fat tissue) showed a distinctive preference for liver and kidney as the main metabolizing and excretory organs. The highest radioactivity concentrations were measured in plasma, and in organs and tissues at one hour after administration. They decreased significantly within the test period of 24 hours. There were no indications of irreversible binding or retention of radioactivity in organs and tissues. The parent compound, major and several minor metabolites were identified in all samples. Identification rates were high with ≥ 95% of radioactivity in urine and approx. 93 - 100% of the radioactivity in plasma, organs and tissues. The metabolic transformation of flupyradifurone was principally oxidative in nature and took place at least at 3 different structural positions of the molecule.
BYI 02960-DFA was by far the dominating metabolite in plasma, organs and tissues. On the other hand, the parent compound was the main constituent in urine. With regard to the extent of metabolism, a clear sex difference was observed since it was higher in male than in female rats. The metabolic degradation of the parent compound was much less pronounced in females as compared to males.

B.6.1.2 Toxicokinetic studies – Repeated dose, oral route

All single dose experiments revealed no indication of a potential for retention, accumulation and/or persistence of the administered radioactivity in organs or tissues. This observation is supported by the low log P_{OW} of BYI 02960 of 1.2. Therefore, in line with paragraph 26 of the OECD Test Guideline 417 (July 22nd, 2010), a repeated dose study was not considered necessary.

Results from expert consultation 2.2.

The RMS considers that from a scientific point of view there is no need for a toxicokinetic study with repeated dosing.

It was noted that after single dose, residual radioactivity was measured in fatty tissues/fat.

Considering the radioactive labelling, it is expected that residues can also be related to metabolites.

The biphasic kinetic was not evident in the different studies available.

Considering all the information available, the experts agreed that there is no potential for bioaccumulation, flupyradifurone being completely absorbed and excreted according to a biphasic kinetic.

The waiving of the repeat dose toxicokinetic study was agreed by the experts.

B.6.1.3 List of identified metabolites

A comprehensive list of major metabolites detected in the rat is provided in the following table.

Report Name	Chemical Structure	IUPAC Name
active substance: BYI 02960		4-[[(6-chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino]furan-2(5H)-one
BYI 02960-OH		4-{[(6-chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino}-5-hydroxyfuran-2(5H)-one
BYI 02960-iso-OH		

BYI 02960-OH-gluA (isomer 1)		
BYI 02960-OH-gluA (isomer 3)		3-{[(6-chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino}-5-oxo-2,5-dihydrofuran-2-yl beta-D-glucopyranosiduronic acid
BYI 02960-hippuric acid		N-[(6-chloropyridin-3-yl)carbonyl]glycine

B.6.1.4 Proposed metabolic pathway

B.6.1.5 Summary and conclusions

Summary

Absorption, distribution, excretion and metabolism of flupyradifurone was investigated using three different labelling positions. The active substance was labelled with ^{14}C in the pyridinylmethylene bridge, in the 4-position of the furanone ring and in the 1-position of the ethyl side chain:

Pyridinylmethyl-^{14}C flupyradifurone

Furanone-4-^{14}C flupyradifurone

Ethyl-1-^{14}C flupyradifurone

The pyridinylmethyl-labelled compound was used in an ADME-study in which male and female rats were orally administered with a single low dose of 2 mg/kg bw or a high dose of 200 mg/kg bw. Due to the high water solubility of flupyradifurone, male rats were also given an intravenous dose of 2 mg/kg bw. In this study, the excretion via urine and faeces was investigated as well as the distribution in the plasma and the radioactivity concentration in organs and tissues at sacrifice. The metabolism was investigated in urine and faeces.

A quantitative whole body autoradiography study was conducted also using the pyridinylmethyl-labelled compound following a single oral dose of 5 mg/kg bw to male and female rats. In this study the excretion of radioactivity was determined in urine, faeces and expired air, as well as the distribution in the plasma and the radioactivity concentration in the organs and tissues at various time points.

The furanone-4-labelled compound was used in an ADME-study in which male and female rats were orally administered with a single dose of 2 mg/kg bw. In this study, the excretion via urine and faeces was investigated as well as the distribution in the plasma and the radioactivity concentration in the organs and tissues at sacrifice. The metabolism was investigated in urine and faeces.

A quantitative whole body autoradiography study was also conducted using the furanone-4-labelled compound following a single oral dose of 5 mg/kg bw to male and female rats. In this study the excretion of radioactivity was determined in urine, faeces and the expired air as well as the distribution in the plasma and the radioactivity concentration in the organs and tissues at various time points.

In an organ metabolism study, male and female rats were orally administered with a single dose of 3 mg/kg bw [furanone-4-^{14}C] flupyradifurone. Animals were sacrificed 6 hours after dosage and the metabolism was investigated in urine, plasma, and in extracts of liver, kidney, muscle and fat tissues.

The ethyl-1-labelled compound was used in an ADME-study in which male rats were orally administered with 2 mg/kg bw. In this study, the excretion via urine, faeces and expired air was investigated as well as the distribution in the plasma and the radioactivity concentration in the organs and tissues at sacrifice. The metabolism was investigated in urine and faeces.

In an organ metabolism study, male and female rats were orally administered with a single dose of 3 mg/kg bw [ethyl-1-^{14}C] flupyradifurone. The animals were sacrificed at 1, 6 or 24 hours after dosing. The total radioactivity was determined over the different time periods in urine, while in plasma, liver, kidney, muscle and fat tissue at different time points after sacrifice. The metabolism was investigated in urine, plasma, and in extracts of liver, kidney, muscle, and fat tissue.

Conclusions

Following oral administration of a single low dose of flupyradifurone to male and female rats, the gastrointestinal absorption of radioactivity was high. It accounted for >80 % of the dose independent of the labelling position used. The value of >80% can be used for further toxicological risk assessment. Excretion was very fast, mainly renal and almost completed after 24 hours. No radioactivity was detected in the expired air after dosing of the pyridinylmethyl- and ethyl-1-labelled compounds, proving the stability of these labelling positions in the molecule. Only after administration of [furanone-4-^{14}C] flupyradifurone between 1% and 3% of the administered radioactivity was exhaled. This demonstrated that for a small portion of the dose (higher in male than in female rats) the furanone ring of the molecule obviously was opened and was subject of biotransformation to C-1 fragments.
The maximum plasma concentration was reached in most cases within 1 or 2 hours after administration of low doses. Only after administration of the high dose of 100 mg/kg bw the peak plasma concentration was observed between 2 and 4 hours after dosage. After reaching the peak concentration, the radioactivity levels in plasma declined steadily by several orders of magnitude in all studies independent of sex or labelling position of the test compound.

Quantitative whole body autoradiography revealed a fast absorption and distribution of the test compound with peak values observed already 1 hour after administration. At this time, the concentrations in liver and kidney were significantly higher than in blood, suggesting a preferred clearance from blood and distribution mainly to these organs which are mainly responsible for metabolism (liver) and excretion (kidney). Higher concentrations than in blood were also detected in the myocardium (heart) and in several glands. Very low levels were found in the brain, spinal cord and renal fat tissue. These results were similar in male and female rats, and independent of the labelling position of the test compound. A fast decline of radioactivity concentrations was observed for all organs and tissues in male and female rats during the entire test period. Concentrations fell for most organs and tissues below 5% of the maximum concentration after one day. After seven days, only very low concentrations were found in a few organs and tissues of rats dosed with the pyridinylmethyl-labelled test compound. In the study using the furanone-4-labelled compound, low radioactive residues were measured in almost all organs and tissues due to the incorporation of C1- or C2-fragments into the endogenous carbon pool. The residues in male rats were higher by a factor of 1.4 to 4.7 as compared to female rats. A similar ratio of approximately 3 (male/female) was also found for the formation of $^{14}CO_2$, and originates presumably from sex related differences in metabolism leading to more C1- and C2-fragments and also higher incorporation of these components into the

endogenous carbon pool in male rats. In general males and female rats exhibited very similar absorption, distribution and excretion behaviour. The results of these studies demonstrate that there is no indication of any accumulation or significant retention of radioactivity in male and female rats. This observation is supported by the low P_{OW} of 1.2. Concentrations of radioactivity detected in tissues and organs at sacrifice were either very low or below the limit of detection.

Flupyradifurone was intensively metabolised in the rat. Numerous metabolites were formed, most of them being minor ones. The parent compound represented the predominant part of the radioactivity in urine of male and female rats. In faeces of male rats, the metabolite BYI 02960-OH was more prominent than the parent compound. Two metabolites, BYI 02960-6-CNA and BYI 02960-hippuric acid were also prominent in male but not in females rats. The organ metabolism study using the ethyl-1-^{14}C label showed that in the 24 hours samples of plasma, and organs and tissues BYI 02960-DFA was by far the dominating metabolite accounting for more than 50% of the radioactivity. In general, the metabolic profiles in urine and faeces were very similar for both sexes but male rats showed a higher rate of metabolite formation as compared to female rats.

The principal metabolic reactions of flupyradifurone in rats were:

- Hydroxylation followed by conjugation with glucuronic acid or sulfate,
- cleavage of the difluoroethyl group forming BYI 02960-des-difluoroethyl, and difluoroacetic acid (BYI 02960-DFA),
- cleavage of the molecule at the pyridinylmethylene bridge forming BYI 02960-6-CNA, which was further conjugated with glycine to BYI 02960-hippuric acid and BYI 02960-difluoroethyl-amino-furanone.
- The figure below schematically shows the sites of the molecule, which are involved in the metabolic reactions:

Summarizing, the results of the metabolism studies conducted in the rat, a proposed metabolic pathway of flupyradifurone can be described as shown in this figure:

B.6.2 ACUTE TOXICITY INCLUDING IRRITANCY AND SKIN SENSITISATION (ANNEX IIA 5.2)

B.6.2.1 Acute toxicity

STUDY 1

Characteristics

reference	:	[redacted]	exposure	:	Once by gavage
type of study	:	Acute oral toxicity study	doses	:	300, 2000 mg/kg bw
year of execution	:	2009	vehicle	:	2% Cremophor EL in tap water
test substance	:	BYI 02960 (batch 2009-000239, purity 96.2%)	GLP statement	:	Yes
route	:	Oral	guideline	:	According to OECD 423
species	:	Rat, Wistar Hsd Cpb:Wu	acceptability	:	Acceptable
group size	:	3f (step 1 and step 2)/dose	**LD50-cut off**	:	>300-2000 mg/kg bw

Study design

The study was performed in accordance with OECD 423.

Four groups of three fasted female Wistar rats (HsdCpb: WU) were given a single oral dose (10 mg/lkg) of BYI 02960 (batch 2009-000239, purity 96.2%) in 2% Cremophor EL. Clinical signs and mortality rates were determined several times on the day of administration and subsequently normally once daily for an observation period of at least 14 days. Weight gain of the animals was checked weekly until the end of the study. Animals which died or were killed in moribund state were weight (except on day of administration) and dissected as soon as possible and examined macroscopically. Surviving animals were sacrificed, dissected and examined macroscopically.

Results

Mortality:

Dose mg/kg bw	Mortality	Time of death
1st 2000	1/3	2h
2nd 2000	3/3	3h
1st 300	0/3	--
2nd 300	0/3	--

Symptoms of toxicity:

In animals dosed with 2000 mg/kg bw clinical signs included decreased motility, tremor, piloerection, labored breathing and clonical cramps. In animals treated with 300 mg/kg bw breathing sounds were noted.

Body weight:

The mean body weights increased throughout the study period.

Pathology:

In animals dosed with 2000 mg/kg that died during the observation period gross pathological findings included black or black spotted liver and hemorrhagic lung. No particular findings were observed in the surviving animals.

Acceptability

This study is considered acceptable.

Conclusions

The LD50-cut off of BYI 02960 was found to be >300-2000 mg/kg bw.

STUDY 2

Characteristics

reference	:	[redacted]	exposure	:	24 hours on a 10% skin area (semi-occlusive)
type of study	:	Acute dermal toxicity study	doses	:	2000 mg/kg bw
year of execution	:	2009	vehicle	:	Distilled water
test substance	:	BYI 02960 (batch 2009-000239, purity 96.2%)	GLP statement	:	Yes
route	:	Percutaneous	guideline	:	According to OECD 402
species	:	Rat, Wistar Hsd Cpb:Wu	acceptability	:	Acceptable
group size	:	5/sex	**LD_{50}**	:	>2000 mg/kg bw

Study design

The study was performed in accordance with OECD 402.

Groups of five animals/sex were exposed to BYI 02960 (batch 2009-000239, purity 96.2%) The test material was moistened and applied to a skin area of 30 cm^2 at a dose of 2000 mg/kg bw under semi-occlusive dressing. After 24 hours the dressings were removed and the area was rinsed. Clinical sign and mortality rates were determined several times on the day of application and subsequently at least once daily for an observation period of at least 14 days. The weight gain of the animals was checked weekly until the end of the study. At the end of the study animals were sacrificed, dissected and examined for gross pathological changes.

Results

Mortality: No mortality was observed

Symptoms of toxicity: No treatment related findings were observed

Body weight: The mean body weights increased throughout the study period.

Pathology: No macroscopic pathologic abnormalities were observed.

Acceptability

The study is considered acceptable.

Conclusions

The acute dermal LD50 of BYI 02960 in male and female rats was found to be greater than 2000 mg/kg bw.

STUDY 3

Characteristics

reference	:	[redacted]	exposure	:	4 hours; nose-only
type of study	:	Acute inhalation toxicity study	doses	:	12.33 mg/L (nominal concentration), 4.7 mg/L (analytical concentration) MMAD 2.04 with GSD 1.86
year of execution	:	2009	vehicle	:	50% (w/w) PEG 400 (Lutrol)
test substance	:	BYI 02960 (batch 2009-000239, purity 96.2%)	GLP statement	:	Yes
route	:	Inhalation	guideline	:	According to OECD 403
species	:	Rat, Wistar Hsd Cpb: WU (SPF)	acceptability	:	Acceptable
group size	:	5/sex	**LC_{50}**	:	> 4.7 mg/L

Study design

The study was performed in accordance with OECD 403 (1981).

Five male and five female Wistar Hsd Cpb:WU (SPF) rats were simultaneously exposed under nose only conditions for 4 h. The test article was aerosolized as 50% (w/w) solution in PEG 400 (Lutrol). Clinical signs and mortality rates were examined several times on the day of exposure and at least once daily for an observation period of 14 days. At the end of the study animals were sacrificed, dissected and gross pathological changes were recorded.

Results

Mortality: No mortality occurred.

Symptoms of toxicity:

Clinical signs after exposure to 4.7 mg/L BYI 02960 included increased breathing, labored breathing patters, irregular breathing, piloerection, motility reduced or increased, anxiety, tremor, limp, gait high-legged, exophthalmia, nose red encrustations, stridor, and abdominal position with uncoordinated movements. 48 hours after exposure all rats were without clinical signs.

Body weight: No treatment related effect on body weight was observed.

Pathology: No macroscopic pathologic abnormalities were observed.

Acceptability

The study is considered acceptable.

Conclusions

Acute LD_{50} for male and female rats was found to be >4.7 mg/L.

B.6.2.2 Irritation and sensitisation

STUDY 1

Characteristics

reference	:	[redacted]	exposure	:	4-hours semi-occlusive
type of study	:	Skin irritation study	doses	:	0.5 g
year of execution	:	2009	vehicle	:	Distilled water
test substance	:	BYI 02960 (Batch 2009-000239, purity 96.2%)	GLP statement	:	Yes
route	:	Dermal	guideline	:	According to OECD 404
species	:	Rabbit, New Zealand White Crl:KBL(NZW)BR	acceptability	:	Acceptable
group size	:	3 females	**Effect**	:	Not skin irritating

Study design

The study was performed in accordance with OECD 404 (2002).

Three New Zealand female rabbits (Crl:KBL(NZW)BR) were exposed to 0.5 g of pulverized BYI 02960 (purity 96.2%) moistened with water on a skin area of 2.5 x 2.5 cm^2 under semi-occlusive dressing. After 4 hours of exposure the patch was removed and the exposed area was washed with water. Dermal irritation was scored at approximately 1, 24, 48 and 72 hours after patch removal.

Results

The results are summarised in the tables below.

Individual irritation scores

Scores observed after	**1 hour**	**24 hours**	**48 hours**	**72 hours**
Erythema	0/0/0	0/0/0	0/0/0	0/0/0
Oedema	0/0/0	0/0/0	0/0/0	0/0/0

Mean value irritation scores

Animal	mean 24-72 hrs	
	erythema	oedema
1	0	0
2	0	0
3	0	0
mean	0	0

Acceptability

The study is considered acceptable.

Conclusions

BYI 02960 was found to be non-irritating to rabbit skin.

STUDY 2

Characteristics

reference	:	[redacted]	exposure	:	single instillation in conjunctival sac
type of study	:	Acute eye irritation study	doses	:	0.1 g
year of execution	:	2009	vehicle	:	none
test substance	:	BYI 02960 (Batch 2009-000239, purity 96.2%)	GLP statement	:	Yes
route	:	Ocular	guideline	:	According to OECD 405
species	:	Rabbit, New Zealand White Crl:KBL(NZW)BR	acceptability	:	Acceptable
group size	:	Three females	**Effect**	:	Not eye irritating

Study design

The study was performed in accordance with OECD 405 (2002).

0.1 g of the pulverized substance was placed into the conjunctival sac of one eye of the first animal. The other eye, which remained untreated, served as control. The eye was rinsed approximately 24 hours following instillation. Eye irritations were scored at 1, 24, 48 and 72 hours post application.

Results

The results are summarised in the tables below.

Individual irritation scores

Scores observed after	**1 hour**	**24 hours**	**48 hours**	**72 hours**
Cornea/opacity	0/0/0	0/0/0	0/0/0	0/0/0
Iris	0/0/0	0/0/0	0/0/0	0/0/0
Conjunctiva redness	2/2/2	2/1/1	0/0/0	0/0/0
Conjunctiva chemosis	0/0/1	1/0/0	0/0/0	0/0/0

Mean value irritation scores

Animal	mean 24-72 hrs			
	Corneal opacity	Iris	Conjunctiva redness	Conjunctiva chemosis
1	0	0	0.7	0.3
2	0	0	0.3	0
3	0	0	0.3	0
mean	0	0	0.4	0.1

Acceptability

The study is considered acceptable.

Conclusions

BYI 02960 is considered not irritating to eyes.

STUDY 3

Characteristics

reference	:	[redacted]	exposure	:	Epicutaneous application dorsal part of both ears; 3 consecutive days
type of study	:	Murine Local Lymph Node Assay (LLNA)	doses	:	0, 2, 10, 50%
year of execution	:	2009	vehicle	:	Dimethylformamide
test substance	:	BYI 02960 (Batch 2009-000239, purity 96.2%)	GLP statement	:	Yes
route	:	Dermal	guideline	:	According to OECD 429 (2002)
species	:	Mouse; Hsd Win: NMRI	acceptability	:	Acceptable
group size	:	6 female/group	**Effect**	:	Not skin sensitising

Study design

The study was performed in accordance with OECD 429 (2002). Instead of ^{3}H-Thymidin or ^{125}I-iododeoxyuridine and 10^{-5}M fluorodeoxyuridine incorporation to measure cell proliferation, cell counts were used as described below.

Six female NMRI mice (Hsd Win:NMRI) per group received 25 µl BYI 02960 (batch 2009-000239, purity 96.2%) at 0%, 2%, 10% and 50% epicutaneously onto the dorsal part of both ears. The treatment was repeated three times. The animals were sacrificed one day after the last application. After sacrifice the weight of the lymph nodes was determined and after crushing the lymph nodes through a sieve into a 12-well plate, the cell counts per ml were determined. Before the first treatment and before sacrifice the thickness of both auricles was measured and on day 4 of the study the ear weight of sacrificed animals was measured.

A concurrent positive control was not included. In the study it is indicated that the last reliability test (November 2008) using Alpha Hexyl Cinnamic Aldehyde formulated in acetone/olive oil (4:1) at concentration of 3%, 10% and 30% showed the sensitizing potential of the test item. However, the data was not included in the study.

Results

No signs of systemic toxicity were noticed. No effect on body weight was observed.

Treatment	Cell count Stimulation Index	Lymph node weight Stimulation Index	Ear weight Stimulation Index	Ear thickness Stimulation Index
Vehicle	1.00	1.00	1.00	1.00
2%	1.21	1.01	1.00	1.00
10%	0.97	0.92	1.00	1.01
50%	0.87	0.93	0.97	1.00

Acceptability

The study is considered acceptable.

Conclusions

BYI 02960 has no sensitising potential in the LLNA test in concentrations up to and including 50%.

B.6.2.3 Summary

The results of the acute toxicity, irritation and sensitisation studies are presented in table 6.2.3.1, 6.2.3.2 and 6.2.3.3, respectively.

Table 6.2.3.1 Acute toxicity, LD_{50}/LC_{50} values

Test substance	LD_{50}/LC_{50}	Species	Route	Vehicle	Reference
BYI 02960	>300-2000 mg/kg bw	Rat	Oral	2% Cremophor EL in tap water	[redacted] U. 2009
BYI 02960	>2000 mg/kg bw	Rat	Dermal	Distilled water	[redacted]
BYI09260	> 4.7 mg/L	Rat	Inhalation	50% PEG 400 (Lutrol)	[redacted] 2010

Table 6.2.3.2 Skin and eye irritation studies

Test substance	Effect/Classification	Species	Route	Vehicle	Reference
BYI 02960	Not skin irritating	Rabbit	Dermal	Distilled water	[redacted] 2009
BYI 02960	Not eye irritating	Rabbit	Occular	None	[redacted] 2009

Table 6.2.3.3 Sensitisation studies

Test substance	Effect/Classification	Species	Route	Vehicle	Reference
BYI 02960	Not skin sensitising (LLNA)	Mouse	Dermal	Dimethylformamide	[redacted]. 2009

Flupyradifurone (BYI 02960) needs to be classified as Acute oral toxic Cat. 4 H302 according to Regulation (EC) 1272/2008. Flupyradifurone does not need to be classified on the basis of its acute dermal, and inhalation toxicology in rats (Category 5/ Unclassified). Flupyradifurone is considered not irritating to skin and eyes (Category 5/ Unclassified). Flupyradifurone does not have sensitising properties in a LLNA (Category 5/ Unclassified).

Table 6.2.3.3 Classification

Type of study	Species	Results	Classification (proposed)
Oral route	Rat	Mortalities observed at 2000 mg/kg; none at 300 mg/kg	Category 4 H302 (LD_{50} cut off >300-2000 mg/kg)
Dermal route	Rat	LD_{50} > 2 000 mg/kg	Category 5 / Unclassified
Inhalation	Rat	LC_{50} at 4 hours > >4.7	Category 5 / Unclassified

		mg/L (nose only) ~~4671 mg/m³~~	
Primary skin irritation	Rabbit	Non irritating	Category 5 / Unclassified
Eye irritation	Rabbit	Slight redness of the conjunctivae, reversed within 48 hours	Category 5 / Unclassified
Skin sensitization	Mouse	Not sensitizing	Category 5 / Unclassified

B.6.3 SHORT-TERM TOXICITY (ANNEX IIA 5.3)

B.6.3.1 28-day oral studies

Study 1

Characteristics

Reference	:	[redacted]	exposure	:	28 days
type of study	:	Exploratory 28-day rat oral toxicity study	dose	:	0, 75, 200, 350 mg/kg bw/day
year of execution	:	2006	vehicle	:	Corn oil supplemented with 10% ethanol and 10% water
test substance	:	BYI 02960 (Batch NLL 7780-11-5, purity 98.3%)	GLP statement	:	No
Route	:	gavage	guideline	:	-
Species	:	Rat, Wistar RJ: WI (IOPS HAN)	acceptability	:	Acceptable as range-finding study
group size	:	5/sex/dose	**NOAEL**	:	75 mg/kg bw/day

Study design

Groups of five animals per sex were orally dosed BYI 02960 (batch NLL 7780-16-5, purity 98.3%) for at least 28 days at dose levels of 75, 200 and 350 mg/kg bw/day. Animals were observed daily for mortality and clinical signs. Body weight and food consumption were recorded once weekly. Selected haematology and clinical chemistry parameters were assayed at the end of the study. At the end of the study, all animals were necropsied, selected organs were weighed and a range of tissues were examined microscopically. A portion of the liver was homogenized for microsomal preparations to determine cytochrome P-450 isoenzyme profile.

Results

Dose (mg/kg bw/day)	**0**		**75**		**200**		**350**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**

Dose (mg/kg bw/day)	**0**		**75**		**200**		**350**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Mortality	Two females at 350 mg/kg bw/day, one female at 200 mg/kg bw							
Clinical signs	Increased salivation in all exposed animals							
Body weight	Slight not statistically significant lower mean body weight (max 8%) in males at 350 mg/kg/day throughout the study and in females in the first week.							
Body weight gain (g)	180	71	170	65	176	76	159	88
Food consumption	Reduced at 350 mg/kg/day in males (17%) and females (29%) between study day 1 and 8, and by 8% in males between day 8 and 15. At 200 mg/kg/day reduced in females by 16% between day 1 and 8.							
Ophthalmology	Not determined							
Urinalysis	Not determined							
FOB	Not determined							
Haematology	No treatment related changes.							
Clin. Chemistry								
-total bilirubin (µmol/L)	1.2	1.6	**0.7*** **(-42%)**	0.9 (-44%)	**0.6*** **(-50%)**	**0.7*** **(-56%)**	**0.4**** **(-67%)**	**0.5**** **(-69%)**
- Glucose concentration	5.42	4.80	4.60 (-15%)	5.07 (+6%)	**3.92**** **(-28%)**	5.12 (+7%)	**2.87**** **(-47%)**	3.57 (-26%)
- Triglyceride concentration	1.14	0.45	1.50 (+32%)	0.46 (+2%)	1.32 (+16%)	0.87 (+93%)	1.89 (+66)	**1.51*** **(+236%)**
- Creatinine concentration	50	51	51 (+2%)	56 (+10%)	**54*** **(+8%)**	**63*** **(+24%)**	53 (+6%)	**63*** **(+24%)**
- Alanine aminotransferase	39	33	43 (+10%)	35 (+6%)	38 (-3%)	**43**** **(+30%)**	46 (+18%)	**51**** **(+55%)**
- Alanine phosphatase	165	95	163 (-1%)	110 (+16%)	166 (+1%)	96 (+1%)	165 (0%)	**126*** **(+36%)**
Organ weights								
-liver weight								
-absolute (g)	10.92	5.86	9.97 (-9%)	6.37 (+9%)	12.01 (+10%)	7.32 (+25%)	12.43 (+14%)	**8.74**** **(+49%)**
- relative to body weight (%)	3.095	2.552	2.913 (-6%)	2.850 (+12%)	3.442 (+11%)	**3.157*** **(+24%)**	**3.814*** **(+23%)**	**3.704**** **(+45%)**
- relative to brain weight	542.7	315.1	482.5 (-11%)	328.8 (+4%)	602.6 (+11%)	382.3 (+21%)	625.6 (+15%)	**460.6**** **(+46%)**

Dose (mg/kg bw/day)	0		75		200		350	
	m	f	m	f	m	f	m	f
Pathology								
Macroscopy (liver)								
- enlarged	0/5	0/5	0/5	0/5	1/5	0/4	3/5	3/3
- prominent lobulation	0/5	0/5	1/5	0/5	2/5	0/4	5/5	2/3
Microscopy								
Centrilobular hepatocellular hypertrophy: diffuse								
- minimal	0	0	0	0	0	2	0	0
- slight	0	0	0	0	4	1	1	2
- moderate	0	0	0	0	1	0	3	1
- marked	0	0	0	0	0	0	1	0
- total	0	0	0	0	5	3	5	3
Follicular cell hypertrophy: diffuse								
- minimal	0	0	0	0	4	0	4	2
- slight	0	0	0	0	0	0	1	1
- total	0	0	0	0	4	0	5	3
Hepatotoxicity testing								
- P-450			X 1.2	NC	X 1.3	X 1.2	NC	X 1.2
- BROD			X 8	NC	X 25	X 8	X 25	X 28
- EROD			NC	NC	NC	**X 4***	**X 3***	**X 4***
- PROD			NC	NC	X 5	NC	X 5	X 4
- Lauric acid			NC	NC	NC	NC	NC	NC

*p≤0.05 **p≤0.01, NC = no significant change

Two females were found dead on Study Day 6 at 350 mg/kg bw/day and one female died at 200 mg/kg bw/day. The only clinical sign found was increased salivation in all animals at all dose levels. Mean body weight was slightly reduced in males by between 5% to 8% (not statistically significant) and in females by 4% on Study Day 8 (not statistically significant). Mean food consumption was reduced by maximally 17% in males and by 29% in females.

Clinical chemistry evaluations showed a decrease in total bilirubin in both sexes at the highest two dose levels. Glucose concentration was only significantly affected in males at 200 and 500 mg/kg bw/day. Triglyceride concentration and alaline phosphatase were significantly increased in high dose females. Both creatinine concentration and alanine aminotransferase were significantly increased in mid and high dose females. Creatine concentration was also significantly increased in males at the

mid dose only. The statistically significant difference observed in total biliribun at 75 mg/kg in males was due to only one animal with a total bilirubin concentration of 0.0 µmol/l, while the mean of the remaining animals was 0.85 µmol/l. Therefore, this effect was considered to not be treatment-related.

Mean absolute and relative liver weights were 14 to 23% higher in males and 45 to 49% higher in females, compared to controls at 500 mg/kg bw/day. Upon macroscopic examination, enlargement and prominent lobulation of the liver were observed in both sexes. The liver effects observed at 200 and 350 mg/kg/day were correlated with microscopic centrilobular hepatocellular hypertrophy. The one incidence of prominent lobulation at 75 mg/kg/day in males were without associated microscopic findings and were considered not to be adverse.

Hepatocellular enzymatic activity assays showed that EROD activity was not found to be clearly affected by treatment. As BROD activity was induced at 350 mg/kg/day in both sexes and at 200 mg/kg/day in males, with no clear induction of PROD activity, BYI 02960 appears to be an inducer of the Cytochrome P-450 3 A family.

Acceptability

The study was not performed under GLP, but it was performed in a GLP-certified laboratory. The study is acceptable as a dose range finding study.

Conclusions

Based on the clinical chemical finding, the increased organ weights and pathological findings the NOAEL is set at 75 mg/kg bw/day.

Study 2

Reference	:	[redacted]	exposure	:	28 days
type of study	:	Exploratory 28-day rat oral study	dose	:	0, 500, 5000 ppm M: 0, 33.6, 385 mg/kg bw/day)
year of execution	:	2007	vehicle	:	none
test substance	:	BYI 02960 (batch NLL 7780-27-1, purity 99.7%)	GLP statement	:	No
Route	:	Oral (Dietary)	guideline	:	-
Species	:	Rat, Wistar Rj:WI (IOPS HAN)	acceptability	:	Acceptable as range-finding study
group size	:	5 males/dose	**NOAEL**	:	33.6 mg/kg bw/d

Study design

Groups of five males were treated with BYI 02960 (batch NLL 7780-27-1, purity 99.7%) via the diet for at least 28 days at concentrations of 500 ppm (actual analyzed dose level of 410 ppm, which equates to 33.6 mg/kg/day BYI 02960) and 5000 ppm (equivalent to 385 mgkg bw/day). All animals were observed for mortality and clinical signs daily, body weight and food consumption were measured weekly. A detailed physical examination was performed weekly throughout the study. Before necropsy a blood sample was collected from the retro-orbital venous plexus of each surviving animal for selected clinical chemistry determinations and hormone analysis. All animals were necropsied, selected organs weighed and a range of tissues were taken, fixed and examined microscopically. The remaining portions of the liver were homogenized for microsomal preparations in order to determine cytochrome P-450 isoenzyme profile.

Results

Dose (ppm)	**0**	**500**	**5000**
Mortality	No mortalities occurred		
Clinical signs	No clinical signs were observed		
Body weight	At 5000 ppm, mean body weight was reduced by between 17 and 19% throughout the study period (statistically significant for the first 3 weeks)		
Body weight gain (g)	186	194	**116***
Food consumption	At 5000 ppm consistently reduced by 12 and 39%, most pronounced between study day 1 and 8.		
Ophthalmology	Not performed		
Urinalysis	Not performed		

Dose (ppm)	**0**	**500**	**5000**
FOB	Not performed		
Haematology	Not performed		
Clin. Chemistry			
- total bilirubin (µmol/L)	1.1	0.7 (-36%)	**0.3*** **(-73%)**
- Glucose concentration	6.91	6.60 (-4%)	**3.76**** **(-46%)**
- Urea	4.98	4.82 (-3%)	**6.83**** **(+37%)**
- Total cholesterol	1.43	1.29 (-10%)	**2.01*** **(+41%)**
- T4	48.5	58.7 (+21%)	39.5 (-19%)
- TSH	9.64	11.23 (+17%)	17.46 (+81%)
Organ weights			
-liver weight			
-absolute (g)	9.79	10.38 (+6%)	11.25 (+15%)
- relative to body weight (%)	2.751	2.873 (+4%)	**3.906**** **(+42%)**
- Thyroid gland			
- absolute (g)	0.0171	0.0165 (-4%)	0.0182 (+6%)
- relative to body weight (%)	0.0048	0.00457 (-5%)	**0.00632**** **(+32%)**
Pathology			
Macroscopy (liver)			
- prominent lobulation	0/5	2/5	4/5
Microscopy			
Centrilobular hepatocellular hypertrophy: diffuse			
- slight	0	0	2
- moderate	0	0	3
- total	0	0	5
Follicular cell hypertrophy: diffuse			

Dose (ppm)	0	500	5000
- minimal	0	0	2
- slight	0	0	3
- total	0	0	5
Hepatotoxicity testing		NC	NC
- P-450		NC	X 2.1
- BROD		NC	NC
- EROD		NC	NC
- PROD		NC	X 1.11
- UDPGT			

*p≤0.05 **p≤0.01 NC = no significant change

At 5000 ppm mean body weight was reduced with a maximum of 19% during the first three weeks of the study. Overall mean cumulative body weight was significantly reduced by 38% compared to controls. Mean food consumption was consistently reduced by maximally 39% with the effect being more pronounced between study day 1 and 8. Total bilirubin and glucose concentration were significantly decreased and urea and total cholesterol were significantly increased at 5000 ppm. Hormone analysis showed a non significant increase in TSH (+81%) and a slight decrease (-19%) in the plasma which was considered treatment related. At the high dose relative liver and thyroid weight were significantly increased. These were associated microscopic findings including slight and moderate centrilobular hepatocellular hypertrophy and minimal and slight follicular cell hypertrophy.

Acceptability

The study was not performed under GLP, but it was performed in a GLP-certified laboratory. The study is acceptable as a dose range finding study.

Conclusion

Based on the clinical chemical findings, the increased relative liver and thyroids weights and the associated pathological findings, the dose level of 500 ppm (33.6 mg/kg/day) is considered to be the NOAEL in this study.

Study 3

Reference	:	[redacted]	exposure	:	28-days
type of study	:	Preliminary 28-day mouse oral toxicity study	dose	:	0, 300, 600, 1200 ppm M: 0, 50, 98, 207 mg/kg bw/day F: 0, 59, 122, 240 mg/kg bw/day
year of execution	:	2007	vehicle	:	none
test substance	:	BYI 02960 (NLL 7780-27-1, purity 99.7%)	GLP statement	:	No
Route	:	(Oral) dietary	guideline	:	-
Species	:	Mouse C57BL/6J	acceptability	:	Acceptable as range-finding study
group size	:	5 sex/dose	**NOAEL**	:	98 mg/kg bw/d ~~166 mg/kg bw/day*~~

~~*Dose adjusted for stability of DYI 02960 in the diet~~

Study design

BYI 02960 was administered continuously via the diet to groups of C57BL/6J mice (five/sex) for at least 28 days at concentrations of 300, 600 and 1200 ppm (equating approximately to 50, 98 and 207 mg/kg bw/day in males and 59, 122, 240 mg/kg bw/day in females). No stability study was performed on the submitted feed. Taking into consideration the available stability data (study SA 07131), the actual concentration received by the treated animals is assumed to be between 80% and 90% of nominal concentrations. Animals were observed daily for mortality and clinical signs. Body weight and food consumption were recorded weekly. Selected clinical chemistry parameters were determined at the end of the study. All animals were subjected to necropsy, selected organs were weighed and a range of tissues were fixed and examined microscopically.

Results

Dose (ppm)	**0**		**300**		**600**		**1200**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Mortality	No mortalities occurred during the study							
Clinical signs	No clinical signs were observed							
Body weight	In males at 1200 ppm, mean body weight was slightly reduced by 6% on study day 8 only.							
Body weight gain (g)	3.3	3.8	3.2	3.9	3.7	3.3	2.8	4.0
Food consumption	No effect on mean food consumption was noted							
Ophthalmology	Not performed							
Urinalysis	Not performed							

Dose (ppm)	**0**		**300**		**600**		**1200**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
FOB	Not performed							
Haematology	Not performed							
Clin. Chemistry								
- Alanine aminotransferase	39	35	39 (0%)	40 (+14%)	30 (-23%)	36 (+3%)	29 (+26%)	**50** (+43%)**
- Alanine phosphatase	112	151	110 (-2%)	174 (+15%)	113 (+1%)	137 (-9%)	118 (+5%)	**183* (+21%)**
Organ weights								
-epididymis								
-absolute (g)	0.10		**0.08* (-20%)**		**0.08* (-20%)**		**0.07** (-30%)**	
- relative to body weight (%)	0.470		**0.384* (-18%)**		0.401 (-15%)		**0.364** (-23%)**	
- Spleen								
-absolute (g)	0.040	0.061	**0.049* (+23%)**	0.053 (-13%)	0.044 (+10%)	0.054 (-11%)	0.044 (+10%)	0.051 (-16%)
- relative to body weight (%)	0.1956	0.3534	**0.2394** (+22%)**	0.3094 (-12%)	0.2148 (+9.8%)	0.3153 (-11%)	0.2214 (+13%)	0.3052 (-14%
Pathology Macroscopy	No treatment related findings							
Microscopy	No treatment related findings							

*p≤0.05 **p≤0.01

There were no mortalities or clinical signs during the course of the study in any treatment group. Mean body weight was slightly reduced by 6% in males at 1200 ppm at study day 8 only. At 1200 ppm mean body weight gain/day was -0.03 g (p≤0.05) compared with 0.14 g/day in the control group between Study Days 1 and 8. Overall cumulative mean body weight gain was reduced by 15% between Study Days 1 and 29. As the effect on body weight was slight and transient and in the absence of other findings, it was considered to be a non-adverse effect of treatment. In historical control data from 28-day mouse study performed in the same laboratory with animals from the same strain and the same age, it can be seen that the range for mean cumulative body weight gain in males is between 2.2 to

3.9 grams. The cumulative body weight gain of 2.8 g for males administered at 1200 ppm is therefore within this range.
When compared to the controls, a higher alanine aminotransferase (+43%, p <0.01) and alkaline phosphatase (+21%, p <0.05) activities were observed in females at 1200 ppm. However in view of the variation of the individual values and the lack of a clear dose response these changes were considered not to be treatment-related.
Lower epididymis weights were found in treated animals when compared to controls but this change was considered not to be relevant since it was not associated with relevant histological findings. Mean absolute and relative spleen weights were statistically significantly higher in males at 300 ppm when compared to controls, but this change was considered not to be relevant since it was not dose-related.

Acceptability
The study was not performed under GLP, but it was performed in a GLP-certified laboratory. The study is acceptable as a dose range finding study.

Conclusion
In conclusion, continuous dietary administration of BYI 02960 to the C57BL/6J mouse for at least 28 days resulted in a No Observed Adverse Effect Level (NOAEL) in males and a No Observed Effect Level (NOEL) in females at a nominal concentration of 1200 ppm. However given the instability of BYI 02960 in rodent diet the actual concentration is considered to be in the region of 960 to 1080 ppm (166 to 186 mg/kg body weight/day for the males and 192 to 216 mg/kg body weight/day for the females, respectively).

Note: The storage stability in the other studies presented in the DAR gave no indication of concern.

Expert consultation 2.5.

The RMS noted that the decreased epididymis weight was within background historical control data. Due to the low number of animals by dose group, the study has to be considered as supplementary.

Considering the reduction of body weight gain at the high dose (15%), this might be considered as a LOAEL. Additionally ALT was also increased at the high dose. Based on these effects the experts agreed on a NOAEL of 98 mg/kg bw per day.

The dose was adjusted for the stability of the diet, and therefore calculated to a NOAEL of 166 mg/kg bw/d. The stability of the diet was not discussed during the expert meeting. Since the study is a preliminary study, and not critical for risk assessment. The RMS can agree with a worst-case NOAEL of 98 mg/kg bw/d.

Data requirement 2.2

Applicant to provide the historical control data regarding epididymis weights for the 28-day study in mice.

Epididymis weights observed in the 28-day toxicity mouse study

In this 28-day mouse study, Flupyradifurone was administered continuously via the diet to groups of C57BL/6J mice (5/sex/group) for at least 28 days at concentrations of 300, 600 and 1200 ppm (equating approximately to 50, 98 and 207 mg/kg bw/day in males and 59, 122 and 240 mg/kg bw/day in females.

Lower epididymis weights were found in treated animals when compared to controls but this change was considered not to be relevant since it was not dose-related and not associated with relevant histological findings. Furthermore the epididymis weights were within historical control data as can be seen in the following table. These historical control data were taken from two 28-day mouse toxicity studies performed in the same laboratory using mice from the same supplier administered with the same diet, A04Cp1-10 from S.A.F.E (SA 00226 performed in 2001 and SA 08036 performed in 2008; more 28-day mouse studies were performed in the laboratory during this period but epididymis weights were not collected).

Table 1: Epididymis weight changes

Dose levels in ppm	Animal Numbers	Absolute organ weights (g)	Organ to body weight ratios (%)	Organ to brain weight ratios (%)
0	RT1M1406	0.10	0.51	23.26
	RT1M1407	0.08	0.39	18.18
	RT1M1408	0.09	0.45	19.57
	RT1M1409	0.11	0.53	25.00
	RT1M1410	0.10	0.47	22.22
	Mean	**0.10**	**0.47**	**21.645**
	SD	**0.01**	**0.054**	**2.761**
300	RT2M1416	0.09	0.45	21.43
	RT2M1417	0.08	0.40	18.60
	RT2M1418	0.08	0.37	17.78
	RT2M1419	0.07	0.36	16.67
	RT2M1420	0.07	0.34	15.91
	Mean	**0.08***	**0.384***	**18.077***
	SD	**0.01**	**0.044**	**2.138**
600	RT3M1426	0.07	0.33	15.91
	RT3M1427	0.09	0.46	20.00
	RT3M1428	0.08	0.38	17.78
	RT3M1429	0.09	0.47	20.45
	RT3M1430	0.08	0.36	17.39
	Mean	**0.08***	**0.401**	**18.307**
	SD	**0.01**	**0.059**	**1.894**
1200	RT4M1436	0.07	0.37	15.56
	RT4M1437	0.07	0.35	15.91
	RT4M1438	0.08	0.41	18.18
	RT4M1439	0.07	0.34	15.56
	RT4M1440	0.07	0.34	15.22

	Mean	**0.07****	**0.364****	**16.084****
	SD	**0.00**	**0.029**	**1.198**
Historical control data				
Mean		0.08	0.376	18.120
Standard deviation		0.01	0.060	3.409
Number of animals		10	10	10
Minimum value		0.07	0.315	14.894
Maximum value		0.10	0.500	23.810
Percentile 5%		0.07	0.321	15.039
Percentile 95%		0.10	0.479	23.560

*: Bartlet, NS -05/Anova, S 05/ Dunnett's P<0.05

**: Bartlet, NS -05/Anova, S 05/ Dunnett's P<0.01

Study 4

Reference	:	[redacted]	exposure	:	Repeated by diet, 28 days
type of study	:	Preliminary 28 day dog oral toxicity study	dose	:	0, 500, 2000, 4000 ppm M: 0, 16, 62, 118 mg/kg bw/day F: 0, 18, 77, 131 mg/kg bw/day
year of execution	:	2008	vehicle	:	none
test substance	:	BYI 02960 (batch NLL 7780-44-6, purity 99.5%)	GLP statement	:	No
Route	:	Oral (dietary)	guideline	:	-
Species	:	Beagles	acceptability	:	Acceptable as range-finding study
group size	:	2/sex/dose	**NOAEL**	:	62 mg/kg bw/day

Study design

Groups of two males and two females received BYI 02960 mixed in their diet at concentrations of 500, 2000 or 4000 ppm (equating approximately to 16, 62, 118 mg/kg body weight/day in males and 18, 77, 131 mg/kg body weight/day in females) for at least 28 days. Mortality and clinical signs were checked at least once daily. Food consumption was recorded daily and body weight was measured weekly. Once during the acclimatization phase and at the end of the treatment, Ophthalmological examination, detailed physical examination, blood analysis (haematology, clinical chemistry) and urinalysis were performed. All animals were subjected to a detailed necropsy. Selected organs were weighed and a range of tissues were taken and processed for histopathological examination.

Results

Dose (ppm)	**0**		**500**		**2000**		**4000**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Mortality	No mortalities occured							
Clinical signs	At 4000 ppm genital discharge between day 15 to 22 in one female (attributed to oestrus)							
Body weight	At 4000 ppm overall body weight loss of 0.2 kg in one male.(-2%)							
Body weight gain (g)	No body weight gain in one male and one female at 4000 ppm (0.5-1.1 kg weight gain in controls)							
Food consumption	At 4000 ppm lower food consumption in all animals.							
Ophthalmology	No treatment-related findings							
Urinalysis	No treatment-related finding							
FOB	Not performed							

Dose (ppm)	**0**		**500**		**2000**		**4000**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Haematology - platelet counts	Increased platelet counts in both females (+43 and +36%, relative to their own pre-study value) and in one of the two males at 4000 ppm (+30%) and in one of the two females at 2000 ppm (+28%).							
Clin. Chemistry	At 4000 ppm, an increase was observed in creatinine concentration in one female (+31%, relative to its own pre-study value).							
Organ weights	No treatment-related findings							
Pathology Macroscopy - enlarged thyroid	0	0	0	0	0	0	0	2
Microscopy **Centrilobular glycogen accumulation** - minimal	0	2	0	2	1	2	0	0
- slight	2	0	2	0	0	0	0	0
- total	2	2	2	2	1	2	0	0

*p≤0.05 **p≤0.01, NC = no significant change

There was an overall body weight loss in one male and an absence of overall weight gain in one female at 4000 ppm. Lower food consumption was observed in both male and female animals compared to the controls.

Hematology assessment revealed an increased platelet counts in both females (+43 and +36%, relative to their own pre-study value) and in one of the two males (+30%, relative to its own pre-study value) at 4000 ppm. At 2000 ppm, an increase was noted in one of the two females (+28%, relative to its own pre-study value). In isolation, these treatment-related changes were not considered to be adverse.

Enlarged thyroid glands were noted in 2/2 females at 4000 ppm but this change was not considered to be treatment-related as there was no effect on thyroid weight and there were no microscopic findings for the thyroid. In the liver, centrilobular glycogen accumulation was decreased in incidence and/or severity at 4000 and 2000 ppm in males and at 4000 ppm in females. This finding was considered to be treatment-related, but not adverse.

Acceptability

The study was not performed under GLP, but it was performed in a GLP-certified laboratory. The study is acceptable as a dose range finding study.

Conclusion

Based on the body weight loss and reduced body weight gain a NOAEL of 2000 ppm is set (equating to 62 mg/kg/day in males and to 77 mg/kg/day in females).

B.6.3.2 28-day dermal studies

Characteristics

reference	:	[redacted]	exposure	:	28 days, 6 h/d, semi-occlusive (10% of the total body surface area)
type of study	:	28-day dermal toxicity study	dose	:	50, 150 or 500 mg/kg bw/d
year of execution	:	2012	vehicle	:	Deionized water
test substance	:	BYI 02960, lot/batch no. 2009-000239, purity 96.2 %, beige powder	GLP statement	:	yes
route	:	Dermal	guideline	:	Partly in accordance with OECD 410
species	:	Rat, Wistar Han CRL: WI (HAN)	acceptability	:	acceptable
group size	:	10 /sex/dose	NOAEL	:	500 mg/kg bw/d

Study design

The study was performed partly in accordance with OECD 410: the highest dose should have resulted in toxic effects, or the limit dose of 1000 m/kg bw/d should have been used as the highest dose level. According to the study report, the high dose of 500 mg/kg bw/d was selected after consideration of the amount of test material that can be reliably applied to the skin. See acceptability. Male and female rats, 10 rats/sex/group (one control and three test substance-dosed groups) were administered vehicle or BYI 02960 (50, 150, or 500 mg/kg/day) daily by dermal application for at least 28 consecutive days (minimum of 6 h/day) and euthanized one day following the last dose. An additional 6 rats were used to test compound wetting suitability. Two rats/group were treated with 200 mg BYI 02960 using 2 mL deionized water, 0.5% methylcellulose, or corn oil. After at least 2 h of exposure, water was found to be an appropriate wetting agent and was chosen to be used to dose in this study.

Results

Table 6.3.2.1

Dose (mg/kg bw/d)	**0**		**50**		**150**		**500**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	No mortalities occurred								
Clinical signs	Clinical signs of toxicity attributable to the test substance were not observed								
Body weight gain	Body weights and body weight gain were not affected by test substance administration.								
Food consumption (g/day)									
- Week 1								dc	
- Week 2				dc				dc	
Haematology	Hematology or coagulation parameter changes attributable to the test substance were not observed								
Clinical chemistry	Clinical chemistry parameter changes attributable to the test substance were not observed								
Organ weights									
- Liver (absolute)								d (6%)	

Dose (mg/kg bw/d)	0		50		150		500		dr
	m	f	m	f	m	f	m	f	
- Liver (relative)								d (9%)	
Pathology									
macroscopy	There were no test substance-related gross lesions observed								
microscopy	There was no test substance-related micropathology change in males or females at any dose level								

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls

Table 6.3.2.2 Food consumption (g/animal/day)

Dosage Level of BYI 02960	0 mg/kg	50 mg/kg	150 mg/kg	500 mg/kg
Males				
Week 1 (Days 0–7) (% Control)	20.87	21.14 (101%)	20.77 (100%)	20.91 (100%)
Week 2 (7-14) (%Control)	22.02	23.25 (106%)	22.27 (101%)	23.07 (105%)
Females				
Week 1 (Days 0–7) (% Control)	17.11	15.78 (92%)	16.64 (97%)	15.12* (88%)
Week 2 (7-14) (%Control)	19.33	17.34* (90%)	18.17 (94%)	17.51* (89%)

* P<=5%

At 500 mg/kg/day

No adverse effects attributable to exposure to the test substance were observed.

In the first and second week of daily dermal administration, females had statistical significantly lower food consumption (g/animal/day) as compared to controls. In addition, food efficiency (g/kg bw/day) was significantly lower in the first week. These findings were not considered to be biologically significant as changes were not consistently affected in a dose-related fashion, were not found in males, and did not impact clinical observations or body weights at any point.

The absolute and relative liver weights at 500 mg/kg/day males were non-statistically decreased by 6% and 9%, respectively, compared to control rats. There were no test substance-related clinical or gross or microscopic pathology changes to correlate this finding.

At 150 mg/kg/day

No adverse effects attributable to exposure to the test substance were observed.

At 50 mg/kg/day

No effects attributable to exposure to the test substance were observed.

Females administered with 50 mg/kg/day had significantly lower food consumption in the second week as compared to controls. This finding was not considered biologically significant as changes were not consistently affected in a dose-related fashion, were not found in males, and did not impact clinical observations or body weights at any point.

Acceptability

The study is considered acceptable despite the fact that not the limit dose of 1000 mg/kg bw/d is used. It is not fully explained in the report why 500 mg/kg bw/d is the amount of test material that can be reliably applied to the skin. However, the study did not indicate any route-specific effects and NOAEL in this dermal study is higher than the oral NOAEL, that is used for the derivation of the reference values.

Conclusions

Based on the lack of adverse findings observed in this study, the 28-day dermal exposure NOAEL in male and female rats dosed with BYI 02960 was 500 mg/kg bw/day.

B.6.3.3 28-day inhalation studies

Based on the results of the acute inhalation toxicity study and the physical properties of BYI 02960 (compound not volatile with an extrapolated vapour pressure of 9.1×10^{-7} Pa at 20 °C), no repeat inhalation study is triggered.

B.6.3.4 Semichronic oral studies

Study 1

Reference	:	[redacted]	exposure	:	Repeated diet, 90 days
type of study	:	90-day rat oral toxicity study	dose	:	0, 100, 500, 2500 ppm M: 0, 6.0, 30.2, 156 mg/kg bw/day F: 0, 7.6, 38.3, 186 mg/kg bw/day
year of execution	:	2008	vehicle	:	none
test substance	:	BYI 02960 (batch NLL 7780-44-6, purity 99.5%)	GLP statement	:	yes
Route	:	diet	guideline	:	OECD 408
Species	:	Rats, Wistar Rj:WI (IOPS HAN)	acceptability	:	Acceptable
group size	:	10/sex/dose	**NOAEL**	:	6 ~~30.2~~ mg/kg bw/day

Study design

Groups of 10 males and 10 females Wistar rats were fed diets containing BYI 02960 (batch NLL 7780-44-6, purity 99.5%) at concentrations of 100, 500, 2500 ppm (equating to approximately 6.0, 30.2, 156 mg/kg bw/day in males and 7.6, 38.3, 186 mg/kg bw/day in females) for at least 90 days. An additional 10 animals per sex were fed control or high dose test diet for at least 90 days and subsequently fed control diet and observed for reversibility or persistence of toxic effects after a post-treatment recovery period of at least 28 days. The stability of the test substance in the diet (concentrations of 20, 100 and 2500 ppm) was checked over a period of at least 92 or 81 days of frozen storage followed by 10 days at room temperature or for at least 102 or 91 days of storage at room temperature. Clinical signs were recorded daily, body weight was measured weekly. Food consumption was measured twice weekly during the first 6 weeks of treatment and weekly thereafter. All surviving animals from the main study

groups were subjected to a neurotoxicity assessment (exploratory locomotor activity, open field observations, sensory reactivity and grip strength) during weeks 12 to 13 of the study. Ophthalmological examinations were performed on all animals during the acclimatization phase and on all animals of the control and high dose groups during week 13. Blood samples were collected from the sublingual vein of the first five suitable animals of each group for further determination of the test substance and its metabolites. Urine samples were collected overnight on the week before scheduled necropsies from all animals. Before scheduled necropsies a blood sample was collected from the retro-orbital venous plexus of each animal for haematology and clinical chemistry determinations. All animals were necropsied, selected organs weighed and a range of tissues were taken, fixed and examined microscopically.

Results

Dose (ppm)	**0**		**100**		**500**		**2500**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Mortality	No mortalities occurred							
Clinical signs	No clinical signs were observed							
Body weight	At 2500 ppm, a lower body weight (6-10%) was observed in both sexes throughout the study (significant in females only).							
Body weight gain (g)	293	110	308	110	287	**97***	**259***	**94****
Food consumption	Slight reduction in both males and females at 2500 ppm							
Ophthalmology	No treatment-related changes							
Urinalysis	No treatment-related changes							
FOB	No treatment-related changes							
Haematology -platelet count	1160	1233	1121 (-3%)	1324 (+7%)	1224 (+6%)	1187 (-4%)	1235 (+6%)	**1415*** **(+15%)**
Clin. Chemistry -total bilirubin (µmol/L)	1.33	2.05	1.1 (-15%)	1.9 (-5%)	1.1 (-15%)	1.8 (-10%)	**0.8**** **(-38%)**	**1.1**** **(-45%)**
- Glucose (mmol/l)	6.59	6.03	6.56 (0%)	5.82 (-3%)	6.19 (-6%)	5.58 (-7%)	**5.23**** **(-21%)**	**4.69**** **(-22%)**

Dose (ppm)	**0**		**100**		**500**		**2500**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
- Total cholesterol (mmol/l)	1.54	1.55	1.78 (+16%)	1.68 (+8%)	1.70 (+10%)	1.86 (+20%)	1.97 (+28%)	**2.26** (+46%)**
- Triglyceride concentration	0.85	0.44	1.03 (+21%)	0.44 (0%)	0.85 (0%)	0.48 (+9%)	1.15 (+35%)	0.73 (+66%)
Organ weights								
-liver weight								
-absolute (g)	10.92	6.18	11.37 (+4%)	5.91 (-4%)	10.92 (0%)	5.93 (-4%)	11.78 (+8%)	6.65 (+8%)
- relative to body weight (%)	2.194	2.290	2.231 (+2%)	2.170 (-5%)	2.255 (+3%)	2.283 (0%)	**2.546** (+16%)**	**2.624** (+15%)**
- thyroid gland								
-absolute (g)	0.0195	0.0162	0.0222 (+14%)	0.0162 (0%)	0.0228 (+17%)	0.0165 (+2%)	0.0234 (+20%)	0.0171 (+6%)
- relative to body weight (%)	0.00393	0.00602	0.00439 (+12%)	0.00599 (0%)	**0.00473* (+20%)**	0.00633 (+5%)	**0.00494** (+26%)**	0.00679 (+13%)
- Heart								
-absolute (g)	1.48	1.05	1.53 (+3%)	1.02 (-3%)	1.51 (+2%)	0.99 (-6%)	1.43 (-4%)	**0.94** (-10%)**
- relative to body weight (%)	0.298	0.388	0.302 (+1)	0.378 (-3%)	0.312 (+5%)	0.380 (2%)	0.305 (+2%)	0.374 (-4%)
- Kidney								
-absolute (g)	2.90	1.88	2.93 (+1%	1.77 (-6%)	2.92 (+1%)	1.75 (-7%)	2.79 (-4%)	**1.71* (-9%)**
- relative to body weight (%)	0.585	0.697	0.581 (-1%)	0.650 (-7%)	0.602 (+3%)	0.674 (-5%)	0.596 (+2%)	0.678 (-3%)
Pathology								
Macroscopy								
- enlarged liver	0/10	0/10	0/10	0/10	0/10	0/10	4/10	1/10
- dark thyroid gland	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10
Microscopy								
Centrilobular hepatocellular hypertrophy: diffuse								
- minimal	0	0	0	0	0	0	6	3
- slight	0	0	0	0	0	0	4	0

Dose (ppm)	0		100		500		2500	
	m	f	m	f	m	f	m	f
- total	0	0	0	0	0	0	10	3
Follicular cell hypertrophy: diffuse								
- minimal	0	0	0	0	0	0	3	0
- total	0	0	0	0	0	0	3	0

*p≤0.05 **p≤0.01

At 2500 ppm, a lower body weight was observed in both sexes throughout the study (6-10%). A reduced mean body weight gain/day was observed in high dose males and in mid and high dose females. At 500 ppm the overall mean body weight gain was 12% lower than the controls in females at the end of the treatment period. In the absence of any other changes in the parameters assessed, this finding was considered not to be adverse.

Ophthalmological examination revealed haemorrhage of the iris and a damaged eye in one male and one female, respectively, exposed to 2500 ppm. In view of the single occurrence, these findings were considered incidental.

Haematological evaluation revealed a higher mean platelet count in high dose females when compared to the control group (+15%). In addition, mean total bilirubin and glucose concentrations were slightly lower in both sexes and mean total cholesterol and triglycerides concentrations were slightly higher when compared to the controls. At the end of the recovery phase, as the mean concentration remained slightly lower. The other treatment-related changes were considered to be reversible as no relevant difference was noted after the recovery period.

At 2500 ppm, mean liver to body weight ratio was statistically significantly higher in both sexes when compared to controls. This change, even if partly due to lower terminal body weight, was associated with relevant histopathological findings and was considered to be treatment-related. Mean thyroid gland to body weight ratio was statistically significantly higher in males when compared to controls and was associated with minimal follicular cell hypertrophy. At the end of the recovery phase, no treatment-related findings were observed in the liver and the thyroid gland. All the treatment-related findings observed at the end of the treatment period were thus considered to be reversible.

Acceptability

The study is considered acceptable

Conclusion

Based on the clinical chemical findings, the increased relative liver and thyroid weights and the associated pathological findings the NOAEL in this study is set at 500 ppm (approximately 30.2 mg/kg bw/day for males and 38.3 mg/kg bw/day for females).

Expert consulatation 2.6

At 500 ppm the overall mean body weight gain was 12% lower than the controls in females at the end of the treatment period. The RMS noted that this was not accompanied by effects on body weight.

With regard to the thyroid weight, the RMS highlighted that the effect was not observed in other studies, and only observed in males. Considering the severity of the effect (20% for the relative weight, statistically significant, and 17% for the absolute weight), the experts agreed that it had to be considered as adverse.

Based on these findings, the agreed NOAEL is 100 ppm, corresponding to 6 mg/kg bw per day.

Study 2

Reference	:	[redacted]	exposure	:	Repeated diet, 90 days
type of study	:	90-day mouse oral toxicity	dose	:	0, 100, 500, 2500 ppm M: 0, 16, 81, 407 mg/kg bw/day F: 0, 19, 98, 473 mg/kg bw/day
year of execution	:	2008	vehicle	:	none
test substance	:	BYI (batch NLL 7780-44-6, purity 99.5%)	GLP statement	:	yes
Route	:	diet	guideline	:	OECD 408
Species	:	Mice C57BL/6J	acceptability	:	Acceptable
group size	:	10/sex/dose	**NOAEL**	:	81 mg/kg bw/day

Study design

The study follows OECD guideline 408 except for that FOB and ophthalmology were not performed.

Mice (10/sex/dose) received BYI 02960 (batch NLL 7780-44-6, purity 99.5%) at dietary concentrations of 100, 500 and 2500 ppm (equating approximately to 16, 81, 407 mg/kg bw/day in males and 19, 98, 473 mg/kg bw/day in females) for at least 90 days. Clinical signs were recorded daily, body weights and food consumption were measured weekly. On the day of necropsy, a blood sample was collected from the retro-orbital venous plexus of each surviving animal for selected clinical chemistry determinations. All animals were necropsied, selected organs weighed and a range of tissues were taken, fixed and examined microscopically.

Results

Dose (ppm)	**0**		**100**		**500**		**2500**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Mortality	No treatment related mortalities occurred							
Clinical signs	No clinical signs were observed							
Body weight	A lower body weight was observed at 2500 ppm in both sexes throughout the study (max 11%).							
Body weight gain (g)	7.2	5.4	6.9	5.7	6.5	5.2	**4.1****	5.0
Food consumption	At 2500 ppm a slight reduction in food consumption (max 11%) between study days 1 and 22 in females only.							
Ophthalmology	Not performed							

Dose (ppm)	**0**		**100**		**500**		**2500**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Urinalysis	Not performed							
FOB	Not performed							
Haematology	Not performed							
Clin. Chemistry								
-total cholesterol (mmol/l)	1.91	1.52	1.87 (-2%)	1.50 (-1%)	1.77 (-7%)	1.42 (-5%)	**1.34** (-30%)**	**1.16** (-24%)**
- Alkaline phosphatise (IU/l)	76	131	74 (-3%)	129 (-2%)	81 (+7%)	134 (+2%)	**105** (+38%)**	144 (+10%)
- Alanine aminotransferase(IU/l)	27	36	32 (+19%)	38 (+6%)	32 (19%)	38 (+6%)	35 (+30%)	**74* (+106%)**
- Aspartate aminotransferase (IU/l)	88	130	91 (+3%)	129 (-1%)	95 (+8%)	132 (2%)	105 (+19%)	177 (+36%)
- Urea (mmol/l)	11.14	12.76	14.04 (+26%)	13.31 (+4%)	13.98 (+25%)	14.79 (+16%)	**16.78** (+51%)**	**15.23* (+19%)**
- Total protein (g/l)	59	58	57 (-3%)	58 (0%)	59 (0%)	57 (-2%)	**56* (-5%)**	**55** (-5%)**
- Albumin (g/l)	34	36	34 (0%)	35 (-3%)	35 (+3%)	35 (-3%)	33 (-3%)	**33** (-8%)**
Organ weights								
-liver weight								
-absolute (g)	0.96	0.82	1.02 (+6%)	0.82 (0%)	0.95 (-1%)	0.84 (+2%)	1.02 (+6%)	**0.92** (+12%)**
- relative to body weight (%)	4.170	4.474	4.422 (+6%)	4.498 (+1%)	4.207 (+1%)	4.609 (+3%)	**4.989** (+20%)**	**5.264** (+18%)**
- kidney weight								
-absolute (g)	0.35	0.26	0.35 (0%)	0.27 (+4%)	0.32 (-9%)	0.26 (0%)	**0.31** (-11%)**	0.25 (-4%)
- relative to body weight (%)	1.541	1.439	1.531 (+1%)	1.474 (+2%)	1.437 (-5%)	1.417 (-2%)	1.515 (0%)	1.425 (-1%)

Dose (ppm)	**0**		**100**		**500**		**2500**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
- brain weight								
-absolute (g)	0.45	0.45	0.46 (+2%)	0.46 (+2%)	0.44 (-2%)	0.44 (-2%)	0.44 (-2%)	0.45 (0%)
- relative to body weight (%)	1.943	2.468	1.985 (+2%)	2.513 (+2%)	1.960 (+1%)	2.415 (-2%)	**2.172** (+12%)**	2.516 (+2%)
- thymus								
-absolute (g)	0.027	0.034	0.028 (+4%)	0.032 (-6%)	0.029 (+7%)	0.032 (-6%)	0.028 (+4%)	0.035 (+3%)
- relative to body weight (%)	0.1156	0.1848	0.1234 (+7%)	0.1740 (-6%)	0.1274 (+10%)	0.1773 (-4%)	**0.1391** (+20%)**	0.1975 (+7%)
Pathology								
Macroscopy								
- pale liver	0/10	0/10	0/10	0/10	0/10	1/10	1/10	6/10
Microscopy								
Hepatocellular vacuolation: diffuse								
- minimal	6	7	3	7	8	6	0	2
- slight	4	3	7	3	2	3	6	3
- moderate	0	0	0	0	0	0	4	5
- total	10	10	10	10	10	9	10	10
Corticoepithelial vacuolation: multifocal/diffuse								
- minimal	7	0	4	0	5	0	0	0
- slight	3	0	2	0	3	0	0	0
- moderate	0	0	2	0	0	0	0	0
- total	10	0	8	0	8	0	0	0

*p≤0.05, **p≤0.01

There were no treatment-related mortalities during the study. One female of the mid dose group was killed for humane reasons on study day 61. Macroscopic examination revealed dilated and tear is esophagus, food content in thoracic cavity, enlarged adrenal glands, dark liver, white foci on stomach, atrophic/small thymus and enlarged bronchial lymph node. The cause of death was due to initial esophageal impaction followed by tearing and a subsequent local inflammation reaction and can therefore be considered incidental.

At 2500 ppm body weight was reduced in both sexes compared to control (max 11%). Mean body weight gain was reduced from study days 1 to 22 in males and between study days 1 and 8 in females. The overall cumulative mean body weight was reduced by 43% in males and 7% in females.

In the high dose animals clinical chemistry evaluation revealed a lower mean total cholesterol and total protein concentration in both sexes, and a higher urea and alkaline concentration in both sexes. Alanine aminotransferase activity was induced in females whereas albumin concentrations were reduced.

Mean absolute and relative liver weights were statistically significantly higher in females. Mean absolute kidney weight were statistically significantly lower in males when compared to controls. At macroscopic examination pale liver was noted in 6/10 females. At microscopic examination, a slight increase in severity of diffuse hepatocellular vacuolation was noted in the liver in both sexes. In the kidney, a loss of the normal multifocal/diffuse cortical epithelial vacuolation was noted in males. Relative brain and thymus weight were significantly increased in high dose males, without corroborating pathological finding these effects are likely due to the decreased body weight and are considered not treatment related.

Acceptability

The study is considered acceptable

Conclusion

Based on the clinical chemical findings, the increased liver and decreased kidney weights and the associated pathological findings the NOAEL in this study is set at 500 ppm (equating to 80.6 mg/kg bw/day in males and 98.1 mg/kg bw/day in females).

Study 3

Reference	:	[redacted]	exposure	:	Repeated diet, 90 days
type of study	:	90-day dog oral toxicity study	Dose	:	0, 400, 1200, 3600/2400* ppm M: 0, 12, 33, 102/85* mg/kg bw/day F: 0, 12, 41, 107/78* mg/kg bw/day
year of execution	:	2009	vehicle	:	Corn oil and acetone
test substance	:	BYI 02960 (batch 2009-000239, purity 96.2%)	GLP statement	:	yes
Route	:	diet	guideline	:	OECD 409
Species	:	Dog, Beagle	acceptability	:	Acceptable
group size	:	4/sex/dose	**NOAEL**	:	12 mg/kg bw/day

**The highest dose (3600 ppm) was reduced to 2400 ppm from study week 9 onward due to clinical signs seen in two dogs on day 44 and continual weight loss in the high-dose group.*

Study design

The study was carried out according to OECD guideline 409.

BYI 02960 was administered via the diet to groups of 4 male and 4 female Beagle dogs at dose levels of 400, 1200 or 3600/2400 ppm for at least 90 days (12, 33 and 102/85 mg/kg bw/ day for males and 12, 41 and 107/78 mg/kg bw/day for females). The 3600 ppm dose group was reduced to 2400 ppm beginning study week 9 due to clinical signs seen in two of the dogs on Day 44 and continual weight loss in the high-dose group. Cage side observations and food consumption were recorded daily and detailed clinical observations and body weights were recorded weekly. Haematology, clinical chemistry, and urinalysis evaluations were performed on all animals once prior to administration of the test substance and from all animals during study weeks 5, 9, and 13. Ophthalmic examinations were performed pre-exposure and pre-sacrifice. A gross necropsy was performed, organ weights were taken, and micropathology was performed.

Results

Dose (ppm)	**0**		**400**		**1200**		**3600/2400**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Mortality	No mortalities occurred							
Clinical signs	In the high dose group: unsteady and stiff back legs and lower back in one male and one female.							
Body weight	-	-	+5%	-5%	-9%	-7%	-11%	-13%
Body weight gain (g)	777	1325	1282	775	**60***	671	**-421***	**1***

Dose (ppm)	**0**		**400**		**1200**		**3600/2400**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Food consumption	Statistically significantly reduced in mid and high dose males during the first ten days and in high dose females during the first seven day.							
Ophthalmology	No treatment-related effects							
Urinalysis	No treatment-related effects							
Haematology								
-RBC								
- day -7	6.99	6.81	7.28	7.10	6.88	6.65	7.50	7.03
- day 28	7.02	6.91	7.13	6.99	6.85	6.41	6.64	6.42
- day 56	6.92	6.95	7.18	7.25	6.67	**6.44***	6.29	**5.48***
- day 84	7.07	6.80	7.36	7.31	6.97	6.53	6.75	6.25
-Hgb								
- day -7	16.3	15.7	17.1	15.9	15.7	15.7	16.8	16.5
- day 28	16.4	16.0	16.5	15.8	15.6	14.9	**14.9***	14.7
- day 56	16.1	16.4	16.8	16.6	15.1	15.3	**14.0***	**13.0***
- day 84	16.3	15.9	17.0	16.9	15.5	15.2	14.9	14.1
- Hct								
- day -7	47.5	45.5	50.0	46.0	45.5	44.6	49.6	47.6
- day 28	47.2	45.8	47.7	45.5	44.3	42.7	**42.3***	42.3
- day 56	46.4	46.2	48.0	47.6	43.2	43.0	**39.9***	**37.7***
- day 84	47.6	45.3	49.5	48.3	45.4	43.6	**43.2***	40.7
- MCV								
- day -7	67.8	66.8	68.7	64.9	66.2	67.2	66.2	67.8
- day 28	67.2	66.3	67.1	65.3	**64.6***	66.7	**63.7***	65.9
- day 56	67.0	66.5	67.0	65.7	64.7	66.7	**63.6***	64.5
- day 84	67.3	66.6	67.3	66.1	65.1	66.7	**64.1***	65.0
Clin. Chemistry study day 56								
-Creatin phosphokinase	242	282	248 (+2%)	**175* (-38%)**	2834# (+1071%)	1538# (+445%)	2944# (+1117%)	**4836* (+1615%)**
- Aspartate aminotransferase (IU/l)	38	42	36 (-5%)	**31* (-26%)**	241 (+534%)	**130* (+210%)**	266 (+600%)	**370* (+780%)**
- Alanine aminotransferase (IU/l)	30	28	36 (+20%)	27 (-4%)	221 (+637%)	**151* (+439%)**	311 (+937%)	**552* (+1871%)**
- Glucose (mg/dl)	89	83	86 (-3%)	87 (+5%)	92 (+3%)	92 (+11%)	88 (-1%)	**103* (24%)**

Dose (ppm)	**0**		**400**		**1200**		**3600/2400**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
- Creatin (mg/dl)	1.0	0.9	0.9 (-10%)	0.9 (-10%)	0.9 (-10%)	0.8 (-20%)	**0.8* (-20%)**	**0.7* (-22%)**
Organ weights								
-liver weight								
-absolute (g)	269	226	260 (-3%)	201 (-11%)	261 (-3%)	225 (0%)	**307* (+14%)**	252 (+12%)
- relative to body weight (%)	2.757	2.892	2.533 (-8%)	2.621 (-9%)	2.911 (+6%)	3.035 (+5%)	**3.525* (+29%)**	**3.596* (+24%)**
- kidney weight								
-absolute (g)	47	38	55 (+17%)	34 (-11%)	51 (+9%)	36 (-5%)	55 (+17%)	38 (0%)
- relative to body weight (%)	0.485	0.469	0.539 (+11%)	0.442 (-6%)	**0.570* (+18%)**	0.488 (+4%)	**0.633* (+31%)**	0.545 (+16%)
- prostate								
-absolute (g)	7.375		9.847 (+34%)		10.410 (+41%)		**12.322* (+67%)**	
- relative to body weight (%)	0.075		0.096 (+28%)		**0.116* (+55%)**		**0.141* (+88%)**	
- lung								
-absolute (g)	90.448	82.549	86.208 (-5%)	68.825 (-17%)	94.794 (+5%)	72.206 (-13%)	91.983 (+2%)	72.190 (-13%
- relative to body weight (%)	0.924	1.025	**0.838* (-9%)**	0.899 (-12%)	**1.039* (+12%)**	0.977 (-5%)	1.052 (+14%)	1.030 (0%)
Pathology	No treatment-related effects							
Macroscopy								
Microscopy								
liverAccumulation of Brown pigment in Kupffer cells								
- minimal	0	0	0	0	0	0	0	2
- total	0	0	0	0	0	0	0	2
Skeletal muscle Myofiber atrophy/degeneration: focal								
- minimal	0	0	0	0	2	3	1	4
- slight	0	0	0	0	0	1	1	0
- total	0	0	0	0	2	4	2	4

*p≤0.05, # Value not statistically significant due to a large SD from dogs that had normal values.

Clinical signs consisted of unsteady and stiff back legs and lower back in one high dose male and one high dose female. Body weight and body weight gain were reduced at 1200 and 3600/2400 ppm.

There was a compound-related reduction in RBC, Hgb, and Hct during the 1, 2, and 3 month hematology evaluations in both sexes at the high dose. In the 1200 and 3600/2400 ppm dose groups, there was a compound-related increase in creatine phosphokinase, aspartate aminotransferase and alanine aminotransferase at the 2-month test interval. The values for CK, AST, and ALT were normal at the 3 month evaluation.

Relative and absolute liver weights were increased in the high dose groups. There was a statistical siginificant compound-related increase in relative kidney weight for males in the mid and high dose group. The statistical increase in the prostate weight for the 3600/2400 ppm dose group males is not considered compound-related as these were young dogs and variations in prostate weight due to maturation can be expected. The statistical significant values for relative lung weight in the 400 and 1200 ppm male groups are not considered to be compound-related as there is no clear dose-response relationship.
Microscopic pathology revealed minimal brown pigments in Kupffers in the liver of high dose females an myofiber atrophy/degeneration in skeletal muscle in both sexes at the mid and high dose.

Historical control data from studies performed in 2010 and 2011:

Sex	Mean ±SD	range
Liver absolute weight (g)		
Males	259.6 ± 37.4	213.2 to 337.1
Females	233.3±45.7	185.4 to 336.5
Liver relative weight		
Males	2.93 ± 0.39	2.47 to 3..78
Females	3.07 ± .0.48	2.34 to 3.99
Prostate absolute weight:		
Males	7.02 ± 3.10	2.30 to 13.31
Prostate relative weight:		
Males	0.08 ± 0.039	0.26 ot 0.171
Females		
Lung absolute weight:		
Males	85.5 ± 7.2	74.6 to 96.2
Females	75.7 ± 12.5	66.9 to 109.6
Lung relative weight:		
Males	0.97 ± 0.09	0.81 to 1.12
Females	1.01 ± 0.25	0.84 to 1.71

Acceptability

The study is considered acceptable.

Conclusion

Based on reduced body weight, reduced body weight gain, clinical chemistry, significant increase in kidney weight and focal myofiber atrophy/degeneration the No Observed Adverse Effect Level is 400 ppm (12 mg/kg bw/day for males and females).

B.6.3.5 1-year toxicity study

Study 1

Reference	:	[redacted]	exposure	:	Repeated diet, 1-year
type of study	:	1-year dog oral toxicity study	Dose	:	0, 150, 300, 1000 ppm M: 0, 4.6, 7.8, 28.1 mg/kg bw/day F: 0, 4.1, 7.8, 28.2 mg/kg bw/day
year of execution	:	2010-2011	vehicle	:	Corn oil and acetone
test substance	:	BYI 02960 (batch 2009-000239, purity 96.2%)	GLP statement	:	yes
Route	:	diet	guideline	:	OECD 452
Species	:	Dog, Beagle	acceptability	:	Acceptable
group size	:	4/sex/dose	**NOAEL**	:	7.8 mg/kg bw/day

Study design

The study was carried out according to OECD guideline 452.

Male and female Beagle dog (4/sex/dose) were fed diet containing BYI 02960 (batch 2009-000239, purity 96.2%) for at least one year at dietary level of 150, 300 and 1000 ppm, equivalent to 4.6, 7.8 and 28.1 mg/kg bwd/day for males and 4.1, 7.8 and 28.2 mg/kg bw/day for females). Body weights were measured weekly and food consumption was measured daily. All animals were generally observed at least daily for clinical signs of toxicity. Detailed clinical observations were preformed on a weekly basis. Ophthalmic exams were conducted prior to initiation of dosing and prior to termination of the study. Clinical chemistry, haematology and urinalysis evaluation were performed once prior to administration and at approximately 3, 6, 9 and 12 months. At the end of the study, a gross necropsy was conducted, organs were weighed and tissues were collected for microscopic examination.

Results

Dose (ppm)	**0**		**150**		**300**		**1000**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Mortality	No mortalities occurred							
Clinical signs	No treatment-related effect							
Body weight	No treatment-related effect							
Body weight gain	Body weight gain reduced in females at 1000 ppm in the first week only (-88%).							

Dose (ppm)	0		150		300		1000	
	m	f	m	f	m	f	m	f
(g)	No overall effect on BWG found.							
Food consumption	No treatment-related effect							
Ophthalmology	No treatment-related effect							
Urinalysis	No treatment-related effect							
Haematology	No treatment-related effect							
Clin. Chemistry	No treatment-related effect							
Organ weights	No treatment-related effect							
Pathology Macroscopy	No treatment-related effect							
Microscopy -skeletal muscle, protocol (gastrocnemius) – degeneration myofiber	0	0	0	0	0	0	2 (1.5)	2 (1.0)
- Muscle, other (biceps femoris) – degeneration, myofiber	0	0	0	0	0	0	3 (1.3)	3 (1.0)

*p≤0.05, **p≤0.01, () = Average severity of dogs with lesion: 1 (minimal) to 5 (severe).

Body weight or overall body weight gain were not affected by treatment. Body weight gain was reduced at 1000 ppm in females in the first week only (-88%). Food consumption, ophthalmology, urinalysis, haematology, clinical chemistry, organ weights and macroscopic pathology evaluations revealed no treatment-related effects. Test substance-related micropathological changes was limited to degeneration noted in skeletal muscle, protocol (gastrocnemius); and muscle, other (biceps femoris). Degeneration of the myofiber comprised of one or more of the following changes: atrophy, necrosis, and/or presence of inflammatory cells around the affected myofiber.

Acceptability

The study is considered acceptable

Conclusion

Based on micropathology findings in males and females, the LOAEL in this study was 1000 ppm, equivalent to 28.1 and 28.2 mg/kg bw/day for male and female dogs, respectively. The NOAEL in this study was 300 ppm, equivalent to 7.8 mg/kg bw/day for both male and female dogs.

B.6.3.6 Summary

The results of the subacute and semichronic toxicity studies are summarised in Tables 6.3.5.1 and 6.3.5.2, respectively.

Table 6.3.5.1 Subacute studies

Duration	Species	Route	NOAEL mg/kg bw/day	LOAEL mg/kg bw/day	Critical effects	Reference
28-day	rat	oral, gavage	75[1]	200[1]	Changes in biochemical parameters, increased liver weight, enlarged liver, prominent lobulation, hepatocellular hypertrophy, follicular cell hypertrophy	[redacted] 2007
28-day	rat	oral, diet	33.6[1]	385[1]	Changes in biochemical parameters, increased relative liver and thyroid weight, prominent lobulation of the liver, hepatocellular hypertrophy, follicular cell hypertrophy	[redacted] 2008
28-day	mouse	oral, diet	98 ~~166~~[1]	-		[redacted] 2007
28-day	dog	oral, diet	62[1]	118[1]	Overall body weight loss and reduced body weight gain	[redacted] 2008
28-day	Rat	Dermal	500	-		[redacted] 2012

1. Dose range finding studies.

Table 6.3.5.2 Semichronic studies

Duration	Species	Route	NOAEL mg/kg bw/day	LOAEL mg/kg bw/day	Critical effects	Reference
90-day study	rat	oral, diet	6.0 ~~30.2~~	30.2 ~~156~~	Changes in biochemical parameters, increased liver and thyroid weight, enlarged liver, dark thyroid gland, hepatocellular hypertrophy, follicular cell hypertrophy.	[redacted]. 2009
90-day study	mouse	oral, diet	81	407	Reduced body weight, changes in biochemical parameters, increased liver weight, decreased kidney weight, pale liver, hepatocellular vacuolation, loss of normal multifocal/diffuse cortical epithelial vacuolation.	[redacted]. 2009
90-day study	dog	oral, diet	12	33	Reduced body weight gain, changes in biochemical parameters, significant increased kidney weight, myofiber atrophy/degeneration.	[redacted]. 2008
1-year	dog	oral,	7.8	28.1	Degeneration of skeletal muscle and other mucle	[redacted]

study		diet			(biceps femoris) degeneration	2012

Oral

Four subacute oral range-finding studies were performed, with rat, mouse and dog.

In a 28-day study with rats exposed to 0, 75, 200 or 350 mg/kg bw/d via gavage, toxicologically relevant effects were clinical chemical findings, increased organ weights and pathological findings in the 200 mg/kg bw/d group. The NOAEL in this study is set at 75 mg/kg bw/d.

In a 28-day study with rats exposed to 0, 33.6 or 385 mg/kg bw/d via diet, toxicologically relevant effects were clinical chemical findings, increased liver and thyroid weights and associated pathological findings in the 385 mg/kg bw/d group. The NOAEL in this study is set at 33.6 mg/kg bw/d.

In a 28-day study with mouse exposed to 300, 600 and 1200 ppm (equating approximately to 50, 98 and 207 mg/kg bw/day in males and 59, 122, 240 mg/kg bw/day in females) via diet, no toxicologically relevant effects were observed. The NOAEL in this study is set at 98 mg/kg bw/d.

In a 28-day study with dogs exposed to 0, 500, 2000 or 4000 ppm (equating approximately to 0, 16, 62, 118 mg/kg body weight/day in males and 0, 18, 77, 131 mg/kg body weight/day in females) via diet, toxicologically relevant effects were body weight loss and reduced body weight gain at the 4000 ppm group. The NOAEL in this study is set at 2000 ppm (62 mg/kg bw/d).

Four semichronic oral studies were performed, with rat, mouse and dog.

In a 90-day toxicity study with rat exposed to 0, 100, 500, 2500 ppm (equating to approximately 0, 6.0, 30.2, 156 mg/kg bw/day in males and 0, 7.6, 38.3, 186 mg/kg bw/day in females) via diet, toxicologically relevant effects were clinical chemical findings, increased relative liver and thyroid weight and associated pathological findings in the 2500 ppm group, and thyroid weight effects in the 500 ppm males. The NOAEL in this study is 100 ~~500~~ ppm (6.0 ~~30.2~~ mg/kg bw/d).

In a 90-day toxicity study with mouse exposed to 0, 100, 500, 2500 ppm (equating approximately to 16, 81, 407 mg/kg bw/day in males and 19, 98, 473 mg/kg bw/day in females) via diet, toxicologically relevant effects were clinical chemical findings, increased relative liver and decreased kidney weights and associated pathological findings in the 2500 ppm group. The NOAEL in this study is 500 ppm (81~~80.6~~ mg/kg bw/d).

In a 90-day toxicity study with dog exposed to 0, 400, 1200 or 3600/2400 ppm (equating approximately to 0, 12, 33 and 102/85 mg/kg bw/ day for males and 0, 12, 41 and 107/78 mg/kg bw/day for females) via diet, toxicologically relevant effects were reduced body weight, reduced body weight gain, clinical chemistry, significant increase in kidney weight and focal myofiber atrophy/degeneration in the 1200 ppm group. The NOAEL in this study is 400 ppm (12 mg/kg bw/d).

In a 1-year toxicity study with dog exposed to 0, 150, 300 and 1000 ppm (equating approximately to 0, 4.6, 7.8 and 28.1 mg/kg bwd/day for males and 0, 4.1, 7.8 and 28.2 mg/kg bw/day for females) via diet, toxicologically relevant effects were micropathology (degeneration of the skeletal muscle (*gastrocnemius* and *biceps femoris* (atrophy, necrosis and/or presence of inflammatory cells)) findings in the 1000 ppm group. The NOAEL in this study is 300 ppm (7.8 mg/kg bw/d).

Dermal

Male and female rats, 10 rats/sex/group (one control and three test substance-dosed groups) were administered BYI 02960 (50, 150, or 500 mg/kg/day) daily by dermal application for at least 28 consecutive days (minimum of 6 h/day) and euthanized one day following the last dose. Based on the lack of adverse findings observed in the study, the 28-day dermal exposure NOAEL in male and female rats dosed with BYI 02960 was 500 mg/kg bw/day.

B.6.4 GENOTOXICITY (ANNEX IIA 5.4)

B.6.4.1 *In vitro*

Study 1

Study design and results

Type of study: Ames test, plate incorporation and preincubation method

Indicator cells	Endpoint	Res. - act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
S. typh. TA 98 TA 100 TA 102 TA 1535 TA 1537	 point mut. point mut. point mut. point mut. point mut.	 - - - - -	 - - - - -	rat liver	Aroclor 1254	16-5000 µg/plate	Herbold, B. (2009)
Test substance: BYI 02960 (batch number 2009-000239; purity 96.2%); tested in DMSO Precipitation: No precipitation of the test substance was reported. Toxicity: at 5000 µg/plate, the substance had only a weak, strain-specific bacteriotoxic effect GLP statement: yes According to OECD 471: yes Adequate positive (+S9: 2-AA; -S9: NaN_3, NF, 4-NPDA, MMC and Cumene) and negative (sterility) controls were included.							

Table 6.4.1-1 Plate incorporation assay with BYI 02960 - Mean number of revertants

Strain	TA 98		TA 100		TA 102		TA 1535		TA 1537	
Metabolic activation	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9
Neg. control 0 µg/plate	25	31	124	198	212	294	8	12	7	10
BYI 02960										
16 µg/plate	26	38	128	182	216	297	8	13	6	8
50 µg/plate	27	25	121	190	218	314	7	10	6	10
158 µg/plate	24	35	145	177	207	335	7	10	5	8
500 µg/plate	24	29	135	213	212	314	7	11	6	8
1581 µg/plate	28	26	131	202	220	288	8	8	5	7
5000 µg/plate	24	35	116	188	223	214	7	7	9	7
Pos. control	68 117	2031 2221	341 406	1719 1881	657 742	613 1183	805 835	90 59	35 52	304 135

Table 6.4.1-2 Pre-incubation assay with BYI 02960 - Mean number of revertants

Strain	TA 98		TA 100		TA 102		TA 1535		TA 1537	
Metabolic activation	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**
Neg. control 0 µg/plate	17	35	128	176	220	263	8	10	7	10
BYI 02960										
16 µg/plate	19	31	113	164	197	224	9	11	7	8
50 µg/plate	17	33	112	150	215	248	8	9	6	10
158 µg/plate	19	32	126	190	227	268	8	9	7	7
500 µg/plate	19	33	131	183	199	311	7	10	7	9
1581 µg/plate	18	22	116	165	197	245	9	9	6	8
5000 µg/plate	18	28	107	116	173	244	8	8	7	9
Pos. control	73 154	917 1065	412 638	1405 1914	418 448	553 1191	629 746	82 60	32 71	309 239

BYI 02960 did not cause any significant increase in the number of revertant colonies in either the presence or absence of metabolic activation. Therefore, BYI 02960 was non-mutagenic with or without S9 mix in the plate incorporation as well as in the pre-incubation modification of the Ames test.

Acceptability

The study is considered acceptable.

Conclusions

BYI 02960 is not mutagenic in the Ames test.

Study 2

Study design and results

Type of study: Ames test, plate incorporation and preincubation method

Indicator cells	Endpoint	Res. - act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
S. typh. TA 98 TA 100 TA 102 TA 1535 TA 1537	 point mut. point mut. point mut. point mut. point mut.	 - - - - -	 - - - - -	rat liver	Phenobarbital/ß-naphthoflavone	3-5000 µg/plate	Sokolowski, A. (2011)

Test substance: BYI 02960 (batch number PFV107N005; batch; purity 97.2%); tested in DMSO
Precipitation: No precipitation of the test substance was reported.
Toxicity: No toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in the test groups with and without metabolic activation. Only in experiment I in strain TA 1537 a minor reduction in the number of revertants (below the indication factor of 0.5), was observed with and without S9 mix at 5000 pg/plate.
GLP statement: yes
According to OECD 471: yes
Adequate positive (+S9: 2-AA; -S9: NaN_3, 4-NOPD, MMS) and negative (sterility and vehicle) controls were included.

Table 6.4.1-3 Plate incorporation assay with BYI 02960 - Mean number of revertants

Strain	TA 98		TA 100		TA 102		TA 1535		TA 1537	
Metabolic activation	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**
Neg. control (DMSO)	29	39	139	174	491	633	16	20	13	13
Neg. control (untreated)	48	37	173	162	485	540	20	17	17	24
BYI 02960										
3 µg/plate	30	40	136	175	493	610	13	19	12	16
10 µg/plate	30	36	141	152	488	611	17	19	14	13
33 µg/plate	31	40	134	159	434	612	18	21	11	14
100 µg/plate	32	39	147	163	452	649	16	21	12	15
333 µg/plate	32	38	156	164	462	622	16	20	12	15
1000 µg/plate	28	39	140	172	455	629	18	20	8	15
2500 µg/plate	31	44	149	153	450	636	13	19	11	14
5000 µg/plate	35	36	132	160	390	543	14	18	4 *	5 *
Pos. control	275	2932	2135	2911	4489	2782	2100	407	63	344

* air bubbles and manual count

Table 6.4.1-4 Pre-incubation assay with BYI 02960 - Mean number of revertants

Strain	TA 98		TA 100		TA 102		TA 1535		TA 1537	
Metabolic activation	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**
Neg. control (DMSO)	29	40	120	122	383	366	12	20	18	20
Neg. control (untreated)	47	42	136	157	338	356	12	15	16	18
BYI 02960										
33 µg/plate	29	42	132	115	354	433	13	24	15	18
100 µg/plate	30	37	106	122	373	385	12	20	19	20
333 µg/plate	27	47	138	122	380	449	13	20	16	22
1000 µg/plate	24	44	96	109	330	487	15	20	18	17
2500 µg/plate	31	34	96	118	321	411	14	18	17	15
5000 µg/plate	26	37	89	106	283	278	15	17	19	19
Pos. control	393	1612	1793	2218	1084	1897	1837	264	93	246

BYI 02960 did not cause any significant increase in the number of revertant colonies in either the presence or absence of metabolic activation. Therefore, BYI 02960 was non-mutagenic with or without S9 mix in the Ames test.

Acceptability

The study is considered acceptable.

Conclusions

BYI 02960 is not mutagenic in the Ames test.

Study 3

Study design and results

Type of study: mammalian cells *in vitro*, cytogenetic assay

Indicator cells	Endpoint	Res. -act.	Res. +act.	Activation		Dose range[a]	Reference
				Tissue	Inducer		
Chinese hamster V79 cells	Chromosome aberration	-	-	rat liver	Aroclor 1254	-S9: 0, 500, 1000, 2000, 2500 and 3000 µg/mL (4 hours treatment, harvest 18 hours after the beginning of treatment) 0, 2000, 2500 and 3000 µg/mL (4 hours treatment, harvest 30 hours after the beginning of treatment) 0, 100, 200, 400, 600 and 800 µg/mL (18 hours treatment, harvest at the same time) +S9: 0, 500, 1000, 2000, 2500 and 3000 µg/mL (4 hours treatment, harvest 18 hours after the beginning of treatment) 0, 2000, 2500 and 3000 µg/mL (4 hours treatment, harvest 30 hours after the beginning of treatment)	Thum, M. (2009)

[a] Based on the results of the survival index and of the mitotic index, the following concentrations were selected for reading:
-S9:
500, 1000 and 2500 µg/mL (4 hours treatment, harvest 18 hours after the beginning of treatment);
2500 µg/mL (4 hours treatment, harvest 30 hours after the beginning of treatment);
200, 400 and 800 µg/mL (18 hours treatment, harvest at the same time).

+S9:
500, 1000 and 3000 µg/mL (4 hours treatment, harvest 18 hours after the beginning of treatment)
3000 µg/mL (4 hours treatment, harvest 30 hours after the beginning of treatment)

Test substance: BYI 02960 (batch number 2009-000239; purity 96.2%); tested in DMSO
Precipitation: No precipitation of the test substance was observed.
Toxicity: Without S9 mix cytotoxic effects were observed at 1000 µg/mL and above after 4 hours treatment and at 400 µg/mL and above after 18 hours treatment. With S9 mix cytotoxic effects were observed at 2000 µg/mL and above.
GLP statement: yes
According to OECD 473: yes
Adequate positive and negative controls were included.

Table 6.4.1-5 Summary of results (metaphases with aberrations, polyploidy metaphases, survival and mitotic index, 4 hours treatment)

Treatment group	Harvest time (hours)	Metaphases with aberrations (%)		Polyploid metaphases[a]	Survival index in %	Mitotic nuclei in 2000 cells[b]	
		incl. gaps	excl. gaps			absolute	in %
without metabolic activation							
DMSO	18	1.0	1.0	13.5	100.0	134	100.0
BYI 02960							
500 µg/mL	18	4.0	3.5	15.5	88.9	148	110.4
1000 µg/mL	18	1.5	1.5	11.0	77.8*	129	96.3
2500 µg/mL	18	4.0	4.0	14.5	77.0*	72	53.7***
Mitomycin C (0.1 µg/mL)	18	56.5	55.5***	8.0	57.1*	100	74.6**
DMSO	30	1.5	1.0	9.0	100.0	123	100.0
BYI 02960							
2500 µg/mL	30	2.0	2.0	12.5	60.6*	86	69.6***
with metabolic activation							
DMSO	18	3.5	2.0	36.0	100.0	128	100.0
BYI 02960							
500 µg/mL	18	2.5	2.0	35.0	81.1	123	96.1
1000 µg/mL	18	3.0	1.5	37.0	81.8	139	108.6
3000 µg/mL	18	3.5	3.5	26.5	65.0*	132	103.1
Cyclophosphamide (2 µg/mL)	18	74.0	71.5***	17.0	49.0*	59	46.1***
DMSO	30	3.0	1.5	19.0	100.0	156	100.0
BYI 02960							
3000 µg/mL	30	3.0	3.0	17.5	46.1*	86	55.1***

[a] The number of polyploid metaphases observed in a culture in addition to 100 metaphases analysed for chromosome aberrations were recorded (2 cultures);
[b] The mitotic index was determined by counting a total of 1000 cells per culture (2 cultures);
* relevant reduction of survival index;
** $p < 0.05$;
*** $p < 0.01$.

Table 6.4.1-6 Summary of results (metaphases with aberrations, polyploidy metaphases, survival and mitotic index, 18 hours treatment)

Treatment group	Harvest time (hours)	Metaphases with aberrations (%)		Polyploid metaphases[a]	Survival index in %	Mitotic nuclei in 2000 cells[b]	
		incl. gaps	excl. gaps			absolute	in %
without metabolic activation							
DMSO	18	3.0	3.0	5.5	100.0	91	100.0
BYI 02960	18						
200 µg/mL	18	4.0	4.0	6.0	80.7	80	87.9
400 µg/mL	18	2.5	2.0	5.0	86.9	80	87.9
800 µg/mL	18	3.5	3.5	8.5	68.2*	63	69.2**
Mitomycin C (0.03 µg/mL)	18	52.0	51.5***	4.5	71.0*	103	113.2

[a] The number of polyploid metaphases observed in a culture in addition to 100 metaphases analysed for chromosome aberrations were recorded (2 cultures);
[b] The mitotic index was determined by counting a total of 1000 cells per culture (2 cultures);

* relevant reduction of survival index;
** $p < 0.05$;
*** $p < 0.01$.

None of the cultures treated with BYI 02960 in the absence or presence of S9 mix showed statistically significant or biologically relevant increases of numbers of metaphases with aberrations. Based on the results of this test, BYI 02960 is considered not to be clastogenic for mammalian cells in vitro.

Acceptability

The study is considered acceptable.

Conclusions

BYI 02960 did not induce structural chromosome aberrations in V79 cells when tested up to and including cytotoxic concentrations.

Study 4

Study design and results

Type of study: mammalian cells *in vitro*, gene mutation test

Indicator cells	Endpoint	Res. –act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
Chinese hamster V79 cells	Gene mutations (HGPRT)	-	-	rat liver	Aroclor 1254	-S9: 0, 46, 92, 184, 368, 736, 1472, 2944 µg/mL; +S9: 0, 46, 92, 184, 368, 736, 1472, 2944 µg/mL	Entian, G. (2009)
Test substance: BYI 02960 (batch number: 2009-000239; purity 96.2%); tested in DMSO Precipitation: precipitation of the test substance was observed in the culture medium at 2944 µg/mL Toxicity: Without and with S9 mix, the test substance induced no decreases in survival to treatment or in relative population growth. The test substance was tested up to 2944 µg/mL, a concentration which slightly exceeded the limit concentration of 10 mM. GLP statement: yes According to OECD 476: yes Adequate positive and negative controls were included.							

Table 6.4.1-7 Gene mutation in mammalian cells, without metabolic activation

Treatment group	Survival to treatment (colony mean)	Population growth (% of NC)	Cloning efficiency (%)	Mutant frequency ($x10^{-6}$)
experiment 1 (5-hour exposure period)				
Untreated control	136	129.7	78.7	1.1
	131	97.9	80.2	9.4
Negative control	121	100	87	5.3
DMSO	128.7	100	91.3	10.0
BYI 02960 46 µg/mL	144	128.4	75.3	0.0
	121.7	106	81.8	5.1
BYI 02960 92 µg/mL	152.3	112.2	73.3	14.2
	128	107.8	81.7	4.6
BYI 02960 184 µg/mL	135.7	120.8	74.3	6.2
	155	120.6	78.7	10.6
BYI 02960 368 µg/mL	114.3	80.6	82.5	1.0
	142.7	135.7	74.3	0.0
BYI 02960 736 µg/mL	148	119.8	87.2	5.7
	154	122.9	83.7	4.5
BYI 02960 1472 µg/mL	130	114.5	79.2	3.7
	165	144.4	80	3.6
BYI 02960 2944 µg/mL [p]	129.3	101.8	73.2	5.1
	63.3	68.8	79	7.4
Positive control (EMS, 900 µg/mL)	126.7	46.8	65.5	628.5*
	102.3	33.9	71.7	751.7*
experiment 2 (5-hour exposure period)				
Untreated control	176.3	62.2	83.2	2.0
	170.7	103.3	78.7	6.4
Negative control	178	100	75.7	2.2
DMSO	206.7	100	76	4.9
BYI 02960 46 µg/mL	163.3	58.7	76.7	6.0
	186.3	91	74.5	5.6
BYI 02960 92 µg/mL	160.7	55.3	74.3	1.7
	194	97.4	71.8	4.6
BYI 02960 184 µg/mL	167	54.5	81.2	8.7
	174.3	94.9	74.3	9.5
BYI 02960 368 µg/mL	147	54.9	74.5	4.5
	147.3	84.9	77.8	1.6
BYI 02960 736 µg/mL	171	56.2	82.5	1.5
	149.7	75.8	75.7	4.4
BYI 02960 1472 µg/mL	162.3	63.3	72.8	4.6
	160.3	87.8	75.2	3.9
BYI 02960 2944 µg/mL [p]	161.3	75	64.3	1.9

	160	60.1	80	6.8
Positive control (EMS, 900 µg/mL)	71.3	19.9	75.2	680.7*
	90.7	37	79.2	495.8*

P precipitation of the test substance
* significant (α = 0.05) increase in the one-sided Dunnett Test.

Table 6.4.1-8 Gene mutation in mammalian cells, with metabolic activation

Treatment group	Survival to treatment (colony mean)	Population growth (% of NC)	Cloning efficiency (%)	Mutant frequency (x10-6)
experiment 1 (5-hour exposure period)				
Untreated control	182.7	128.3	64.3	9.7
	144.7	130	69.7	0.6
Negative control	173.3	100	68.8	1.2
DMSO	179	100	77.3	1.4
BYI 02960 46 µg/mL	169.7	106.1	70.3	1.8
	172	127	76.7	4.9
BYI 02960 92 µg/mL	143	89.4	78.3	1.6
	164.7	124.1	76.7	[c]
BYI 02960 184 µg/mL	155.3	103.4	72.5	2.3
	145	137	73	9.7
BYI 02960 368 µg/mL	181.7	127.7	81	3.1
	160.7	134.1	73.3	5.7
BYI 02960 736 µg/mL	185.3	111.7	71.2	9.4
	166.7	102.2	83.8	6.5
BYI 02960 1472 µg/mL	167	90.4	80.7	8.3
	177.3	137.5	80.8	8.2
BYI 02960 2944 µg/mL [p]	157.3	98.3	76.7	2.7
Positive control (DMBA, 20 µg/mL)	175	57.1	79.7	161.6*
	100	22.4	84.5	150.4*
experiment 2 (5-hour exposure period)				
Untreated control	149.3	105.4	68.2	0.6
	161	78.1	83.5	0.5
Negative control	171.7	100	93.8	0.9
DMSO	181.3	100	78	2.1
BYI 02960 46 µg/mL	162.3	114.3	83.3	2.0
	139.3	49.1	82.5	0.5
BYI 02960 92 µg/mL	170.7	94.4	82	2.0
	156.3	55.7	67	0.0
BYI 02960 184 µg/mL	158.7	114	80.8	2.6
	194.3	45.8	74.7	1.1
BYI 02960 368 µg/mL	170.3	99.3	78.5	0.5
	192.3	67.4	79.8	1.6
BYI 02960 736 µg/mL	180.3	85.8		[c]
	189.3	38.3	80.7	1.0
BYI 02960 1472 µg/mL	187.7	116.6	82	0.0
	181.3	33.6	93.5	0.9
BYI 02960 2944 µg/mL [p]	161.3	82.4	76	2.7
	162.3	48.8	85.7	0.5

Positive control (DMBA, 20 µg/mL)	63	22.8	76	121.7*
	33.7	12.6	79.5	139.9*

[c] Due to contamination in one cell culture, for only one culture the mutant frequency was determined.
[P] precipitation of the test substance
* significant (α = 0.05) increase in the one-sided Dunnett Test.

BYI 02960 was tested up to 2944 µg/mL, a concentration which exceeded the requested limit concentration of 10 mM. Under both activation conditions, no cytotoxic effects were induced. There were no indications of mutagenic effects of BYI 02960 in the V79/HPRT forward mutation assay either without or with S9 mix.

Acceptability

The study is considered acceptable.

Conclusions

In this study, BYI 02960 has no mutagenic potency in vitro in the CHO/HPRT assay.

~~The test substance did not induce unscheduled DNA synthesis in mammalian cells.~~

B.6.4.2 *In vivo*

Study 1

Study design and results

Type of study: mouse micronucleus test

Indicator cells	Endpoint	Result	Dose range	Reference
Mouse, Crl:NMRI Two intraperitoneal injections separated by 24 hours: 5/male/dose (vehicle and positive control, low, mid and high dose)	Micronuclei (bone marrow)	-	Pretest dose selection: 10, 40, 100 and 1000 mg/kg bw (m/f) Main test: 10, 20 and 40 mg/kg bw Dosing route: intraperitoneal Dosing volume: 10 mL/kg bw Vehicle (corn oil) and an adequate positive control was included	[redacted] (2009)
Test substance: BYI 02960 (batch number: 2009-000239; purity 96.2%); tested in corn oil Toxicity: at all tested dose levels, apathy, roughened fur, loss of weight, sternal recumbency, spasm, periodically stretching of body and difficulty in breathing GLP statement: yes According to OECD 474: yes				

Table 6.4.2-1 Results of the micronucleus test with BYI 02960

Treatment group	Number of evaluated PCE	Number of NCE per 2000 PCE	MNNCE per 2000 NCE	MNPCE per 2000 PCE
Negative control (corn oil)	10,000	3800	1.5	3.4
BYI 02960				
10 mg/kg bw	10,000	3134	1.0	1.4
20 mg/kg bw	10,000	3139	1.9	3.0
40 mg/kg bw	10,000	3008	1.0	3.8
Positive control				
Cyclophosphamide (20 mg/kg bw)	10,000	3308	1.9	20.6*

* P < 0.01 in non-parametric Wilcoxon ranking test

There was no indication of a clastogenic effect of intraperitoneally administered BYI 02960 in the micronucleus test on the male mouse, i.e. in a somatic test system in vivo.

Acceptability

The study is considered acceptable.

Conclusions

BYI 02960 has no potential to induce micronuclei in mouse bone marrow cells.

Study 2

Study design and results

Type of study: mouse micronucleus test

Indicator cells	Endpoint	Result	Dose range	Reference
Mouse, Crl:NMRI Two intraperitoneal injections separated by 24 hours: 7/female/dose (vehicle and positive control, low, mid and high dose)	Micronuclei (bone marrow)	-	Pretest dose selection: 10, 50, 75 and 100 mg/kg bw (f) Main test: 12.5, 25 and 50 mg/kg bw Dosing route: intraperitoneal Dosing volume: 10 mL/kg bw Vehicle (10% DMSO / 90% corn oil) and an adequate positive control was included	[redacted] (2011)
Test substance: BYI 02960 (batch number: PVF107N005; purity 97.2%); tested in 10% DMSO / 90% corn oil Toxicity: at 25 mg/kg bw, reduction of spontaneous activity, eyelid closure and ruffled fur were observed; at 50 mg/kg bw reduction of spontaneous activity, eyelid closure, ruffled fur and abdominal position were observed GLP statement: yes According to OECD 474: yes				

Table 6.4.2-2 Results of the micronucleus test with BYI 02960

Treatment group	Number of evaluated PCE	PCE per 2000 erythrocytes	PCEs with micronuclei (%)
Negative control (corn oil)	14,000	1229	0.121
BYI 02960			
12.5 mg/kg bw	14,000	1250	0.114
25 mg/kg bw	14,000	1234	0.143
50 mg/kg bw	14,000	1177	0.093
Positive control			
Cyclophosphamide (40 mg/kg bw)	14,000	1125	2.264*

* P < 0.05 in non-parametric Mann-Whitney test

There was no indication of a clastogenic effect of intraperitoneally administered BYI 02960 in the micronucleus test on the female mouse.

Acceptability

The study is considered acceptable.

Conclusions

BYI 02960 has no potential to induce micronuclei in mouse bone marrow cells.

B.6.4.3 Summary

The results from the *in vitro* and *in vivo* genotoxicity studies are summarized in table 6.4.3-1 and 6.4.3-2, respectively.

Table 6.4.3-1 *In vitro* genotoxicity studies

Test substance	Type of study		Result		Reference
	Indicator cells	Endpoint	without activation	with activation	
BYI 02960	S. typh. TA 98 TA 100 TA 102 TA 1535 TA 1537	Point mutation	-	-	Herbold, B. (2009)
BYI 02960	S. typh. TA 98 TA 100 TA 102 TA 1535	Point mutation	-	-	Sokolowski, A. (2011)

Test substance	Type of study		Result		Reference
	Indicator cells	Endpoint	without activation	with activation	
	TA 1537				
BYI 02960	Chinese hamster V79 cells	Chromosome aberration	-	-	Thum, M. (2009)
BYI 02960	Chinese hamster V79 cells	Gene mutations (HGPRT)	-	-	Entian, G. (2009)

Table 6.4.3-2 In vivo genotoxicity studies

Test substance	Type of study		Result	Reference
	Species	Endpoint		
BYI 02960	Mouse, Crl:NMRI	Micronuclei (bone marrow)	-	[redacted] (2009)
BYI 02960	Mouse, Crl:NMRI	Micronuclei (bone marrow)	-	[redacted] (2011)

BYI 02960 was not mutagenic in two Ames tests.
BYI 02960 did not induce structural chromosome aberrations in V79 cells when tested up to and including cytotoxic concentrations and showed no mutagenic potency in vitro in the CHO/HPRT assay.
BYI 02960 did not induce micronuclei in mouse bone marrow cells in two separate in vivo studies.
Overall it can be concluded that BYI 02960 has no mutagenic or clastogenic potential.

B.6.5 LONG-TERM TOXICITY AND CARCINOGENICITY (ANNEX IIA 5.5)

B.6.5.1 Chronic toxicity and carcinogenicity

STUDY 1

Data requirement:
Applicant to provide the tabled results of body weight/body weight gain and haematological findings of the 2-year rat study and relevant historical control data.

Body weight and body weight gain changes
The body weight and body weight gains have been reported as follows in the study report.
At 2000 ppm in males, the mean cumulative body weight gain was significantly lower than controls during the first week of treatment (- 27%, p≤0.01) and over the first three months of the study (-13%, p≤0.01). Thereafter, mean cumulative body weight gain was comparable to controls. The mean body weight was lower compared to controls from Week 1 to Week 54 (statistically significant in most occasions). Thereafter the mean body weight was comparable to controls. In females, mean body weight and cumulative body weight gains

were lower compared to controls throughout the study (-5% to -17%; p≤0.01 or 0.001 for body weight and -18% to -62%; p≤0.001, 0.01 or 0.05 for cumulative body weight gains. Overall, the mean cumulative body weight gain was decreased by 23% when compared to controls at the end of the study, the mean body weight was 13% lower.

At 400 ppm, mean cumulative body weight gain was statistically significantly lower than in controls during the first week of treatment for males (-6%, p≤0.05) and females (-12%, p≤0.05) and was comparable to control thereafter. Mean body weight was not affected throughout the study.

At 80 ppm, there was no treatment-related effect on body weight parameters in either sex.

Table 1: mean body weights (BW and cumulative body weight gains (BWG))

Group mean body weights (BW) and cumulative body weight gains (BWG) (g)				
BYI 02960 dosage level (ppm)	0	80	400	2000
Males				
Initial BW (Day 1) (%C)	**237**	**237 (100)**	**237 (100)**	**237 (100)**
BW Week 2 (Day 8) (%C)	296	295 (100)	293 (99)	280 ** (95)
BW Week 14 (Day 92) (%C)	544	537 (99)	540 (99)	506 ** (93)
BW Week 26 (Day 176) (%C)	623	612 (98)	615 (99)	585 ** (94)
BW Week 54 (Day 372) (%C)	714	699 (98)	712 (100)	680 * (95)
BW Week 78 (Day 540) (%C)	749	730 (97)	752 (100)	721 (96)
BW Week 106 (Day 736) (%C)	**653**	**623 (95)**	**655 (100)**	**616 (94)**
BWG Weeks 1-2 (Days 1 to 8) (%C)	59	58 (98)	56 * (94)	43 ** (73)
BWG Weeks 1-14 (Days 1 to 92) (%C)	308	301 (98)	303 (98)	269 ** (87)
BWG Weeks 14-26 (Days 92 to 176) (%C)	78	75 (96)	75 (96)	80 (103)
BWG Weeks 26-54 (Days 176 to 372) (%C)	89	90 (101)	95 (107)	93 (104)
BWG Weeks 54-78 (Days 372 to 540) (%C)	31	42 (135)	39 (126)	39 (126)
BWG Weeks 78-106 (Days 540 to 736) (%C)	-91	-84 (nc)	-82 (nc)	-113 (nc)
Overall BWG Weeks 1-106 (Days 1 to 736) (%C)	**416**	**392 (94)**	**420 (101)**	**379 (91)**
Females				
Initial BW (Day 1) (%C)	**167**	**168 (100)**	**168 (100)**	**167 (100)**
BW Week 2 (Day 8) (%C)	192	191 (100)	190 (99)	181 ** (95)
BW Week 14 (Day 92) (%C)	290	288 (99)	289 (100)	267 ** (92)
BW Week 26 (Day 176) (%C)	319	313 (98)	316 (99)	289 *** (91)
BW Week 54 (Day 372) (%C)	364	354 (97)	355 (97)	317 ** (87)
BW Week 78 (Day 540) (%C)	421	430 (102)	417 (99)	349 ** (83)
BW Week 106 (Day 736) (%C)	**421**	**437 (104)**	**444 (105)**	**364 ** (87)**
BWG Weeks 1-2 (Days 1 to 8) (%C)	25	23 (92)	22 * (88)	15 ** (60)
BWG Weeks 1-14 (Days 1 to 92) (%C)	122	120 (98)	122 (100)	100 ** (82)
BWG Weeks 14-26 (Days 92 to 176) (%C)	29	26 (90)	27 (93)	22 ** (76)
BWG Weeks 26-54 (Days 176 to 372) (%C)	48	39 (81)	37 (77)	28 *** (58)
BWG Weeks 54-78 (Days 372 to 540) (%C)	59	76 (129)	62 (105)	34 ** (58)
BWG Weeks 78-106 (Days 540 to 736) (%C)	39	21 (54)	35 (90)	15 * (38)
Overall BWG Weeks 1-106 (Days 1 to 736) (%C)	**256**	**271 (106)**	**275 (107)**	**198 *** (77)**

%C: % *vs* control C = control nc = not calculated
** Statistically different (p□0.01) from the control
* Statistically different (p□0.05) from the control
*** Statistically different (p□0.001) from the control

These data can be summarized as follows in the Table 2.

Table 2 : Body weight and body weight gain additional data

Dose (ppm)	0		80		400		2000		dr
	m	f	m	f	m	f	m	f	
BW W106	653	421	623 (-5%)	437 (+4%)	655	444 (+5%)	616 (-6%)	364 ds (-13%)	
BWG W1-106	416	256	392 (-6%)	271 (+6%)	420 (+1%)	275 (+7%)	379 (-9%)	198 ds (-23%)	

The historical control body weight data have been collected from carcinogenicity studies performed at Bayer CropScience (Centre of Toxicology, Sophia-Antipolis, France), initiated between February 2005 and January 2010. Only the studies in which the age of animals was identical at the start of the study were considered. This represents a group of 5 studies. For technical reasons, some body weight sessions were not recorded on the exact same day for all studies, therefore when possible the most approaching data were taken instead (details were provided and evaluated by the RMS).

Table 3: Historical body weight control data from carcinogenicity studies performed on Wistar rats initiated from February 2005 to January 2010

Sex	Ref Session (Days)	MEAN	NUMBER	STANDARD DEVIATION	MINIMUM	MAXIMUM
F	1 (Day 1)	162	290	11.1	132	193
F	2 (Day 8)	188	290	13.5	150	224
F	3 (Day 92)	287	289	23.1	227	346
F	4 (Day 176)	313	285	26.9	251	386
F	5 (Day 372)	357	250	44.9	269	509
F	6 (Day 540)	415	221	66.9	280	610
F	7 (Day 736)	437	154	77.5	292	676
M	1 (Day 1)	221	360	14.0	178	254
M	2 (Day 8)	278	360	17.1	222	325
M	3 (Day 92)	535	358	44.2	424	672
M	4 (Day 176)	613	355	53.1	499	767
M	5 (Day 372)	708	314	69.7	549	888
M	6 (Day 540)	739	254	82.3	546	982
M	7 (Day 736)	672	137	83.2	485	892

Table 4: Historical body weight gain control data from carcinogenicity studies performed on Wistar rats initiated from February 2005 to January 2010

Sex	SESSION	MEAN	NUMBER	STANDARD DEVIATION	MINIMUM	MAXIMUM

F	S 1-2	26	290	7	8	50
F	S 1-3	125	289	18	80	174
F	S 3-4	27	285	11	-1	74
F	S 4-5	42	250	27	-9	145
F	S 5-6	58	221	38	-39	199
F	S 6-7	32	154	42	-91	186
F	S 1-7	275	154	74	135	505
M	S 1-2	57	360	7	18	76
M	S 1-3	314	358	41	191	451
M	S 3-4	78	355	20	28	146
M	S 4-5	92	314	34	-83	179
M	S 5-6	27	254	36	-116	126
M	S 6-7	-53	137	60	-263	114
M	S 1-7	453	137	83	266	659

Haematological findings

The haemaological changes were reported as follows in the report M-428257-01-1:

Effects were observed at 2000 ppm only.
At 2000 ppm in males, relative to the controls, mean leucocyte counts were higher at Month 12 (+22%, p≤0.01), 18 (+31%, p≤0.01) and 24 (+63%, p≤0.05). This variation was associated with higher mean absolute lymphocyte count at Month 12 (+19%, p≤0.01) and 18 (+32%, p≤0.01) and with higher mean absolute neutrophil count at Month 12 (+36%, p≤0.05) and 24 (+143%, p≤0.05).
At 2000 ppm in females, throughout the first year of treatment, statistically significant differences were noted in some erythrocyte parameters. Mean hemoglobin concentration and/or mean corpuscular volume were lower; as a consequence mean hematocrit and/or mean corpuscular hemoglobin were lower too. These variations were very slight (≤ -4%, relative to the controls).
In this group, mean platelet counts were slightly higher (< +20%, relative to the controls) during the first year and mean total leucocyte count and mean absolute lymphocyte count were slightly higher at Month 4 (+31% and +34% respectively, relative to the controls) and at Month 6 (+34% and +42% respectively, relative to the controls).
Altogether these changes were considered not to be toxicologically relevant in view of their low magnitude and their transient occurrence.
The few other differences observed, even if statistically significant, were considered to be incidental and not treatment-related.

Haematological findings in males are tabulated in the table 5

Table 5: Haematological changes in the males during the rat carcinogenicity study with flupyradifurone

Parameters	Dose levels in ppm				Historical control data
	0	80	400	2000	
Month 4					
White blood cell count	14.79	14.66	14.58	15.67	13.15 ±2.89 [5.2-24.5]
Neutrophil count	2.68	2.17	2.14	2.38	1.96 ±0.653 [0.8-7.5]
Lymphocyte count	11.30	11.75	11.62	12.51	10.42 ± 2.5 [3.9-20.1]
Month 6					
White blood cell count	12.13	12.39	12.91	14.07	12.43 ± 2.433 [7.5- 23.0]
Neutrophil count	1.85	2.00	1.94	2.28	1.98 ± 0.558 [1.0-4.2]
Lymphocyte count	9.56	9.60	10.17	10.93	9.73 ± 2.108 [5.6-19.4]
Month 12					
White blood cell count	10.55	11.27	11.73	12.91 ** (+ 22.4%)	10.95 ± 2.262 [6.5- 17.3]
Neutrophil count	2.05	2.18	2.19	2.79* (+36.1%)	2.16 ± 0.670 [4.3-13.4]
Lymphocyte count	7.8	8.44	8.86	9.31++ (+19.4%)	8.07 ± 1.915 [4.3-13.4]
Month 18					
White blood cell count	8.92	10.01	9.08	11.68 ** (+30.9)	10.60 ± 2.404 [5.8-17.5]
Neutrophil count	2.07	2.16	1.87	2.63	2.67 ± 0.763 [1.2 – 4.6]
Lymphocyte count	6.32	7.25	6.70	8.37 ** (+32.4%)	7.24 ± 1.828 [3.9 – 13.0]
Month 24					
White blood cell count	11.10	11.10 11.68	11.68	18.06 ++ (+62.7%)	11.57 ± 4.645 [5.1 – 29.3]
Neutrophil count	3.58	3.60	4.11	8.69 + (143%)	4.04 ± 2.343 [1.4 – 13.7]
Lymphocyte count	6.81	6.72	6.80	8.38	6.70 ± 2.822 [2.2- 19.9]

Counts expressed in 10^9/L
**: Dunnett LSD Test significant at 0.01 level
*: Dunnett LSD Test significant at 0.05 level
++: Dunn Rank Sum Test significant at 0.01 level
+: Dunn Rank Sum Test significant at 0.05 level

All the data are within historical control data except for the white blood cell and neutrophil counts after 24 hours, but the mean values are within the range of historical control data.

Haematological findings in females are tabulated in table 6:

Table 6: haematological changes in the females during the rat carcinogenicity study with flupyradifurone

Parameters	Dose levels in ppm				Historical control data
	0	80	400	2000	
Month 4					
HGB (g/dL)	15.55	15.77	15.55	15.46	15.68± 0.546 [14.2-17.4]
HCT (L/L)	0.4518	0.4555	0.4493	0.4518	0.4672 ±0.0219 [0.408-0.545]
MCV (fL)	52.4	52.2	52.2	51.3* (-2%)	53.5± 1.77 [49-58]
MCH (pg)	18.03	18.06	18.11	17.57 ++ (-2.6%)	17.94± 0.591 [16.3-19.4]
Platelet (10^9/L)	1192.8	1190.8	1182.6	1390.1 * (+16.5%)	1204.6± 158.01 [732-1603]
White blood cell count (10^9/L)	8.12	8.43	8.84	10.64 ** (+31%)	8.33± 2.289 [3.7-19.8]
Lymphocytes (10^9/L)	6.65	6.69	7.19	8.90 ** (+33.8%)	6.63± 1.965 [2.6-17.3]
Month 6					
HGB (g/dL)	15.14	15.36	15.33	14.48 ** (-6.3%)	15.33 ± 0.655 [13.8-16.9]
HCT (L/L)	0.4453	0.4537	0.4535	0.4323 + (-2.9%)	0.4555 ± 0.0201 [0.408-0.512]
MCV (fL)	53.4	53.2	53.5	52.3* (-2.07%)	53.3 ± 1.69 [49 – 59]
MCH (pg)	18.16	18.04	18.08	17.49 ** (-3.7%)	17.92 ± 0.576 [16.6 – 19.5]
Platelet (10^9/L)	1238.8	1214.9	1199.9	1451.8 + (+17.2%)	1209.1 ± 181.33 [111-1663]
White blood cell count (10^9/L)	6.33	7.17	7.02	8.51 ** (+34.44%)	7.12 ± 1.855 [3.4-13.1]
Lymphocytes (10^9/L)	4.84	5.64	5.40	6.85 ** (+41.53%)	5.57 ± 1.587 [2.5-11.2]
Month 12					
HGB (g/dL)	14.59	14.86	15.01	14.04 * (-3.8%)	15.72± 0.597 [13.2-17.3]
HCT (L/L)	0.4296	0.4380	0.4389	0.4144* (-3.5%)	0.4730 ±0.0189 [0.418-0.515]
MCV (fL)	52.7	52.5	52.4	51.1 * (-3.04%)	52.0 ± 1.55 [48-55]
MCH (pg)	17.88	17.83	17.93	17.32++ (-3.13%)	17.26 ± 0.669 [15.6-19.3]
Platelet (10^9/L)	1101.9	1127.2	1171.7	1306.4** (+18.6%)	1168.2 ± 193.78 [597-1693]
White blood cell count (10^9/L)	5.77	6.09	6.33	6.44	10.95 ± 2.262 [6.5-17.3]
Lymphocytes (10^9/L)	4.08	4.27	4.58	4.64	8.07 ± 1.915 [4.3-13.4]

*: Dunnett LSD Test significant at 0.05 level
++: Dunn Rank Sum Test significant at 0.01 level
+: Dunn Rank Sum Test significant at 0.05 level

All data are within historical control data except for the lymphocyte counts after 4 months and haemoglobin concentration and haematocrit after 12 months but the mean values are within the range of historical control data.

Characteristics

reference	:	[redacted]	exposure	:	Main groups: 104 weeks Satellite groups: 52 weeks
type of study	:	Combined chronic toxicity and carcinogenicity study	doses	:	0, 80, 400 and 2000 ppm **Average daily intake:** 0-104 weeks: Males: 0, 3.17, 15.8, 80.9 mg/kg bw/day Females: 0, 4.48, 22.5, 120 mg/kg bw/day 0-52 weeks: Males: 3.57, 18.5 and 95.1 mg/kg bw/day Females: 5.97, 25.3, 136 mg/kg bw/day
year of execution	:	2009-2012	vehicle	:	None
test substance	:	BYI 02960 (batch number 2009-000239, purity 96.2%)	GLP statement	:	Yes
route	:	Oral (diet)	guideline	:	According to OECD 453
species	:	Rat, Wistar Ri:WI (IOPS HAN)	acceptability	:	Acceptable
group size	:	Main group: 60/sex/dose Satellite group (interim sacrifice 52 weeks): 10/sex/dose	**NOAEL**toxicity (104 weeks) **NOAEL**toxicity (52 weeks) **NOAEL**carcinogenicity (104 weeks)	:	15.8 mg/kg bw/day 18.5 mg/kg bw/day 80.9 mg/kg bw/day

Study design

The study is performed according to OECD 453.

Results

The results are summarised in Table 6.5.1-1. Additional information on the observed effects is provided in Tables 6.5.1-2.-6.5.1-6.

Table 6.5.1-1

Dose (ppm in diet)	**0**		**80**		**400**		**2000**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	35	31	39	30	30	28	33	14	
Clinical signs - hair loss - soiled fur							 i i		
Body weight								ds (-13%)	
Body weight gain								ds (-22%)	
Food consumption								d	

Dose (ppm in diet)	0		80		400		2000		dr
	m	f	m	f	m	f	m	f	
Ophthalmoscopy - iris mydriasis - retina, fundus abnormal colour, pale - lens opacity								i i i	
Haematology	No substance-related findings								
Urinalysis	No substance-related findings								
Clinical chemistry - cholesterol - bilirubin								i d ds	
Organ weights *Satellite group*									
- terminal BW (g)				d		d	d	ds	
- liver - relative							is	is	
Main group									
- terminal BW (g)							d	ds	
- liver									
- relative							is	is	
Pathology									
Macroscopy *Satellite group* - liver: enlarged, pale							i		
Macroscopy *Main group* - lung: white foci - ovarian cyst								i i	
Microscopy	See table below								
Neoplastic lesions	No substance-related findings								

dr dose related
ds/is statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls

Table 6.5.1-2 Treatment-related ophthalmological findings at the second year examination (main groups).

Sex	Males				Females			
Dose level of BYI 02960 (ppm)	0	80	400	2000	0	80	400	2000
Group size	26	24	31	31	30	32	33	48
Iris mydriasis	3 (11.5%)	2 (8.3%)	3 (9.7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (8.3%)
Lens opacity	23 (88%)	22 (92%)	29 (94%)	29 (94%)	15 (50%)	22 (69%)	27 (82%)	46 (96%)
HCD – Mean % (Min-Max)					92.0% (81.4 – 100.0)			
Retina, fundus abnormal color, pale	0 (0%)	1 (4.2%)	1 (3.2%)	1 (3.2%)	0 (0%)	0 (0%)	0 (0%)	3 (6.3%)

HCD= Historical Control Data

Table 6.5.1-3 Mean liver weight ± SD at the 24-month scheduled sacrifice (% change when compared to the controls).

MEAN LIVER WEIGHT ±SD AT 24-MONTH SCHEDULED SACRIFICE (% CHANGE WHEN COMPARED TO CONTROLS)								
Sex	Males				Females			
BYI 02960 Dose-level (ppm)	0	80	400	2000	0	80	400	2000
Absolute liver weight (g)	12.401 ±1.9940	12.065 ±1.8556 (-3%)	11.971 ±1.8261 (-3%)	12.915 ±1.4947 (+4%)	8.878 ±1.7052	9.246 ±1.6570 (+4%)	9.406 ±2.1925 (+6%)	8.308 ±1.3457 (-6%)
Liver to body weight ratio (%)	1.971 ±0.2142	2.008 ±0.2758 (+2%)	1.941 ±0.1859 (-2%)	2.215** ±0.3085 (+12%)	2.242 ±0.2658	2.291 ±0.3077 (+2%)	2.278 ±0.3116 (+2%)	2.458** ±0.2783 (+10%)
Liver to brain weight ratio (%)	530.945 ±90.8128	518.885 ±81.6223 (-2%)	514.364 ±79.8667 (-3%)	546.361 ±64.5187 (+3%)	418.821 ±79.1927	440.047 ±79.7220 (+5%)	449.204 ±105.7920 (+7%)	392.895 ±67.0007 (-6%)

**: $p \leq 0.01$

Table 6.5.1-4 Incidence and severity of microscopic changes in the liver at 24-month scheduled sacrifice

INCIDENCE AND SEVERITY OF MICROSCOPIC CHANGES IN THE LIVER, ALL ANIMALS OF THE CARCINOGENICITY PHASE								
Sex	Male				Female			
BYI 02960 Dose-level (ppm)	0	80	400	2000	0	80	400	2000
Number of examined animals	60	60	60	60	60	60	60	60
Eosinophilic focus(i) of hepatocellular alteration								
Minimal	22	21	19	28	14	9	8	16
Slight	4	6	9	9	2	3	1	6
Moderate	1	0	2	7	0	0	1	1
Marked	0	0	0	1	0	0	0	0
Total	**27****	**27**	**30**	**45****	**16**	**12**	**10**	**23**
Tigroid focus(i) of hepatocellular alteration								
Minimal	29	28	32	42	30	30	24	36
Slight	5	8	7	3	5	7	5	5
Moderate	0	0	0	0	1	0	1	0
Total	**34**	**36**	**39**	**45**	**36**	**37**	**30**	**41**
Mixed focus(i) of hepatocellular alteration								
Minimal	0	0	0	3	0	0	0	0
Total	**0***	**0**	**0**	**3**	**0**	**0**	**0**	**0**
Hepatocellular hypertrophy: centrilobular								
Minimal	0	0	6	23	0	0	0	27
Slight	0	0	0	2	0	0	0	1
Total	**0****	**0**	**6***	**25****	**0****	**0**	**0**	**28****
Hepatocellular macrovacuolation: centrilobular: diffuse								
Minimal	3	4	3	18	1	0	0	19
Slight	0	0	1	6	0	1	0	5
Moderate	0	1	0	0	1	0	0	0
Total	**3****	**5**	**4**	**24****	**2****	**1**	**0**	**24****
Hepatocellular macrovacuolation: periportal: diffuse								
Minimal	5	4	2	2	18	22	12	11
Slight	2	3	1	4	8	11	13	2
Moderate	2	1	1	0	2	3	1	0
Marked	0	0	1	1	0	0	0	0
Total	**9**	**8**	**5**	**7**	**28****	**36**	**26**	**13****
Hepatocellular brown pigment : focal								
Minimal	0	0	0	0	1	0	1	13
Slight	0	0	0	0	0	0	0	3
Total	**0**	**0**	**0**	**0**	**1****	**0**	**1**	**16****

*: Significant ($p \leq 0.05$) **: Significant ($p \leq 0.01$)

Statistical significances in the control findings are considered an error.

(to be continued)

INCIDENCE AND SEVERITY OF MICROSCOPIC CHANGES IN THE LIVER, ALL ANIMALS OF THE CARCINOGENICITY PHASE								
Sex	Male				Female			
BYI 02960 Dose-level (ppm)	0	80	400	2000	0	80	400	2000
Number of examined animals	60	60	60	60	60	60	60	60
Accumulation of brown pigment in Kupffer cells								
Minimal	8	11	10	9	10	9	14	18
Slight	2	5	8	4	4	4	1	8
Moderate	0	0	0	1	1	1	1	1
Total	**10**	**16**	**18**	**14**	**15***	**14**	**16**	**27***
Interstitial mononuclear cell infiltrate: focal								
Minimal	20	19	21	24	22	28	29	35
Slight	1	0	1	2	1	4	0	1
Moderate	0	0	0	0	1	0	0	0
Total	**21**	**19**	**22**	**26**	**24**	**32**	**29**	**36***

*: Significant (p≤0.05) **: Significant (p≤0.01)

Statistical significances in the control findings are considered an error.

Table 6.5.1-5 Incidence and severity of microscopic changes in the lung at the 24-month scheduled sacrifice

INCIDENCE AND SEVERITY OF MICROSCOPIC CHANGES IN THE LUNG, ALL ANIMALS OF THE CARCINOGENICITY PHASE								
Sex	Male				Female			
BYI 02960 Dose-level (ppm)	0	80	400	2000	0	80	400	2000
Number of examined animals	60	60	60	60	60	60	60	60
Alveolar foamy macrophages: focal								
Minimal	18	24	22	25	27	18	11	25
Slight	10	12	8	7	2	11	16	18
Moderate	2	4	4	3	1	0	2	6
Marked	0	0	0	0	0	0	0	1
Total	**30**	**40***	**34**	**35**	**30****	**29**	**19***	**50****
Chronic interstitial inflammation: focal								
Minimal	1	4	4	3	2	3	5	10
Slight	0	1	0	0	0	0	0	0
Moderate	0	0	1	0	0	0	0	0
Total	**1**	**5**	**5**	**3**	**2***	**3**	**5**	**10***
Perivascular inflammation: focal								
Minimal	23	20	30	20	17	22	17	35
Slight	1	3	2	1	0	1	0	1
Total	**24**	**23**	**32**	**21**	**17****	**23**	**17**	**36****

*: Significant (p≤0.05) **: Significant (p≤0.01)

Statistical significances in the control findings are considered an error.

Table 6.5.1-6 Incidence and severity of microscopic changes in the thyroid gland at the 24-month scheduled sacrifice

INCIDENCE AND SEVERITY OF MICROSCOPIC CHANGES IN THE THYROID GLAND, ALL ANIMALS OF THE CARCINOGENICITY PHASE								
Sex	Male				Female			
BYI 02960 Dose-level (ppm)	0	80	400	2000	0	80	400	2000
Number of examined animals	60	60	60	60	60	60	60	60
Colloid alteration								
Minimal	14	12	28	18	11	10	10	11
Slight	7	9	9	18	1	3	0	4
Moderate	0	1	1	4	0	1	0	0
Marked	0	0	0	0	1	0	0	0
Total	**21****	**22**	**38****	**40****	**13**	**14**	**10**	**15**
Follicular cell hypertrophy: diffuse								
Minimal	1	0	1	3	0	1	0	3
Total	**1**	**0**	**1**	**3**	**0**	**1**	**0**	**3**
Brown pigments: follicular cells								
Minimal	16	10	18	22	8	7	5	17
Slight	1	0	1	1	0	0	0	0
Moderate	0	0	1	0	0	0	0	0
Total	**17**	**10**	**20**	**23**	**8**	**7**	**5**	**17**

** : Significant ($p \leq 0.01$)

Statistical significances in the control findings are considered an error.

No effects of the treatment on the mortality were observed. Clinical signs were limited soiled fur, hyper-reactivity to external stimuli and resistance to handling, observed during the first year of treatment, and soiled fur and hair loss observed during the second year of treatment in high-dose males.

In high-dose females the mean body weight and cumulative body weight gain were lower compared to controls throughout the study (statistically significant). Overall the mean cumulative body weight gain was decreased by 23% when compared to the controls at the end of the study, the mean body weight was 13% lower. In high-dose males, mean body weight was lower compared to controls from week 1 to week 54 (statistically significant on most occasions); thereafter the mean body weight was comparable to the controls.

In high-dose females, food consumption was marginally lower than control in many occasions throughout the study. In all other groups mean food consumption was considered to be comparable to the controls throughout the study.

Iris mydriatis and an abnormal pale colour of fundus of the retina was noted in 4/48 and 3/48 high-dose females, respectively, compared to 0/30 in controls. In addition, higher incidences of lens opacity was observed at this dose level in comparison to the controls. However, lens opacity is a common finding in rats of this age and strain, and the values were well within the historical control data, while the value in the control group was particularly low and outside the historical control data of the test lab. Therefore this change was considered to be not treatment-related.

The transient haematological changes observed were considered to be not treatment-related in view of their low magnitude and transient occurrence.

Slightly lower total bilirubin concentrations were observed at 2000 ppm in both sexes. Slightly higher mean total cholesterol was observed throughout the study in high-dose females. No relevant changes were noted in other groups and at other dose levels.

No treatment-related findings were observed at urinalysis.

At the interim sacrifice and at the end of the study duration, mean liver to body weight ratios were statistically significantly higher when compared to controls at 2000 ppm in both sexes. Enlarged and pale liver was noted in 3/10 high-dose males at interim sacrifice and was considered to be associated with the treatment. At the completion of the 2-year study period white foci in the lung were noted in 21/46 of high-dose females, which were considered to be associated with the treatment. Also higher incidence of ovarian cysts was noted in 18/46 females, when compared to 8/29 in the controls. However, as microscopically there were no differences in terms of percentages in ovarian cysts between the control group (18/29, 62%) and high-dose group (31/46, 67%), this finding was considered to be not treatment-related.

At the interim sacrifice, higher incidences of eosinophilic and tigroid foci of altered hepatocytes were observed in high-dose males. Centrilobular hypertrophy was observed in both sexes at 2000 ppm and also at 400 ppm in 2/10 males (dose-related effects). However, in the absence of any increase of incidences of foci of alterations or hepatocellular macrovacuolation, this change at 400 ppm was considered to be not an adverse effect. Centrilobular hepatocellular macrovacuolation and lower incidences of periportal hepatocellular vacuolation were noted in both sexes at 2000 ppm. In the thyroid gland, colloid alteration was found to be increased in incidence and severity in both sexes at 2000 pm and in males at 400 ppm. At the terminal sacrifice, higher incidences of eosinophilic, mixed and tigroid foci of altered hepatocytes were observed in males at 2000 ppm. Centrilobular hypertrophy and higher incidences of centrilobular hepatocellular macrovacuolation were observed in both sexes of high-dose group. Higher incidences of hepatocellular brown pigments and brown pigments in Kupffer cells, higher incidence of interstitial mononuclear cell infiltrate and lower incidences of periportal hepatocellular macrovacuolation were noted in high-dose females. No adverse effects was noted in both sexes at 400 ppm; there was only minimal centrilobular hypertrophy in 6 male animals without any increase of foci of alteration or hepatocellular macrovacuolation.

In the lung higher incidences of foamy macrophages, chronic interstitial inflammation and perivascular inflammation were observed in high-dose females at the terminal sacrifice.

In the thyroid, increased incidences of colloid alteration were noted in males at 2000 and 400 ppm. The effect at 400 ppm was not considered to be adverse, as increased incidence of colloid alteration occurs naturally in aging rats and was not associated with relevant follicular hypertrophy at this dose level.

No neoplastic lesions were observed at any dose levels.

Historical control data are provided only for ophthalmologic findings and are based on selected 2-year studies performed in-house between February 2005 and February 2008.

Acceptability

The study is considered acceptable.

Conclusions

No neoplastic lesions were observed in the study at any dose level. The NOAEL for carcinogenicity was set at the highest dose level of 2000 ppm, equal to the average daily intake of 80.9 and 120 mg/kg bw/day over 104 weeks for male and female rats, respectively.

Based on effects in the liver and in a less extend in the thyroid and lung (females), the NOAEL for general toxicity was set at 400 ppm in diet, equal to the average daily intake of 15.8 and 22.5 mg/kg bw/day over 104 weeks for male and female rats, respectively (18.5 and 25.3 mg/kg bw/day, respectively, over 52 weeks).

Study 2

Characteristics

reference	:	[redacted]	exposure	:	Main groups: at least 78 weeks Satellite groups: 52 weeks
type of study	:	Carcinogenicity study	doses	:	0, 70, 300 and 1500 ppm **Average daily intake:** 0-78 weeks: Males: 0, 10.0, 43 and 224 mg/kg bw/day Females: 0, 12.2, 53 and 263 mg/kg bw/day 0-52 weeks: Males: 10.5, 45 and 232 mg/kg bw/day Females: 12.9, 56 and 263 mg/kg bw/day
year of execution	:	2009-2012	vehicle	:	None
test substance	:	BYI 02960 (batch number 2009-000239, purity 96.2%)	GLP statement	:	Yes
route	:	Oral (diet)	guideline	:	According to OECD 451
species	:	Mouse, C57BL/6J	acceptability	:	Acceptable
group size	:	Main group: 50/sex/dose Satellite group (interim sacrifice 52 weeks): 10/sex/dose	**NOAEL**toxicity **NOAEL**carcinogenicity	:	43 mg/kg bw/day 224 mg/kg bw/day

Study design

The study is performed according to OECD 451

Results

The results are summarised in Table 6.5.1-7. Additional information on the observed effects is provided in Tables 6.5.1-8 – 6.5.1-12.

Table 6.5.1-7

Dose (ppm in diet)	**0**		**70**		**300**		**1500**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	11	8	5	10	9	5	8	7	
Clinical signs	No treatment-related findings								
Body weight							ds	d	
Body weight gain					d	d	ds	ds	
Food consumption								d	
Haematology	No substance-related findings								
Urinalysis	Not evaluated in the study								
Clinical chemistry	Not evaluated in the study								
Organ weights *Satellite group*									
- terminal BW (g)							d		
kidney - absolute - relative to brain weight							ds ds		
brain - relative to body weight							is		
Main group									
terminal BW (g)							ds	d	
liver - absolute - relative to body weight							is is	 is	
kidney - absolute - relative							d d		
brain - relative to body weight							is	is	
Pathology									
Macroscopy *Satellite group*	No treatment-related changes								
Macroscopy *Main group* **kidney**: - atrophic/small							 is		
Microscopy	See table below.								
Neoplastic lesions	No substance-related findings								

dr dose related
ds/is statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls

Appendix Table 6.5.1-7: Body weight and body weight gain additional data

Dose (ppm)	0	70	300	1500	dr

	m	f	m	f	m	f	m	f	
BW W78	31.2	28.5	30.9 (-1%)	28.4	30.7 (-1%)	27.1 (-5%)	29.4 ds (-6%)	26.6 d (-7%)	
BWG W1-78	10.8	11.2	10.4 (-4%)	11.5 (+3%)	10.2 d (-6%)	10.1 d (-10%)	8.7 ds (-19%)	9.7 ds (-13%)	

Table 6.5.1-8 Mean liver weight ± SD at the 18-month scheduled sacrifice

MEAN LIVER WEIGHT ± SD AT SCHEDULED SACRIFICE (% CHANGE WHEN COMPARED TO CONTROLS)								
Sex	Males				Females			
Dose-level of BYI 02960 (ppm)	0	70	300	1500	0	70	300	1500
Absolute liver weight (g)	1.128 ± 0.0784	1.128 ± 0.0846 (0%)	1.137 ± 0.0727 (+1%)	1.233 ** ± 0.0861 (+9%)	1.285 ± 0.1749	1.287 ± 0.1751 (0%)	1.220 ± 0.2035 (-5%)	1.294 ± 0.1899 (+1%)
Liver to body weight ratio (%)	4.166 ± 0.2381	4.171 ± 0.2616 (0%)	4.172 ± 0.2388 (0%)	4.759 ** ± 0.2743 (+14%)	5.110 ± 0.3489	5.167 ± 0.4445 (+1%)	4.986 ± 0.6718 (-2%)	5.509 ** ± 0.8036 (+8%)
Liver to brain weight ratio (%)	249.155 ± 16.3057	248.368 ± 19.3286 (0%)	251.190 ± 15.1611 (+1%)	270.814 ** ± 16.0455 (+9%)	272.958 ± 34.0733	270.002 ± 33.9804 (-1%)	257.339 ± 44.1777 (-6%)	273.926 ± 39.0896 (0%)

**:p≤0.01

Table 6.5.1-9 Mean kidney weight ± SD at the 18-month scheduled sacrifice

MEAN KIDNEY WEIGHT ± SD AT SCHEDULED SACRIFICE (% CHANGE WHEN COMPARED TO CONTROLS)								
Sex	Males				Females			
Dose-level of BYI 02960 (ppm)	0	70	300	1500	0	70	300	1500
Absolute kidney weight (g)	0.4959 ± 0.0482	0.5148 ± 0.0489 (+4%)	0.4932 ± 0.0505 (-1%)	0.4290 ** ± 0.0384 (-13%)	0.4424 ± 0.0497	0.4533 ± 0.0832 (+2%)	0.4381 ± 0.0455 (-1%)	0.4187 ± 0.0420 (-5%)
Kidney to body weight ratio (%)	1.8295 ± 0.1406	1.9004 ± 0.1321 (+4%)	1.8073 ± 0.1583 (-1%)	1.6538 *** ± 0.0966 (-10%)	1.7650 ± 0.1179	1.8233 ± 0.3017 (+3%)	1.7957 ± 0.1407 (+2%)	1.7800 ± 0.1246 (+1%)
Kidney to brain weight ratio (%)	109.5244 ± 10.1927	113.2376 ± 10.4639 (+3%)	108.8135 ± 9.7886 (-1%)	94.2427 ** ± 7.5710 (-14%)	93.9341 ± 8.6909	95.1333 ± 17.5359 (+1%)	92.2409 ± 8.8349 (-2%)	88.5742 ± 7.4842 (-6%)

**:p≤0.01; ***:p≤0.001

Table 6.5.1-10 Incidence of macroscopic changes in the kidney at the 18-month scheduled sacrifice

INCIDENCE OF MACROSCOPIC CHANGES IN THE KIDNEY, SCHEDULED SACRIFICE, CARCINOGENICITY PHASE								
Sex	Males				Females			
BYI 02960 Dose level (ppm)	0	70	300	1500	0	70	300	1500
Atrophic/small	0/38	0/45	0/41	5/42 *	0/42	0/39	0/45	0/42

*:p≤0.05; **:p≤0.01 (Appendix M)

Table 6.5.1-11 Incidence and severity of microscopic changes in the liver at the 18-month scheduled sacrifice

Incidence and severity of microscopic changes in the liver (all animals) 18-month carcinogenicity phase								
Sex	Males				Females			
Dose level of BYI 02960 (ppm)	0	70	300	1500	0	70	300	1500
Number of animals examined	50	50	50	50	50	50	50	50
Hepatocellular macrovacuolation : mainly periportal: diffuse								
Minimal	0	0	0	0	32	28	28	13
Slight	0	0	0	0	2	1	1	0
Total	**0**	**0**	**0**	**0**	**34**	**29**	**29**	**13****
Hepatocellular vacuolation : mainly centrilobular: diffuse								
Minimal	10	16	2	0	0	0	0	0
Slight	16	16	22	5	0	1	0	1
Moderate	2	2	12	31	0	1	0	3
Marked	0	0	0	5	1	0	0	0
Total	**28**	**34**	**36**	**41****	**1**	**2**	**0**	**4**

**: p≤0.01

Table 6.5.1-12 Incidence and severity of microscopic changes in the kidney at the 18-month scheduled sacrifice

Incidence and severity of microscopic changes in the kidney (all animals) 18-month carcinogenicity phase								
Sex	Males				Females			
BYI 02960 Dose level (ppm)	0	70	300	1500	0	70	300	1500
Number of animals examined	50	50	50	50	50	50	50	50
Basophilic tubules : bilateral								
Minimal	23	23	18	3	3	2	3	1
Slight	4	2	3	0	0	0	0	0
Total	**27**	**25**	**21**	**3****	**3**	**2**	**3**	**1**
Cortical mineralization: focal								
Minimal	12	19	13	1	1	0	0	0
Moderate	0	0	1	0	0	0	0	0
Total	**12**	**19**	**14**	**1****	**1**	**0**	**0**	**0**
Corticoepithelial vacuolation								
Minimal	3	2	5	19	0	0	0	0
Slight	4	3	7	9	0	0	0	0
Moderate	29	26	31	4	0	0	0	0
Marked	13	14	4	0	0	0	0	0
Total	**49**	**45**	**47**	**32****	**0**	**0**	**0**	**0**

**: p≤0.01

No test-substance related mortality or clinical signs were observed. Reduced body weight was observed in both sexes at the highest dose level (statistically significant in males). Mean cumulative body weight gain over the whole study period was also significantly reduced in males and females of the high-dose group and was 19% lower than in controls in males and 13% lower than in controls for females. Mean body weight gain over the whole study period was also slightly reduced in mid-dose

animals of both sexes (not statistically significant). Food consumption was decreased on several occasions throughout the study in high-dose females (average 3% below controls).

At the interim sacrifice, absolute kidney weight and kidney to brain weight ratio were 13 to 15% lower than controls in high-dose males (statistically significant). Mean brain to body weight ratio was 9% higher than controls in high-dose males ($p \leq 0.05$), but this effect was considered to be related to the decreased body weight. At the macroscopic observation, no treatment-related changes were noted in either sex at any dose level. Histopathology was not performed at the interim sacrifice.

At the terminal sacrifice, absolute and relative liver weights were 9 to 14% higher than controls in high-dose males ($p \leq 0.01$). Relative liver weight was also increased in high-dose females ($p \leq 0.01$), but this effect was considered to be related to the reduced body weight. Statistically significantly reduced absolute and relative kidney weights were observed in high-dose males. Relative brain weight was slightly higher than controls in both sexes of the high-dose group (+5% in males and +7% in females, $p \leq 0.01$), but this effect was considered to be related to the reduced body weight.

At the macroscopic observation, atrophic/small kidneys were noted in 5/42 high-dose males (in comparison to 0/38 in controls). At histopathological examination increased incidence of centrilobular hepatocellular vacuolation was noted in high-dose males (statistically significant), whilst a decreased incidence of periportal hepatocellular macrovacuolation was noted in high-dose females (statistically significant). Not statistically significant increase in the incidence of centrolobular hepatocellular vacuolation was also noted in mid-dose males. These changes in the liver were considered to be treatment-related but not adverse due to the lack of associated degenerative changes. In the kidney, decreased incidence and severity of bilateral basophilic tubules, focal cortical mineralization and corticoepithelial vacuolation were noted in high-dose males (all statistically significant). These changes in the kidney were considered to be treatment-related but not adverse, as they occurred with lower incidence and severity than in control animals).

No neoplastic changes were evidenced at any dose level in either sex.

Historical control data for histopathological findings were provided from 11 in-house studies with C57BL/6J mice performed in the period October 2000 – March 2007.

Acceptability

The study is considered acceptable.

Conclusions

No neoplastic changes were evidenced in any group at any dose level. The NOAEL for carcinogenicity was set at the highest dose level of 1500 ppm, equal to the average daily intake of 224 and 263 mg/kg bw/day over 18 months for male and female mice, respectively.

Based on effects in the liver and in de kidney (male) the NOAEL for general toxicity was set at 300 ppm in diet, equal to the average daily intake of 45 and 53 mg/kg bw/day over 18 months for male and female mice, respectively.

B.6.5.2 Summary

In Table 6.5.2.1 the results of the chronic toxicity and carcinogenicity studies with the test substance are summarised.

Table 6.5.2.1 Oral chronic toxicity and carcinogenicity studies

Duration (weeks)	Species	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Critical effects	Reference
104	rat	15.8	80.9	Effects on liver, thyroid and lung	[redacted], 2012
78	mouse	43	224	Effects on liver and kidney	[redacted] 2012

In a two-year toxicity/carcinogenicity study with rats, effects in the liver and in a less extend in the thyroid and lung (females) were observed in the 2000 ppm dose group. The NOAEL for general toxicity was set at 400 ppm in diet, equal to the average daily intake of 15.8 mg/kg bw/d. No neoplastic lesions were observed in the study at any dose level.
In an 18-months carcinogenicity study with mice, effects in the liver and in de kidney (male) were observed in the 1500 ppm dose group. the NOAEL for general toxicity was set at 300 ppm equal to the average daily intake of 45 mg/kg bw/day.

B.6.6 REPRODUCTIVE TOXICITY (ANNEX IIA 5.6)

B.6.6.1 Reproductive toxicity

STUDY 1

Characteristics

reference	:	[redacted] 2010	exposure	:	Throughout the entire in-life phase of the study P: 10 weeks before mating, 7 days mating, 22 days gestation, 21 days lactation
type of study	:	One generation reproductive toxicity study (dose range-finding)	doses	:	Nominal: 0, 200, 700 and 2000 ppm in diet Actual daily intake: See study design
year of execution	:	2009/2010	vehicle	:	none
test substance	:	BYI 02960 (batch number 2009-000239; purity 96.2%)	GLP statement	:	Yes
route	:	Oral (diet)	guideline	:	None – pilot study
species	:	Rat, Wistar Crl:WI (Han)	acceptability	:	Acceptable
group size	:	10/sex/dose	**NOAEL**par toxicity	:	17.5 mg/kg bw/day (f).
			NOAELpar reproductive effects	:	147.5 mg/kg bw/day (m, highest dose tested)
			NOAELoffspring	:	17.5 mg/kg bw/day (f)

Study design

The study was performed in order to determine appropriate dietary levels for a definitive (guideline) two-generation reproductive toxicity study with BYI 02960. In this study, BYI 02960 was administered continuously in the feed to the Wistar rat (10 animals/dose/sex) at nominal dietary concentrations of 0, 200, 700, and 2000 ppm. All test diets (including control) were available for *ad libitum* consumption; the homogeneity and stability of BYI 02960 as a dietary admixture was confirmed. Body weight and food consumption determinations and detailed clinical examinations of each animal were conducted weekly throughout the study, as well as, an evaluation of multiple reproductive parameters. All animals placed on study were subject to a postmortem examination, which included documenting and saving all gross lesions, weighing designated organs and, collecting representative tissue specimens. Micropathology was not performed on any tissue collected in this study.

The mean values of the actual substance intake from the intended test material dose levels for the parental generation are shown in Table 6.6.1-1.

Table 6.6.1-1 Mean values of the actual substance intake by parental animals during premating, gestation and lactation

Phase of Study	200 ppm in mg/kg/day[a]	700 ppm in mg/kg/day[a]	2000 ppm in mg/kg/day[a]
Premating (P-gen) - Male	14.5	50.1	147.5
Premating (P-gen) - Female	17.5	60.0	168.9
Gestation (P-gen) - Female	15.8	48.8	164.4
Lactation (P-gen) - Female	17.5	60.9	182.3

[a] Individual values were based on means for each particular phase.

Results

The results of the study are summarised in table 6.6.1-2. Additionally, information on parental body weights, body weight gains, food consumption, reproductive performance, litter parameters and pup weights is presented in Tables 6.6.1-3 -6.6.1-8.

Table 6.6.1-2

Dose (ppm in diet)	**0**		**200**		**700**		**2000**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
F0 animals									
Mortality	No substance-related mortality								
Clinical signs	No substance-related findings								
Body weight					d (g) ds (l)			ds (pm, g, l)	
Body weight gain					d (pm)		d (pm)	d (pm)	
Food consumption							d (pm)		
Mating/fertility/gestation	No substance-related findings								
Oestrus cycle	Not investigated								
Sperm evaluation	Not investigated								
Organ weight -liver -spleen					 i		 i i(a/r)	 ds (a/r)	
Pathology									
Macroscopy	No substance-related findings								
Microscopy	Not investigated								
F1 pups									
Litter size	No substance-related findings								
Viability index	No substance-related findings								
Lactation index	No substance-related findings								
Sex ratio	No substance-related findings								
Clinical signs	No substance-related findings								
Body weight - day 14 - day 21					 d d	 ds d	 d d	 ds ds	
Physical development	No substance-related findings								
Organ weight - brain						d (a) i (r)		ds (a) is (r)	
Pathology									
Macroscopy	No substance-related findings								

dr dose related
ds/is statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a/r absolute/relative organ weight
pm premating

l lactation
g gestation

Table 6.6.1-3 Mean (S.E.) body weight and food consumption during premating.

Observations/study week	Dose Group			
	Control 0 ppm	LDT 200 ppm	MDT 700 ppm	HDT 2000 ppm
P Generation Males				
Mean body weight (g) - Week 15 S.E.	452.9 13.42	463.2 14.30	454.6 11.51	432.6 14.36
Mean weight gain (g) Weeks 1-15	232.2	242.3	235.3	210.7
Mean food consumption (g/animal/day) Weeks 1-10	23.9	24.3	23.8	23.6
Mean food consumption (g/kg/day) Weeks 1-10	72.6	73.2	73.5	75.4
P Generation Females - Pre-mating				
Mean body weight (g) - Week 10 S.E.	231.0 3.87	230.3 5.22	221.7 6.00	207.0** 3.27
Mean weight gain (g) Weeks 1-10	79.0	79.5	69.3	52.6
Mean food consumption (g/animal/day) Weeks 1-10	17.1	17.2	16.8	15.6
Mean food consumption (g/kg/day) Weeks 1-10	86.8	88.4	88.4	85.7

[a] Data obtained from Tables 2, 3, and 4 in the study report
** Statistically different from control, $p \leq 0.01$

Table 6.6.1-4 Mean (S.E.) body weight and food consumption during gestation (amended table, open point 2(26))

P Generation Females - Gestation				
Observations/study week	Dose Group			
	Control 0 ppm	LDT 200 ppm	MDT 700 ppm	HDT 2000 ppm
Mean body weight (g) - Day 0 S.E.	233.5 4.33	231.3 6.22	225.5 4.69	206.4** 4.47
Mean body weight (g) - Day 6 S.E.	249.4 5.26	251.8 6.06	241.2 4.09	225.0** 3.85
Mean body weight (g) - Day 13 S.E.	274.7 5.15	274.8 7.39	261.4 4.26	241.6** 4.38
Mean body weight (g) - Day 20 S.E.	334.8 6.45	337.8 10.78	315.5 6.10	295.9** 4.42
Mean weight gain (g) - Days 0-20 S.E.	101.4 5.30	106.5 6.14	90.0 3.98	89.5 3.15
Mean food consumption (g/animal/day) Days 0–20	18.6	20.1	17.4	18.6
Mean food consumption (g/kg/day) Days 0–20	73.8	80.1	71.8	83.4

**: Statistically different from control, p≤0.01

Table 6.6.1-5 Mean (S.E.) body weight and food consumption during lactation

P Generation Females - Lactation				
Observations/study week	Dose Group			
	Control 0 ppm	LDT 200 ppm	MDT 700 ppm	HDT 2000 ppm
Mean body weight (g) - Day 0 S.E.	255.2 5.94	253.2 7.97	242.1 2.99	223.9** 5.85
Mean body weight (g) - Day 4 S.E.	266.1 4.96	270.0 7.13	253.6 4.16	241.6* 5.23
Mean body weight (g) - Day 7 S.E.	274.9 4.22	275.9 5.84	266.5 3.43	252.3* 5.39
Mean body weight (g) - Day 14 S.E.	296.6 4.78	296.4 6.25	276.8* 3.77	262.5** 6.28
Mean body weight (g) - Day 21 S.E.	290.1 4.80	287.1 10.04	272.8* 2.87	261.8** 3.95
Mean food consumption (g/animal/day) Days 0–21	49.3	47.3	45.0	45.3
Mean food consumption (g/kg/day) Days 0–21	179.3	171.3	171.5	182.7

[1] Data obtained from Tables 13, 14 and 15 in the study report
* Statistically different from control, $p \leq 0.05$ ** Statistically different from control, $p \leq 0.01$

Table 6.6.1-6 Reproductive performance

Observation	Dose Group (ppm)			
	Control 0 ppm	LDT 200 ppm	MDT 700 ppm	HDT 2000 ppm
P Generation – F_1 Offspring				
Number Cohoused	10	10	10	10
Number Mated	10	9	9	9
Number of Animals Delivered	8	8	9	9
Number of Animals with Implants	9	8	9	9
Mating Index	100.0	90.0	90.0	90.0
Fertility Index	90.0	88.9	100.0	100.0
Gestation Index	88.9	100.0	100.0	100.0
Mean Number Days to Insemination (S.E.)	2.8 (0.32)	1.9 (0.26)	2.3 (0.33)	2.2 (0.28)
Mean Gestation Length (days) (S.E.) Median Gestation Length (days)	21.9 (0.23) 22.0	22.3 (0.16) 22.0	22.0 (0.24) 22.0	22.2 (0.28) 22.0

[a] Data obtained from Table 6 in the study report

Table 6.6.1-7 Litter parameters

Observation	Dose Group (ppm)			
	Control 0 ppm	LDT 200 ppm	MDT 700 ppm	HDT 2000 ppm
P Generation				
Total number of implantation sites	96	99	101	94
Total number of pups born	91	97	100	92
Number stillborn	0	4	0	0
Sex Ratio Day 0 (% male)	55.3	45.5	48.0	54.2
Mean litter size Day 0	11.4	12.1	11.1	10.2
Birth index	87.0	98.1	99.0	98.0
Live birth index	100.0	96.2	100.0	100.0
Viability index	100.0	98.2	99.1	100.0
Lactation index	100.0	100.0	100.0	100.0

[a] Data obtained from Table 6 and 20 in the study report

Table 6.6.1-8 Mean (SE) combined male and female pup weights (g).

F_1 Generation				
Lactation Day	Control 0 ppm	LDT 200 ppm	MDT 700 ppm	HDT 2000 ppm
0 S.E	6.1 0.18	6.0 0.18	5.7 0.11	6.0 0.23
4[b] S.E.	10.2 0.48	10.0 0.39	9.4 0.22	10.0 0.43
4[c] S.E.	10.1 0.51	10.0 0.39	9.4 0.22	10.0 0.41
7 S.E.	16.2 0.71	16.2 0.51	15.0 0.25	15.3 0.45
14 S.E.	33.2 0.86	32.1 0.83	29.9* 0.57	29.9* 0.71
21 S.E.	50.6 1.42	48.4 1.05	45.9 1.11	45.7* 1.11
GAIN	44.5	42.4	40.2*	39.7*

[a] Data obtained from Table 18 and 20 in the study report
[b] Before standardization (culling)
[c] After standardization (culling)
* Statistically different from control, $p \leq 0.05$

There were no test substance-related mortalities or clinical observations observed during the course of the study at any dietary level tested.

In males, no effects on body weight were observed at any dose level. In females, declines in absolute body weight were observed during premating in 2000 ppm group (9.4% decline when compared to controls), during gestation in 700 and 2000 ppm groups (4.5% and 11.3% respectively, value in 2000 ppm group reached statistical significance) and during lactation in 700 and 2000 ppm groups (10.6% during days 14-21 in 700 ppm group, 10.2% during days 0-21 in 2000 ppm groups, both statistically significant).

In males a very slight decrease in body weight gain during premating was observed in the 2000 ppm dose group (9.3% decline when compared to controls). In females, declines in body weight gain were noted during premating in 700 and 2000 ppm group (12.3% and 33.4%, respectively). Food consumption was decreased 8.8% in females at 2000 ppm during premating. No further changes on body weight gain and food consumption were noted.

There were no test substance-related effects on any reproductive parameter (e.g. mating, fertility, or gestation indices, days to insemination, gestation length, or the median number of implants) at any dietary level tested.

In males, equivocal increases in liver weights were observed in both the 700 and 2000 ppm dose groups. In females, test substance-related decreases in absolute and relative spleen weight were observed in the 2000 ppm dose level.

There was no test substance-related effects observed on the viability of the pups after delivery at any dietary level tested. No test substance-related clinical observations were observed at any dietary level tested. Declines in absolute pup weight were observed beginning post-natal day (PND) 14 and continuing to PND 21 in the 700 and 2000 ppm dietary groups with significance only observed for the females. Body weight gain for the males and females was declined in both the 700 and 2000 ppm dose group (9.7% and 10.8%, respectively).
Significant changes in brain weight in the 2000 ppm dose group and slight changes in the 700 ppm dose group were observed (decreased absolute and increased relative) and are considered to be secondary to body weight declines observed in the pups at these same dose groups. There were no test substance-related gross necropsy findings observed at any dietary level tested.

Historical control data were obtained from reproduction studies performed in the same test laboratory in the period of 1998-2008 in the Wistar rat.

Acceptability
The study is considered acceptable.

Conclusion
The parental systemic LOAEL is 700 ppm (60.0 mg /kg bw/day females), based on decreased body weight, decreased body weight gain, alterations in food consumption during premating, and decreased spleen weights in females. The parental systemic NOAEL is 200 ppm (17.5 mg /kg bw/day).
The reproductive NOAEL is 147.5 mg /kg bw/day based on no reproductive findings observed at the highest dose tested.
The offspring LOAEL is 700 ppm (60.9 mg /kg bw/day). The LOAEL is based on maternal effects leading to secondarily-mediated effects on pup weight, pup weight gain, and organ weight changes (brain). The offspring NOAEL is 200 ppm (17.5 mg /kg bw/day).

Study 2

Data requirement:
Applicant to provide clarification on the possible endocrine-mediated MoA of flupyradifurone. The information *embedded in column 3 of the reporting table should be included.*

In the rat two-generation reproduction study conducted on Flupyradifurone changes were observed in offspring at the high dietary concentration of 1800 ppm. Effects seen on PPS and VO, two well-established markers of pubertal development, included a statistically significant delay (PPS) and a slight, non-statistical delay (VO) in F1 generation animals. In addition, variations in brain, thymus, and spleen weights in offspring of

the 500 ppm (F2 generation) and 1800 ppm (F1 and F2 generations) dose groups were also observed. A slight, albeit significant, decrease in median implantation sites (and thus decrease in litter size) in the F1-generation was observed with 1800 ppm Flupyradifurone. It is the position of both Bayer CropScience (BCS) and the performing laboratory that the aforementioned effects are likely a result of treatment-induced reductions in BW and BWG rather than a direct, endocrine-mediated MOA. The discussion that follows defends this position for each of the observed changes in the context of available data on Flupyradifurone and in the publicly available literature (see below). This position from BCS should be taken into account during the expert consultation.

Preputial Separation

Toxicity testing guidelines for the rat two-generation reproduction toxicity study require that systemic toxicity is evident at the highest dose level, and dose levels should be chosen with the aim to induce some reproductive and/or systemic toxicity, but not death or severe suffering (OECD 416; OCSPP 870.3200). As such, effects on BW (reductions >10%) are the most common effects noted at maximum tolerated dose levels, and it is reasonable that delays in preputial separation may be observed as a result of general systemic toxicity rather than a consequence of an endocrine-mediated MOA (Stump et al., 2012). In the present study, animals exposed to 100 ppm or 500 ppm.

Flupyradifurone achieved PPS in a time latency similar to concurrent controls and well within historical control data for Wistar Han rats in the performing laboratory (see Appendix 1). At the high dietary level of 1800 ppm, however, a statistically significant delay in PPS (3.9 days) was observed compared to controls (Table 1). The observed delay in PPS in animals at 1800 ppm is likely due to significant BW loss and food consumption and not a result of an endocrine MOA associated with delayed PPS.

Table 1: Mean Day to Criterion and Body Weight (g) at Preputial Separation

	Dose levels in ppm			
	0	100	500	1800
Days to criterion				
Mean	42.6±0.38	42.7±0.44	43.3±0.31	46.5**±0.50
N	35	35	35	35
BW at criterion				
Mean	178±2.6	180±2.2	176±2.9	180±2.9
N	35	35	35	35

Statistically significant compared to control animals (p≤0.05).

A recent study concluded that while treatment-related delays in PPS may be indicative of specific anti-androgenic activity, impaired growth could also alter the onset of puberty (Melching-Kollmuss et al., 2014). The mean BW at the day of complete PPS was similar between the control animals (178 g) and 1800 ppm group (180 g) (Table 1, Figure 1), however, the mean BW at PND 21 was significantly decreased (-12.5%) compared to control animals (see Appendix 2); BW at PND 21 is often used as a covariate for evaluating the age at complete PPS (Stoker et al., 2000b). This significant decrease in BW at PND 21 was accompanied by significant

decreases observed in maternal BW of the 1800 ppm group throughout gestation (GDs 0, 6, 13, and 20; see Appendix 3). As other sensitive anti-androgenic parameters investigated in this study such as male fertility, male reproductive organs (weights and histopathology) and sperm parameters (count, motility, or progressive sperm) were not affected at any dietary level of Flupyradifurone, it is likely that the effect on PPS was due to a non-specific effect linked to overt toxicity with decreased BW and retarded growth.

Figure 1: Mean Body Weight at Day of Complete Preputial Separation

The influence of BW loss on reproductive endpoints has also been evaluated in rats by diet restriction. A significant delay in PPS (2.1 days) was observed in Wistar rats that were food-restricted and exhibited a mean body weight loss of 13% (Stoker et al., 2000), and Ashby and LeFevre (2000) reported that the day of PPS was dependent upon the BW at weaning, and not specifically to BW at the time of complete PPS. Consistent with these diet restriction studies, P-generation females in the high dietary dose group exhibited significantly reduced food consumption throughout premating, which is likely responsible for the observed decreases in BW and BWG in these dams and delayed PPS in their male offspring. By comparison, direct-acting anti-androgens do not inhibit food consumption or slow growth (Anderson et al., 1995), but they delay PPS and reduce the weights of the ventral prostate, seminal vesicle, epididymides, Cowper's glands, and levator ani plus bulbocavernosus muscles (Stoker, 2000b; Owens et al., 2006). Compounds that act as anti-androgens may also delay PPS via hypothalamic-pituitary or steroid hormone synthesis disruptions (for review see Stoker et al., 2000b), but in these cases, changes in testicular weights, testosterone and/or luteinizing hormone concentrations (not evaluated in the current study), and changes in accessory sex gland tissue weights would have accompanied the observed delay in PPS. Furthermore, anti-androgens such as vinclozolin, can decrease male pup anogenital distance (AGD) to an extent similar to female pups (Gray et al., 2001). None of these anti-androgenic changes were observed in the two-generation reproduction study or the rat developmental neurotoxicity study (Gilmore, 2012) conducted on Flupyradifurone.

These data strongly support the conclusion that the delay in PPS observed in F1-generation males after exposure with high dietary concentrations of Flupyradifurone is due to significant BW loss and reduced food consumption and BWG over the exposure period, and not due to an endocrine-mediated MOA.

Vaginal Patency

F1-generation females showed a slight, non-significant delay in VO (1.0 day) in animals in the 1800 ppm dietary concentration group. The mean for complete VO was 34.8 days for the control females and 35.8 days for the high dietary level females (Table 2, Figure 2). It should be noted, however, that the day means to complete VO were within the historical control range (33.4 – 36.7 days, see Appendix 1) for all dietary levels of Flupyradifurone. The F1-generation females achieved VO with significantly reduced BW and BWG (-16.6% and -19%, respectively, compared to controls) during lactation, and no other disruptions in estrogen sensitive endpoints (i.e. tissue weights, estrous cyclicity, histopathological analysis, mating parameters) were observed in the remainder of the study. Furthermore, no other estrogen sensitive parameters were affected by Flupyradifurone in the rat developmental neurotoxicity study (Gilmore, 2012), the developmental toxicity studies (Langrand-Lerche, 2010 ; Kennel, 2012), or the chronic toxicity/carcinogenicity studies (Garcin, 2012 ; Kennel, 2012). Therefore, the slight delay of 1 day in VO after high dietary exposure to Flupyradifurone is most likely the result of decreased BW and BWG during lactation, as well as decreased maternal BW and BWG throughout premating, gestation, and lactation. Delays in VO, concurrent with observations of delayed PPS in males, is understood to indicate a general growth delay, rather than an endocrine MOA (Stump et al., 2012). A slight, but statistical, decrease in the number of estrous cycles in the F1-generation was observed, but is also attributable to BW loss, as irregular cycles are often noted in the immediate post-pubertal period (Goldman et al., 1985). Taken together, these points indicate that the non-statistically significant delay VO is not endocrine-mediated and is of no biological significance.

Table 2: Mean Day to Criterion and Body Weight (g) at Vaginal Patency

	Dose levels in ppm			
	0	100	500	1800
Days to criterion				
Mean	34.8±0.78	34.1±0.48	34.4±0.43	35.8±0.60
N	34	35	35	35
BW at criterion				
Mean	113±3.5	106±2.2	102*±1.8	103*±2.0
N	34	35	35	35

*Statistically significant compared to control animals (p≤0.05).

Figure 2 : Mean Body Weight at Day of Complete Vaginal Patency

Organ Weight Changes in Pups

Organ weight changes in pups were observed in brain, thymus, and spleen in pups exposed to 1800 ppm Flupyradifurone. Similar to changes observed in PPS and VO, the pup organ weight changes were due to significant BW loss over the exposure period, and not due to an endocrine MOA. In F1-generation pups, birth weight was significantly decreased in both sexes, and an overall decrease in BWG throughout lactation was observed in F1- (-13.7%) and F2-generation (-13.8%) pups at the high dietary level. Pup terminal BWs were also decreased in males (-10%) and females (-12%). In both sexes and generations, absolute brain weights were decreased, while brain weight relative to BW was increased. A similar trend was observed thymus weights, while absolute spleen weights were decreased in F1 males and both sexes in the F2 generation.

Brain weights (see Appendix 4):

In F1 pups on PND21, a slight decrease in absolute brain weight was observed in males (-3.93%) and females (-3.3%) at 1800 ppm (only one male was outside the control range) and a slight increase in brain weight to body weight ratio was observed (+9.5% in males and +9.07% in females) due to lower body weight. Nine males from 7 litters and 12 females from 7 litters were outside the control range but they had lower body weight compared to control animals.

In F2 pups, a statistically significant decrease in absolute brain weight was observed in females only (-3%) with only one animal outside the control range. A statistically significant increase in brain weight to body weight ratio was observed in males (+11.5%) with 6 animals from the same litter outside the control range due to lower body weight and in females (+11.4%) with only one animal outside the control range.

Thymus weights (see Appendix 5):

F1 pups at 1800 ppm: Statistically significant thymus weight changes were observed in males for absolute weights (-11.3%, all animals within control range) and in females for thymus weight to body weight ratio (+6.8% with 3 animals outside the control range due to lower body weight).

F2 pups at 1800 ppm: A statistically significant decrease in absolute thymus weight in females (- 10.96%) was noted with 12 animals from 5 litters outside the control range due to lower body weight. No significant changes were observed in thymus weight to body weight ratio.

Spleen weight (See Appendix 6):

F1 pups at 1800 ppm: A statistically significant decrease in absolute spleen weight was observed in males only (-17.98%) with all animals within the control range and no significant effect on spleen weight to body weight ratio.

F2 pups at 1800 ppm: A statistically significant decrease in absolute spleen weight was observed in both males (-14.98%) with 6 animals from one litter outside the control range due to lower body weight and females (-15.48%) with 6 animals from 3 litters outside the control range due to lower body weight. No significant effect on spleen weight to body weight ratio in both sexes.

The organ weight changes and reduction in pup terminal BWs seen in the present study are consistent with changes in the brain, thymus, and spleen observed in a food restriction reproduction study in which animals were given 30% less food daily relative to control groups (Carney et al., 2004). Animals receiving 30% less food (equivalent to 6-19% BW decrease) showed similar reductions in absolute organ weights with concomitant increases in relative organ weights in both male and female rats ; animals receiving only 10% less food, which did not change BW, showed no organ weight changes compared to control groups (Carney et al., 2004). Animals receiving 30% less food exhibited BW reductions similar in magnitude to those found in animals exposed to the high dietary level of Flupyradifurone in the two-generation reproduction study. These data support the notion that significant reductions in BW can influence organ weights. In addition, no microscopic changes were observed in the brain, thymus, or spleen in animals of either sex in the Flupyradifurone two-generation reproduction study. The rat DNT study (Gilmore, 2012) and rat subchronic neurotoxicity study (Garcin, 2011) conducted with Flupyradifurone did not produce any brain weight changes up to the high dietary levels of 1200 ppm and 2500 ppm, respectively. In both studies, BW and BWG declines were not as severe in magnitude as those seen in the two-generation reproduction study, supporting the conclusion that the organ weight changes observed in the reproduction study are likely due to reductions in BW and BWG. In a rat immunotoxicity study conducted with Flupyradifurone (Repetto, 2011), significant decreases in the absolute weight of the spleen and thymus were observed, but relative weights were unchanged for both organs. These results are consistent with other repeat-dose dietary toxicity studies conducted with Flupyradifurone and support the conclusion that the observed organ weight changes in pups are likely due to general systemic toxicity and decreased BW and BWG of the animals. The lack of response in endocrine-sensitive tissues in both sexes further supports these organ weight changes are due to significant decreases in BW and not an endocrine-mediated event.

Reduced Number of Implantation Sites

A slight, albeit significant, decrease in median implantation sites (and thus decrease in litter size) in the F1-generation was observed with 1800 ppm Flupyradifurone. This effect, however, was not observed in the P0-generation dams (Table 3), and the total number of implantation sites between the P0- and F1-generations in the control (311 and 305, P0 and F1, respectively) and high dose (289 and 281, P0 and F1, respectively) are similar. This slight decrease is likely due to the decreased BW in the P0 females, which was observed as early as day 7 after initiation of exposure. Significant BW decreases were maintained for the duration of the study, including the F1 females at birth, and continuing through mating and gestation of the F2 animals (see Appendix 3). Exposure to exogenous chemicals that produce a reduction in the amount of BW gained during the first 9 days of pregnancy is an indication of the inherent toxicity of the chemical and may contribute to maternal toxicity and pregnancy loss (Cummings et al., 2000). No other effects were noted in female organ weights or histopathology in the two-generation reproduction study. Developmental toxicity studies in rats (Langrand-Lerche, 2010) and rabbits (Kennel, 2012) for Flupyradifurone did not show any significant decreases in the number of implantation sites in dams that showed a significant decrease in BW, nor in any other changes in reproductive indices, tissue weights, or histopathology. Taken together, the data supports that this finding was due to a decreased BW and not an endocrine-mediated MOA.

Table 3: Total Number of Implantation Sites Across Generations and Dietary Levels of Flupyradifurone

Observations	Dose levels in ppm			
	0	100	500	1800
	Po-generation			
Total number of implantation sites (Median)	311 (11.0)	285 (11.0)	298 (10.5)	289 (10.0)
	Fi-generation			
Total number of implantation sites (Median)	305 (12.0)	314 (11.0)	323 (11.0)	281 (10.0**)

Statistically different from control, $p < 0.01$

Summary and Conclusion

The two-generation reproduction study conducted in rats with Flupyradifurone reported changes in a few endpoints that are known to be influenced by endocrine MOAs. In light of this, EFSA questioned whether observed delays in PPS and VO, pup organ weight changes, and reductions in implantation sites observed at the highest dietary level of 1800 ppm might be endocrine mediated. Thorough evaluation of the available data on Flupyradifone and the peer-reviewed literature confirmed the initial position of BCS and the performing laboratory that these changes were secondary to decreases in BW and BWG. Several publications reporting studies that utilized food restriction to evaluate the effect of reduced BW and BWG on developmental and reproductive endpoints support this position. The mammalian toxicity database for Flupyradifurone does not indicate any anti-androgenic or anti-estrogenic activity, two known endocrine-mediated MOAs. Thus, the changes seen in the endpoints in question appear to occur secondary to

overt toxicity associated with exposure to Flupyradifurone as evidenced by reductions in BW and BWG throughout all stages, including critical periods of development, of the two-generation reproduction study.

Appendix 1: Historical Control of Preputial Separation and Vaginal Patency for the Wistar Han Rat (Days)

*Study #	Vehicle/Excipient (Route)	Study Conduct (Start of exposure – termination of in	Preputial Separation		Vagina 1 Patency	
			FI	F2	FI	F2
03-R72-PZ	Ethanol (Dietary)	6/03 - 4/04	40.7	40.7	33.4	33.6
03-R72-PT	Acetone (Dietary)	4/03 - 3/04	41.8	41.6	33.7	35.3
03-P72-SI	Dry Mix (Dietary)	9/03 - 3/04	42.3	-	33.8	-
04-R72-SJ	Dry Mix (Dietary)	4/04 - 2/05	42.4	-	35.1	-
06-R72-DI	Dry Mix (Dietary)	1/06 - 10/06	43.8	-	35.5	-
06-R72-DX	Acetone (Dietary)	3/06 - 12/06	41.0	-	34.8	-
06-R72-GY	Acetone (Dietary)	10/06 - 7/07	42.0	-	34.3	-
06-R72-HW	Dry Mix (Dietary)	11/06 - 8/07	42.6	-	34.0	-
07-R72-IH	Dry Mix (Dietary)	1/07 - 12/07	42.5	43.2	34.1	34.4
07-R72-MK	Acetone (Dietary)	11/07 - 10/08	43.5	44.0	35.5	36.7
08-R72-MQ	Dry Mix (Dietary)	1/08 - 10/08	44.3	-	36.0	-
08-R72-MX	Acetone (Dietary)	2/08 - 10/08	43.6	-	35.4	-
08-R72-OB	Acetone (Dietary)	7/08 - 4/09	44.4	-	34.8	-
Range			**40.7 - 44.4**		**33.4 - 36.7**	

Not applicable, either a one-generation study or only performed in the first generation. = All animals supplied by Charles River, Raleigh, NC.

Appendix 2: Mean (± Standard Error) pup weights (g) with percentage compared to control group mean

Males								
Lactation Day	FI-generation				F2-generation			
	Dose levels in ppm				Dose levels in ppm			
	0	100	500	1800	0	100	500	1800
0	6.2±0.11	6.2±0.11 (100%)	6.0±0.10 (100%)	5.7**±0.12 (92%)	6.2±0.10	6.0±0.11 (97%)	6.0±0.11 (97%)	6.0±0.08 (97%)
4[a]	10.4±0.24	10.4±0.24 (100%)	9.9±0.28 (95%)	9.4*±0.29 (90%)	10.3±0.23	9.7±0.23 (94%)	9.8±0.26 (95%)	9.8±0.22 (95%)
4b	10.4±0.25	10.4±0.24 (100%)	9.9±0.28 (95%)	9.4*±0.29 (90%)	10.3±0.23	9.7±0.22 (94%)	9.8±0.26 (95%)	9.8±0.21 (95%)
7	16.2±0.30	16.3±0.34 (101%)	15.5±0.41 (96%)	14.4**±0.39 (89%)	16.3±0.33	15.8±0.29 (97%)	15.5±0.38 (95%)	15.0±0.40 (92%)
14	32.5±0.42	32.1±0.62 (99%)	30.8±0.70 (95%)	28.2**±0.53 (87%)	32.4±0.64	31.5±0.48 (97%)	30.3*±0.51 (94%)	28.4**±0.76 (88%)
21	49.8±0.59	49.3±0.96 (99%)	48.0±1.00 (98%)	43.5**±0.78 (87%)	49.6±0.80	48.1± 0.86 (97%)	45.9**±0.74 (93%)	43.8**±1.19 (88%)

Females

Lactation Day	Fl-generation				F2-generation			
	Dose levels in ppm				Dose levels in ppm			
	0	100	500	1800	0	100	500	1800
0	5.9±0.12	5.9±0.12 (100%)	5.7±0.11 (97%)	5.4*±0.11 (92%)	5.9±0.11	5.8±0.09 (98%)	5.7±0.10 (97%)	5.6±0.08 (95%)
4[a]	10.0±0.28	10.1±0.28 (101%)	9.7±0.29 (97%)	9.1±0.27 (99%)	9.9±0.24	9.6±0.21 (97%)	9.4±0.24 (95%)	9.5±0.25 (96%)
4b	10.0±0.29	10.1±0.28 (101%)	9.7±0.29 (97%)	9.1±0.26 (99%)	9.9±0.24	9.6±0.21 (97%)	9.4±0.24 (95%)	9.5±0.25 (96%)
7	15.8±0.36	15.8±0.39 (100%)	15.1±0.41 (96%)	14.0**±0.35 (89%)	15.7±0.34	15.4±0.22 (98%)	15.0±0.35 (95%)	14.4*±0.39 (92%)
14	31.9±0.52	31.3±0.60 (98%)	30.0±0.71 (94%)	27.7**±0.51 (87%)	31.8±0.57	30.9±0.40 (97%)	29.6*±0.52 (93%)	27.5**±0.76 (84%)
21	48.4±0.72	47.8±0.80 (99%)	46.1±1.05 (95%)	42.2**±0.70 (87%)	47.8±0.77	46.8±0.71 (98%)	44.4**±0.74 (93%)	41.8**±1.07 (87%)

a: Before standardization (culling) b : After standardization (culling)

* Statistically different from control, $p < 0.05$ ** Statistically different from control, $p < 0.01$

Appendix 3: Mean body weight and body weight gains (± Standard Error) during premating, gestation and lactation with percentage compared to control group

Premating:

Observations/Study Week	Dose levels of Flupyradifurone			
	0	100	500	1800
P-generation Males				
Mean body weight (g) – Week 14	436.0±7.81	436.0±5.96 (100%)	441.3±7.77 (101%)	438.4±7.54 (100%)
Mean weight gain (g)	200.8	201.9 (100%)	200.0 (100%)	198.0 (99%)
P-generation Females				
Mean body weight (g) – Week 14 SD	237.4±3.33	235.5±3.09 (99%)	229.0±2.92 (96%)	213.3**±2.73 (90%)
Mean weight gain (g)	63.4	61.8 (97%)	50.4 (79%)	36.3 (57%)
Fl-generation Males				
Mean body weight (g) – Week 14 SD	454.5±8.27	456.0±7.51 (100%)	452.3±8.28 (99%)	410.8**±7.53 (90%)
Mean weight gain (g)	152.3	152.0 (100%)	166.2 (109%)	145.1 (95%)
Fl-generation Females				
Mean body weight (g) – Week 14 SD	242.1±3.04	237.3±4.06 (98%)	224.2**±2.90 (93%)	203.5**±2.49 (84%)
Mean weight gain (g)	52.2	51.5 (99%)	43.7 (84%)	41.2 (79%)

**Statistically different from control, $p < 0.01$

Gestation:

Observations/Study Day	Dose levels of Flupyradifurone			
	0	100	500	1800

P-generation Females				
Mean body weight (g) – Day 0	238.l±4.27	235.2±3.25 (99%)	232.2±3.10 (98%)	214.8**±3.06 (90%)
Mean body weight (g) – Day 6	255.0±3.65	250.2±3.07 (98%)	248.6±3.22 (97%)	228.1**±3.25 (89%)
Mean body weight (g) – Day 13	276.0±3.54	272.3±3.57 (99%)	267.9±3.63 (97%)	247.1**±3.06 (89%)
Mean body weight (g) – Day 20	333.6±5.68	326.5±4.28 (98%)	322.l±5.41 (97%)	301.1**±4.43 (90%)
Mean weight gain (g) – Days 0-20	95.6±3.36	91.3±2.59 (96%)	89.9±3.74 (94%)	86.4±2.87 (90%)
Fl-generation Females				
Mean body weight (g) – Day 0	243.4±3.27	238.3±4.82 (98%)	224.4**±3.01 (92%)	202.6**±2.85 (83%)
Mean body weight (g) – Day 6	257.6±3.09	251.0±4.57 (97%)	238.7**±2.86 (93%)	214.0**±3.23 (83%)
Mean body weight (g) – Day 13	277.8±3.33	270.0±4.54 (97%)	258.3**±2.80 (93%)	230.8**±2.98 (83%)
Mean body weight (g) – Day 20	335.l±4.52	327.4±5.28 (98%)	313.8**±4.01 (94%)	277.2**±4.28 (83%)
Mean weight gain (g) – Days 0-20	91.8±2.59	89.l±3.50 (97%)	89.4±2.86 (97%)	74.7**±2.11 (81%)

Statistically different from control, $p < 0.01$

Lactation:

Observations/Study Day	Dose levels of Flup		yradifurone (in	
	0	100	ppm) 500	1800
P-generation Females				
Mean body weight (g) – Day 0	262.8±3.22	256.l±2.95 (97%)	250.8±4.30 (95%)	233.3**±3.24 (89%)
Mean body weight (g) – Day 4	271.3±3.12	271.6±3.36 (100%)	265.8±3.91 (98%)	245.7**±3.66 (91%)
Mean body weight (g) – Day 7	277.4±2.81	276.7±3.05 (100%)	271.2±3.90 (98%)	251.6**±3.20 (91%)
Mean body weight (g) – Day 14	292.6±3.81	292.3±2.77 (100%)	287.2±4.22 (98%)	267.1**±3.17 (91%)
Mean body weight (g) – Day 21	285.8±3.67	281.4±2.80 (98%)	280.l±3.59 (98%)	264.3**±3.15 (92%)
Fl-generation Females				
Mean body weight (g) – Day 0	263.0±3.38	254.5±4.53 (97%)	242.5**±3.27 (92%)	218.4**±2.84 (83%)
Mean body weight (g) – Day 4	277.3±3.24	265.9±4.13 (96%)	256.0**±3.51 (92%)	225.1**±3.69 (81%)
Mean body weight (g) – Day 7	278.7±3.23	268.5±4.02 (94%)	260.9**±3.43 (94%)	231.3**±3.02 (83%)
Mean body weight (g) – Day 14	294.8±3.54	282.8±3.64 (96%)	272.2**±3.72 (92%)	244.3**±3.53 (83%)
Mean body weight (g) – Day 21	283.8±3.45	278.5±3.91 (98%)	261.6**±4.17 (92%)	247.1**±3.22 (87%)

Statistically different from control, $p < 0.01$

Appendix 4: Statistically significant brain weight changes in pups on day 21

F1 pups				
Absolute Brain weights				
Groups	Sex	MeantS.D. (N)	range	Comments
Control	Males	1.525 ± 0.0128 (28)	[1.312 - 1.721]	
	Females	1.475 ± 0.0118 (27)	[1.278 - 1.605]	
1800 ppm	Males	1.465** ± 0.0130 (25)	[1.312 - 1.735]	1 animal outside the control range
	Females	1.426* ± 0.0114 (27)	[1.315 - 1.587]	All animals within the control range
Brain weight to body weight ratio				
Control	Males	3.089 ± 0.0364 (28)	[2.557 - 3.665]	
	Females	3.088 ± 0.0462 (27)	[2.485 - 3.764]	
1800 ppm	Males	3.383** ± 0.0449 (25)	[2.808 - 3.906]	9 animals outside the control range (from 7 litters) due to lower body weight
	Females	3.396** ± 0.0549 (27)	[2.818 - 4.247]	12 animals outside the control range (from 7 litters) due to lower body weight
F2 pups				
Absolute Brain weight				
Groups	Sex	Mean ±S.D. (N)	range	Comments
Control	Males	1.505 ± 0.0085 (27)	[1.370 - 1.683]	
	Females	1.469 ± 0.0092 (27)	[1.264 - 1.594]	
1800 ppm	Males	1.474 ± 0.0115 (29)	[1.295 - 1.613]	8 animals outside the control range (from 4 litters) due to lower body weight
	Females	1.425* ± 0.0116 (29)	[1.256 - 1.576]	1 animal outside the control range
Brain weight to body weight ratio				
Control	Males	3.073 ±0.0476 (27)	[2.597 - 4.376]	
	Females	3.114 ± 0.0629 (27)	[2.490 - 6.117]	
1800 ppm	Males	3.427** ± 0.0867 (29)	[2.656 - 5.283]	6 animals outside the control range from the same litter due to lower body weight
	Females	3.468** ± 0.0773 (29)	[2.471 - 4.812]	1 animal outside the control range

Appendix 5: Statistically significant thymus weight changes in pups on day 21

F1 pups				
Absolute Thymus weight				
Groups	Sex	MeantS.D. (N)	range	Comments
Control	Males	0.222 ± 0.0057 (28)	[0.135 - 0.296]	
	Females	0.219 ± 0.0052 (27)	[0.113 - 0.298]	
1800 ppm	Males	0.197* ± 0.0064 (25)	[0.160 - 0.294]	All animals within the control range
	Females	0.206 ± 0.0056 (27)	[0.150 - 0.299]	1 animal outside the control range
Thymus weight to body weight ratio				
Groups	Sex	Mean ±S.D.(N)	range	Comments
Control	Males	0.448 ± 0.0103 (28)	[0.340 - 0.556]	

	Females	0.455 ± 0.0090 (27)	[0.314 - 0.582]	
1800 ppm	Males	0.452 ± 0.0106 (25)	[0.346 - 0.593]	3 animals outside the control range due to lower body weight
	Females	0.486* ± 0.0090 (27)	[0.333 - 0.669]	3 animals outside the control range due to lower body weight
F2 pups				
Absolute Thymus weight				
Groups	Sex	Mean ±S.D. (N)	range	Comments
Control	Females	0.219 ± 0.0054 (27)	[0.149 - 0.320]	
1800 ppm	Females	0.195** ± 0.0056 (29)	[0.114 - 0.250]	12 animals outside the control range from 5 litters dam due to lower body weight – No significant effects on thymus weight to body weight ratio

Appendix 6: Statistically significant spleen weight changes in pups on day 21

F1 pups				
Absolute Spleen weight				
Groups	Sex	MeantS.D. (N)	range	Comments
Control	Males	0.228 ± 0.0082 (28)	[0.061 - 0.348]	
1800 ppm	Males	0.187** ± 0.0069 (25)	[0.092 - 0.325]	All animals within the control range
Spleen weight to body weight ratio				
Control	Males	0.456 ± 0.128 (28)	[0.170 - 0.684]	
1800 ppm	Males	0.428 ± 0.0121 (25)	[0.337 - 0.661]	All animals within the control range
F2 pups				
Absolute Spleen weight				
Groups	Sex	Mean ±S.D.(N)	range	Comments
Control	Males	0.227 ± 0.0062 (27)	[0.118 - 0.361]	
	Females	0.226 ± 0.0065 (27)	[0.126 - 0.320]	
1800 ppm	Males	0.193** ± 0.0082 (29)	[0.070 - 0.327]	6 animals outside the control range from one litter due to lower body weight - No significant effects on spleen weight to body weight ratio
	Females	0.191** ± 0.0088 (29)	[0.074 - 0.348]	6 animals outside the control range from 3 litters due to lower body weight - No significant effects on spleen weight to body weight ratio

General Comment

Effects unrelated to the endocrine system observed after chemical treatment often confound the interpretation of results of endpoints evaluated in standard reproductive toxicology studies (Carney et al., 2004; Laws et al., 2007; Marty et al., 2007; Laws et al., 2000; Stoker et al., 2000a; for review see Stoker et al.,

2000b). For example, significant body weight loss, altered feed intake, and stress responses can contribute to changes in study endpoints that may be inaccurately attributed to an endocrine-mediated mechanism. Several peer-reviewed publications report studies that have been conducted utilizing food restriction, resulting in varying degrees of BW loss, to determine the influence of BW reduction on *in utero* and postnatal developmental endpoints (Carney et a., 2004; Chapin et al., 1993; Laws et al., 2000; Laws et al., 2007; Marty at al., 2007; Stoker et al., 2000a). Endocrine-related endpoints evaluated in these studies include PPS and sperm parameters in males, VO and estrous cyclicity in females, and organ weights and hormone concentrations in both sexes.

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Characteristics

reference	:	[redacted]	exposure	:	Throughout the entire in-life phase of the study P: 10 weeks before mating, 14 days mating, 22 days gestation, 21 days lactation F1: 3-4 weeks after weaning before mating, 14 days mating, 22 days gestation, 21 days lactation
type of study	:	Two-Generation Reproductive Toxicity Study	doses	:	Nominal: 0, 100, 500 and 1800 ppm Actual daily intake: See study design.
year of execution	:	2009/2011	vehicle	:	acetone
test substance	:	BYI 02960 (batch number 2009-000239; purity 96.2%)	GLP statement	:	Yes
route	:	Oral (diet)	guideline	:	OECD guideline 416
species	:	Rat, Wistar Crl:WI (Han)	acceptability	:	Acceptable
group size	:	30/sex/dose	**NOAEL**par toxicity	:	~~32 mg/kg bw/day~~. 6.4 ~~7.7~~ mg / kg bw/d
			NOAELpar reproductive effects	:	34 mg/kg bw/day
			NOAELoffspring	:	34 mg/kg bw/day

Study design

The study was performed in accordance with OECD guideline 416.

The mean values of the actual substance intake from the intended test material dose levels for the P and the F1 generation are shown in Table 6.6.1-8.

Table 6.6.1-8. Mean values of the actual substance intake by parental animals during premating, gestation and lactation

Phase of Study	100 ppm in mg/kg/day[a]	500 ppm in mg/kg/day[a]	1800 ppm in mg/kg/day[a]
Premating (P-gen) - Male	6.6	32.5	117.4
Premating (F_1-gen) - Male	6.4	32.0	122.1
Mean P- and F_1-gen - Males	**6.5**	**32.3**	**119.8**
Premating (P-gen) - Female	7.7	38.7	137.0
Premating (F_1-gen) - Female	7.8	39.6	143.4
Mean P- and F_1-gen - Females	**7.8**	**39.2**	**140.2**
Gestation (P-gen) – Female	6.9	34.3	134.0
Gestation (F_1-gen) – Female	7.0	36.6	168.8
Mean Gestation P and F_1	**7.0**	**35.5**	**151.4**
Lactation (P-gen) - Female	7.8	37.4	140.4
Lactation (F_1-gen) - Female	7.7	42.2	160.3
Mean Lactation P and F_1	**7.8**	**39.8**	**150.4**

[a] Individual values were based on the means for each particular phase

Results

The results of the study are summarised in table 6.6.1-9. Additionally, data on the mean body weight and food consumption during premating, gestation and lactation, estrous cycle, sperm measures, reproductive performance, litter parameters and pup weights are presented in tables 6.6.1-10 – 6.6.1-18.

Table 6.6.1-9.

Dose (mg/kg bw/day)	**0**		**100**		**500**		**1800**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
F0 animals									
Mortality	No substance-related mortality								
Clinical signs	No substance-related findings								
Body weight								ds (pm, g, l)	
Body weight gain						d (-21%) (pm)		d (pm, g)	
Food consumption								ds (pm), i (g)	
Mating/fertility/gestation	No substance-related findings								
Oestrus cycle	No substance-related findings								
Sperm evaluation	No substance-related findings								
Organ weight -liver -thyroid							i (a/r) i (a)		
Pathology Macroscopy	No substance-related findings								
Microscopy - centrilobular hypertrophy							i		
F1 pups									
Litter size	No substance-related findings								
Viability index	No substance-related findings								
Lactation index	No substance-related findings								
Sex ratio	No substance-related findings								
Clinical signs	No substance-related findings								
Body weight - birth - day 7 - day 14 - day 21							ds ds ds ds	ds ds ds ds	

Dose (mg/kg bw/day)	0		100		500		1800		dr
	m	f	m	f	m	f	m	f	
Physical development - delay in prepuital separation - delay in vaginal patency								i i	
Organ weight	No substance-related findings								
Pathology Macroscopy	No substance-related findings								
F1 animals **Mortality**	No substance-related mortality								
Clinical signs	No substance-related findings								
Body weight **- premating** **- gestation** **- lactation**						ds ds ds	ds	ds ds ds	
Body weight gain **- premating** **- gestation** **- lactation**					ds (-21%)	d (-16%)	ds (-43%)	d (-21%) ds (-19%)	
Food consumption							is (11%)	ds (pm) is (g)	
Mating/fertility/gestation	No substance-related findings								
Number of implantation sites								ds	
Oestrus cycle **-** number								ds	
Sperm evaluation	No substance-related findings								
Organ weights	No substance-related findings								
Pathology Macroscopy	No substance-related findings								
Microscopy	No substance-related findings								
F2 pups **Litter size**							ds	ds	
Viability index	No substance-related findings								
Sex ratio	No substance-related findings								
Clinical signs	No substance-related findings								
Body weight - Birth - day 7 - day 14 - day 21					ds -6% ds -7%	ds -7% ds -7%	d -8% ds -12% ds -12%	ds -8% ds -14% ds -13%	

Dose (mg/kg bw/day)	0		100		500		1800		dr
	m	f	m	f	m	f	m	f	
Organ weight	No substance-related findings								
Pathology Macroscopy	No substance-related findings								

dr dose related
ds/is statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a/r absolute/relative organ weight

Table 6.6.1-10. Mean (S.E.) body weights and food consumption during premating (P and F1 animals)

Observations/Study Week	Dose Group			
	Control 0 ppm	LDT 100 ppm	MDT 500 ppm	HDT 1800 ppm
P-Generation Males				
Mean body weight (g) - Week 14 S.E.	436.0 7.81	436.0 5.96	441.3 7.77	438.4 7.54
Mean weight gain (g) Weeks 1–14	200.8	201.9	200.0	198.0
Mean food consumption (g/animal/day) Weeks 1–10	21.4	21.9	21.9	21.7
Mean food consumption (g/kg/day) Weeks 1–10	65.4	67.2	65.6	65.5
P-Generation Females - Pre-mating				
Mean body weight (g) - Week 10 S.E.	237.4 3.33	235.5 3.09	229.0 2.92	213.3** 2.73
Mean weight gain (g) Weeks 1–10	63.4	61.8	50.4	36.3
Mean food consumption (g/animal/day) Weeks 1–10	16.3	16.0	16.0	14.7**
Mean food consumption (g/kg/day) Weeks 1-10	78.5	77.6	78.1	76.4
F_1-Generation Males				
Mean body weight (g) – Week 14 S.E.	454.5 8.27	456.0 7.51	452.3 8.28	410.8** 7.53
Mean weight gain (g) Weeks 1–14	152.3	152.0	166.2	145.1
Mean food consumption (g/animal/day) Weeks 1–10	23.4	23.8	23.4	22.8
Mean food consumption (g/kg/day) Weeks 1–10	62.2	63.7	64.5	68.8**
F_1-Generation Females - Pre-mating				
Mean body weight (g) - Week 10 S.E.	242.1 3.04	237.3 4.06	224.2** 2.90	203.5** 2.49
Mean weight gain (g) Weeks 1–10	52.2	51.5	43.7	41.2
Mean food consumption (g/animal/day) Weeks 1–10	16.8	16.3	16.3	14.8**
Mean food consumption (g/kg/day) Weeks 1–10	77.7	76.8	79.8	80.7

[a]Data obtained from Tables 2, 3, and 4 in the study report. Food consumption data represents grand means.
**Statistically different from control, $p \leq 0.01$

Table 6.6.1-11 Mean (S.E.) body weights and food consumption during gestation (P animals)

Observations/Study Week	Dose Group			
	Control 0 ppm	LDT 100 ppm	MDT 500 ppm	HDT 1800 ppm
P- Generation Females - Gestation				
Mean body weight (g) - Day 0 S.E.	238.1 4.27	235.2 3.25	232.2 3.10	214.8** 3.06
Mean body weight (g) - Day 6 S.E.	255.0 3.65	250.2 3.07	248.6 3.22	228.1** 3.25
Mean body weight (g) - Day 13 S.E.	276.0 3.54	272.3 3.57	267.9 3.63	247.1** 3.06
Mean body weight (g) - Day 20 S.E.	333.6 5.68	326.5 4.28	322.1 5.41	301.1** 4.43
Mean weight gain (g) - Days 0–20 S.E.	95.6 3.63	91.3 2.59	89.9 3.74	86.4 2.87
Mean food consumption (g/animal/day) Days 0–20	18.1	17.9	17.9	17.5
Mean food consumption (g/kg/day) Days 0–20	70.6	70.9	71.6	76.2

[a]Data obtained from Tables 8, 9, and 10 in the study report. Food consumption data represents grand means.
** Statistically different from control, $p \leq 0.01$

Table 6.6.1-12 Mean (S.E.) body weights and food consumption during gestation (F1 animals)

Observations/Study Week	Dose Group			
	Control 0 ppm	LDT 100 ppm	MDT 500 ppm	HDT 1800 ppm
F_1-Generation Females - Gestation				
Mean body weight (g) - Day 0 S.E.	243.4 3.27	238.3 4.82	224.4** 3.01	202.6** 2.85
Mean body weight (g) - Day 6 S.E.	257.6 3.09	251.0 4.57	238.7** 2.86	214.0** 3.23
Mean body weight (g) - Day 13 S.E.	277.8 3.33	270.0 4.54	258.3** 2.80	230.8** 2.98
Mean body weight (g) - Day 20 S.E.	335.1 4.52	327.4 5.28	313.8** 4.01	277.2** 4.28
Mean weight gain (g) - Days 0–20 S.E.	91.8 2.59	89.1 3.50	89.4 2.86	74.7** 2.11
Mean food consumption (g/animal/day) Days 0–20	18.2	17.6	17.8	19.0
Mean food consumption (g/kg/day) Days 0–20	70.2	69.7	74.3	89.3**

[a]Data obtained from Tables 8, 9, and 10 in the study report.. Food consumption data represents grand means.
* Statistically different from control, $p \leq 0.05$; ** Statistically different from control, $p \leq 0.01$

Table 6.6.1-13 Mean (S.E.) body weights and food consumption during lactation (P and F1 animals)

Observations/Study Week	Dose Group			
	Control 0 ppm	LDT 100 ppm	MDT 500 ppm	HDT 1800 ppm
P-Generation Females - Lactation				
Mean body weight (g) - Day 0 S.E.	262.8 3.22	256.1 2.95	250.8 4.30	233.3** 3.24
Mean body weight (g) - Day 4 S.E.	271.3 3.12	271.6 3.36	265.8 3.91	245.7** 3.66
Mean body weight (g) - Day 7 S.E.	277.4 2.81	276.7 3.05	271.2 3.90	251.6** 3.20
Mean body weight (g) - Day 14 S.E.	292.6 3.81	292.3 2.77	287.2 4.22	267.1** 3.17
Mean body weight (g) - Day 21 S.E.	285.8 3.67	281.4 2.80	280.1 3.59	264.3** 3.15
Mean food consumption (g/animal/day) Days 0–21	41.5	43.1	41.6	40.2
Mean food consumption (g/kg/day) Days 0–21	149.2	155.9	153.3	159.8
F_1-Generation Females - Lactation				
Mean body weight (g) - Day 0 S.E.	263.0 3.38	254.5 4.53	242.5** 3.27	218.4** 2.84
Mean body weight (g) - Day 4 S.E.	277.3 3.24	265.9 4.13	256.0** 3.51	225.1** 3.69
Mean body weight (g) - Day 7 S.E.	278.7 3.23	268.5 4.02	260.9** 3.43	231.3** 3.02
Mean body weight (g) - Day 14 S.E.	294.8 3.54	282.8 3.64	272.2** 3.72	244.3** 3.53
Mean body weight (g) - Day 21 S.E.	283.8 3.45	278.5 3.91	261.6** 4.17	247.1** 3.22
Mean food consumption (g/animal/day) Days 0–21	45.3	42.5	44.0	40.1
Mean food consumption (g/kg/day) Days 0–21	162.0	158.5	169.4	173.2

[a]Data obtained from Tables 13, 14, and 15 in the study report. Food consumption data represents grand means.
* Statistically different from control, $p \leq 0.05$; ** Statistically different from control, $p \leq 0.01$

Table 6.6.1-14 Estrous Cycle Length and Periodicity

Observation	Dose Group (ppm)			
	Control 0 ppm	LDT 100 ppm	MDT 500 ppm	HDT 1800 ppm
P-Generation				
Number of Estrous Cycles (S.E.)	3.5 (0.1)	3.7 (0.1)	3.4 (0.2)	3.4 (0.1)
Estrous Cycle Length (S.E.)	4.4 (0.3)	4.3 (0.1)	4.3 (0.2)	4.3 (0.1)
F_1-Generation				
Number of Estrous Cycles (S.E.)	3.5 (0.2)	3.3 (0.2)	3.3 (0.2)	2.9* (0.2)
Estrous Cycle Length (S.E.)	4.0 (0.2)	4.1 (0.1)	4.4 (0.2)	4.4 (0.1)

* Statistically different from control, $p \leq 0.05$ Data taken from Table 6 in the study report.

Table 6.6.1-15 Sperm measures

Sperm Analysis		Dose Group (ppm)			
		Control 0 ppm	LDT 100 ppm	MDT 500 ppm	HDT 1800 ppm
P Generation Males					
Sperm Motility	% Motile	92.4	90.9	93.3	90.7
	% Progressive	62.1	60.7	63.3	59.2
Sperm Counts (sperm/gram)	Testis	26.9	N/A	N/A	23.7
	Epididymis	158.8	N/A	N/A	138.7
Sperm Morphology (mean total number)	Normal	193.2	N/A	N/A	197.0
	Abnormal	2.7	N/A	N/A	2.2
	Detached Head	4.1	N/A	N/A	0.8
F_1-Generation Males					
Sperm Motility	% Motile	90.7	94.5	92.0	92.7
	% Progressive	64.0	66.4	64.8	63.3
Sperm Counts (sperm/gram)	Testis	29.4	N/A	N/A	30.9
	Epididymis	158.8	N/A	N/A	141.8
Sperm Morphology (mean total number)	Normal	189.1	N/A	N/A	195.2
	Abnormal	3.8	N/A	N/A	4.2
	Detached Head	0.4	N/A	N/A	0.6

a Data obtained from Table 24 in the study report N/A = Not Applicable

Table 6.6.1-16. Reproductive performance

Observation	Dose Group (ppm)			
	Control 0 ppm	LDT 100 ppm	MDT 500 ppm	HDT 1800 ppm
P-Generation – F_1-Offspring				
Number Cohoused	30	30	30	30
Number Mated	30	29	29	30
Number of Animals Delivered	29	27	28	28
Number of Animals with Implants	29	27	28	28
Mating Index	100.0	96.7	96.7	100.0
Fertility Index	96.7	93.1	96.6	93.3
Gestation Index	100.0	100.0	100.0	100.0
Mean Number Days to Insemination (S.E.) Median	3.4 (0.67) 3.0	3.3 (0.59) 3.0	3.2 (0.52) 3.0	3.1 (0.59) 3.0
Mean Gestation Length (days) (S.E.) Median Gestation Length (days)	22.1 (0.11) 22.0	22.1 (0.12) 22.0	22.1 (0.12) 22.0	22.0 (0.12) 22.0
Total number of implantation sites (Median)	311 (11.0)	285 (11.0)	298 (10.5)	289 (10.0)
F_1-Generation – F_2-Offspring				
Number Cohoused	30	30	30	30
Number Mated	29	30	29	30
Number of Animals Delivered	27	28	28	29
Number of Animals with Implants	27	28	28	29
Mating Index	96.7	100.0	96.7	100.0
Fertility Index	93.1	93.3	96.6	96.7
Gestation Index	100.0	100.0	100.0	100.0
Mean Number Days to Insemination (S.E.) Median	2.3 (0.23) 2.0	3.7 (0.45) 4.0**	2.6 (0.24) 2.5	2.1 (0.18) 2.0
Mean Gestation Length (days) (S.E.) Median Gestation Length (days)	22.0 (0.09) 22.0	22.1 (0.15) 22.0	21.9 (0.09) 22.0	22.0 (0.08) 22.0
Total number of implantation sites (Median)	305 (12.0)	314 (11.0)	323 (11.0)	281 (10.0**)

[a]Data obtained from Table 6 in the study report ** Statistically different from control, $p \leq 0.01$

Table 6.6.1-17 Litter parameters

Observation	Dose Group (ppm)			
	Control 0 ppm	LDT 100 ppm	MDT 500 ppm	HDT 1800 ppm
P Generation				
Total number of pups born	297	277	282	281
Number stillborn	0	2	0	0
Sex Ratio Day 0 (% male)	55.1	53.0	54.7	49.6
Mean litter size Day 0 Median	10.2 11.0	10.3 11.0	10.1 10.0	10.0 10.0
Birth index	95.0	97.1	93.3	97.4
Live birth index	100.0	99.0	100.0	100.0
Viability index	98.0	99.3	99.7	98.1
Lactation index	99.1	99.5	100.0	99.1
F_1-Generation				
Total number of pups born	291	300	311	266
Number stillborn	1	0	1	4
Sex Ratio Day 0 (% male)	51.6	50.6	50.6	47.7
Mean litter size Day 0 Median	10.8 11.0	10.7 11.0	11.1 10.5	9.2 **10.0***
Birth index	95.6	92.7	96.4	94.8
Live birth index	99.7	99.7	99.2	98.2
Viability index	99.3	99.7	99.4	97.2
Lactation index	99.1	100.0	100.0	98.7

[a] Data obtained from Table 6 and 20 in the study report * Statistically different from control, $p \leq 0.05$

Table 6.6.1-18 Mean (S.E.) combined male and female pup weights (g)

F_1-Generation					F_2-Generation				
Lactation Day	Control 0 ppm	LDT 100 ppm	MDT 500 ppm	HDT 1800 ppm	Lactation Day	Control 0 ppm	LDT 100 ppm	MDT 500 ppm	HDT 1800 ppm
0	6.1	6.0	5.9	5.6**	**0**	6.1	6.0	5.9	5.8
S.E	0.11	0.11	0.11	0.11	**S.E**	0.10	0.10	0.10	0.08
4[b]	10.2	10.3	9.8	9.3*	**4[b]**	10.1	9.8	9.6	9.6
S.E.	0.26	0.25	0.28	0.27	**S.E.**	0.23	0.21	0.25	0.22
4[c]	10.3	10.3	9.8	9.3*	**4[c]**	10.1	9.7	9.7	9.7
S.E.	0.26	0.25	0.28	0.26	**S.E.**	0.23	0.21	0.25	0.22
7	16.1	16.1	15.3	14.2**	**7**	16.0	15.6	15.3	14.7*
S.E.	0.32	0.35	0.40	0.36	**S.E.**	0.32	0.23	0.36	0.38
14	32.3	31.9	30.6	28.0**	**14**	32.1	31.2	29.9*	27.9**
S.E.	0.46	0.61	0.70	0.49	**S.E.**	0.60	0.41	0.51	0.75
21	49.3	48.9	47.3	42.8**	**21**	48.8	47.4	45.2**	42.7**
S.E.	0.63	0.88	1.00	0.69	**S.E.**	0.77	0.72	0.72	1.10
GAIN[d]	43.2	42.9	41.4	37.3**	**GAIN[d]**	42.7	41.5	39.4**	36.8**

[a] Data obtained from Table 18 in the study report [b] Before standardization (culling) [c] After standardization (culling)
[d] GAIN taken from Table 20 in the study report * Statistically different from control, $p \leq 0.05$ ** Statistically different from control, $p \leq 0.01$

Parental animals (P and F1 adults)

At the highest dose level, statistically significant decreases in body weight were observed in parental P and F1 females during premating, gestation and lactation. In F1 males, at the highest dose level significant decreases in body weight were observed beginning on Day 0 and continued throughout exposure. Statistically significant decreases in body weight during premating, gestation and lactation were observed also in F1 females at the mid-dose level however the weight loss was less than 10%.

Body weight gains were decreased in P and F1 females during premating and gestation in the high-dose groups, and non significant during pre-mating in the mid-dose group (21% for P-generation females and 16% for F1 generation females). Also increased food consumption was observed in the high-dose F1 females during gestation.

In the high-dose P males, increased absolute and relative liver weight (9% relative to controls) and increased absolute thyroid weights (13-21% relative to controls) were observed. Minimal centrilobular hypertrophy of the liver was observed in the high-dose P males, correlated with the increased liver weights. No other organ weight changes and microscopic changes were noted at any dose levels in both P and F1 generations.

No adverse effects on reproductive performance, sperm parameters or oestrous cycle were noted in P generation at any dose level. In the F1 generation, a significant decrease in the number of oestrous cycles was observed in the high-dose females (see Table 2.6). This finding paralleled the significant weight loss observed in these females. A statistically significant decline in the total number of implantation sites was also observed in the high-dose F1 females (see Table 2.8). No clear dose-response was obvious. These findings are considered to be secondary to the maternal toxicity, as evidenced from the significant weight loss, rather than true adverse reprotoxic effects.

Off-spring (F1and F2 pups)

No effects on the litter size, pup viability, sex ratio and lactation index were observed in F1 pups at any dose level. A slight decrease in the litter size was noted in the high-dose F2 pups (see Table 2.9). This decrease paralleled the reduced body weight gain during gestation and a decreased number of implantation sites in the high-dose F1 females. There were no substance-related effects on the pup viability, sex ratio and lactation index.

A significant decrease in birth weight was observed (8.2% compared to the controls) in the F1 pups of the highest dose level. This decrease continued and increased by magnitude by post-natal day (PND) 21. A significant delay in preputial separation and a slight nonstatistical delay in vaginal patency were observed in parallel with the decreased pup weight exhibited during lactation in the same dose group. Reduced absolute and relative brain (statistically significant), thymus (statistically significant only in males) and spleen weights (statistically significant) were observed in high-dose F1 pups, but were

considered to be secondary to the decreased body weights at the same dose level and not a direct test substance effect.

The body weights at birth were comparable to controls in the F2 pups at all dose levels. Pup weight declines were observed beginning on PND 7 (declined 8.1%) and continued to Day 21 (declined 12.5%) at the high-dose level and on PND 14 to Day 21 at the mid-dose level. No effect on anogenital distance was observed in the F2-generation pups at any dose level. Reduced absolute and relative brain (statistically significant only in females), thymus and spleen weights (statistically significant) were observed in high-dose F2 pups, but were considered to be secondary to the decreased body weights at the same dose level and not a direct test substance effect. Significantly reduced thymus weight was also observed in mid-dose F2 female pups.

Acceptability

The study is considered acceptable.

Conclusions

A significant decrease in body weight, but <10% was observed in the mid-dose of the F1 females and a non significant decrease in body weight gain was found in the parental animals (21 % in females) and the F1 animals (16 % in females). Therefore the parental systemic LOAEL is considered 500 ppm (32 mg/kg bw/d) based on decreased body weight gain in females. The parental systemic NOAEL is 100 ppm (7.7 mg/kg bw/d). ~~Therefore the parental systemic LOAEL is considered 1800 ppm (117.4 mg BYI 02960/kg bw/day) based on increased liver and thyroid weights, and minimal centrilobular hypertrophy in males decreased body weight (>10%)(premating, gestation, and lactation), body weight gain (premating; P and F_1). The parental systemic NOAEL was 500 ppm (32.0 mg BYI 02960/kg bw/day).~~

The reproductive NOAEL was 500 ppm (34.3 mg BYI 02960/kg bw/day females) based on decreased estrous cycle number, litter size, and the number of implants observed in the F_1 generation at the highest dietary level tested (LOAEL = 1800 ppm (134.0 mg BYI 02960/kg bw/day in females).

A significant decrease in body weight, but <10% was observed in the mid-dose of the F2 pups. The offspring LOAEL is therefore 1800 ppm (134.0 mg BYI 02960/kg bw/day) based on maternal effects leading to secondary effects on pup weight, and physical development. The offspring NOAEL is 500 ppm (34.3 mg BYI 02960/kg bw/day).

Expert consulatation 2.8

With regard to the parental toxicity, the RMS proposed a revised NOAEL of 100 ppm (equivalent to 6.4 mg/kg bw per day), considering significant decrease in body weight (<10% observed in the mid-dose of the F_1 females) and a non-significant decrease in body weight gain found in the parental animals (21% in females) and the F_1 animals (16% in females). This was agreed by the experts.

The reproductive NOAEL was set at 500 ppm (equivalent to 32 mg/kg bw per day) based on decreased oestrous cycle number, litter size, and the number of implantation sites observed in the F_1 generation. This was agreed by the experts.

The offspring NOAEL was set at 500 ppm based on significant decrease in pup weight in F_1 generation (21%). Considering the effect in the F_2 pups on body weight, the experts agreed on a NOAEL of 100 ppm for the pup development (reduced weight gain).

Some findings could be considered as endocrine-mediated, even though the notifier claimed that they were within historical control data. It was noted that historical control data were not fully appropriate and more weight should be given to the concurrent control data.

The experts agreed that there was not sufficient evidence demonstrating that the mode of action was not endocrine-mediated and set a data gap for level 2 studies according to OECD Conceptual Framework.

B.6.6.2 Teratogenicity studies

STUDY 1

Characteristics

reference	:	[redacted] 2010	exposure	:	Once daily, days 6-20 of gestation
type of study	:	Developmental toxicity study	doses	:	0, 15, 50 and 150 mg/kg bw/day
year of execution	:	2009/2010	vehicle	:	0.5% aqueous methylcellulose 400
test substance	:	BYI 02960 (batch number 2009-000239; purity 96.2%)	GLP statement	:	Yes
route	:	Oral (gavage)	guideline	:	OECD guideline 414
species	:	Rat, Sprague-Dawley Crl:CD (SD)	acceptability	:	Acceptable
group size	:	23 females/dose	**NOAEL**maternal toxicity	:	50 mg/kg bw/day
			NOAELdevelopmental toxicity	:	50 mg/kg bw/day

Study design

The study was performed in accordance with OECD guideline 414.

Results

The results of the study are summarised in Table 6.6.2-1. Additionally, information on maternal body weights, body weight gains, food consumption, reproductive performance, litter parameters and pup weights is presented in Tables 6.6.2-2 -6.6.2-4.

Table 6.6.2-1.

Dose (mg/kg bw/day)	**0**	**15**	**50**	**150**	**dr**
Maternal effects					
Mortality	No substance-related mortality				
Clinical signs					

Dose (mg/kg bw/day)	0	15	50	150	dr
- salivation				i	
Pregnant animals	22	22	22	23	
Body weight (gain)				d	
Food consumption			ds	ds	
Uterus weight	No substance-related findings				
Pathology - liver weight				is	
Macroscopy					
Litter response					
Number of dams examined	22	22	22	23	
Pregnancy rate	96%	96%	96%	100%	
Resorptions - early - late - litters with total resorption	 21 4 0	 28 2 0	 21 0 0	 30 1 0	
Corpora lutea/dam	No substance-related findings				
Dams with live foetuses	22	22	22	23	
Live foetuses/dam	13.8 ± 3.0	14.5 ± 2.2	14.2 ± 2.3	13.7 ± 2.5	
Foetal weight	No substance-related findings				
Post implantation loss (mean %)	7.9 ± 10.3	8.6 ± 8.4	6.5 ± 9.3	9.0 ± 8.3	
Sex ratio, % male	51.3 ± 12.3	52.9 ± 14.1	46.7 ± 15.4	51.7 ± 10.3	
Examination of the foetuses					
External observations	No substance-related findings				
Skeletal findings (incidence) -evaluated foetuses/litter -# parietal (uni/bi): incomplete ossification (number litter/fetuses affected)	 158/22 0/0	 165/22 2/6*	 161/22 0/0	 162/23 4/9**	
- # hyoid centrum: incomplete ossification (number litter/fetuses affected)	0/0	2/2	1/1	4/9**	
- # 7th cervical centrum; unossified (number litter/fetuses affected)	2/6	2/7	4/4	7/11	
- # 5th sternebra: uncomplete ossification	10/17	11/21	7/8*	12/24	
- 13th costal cartilage (uni): short	0/0	0/0	0/0	2/2	

Dose (mg/kg bw/day)	0	15	50	150	dr
Visceral findings	No substance-related findings				

dr dose related
ds/is statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a/r absolute/relative organ weight
* statistically significant (p ≤ 0.05)
** statistically significant (p ≤ 0.01)

Table 6.6.2-2. Mean (±SD) maternal body weight gain (g)

Interval	Dose level of BYI 02960 in mg/kg/day				HCD#
	0	15	50	150	
Number of dams (pregnant)	22	22	22	23	
Pretreatment, GD 0-6:	29.4 ± 8.08	25.3 ± 7.50	27.6 ± 5.53	28.5 ± 5.44	28.05 - 39.25
Treatment, GD 6-8:	5.9 ± 3.82	5.9 ± 2.78	3.0 ± 3.75	-5.7 ± 5.18**	4.05- 8.48
Treatment, GD 8-10:	9.1 ± 1.96	7.4 ± 2.28*	8.5 ± 2.56	6.9 ± 3.48*	7.78 - 11.30
Treatment, GD 10-14:	16.8 ± 3.84	17.1 ± 7.81	16.1 ± 5.22	19.5 ± 4.09	17.17 - 22.33
Treatment, GD 14-18:	41.0 ± 8.88	43.8 ± 7.06	44.9 ± 6.48	41.7 ± 7.64	41.48 - 48.21
Treatment, GD 18-21:	43.6 ± 9.61	44.9 ± 17.09	47.5 ± 7.59	45.7 ± 8.89	47.09 - 58.21
Treatment, GD 6-21	116.4 ± 18.77	119.1 ± 25.52	120.1 ± 16.94	108.2 ± 16.75	122.55 - 143.29
Corrected BW gain	44.6 ± 16.80	40.9 ± 18.89	44.0 ± 12.81	37.3 ± 8.89	49.56 – 75.43

a Data obtained from Table 4 in the Tables and Appendices section of the study report.
HCD: Historical control range (lowest – highest) from Att.3 in the Tables and Appendices section of the study report.
**: p≤0.01 *: p≤0.05

Table 6.6.2-3 Cesarean section observations

Observation	Dose Level of BYI 02960 (mg/kg/day)				HCD
	0	15	50	150	
Maternal data:[a]					
No. Animals assigned	23	23	23	23	NA
No. Animals pregnant	22	22	22	23	NA
Pregnancy rate, %	96	96	96	100	NA
No. Animals non-pregnant	1	1	1	0	NA
Maternal wastage					
No. died (total)	0	0	0	0	NA
No. died pregnant	0	0	0	0	NA
No. died non-pregnant	0	0	0	0	NA
No. premature delivery	0	0	0	0	NA
Uterine data:[b]					
Total No. corpora lutea[c]	370	380	361	377	NA
Corpora lutea / dam	16.8±2.0	17.3±2.4	16.4±1.7	16.4±2.2	15.74-17.83
Total No. implantations[c]	329	349	334	345	NA
Implantations / dam	15.0±2.8	15.9±1.8	15.2±1.8	15.0±2.4	14.38-16.04
Total No. litters[c]	22	22	22	23	
Total No. live fetuses[c]	304	319	313	314	NA
Live fetuses / dam[c]	13.8±3.0	14.5±2.2	14.2±2.3	13.7±2.5	13.52-15.04
Total No. dead fetuses	0	0	0	0	NA
Dead fetuses / dam[c], %	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0-0.30
Total No. early resorptions[c]	21	28	21	30	NA
Total No. late resorptions[c]	4	2	0	1	NA
Early resorptions / dam	1.0±1.1	1.3±1.3	1.0±1.3	1.3±1.3	0.474-2.042
Late resorptions / dam	0.2±0.4	0.1±0.3	0.0±0.0	0.0±0.2	0.0-0.217
Litters with total resorptions[c]	0	0	0	0	NA
Mean fetal weight, combined sexes, g	5.41±0.28	5.27±0.60	5.35±0.24	5.27±0.25	5.30-5.57
Mean fetal weight, males, g	5.54±0.28	5.36±0.60	5.46±0.29	5.40±0.29	5.45-5.70
Mean fetal weight, females, g	5.26±0.29	5.18±0.59	5.23±0.24	5.14±0.25	5.16-5.42
Sex ratio, % male	51.3±12.3	52.9±14.1	46.7±15.4	51.7±10.3	46.2-53.5
Preimplantation loss per litter, %	10.5±16.2	7.4±9.2	7.2±9.3	8.3±10.6	3.32-13.21
Postimplantation loss per litter, %	7.9±10.3	8.6±8.4	6.5±9.3	9.0±8.3	3.95-13.15

a Data obtained from Table 1 in the Tables and Appendices section of the study report.
b Data obtained from Table 8 and Appendix F in the Tables and Appendices section of the study report.
* Statistically different ($p \leq 0.05$) from the control.
** Statistically different ($p \leq 0.01$) from the control.
c Statistical analysis was not conducted on this endpoint
HCD Historical control range (lowest – highest) of main uterine parameters from Att 3 in the Tables and Appendices section of the study report
NA not applicable

Table 6.6.2-4. Skeletal examinations

Dose level of BYI 02960 (mg/kg/day)	0	15	50	150	Historical Control Range	0	15	50	150	Historical Control Range
	Number of litters examined					Number of fetuses examined				
	22	22	22	23		158	165	161	162	
OBSERVATIONS	Number of litters affected (% of litters affected)					Number of fetuses affected (% of fetuses affected)				
Variations # Parietal (uni/bi) : incomplete ossification.	0 (0.0)	2 (9.1)	0 (0.0)	4 (17.4)	(0.0 – 9.1)	0 (0.0)	6 (3.6)*	0 (0.0)	9 (5.6)**	(0.0 – 1.3)
# Hyoid centrum : incomplete ossification.	0 (0.0)	2 (9.1)	1 (4.5)	4 (17.4)	(0.0 – 12.5)	0 (0.0)	2 (1.2)	1 (0.6)	9 (5.6)**	(0.0 – 1.9)
# 7th cervical centrum : unossified.	2 (9.1)	2 (9.1)	4 (18.2)	7 (30.4)	(0.0 – 41.7)	6 (3.8)	7 (4.2)	4 (2.5)	11 (6.8)	(0.0 – 12.2)
# 5th sternebra: incomplete ossification.	10 (45.5)	11 (50.0)	7 (31.8)	12 (52.2)	(19.0 – 70.8)	17 (10.8)	21 (12.7)	8 (5.0)*	24 (14.8)	(3.1 – 19.9)
13th costal cartilage (uni) : short.	0 (0.0)	0 (0.0)	0 (0.0)	2 (8.7)	(0.0 – 0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.2)	(0.0 – 0.0)

a Data obtained from Table 12 in the Tables and Appendices section of the study report.
 # : Statistical analysis was conducted on this observation (App M)
*** Statistically different (p≤ 0.05) from the control.**
**** Statistically different (p≤ 0.01) from the control.**

There were no mortalities during the study. At 150 mg/kg bw/day, increased salivation was noted in 20/23 animals on one or several occasions between gestation days (GD) 11 and 21 and soiling around the mouth was observed in 1/23 females on GD 21.

In the high-dose females a mean maternal body weight gain and the maternal corrected body weight change were decreased between GD 6-21 (not statistically significant). The mean body weight changes were significantly lower than controls by 14 to 91% on all intervals between GD 6 and GD 18 (p ≤ 0.01). The food consumption was significantly decreased between GD 6 and 12. At 50 mg/kg bw/day the mean maternal body weight gain was reduced by 49% between GD 6-8, and the mean maternal body weight change was significantly lower than controls by 23% between GD 6 and GD 10. The mean food consumption was reduced by 8% between GD 6-8 (p ≤ 0.05). No treatment-related body weight changes were observed in the low-dose group.

At 150 mg/kg bw/day the mean absolute liver weight was 13% higher than in the controls (p ≤ 0.01). No other treatment-related findings were observed at the macroscopic examination at any dose level.

The pregnancy rate was at least 96% in all dose groups and was unaffected by treatment. The number of live fetuses, the number of implant sites per dam, the percentages of pre and post

implantation losses, the number of early and late resorptions, the fetal death status and the percentage of male fetuses were unaffected by treatment in any dose group.

At 150 mg/kg/day, mean fetal body weights for combined sexes and females were reduced by 2 to 3%, compared to the controls (not statistically significant). No other treatment-related changes on fetal body weights were noted.

There were no treatment-related malformations or variations noted at the fetal external examination and fetal visceral examination. At 150 mg/kg bw/day, the incidence of parietal and hyoid centrum incomplete ossification were statistically significantly higher than in the control group ($p \leq 0.01$). No clear dose-response was obvious. The incidence of unossified 7th cervical centrum and 5th sternebra incomplete ossification were also higher in 150 mg/kg bw/day group than in the controls, but the incidence was not statistically significant and the observed values within the in-house historical control data. The incidence of the short 13th costal cartilage was also higher in the 150 mg/kg bw/day group than in the controls and was outside the in-house historical control data, but as the observation was noted unilaterally only and the incidence was low, the finding was considered to be not treatment-related.

The historical control data were generated from fifteen in-house Sprague-Dawley Crl:CD (SD) rat studies conducted between 2001-2008.

Conclusions

Minimal maternal toxicity was observed at the high-dose level, manifested as decreased body weight gain and reduced food consumption. The mean absolute liver weight was increased in the high dose group. Based on these findings the NOAEL for maternal toxicity was set at 50 mg/kg bw/day.

The increased incidence of parietal incomplete ossification showed no dose response, the unossified cervical centrum and the incomplete ossification of the sternebra was within the historical background. A increase of a short costal cartilage was seen at the high-dose. Based on these findings, the NOAEL for developmental toxicity was set at 50 mg/kg bw/day.

STUDY 2

Characteristics

reference	:	[redacted] 2012	exposure	:	Gestation days (GD) 6-20
type of study	:	Complementary maternal tolerability study	doses	:	0, 20 and 30 mg/kg bw/day
year of execution	:	2011/2012	vehicle	:	0.5% aqueous methylcellulose 400
test substance	:	BYI 02960 (batch number 2009-000239; purity 96.2%)	GLP statement	:	Yes
route	:	Oral (gavage)	guideline	:	None (complementary tolerability study); however, the in-life portion of the study was conducted according to OECD guideline 414.
species	:	Rat, Sprague-Dawley Crl:CD (SD)	acceptability	:	Acceptable
group size	:	23/sex/dose	**NOAEL**maternal toxicity	: : :	30 mg/kg bw/day

Characteristics

The study was performed to provide additional information on the maternal tolerability of BYI 02960 and to determine more precisely the NOAEL for maternal toxicity. Groups of 23 sperm-positive female Sprague-Dawey rats were exposed to BYI 02960 by oral gavage from gestation day (GD) 6 to 20, the sperm-positive day being GD 0. The doses given were 0, 20 and 30 mg/kg bw/day in aqueous solution of 0.5% methylcellulose 400. Clinical observations were recorded daily. Maternal body weights were recorded for all females on GD 0, 6, 8, 10, 12, 14, 16, 18 and 21. Food consumption was also measured for all the females during the intervals GD 1-6, 6-8, 8-10, 10-12, 12-14, 14-16, 16-18 and 18-21. At scheduled sacrifice, on GD 21, a macroscopic examination of the visceral organs was performed, the gravid uterine weight was recorded and the live fetuses were euthanized. In addition, the liver was weighed at scheduled sacrifice for all pregnant females. A portion of liver was retained in 10% neutral buffered formalin from all females on study for possible histological examination. Specimens were not examined and are retained in the archives.

Results

The results of the study are summarized in Table 6.6.2-5. Additional information on maternal body weight gain and caesarean section observations is provided in Tables 6.6.2-6 – 6.6.2-7.

Table 6.6.2-5

Dose (mg/kg bw/day)	**0**	**20**	**30**	**dr**
<u>Maternal effects</u>				
Mortality	No substance-related mortality			
Clinical signs	No substance-related clinical signs			
Pregnant animals	21	23	23	
Body weight (gain)	No treatment-related effects			
Food consumption	No treatment-related effects			
Pathology	No treatment-related findings			
Pregnancy rate	91%	100%	100%	
Number of animals died pregnant	0	1	0	

Dose (mg/kg bw/day)	0	20	30	dr
Number of animals died non-pregnant	0	0	0	
Number of animals with premature delivery	0	0	0	

dr dose related

Table 6.6.2-6 Mean (± SD) maternal body weight gain (g)

Interval	Dose level of BYI 02960 in mg/kg/day		
	0	20	30
Number of dams (pregnant)	21	23	23
Pretreatment, GD 0-6:	29 ± 5	28 ± 6	30 ± 8
Treatment, GD 6-8:	4 ± 4	4 ± 4	3 ± 4
Treatment, GD 8-10:	10 ± 4	10 ± 4	9 ± 4
Treatment, GD 10-14:	20 ± 5	19 ± 5	21 ± 5
Treatment, GD 14-18:	46 ± 9	46 ± 8	47 ± 8
Treatment, GD 18-21:	53 ± 9	52 ± 9	53 ± 10
Treatment, GD 6-21	133 ± 19	131 ± 18	133 ± 20
Corrected BW gain	55.6 ± 12.0	53.9 ± 8.5	58.0 ± 13.9

a Data obtained from Table 4 and 8 in the Tables and Appendices section of the study report.

Table 6.6.2-7 Caesarean section observations

Observation	Dose Level of BYI 02960 (mg/kg/day)		
	0	20	30
Maternal data:			
No. Animals assigned	23	23	23
No. Animals pregnant	21	23	23
Pregnancy rate, %	91	100	100
No. Animals non-pregnant	2	0	0
Maternal wastage			
No. died (total)	0	1	0
No. died pregnant	0	1	0
No. died non-pregnant	0	0	0
No. premature delivery	0	0	0

Data obtained from Table 1 and Appendix G in the Tables and Appendices section of the study report.

No treatment-related mortality or clinical signs were observed in the study. One animal of the mid-dose group died on GD 20 after having been dosed. Necropsy revealed red dark lungs and presence of foam in lungs and trachea, indicating of a gavage error. Body weight parameters and food consumption were unaffected by treatment. There were no treatment-related macroscopic findings at necropsy and no changes in liver weights at any dose level.

Historical control data on the maternal parameters were obtained from the in-house developmental toxicity studies with Sprague-Dawley Crl:CD (SD) rats performed in the period June 2001-April 2009.

Acceptability

The study is considered acceptable.

Conclusions

Based on the lack of adverse effects on mortality, clinical signs, body weight changes, food consumption, gross pathology and caesarean section data the highest dose level of 30 mg/kg bw/day was considered a NOAEL for maternal toxicity in this study.

STUDY 3

Characteristics

reference	:	[redacted] 2012	exposure	:	Gestation days (GD) 6-28
type of study	:	Developmental toxicity study	doses	:	0, 7.5, 15 and 40 mg/kg bw/day
year of execution	:	2011/2012	vehicle	:	0.5% aqueous methylcellulose 400
test substance	:	BYI 02960 (batch number 2009-000239; purity 96.2%)	GLP statement	:	Yes
route	:	Oral (gavage)	guideline	:	OECD guideline 414.
species	:	Rabbit, New Zealand White Crl: KBL (NZW)	acceptability	:	Acceptable
group size	:	23 females/dose	**NOAEL**maternal toxicity	:	40 15 mg/kg bw/day
			NOAELdevelopmental toxicity	:	40 mg/kg bw/day

Study design

The study was performed in accordance with OECD guideline 414.

Results

The results of the study are summarised in Table 6.6.2-8. Additionally, information on maternal body weights, body weight gains, food consumption, reproductive performance, litter parameters and pup weights is presented in Tables 6.6.2-9 – 6.6.2-11.

Table 6.6.2-8

Dose (mg/kg bw/day)	**0**	**7.5**	**15**	**40**	**dr**
Maternal effects					
Mortality	No substance-related mortality				
Clinical signs	No treatment-related clinical signs				
Pregnant animals	23	22	23	23	
Body weight (gain)				d	
Food consumption				d	
Uterus weight	No substance-related findings				
Pathology	No substance-related findings				
Litter response					
Number of dams examined	23	22	23	22	
Pregnancy rate	100%	96%	100%	96%	

Dose (mg/kg bw/day)	0	7.5	15	40	dr
Resorptions					
- early	7	9	9	13	
- late	3	2	3	3	
- litters with total resorption	0	0	0	0	
Corpora lutea/dam	No substance-related findings				
Dams with live foetuses	22	22	23	22	
Number of implantations/dam	10.5 ± 2.1	10.9 ± 2.3	10.2 ± 2.0	11.5 ± 2.9	
Live foetuses/dam	9.7 ± 2.5	10.1 ± 2.1	9.4 ± 1.8	10.4 ± 2.4	
Foetal weight	No substance-related findings				
Post implantation loss (mean %)	8.7 ± 13.0	6.1 ± 9.2	7.4 ± 9.2	9.7 ± 9.5	
Sex ratio, % male	48.4 ± 18.6	52.4 ± 15.2	50.0 ± 16.4	47.4 ± 20.7	
Examination of the foetuses					
External observations	No substance-related findings				
Skeletal findings (incidence)					
-evaluated foetuses/litter	213/22	223/22	216/23	228/22	
- # hyoid centrum: incomplete ossification (number litter/fetuses affected)	9/20	10/13	11/16	12/23	
- # 5th and 6th sternebra: uncomplete ossification	8/18	8/11	7/10	10/19	
- pubis (uni/bi): incomplete ossification	1/2	2/2	0/0	3/5	
- insertion point (uni/bi) of pelvic girdle on 2nd sacral vertebra	21/85	22/87	19/85	17/95	
Visceral findings	No substance-related findings				

dr dose related

d/i decreased/increased, but not statistically significantly compared to the controls

Table 6.6.2-9 Mean maternal body weight gain (kg)

Interval	Dose level of BYI 02960 (mg/kg/day)				Historical control range
	0	7.5	15	40	
Number of dams (pregnant)	23	22	23	22	
Pretreatment, GD 3-6:	0.03 (±0.076)	0.03 (±0.073)	0.01 (±0.075)	0.02 (±0.070)	-0.067 to 0.046
Treatment, GD 6-8:	0.02 (±0.042)	0.02 (±0.035)	0.00 (±0.031)	-0.01 (±0.060)	-0.026 to 0.025
Treatment, GD 8-10:	0.04 (±0.046)	0.04 (±0.033)	0.03 (±0.025)	0.03 (±0.039)	-0.003 to 0.048
Treatment, GD 10-14:	0.03 (±0.081)	0.09 (±0.056) **	0.07 (±0.049) *	0.06 (±0.054)	0.023 to 0.088
Treatment, GD 14-18:	0.03 (±0.075)	0.05 (±0.054)	0.07 (±0.045)	0.04 (±0.049)	0.046 to 0.101
Treatment, GD 18-22:	0.04 (±0.046)	0.05 (±0.046)	0.03 (±0.042)	0.06 (±0.061)	0.031 to 0.083
Treatment, GD 22-26:	0.06 (±0.080)	0.04 (±0.067)	0.04 (±0.050)	0.07 (±0.058)	0.000 to 0.087
Treatment, GD 26-29:	0.06 (±0.069)	0.04 (±0.059)	0.03 (±0.049)	0.04 (±0.076)	-0.002 to 0.065
Treatment, GD 6-29	0.28 (±0.228)	0.31 (±0.111)	0.28 (±0.160)	0.28 (±0.116)	0.221 to 0.439
Corrected BW change	-0.25 (±0.177)	-0.24 (±0.110)	-0.25 (±0.157)	-0.28 (±0.126)	-0.25 to -0.08

a Data obtained from Table 3 in the study report.
* Statistically different ($p \leq 0.05$) from the control.
** Statistically different ($p \leq 0.01$) from the control.

Table 6.6.2-10 Ceasarean section observations

Observation	Dose Level of BYI 02960 (mg/kg/day)				Historical control range
	0	7.5	15	40	
Maternal data:[a]					
No. Animals assigned	23	23	23	23	NA
No. Animals pregnant	23	22	23	22	NA
Pregnancy rate, %	100	96	100	96	NA
No. Animals non-pregnant	0	1	0	1	NA
Maternal wastage					
Total No. intercurrent death or sacrifice (pregnant & non pregnant)	0	0	0	0	NA
Total No. intercurrent death or sacrifice (pregnant)	0	0	0	0	NA
Total No. intercurrent death or sacrifice (non pregnant)	0	0	0	0	NA
No. premature delivery	0	0	0	0	NA
No. abortion	1	0	0	0	NA
Uterine data at scheduled sacrifice:[b]					
Total No. corpora lutea[c]	253	280	265	283	NA
Corpora lutea / dam	11.5 ± 1.8	12.7 ± 1.9	11.5 ± 1.6	12.9 ± 2.6	10.79 - 13.21
Total No. implantations[c]	231	239	235	254	NA
Implantations / dam	10.5 ± 2.1	10.9 ± 2.3	10.2 ± 2.0	11.5 ± 2.9	9.10 - 10.95
Total No. litters[c]	22	22	23	22	NA
Total No. live fetuses[c]	213	223	216	228	NA
Live fetuses / dam[c]	9.7 ± 2.5	10.1 ± 2.1	9.4 ± 1.8	10.4 ± 2.4	8.23 - 9.90
Total No. dead fetuses	8	5	7	10	NA
Dead fetuses / dam[c], %	3.2 ± 6.8	2.0 ± 4.3	2.9 ± 5.4	3.8 ± 6.0	0.74 - 6.58
Total No. early resorptions[c]	7	9	9	13	NA
Total No. late resorptions[c]	3	2	3	3	NA
Early resorptions / dam	0.3 ± 0.8	0.4 ± 0.7	0.4 ± 0.5	0.6 ± 0.6	0.167 - 1.000
Late resorptions / dam	0.1 ± 0.5	0.1 ± 0.3	0.1 ± 0.5	0.1 ± 0.5	0.000 - 0.333
Litters with total resorptions[c, d]	0	0	0	0	NA
Pre-implantation loss per litter, %	9.2 ± 8.5	14.5 ± 13.6	11.0 ± 14.1	10.9 ± 11.0	9.08 - 26.73
Post-implantation loss per litter, %	8.7 ± 13.0	6.1 ± 9.2	7.4 ± 9.2	9.7 ± 9.5	4.07 - 19.25
Mean fetal weight, combined sexes, g	37.23 ± 6.20	38.07 ± 3.44	39.14 ± 4.26	37.89 ± 4.69	34.96 - 41.37
Mean fetal weight, males, g	37.31 ± 6.49	38.66 ± 4.05	39.48 ± 4.50	38.04 ± 5.77	34.92 - 42.33
Mean fetal weight, females, g	36.52 ± 6.86	37.60 ± 3.54	38.81 ± 4.41	37.75 ± 4.57	34.57 - 40.85
Sex ratio, % male	48.4 ± 18.6	52.4 ± 15.2	50.0 ± 16.4	47.4 ± 20.7	44.0 - 53.8

a Data obtained from Table 1 in the Tables and Appendices section of the study report.
b Data obtained from Tables 8 and 9 and Appendix F in the Tables and Appendices section of the study report.
* Statistically different (p <0.05) from the control.
** Statistically different (p <0.01) from the control.
c Statistical analysis was not conducted on this endpoint.
d Also includes litters with dead fetuses only or dead fetuses and resorptions.
HCD Historical control range (lowest – highest) of main uterine parameters from Attachment 3 in the Tables and Appendices section of the study report.
NA Not applicable.

Table 6.6.2-11 Skeletal examinations

Dose level of BYI 02960 (mg/kg/day)	0	7.5	15	40	Historical Control Range	0	7.5	15	40	Historical Control Range
	Number of litters examined					Number of fetuses examined				
	22	22	23	22		213	223	216	228	
	Number of litters examined					Number of heads examined				
	22	22	23	22		112	117	115	119	
Observations #	Number of litters affected (% of litters affected)					Number of fetuses affected (% of fetuses affected)				
Variations Hyoid centrum: incomplete ossification or unossified.	9 (40.9)	10 (45.5)	11 (47.8)	12 (54.5)	(4.8 – 63.6)	20 (17.9)	13 (11.1)	16 (13.9)	23 (19.3)	(1.1 – 28.6)
5th and 6th sternebrae: incomplete ossification.	8 (36.4)	8 (36.4)	7 (30.4)	10 (45.5)	(14.3 – 45.8)	18 (8.5)	11 (4.9)	10 (4.6)	19 (8.3)	(2.1 – 12.6)
Pubis (uni/bi): incomplete ossification.	1 (4.5)	2 (9.1)	0 (0.0)	3 (13.6)	(0.0 – 27.3)	2 (0.9)	2 (0.9)	0 (0.0)	5 (2.2)	(0.0 – 4.3)
Insertion point (uni/bi) of pelvic girdle on 2nd sacral vertebra.	21 (95.5)	22 (100)	19 (82.6)	17 (77.3)	(66.7 – 95.8)	85 (39.9)	87 (39.0)	85 (39.4)	95 (41.7)	(28.2 – 56.7)

a Study data obtained from Table 13 and historical control range from Attachment 4 in the Tables and Appendices section of the study report. Statistical analysis was conducted on all these observations.
Statistical analysis was conducted on the observations (Appendix M).
* Statistically different ($p \leq 0.05$) from the control.
** Statistically different ($p \leq 0.01$) from the control.

There were no treatment-related mortalities or clinical signs at any dose level tested.

In the high-dose group mean maternal body weight between GD 6 and 10 was lower than in controls ($p \leq 0.05$). Thereafter mean body weight gain was comparable to the controls throughout all intervals. Mean maternal corrected body weight change was reduced by 12% compared to the controls (not statistically significant) and slightly outside the range of in-house historical control data. Food consumption was transiently reduced by 20% between GD 6 and 8 ($p \leq 0.01$) and by 11% between GD 8 and 10 (not statistically significant). These effects on (corrected) body weight gain and food consumption were slight, and mainly transient.

At 15 and 7.5 mg/kg bw/day no treatment-related effects were observed on mean maternal body weights, body weight gains and maternal corrected body weight change. Mean maternal food consumption was similar to the controls.

There were no treatment-related macroscopic changes in the dams of any dose levels observed at necropsy. Mean liver weight was similar to the controls at all dose levels.

Pregnancy rate was similar in all groups. No treatment-related changes were noted on litter parameters, including the number of live fetuses, the number of implant sites per dam, the percentages of pre- and post-implantation losses, the number of early and late resorptions, the fetal death status, the fetal body weight and sex ratio.

There were no treatment-related malformations or variations noted at the fetal external examination. At 40 mg/kg/day, the incidence of the variation “eye bulge (uni/bi): protruding” was higher than in the control group and was outside the range of in-house HCD at the fetal level. However, as the observation was noted in only one litter and as the finding was not corroborated at the internal and skeletal observations, this increased incidence was considered to have occurred by chance.

There were no treatment-related malformations or variations noted at the fetal visceral and skeletal examinations. The observed skeletal variations (see Table 5.2) were not statistically different from the controls and were well within the in-house historical control data. Therefore they were not considered to be treatment-related.

Historical control data were generated from 14 developmental toxicity studies with New Zealand White Crl: KBL (NZW) rabbits performed by the testing laboratory in the period of March 2000 – October 2009.

Acceptability

The study is considered acceptable.

Conclusions

The effects on (corrected) body weight gain and food consumption were slight and mainly transient, however considered adverse (>10%) ~~and therefore considered not adverse~~.The NOAEL for maternal toxicity and developmental toxicity was set at 15 ~~40~~ mg/kg bw/day.

B.6.6.3 Summary

The results of the reproduction toxicity and teratogenicity studies are summarised in table 6.6.3-1.

Table 6.6.3-1 Summary of reproduction toxicity and teratogenicity studies

Type of study	Species		NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Critical effects	Reference
Reproduction toxicity						
A range-finding 1-generation toxicity study	rat	parental	17.5	60	Body weight (gain) ↓, food consumption ↓ , spleen weight ↓	[redacted] 2010
		reproduction	147.5	-	-	

Type of study	Species		NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Critical effects	Reference
		Offspring	17.5	61	Body weight (gain) ↓, brain weight ↓	
2-generation toxicity study	Rat	parental	6.4 ~~7.7~~ ~~32~~	32 ~~117~~	Body weight gain ↓ Liver and thyroid weight ↑, centrilobular hypertrophy ↑	[redacted]. 2011
		reproduction	34	134	Estrous cycle ↓, litter size ↓, implants ↓	
		Offspring	34	134	Body weight ↓, physical development ↓	
Embryo/fetal toxicity and teratogenicity						
teratogenicity study	rat	maternal	50	150	Body weight ↓, liver weight ↑	[redacted]. 2010
		developmental	50	150	short costal cartilage ↑	
Maternal tolerability study	rat	maternal	30	-	-	[redacted], 2012
teratogenicity study	rabbit	maternal	1540	40 -	Body weight gain ↓	[redacted] 2012
		developmental	40	-	-	

In the rangefinder one generation rat reproduction study, BYI 02960 was administered continuously in the feed to Wistar rats (10 animals/dose/sex) at nominal dietary concentrations of 0, 200, 700, and 2000 ppm. The parental systemic LOAEL is 700 ppm (60.0 mg/kg bw/day females), based on decreased body weight, decreased body weight gain, alterations in food consumption during premating, and decreased spleen weights in females. The parental systemic NOAEL is 200 ppm (17.5 mg/kg bw/day).
The reproductive NOAEL is 147.5 mg/kg bw/day based on no reproductive findings observed at the highest dose tested.
The offspring LOAEL is 700 ppm (60.9 mg/kg bw/day). The LOAEL is based on maternal effects leading to secondarily-mediated effects on pup weight, pup weight gain, and organ weight changes (brain). The offspring NOAEL is 200 ppm (17.5 mg/kg bw/day).

In the rat two-generation reproduction study, BYI 02960 was administered continuously in the diet to Wistar rats (30 animals/dose/sex) at nominal dietary concentrations of 0, 100, 500, and 1800 ppm. A significant decrease in body weight, but <10% was observed in the mid-dose of the F1 females and a non significant decrease in body weight gain was found in the parental animals (21 % in females) and the F1 animals (16 % in females). Therefore the parental systemic LOAEL is considered 500 ppm (32 mg/kg bw/d) based on decreased body weight gain in females. The parental systemic NOAEL is 100 ppm (6.4 ~~7.7~~ mg/kg bw/d). ~~Therefore the parental systemic LOAEL is considered 1800 ppm (117.4 mg BYI 02960/kg bw/day) based on increased liver and thyroid weights, and minimal centrilobular hypertrophy in males decreased body weight (>10%)(premating, gestation, and lactation), body weight~~

~~gain (premating; P and F_1). The parental systemic NOAEL was 500 ppm (32.0 mg BYI 02960/kg bw/day).~~ The reproductive NOAEL was 500 ppm (34.3 mg/kg bw/day females) based on decreased estrous cycle number, litter size, and the number of implants observed in the F_1 generation at the highest dietary level tested (1800 ppm =134.0 mg/kg bw/day in females). A significant decrease in body weight, but <10% was observed in the mid-dose of the F2 pups. The offspring LOAEL is therefore 1800 ppm (134.0 mg/kg bw/day) based on maternal effects leading to secondary effects on pup weight, and physical development. The offspring NOAEL is 500 ppm (34.3 mg/kg bw/day).

In a rat developmental study, BYI 02960 was administered daily by gavage to groups of 25 pregnant Sprague-Dawley female rats per dose-group at 0, 15, 50 and 150 mg/kg/day from gestation day (GD) 6 to 20. Minimal maternal toxicity was observed at the high-dose level, manifested as decreased body weight gain and reduced food consumption. The mean absolute liver weight was increased in the high dose group.Based on these findings the NOAEL for maternal toxicity was set at 50 mg/kg bw/day. The increased incidence of parietal incomplete ossification showed no dose response, the unossified cervical centrum and the incomplete ossification of the sternebra was within the historical background. A increase of a short costal cartilage was seen at the high-dose. Based on these findings, the NOAEL for developmental toxicity was set at 50 mg/kg bw/day.

In a complementary study, where groups of 23 sperm-positive female Sprague-Dawley rats were exposed to BYI 02960 by oral gavage from gestation day (GD) 6 to 20 at 0, 20 and 30 mg/kg/day, no maternal toxicity was observed up to 30 mg/kg/day. Therefore, based on these two studies, it can be concluded that the NOEL for maternal toxicity was 30 mg/kg/day.

In a rabbit developmental study, groups of 23 time-mated pregnant female New Zealand White rabbits were administered BYI 02960 by oral gavage from gestation day (GD) 6 to 28 at 0, 7.5, 15 and 40 mg/kg/day. The effects on (corrected) body weight gain and food consumption were slight and mainly transient, however considered adverse (>10%).The NOAEL for maternal toxicity and developmental toxicity was set at 15 mg/kg bw/day.~~There were no treatment-related findings for maternal and developmental toxicity therefore the NOAEL for maternal toxicity and developmental toxicity was set at 40 mg/kg bw/day.~~

B.6.7 NEUROTOXICITY / DELAYED NEUROTOXICITY (ANNEX IIA 5.7)

B.6.7.1 Acute oral neurotoxicity

STUDY 1

Characteristics

reference	:	[redacted]	exposure	:	Once by gavage
type of study	:	Acute neurotoxicity study	doses	:	Initial study : 0, 50, 200 and 800

					mg/kg bw Follow-up study : 0, 20 and 35 mg/kg bw
year of execution	:	2011	vehicle	:	methylcellulose 400 (0.5%)
test substance	:	BYI 02960 (batch number 2009-000239; purity 96.2%)	GLP statement	:	Yes
route	:	Oral	guideline	:	According to OECD 424
species	:	Rat, Wistar Rj:WI (IOPS HAN)	acceptability	:	Acceptable
group size	:	Initial study: 12/sex/dose Follow-up study: 12 females/dose	**NOAEL**	:	35 mg/kg bw

Study design

The study was performed according to OECD 424. Initial study was performed with dose levels of 0, 50, 200 and 800 mg/kg bw with male and female rats (12/sex/dose). The follow-up study was performed in order to establish the NOAEL for the effects observed at all dose levels in the initial study, using the dose levels of 0, 20 and 35 mg/kg bw. Based on the higher sensitivity of females in the initial study, the follow-up study was performed with females only (12/dose). The animals in the follow-up study were sacrificed without necropsy.

Results

The results are summarised in Tables 6.7.1-1 (initial study) and 6.7.1-2 (follow-up study).

Table 6.7.1-1 The results of the initial study

Dose (mg/kg bw/d)	**0**		**50**		**200**		**800**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality								2/12	
Clinical signs	No substance-related findings								
Body weight (gain)	No substance-related findings								
Functional observation battery	See table below								
Motor activity measurements - *spontaneous motor activity, 60-min session, day 1*									
- 10 minutes					ds	ds	ds	ds	
- 60 minutes							ds	ds	
Pathology	No substance-related findings								
macroscopy	No substance-related findings								
microscopy	No substance-related findings								
Neuropathology	No substance-related findings								

i/d: increased/decreased
is/ds: statistically significantly increased/decreased
dr: dose response

Table 6.7.1-2 The results of the follow-up study

Dose (mg/kg food)	**0**	**20**	**35**

	f	f	f
Mortality	No mortality		
Clinical signs	No substance-related findings		
Body weight (gain)	Not evaluated in the study		
Functional observation battery			
- quantitative parameters	No substance-related findings		
- home cage	No substance-related findings		
- open field	No substance-related findings		
- sensorimotor	No substance-related findings		
Motor activity measurements	No substance-related findings		
Pathology			
macroscopy	Not evaluated in the study		
Neuropathology	Not evaluated in the study		

Initial study

During the initial study, one high-dose female died during neurobehavioral testing conducted at the time of peak effect after dosing. Another high-dose female was found dead on study day 5.

One week after dosing, mean absolute body weight gain was statistically significantly lower in high-dose animals of both sexes (-40% and -46% in males and females, respectively, compared to controls). Body weight gain recovered after day 7 and overall, was comparable to controls for the duration of the study.

In the high-dose animals, findings associated with treatment were observed at the time-of-peak effect (2 hours after dosing), including piloerection, lower muscle tone, rapid respiration, low arousal, tremors, myoclonic jerks, chewing, repetitive licking of lips, gait incoordination, flattened or hunched posture, dilated pupils, impaired (uncoordinated or slow) righting reflex, impaired flexor and tail pinch responses and reduced rectal temperature. Automated measures of motor activity were also reduced in both sexes, compared to controls.

In the mid-dose group, at the time-of-peak effect after dosing, treatment-related observations included piloerection, rapid respiration, gait incoordination and flattened body posture in both sexes, with a higher incidence of tremors in both sexes. In addition, automated measures of motor activity were reduced during the first 10-min interval of the session, while activity for the entire test session was comparable to controls.

At the low-dose level, the only treatment-related effects were limited to higher incidences of piloerection (both sexes) and dilated pupils (females only) at the time-of-peak effect after dosing.

All effects were reversible, with none observed at later time points of the study.

There were no macroscopic or microscopic treatment-related observations in either sex at any dose level.

Table 6.7.1-3: Summary of significant changes in open field observations during the initial study at time peak-effect

BYI 02960 Dose levels (mg/kg)	0	50	200	800
Male				
Piloerection	4/12	8/12	12/12	12/12
Rapid respiration	1/12	0/12	9/12	12/12
Tremors	0/12	0/12	4/12	8/12
Myoclonic jerks	0/12	0/12	1/12	5/12
Chewing	0/12	0/12	0/12	5/12
Convulsions	0/12	0/12	0/12	0/12
Gait incoordination	0/12	0/12	3/12	7/12
Rearing (mean ± S.D.)	7.2 ± 2.4	7.8 ± 3.4	4.3 ± 3.6	2.7 ± 2.8
Low arousal	1/12	1/12	6/12	11/12
Repetitive licking of lips	1/12	0/12	0/12	2/12
Flattened or hunched posture	3/12	2/12	8/12	11/12
Female				
Piloerection	4/12	7/12	11/12	12/12
Rapid respiration	0/12	0/12	6/12	12/12
Tremors	0/12	0/12	4/12	10/12
Myoclonic jerks	0/12	0/12	0/12	7/12
Chewing	0/12	0/12	1/12	4/12
Convulsions	0/12	0/12	0/12	1/12
Gait incoordination	0/12	0/12	5/12	5/12
Rearing	12.3 ± 4.6	10.7 ± 3.3	9.9 ± 4.6	2.8 ± 2.8
Low arousal	0/12	0/12	1/12	8/12
Repetitive licking of lips	0/12	1/12	3/12	8/12
Flattened or hunched posture	0/12	0/12	3/12	11/12

Table 6.7.1-4: Summary of significant changes in sensory reactivity during the initial study at time peak-effect

BYI 02960 Dose levels (mg/kg)	0	50	200	800
Male				
Right pupil: dilated	0/12	1/12	4/12	6/12
Left pupil: dilated	0/12	1/12	4/12	6/12
Incoordianted or slow surface righting reflex	0/12	0/12	4/12	6/12
Abnormal tail pinch response	1/12	4/12	2/12	4/12
Female				
Right pupil: dilated	1/12	6/12	7/12	10/12
Left pupil: dilated	1/12	6/12	7/12	10/12
Incoordianted or slow surface	0/12	1/12	2/12	5/12

righting reflex				
Abnormal tail pinch response	3/12	2/12	4/12	8/11

Table 6.7.1-5: Significant changes in motor activity during the initial study at time peak-effect

BYI 02960 Dose levels (mg/kg)	0	50	200	800
Male				
First interval (10 minutes) (mean ± S.D.)	107 ± 37	120 ± 37	64 ± 32 *	31 ± 17 *
Total activity (60 minutes) (mean ± S.D.)	194 ± 47	254 ± 118	163 ± 92	97 ± 29 *
Female				
First interval (10 minutes) (mean ± S.D.)	136 ± 31	111 ± 28	72 ± 29 *	37 ± 23 *
Total activity (60 minutes) (mean ± S.D.)	293 ± 156	272 ± 154	199 ± 125	115 ± 72 *

* : Statistically different (p ≤0.05) from the control

Follow-up study

No treatment-related effects were evident at either dose tested in the follow-up study that was performed to establish a NOAEL for effects observed at 50 mg/kg.

Acceptability

The study is considered acceptable.

Conclusions

Based on piloerection and dilated pupil in the initial study a LOAEL is 50 mg/kg. The NOAEL is therefore based on the follow-up study and set at 35 mg/kg bw in rats.

B.6.7.2 Semichronic oral neurotoxicity

STUDY 1

Characteristics

reference	:	[redacted]. 2011	exposure	:	Dietary 90 days
type of study	:	90-day Neurotoxicity study	doses	:	0, 5.7/6.9, 29.4/34.8, 143/173 mg/kg bw/d (M/F)
year of execution	:	2011	vehicle	:	None
test substance	:	BYI 02960 (batch number 2009-000239; purity 96.2%)	GLP statement	:	Yes
route	:	Oral (diet)	guideline	:	According to OECD 424
species	:	Rat, Wistar Rj:WI (IOPS HAN)	acceptability	:	Acceptable
group size	:	12/sex/dose	**NOAEL sys** **NOAEL neuro**	:	29.4 mg/kbg bw/d 143 mg/kg bw/d

Study design

The study was performed according to OECD 424. The study was performed with dose levels of 0, 5.7/6.9, 29.4/34.8, 143/173 (M/F) mg/kg bw/d with male and female rats (12/sex/dose). Animals were observed for clinical signs daily, with body weight and food consumption measured weekly. A detailed

physical examination was performed once during the acclimatization phase and weekly throughout the study. Neurotoxicity assessment, consisting of a Functional Observational Battery (FOB) and automated measurement of motor activity, was performed on all animals on 5 occasions (pre-study, Study weeks 2, 4, 8 and 13 - 14). Ophthalmological examinations were performed on all animals during the acclimatization phase and on all animals of all dose groups during Week 13. All animals were subjected to a complete necropsy (macroscopic examinations). At least 6 animals from each group were perfused for neuropathological investigation. The brain was weighed and a range of tissues from the nervous system was collected, fixed and examined microscopically.

Results

Table 6.7.2-1 The results of the study

Dose (mg/kg bw/d) (M/F)	**0**		**5.7/6.9**		**29.4/34.8**		**143/173**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	No mortality throughout the study								
Clinical signs	No substance-related findings								
Body weight (gain)							d	ds	
Food consumption							d	d	
Ophthalmological examination	No substance-related findings								
Neurobehavioral assessment	No substance-related findings								
Motor activity measurements *- spontaneous motor activity*	No substance-related findings								
(Neuro-) Pathology *-Terminal body weight* *- brain to terminal body weight ratio* *- enlarged liver*							d	d is 4/6	
macroscopy	No treatment-related macroscopic findings were observed.								
microscopy	No treatment-related microscopic findings were observed.								

i/d: increased/decreased
is/ds: statistically significantly increased/decreased
dr: dose response

Up to and including the highest dose tested of 2500 ppm, there was no treatment-related mortality, clinical signs or ophthalmological changes during the study and no treatment-related effects were observed in any of the neurotoxicology endpoints, including neuropathological examinations, in either sex.

There were no treatment-related effects in either sex at 5.7/6.9 and 29.4/34.8 mg/kg bw/d.

At 143/173 mg/kg bw/d body weight parameters were clearly affected in both sexes, especially during the first week of treatment, when mean body weight remained static in females and the mean body

weight gain was 50% lower in males as compared to controls. Overall at the end of the study, mean body weight gain at this dose level represented 85% and 79% of the control values in males and females, respectively. Consequently, mean body weight remained lower compared to controls throughout the study in both sexes (- 7% to - 10% in males and - 5% to - 9% in females).
Mean food consumption was significantly lower in both sexes during the first week of treatment (- 18% and - 29% in males and females, respectively) and remained slightly lower in both sexes throughout the study (up to - 14% and up to - 13% in males and females, respectively, the effect being statistically significant on several occasions).
At necropsy, mean terminal body weight was 7% lower in both sexes, compared to controls. In remaining animals not used for neuropathology, enlarged liver was observed in 4/6 females.

Acceptability

The study is considered acceptable.

Conclusions

Body weight and food intake effects were seen at the highest dosage. The systemic NOAEL is therefore 29.4/34.8 mg/kg bw/d for males and females respectively.
There was no evidence of neurotoxicity at dietary levels as high as 143 mg/kg bw/d (males), therefore this is considered to be a NOAEL for neurotoxicology.

B.6.7.3 Developmental neurotoxicity

Study 1

Characteristics

reference	:	[redacted].; 2012	exposure	:	Dietary, GD 6 -LD 21
type of study	:	A Developmental Neurotoxicity Study	doses	:	0, 120, 500 and 1200 ppm
year of execution	:	2012	vehicle	:	none
test substance	:	BYI 02960 (batch number 2009-000239; purity 96.2%)	GLP statement	:	Yes
route	:	Oral	guideline	:	According to OECD 426
species	:	Rat, Wistar Rj:WI (IOPS HAN)	acceptability	:	Acceptable
group size	:	30 females/dose	**NOAEL maternal**	:	500 ppm (42.4 mg/kg bw/d)
			NOAEL developmental		500 ppm (42.4 mg/kg bw/d)
			NOAEL developmental neurotoxicity		500 ppm (42.4 mg/kg bw/d)

Study design

The study was performed according to OECD 426. BYI 02960 was administered via the diet from gestation Day (GD) 6 through lactation Day (LD) 21 to mated female Wistar rats at nominal concentrations of 0, 120, 500, or 1200 ppm with adjustment during lactation to maintain a more consistent dosage throughout the period of exposure. The average mean daily intake of the test substance (mg BYI 02960/kg bw/day) based on the average dietary consumption for the last two weeks of gestation and three weeks of lactation at 120, 500, or 1200 ppm, respectively, was 0, 10.3, 42.4, and 102 mg/kg bw/day. All test diets (including control) were provided for ad libitum

consumption throughout the study, except during neurobehavioral testing. The Parental (P)-generation females were evaluated by cage-side and detailed clinical observations, body weight, food consumption, and reproductive endpoints. On postnatal Day (PND) 4, litters with a minimum of seven pups, including at least three per sex, were culled to yield, as closely as possible, four males and four females. Subsets of surviving offspring, representing 19–20 litters per dietary level, were subjected to evaluation using the following observations and measurements: detailed clinical observations and a detailed observational battery, pupil response, surface righting, balanopreputial separation or vaginal patency, body weight, food consumption, automated measures of activity (figure-eight maze), auditory startle habituation, learning and memory (passive avoidance after weaning and a water maze task beginning on PND 60 ± 2 days), and an ophthalmic examination. Neural tissues were collected from 10 rats/sex/dietary level (representing 20 litters) on PND 21 (brain only) and at study termination (approximately 75 days of age) for microscopic examination and morphometry.

Results

The results are summarised in Tables 6.7.3-1 (parental animals) and 6.7.3-2 (offspring).

Parental animals

Table 6.7.3-1 The results of the parental females

Dose (ppm)	**0**	**120**	**500**	**1200**
	f	**f**	**f**	**f**
Mortality	No P-generation females were found dead during gestation or lactation.			
Clinical signs				
Gestation (Days 6–24)				
- Red Vaginal Discharge	0/30	0/30	1/30	0/30
- Hair loss	1/30	1/30	2/30	3/30
- Fused Digits	1/30	0/30	0/30	0/30
Lactation (Days 0 – 21)				
- Hair loss	1/27	1/29	3/29	4/30
- Fused Digits	1/27	0/29	0/29	0/30
Detailed observational battery	No test substance-related findings			
Body weight (g) (Gestation)				
GD 20				ds (-7%)
GD 0-20				ds (-21%)
Body weight (g) (Lactation)				
LD 0				ds (-4%)
LD 4				ds (-6%)
Reproductive performance	No test substance-related findings			
-				

i/d: increased/decreased
is/ds: statistically significantly increased/decreased

Table 6.7.3-1.1: Mean (±S.E.) Maternal Body Weight and Food Consumption

Observations/Study Week	Dietary Level of BYI 02960			
	Control	120 ppm	500 ppm	1200 ppm
Gestation				
Mean Body Weight (g) GD 0	206.4±2.18 (27)	208.0±2.54 (29)	209.6±2.36 (29)	209.8±2.59 (30)
Mean Body Weight (g) GD 6	229.1±2.71 (27)	230.8±2.96 (29)	231.2±3.46 (29)	233.1±2.72 (30)
Mean Body Weight (g) GD 13	254.5±3.74 (27)	256.0±3.58 (29)	256.0±3.27 (29)	245.3±2.92 (30)
Mean Body Weight (g) GD 20	320.9±4.09 (27)	314.7±5.14 (28)	318.7±4.26 (29)	299.9**±4.05 (29)4%)
Mean Weight Gain (g) GD 0–20	114.5±2.80 (27)	107.5±3.38 (28)	109.1±2.59 (29)	90.3**±2.57 (29)
Mean Food Consumption (g/animal/day) GD 6–13	20.1±0.73 (27)	19.1±0.47 (28)	18.9±0.47 (29)	18.4±0.59 (30)
Mean Food Consumption (g/animal/day) GD 13–20	20.3±0.37 (27)	20.1±0.43 (27)	20.2±0.40 (29)	19.2±0.48 (29)
Lactation				
Mean body weight (g) LD 0	244.0±3.30 (27)	245.2±3.85 (29)	242.9±3.43 (29)	233.3*±2.76 (30)
Mean body weight (g) LD 4	262.0±3.19 (25)	256.7±4.48 (23)	256.1±3.16 (26)	247.2**±3.62 (25)
Mean body weight (g) LD 7	265.4±2.98 (23)	263.2±3.89 (23)	263.4±3.36 (23)	255.7±3.46 (23)
Mean body weight (g) LD 14	279.6±3.17 (23)	274.6±4.05 (23)	275.2±3.30 (23)	266.6±2.86 (23)
Mean body weight (g) LD 21	261.1±5.87 (23)	264.1±4.50 (23)	254.2±3.88 (23)	246.5±6.38 (23)
Mean food consumption (g/animal/day) LD 0–7	36.0±1.05 (23)	35.3±1.01 (23)	34.1±1.14 (23)	34.0±0.80 (23)
Mean food consumption (g/animal/day) LD 7–14	50.8±0.84 (23)	50.3±1.08 (23)	50.7±0.86 (23)	50.3±1.00 (23)
Mean food consumption (g/animal/day) LD 14–21	55.9±1.76 (23)	58.1±1.12 (23)	54.7±1.06 (23)	54.5±1.35 (23)

Values are mean ± S.E. (*n*). Means for gestation include only dams found sperm positive and with pups at termination of gestation.
* Statistically different from control, Dunnett's Test $p \leq 0.05$; ** Statistically different from control, Dunnett's Test $p \leq 0.01$

Table 6.7.3-1.2 : Reproductive Performance

Observation	Dietary Level of BYI 02960			
	Control	120 ppm	500 ppm	1200 ppm
No. of Animals Co-housed [a]	30	30	30	30
No. of Animals Mated	30	30	30	30
Maternal Wastage				
No. of Dams not Pregnant	3	1	1	0
No. of Dams that Delivered Dead Pups	1	0	0	2
No. of Dams with Pre-Mature Delivery	0	0	0	0
Mating Index	100.0	100.0	100.0	100.0
Fertility Index (No. of pregnant females/No. of inseminated females X 100)	90.0	96.7	96.7	100.0
Gestation Length (days) [b]	21.7±0.10 [22.0] (21.0–22.0)	21.7±0.13 [22.0] (20.0–23.0)	21.8±0.13 [22.0] (21.0–23.0)	21.9±0.16 [22.0] (20.0–23.0)

[a] Number of animals assigned to each dietary level.
[b] Values are mean ± S.E., [median], and (range).
Values were not statistically different from control, $p \leq 0.05$ or $p \leq 0.01$

During gestation, findings considered incidental and unrelated to the test substance included red vaginal discharge on GD 13 (1 mid-dose dam), fused digits (1 control dam), and hair loss (1 to 3 dams from all dietary levels, including controls), which is a common finding associated with nest-building

behavior in pregnant rats. During lactation, findings that are considered incidental and unrelated to the test substance included fused digits (1 control dam) and areas of hair loss (1 to 4 dams at all dietary levels, including control).

During gestation, there was a statistical decrease (-7%), relative to controls, in mean body weight on GD 20 and a statistical decrease (-21%) in overall mean weight gain during gestation (GD 0-20) at the 1200 ppm dietary level. There were statistical decreases in body weights on LD 0 and 4 (-4% and -6%, respectively), relative to controls, at the 1200 ppm dietary level, and non-statistical decreases (-4% to -6%) that continued through termination on LD 21 at the 1200 ppm dietary level. There were no effects on body weight or body weight gain during gestation or lactation at any other dietary level.

Offspring

Table 6.7.3-2 The results of the offspring

Dose (ppm)	**0**		**120**		**500**		**1200**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Litter size and viability	No substance-related findings							
Clinical signs	There were no test substance-related clinical signs during lactation or postweaning in males or females at any dietary level.							
Body weight								
PND 17							d (-5%)	d (-5%)
PND 21							d (-5%)	d (-4%)
PND 4-17							ds(-7%)	d (-6%)
PND 11-17							ds(-10%)	ds (-7%)
Body weight Post weaning (PND 28-70)	No difference in body weight after weaning for males and females							
Developmental landmarks	No substance-related findings							
- Balanopreputial separation								
- Vaginal opening								
- Surface righting								
- Pupil constriction								
Detailed observational battery	No substance-related findings							
Motor activity								
PND 13				d			i	
PND 17			i				i	
PND 21			i	i			i	
PND 60								i
Auditory startle habituation								
PND 23								
PND 60								is
Learning and memory	There were no differences in acquisition or retention attributable to exposure to the test substance in either sex at any dietary level							
- Passive avoidance								
- Water maze								

Dose (ppm)	0		120		500		1200	
	m	f	m	f	m	f	m	f
Ophthalmology	No substance-related findings							
Gross Pathology	No substance-related findings							
Terminal body weight								
PND 21				d (-4%)		d (-7%)	d (-8%)	d (-8%)
PND 75 (non-perfused)				d (-5%)			d (-5%)	d (-5%)
Brain weight								
PND 75 (non-perfused)							d (-5%)	
Gross Brain measurements	No substance-related findings							
Micropathology brain measurements	No substance-related findings							

i/d: increased/decreased
is/ds: statistically significantly increased/decreased

Table 6.7.3-2.1: Litter Size and Viability

Observation	Dietary Level of BYI 02960			
	Control	120 ppm	500 ppm	1200 ppm
No. of Litters	23	23	23	23
Total No. of Pups Born	269	265	275	255
Total No. of Pups Missing	0	0	0	1
Litters with Pups Missing	0	0	0	1
Total No. of Pups Found Dead	2	2	0	0
Litters with Pups Found Dead	2	2	0	0
Total No. of Pups Cannibalized	0	0	0	0
Litter with Pups Cannibalized	0	0	0	0
Litter Size	11.7±0.34 [12.0] (8.0–15.0)	11.5±0.39 [12.0] (8.0–14.0)	12.0±0.36 [12.0] (8.0–15.0)	11.1±0.38 [11.0] (7.0–14.0)
Stillborn Pups Number % Mean±S.E. [Median] (Range)	 1 0.4 0.0±0.04 [0.0] (0.0–1.0)	 0 0.0 0.0±0.00 [0.0] (0.0–0.0)	 0 0.0 0.0±0.00 [0.0] (0.0–0.0)	 2 0.8 0.1±0.06 [0.0] (0.0–1.0)
Mean No. of Viable Pups Birth Day 4 (Pre-cull) [a] Day 4 (Post-cull) [b] Day 21	 12 12 8 8	 12 11 8 8	 12 12 8 8	 11 11 8 8
Live Birth Index [c]	99.6±0.43 [100.0] (90-100)	100.0±0.00 [100.0] (100-100)	100.0±0.00 [100.0] (100-100)	99.3±0.51 [100.0] (91–100)
Viability Index [c]	99.3±0.51 [100.0] (91–100)	99.1±0.64 [100.0] (89–100)	100.0±0.00 [100.0] (100–100)	100.0±0.00 [100.0] (100–100)
Lactation Index [c]	100.0±0.00 [100.0] (100–100)	100.0±0.00 [100.0] (100–100)	100.0±0.00 [100.0] (100–100)	99.5±0.54 [100.0] (88–100)

[a] Before standardization (culling) [b] After standardization (culling) [c] Values are mean ± S.E., [median], (range)
Values were not statistically different from control, $p \leq 0.05$ or $p \leq 0.01$

Table 6.7.3-2.2: Mean (± S.E.) Preweaning Pup Body Weights and Body Weight Change (g)

Postnatal Day	Dietary Level of BYI 02960							
	Control	120 ppm	500 ppm	1200 ppm	Control	120 ppm	500 ppm	1200 ppm
	Males				Females			
0	5.9±0.13 (23)	5.9±0.10 (23)	6.0±0.10 (23)	6.0±0.10 (23)	5.6±0.11 (23)	5.7±0.10 (23)	5.7±0.08 (23)	5.7±0.11 (23)
4 [a]	9.9±0.25 (23)	9.8±0.20 (23)	9.9±0.24 (23)	10.0±0.26 (23)	9.5±0.23 (23)	9.5±0.20 (23)	9.4±0.21 (23)	9.5±0.28 (23)
4 [b]	10.0±0.25 (23)	9.8±0.20 (23)	9.9±0.24 (23)	9.9±0.26 (23)	9.5±0.24 (23)	9.5±0.20 (23)	9.4±0.21 (23)	9.4±0.28 (23)
11	25.4±0.48 (23)	24.9±0.46 (23)	25.7±0.44 (23)	24.5±0.49 (23)	24.5±0.44 (23)	24.3±0.46 (23)	24.6±0.42 (23)	23.7±0.47 (23)
17	38.9±0.65 (23)	37.9±0.55 (23)	39.2±0.56 (23)	36.8±0.60 (23)	37.5±0.56 (23)	37.0±0.59 (23)	37.6±0.47 (23)	35.8±0.54 (23)
21	47.9±1.04 (23)	48.4±0.85 (23)	47.6±0.66 (23)	45.6±1.02 (23)	46.3±1.01 (23)	47.2±0.96 (23)	45.4±0.65 (23)	44.4±0.83 (23)
Body Weight Change								
	Males				Females			
4–17	28.9±0.56 (23)	28.1±0.45 (23)	29.3±0.41 (23)	26.9±0.51* (23)	27.9±0.50 (23)	27.5±0.48 (23)	28.2±0.33 (23)	26.3±0.43 (23)
4–21	37.9±0.88 (23)	38.6±0.73 (23)	37.7±0.57 (23)	35.7±0.87 (23)	36.8±0.88 (23)	37.7±0.82 (23)	36.0±0.56 (23)	34.9±0.68 (23)
11–17	13.6±0.31 (23)	13.0±0.20 (23)	13.5±0.23 (23)	12.3±0.25** (23)	13.0±0.29 (23)	12.7±0.22 (23)	13.0±0.21 (23)	12.1±0.18* (23)
11–21	22.5±0.75	23.5±0.47	22.0±0.52	21.1±0.68	21.9±0.77	22.9±0.56	20.8±0.51	20.7±0.58

	(23)	(23)	(23)	(23)	(23)	(23)	(23)	(23)

Values are mean ± S.E. (*n*)
[a] Before standardization (culling). * Values were statistically different from control, *p* ≤ 0.05
[b] After standardization (culling). ** Values were statistically different from control, *p* ≤ 0.01

Table 6.7.3-2.3: Mean (±S.D.) Motor Activity Data (total activity counts for session)

Test Day	Dietary Level of BYI 02960			
	Control	120 ppm	500 ppm	1200 ppm
Males				
PND 13	55±55 (20)	66±82 (20)	63±66 (20)	114±130 (20)
PND 17	146±117 (20)	260±104 (20)	173±114 (20)	221±144 (20)
PND 21	307±119 (20)	367±132 (20)	331±149 (20)	367±123 (20)
PND 60	503±142 (20)	518±108 (20)	505±121 (20)	529±111 (20)
Females				
PND 13	74±141 (20)	88±81 (19)	68±60 (20)	62±40 (20)
PND 17	201±152 (20)	149±112 (19)	243±121 (20)	227±200 (20)
PND 21	303±88 (20)	333±140 (19)	308±132 (20)	322±119 (20)
PND 60	656±143 (20)	666±233 (19)	624±202 (20)	790±232 (20)

Values are mean ± S.D. (*n*) Values were not statistically different from control, *p* ≤ 0.05

Table 6.7.3-2.4. Auditory Startle Reflex Peak Amplitude Data (g, mean ± S.D.)

Block		Dietary Level of BYI 02960			
		Control	120 ppm	500 ppm	1200 ppm
Males					
PND 23	Block 1	44±18	48±13	43±18	46±22
	Block 2	43±16	50±13	42±23	41±15
	Block 3	39±16	48±15	40±23	42±14
	Block 4	40±17	48±14	38±17	41±15
	Block 5	37±16	45±15	37±14	35±12
	Avg. For Total Session	41±15	48±12	40±18	41±13
	No. Of Animals	20	20	20	20
	Body Weight	60	60	60	57
PND 60	Block 1	270±115	301±159	241±151	324±162
	Block 2	255±161	299±190	203±141	289±215
	Block 3	200±140	245±157	175±136	214±183
	Block 4	187±119	214±140	152±130	177±163
	Block 5	145±92	187±131	134±116	144±108
	Avg. For Total Session	211±115	249±145	181±127	230±153
	No. Of Animals	20	20	20	20
	Body Weight	292	291	280	285
Females					
PND 23	Block 1	39±22	38±16	35±21	45±17
	Block 2	39±22	36±16	36±18	43±17
	Block 3	36±19	37±18	34±18	43±17
	Block 4	32±14	37±17	31±18	39±16

Block		Dietary Level of BYI 02960			
		Control	120 ppm	500 ppm	1200 ppm
	Block 5	29±15	34±15	28±14	36±16
	Avg. For Total Session	35±17	36±14	33±17	41±15
	No. Of Animals	20	20	20	20
	Body Weight	57	58	56	57
PND 60	Block 1	102±74	133±75	140±91	186±129
	Block 2	94±82	121±94	127±84	181±126 ***
	Block 3	71±73	108±86	113±76	160±116 ***
	Block 4	68±54	84±58	96±65	132±87 ***
	Block 5	58±33	74±46	76±50	122±83 ***
	Avg. For Total Session	78±57	104±65	110±68	156±102 ***
	No. Of Animals	20	20	20	20
	Body Weight	185	180	184	174

Values are mean ± S.D. *** Statistically different from control, ANOVA $p \leq 0.05$.

Body weight was non-statistically decreased in high-dose males and females on PND 17 and 21 (-4% to -5%). Although these differences from control are small and not statistically significant, they are attributed to the test substance because of the relationship with dose (both genders and at multiple time points). These lower body weights were comparable to the 4–6% lower body weight for the high-dose dams throughout lactation. Body weight was not affected by the test substance in either sex at any other dietary levels. Body weight gain for PND 4–17 was statistically decreased in high-dose males (-7%). Body weight gain for the shorter period of PND 11–17 was statistically decreased in high-dose males (-10%), females (-7%). There were similar non-statistical trends in body weight gain for PND 4–17 in high-dose females (-6%), for PND 4–21 and PND 11–21 in both sexes. These differences from control are attributed to the test substance. Body weight gain was not affected by the test substance in either sex at the 120 and 500 ppm dietary levels. There was no difference in body weight after weaning for males and females at any dietary level.

A non-statistical increase in both motor and locomotor activity was measured in high-dose males on PND 13. These differences from control are considered test substance-related, but are also partially attributed to a low mean average activity for controls (55 and 5, for motor and locomotor, respectively), which was below the range for historical controls (59–72 and 6–11, respectively) in the last five studies conducted at this laboratory. An increase in motor activity was measured in low- and high-dose males on PND 17. These differences from control are not attributed to the test substance, since there was no dose-related pattern and the increases were not observed in mid-dose males or in females. Additionally, the increases were not observed on any subsequent test occasions, and means for all groups were within the range of historical controls. An increase in locomotor activity was measured in low-, mid-, and high-dose males on PND 17. These differences from control are not attributed to the test substance, since there was no dose-related trend, increases were not observed in females and the means were within the range of historical controls. Lastly, very slight increases in locomotor activity were measured in low-dose males on PND 21 and high-dose females on PND 60. These are

not considered test substance-related since motor activity for these same animals was generally comparable to controls, other dose levels were not similarly affected, and the differences were seen in only one sex.

In the auditory startle habituation test startle amplitude for all 50 trials was statistically increased two-fold in high-dose females (156 g), compared to controls (78 g) on PND 60. This was reflected as a non-statistical increase in response amplitude for the first block of trials, and a statistical increase in blocks 2–5, relative to control. This finding is considered to be related to the test substance. There was a non-statistical trend involving a slight increase in average startle amplitude in all five blocks of trials, in females at the low- and mid-dose, relative to controls, on PND 60. This difference from control is not thought to be test substance-related since the slight increase was similar in both dose levels with a 4-fold increase in dose and the mean average for startle response amplitude in control animals (78 g) is below the range for historical controls (87–116 g) in the last five studies conducted in this laboratory. There were no differences in startle amplitude in male and female weanlings or in adult males at any dietary level age. Startle latency and habituation were not affected by the test substance in males or females at any dietary level, on any test occasion.

Test substance-related mean terminal body weight changes for PND 21 pups were noted in males at 1200 ppm dietary level (non-statistically decreased, 8%, as compared to controls) and in females at 120, 500, and 1200 ppm dietary levels (non-statistically decreased, -5, -7%, and -8%, respectively, as compared to controls).
Terminal body weights are collected once at termination from a sub-group of animals in each dose level (i.e., those animals scheduled for necropsy; 10/sex/dietary level). In-life body weights are collected from all animals at selected time points throughout the in-life phase of the study (e.g., mean litter weight is calculated using 23 litters/level). Although the sub-group of animals weighed at termination did indicate an apparent effect on body weight, in-life data clearly indicates no effect on body weight (compared to controls) in females from the 120 and 500 ppm dietary levels.
There was no test substance-related terminal body weight change noted at any dietary level in the PND 75 (± 5 days) perfused rats.
There was a slight trend of decline in terminal body weights at 1200 ppm males (-5%, as compared to controls) in the PND 75 (± 5 days) non-perfused rats. There was a decrease (-5%) in terminal body weights observed in 120 and 1200 ppm females; however, these changes were not considered test substance-related due to lack of dietary relationship or the changes were small.

There was a decrease (-5%) in fresh brain weights at 1200 ppm males as compared to controls in the PND 75 (± 5 days) Rat**,** however, this change was not considered directly related to test substance administration since it was related to the decrease in terminal body weights.
The brain weight range for the non-perfused males was 1.769 g to 2.050 g and the range for the non-perfused 1200 ppm treated males was 1.739 g to 1.993 g with only two animals with a brain weight outside the control range.

Acceptability

The study is considered acceptable.

Conclusions

Dietary exposure to BYI 02960 from GD 6 through LD 21 to mated female Wistar rats resulted in treatment-related effects (maternal: decreased body weight gain. Offspring: decreased body weight gain, slightly increased in motor and locomotor activity, slightly increased auditory startle habituation) evident only at 1200 ppm (102 mg/kg bw/day) in maternal animals and offspring. ~~Therefore, the NOAEL for both maternal animals and offspring is 500 ppm (42.4 mg/kg bw/day) in this developmental neurotoxicity study.~~

The maternal NOAEL was 500 ppm (42.4 mg/kg bw/d) based on decreased body weight and body weight gain. The developmental NOAEL was 500 ppm (42.4 mg/kg bw/d) based on decreased offspring body weight and body weight gain. The developmental neurotoxicity NOAEL was 500 ppm (42.4 mg/kg bw/d) based on increased startle amplitude in females only on PND 60 and increased motor and locomotor activity on PND 13 in males only.

B.6.7.4 Summary

The results of the neurotoxicity studies are summarised in table 6.6.3-1.

Table 6.6.3-1 Summary of neurotoxicity studies

Type of study	Species	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Critical effects	Reference
Acute neurotoxicity in the rat	Rat	35	50	Piloerection, dilated pupil	[redacted], 2011
90-day neurotoxicity in the rat	Rat	29.4 (systemic) 143 (neuro)	143 -	Reduced body weight and food intake. None	[redacted], 2011
Developmental neurotoxicity in the rat	Rat	42.4 (maternal) 42.4 (developmental) 42.4 (developmental neurotoxicity)	102 102 102	Maternal: decreased body weight gain Offspring: decreased body weight gain Develompental neurotoxicity: slightly increased motor/locomotor activity and startle habituation.	[redacted]; 2012

In an acute neurotoxicity study, BYI 02960 was administered by gavage in a single dose to non-fasted young adult Wistar rats at 0, 50, 200 and 800 mg/kg bw. Compound-related effects were observed at all dose concentrations in both sexes. The only treatment-related effects at 50 mg/kg were limited to higher incidences of piloerection in both sexes and dilated pupils in females only. A follow-up study was performed in order to establish a clear NOAEL for findings observed at all dose levels in the initial

study. In this follow-up study, females only were used as they were equal or more sensitive than males at higher dose levels and were administered BYI 02960 at 0, 20 or 35 mg/kg. No treatment-related effects were evident at either dose tested. The dose level of 35 mg/kg of BYI 02960 was considered to be the overall NOAEL for both sexes.

In a 90-day neurotoxicity study, through approximately 13 weeks of continuous dietary exposure to BYI 02960 at 0, 100, 500 or 2500 ppm, there were no neurotoxic treatment-related findings apparent at any dietary level in either sex. Based on these findings, a NOAEL of 2500 ppm was established for the rat (equating to 143 mg/kg bw/day for males). Body weight and food intake effects were seen at the highest dosage. The systemic NOAEL is therefore 29.4/34.8 mg/kg bw/d for males and females respectively.

In a developmental neurotoxicity study, dietary exposure to BYI 02960 from GD 6 through LD 21 to mated female Wistar rats resulted in treatment-related effects (maternal: decreased body weight gain. Offspring: decreased body weight gain, slightly increased in motor and locomotor activity, slightly increased auditory startle habituation) evident only at 1200 ppm (102 mg/kg bw/day) in maternal animals and offspring. ~~Therefore, the NOAEL for both maternal animals and offspring is 500 ppm (42.4 mg/kg bw/day) in this developmental neurotoxicity study.~~

The maternal NOAEL was 500 ppm (42.4 mg/kg bw/d) based on decreased body weight and body weight gain. The developmental NOAEL was 500 ppm (42.4 mg/kg bw/d) based on decreased offspring body weight and body weight gain. The developmental neurotoxicity NOAEL was 500 ppm (42.4 mg/kg bw/d) based on increased startle amplitude in females only on PND 60 and increased motor and locomotor activity on PND 13 in males only.

B.6.8 OTHER TOXICOLOGICAL STUDIES (ANNEX IIA 5.8)

Literature search

With respect to Article 8(5) of Regulation (EC) No 1107/2009 a literature search has been conducted for flupyradifurone and its metabolites.

The following list gives an overview of all bibliographic databases searched. The time frame that was considered ranged from January 1, 2012 until April 23, 2011. As requested in the EFSA Guidance Document, all scientific peer-reviewed open literature has been considered and downloaded. Publications related to patents were not considered in the literature search.

Bayer CropScience has searched what it considers a broad collection of relevant databases (see list below). STN was selected as the preferred provider, because it offers a convenient search service that includes all databases considered relevant to cover the requirements established under Article 8(5) of Regulation (EC) 1107/2009.

The databases used in the literature search of flupyradifurone were:

Agricola	Biosis	CABA	Chemical Abstracts
Derwent Crop Protection (CROPU)		Derwent Drug File (DRUGB, DRUGU)	
EMBAL/ EMBASE	Esbiobase	IPA	Lifesci
Medline	Pascal	Registry	Scisearch
Toxcenter	Ulidat	FSTA	

The data that was used as input parameter for the database search included: active substance and its metabolites, including report name, structure, IUPAC name, CAS name/number, molecular formula, molar mass and other names/codes, as far as available. The categories/keywords which were used as text mining filter is very extensive and therefore not reported in the DAR.
All references were reviewed by the scientific experts, categorised according to the corresponding OECD dossier sections – if possible- and evaluated for their relevance.

A total of 342 publications were found for flupyradifurone and its metabolites. When using the keyword list as a text mining filter, 136 references were identified, 206 references were not related to any of the keywords. From those references which could not be assigned to any of the categories "health", "environment" or "non target species", none was identified by the scientific experts to provide relevant information for regulatory purposes, *i.e.* no false negative findings were identified. This indicates that the text mining filter may be considered as an appropriate tool for a first step of future literature reviews.

No unknown reference turned out to be relevant according to the definition under which this literature review has been carried out ("dealing with side-effects on health, the environment and non-target species"). No information was identified that was considered to have an impact on an endpoint or would result in the need to adapt a risk assessment in the flupyradifurone dossier .

Since no unknown publication turned out as relevant, Bayer CropScience does not consider it necessary to have a separate discussion of individual publications in appropriate Annex points of the dossier. Instead, we think it is sufficient to list all references that have become available as a part of this literature report (see Appendix C).

Even though the filter identified a large number of references as potentially relevant by comparing keywords in the list with the title and abstract of the publication, in the area of toxicology only one, but already known reference eventually was considered by Bayer CropScience development experts as relevant under the definition of criteria for relevance with respect to Article 8(5) of Regulation (EC) No 1107/2009.

List of relevant references

Database Accession Number*	Compound	Publi-cation Year*	Author/Inventor*	Title*	Source*	Recognized by keyword filter	Considered relevant by development experts
2002:917911	Difluoroacetic acid	2002	Lantum, Hoffman B. M.; Baggs, Raymond B.; Krenitsky, Daria M.; Anders, M. W.	Nephrotoxicity of Chlorofluoroacetic Acid in Rats	Toxicological Sciences (2002), 70(2), 261-268	Y	Y

Remarks RMS

A literature search and review was provided by the applicant. The search has been conducted in line with the EFSA guidance. Of all identified papers (342) the relevant ones (136) have been selected by the applicant in a transparent way using a text mining filter. The applicant provided a general justification for evaluating the relevant papers. As no study-by-study justifications were given the RMS can not properly verify whether the 136 relevant papers were correctly evaluated in terms of reliability/validity/acceptability for regulatory use. The one study that was considered relevant by the applicant is evaluated in the DAR. RMS notes that flupyradifurone is a new active substance worldwide. Therefore, public literature is not expected to be an important new information source for the active substance itself.

B.6.8.1 Biokinetic study

The biokinetic study below is performed to assess if the Cmax concentration of flupyradifurone can be determined in a certain period.

Study 1

Characteristics

Reference	:	[redacted]., 2010	exposure	:	diet 7-days
type of study	:	Biokinetic in the plasma of rats following 7 day exposure through the diet	dose	:	400 ppm (equivalent to app. 22.6 mg/kg bw/d in males and 32.4 mg/kg bw/d in females)
year of execution	:	2009-2010	vehicle	:	- (dry mixing with the diet)
test substance	:	flupyradifurone (Batch 2009-000239, purity 96.2%)	GLP statement	:	Not GLP but in a GLP laboratory
Route	:	Oral	guideline	:	-
Species	:	Rat Rj: WI (IOPS HAN)	acceptability	:	Acceptable as indicative study
group size	:	5 male, 5 female	**NOAEL**	:	-

Study design

The plasma concentration of flupyradifurone was assessed at three time points after continuous dietary administration for seven days. Groups of 5 male and 5 female Wistar rats received the diet admixed with the test substance at a concentration of 400 ppm (equating approximately to 22.6 mg/kg body weight/day in males and 32.4 mg/kg body weight/day in females).

During the first 15 days of acclimatization, the daily light/dark rhythm was 7 a.m. - 7 p.m. Rats being known to preferably eat during the dark period, the daily light/dark rhythm of rats was changed from pre-study Day 16, *i.e.* 14 days prior to first day of treatment, onwards, *i.e.* dark period was from 2 a.m. to 2 p.m. and light period was from 2 p.m. to 2 a.m., in order to allow the blood sampling during the working day. Clinical signs were observed daily. Body weights were measured just prior to the start of admixed food administration (Study Day 1) and on Study Day 8. Food consumption was recorded on Study Days 1 - 4, 4 - 7 and 7 - 8.

Whole blood was collected on heparin for plasma preparation from treated animals at three time points on Study Day 8, 6 hours after the start of feeding (8 am), 12 hours (2 pm) and 15 hours (5 pm). As the compound is rapidly absorbed, this should correspond to the period where the blood concentration in BYI 02960 should be higher. Blood samples collected at later time points should be lower. This should provide enough information to know when blood samples should be collected during subchronic or long term studies.The day after the end of the treatment period, animals were sacrificed without necropsy.

Results

There were no mortalities and no treatment-related clinical signs observed during the study. The few clinical signs observed (hair loss observed on two females) were considered not to be related to

flupyradifurone administration. Body weight evolution and food consumption were similar to the one observed in the other repeated dose studies.

The individual and mean concentrations of flupyradifurone in plasma samples were used to identify the appropriate maximal concentration (Cmax) and the corresponding time point of collection for the long-term studies.
The results obtained indicated a small sex difference in the plasma concentrations of flupyradifurone, values being slightly higher for females than for males.
In male rats, a Cmax of 8.3 mg/L was measured at the time of collection of 8 a.m. In female rats, a Cmax of 9.4 mg/L was measured at the time collection of 2 p.m. However, in view of the inter-individual variability, the concentrations of flupyradifurone in plasma were considered similar between the three times of blood collection for both sexes (between 7.8 and 8.3 mg/L for males and between 8.8 and 9.4 mg/L for females).

Table 6.8.1-1: Plasma concentration of flupyradifurone in male

Animal reference	Time collection	Plasma concentration of flupyradifurone		
		Individual data (mg/L)	Mean (mg/L)	SD
TT1M4982	8 a.m.	6.14	8.3	1.3
TT1M4983		8.63		
TT1M4984		8.59		
TT1M4985		8.56		
TT1M4986		9.77		
TT1M4982	2 p.m.	5.87	7.8	1.3
TT1M4983		7.14		
TT1M4984		8.73		
TT1M4985		8.36		
TT1M4986		8.90		
TT1M4982	5 p.m.	4.73	8.0	2.0
TT1M4983		9.98		
TT1M4984		8.30		
TT1M4985		7.77		
TT1M4986		9.05		

Table 5.10-03: Plasma concentration of flupyradifurone in female

Animal reference	Time collection	Plasma concentration of flupyradifurone		
		Individual data (mg/L)	Mean (mg/L)	SD
TT1F4987	8 a.m.	7.76	9.0	1.3
TT1F4988		9.33		
TT1F4989		8.66		
TT1F4990		11.0		
TT1F4991		8.28		

TT1F4987	2 p.m.	8.16	9.4	1.2
TT1F4988		9.86		
TT1F4989		9.15		
TT1F4990		11.1		
TT1F4991		8.56		
TT1F4987	5 p.m.	7.56	8.8	1.6
TT1F4988		9.85		
TT1F4989		7.91		
TT1F4990		11.2		
TT1F4991		7.67		

Using the experimental study design, blood samples collected between 8 a.m. and 5 p.m. are adequate for measuring flupyradifurone concentrations in plasma around the C_{max} for both sexes.

Acceptability

The study is not performed in GLP compliance. It was performed according to standard operation procedures of the GLP compliant laboratory but the results were not subjected to specific quality assurance inspections. The study is considered acceptable as supplementary study.

Conclusions

In conclusion, in the environmental conditions of the long-term studies (light: 7 a.m. to 7 p.m. & dark period: 7 p.m. to 7 a.m.) and since animals start eating food when the light is switched off, the blood collection can be performed between 1 a.m. and 10 a.m. in order to measure the maximum concentration of flupyradifurone in the ADME studies.

B.6.8.2 Immunotoxicity

Study 1

Characteristics

Reference	:	[redacted], 2011	exposure	:	diet 28-days
type of study	:	28-day Immunotoxicity study	dose	:	0, 125, 600 and 3000 ppm (equivalent to app. 0, 10, 50 and 230 mg/kg bw/d)
year of execution	:	2011	vehicle	:	acetone (positive control: cyclophosphamide)
test substance	:	Flupyradifurone (Batch 2009-000239, purity 96.2%)	GLP statement	:	Yes
Route	:	Oral	guideline	:	U.S.E.P.A., OPPTS Series 870, Health Effects Testing Guidelines, N° 870.7800 (August 1998)
Species	:	Female Wistar rats	acceptability	:	Acceptable
group size	:	10 females/dose	**NOAEL**	:	Systemic NOAEL of 50 mg/kg bw/d Immunotoxic NOAEL of >230 mg/kg

bw/d

Study design

This study was conducted according to U.S. E.P.A., OPPTS Series 870, Health Effects Testing Guidelines N° 870.7800 (August 1998). Female Wistar rats (10/group) were administered flupyradifurone continuously via dietary administration for at least 28 days at concentrations of 0, 125, 600 and 3000 ppm (equivalent to approximately 0, 10, 50 and 230 mg/kg bw/d). A similarly constituted group received untreated diet and a similarly constituted group received the positive control 3.5 mg/kg bw/d cyclophosphamide (immunosuppressive agent) for at least 28 days.

All animals were immunized with Sheeps Red Blood Cell (SRBC) antigen by intravenous injection of 2.5 x 10^8 SRBC/animal via the tail vain.

Mortality and clinical signs were observed daily, food consumption and body weight were recorded once weekly. Blood samples were collected of each surviving animal on study day 30 (just before necropsy) for specific anti-SRBC immunoglobulin M (IgM) analysis. All animals were necropsied, gross pathology observations were performed and spleen and thymus weighed.

Results

The flupyradifurone was homogeneous distributed through the diet and the concentrations were acceptable in the diet, in addition the formulation at 3000 ppm was found stable up to 34 days at room temperature in rodent diet.

One animal showed automutilation of both forelimbs and was killed for humane reasons.

Table 6.8.2-1

Dose (mg/kg bw/d)	**0**	**10**	**50**	**230**
n=10	**f**	**f**	**f**	**f**
Mortality	No treatment related mortality			
Clinical signs	No substance-related findings			
Body weight (mean)				
Day 1				
Day 8				**ds** (11%)
Day 15				**ds** (8.3%)
Day 22				**d**
Day 29				**d**
Food consumption				
Day 8				**d**
Day 15				**d**
Day 22				**d**
Day 29				**d**
Gross Pathology				
macroscopy	No macroscopic finding was noticed.			
Mean spleen weight	No substance-related findings			
Mean thymus weight				
Clinical pathology				
SRBC-specific Ig-M	No substance-related findings			

CPP = cyclophosphamide (positive control)
ds/is statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls

Mean body weight at 3000 ppm was reduced by 6 to 11% from Study Day 8 to 29 when compared to the control group. The effect was statistically significant on the intervals Day 1 to 8 and Day 8 to 15. There was no body weight gain/day between Study Day 1 and 8. Body weight gain was then similar to control animals at the following intervals. Between Study Day 1 and 29, the cumulative body weight was reduced by 23%, when compared to the control group.
At 600 and 125 ppm, body weight parameters were unaffected by treatment.

At 3000 ppm, mean food consumption was reduced by approximately 34% on Study Day 8 and by 9 to 13% onwards, when compared to control group although not statistically significant.
At 600 and 125 ppm, food consumption wasunaffected by treatment.

The spleen and thymus weight changes were considered to be incidental and not treatment-related (no dose response in the organ to bw ratio).

Table 6.8.2-2: Mean organ weights and organ/terminal body weight ratios.

Mean organ weight ±SD at scheduled sacrifice (% change when compared to controls)				
Sex	**Female**			
Dose level mg/kg bw/d	**0**	**10**	**50**	**230**
Mean absolute spleen weight (g)	0.762 ±0.122	0.743 ± 0.110	0.732 ± 0.135	0.720 ± 0.096
Mean spleen to body weight ratio (%)	0.316 ± 0.038	0.312 ±0.037	0.301 ± 0.039	0.319 ±0.036
Mean absolute thymus weight (g)	0.544 ±0.130	0.505 ± 0.075	0.591 ±0.154	0.522 ± 0.104
Mean thymus to body weight ratio (%)	0.226 ±0.048	0.212 ± 0.033	0.242 ± 0.053	0.230 ± 0.036

No statistically significant difference was noted in anti-SRBC IgM concentrations up to 3000 ppm. A high inter-individual variability was noted in all the groups as usually observed with SRBC sensitization. No dose-effect relationship was seen, the variations observed were due to only few animals.

Table 6.8.2-3: Mean SRBC-specific IgM

SRBC-specific IgM (u/mL) mean ± standard deviation (% change when compared to controls)				
Dose level mg/kg bw/d	**0**	**10**	**50**	**230**
Study Day 30	**40516 ± 23875**	**62920 ± 41649 (+ 55%)**	**34286 ± 30710 (- 15%)**	**33354 ± 26016 (- 18%)**

In the positive controls (cyclophosphamide at a dose level of 3.5 mg/kg/day) there was no change in mean terminal body weight in treated animals when compared to the controls. The principal findings noted at the macroscopic examination were atrophic/small spleen and/or thymus (6/10 and 5/10

respectively) which correlated with significantly lower mean absolute and relative spleen and thymus weights (- 34% to - 32% and - 28% to - 27% respectively).

Table 6.8.2-4: Mean spleen weights

Mean spleen weight ±SD at scheduled sacrifice (% change when compared to controls)		
Sex	**Female**	
Dose level of Cyclophosphamide (mg/kg/day)	0	3.5
Mean absolute spleen weight (g)	0.762 ± 0.122	0.500** ± 0.049 (- 34%)
Mean spleen to body weight ratio (%)	0.3161 ± 0.0380	0.2136** ± 0.0176 (- 32%)

** : p ≤0.01

Table 6.8.2-5: Mean thymus weights

Mean thymus weight ±SD at scheduled sacrifice (% change when compared to controls)		
Sex	**Females**	
Dose level of Cyclophosphamide (mg/kg/day)	0	3.5
Mean absolute thymus weight (g)	0.544 ± 0.130	0.389** ± 0.063 (- 28%)
Mean thymus to body weight ratio (%)	0.2259 ± 0.0482	0.1656** ± 0.0202 (- 27%)

** : p ≤0.01

In the positive controls the mean anti-SRBC IgM concentration was markedly lower when compared to controls (- 93%).

Table 6.8.2-6: Mean SRBC-specific IgM

SRBC-specific IgM (u/mL) mean ± standard deviation (% change when compared to controls)		
Dose level of Cyclophosphamide (mg/kg/day)	0	3.5
Study Day 30	**40516 ± 23875**	**2732 ± 1737** **(- 93%)**

Acceptability

The study is considered acceptable.

Conclusions

The mean body weight and mean food consumption was reduced at 3000 ppm (230 mg/kg bw/d). For the immunological response, no relevant change was noted in anti-SRBC IgM concentrations up to 3000 ppm (230 mg/kg bw/d) and no significant change was noted in the spleen and thymus weight. The systemic flupyradifurone NOAEL is 600 ppm (equivalent to approximately 50 mg/kg bw/d), and the immunotoxic NOAEL is >3000 ppm (equivalent to approximately 230 mg/kg bw/d).

B.6.9 STUDIES ON METABOLITES

The toxicological properties of two metabolites specific to BYI 02960 and two additional metabolites which are also formed from other agrochemicals are reported in this section.

Difluoroacetic acid (DFA, BCS-AA56716, M44) is a major soil, water, plant and livestock metabolite of BYI 02960. In the rat ADME study, it was found in urine at around 6% of the administered dose and the organ metabolism study showed that DFA was by far the dominating metabolite in the 24 hours samples of plasma, organs and tissues, accounting for more than 50% of the radioactivity

BYI 02960-difluoroethyl-amino-furanone (BCS-CC98193, BYI 02960-DFEAF, M34) is a minor plant metabolite of BYI 02960 and was also observed in the rat ADME study. It accounted for less than 10% of the administered dose in the rat urine. Since the confined rotational crop study with [furanone-4-^{14}C]BYI 02960 indicated rather high residue levels of BYI 02960-difluoroethyl-amino-furanone in leafy crops and the absence of other suitable markers, this metabolite is included in the residue definition for the data collection method for target and rotational crops (see residue section).

BYI 02960-CHMP (6-chloropyridin-3-ylmethanol, IM-O, M21) and **BYI 02960-6-CNA (6-chloronicotinic acid, IC-0, M27)** are metabolites common to other pesticides. In rat BYI 02960-CHMP was not found and BYI 02960-6-CNA was found up to 6% of the applied dose. Both metabolites are plant metabolites whereas BYI 02960-6-CNA is also found in soil.

Expert consultation 2.9

For the metabolite DFA:

The decreased glucose concentration was the only treatment-related effect at all doses in the **14-day study**. In the absence of other findings, the RMS considered this change as not adverse and proposed a NOAEL of 500 ppm based on effects on body weight (-12% in females). Bilirubin decrease (in females only) and increased urea were observed in all dose groups without dose response relationship or concurrent findings in organs. The experts agreed on a NOAEL of 500 ppm corresponding to 51 mg/kg bw per day.

In the **90-day study**, bilirubin and glucose effects were observed in all dose groups. No dose-response relationship was observed in the clinical chemistry parameters. The RMS proposed a

NOAEL of 200 ppm corresponding to 12.7 mg/kg bw per day based on reduced body weight gain. This was agreed by the experts.

In the rat metabolism, DFA did not appear as a major metabolite.

Comparison with the toxicity profile of the parent could not be concluded.

The experts considered the use of the reference value of the parent or reference values derived from specific data with the metabolite. The majority agreed to use the reference values of the parent.

For the metabolite BYI 02960-difluoroethyl-amino-furanone:

In the **14-day rat** dietary study, the proposed NOAEL is 1280 ppm corresponding to 135 mg/kg bw per day based on reduced bw gain and lower blood glucose concentration in females.

In the **28-day rat** dietary study, the proposed NOAEL is 3000 ppm corresponding to 243 mg/kg bw per day.

The experts agreed with the RMS conclusions.

Historical control data were provided for the positive *in vitro* chromosome aberration test; negative results were obtained in a micronucleus test where exposure of the bone marrow was indirectly demonstrated by signs of general toxicity. In the absence of toxicokinetic data for the metabolite, this might not be considered as a direct sign of exposure of the bone marrow. An *in vivo* UDS test provided also negative results but might not be the most appropriate follow up (nowadays a Comet assay would be required).

In the ADME studies of the parent, this metabolite has been found in urine, plasma, kidney, muscle.

The experts agreed that negative results in micronucleus and UDS tests *in vivo* were sufficient to conclude on the absence of genotoxic potential for this metabolite.

The RMS proposed that the reference values of the parent should be applied to this metabolite as well. This approach was agreed by the experts.

B.6.9.1 Difluoroacetic acid (DFA, BCS-AA56716, M44)

B.6.9.1.1 Acute toxicity Difluoroacetic acid (DFA, BCS-AA56716, M44)

Study 1

Characteristics

reference	:	[redacted]. 2010	exposure	:	Once by gavage
type of study	:	Acute oral toxicity study	doses	:	300, 2000 mg/kg bw
year of execution	:	2009	vehicle	:	Purified water
test substance	:	BCS-AA56716 (batch BCOO 5984-4-11, purity 96,7%)	GLP statement	:	Yes
route	:	Oral	guideline	:	According to OECD 423

species	:	Nulliparous and non-pregnant female rats Sprague-Dawley Rj: SD (IOPS Han)	acceptability	:	Acceptable
group size	:	3f/dose	**LD50-cut off**	:	Between 300-2000 mg/kg bw

Study design

The study was performed in accordance with OECD 423.

Three groups of three female rats Sprague-Dawley were given a single oral dose (10 mg/lkg) of BCS-AA56716 (batch BCOO 5984-4-11, purity 96.7%). The substance was tested using a stepwise procedure, each step using three female rats. The animals were assigned to their groups by randomization based on evenly distributed chance numbers. Following an overnight fast (18 hours), the first group received a single dose of 300 mg/kg of BCS-AA56716 (96.7% purity) by gavage. The test substance was administered in purified water at a volume of 10 mL/kg bw. After the first assay, as no deaths occurred, another assay was carried out on three animals at the next higher dose-level (2000 mg/kg). After the second assay, as 2/3 animals died, the results were confirmed in three other females at the dose-level of 300 mg/kg. Clinical signs and mortality rates were determined several times on the day of administration and subsequently at least once daily for an observation period of at least 14 days. Body weights were recorded on days 1, 8 and 15. On day 15, surviving animals were sacrificed and all animals were necropsied and examined for gross pathological changes.

Results

Table 6.9.1.1-1 Mortality:

Dose mg/kg bw	Mortality	Time of death
1st 300	0/3	--
2nd 2000	2 ~~1~~/3	1h
3rd 300	0/3	--

Symptoms of toxicity:

At the 2000 mg/kg dose-level (three females), two females were found dead 1 hour after treatment. Hypoactivity, dyspnea and/or lateral recumbency were observed within 25 minutes prior to their deaths. In the surviving animal at 2000 mg/kg, sedation then hypoactivity, dyspnea, lateral recumbency, piloerection and staggering gait were noted from day 1 until day 9. At 300 mg/kg, loud breathing was recorded in 1/6 females on day 3. No other clinical signs were noted.

Body weight:

When compared to CIT historical control data, a lower body weight gain (28 g *vs.* 41 ± 9 g in control data base) was noted in 1/6 females given 300 mg/kg between day 1 and day 8. The body weight gain of this female returned to normal thereafter. The body weight gain of the other animals was not affected by the test item-treatment.

A body weight loss of 8% (185 g *vs.* 201 g) was noted in the surviving female given 2000 mg/kg, between day 1 and day 8. The body weight gain of this female return to normal thereafter.

Necropsy:

No abnormalities were observed at gross necropsy.

Acceptability

This study is considered acceptable.

Conclusions

The LD50-cut off of BCS-AA56716 was found to be between 300-2000 mg/kg bw.

B.6.9.1.2 Genotoxicity Difluoroacetic acid (DFA, BCS-AA56716, M44)

Study 1

Study design and results

Type of study: Ames test, plate incorporation and preincubation method

Indicator cells	Endpoint	Res. - act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
S. typh. TA 98 TA 100 TA 102 TA 1535 TA 1537	 point mut. point mut. point mut. point mut. point mut.	 - - - - -	 - - - - -	rat liver	Phenobarbital/ß-Naphthoflavone	3-5000 µg/plate	Sokolowski, A. (2010)

Test substance: BCS-AA56716 (batch number BCOO 5984-4-11; purity 96.7%); tested in DMSO
Precipitation: No precipitation of the test substance was reported.
Toxicity: The plates incubated with the test substance showed normal background growth in all strains in both independent experiments with and without S9 mix. No toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in the treatment groups with and without metabolic activation.
GLP statement: yes
According to OECD 471: yes
Adequate positive (+S9: 2-AA; -S9: NaN3, 4-NOPD, MMS) and negative (sterility and vehicle) controls were included.

Table 6.9.1.2-1 Plate incorporation assay with BCS-AA56716 - Mean number of revertants

Strain	**TA 98**		**TA 100**		**TA 102**		**TA 1535**		**TA 1537**	
Metabolic activation	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9
Neg. control (DMSO)	25	42	120	138	388	587	13	18	11	18
Neg. control (untreated)	29	41	125	155	421	569	17	24	11	13
BCS-AA56716										
3 µg/plate	27	42	121	153	371	595	15	17	10	19
10 µg/plate	28	36	128	140	433	608	13	17	13	16
33 µg/plate	28	49	115	144	421	641	14	16	12	18
100 µg/plate	29	41	129	143	411	613	13	20	13	15
333 µg/plate	32	47	116	126	379	562	14	20	9	15
1000 µg/plate	34	38	115	142	408	592	13	23	12	15
2500 µg/plate	36	45	111	152	427	620	15	20	10	16
5000 µg/plate	25	48	123	147	418	578	18	20	7	15
Pos. control	320	2861	2188	3435	3613	2457	1870	437	77	429

Table 6.9.1.2-2 Pre-incubation assay with BCS-AA56716 - Mean number of revertants

Strain	**TA 98**		**TA 100**		**TA 102**		**TA 1535**		**TA 1537**	
Metabolic activation	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9

Table 6.9.1.2-2 Pre-incubation assay with BCS-AA56716 - Mean number of revertants

Strain	TA 98		TA 100		TA 102		TA 1535		TA 1537	
Neg. control (DMSO)	31	37	114	138	393	484	15	17	12	12
Neg. control (untreated)	38	41	147	173	401	565	18	24	9	15
BCS-AA56716										
33 µg/plate	31	36	122	145	396	562	17	19	10	14
100 µg/plate	33	36	106	138	415	574	15	20	15	11
333 µg/plate	25	37	126	133	371	510	19	14	12	15
1000 µg/plate	36	40	116	126	416	521	11	25	7	12
2500 µg/plate	35	40	115	135	429	531	14	18	13	18
5000 µg/plate	30	41	118	141	367	482	16	28	13	15
Pos. control	408	1730	1684	2007	1956	2739	1631	367	79	230

No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment at any concentration level, neither in the presence nor absence of metabolic activation.

Acceptability

The study is considered acceptable.

Conclusions

BCS-AA56716 is not mutagenic in the Ames test.

<u>Study 2</u>

Study design and results
Type of study: mammalian cells in vitro, cytogenetic assay

Indicator cells	Endpoint	Res. -act.	Res. +act.	Activation		Dose rangea	Reference
				Tissue	Inducer		
Chinese hamster V79 cells	Chromosome aberration	-	-	rat liver	Phenobarbital/ß -naphthoflavone	-S9: 0, 3.8, 7.5, 15.0, 30.0, 60.0, 120.0, 240.0, 480.0, 960.0 µg/mL (4 hours treatment, harvest 18 hours after the beginning of treatment) 0, 60.0, 120.0, 240.0, 480.0, 960.0 µg/mL (18 hours treatment, harvest at the same time) +S9: 0, 3.8, 7.5, 15.0, 30.0, 60.0, 120.0, 240.0, 480.0, 960.0 µg/mL (4 hours treatment, harvest 18 hours after the beginning of treatment) 0, 60.0, 120.0, 240.0, 480.0, 960.0 µg/mL (18 hours treatment,	Hall, C. (2010)

Indicator cells	Endpoint	Res. -act.	Res. +act.	Activation		Dose rangea	Reference
				Tissue	Inducer		
						harvest at the same time)	

a The guideline requires three analysable concentrations, in this study the concentrations of 240.0, 480.0 and 960.0 µg/mL were selected.

Test substance: BCS-AA56716 (batch number BCOO 5984-4-11; batch code: BCS-AA56716-01-02; purity 96.7%); tested in DMSO
Precipitation: No precipitation of the test substance was observed.
Toxicity: No relevant cytotoxic effects indicated by reduced mitotic indices or reduced ceil numbers to approximately 50% were observed after treatment with the test substance. The highest tested concentration (960.0 µg/mL) was equal to a molar concentration of about 10 mM.
GLP statement: yes
According to OECD 473: yes
Adequate positive and negative controls were included.

Table 6.9.1.2-3 Summary of results of the cytogenetic assay

Treatment group	**Exposure period / Recovery (hrs)**	**Cell numbers in % of control**	**Mitotic indices in % of control**	**Aberrant cells in %**		
				incl. gapsa	excl. gapsa	with exchanges
without metabolic activation						
DMSO	4 / 14	100.0	100.0	3.0	3.0	1.5
BCS-AA56716	4 / 14					
240.0 µg/mLb	4 / 14	111.8	91.7	3.8	3.5	0.8
480.0 µg/mLb	4 / 14	113.7	106.0	4.8	4.5	1.5
960.0 µg/mL	4 / 14	115.5	90.5	2.5	2.0	0.0
EMS (1000.0 µg/mL)c	4 / 14	not tested	94.0	39.0	37.0d	22.0
DMSO	18 / -	100.0	100.0	2.5	2.0	0.0
BCS-AA56716	18 / -					
240.0 µg/mL	18 / -	87.3	107.5	1.0	0.5	0.0
480.0 µg/mL	18 / -	84.0	106.3	0.0	0.0	0.0
960.0 µg/mL	18 / -	107.1	108.2	1.5	1.5	0.0
EMS (600.0 µg/mL)	18 / -	not tested	89.9	17.5	17.0d	6.0
with metabolic activation						
DMSO	4 / 14	100.0	100.0	3.5	3.5	0.0
BCS-AA56716	4 / 14					
240.0 µg/mLb	4 / 14	121.3	106.9	1.5	1.0	0.0
480.0 µg/mLb	4 / 14	99.7	112.8	1.0	1.0	0.0
960.0 µg/mL	4 / 14	119.8	103.9	3.5	3.5	0.0
CPA (1.4 µg/mL)	4 / 14	not tested	71.4	9.0	8.5c	4.5
DMSO	4 / 14	100.0	100.0	2.5	2.0	0.5
BCS-AA56716	4 / 14					
240.0 µg/mL	4 / 14	93.9	102.7	2.0	2.0	0.5
480.0 µg/mL	4 / 14	85.5	100.5	2.0	2.0	1.0
960.0 µg/mL	4 / 14	64.9	87.7	1.5	1.0	0.0

Table 6.9.1.2-3 Summary of results of the cytogenetic assay

Treatment group	Exposure period / Recovery (hrs)	Cell numbers in % of control	Mitotic indices in % of control	Aberrant cells in %		
				incl. gapsa	excl. gapsa	with exchanges
CPA (1.4 µg/mL)	4 / 14	not tested	98.2	15.0	12.0c	4.0

a Inclusive cells carrying exchanges;
b Evaluation of 200 metaphases per culture due to inhomogeneous data;
c Evaluation of 50 metaphases per culture due to strong clastogenic effects;
d Aberration frequency statistically significant higher than corresponding control values.

In both experiments, in the absence and presence of S9 mix, no biologically relevant increase in the number of cells carrying structural chromosome aberrations was observed. In experiment I in the absence of S9 mix an isolated single increase in the number of aberrant cells, excluding gaps (4.5%) slightly exceeding the total laboratory's historical solvent control data range (0.0 - 4.0% aberrant cells, excluding gaps) was observed after treatment with 480.0 µg/mL This observation was regarded as biologically irrelevant, since the value was not statistically significantly increased and no increase was observed at the highest applied dose of 960 µg/mL.
No relevant evidence of an increase in polyploid metaphases was noticed after treatment with the test item as compared to the controls (information provided only as text, no table with results regarding polyploid metaphases was shown in the report).

Acceptability

The study is considered acceptable.

Conclusions

BCS-AA56716 did not induce structural chromosome aberrations in V79 cells when tested up to and including cytotoxic concentrations. Furthermore, no relevant evidence of an increase in polyploid metaphases was noticed after treatment with the test substance as compared to the controls.

<u>Study 3</u>

Study design and results

Type of study: mammalian cells in vitro, gene mutation test

Indicator cells	Endpoint	Res. –act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
Chinese hamster V79 cells	Gene mutations (HGPRT)	-	-	rat liver	Phenobarbital/ ß-naphthoflavone	Experiment I: - S9 (exposure period: 4 hours): 60.0, 120.0, 240.0, 480.0, 960.0) + S9 (exposure period: 4 hours): 60.0, 120.0, 240.0, 480.0, 960.0) Experiment II: - S9 (exposure period: 24	Hall, C. (2010)

Indicator cells	Endpoint	Res. –act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
						hours): 30.0, 60.0, 120.0, 240.0, 960.0) - S9 (exposure period: 4 hours): 120.0, 240.0, 480.0, 960.0)	

Test substance: BCS-AA56716 (batch number BCOO 5984-4-11; purity 96.7%); tested in DMSO
Precipitation: No precipitation of the test substance was observed.
Toxicity: Moderate cytotoxic effects occurred in experiment II without S9 mix and with S9 mix in culture II. The maximum concentration of the test substance (960.0 µg/mL) applied in the main experiments equals a molar concentration of 10 mM.
GLP statement: yes
According to OECD 476: yes
Adequate positive and negative controls were included.

Table 6.9.1.2-4 Gene mutation in mammalian cells - 1st experiment

Treatment group	Relative cloning efficiency Ia	Relative cell density	Relative cloning efficiency IIb	Mutant colonies / 106 cells	Induction factor
Without metabolic activation (culture I)					
Solvent control	100.0	100.0	100.0	5.8	1.0
BCS-AA56716					
30 µg/mL	70.8	n.c.	n.c.	n.c.	n.c.
60 µg/mL	87.7	87.0	83.3	34.5	6.0
120 µg/mL	92.1	71.2	88.5	38.5	6.7
240 µg/mL	100.2	84.8	91.9	27.2	4.7
480 µg/mL	87.7	60.4	103.8	38.3	6.6
960 µg/mL	82.2	101.8	96.4	24.0	4.1
Positive control EMS					
150 µg/mL	79.9	81.2	84.3	268.6	46.4
Without metabolic activation (culture II)					
Solvent control	100.0	100.0	100.0	24.8	1.0
BCS-AA56716					
30 µg/mL	60.7	n.c.	n.c.	n.c.	n.c.
60 µg/mL	89.4	92.0	90.1	22.8	0.9
120 µg/mL	77.1	89.6	93.8	35.5	1.4
240 µg/mL	92.0	114.9	89.5	17.1	0.7
480 µg/mL	98.8	115.8	76.3	16.4	0.7
960 µg/mL	95.9	126.0	64.1	22.1	0.9
Positive control EMS					
150 µg/mL	75.4	116.3	77.1	168.9	6.8
With metabolic activation (culture I)					
Solvent control	100.0	100.0	100.0	45.3	1.0
BCS-AA56716					
30 µg/mL	108.8	n.c.	n.c.	n.c.	n.c.
60 µg/mL	97.2	90.0	87.8	18.4	0.4
120 µg/mL	98.5	85.3	102.8	8.6	0.2
240 µg/mL	104.9	97.9	90.8	20.9	0.5
480 µg/mL	105.4	97.5	97.5	34.5	0.8
960 µg/mL	110.1	104.9	87.1	41.1	0.9
Positive control DMBA					
1.1 µg/mL	33.6	67.9	82.8	1232.2	27.2
With metabolic activation (culture II)					
Solvent control	100.0	100.0	100.0	25.6	1.0
BCS-AA56716					
30 µg/mL	87.7	n.c.	n.c.	n.c.	n.c.
60 µg/mL	86.3	129.9	101.9	54.4	2.1
120 µg/mL	93.5	125.4	107.5	21.2	0.8
240 µg/mL	92.6	112.6	106.7	37.9	1.5

Table 6.9.1.2-4 Gene mutation in mammalian cells - 1st experiment

Treatment group	Relative cloning efficiency Ia	Relative cell density	Relative cloning efficiency IIb	Mutant colonies / 106 cells	Induction factor
480 µg/mL	86.1	111.4	114.8	10.1	0.4
960 µg/mL	82.4	105.6	92.4	35.6	1.4
Positive control DMBA					
1.1 µg/mL	38.1	97.6	96.8	1000.4	39.0

a cloning efficiency determined immediately after treatment to measure toxicity;
b cloning efficiency determined after the expression period to measure viability of the cells without selective agent.
n.c.: culture was not continued since a minimum of only four analysable concentrations is required.

Table 6.9.1.2-5 Gene mutation in mammalian cells - 2nd experiment

Treatment group	Relative cloning efficiency Ia	Relative cell density	Relative cloning efficiency IIb	Mutant colonies / 106 cells	Induction factor
Without metabolic activation (culture I)					
Solvent control	100.0	100.0	100.0	38.4	1.0
BCS-AA56716					
30 µg/mL	84.3	81.0	106.4	28.8	0.8
60 µg/mL	101.2	82.3	111.2	22.7	0.6
120 µg/mL	95.2	93.6	101.7	22.3	0.6
240 µg/mL	94.9	88.1	95.7	25.3	0.7
480 µg/mL	86.4	n.c.c.	n.c.c.	n.c.c.	n.c.c.
960 µg/mL	83.6	68.0	102.7	22.3	0.6
Positive control EMS					
150 µg/mL	90.2	88.7	81.7	534.4	13.9
Without metabolic activation (culture II)					
Solvent control	100.0	100.0	100.0	14.5	1.0
BCS-AA56716					
30 µg/mL	91.2	99.8	95.5	13.7	0.9
60 µg/mL	104.2	103.7	95.7	23.4	1.6
120 µg/mL	85.6	107.0	89.7	11.8	0.8
240 µg/mL	97.0	79.6	96.7	28.2	1.9
480 µg/mL	87.0	n.c.c.	n.c.c.	n.c.c.	n.c.c.
960 µg/mL	75.0	41.7	94.8	22.1	1.5
Positive control EMS					
150 µg/mL	102.6	100.0	91.5	410.3	28.3
With metabolic activation (culture I)					
Solvent control	100.0	100.0	100.0	24.5	1.0
BCS-AA56716					
30 µg/mL	99.0	n.c.	n.c.	n.c.	n.c.
60 µg/mL	105.4	n.c.	n.c.	n.c.	n.c.
120 µg/mL	110.4	98.1	105.2	23.1	0.9
240 µg/mL	102.8	139.2	100.3	26.5	1.1
480 µg/mL	112.0	144.1	101.7	52.8	2.2
960 µg/mL	115.3	104.5	103.0	33.9	1.4
Positive control DMBA					
1.1 µg/mL	27.0	44.2	70.3	1337.5	54.7
With metabolic activation (culture II)					
Solvent control	100.0	100.0	100.0	27.0	1.0
BCS-AA56716					
30 µg/mL	85.2	n.c.	n.c.	n.c.	n.c.
60 µg/mL	86.4	n.c.	n.c.	n.c.	n.c.
120 µg/mL	87.0	66.2	100.3	24.1	0.9
240 µg/mL	94.8	56.4	99.3	37.1	1.4
480 µg/mL	87.6	47.8	95.0	27.0	1.0
960 µg/mL	100.7	51.0	96.6	27.0	1.0

Table 6.9.1.2-5 Gene mutation in mammalian cells - 2nd experiment

Treatment group	Relative cloning efficiency Ia	Relative cell density	Relative cloning efficiency IIb	Mutant colonies / 106 cells	Induction factor
Positive control DMBA					
1.1 µg/mL	21.2	37.2	58.2	1185.9	43.9

a cloning efficiency determined immediately after treatment to measure toxicity;
b cloning efficiency determined after the expression period to measure viability of the cells without selective agent.
n.c.: culture was not continued since a minimum of only four analysable concentrations is required.
n.c.c.: culture not continued due to contamination.

No relevant and reproducible increase in mutant colony numbers/106 cells was observed in the main experiments up to the maximum concentration. The induction factor exceeded the threshold of three times the corresponding solvent control in the first culture of the first experiment without metabolic activation in all dose groups. This effect however, was judged to be due to the rather low solvent controls of 5.8 mutant colonies/106 cells. The induction factor was well below 3.0 in the respective parallel culture. Additionally, several mutant frequencies (33.9 - 54.4 mutant colonies/106 cells) exceeded the respective historical range of solvent controls. This effect is considered as not being biologically relevant as the mutation frequencies did not exceed the historical data range in the respective parallel culture or the induction factor did not exceed the threshold of three times the solvent control on a reproducible basis.

A linear regression analysis (least squares) was performed to assess a possible dose dependent increase of mutant frequency. No significant dose dependent trend of the mutation frequency indicated by a probability value of <0.05 was determined in any of the experimental groups.

In both experiments of this study (with and without metabolic activation) the range of the solvent controls was from 5.8 up to 45.3 mutant colonies per 106 cells; the range of the groups treated with the test item was from 8.6 up to 54.4 mutant colonies per 106 cells. The highest solvent control after treatment with S9 mix in experiment I and without S9 mix in experiment II (45.3 and 38.4 mutant colonies per 106 cells, respectively) exceeded the historical range of solvent control slightly (3.0 - 33.2 and 3.9 - 31.5 colonies per 106 cells). However, this effect was judged as irrelevant since it is very minor and the corresponding solvent control remained well within the range of historical controls.

Acceptability

The study is considered acceptable.

Conclusions

In this study, BCS-AA56716 has no mutagenic potency in vitro in the CHO/HGPRT assay.

Summary

The results from the available in vitro genotoxicity studies with BCS-AA56716 are summarised in table 6.8.1.1-6, respectively.

Table 6.9.1.2-6 In vitro genotoxicity studies

Test substance	Type of study		Result		Reference
	Indicator cells	Endpoint	without activation	with activation	
BCS-AA56716	S. typh. TA 98 TA 100 TA 102 TA 1535 TA 1537	Point mutation	-	-	Sokolowski, A. (2010)
BCS-AA56716	Chinese hamster V79 cells	Chromosome aberration	-	-	Hall, C. (2010)
BCS-AA56716	Chinese hamster V79 cells	Gene mutations (HGPRT)	-	-	Hall, C. (2010)

BCS-AA56716 was not mutagenic in the Ames test.

BCS-AA56716 did not induce structural chromosome aberrations in V79 cells and showed no mutagenic potency in vitro in the CHO/HPRT assay.

B.6.9.1.3 Short-term studies Difluoroacetic acid (DFA, BCS-AA56716, M44)

Study 1

Characteristics

Reference	:	[redacted]2011	exposure	:	14 days
type of study	:	Preliminary 14-day study	dose	:	0, 500, 2000 and 8000 ppm M: 0, 48, 187, 745 mg/kg bw/d F: 0, 51, 201, 800 mg/kg bw/d
year of execution	:	2011	vehicle	:	none
test substance	:	BCS-AA56716 (batch BCOO 5984-4-11, purity 96,7%)	GLP statement	:	Study not performed under GLP, but laboratory GLP-certified
Route	:	diet	guideline	:	Not applicable (only preliminary and explorative study design)
Species	:	Rat, Wistar RJ: WI (IOPS HAN)	acceptability	:	Yes as preliminary
group size	:	5/sex/dose	**NOAEL**	:	500 ppm (51 mg/kg bw/day)

Study design

5 rats per sex per dose group were assigned to dose groups using a randomization by weight. BCS-AA56716 was administered in the diet for 14 days to Wistar rats at the following doses: 0, 500, 2000 and 8000 ppm (equating to approximately 0, 48, 187 and 745 mg/kg/day in males and 0, 51, 201 and 800 mg/kg/day in females). A negative control group received plain diet.

Animals were observed daily for mortality and clinical signs. Physical examinations were performed at least weekly. Body weight and food consumption were recorded once weekly. Hematology and clinical chemistry parameters were determined at the end of the study. All animals were necropsied, selected organs weighed and a range of tissues were taken, fixed and examined microscopically.

Results

Table 6.8.1.3-1

Dose (ppm)	**0**		**500**		**2000**		**8000**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Mortality	No mortalities							
Clinical signs	No clinical signs during the course of the study							
Body weight	Slight not statistically significant lower mean body weight at 8000 mg/kg/day throughout the study in males and females and at 2000 mg/kg/day throughout the study in females (-12%).							
Body weight gain (g)	102	43	107	42	101	39	89	38
Food consumption	Mean food consumption was unaffected by treatment in either sex.							
Ophthalmology	Not determined							
Urinalysis	Not determined							
FOB	Not determined							
Haematology	No treatment related changes.							
Clin. Chemistry -total bilirubin (µmol/L)	0.9	1.1	0.6	0.6* (-45%	0.5	0.9	0.7	0.6* (-45%)
- Glucose concentration	5.47	4.89	3.62** (-34%)	2.86** (-42%)	3.25** (-41%0	2.53** (-48%)	2.5** (-54%)	2.68** (-45%)
- Triglyceride concentration	0.72	0.51	0.72	0.56	0.67	0.58	0.64	0.54
- Urea concentration	5.43	5.31	6.46 (+19%)	6.54 (+23%)	5.91 (+9%)	6.63 (+25%)	5.78 (+6%)	6.77 (+27%)
- Creatinine concentration	44	42	43	42	43	42	45	43
- Alanine aminotransferase	31	24	30	31	33	30	32	32
- Alanine phosphatase	187	137	186	102	162	143	191	127
Organ weights	No treatment related changes.							

Dose (ppm)	0		500		2000		8000	
	m	f	m	f	m	f	m	f
Pathology (gross and microscopic)	All changes were considered to be incidental and not treatment-related							

*p≤0.05 **p≤0.01

BCS-AA56716 dietary administration at dose levels of 0, 500, 2000 and 8000 ppm to males and females Wistar rats for at least 14 days induced no treatment-related changes with regard to survival, clinical signs, food consumption, hematological parameters, macroscopic observation and microscopic examination.

At 8000 ppm, mean body weight was similar to the controls in both sexes on study Day 8 and was slightly reduced by 4% in males and 3% in females on study Day 15 (not statistically significant) in comparison to the controls. This slight effect was attributable to a mean cumulative body weight gain reduced by 13% in males and 12% in females throughout the study (not statistically significant) when compared to the controls. Clinical chemistry revealed a mean glucose concentration reduced by 54% in males and 45% in females (p <0.01) compared to the controls. In addition, mean urea concentration was 27% higher in females (not statistically significant) in comparison to the controls. At necropsy, mean terminal body weight was lower by 6% in males and 7% in females (not statistically significant), compared to the controls. Lower mean total bilirubin concentrations (- 45%, p <0.05) were also noted at 8000 and 500 ppm. In the absence of a dose-effect relationship, these variations were considered not to be treatment related.

At 2000 ppm, mean body weight parameters were unaffected by treatment in males, whilst in females a marginal reduction in mean cumulative body weight gain was noted throughout the study (- 12% compared to the controls, not statistically significant). Clinical chemistry revealed a mean glucose concentration reduced by 41% in males and 48% in females (p <0.01) compared to the controls. In addition, mean urea concentration was 25% higher in females (not statistically significant) in comparison to the controls. At necropsy, mean terminal body weight was lower by 6% in females only (not statistically significant) compared to the controls. The change observed in males at this dose level was considered not to be adverse in view of its isolated occurrence and absence of associated histological findings.

At 500 ppm, the only changes noted for clinical chemistry consisted of a mean glucose concentration reduced by 34% in males and 42% in females (p <0.01), together with mean urea concentration higher by 23% in females (not statistically significant) in comparison to the controls. These few changes were considered not to be adverse in view of their isolated occurrence and absence of associated histological findings.

Acceptability

The study was not performed under GLP, but it was performed in a GLP-certified laboratory.

Conclusions

500 ppm (corresponding to 51 mg/kg body weight/day) was considered to be a NOAEL in females based on clinical chemistry changes.

Study 2

Reference	:	[redacted].2012	exposure	:	28 days
type of study	:	90 -days toxicity study	dose	:	0, 200, 1000 and 6000 ppm M: 0, 12.7, 66.2, 380 mg/kg bw/d F: 0, 15.6, 78.7, 472 mg/kg bw/d
year of execution	:	2011	vehicle	:	none
test substance	:	BCS-AA56716 (batch BCOO 5984-5-8, purity 97.1%)	GLP statement	:	Yes
Route	:	Dietary	guideline	:	OECD 408
Species	:	Rat, Wistar Rj:WI (IOPS HAN)	acceptability	:	Yes
group size	:	10/sex/group	**NOAEL**	:	200 ppm (12.7 mg/kg bw/d)

Study design

There were 10 animals of each sex per dose group. Animals were assigned to dose groups using a randomization by weight. BCS-AA56716 was administered in the diet for at least 90 days to Wistar rats at the following doses – 0, 200, 1000 and 6000 ppm (equating to approximately 0, 12.7, 66.2, 380 mg/kg body weight/day in males and 0, 15.6, 78.7, 472 mg/kg body weight/day in females). A negative control group received plain diet.

Clinical signs were recorded daily, and body weight was measured weekly. Food consumption was measured twice weekly during the first 6 weeks of treatment and weekly thereafter. A detailed physical examination was performed once during the acclimatization phase and weekly throughout the study. All surviving animals were subjected to a neurotoxicity assessment (functional observational battery and spontaneous motor activity)) during Week 11 of the study. Ophthalmological examinations were performed on all animals during the acclimatization phase and on all surviving animals of the control and high dose groups during Week 12. Urine samples were collected overnight during the week before necropsy from all surviving animals. Before necropsy a blood sample was collected from the retro-orbital venous plexus of each animal for haematology and clinical chemistry investigations. All animals were necropsied, selected organs weighed and a range of tissues were taken, fixed and examined microscopically.

Results

Table 6.9.1.3-2

Dose (ppm)	**0**		**200**		**1000**		**6000**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Mortality	No mortalities							
Clinical signs	No clinical signs during the course of the study							
Body weight	Slight progressive statistically significant (on several occasions) lower mean body weight throughout the study in males and females (see table below) at 1000 and 6000 ppm.							

Dose (ppm)	0		200		1000		6000	
	m	f	m	f	m	f	m	f
Body weight gain (g)	317	133	335	131	255**	121	283	106**
Food consumption	Mean food consumption was affected by treatment in either sex at 1000 and 6000 ppm.							
Ophthalmology	no treatment-related changes at the ophthalmological examinations.							
Urinalysis	Urinalysis revealed higher urinary volumes and lower mean refractive indices in both sexes, lower protein levels in females and lower amounts of usually observed crystals in males, compared to controls. Despite the higher urinary volumes, higher ketone levels were noted in both sexes, in correlation with the low glucose concentrations observed in the serum (See table below).							
FOB	no treatment-related changes at the neurological examinations.							
Haematology	Lower statistical significant hemoglobin concentration and lower mean corpuscular volume were observed at 6000 (-8%; -5% resp.) and 1000 ppm (-9%; -6% resp.) in females only. As a consequence, lower mean corpuscular hemoglobin and lower hematocrit were also noted. These changes were observed with no clear dose-effect relationship.							
Clin. Chemistry								
-total bilirubin (µmol/L)	1.47	2.45	1.07* (-27%)	2.16 (-12%)	0.7** (-52%)	1.55** (-37%)	0.65** (-56%)	1.3** (-47%)
- Glucose concentration	5.677	6.036	4.003** (-29%)	4.423** (-27%)	3.141** (-45%)	3.065** (-49%)	3.123** (-45%)	2.815** (-53%)
- Triglyceride concentration	0.718	0.461	0.927**	0.582	0.701	0.487	0.809	0.567
- Urea concentration	4.831	5.509	5.569* (+15%)	6.253* (+14%)	6.076** (+26%)	6.764** (+23%)	5.702* (+18%)	6.165 (+12%)
- Creatinine concentration	57.2	58.2	53.7	56.4	55.9	55.2	54.4	53.9**
- Inorganic phosphorus	2.173	1.837	2.337	1.941	2.334	1.948	2.477**	2.103**
- Alanine aminotransferase	32.3	28.7	34.8	32.6	37.4	34.1	37.7	43.5**
- Alanine phosphatase	94.2	56.2	84.6	61.8	84.0	54.6	91.1	52.2
Organ weights	The few organ weight changes noted were considered to be incidental or attributable to the lower mean terminal body weight							
Pathology								
Macroscopy (stomach)								
- black foci in glandular part of the stomach	0/10	1/10	0/10	0/10	1/10	2/10	3/10	2/10
Microscopy								

Dose (ppm)	0		200		1000		6000	
	m	f	m	f	m	f	m	f
Incidence and severity of microscopic changes in the stomach - Glandular erosion/necrosis: focal	0/10	0/10	0/10	0/10	1/10	1/10	2/10	1/10

*p≤0.05 **p≤0.01

Table 6.9.1.3-3: Mean body weights (BW) and cumulative body weight gains (BWG)

Dosage level of BCS-AA56716 (ppm)	0	200	1000	6000
Male				
Initial BW (Day 1) (%C)	195	196 (101)	195 (100)	196 (101)
BW Week 2 (Day 8) (%C)	257	259 (101)	247 (96)	255 (99)
BW Week 4 (Day 29) (%C)	380	379 (100)	348 (91)**	364 (96)
BW Week 8 (Day 57) (%C)	465	472 (102)	408 (88)**	435 (94)
Final BW Week 13 (Day 92) (%C)	**512**	**531 (104)**	**450 (88)****	**479 (94)**
BWG Weeks 1-4 (Days 1 to 29) (%C)	186	183 (97)	153 (82)**	168 (90)
BWG Weeks 1-8 (Days 1 to 57) (%C)	270	276 (102)	213 (79)**	239 (88)
Overall BWG Weeks 1-13 (Days 1-92) (%C)	**317**	**335 (105)**	**255 (80)****	**283 (89)**
Female				
Initial BW (Day 1) (%C)	**162**	**164 (101)**	**161 (100)**	**162 (100)**
BW Week 1 (Day 8) (%C)	188	189 (101)	188 (100)	184 (98)
BW Week 4 (Day 29) (%C)	240	242 (101)	235 (98)	225 (94)**
BW Week 8 (Day 57) (%C)	274	276 (101)	260 (95)	255 (93)**
Final BW Week 13 (Day 92) (%C)	**295**	**295 (100)**	**283 (96)**	**268 (91)****
BWG Weeks 1-4 (Days 1 to 29) (%C)	78	78 (100)	74 (94)	63 (81)**
BWG Weeks 1-8 (Days 1 to 57) (%C)	112	112 (100)	99 (88)*	93 (83)**
Overall BWG Weeks 1-13 (Days 1-92) (%C)	**133**	**131 (99)**	**121 (91)**	**106 (80)****

% C : % *vs.* control, calculated on raw data
* : Statistically different (p ≤0.05) from the control
** : Statistically different (p ≤0.01) from the control

Table 6.9.1.3-4: Urinary volume (mL) - Mean ± standard deviation (% change when compared to controls)

BCS-AA56716 Dose level (ppm)	0	200	1000	6000
Male	3.50 ± 1.084	7.43 ± 1.730 (+ 112%)	11.91 ± 5.731 *** (+ 240%)	12.80 ± 4.508 *** (+ 266%)
Female	1.66 ± 1.727	2.13 ± 1.286 (+ 28%)	3.41 ± 1.559 (+ 105%)	4.24 ± 1.620 ** (+ 155%)

** : p ≤0.01 *** : p ≤0.001

Table 6.9.1.3-5: Urinary ketone level - (number of urine samples observed per grade)

BCS-AA56716 Dose level (ppm)		Control	200	1000	6000
Male	Grade 0	0	0	0	0
	1	7	2	0	0
	2	3	8	0	0
	3	0	0	0	0
	4	0	0	10	10
	total	10	10	10	10
Female	Grade 0	6	6	0	0
	1	1	2	0	0
	2	0	0	5	0
	3	0	0	4	7
	4	0	0	0	2
	total	7	8	9	9

At 6000 ppm

Mean body weight was decreased by up to 7% in males and 9% in females compared to controls (statistically significant on several occasions for females), the effect being progressive throughout the study. At the end of the treatment (study Day 92), the overall mean body weight gain was reduced by 11% in males and 20% in females (p ≤0.01 for females) compared to controls. Mean food consumption was decreased by up to 9% in males (maximal effect observed on study Day 43) and 13% in females (maximal effect observed on study Day 50) throughout the study, compared to controls. The average decrease over weeks 1 to 13 was of 5% in males and 7% in females.
At the hematological evaluation, lower hemoglobin concentration (- 8%, p ≤0.01) and lower mean corpuscular volume (- 5%, p ≤0.01) were observed in females, together with lower mean corpuscular hemoglobin (- 7%, p ≤0.01) and lower hematocrit (- 6%, p ≤0.01), compared to controls. Clinical chemistry determination revealed lower mean glucose concentrations in both sexes (- 45 and - 53% in males and females, respectively, p ≤0.01), lower total bilirubin concentrations in both sexes (- 56 and - 47% in males and females, respectively, p ≤0.01), slightly higher mean inorganic phosphorus concentrations in both sexes (+ 14%, p ≤0.01 or 0.05) and slightly higher mean urea concentrations in both sexes (+ 18 and + 12% in males and females, respectively, p ≤0.05 in males), compared to controls. Urinalysis revealed higher urinary volumes and lower mean refractive indices in both sexes, lower protein levels in females and lower amounts of usually observed crystals in males, compared to controls. Despite the higher urinary volumes, higher ketone levels were noted in both sexes, in correlation with the low glucose concentrations observed in the serum.
At necropsy, a lower mean terminal body weight was observed in males (- 9%, p≤0.05) and females (- 10%, p≤0.01) when compared to the controls. The few organ weight changes noted were considered to be incidental or attributable to the lower mean terminal body weight. At the macroscopic observation, a few black foci were noted in the glandular part of the stomach in both sexes, in correlation with a few cases of focal glandular erosion/necrosis observed at the microscopic examination.

At 1000 ppm

Mean body weight was decreased by up to 13% in males and 6% in females compared to controls (statistically significant on several occasions). The effect was progressive in both sexes throughout the study, but was slightly more marked in this mid dose male group than at the high dose. At the end of the treatment (study Day 92), the overall mean body weight gain was reduced by 20% in males and 7% in females (p ≤0.01 for males) compared to controls. Mean food consumption was decreased by up to 7% in males (maximal effect observed on study Day 43) and 10% in females (maximal effect observed on study Day 50) throughout the study, compared to controls. The average decrease over weeks 1 to 13 was of 5% in males and 4% in females.
At the hematological evaluation, lower hemoglobin concentration (- 9%, p ≤0.01) and lower mean corpuscular volume (- 6%, p ≤0.01) were observed in females, together with lower mean corpuscular hemoglobin (- 8%, p ≤0.01) and lower hematocrit (- 7%, p ≤0.01). These changes were observed with no clear dose-effect relationship. Clinical chemistry determination revealed lower mean glucose concentrations in both sexes (- 45 and - 49% in males and females, respectively, p ≤0.01), lower total bilirubin concentrations in both sexes (- 52 and - 37% in males and females, respectively, p ≤0.01) and slightly higher mean urea concentrations in both sexes (+ 26 and + 23% in males and females, respectively, p ≤0.01) with no clear dose-effect relationship when compared to controls. Urinalysis revealed higher urinary volumes and lower mean refractive indices in both sexes, lower protein levels in females and lower amounts of usually observed crystals in males compared to controls. Despite the higher urinary volumes, higher ketone levels were noted in both sexes, in correlation with the low glucose concentrations observed in the serum.
At necropsy, a lower mean terminal body weight was observed in males (- 15%, p≤0.01) and females (- 7%, p≤0.05) when compared to the controls. The few organ weight changes noted were considered to be incidental or attributable to the lower mean terminal body weight. At the macroscopic observation, a few black foci were noted in the glandular part of the stomach in one or two animals from both sexes (including one control female), in correlation with focal glandular erosion/necrosis observed at the microscopic examination.

<u>At 200 ppm</u>
Treatment-related effects were noted only in the clinical chemistry determination, which revealed lower mean glucose concentrations in both sexes (- 29 and - 27% in males and females, respectively, p ≤0.01), lower total bilirubin concentrations in males (- 27%, p ≤0.05) and slightly higher mean urea concentrations in both sexes (+ 15 and + 14% in males and females, respectively, p ≤0.05). However, these changes are considered not to be adverse effects of the test substance as they do not represent any functional impairment in the test organism since no concomitant change was noted in creatinine concentrations or at the histological examination.

Acceptability
This study is considered acceptable.

Conclusion

The dose level of 200 ppm (equating to 12.7 and 15.6 mg/kg body weight/day in males and females, respectively) was considered to be a NOAEL in male and female Wistar rats, based on the minor changes noted at the clinical chemistry determination.

B.6.9.2 BYI 02960-difluoroethyl-amino-furanone (BCS-CC98193, BYI 02960-DFEAF, M34)

B.6.9.2.1 Acute toxicity BYI 02960-difluoroethyl-amino-furanone (BCS-CC98193, BYI 02960-DFEAF, M34)

Characteristics

reference	:	[redacted]. 2011	exposure	:	Gavage
type of study	:	Acute oral toxicity study	doses	:	300, 2000 mg/kg bw
year of execution	:	2011	vehicle	:	Purified water
test substance	:	BYI 02960-difluoroethyl-amino-furanone (batch NLL 8671-6-1, 98.5% purity	GLP statement	:	Yes
route	:	Oral	guideline	:	According to OECD 423
species	:	Rj Han: SD (IOPS Han)	acceptability	:	Acceptable
group size	:	3f/dose	**LD50-cut off**	:	>2000 mg/kg bw

Study design

The study was performed in accordance with OECD 423.

Three groups of three fasted, young adult female Sprague Dawley rats were given successively a single oral dose of BYI 02960-difluoroethyl-amino-furanone (batch NLL 8671-6-1, 98.5% purity) in purified water of 300, 2 000 and then 2000 mg/kg bw and were observed for 14 days. The substance was tested using a stepwise procedure, each step using three female rats. The animals were assigned to their groups by randomization based on a computerized random procedure. Following an overnight fast (18 hours), the first group received a single dose of 300 mg/kg of BYI 02960-difluoroethyl-amino-furanone (98.5% purity) by gavage. The test substance was administered in drinking water treated by reverse osmosis at a volume of 10 mL/kg bw. After the first assay, as no deaths occurred, another assay was carried out on three animals at the next higher dose-level (2000 mg/kg). After the second assay, as no deaths occurred, the results were confirmed in three other females at the dose-level of 2000 mg/kg. Clinical signs and mortality rates were determined several times on the day of administration and subsequently at least once daily for an observation period of at least 14 days. Body weights were recorded on days 1, 8 and 15. On day 15, surviving animals were sacrificed and all animals were necropsied and examined for gross pathological changes.

Results

Table 6.9.2.1-1 Mortality:

Dose mg/kg bw	Mortality	Time of death

1st 300	-	-
2nd 2000	-	-
3rd 2000	-	-

Clinical observations

At 2000 mg/kg, hypoactivity was noted in one female (first assay) between 1 hour and 4 hours after treatment. In the confirmatory assay, piloerection was observed in three females 4 hours after treatment, associated with chromorhynorrhea in one female. No clinical signs persisted on day 2 in these animals.

At 300 mg/kg, no clinical signs were noted in any animals.

Body weight

At 2000 mg/kg, lower mean body weight gain was observed in groups 2 females all over the study period (-28%) and in group 3 females between days 1 and 8 (-19%), when compared to CIT historical control data. These variations were due to lower body weight gain in 2/3 females (first assay) and 1/3 females (confirmatory assay).
Body weight of the animals treated at 300 mg/kg was unaffected by test item treatment, when compared to CIT historical control data.

Necropsy

The few macroscopic findings noted at the end of the treatment period were of those commonly recorded in the Sprague-Dawley rat and none were considered to be related to the test item administration.

Acceptability

This study is considered acceptable.

Conclusions

The LD50-cut off of BCS-AA56716 was found to be between >2000 mg/kg bw.

B.6.9.2.2 Genotoxicity BYI 02960-difluoroethyl-amino-furanone (BCS-CC98193, BYI 02960-DFEAF, M34)

Study 1

Study design and results

Type of study: Ames test, plate incorporation and preincubation method

Indicator cells	Endpoint	Res. - act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
S. typh. TA 98 TA 100 TA 102	 point mut. point mut. point mut.	 - - -	 - - -	rat liver	Phenobarbital/ß-Naphthoflavone	3-5000 µg/plate	Sokolowski, A. (2011)

Indicator cells	Endpoint	Res. - act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
TA 1535 TA 1537	point mut. point mut.	- -	- -				

Test substance: BYI 02960-difluoroethyl-amino-furanone (batch number: NLL 8671-6-1; purity 98.5%); tested in DMSO
Precipitation: No precipitation of the test substance was reported.
Toxicity: The plates incubated with the test substance showed normal background growth up to 5000 µg/plate with and without S9 mix in all strains used. No toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in the treatment groups with and without metabolic activation.
GLP statement: yes
According to OECD 471: yes
Adequate positive (+S9: 2-AA; -S9: NaN3, 4-NOPD, MMS) and negative (sterility and vehicle) controls were included.

Table 6.9.2.2-1 Plate incorporation assay with BYI 02960-difluoroethyl-amino-furanone - Mean number of revertants

Strain	**TA 98**		**TA 100**		**TA 102**		**TA 1535**		**TA 1537**	
Metabolic activation	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9
Neg. control (DMSO)	28	42	141	149	330	357	13	27	16	24
Neg. control (untreated)	26	51	144	147	320	324	13	20	20	25
BYI 02960-difluoroethyl-amino-furanone										
3 µg/plate	30	38	127	138	307	371	13	26	15	22
10 µg/plate	31	41	136	159	340	390	11	22	16	24
33 µg/plate	30	39	137	152	360	395	14	26	19	26
100 µg/plate	29	41	137	154	351	393	15	23	19	25
333 µg/plate	26	45	124	147	313	377	13	24	17	27
1000 µg/plate	29	47	135	153	328	423	12	25	16	22
2500 µg/plate	28	34	131	150	326	380	14	27	15	24
5000 µg/plate	27	41	143	160	320	386	17	29	13	30
Pos. control	408	2409	2167	2583	4464	2553	1857	301	65	298

Table 6.9.2.2-2 Pre-incubation assay with BYI 02960-difluoroethyl-amino-furanone - Mean number of revertants

Strain	**TA 98**		**TA 100**		**TA 102**		**TA 1535**		**TA 1537**	
Metabolic activation	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9
Neg. control (DMSO)	26a	40a	156	160	432	632	16	21	19	20
Neg. control (untreated)	27a	38a	186	216	445	650	13	22	20	26
BYI 02960-difluoroethyl-amino-furanone										
33 µg/plate	27a	40a	142	166	477	595	17	19	20	24
100 µg/plate	23a	40a	141	173	459	641	16	20	22	18
333 µg/plate	26a	41a	151	196	476	670	18	22	26	21
1000 µg/plate	30a	37a	158	174	427	644	17	19	20	22
2500 µg/plate	28a	40a	158	169	456	648	19	20	21	25
5000 µg/plate	28a	35a	152	186	473	655	16	21	20	25
Pos. control	382a	2532a	2464	2402	2091	4314	2237	403	106	302

a extensive bacterial growth, manual count

No indication of mutagenic effects of BYI 02960-difluoroethyl-amino-furanone could be found at assessable doses of up to 5000 g/plate in any of the Salmonella typhimurium strains used in the assay.

Acceptability

The study is considered acceptable.

Conclusions

BYI 02960-difluoroethyl-amino-furanone is not mutagenic in the Ames test.

Study 2

Study design and results

Type of study: mammalian cells in vitro, cytogenetic assay

Indicator cells	Endpoint	Res. -act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
Chinese hamster V79 cells	Chromosome aberration	-	-	rat liver	Phenobarbital/ß-naphthoflavone	-S9: 6.4, 12.8, 25.6, 51.1, 102.3, 204.5, 409.0, 818.0, 1636.0 µg/mL (4 hours treatment, harvest 18 hours after the beginning of treatment) +S9: 6.4, 12.8, 25.6, 51.1, 102.3, 204.5, 409.0, 818.0, 1636.0 µg/mL (4 hours treatment, harvest 18 hours after the beginning of treatment)	Hall, C. (2011)
Test substance: BYI 02960-difluoroethyl-amino-furanone (batch number: NLL 8671-6-1; purity 98.5%); tested in deionised water Precipitation: No precipitation of the test substance was observed. Toxicity: No cytotoxicity indicated by reduced cell numbers or mitotic indices were observed after 4 hours treatment with the test substance up to the maximum applied concentration. GLP statement: yes According to OECD 473: yes Adequate positive and negative controls were included.							

Table 6.9.2.2-3 Summary of results of the cytogenetic assay

Treatment group	Endomitotic cells in %	Polyploid cells in %	Cell numbers in % of control	Mitotic indices in % of control	Aberrant cells in %		
					incl. gapsa	excl. gapsa	with exchanges
without metabolic activation							
Deionised water	0.5	4.4	100.0	100.0	1.0	1.0	0.5
BYI 02960-difluoroethyl-amino-furanone							
6.4 µg/mL	0.6	4.3	88.6	121.7	4.0	4.0c	2.0
12.8 µg/mL	0.3	5.2	97.2	112.1	4.5	4.5c	2.0
25.6 µg/mL	0.6	5.0	95.0	116.5	9.0	8.5c	5.0
51.1 µg/mL	0.3	4.0	105.7	99.6	8.5	8.0c	2.0
102.3 µg/mL	1.0	6.4	96.6	92.6	5.5	5.5c	3.0
204.5 µg/mL	1.7	9.3	89.3	117.3	4.5	4.0c	2.5
409.0 µg/mL	0.5	8.9	102.7	86.0	5.0	4.5c	3.5
818.0 µg/mL	0.4	8.1	91.1	87.1	7.5	7.5c	4.5
1636.0 µg/mL	0.9	6.8	85.8	88.6	8.0	7.5c	4.0
EMS (1000.0 µg/mL)b	0.0	3.8	not tested	94.9	39.0	39.0c	36.0
with metabolic activation							

Table 6.9.2.2-3 Summary of results of the cytogenetic assay

Treatment group	Endomitotic cells in %	Polyploid cells in %	Cell numbers in % of control	Mitotic indices in % of control	Aberrant cells in %		
					incl. gapsa	excl. gapsa	with exchanges
Deionised water	0.3	4.5	100.0	100.0	3.0	3.0	0.5
BYI 02960-difluoroethyl-amino-furanone							
409.0 µg/mL	0.0	2.7	91.0	106.7	0.5	0.5	0.0
818.0 µg/mL	0.4	4.5	88.0	112.5	1.5	1.0	0.0
1636.0 µg/mL	0.1	3.5	103.6	97.9	2.0	2.0	1.0
CPA (1.4 µg/mL)	0.0	3.9	not tested	65.0	12.5	12.0c	2.5

a Inclusive cells carrying exchanges;
b Evaluation of 50 metaphases per culture due to strong clastogenic effects;
c Aberration frequency statistically significant higher than corresponding control values.

In the absence of S9 mix the aberration rates were statistically and significantly increased in all dose groups (6.4 to 1636 µg/mL): 4.0 to 8.5 % aberrant cells, excluding gaps were determined in comparison to the corresponding controls showing 1.0% aberrant cells, excluding gaps. The values of historical control data for chromosomal aberration showed a range from 0.0 to 4.0% aberrant cells, excluding gaps. Therefore, the two test item concentrations showing values of 4.0% aberrant cells, excluding gaps were not biologically relevant. However, all other values clearly exceeded the laboratory's historical data range without showing a clear dose-dependency. Also the number of cells carrying exchanges was distinctly increased after treatment with the test item in the absence of metabolic activation (2.0 to 5.0%) as compared to the solvent control (0.5%).
In the presence of S9 mix the aberration rates were lower than the respective solvent control and remained well within the laboratory's historical data range.

Single increased values of polyploidy metaphases (5.0 to 9.3%) and endomitotic cells (0.9 to 1.7%) were observed in different dose groups as compared to the rates of the solvent controls (4.4 and 0.5%).

Acceptability

The study is considered acceptable.

Conclusions

BYI 02960-difluoroethyl-amino-furanone induced structural chromosome aberrations in V79 cells (Chinese hamster cell line) in the absence of metabolic activation at all tested concentrations.

<u>Study 3</u>

Study design and results

Type of study: mammalian cells in vitro, gene mutation test

Indicator cells	Endpoint	Res. –act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
Chinese	Gene	-	-	rat liver	phenobarbital	-S9: 0, 51.3, 102.5, 205, 410,	Wollney, H.

Indicator cells	Endpoint	Res. –act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
hamster V79 cells	mutations (HGPRT)					820, 1640 µg/mL; +S9: 0, 51.3, 102.5, 205, 410, 820, 1640 µg/mL	(2011)
Test substance: BYI 02960-difluoroethyl-amino-furanone (batch number: NLL 8671-61-1; purity 98.5%); tested in deionised water Precipitation: precipitation of the test substance was not observed in the culture medium up to the maximum concentration tested with and without metabolic activation. Toxicity: no cytotoxic effects occurred up to the maximum concentration tested with and without metabolic activation. GLP statement: yes According to OECD 476: yes Adequate positive and negative controls were included.							

Table 6.9.2.2-4 Gene mutation in mammalian cells - 1st experiment

Treatment group	Relative cloning efficiency Ia	Relative cell density	Relative cloning efficiency IIb	Mutant colonies / 106 cells	Induction factor
Without metabolic activation (culture I)					
Solvent control with water	100.0	100.0	100.0	5.4	1.0
BYI 02960-difluoroethyl-amino-furanone					
51.3 µg/mL	98.3	n.c.	n.c.	n.c.	n.c.
102.5 µg/mL	97.1	102.9	109.8	5.7	0.9
205.0 µg/mL	91.7	109.6	99.7	13.5	2.1
410.0 µg/mL	97.1	107.9	69.8	26.8	4.2
820.0 µg/mL	94.7	108.1	75.7	19.8	3.1
1640.0 µg/mL	100.4	110.8	75.5	15.2	2.4
Positive control EMS					
150 µg/mL	51.2	99.5	95.1	100.4	15.8
Without metabolic activation (culture II)					
Solvent control with water	100.0	100.0	100.0	9.6	1.0
BYI 02960-difluoroethyl-amino-furanone					
51.3 µg/mL	99.6	n.c.	n.c.	n.c.	n.c.
102.5 µg/mL	98.1	123.9	101.0	11.5	1.2
205.0 µg/mL	97.1	116.4	90.3	13.7	1.4
410.0 µg/mL	98.5	120.2	100.1	6.4	0.7
820.0 µg/mL	96.7	118.4	72.8	11.0	1.1
1640.0 µg/mL	96.2	111.7	92.0	16.4	1.7
Positive control EMS					
150 µg/mL	50.1	117.5	117.4	68.7	7.1
With metabolic activation (culture I)					
Solvent control with water	100.0	100.0	100.0	7.4	1.0
BYI 02960-difluoroethyl-amino-furanone					
51.3 µg/mL	94.6	n.c.	n.c.	n.c.	n.c.
102.5 µg/mL	100.0	90.6	92.0	20.7	2.8
205.0 µg/mL	96.6	100.9	92.7	17.9	2.4
410.0 µg/mL	95.0	101.2	90.9	18.0	2.4
820.0 µg/mL	98.0	101.3	92.0	10.5	1.4
1640.0 µg/mL	97.2	80.6	87.3	14.1	1.9
Positive control DMBA					
1.1 µg/mL	36.7	45.0	81.4	752.9	102.2
With metabolic activation (culture II)					

Table 6.9.2.2-4 Gene mutation in mammalian cells - 1st experiment

Treatment group	Relative cloning efficiency Ia	Relative cell density	Relative cloning efficiency IIb	Mutant colonies / 106 cells	Induction factor
Solvent control with water	100.0	100.0	100.0	18.9	1.0
BYI 02960-difluoroethyl-amino-furanone					
51.3 µg/mL	99.5	n.c.	n.c.	n.c.	n.c.
102.5 µg/mL	92.2	97.0	96.2	11.2	0.6
205.0 µg/mL	104.6	82.4	94.6	15.7	0.8
410.0 µg/mL	95.8	72.0	114.1	9.5	0.5
820.0 µg/mL	99.0	97.4	90.2	15.9	0.8
1640.0 µg/mL	101.6	101.0	130.5	8.1	0.4
Positive control DMBA					
1.1 µg/mL	46.3	41.6	103.1	640.2	33.9

a cloning efficiency determined immediately after treatment to measure toxicity;
b cloning efficiency determined after the expression period to measure viability of the cells without selective agent.
n.c.: culture was not continued since a minimum of only four analysable concentrations is required.

Table 6.9.2.2-5 Gene mutation in mammalian cells - 2nd experiment

Treatment group	Relative cloning efficiency Ia	Relative cell density	Relative cloning efficiency IIb	Mutant colonies / 106 cells	Induction factor
Without metabolic activation (culture I)					
Solvent control with water	100.0	100.0	100.0	17.5	1.0
BYI 02960-difluoroethyl-amino-furanone					
51.3 µg/mL	88.6	n.c.	n.c.	n.c.	n.c.
102.5 µg/mL	91.3	65.5	86.3	15.9	0.9
205.0 µg/mL	96.3	101.1	154.5	16.4	0.9
410.0 µg/mL	90.2	101.7	116.0	22.3	1.3
820.0 µg/mL	96.0	92.2	107.3	16.7	1.0
1640.0 µg/mL	106.2	109.7	159.9	31.6	1.8
Positive control EMS					
150 µg/mL	91.8	95.5	114.1	372.5	21.2
Without metabolic activation (culture II)					
Solvent control with water	100.0	100.0	100.0	30.0	1.0
BYI 02960-difluoroethyl-amino-furanone					
51.3 µg/mL	97.8	n.c.	n.c.	n.c.	n.c.
102.5 µg/mL	94.2	106.9	83.7	21.1	0.7
205.0 µg/mL	91.0	101.9	84.8	21.5	0.7
410.0 µg/mL	93.0	86.9	91.3	16.9	0.6
820.0 µg/mL	91.0	86.1	85.2	16.7	0.6
1640.0 µg/mL	103.6	93.8	79.9	22.3	0.7
Positive control EMS					
150 µg/mL	82.6	86.7	81.7	381.8	12.7
With metabolic activation (culture I)					
Solvent control with water	100.0	100.0	100.0	24.2	1.0
BYI 02960-difluoroethyl-amino-furanone					

Table 6.9.2.2-5 Gene mutation in mammalian cells - 2nd experiment

Treatment group	Relative cloning efficiency Ia	Relative cell density	Relative cloning efficiency IIb	Mutant colonies / 106 cells	Induction factor
51.3 µg/mL	107.9	n.c.	n.c.	n.c.	n.c.
102.5 µg/mL	100.9	100.4	93.7	14.3	0.6
205.0 µg/mL	99.9	92.4	95.2	31.9	1.3
410.0 µg/mL	103.2	126.1	93.1	33.2	1.4
820.0 µg/mL	95.3	112.2	85.1	21.8	0.9
1640.0 µg/mL	97.0	125.8	92.8	27.4	1.1
Positive control DMBA					
1.1 µg/mL	48.5	47.2	65.9	773.8	32.0
With metabolic activation (culture II)					
Solvent control with water	100.0	100.0	100.0	8.2	1.0
BYI 02960-difluoroethyl-amino-furanone					
51.3 µg/mL	113.1	n.c.	n.c.	n.c.	n.c.
102.5 µg/mL	107.0	110.1	80.2	11.6	1.4
205.0 µg/mL	111.6	91.1	70.0	11.6	1.4
410.0 µg/mL	113.6	94.6	76.5	11.8	1.4
820.0 µg/mL	104.5	97.9	72.5	12.4	1.5
1640.0 µg/mL	114.7	109.7	73.5	15.0	1.8
Positive control DMBA					
1.1 µg/mL	48.7	44.8	46.5	596.2	72.3

a cloning efficiency determined immediately after treatment to measure toxicity;
b cloning efficiency determined after the expression period to measure viability of the cells without selective agent.
n.c.: culture was not continued since a minimum of only four analysable concentrations is required.

No relevant and reproducible increase in mutant colony numbers/106 cells was observed in the main experiments up to the maximum concentration. The mutant frequency generally remained well within the historical range of solvent controls (see below). The induction factor exceeded the threshold of three times the mutant frequency of the corresponding solvent control at 410 and 820 µg/mL in the first culture of the first experiment without metabolic activation. This effect however, was judged as biologically irrelevant since all absolute values of the mutation frequency remained within the range of historical solvent controls. Furthermore, the increase was not reproduced in the parallel culture performed under identical experimental conditions and was not dose dependent as indicated by the lacking statistical significance.

A linear regression analysis (least squares) was performed to assess a possible dose dependent increase of mutant frequency. A significant dose dependent trend of the mutation frequency indicated by a probability value of <0.05 was determined in the first culture of the second experiment without metabolic activation and in the second culture of the second experiment with metabolic activation. Both trends were judged as irrelevant however, as all of the individual levels of the mutation frequency remained well within the range of historical solvent controls.

In both experiments of this study (with and without metabolic activation) the range of the solvent controls was from 6.4 up to 30.0 mutant colonies per 106 cells; the range of the groups treated with the test item was from 5.7 up to 33.2 mutant colonies per 106 cells.

Historical controls:

These values represent the historical control data from 2009 – 2010

Number of mutant colonies per 10^6 cells		
without metabolic activation (4 hours treatment time)		
	Positive control EMS 150 µg/mL	Solvent control (medium, water, DMSO, ethanol, THF)
Range:	65.0 – 1386.4	4.4 – 42.9
Mean value:	181.8	16.7
Standard deviation:	170.5	8.2
Number of studies:	59	60
with metabolic activation (4 hours treatment time)		
	Positive control DMBA 1.1 - 2.0 µg/mL	Solvent control (medium, water, DMSO, ethanol, THF)
Range:	64.3 – 2634.0	3.3 – 45.3
Mean value:	939.4	17.1
Standard deviation:	406.1	7.6
Number of studies:	59	59
without metabolic activation (24 hours treatment time)		
	Positive control EMS 75 – 300 µg/mL	Solvent control (medium, water, DMSO, ethanol, THF)
Range:	51-6 – 1925.5	2.6 – 40.3
Mean value:	358.2	18.0
Standard deviation:	246.6	8.2
Number of studies:	48	48

Acceptability

The study is considered acceptable.

Conclusions

In this study, BYI 02960-difluoroethyl-amino-furanone has no mutagenic potency in vitro in the CHO/HGPRT assay.

Study 4

Datarequirement:

Applicant to provide historical control data for the in vivo micronucleus assay performed with BYI 02960-difluoroethyl-amino-furanone.

In the main study, groups of 7 male mice received twice intraperitoneally administrations of BYI 02960-difluoroethyl-amino-furanone at 125, 250 and 500 mg/kg bw, dissolved in sterile water at 24 hours interval. The volume administered was 10 mL/kg bw. No clinical signs were recorded at 125 mg/kg bw. Clinical signs recorded in animals treated at 250 mg/kg bw were reduction of spontaneous activity, eyelid closure and ruffled fur mainly observed after the first administration. The same clinical signs were recorded in the animals treated at 500 mg/kg bw but after both administration.

In comparison to the corresponding vehicle control values there was no biologically or statistically relevant enhancement in the frequency of the detected micronuclei at any preparation interval and dose level after administration of the test item. The mean values of micronuclei observed after treatment with BYI 02960-difluoroethyl-aminofuranone were below the value of the vehicle control group for the low dose. A dose dependent increase occurred within the three test item dose groups resulting in micronuclei frequencies above the concurrent vehicle group in the mid and high dose groups. However, all values were within the laboratory's historical control data range and additionally, one isolated increased value of micronuclei was observed in the high dose group (animal no. 26) which was still within individual control data and below the lowest value obtained in the positive control data. Therefore, the effect was considered to be not biologically relevant.

Table 1: Micronuclei in polychromatic erythrocytes (PCE) (scoring 24 hours after treatment).

Test substance	Dose in mg/kg bw	Animal N°	Micronucleated cells per 2000 PCEs per animal
Sterile water	0	1	0
		2	1
		3	4
		4	4
		5	1
		6	1
		7	3
		Mean	2.0
		% cells with micronuclei	0.100
BYI 02960-difluoroethyl-aminofuranone	125	8	4
		9	0
		10	3
		11	1
		12	2
		13	2
		14	0
		Mean	1.7
		% cells with micronuclei	0.086
	250	15	3
		16	3
		17	3
		18	3

		19	2
		20	1
		21	1
		Mean	2.3
		% cells with micronuclei	0.114
	500	22	2
		23	3
		24	2
		25	4
		26	7
		27	2
		28	3
		Mean	3.3
		% cells with micronuclei	0.164
Cyclophosphamide	40	29	34
		30	45
		31	41
		32	30
		33	42
		34	28
		35	55
		Mean	39.3
		% cells with micronuclei	1.964

Table 2: Historical control data from studies performed between 2006 and 2010

Micronucleated cells	Negative Controls Males	Positive Controls (CPA) Males
Mean ± S.D. (%)	0.11 ± 0.04	2.56 ± 0.63
Range of mean group value (%)	0.04 ± 0.25	0.85 ± 4.52
Range (individual animal data)	0 - 12	10 - 139
Number of Experiments	186	184

Study design and results

Type of study: mouse micronucleus test

Indicator cells	Endpoint	Result	Dose range	Reference
Mouse, Crl:NMRI Two intraperitoneal injections separated by 24 hours: 7/male/dose (vehicle and positive control, low, mid and high dose)	Micronuclei (bone marrow)	-	Pretest dose selection: 500, 750 and 1000 mg/kg bw (m/f) Main test: 125, 250 and 500 mg/kg bw Dosing route: intraperitoneal Dosing volume: 10 mL/kg bw Vehicle (sterile water) and an adequate positive control was included	Hall, C. (2011)
Test substance: BYI 02960-difluoroethyl-amino-furanone (batch number NLL8671-6-1; purity 98.5%); tested in sterile water Toxicity: at 250 and 500 mg/kg bw, reduction of spontaneous activity, abdominal position, eyelid closure and ruffled fur were observed GLP statement: yes According to OECD 474: yes				

Table 6.9.2.2-6 Results of the micronucleus test with BYI 02960-difluoroethyl-amino-furanone

Treatment group	Number of evaluated PCE	PCE per 2000 erythrocytes	PCEs with micronuclei (%)
Negative control (sterile water)	14,000	1158	0.100
BYI 02960-difluoroethyl-amino-furanone			
125 mg/kg bw	14,000	1120	0.086
250 mg/kg bw	14,000	1118	0.114
500 mg/kg bw	14,000	1046	0.164
Positive control			
Cyclophosphamide (40 mg/kg bw)	14,000	1039	1.964*

* $p < 0.05$ in non-parametric Mann-Whitney test

In comparison to the corresponding vehicle control values there was no biologically relevant enhancement in the frequency of the detected micronuclei at any dose level after administration of the test item. The mean values of micronuclei observed after treatment with BYI 02960-difluoroethyl-amino-furanone were below the value of the vehicle control group for the low dose. A dose dependent increase occurred within the three test item dose groups resulting in micronuclei frequencies above the concurrent vehicle group in the mid and high dose groups. However, all values were within the laboratory's historical control data range and additionally, one isolated increased value of micronuclei was observed in the high dose group. Therefore, the effect is considered to be not biologically relevant.

Acceptability

The study is considered acceptable.

Conclusions

BYI 02960-difluoroethyl-amino-furanone has no potential to induce micronuclei in mouse bone marrow cells.

<u>Study 5</u>

Study design and results

Type of study: unscheduled DNA synthesis in rat hepatocytes

Indicator cells	Endpoint	Result	Dose range	Reference
Rat (Wistar), single oral gavage treatment: 4/male/dose (negative and positive control, low and high dose) per preparation interval (4 and 16 hours)	unscheduled DNA synthesis (UDS)	-	Main test: 1000 and 2000 mg/kg bw Dosing route: oral (gavage) Dosing volume: 10 mL/kg bw Vehicle control and an adequate positive control were included	Hall, C. (2011)

Indicator cells	Endpoint	Result	Dose range	Reference
Test substance: BYI 02960-difluoroethyl-amino-furanone (batch number NLL8671-6-1; purity 98.5%); tested in sterile water Toxicity: at 1000 and 2000 mg/kg bw, reduction of spontaneous activity and ruffled fur were observed GLP statement: yes According to OECD 486: yes				

Table 6.9.2.2-7 Results of the UDS test with BYI 02960-difluoroethyl-amino-furanone 4 hours treatment experiment

Treatment group	Animal number	Mean Nuclear Grain Count (mean ± SD)	Mean Cytoplasmic Grain Count (mean ± SD)	Mean Net grain count (mean ± SD)	Mean nuclear grains of cells in Repair (mean ± SD)	% Cells in repair
Vehicle control (sterile water)	1	12.37 ± 6.56	30.39 ± 9.21	- 6.55 ± 6.12	7.33 ± 1.53	3
	2	11.56 ± 5.87	24.07 ± 8.81	- 7.90 ± 5.69	9.00 ± 0.00	2
	3	14.88 ± 6.06	32.80 ± 13.40	- 13.08 ± 8.54	0.00 ± 0.00	0
	4	11.30 ± 5.98	20.74 ± 7.15	- 8.79 ± 7.92	10.00 ± 7.07	2
BYI 02960-difluoroethyl-amino-furanone (1000 mg/kg bw)	5	13.74 ± 15.58	45.41 ± 17.04	- 8.96 ± 9.86	6.80 ± 2.49	5
	6	10.05 ± 3.98	35.42 ± 18.12	- 7.61 ± 5.39	0.00 ± 0.00	0
	7	15.92 ± 6.35	30.38 ± 11.26	- 7.47 ± 6.65	6.50 ± 0.71	2
	8	13.01 ± 5.44	30.73 ± 10.14	- 11.06 ± 7.72	11.00 ± 0.00	1
BYI 02960-difluoroethyl-amino-furanone (2000 mg/kg bw)	9	12.15 ± 4.67	29.01 ± 9.72	- 9.42 ± 6.81	0.00 ± 0.00	0
	10	17.15 ± 7.86	36.37 ± 13.08	- 10.21 ± 8.07	8.33 ± 3.06	3
	11	43.06 ± 17.55	31.43 ± 6.67	- 16.47 ± 13.71	9.25 ± 2.50	4
	12	19.23 ± 11.67	21.85 ± 6.75	- 10.05 ± 8.08	6.33 ± 0.58	3
Positive control (DMH 80 mg/kg bw)	13	46.91 ± 18.57	13.80 ± 8.39	32.74 ± 15.84	33.05 ± 15.61	99
	14	67.28 ± 19.63	21.41 ± 8.97	49.41 ± 18.41	49.41 ± 18.41	100
	15	55.58 ± 13.17	31.23 ± 9.59	27.67 ± 12.02	27.67 ± 12.02	100
	16	57.73 ± 23.86	50.49 ± 11.14	37.16 ± 23.21	40.45 ± 21.15	92

Table 6.9.2.2-8 Results of the UDS test with BYI 02960-difluoroethyl-amino-furanone 16 hours treatment experiment

Test group	Animal number	Mean Nuclear Grain Count (mean ± SD)	Mean Cytoplasmic Grain Count (mean ± SD)	Mean Net grain count (mean ± SD)	Mean nuclear grains of cells in Repair (mean ± SD)	% Cells in repair
Vehicle control (sterile water)	17	15.17 ± 6.05	30.39 ± 9.21	- 15.22 ± 7.62	7.00 ± 0.00	1
	18	12.98 ± 5.57	24.07 ± 8.81	- 11.09 ± 7.50	0.00 ± 0.00	0
	19	19.81 ± 8.98	32.80 ± 13.40	- 12.99 ± 9.40	5.00 ± 0.00	2
	20	11.87 ± 5.77	20.74 ± 7.15	- 8.87 ± 6.30	13.00 ± 0.00	1
BYI 02960-difluoroethyl-amino-furanone (2000 mg/kg bw)	21	35.74 ± 13.50	45.41 ± 17.04	- 9.67 ± 10.76	8.86 ± 2.34	7
	22	25.89 ± 14.32	35.42 ± 18.12	- 9.53 ± 8.27	9.00 ± 0.00	1
	23	20.15 ± 7.39	30.38 ± 11.26	- 10.23 ± 8.68	11.00 ± 0.00	1
	24	20.78 ± 8.01	30.73 ± 10.14	-9.95 ± 8.97	6.50 ± 1.00	4
BYI 02960-difluoroethyl-amino-furanone (2000 mg/kg bw)	25	14.36 ± 6.35	29.01 ± 9.72	- 14.65 ± 9.28	0.00 ± 0.00	0
	26	19.37 ± 8.90	36.37 ± 13.08	-17.00 ± 10.20	0.00 ± 0.00	0
	27	17.15 ± 6.48	31.43 ± 6.67	- 14.28 ± 8.39	8.00 ± 0.00	1
	28	11.58 ± 5.19	21.85 ± 6.75	- 10.27 ± 6.52	5.00 ± 0.00	1

Positive control (2-AAF 100 mg/kg bw)	29	30.63 0 ± 12.46	13.80 ± 8.39	16.83 ± 10.08	18.19 ± 8.92	93
	30	34.38 ± 10.82	21.41 ± 8.97	12.97 ± 8.16	14.85 ± 7.11	86
	31	43.39 ± 10.82	31.23 ± 9.59	12.16 ± 7.88	14.21 ± 6.26	86
	32	64.54 ± 11.94	50.49 ± 11.14	14.05 ± 8.83	16.76 ± 6.76	83

The viability of the hepatocytes was not substantially affected by the in vivo treatment with the test item at any of the treatment periods or dose groups. The inter-individual variations obtained for the numbers and the viabilities of the isolated hepatocytes were in the range of the historical laboratory control.

No dose level of the test item revealed UDS induction in the hepatocytes of the treated animals as compared to the current vehicle controls. Neither the nuclear grain counts nor the resulting net grain counts were distinctly increased after in vivo treatment of the animals with the test item at 4 hours or 16 hours, respectively. Net grain counts obtained after treatment with the test item remained consistently negative. In addition, no substantial shift to higher values of percentage of cells in repair was reported.

Acceptability

The study is considered acceptable.

Conclusions

BYI 02960-difluoroethyl-amino-furanone has no potential to induce unscheduled DNA synthesis in hepatocytes of treated rats.

Summary

The results from the in vitro and in vivo genotoxicity studies with BYI 02960-difluoroethyl-amino-furanone are summarised in table 6.8.1.2-8 and 6.8.1.2-9, respectively.

Table 6.9.2.2-9 In vitro genotoxicity studies

Test substance	Type of study		Result		Reference
	Indicator cells	Endpoint	without activation	with activation	
BYI 02960-difluoroethyl-amino-furanone	S. typh. TA 98 TA 100 TA 102 TA 1535 TA 1537	Point mutation	-	-	Sokolowski, A. (2011)
BYI 02960-difluoroethyl-amino-furanone	Chinese hamster V79 cells	Chromosome aberration	+	-	Hall, C. (2011)
BYI 02960-difluoroethyl-amino-furanone	Chinese hamster V79 cells	Gene mutations (HGPRT)	-	-	Wollney, H. (2011)

Table 6.9.2.2-10 In vivo genotoxicity studies

Test substance	Type of study		Result	Reference
	Species	Endpoint		
BYI 02960-difluoroethyl-amino-furanone	Mouse, Crl:NMRI	Micronuclei (bone marrow)	-	[redacted] (2011)

BYI 02960-difluoroethyl-amino-furanone	Rat, Wistar	unscheduled DNA synthesis (UDS)	-	[redacted] (2011)

BYI 02960-difluoroethyl-amino-furanone was not mutagenic in the Ames test.
BYI 02960-difluoroethyl-amino-furanone induced structural chromosome aberrations in V79 cells (Chinese hamster cell line) in the absence of metabolic activation at all tested concentrations.
BYI 02960-difluoroethyl-amino-furanone has no mutagenic potency in vitro in the CHO/HGPRT assay.

BYI 02960-difluoroethyl-amino-furanone has no potential to induce micronuclei in mouse bone marrow cells.
BYI 02960-difluoroethyl-amino-furanone has no potential to induce unscheduled DNA synthesis in hepatocytes of treated rats.

Overall, it can be concluded that BYI 02960-difluoroethyl-amino-furanone has not potential to induce genotoxic effects in vivo.

B.6.9.2.3 Short-term studies

Study 1

Characteristics

Reference	:	[redacted].; 2012	exposure	:	14 days
type of study	:	14-day dose range finding toxicity/palatability study	dose	:	0, 1280, 3200, 8000 and 20000 ppm (equating to approximately 0, 135, 339, 736 and 1226 mg/kg bw/d in males and 0, 135, 335, 741 and 2254 mg/kg bw/d in females
year of execution	:	2011	vehicle	:	none
test substance	:	BYI02960-difluoroethyl aminofuranone, a metabolite of BYI 02960 (batch number NLL 8671-12-1: a white powder, 98.9 % w/w purity)	GLP statement	:	Study not performed under GLP, but laboratory GLP-certified
Route	:	diet	guideline	:	Not applicable (only preliminary and explorative study design)
Species	:	Rat, Crl:WI rats	acceptability	:	Yes as preliminary
group size	:	5/sex/dose	**NOAEL**	:	1280 ppm (equating to 135 mg/kg bw/d)

Study design

BYI 02960-difluoroethyl aminofuranone, a metabolite of BYI 02960 (batch number NLL 8671-12-1: a white powder, 98.9 % w/w purity) was administered continuously via the diet to groups of Wistar rats (5/sex/group) for at least 14 days at concentrations of 0, 1280, 3200, 8000 and 20000 ppm (equating to approximately 0, 135, 339, 736 and 1226 mg/kg body weight/day in males and 0, 135, 335, 741 and 2254 mg/kg body weight/day in females). A similarly constituted group received untreated diet and served as a control. Animals were observed daily for mortality and clinical signs. Physical examinations were performed at least weekly. Body weight was recorded on days -7, 0, 1, 3, 6, 7, 10, 13 and 14. Full feeders were weighed on days -7, 0, 1, 3, 6, 7 and 10 and empty feeders were

weighed on days 0, 1, 3, 6, 7, 10 and 13. Hematology and clinical chemistry parameters were determined at the end of the study. All animals were necropsied, selected organs weighed and a range of tissues were retained in fixative. No histopathological examination was performed.

Results

Table 6.9.2.3-1

Dose (ppm)	**0**		**1280**		**3200**		**8000**		**20000**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Mortality	No mortalities									
Clinical signs	No clinical signs during the course of the study									
Body weight	Statistically significant lower mean body weight at 3200, 8000 and 20000 mg/kg/day throughout the study in females and at 8000 and 20000 mg/kg/day throughout the study in males. (See table below)									
Body weight (%)			-0.33	-3.56	-4.5	-11.93**	-16.48**	-15.88**	-32.43**	-30.03**
Food consumption	Mean food consumption was affected by treatment in either sex (- 59% lower in the males and up to - 25% in the females at 20000 ppm)									
Ophthalmology	Not determined									
Urinalysis	Not determined									
FOB	Not determined									
Haematology	Equivocal test related effects (no similar statistical differences in males and females and variations generally comparable with the physiological ranges (as reticulocyte mean values, RBC, HGB and PLT).									
Clin. Chemistry -total bilirubin (µmol/L)	6.86	7.02	7.04 (+3%)	7.16 (+2%)	6.66 (-3%)	6.94 (-1%)	7.14 (+4%)	8.64 (+23%)	8.62* (+26%)	9.73 (+39%)
- Glucose concentration (mmol/L)	4.24	5.54	3.92 (-7%)	4.35** (-21%0	2.98** (-30%)	4.13** (-25%)	2.15** (-49%)	2.66 (-52%)	2.61** (-38%)	2.93** (-47%)
- Urea concentration (mmol/L)	6.37	8.59	5.57 (-13%)	8.50 (-1%)	5.91 (-7%)	8.61 (0%)	6.21 (-2%)	9.90 (+15%)	8.41** (-32%)	10.35 (+20%)
- Chol. (mmol/L)	1.85	2.11	2.02 (10%)	2.05 (-3%)	2.42 (+31%)	2.71 (+29%)	2.55* (+38%)	2.60 (+23%)	3.06** (+65%	3.15** (+49%)
- Alb.	29.46	33.08	30.30 (+3%)	32.70 (-1%)	30.38 (+3%)	35.00 (+6%)	32.36** (+10%)	35.04 (+6%)	36.56** (+24%)	36.25 (+10%)

Dose (ppm)	0		1280		3200		8000		20000	
	m	f	m	f	m	f	m	f	m	f
Organ weights	Due to highly significant effect on body weight there were no relevant differences in organ weights (only for the heart, brain, kidney, liver, testes and epididymides and in males for the thymus, and prostate in the high doses after adjustement for body weight).									
Pathology Macroscopy	Small prostate and seminal vesicles in 5/5 and small spleen in 2/5 males associated with the potential test item related changes in the terminal body weights and correlated with the organ changes.									
Microscopy	Not determined									

*p≤0.05 **p≤0.01

Table 6.9.2.3-2: Summary of body weight and body weight gain data (g)

Dose levels of Test item (ppm)	0	1280	3200	8000	20000
Male					
Initial BW (Day 0) (%C)	207.6	211.0(102%)	210.6(101%)	209.0(101%)	208.0(100%)
BW (Day 1) (%C)	215.0	218.8 (102%)	212.0 (99%)	202.2 (94%)	198.0* (92%)
BW (Day 7) (%C)	265.0	266.0 (100%)	255.6 (96%)	235.2** (89%)	196.2** (74%)
Final BW (Day 14) (%C)	302.2	301.2 (100%)	288.6 (95%)	252.4** (84%)	204.2** (68%)
BWG (Day 0-1) (%C)	7.4	7.8 (105%)	1.4* (19%)	- 6.8** (-192%)	- 10.0** (-235%)
BWG (Day 1-3) (%C)	17.6	15.4 (87%)	16.2 (92%)	6.8** (39%)	-9.4** (-53%)
BWG (Day 7-10) (%C)	30.2	26.2 (87%)	25.4 (84%)	16.0** (53%)	11.2** (37%)
Overall BWG (Days 0-14) (%C)	94.6	90.2 (95%)	78.0 (82%)	43.4** (46%)	- 3.8** (-104%)
Female					
Initial BW (Day 0)(%C)	163.4	160.2 (98%)	156.8 (96%)	158.8 (97%)	155.4 (95%)
BW (Day 1) (%C)	163.0	164.2 (101%)	157.0 (96%)	153.8 (94%)	143.8** (88%)
BW (Day 7) (%C)	187.8	186.6 (99%)	174.0 (93%)	168.6* (90%)	147.4** (78%)
Final BW (Day 14) (%C)	207.8	200.4 (96%)	183.0** (88%)	174.8** (84%)	145.4** (70%)
BWG (Day 0-1) (%C)	-0.4	4.0 (900%)	0.2 (150%)	- 5.0 (-1350%)	- 11.6** (3000%)
BWG (Day 1-3) (%C)	10.0	8.2 (82%)	8.0 (80%)	6.4 (64%)	- 2.6** (-126%)
BWG (Day 7-10) (%C)	18.2	10.6* (58%)	11.2 (62%)	13.4 (74%)	3.6** (20%)
Overall BWG (Days 0-14) (%C)	44.4	40.2 (90%)	26.2** (59%)	16.0** (36%)	- 10.0** (-123%)

* : p <0.05 ** : p <0.01

There was no unscheduled mortality during the study. There were no clinical signs during the 14-day administration in either the treated, or control animals.

Adverse effects considered related to BYI 02960-difluoroethyl aminofuranone administration under the conditions of this study were noted on the animal body weight and/or body weight gain values in the 3200, 8000 and/or 20000 ppm male and/or female animals, with an apparent dose response.

At 3200 ppm, statistically lower body weight gain was noted in males between Days 0 and 1 (1.4 g *vs.* 7.4 g) as well as when calculated for the whole duration of the study (Days 0 to 14, 78 g *vs.* 94.6 g, p <0.05, 37.04% *vs.* 45.67 %). In the Low-mid dose 3200 ppm Group 3 females, statistically lower

body weights up to -11.93% were recorded approximately after 1 week of treatment on Day 6 and towards the end of the treatment on Days 10, 13 and 14.

At 8000 ppm, statistically lower mean body weights up to -16.48% were noted in the Group 4 males after Day 3 of the treatment, and up to -15.88%, in the females, after Day 6, until its completion on Day 14.
In the 20000 ppm Group 5 animals, body weight loss was noted in both males and females between Days 0 and 14.

The mean daily food consumption evaluated based on the cage-based weekly food consumption of male and female rats was lower than control in the 8000 and 20000 ppm Groups 4 and 5, up to - 59% lower in the males and up to - 25% in the females at 20000 ppm, effects considered possibly associated with test item administration. However, spillage was noted in the High dose Group 5 females and the dose response was not clear.

Statistically lower than control reticulocyte mean values were noted in the 20000 ppm males and 8000 and 20000 ppm females and were regarded as possibly associated with BYI 02960-difluoroethyl aminofuranone administration.

Lower than control glucose blood levels were noted in both males and females in all the dose groups, attaining statistical significance at above and including 3200 ppm in the males, with mean values up to - 49% lower than control, and at all the dose levels tested in the females, with mean values of up to - 52%, $p < 0.01$. Cholesterol mean values increased in the 3200 ppm group and above to up to 65% in the males, and up to 49% in the females. Albumin and A/G ratio were slightly higher than control, attaining statistical significance at 8000 and/or 20000 ppm in the males and in both males and females, respectively.

In addition, higher urea was noted in the 20000 ppm males, and in the 20000, and 8000 ppm females. Potentially test item-related macroscopic changes were observed at necropsy in the High dose group fed diet with 20000 ppm BYI 02960-difluoroethyl aminofuranone. These consisted of small prostate and seminal vesicles in 5/5 males and small spleen, in 2/5 males, were associated with the potential test item related changes in the terminal body weights and correlated with the organ weight changes, however, a definitive attribution cannot be made without histopathological evaluation.

Acceptability

The study was not performed under GLP, but it was performed in a GLP-certified laboratory.

Conclusions

In conclusion, based on the effects noted in the current 14-day preliminary study a NOEL is set at 1280 ppm (equating to 135 mg/kg bw/d) based on the lower blood glucose concentrations in females.

The dose levels selected for the upcoming 28-day dietary study with BYI 02960-difluoroethyl aminofuranone were 200, 800 and 3000 ppm.

Study 2

Reference	:	[redacted].; 2012	exposure	:	28 days
type of study	:	A 28-day dietary toxicity study	dose	:	0, 200, 800 and 3000 ppm (equating to approximately 0, 17, 68 and 243 mg/kg bw/d in males and 19, 76 and 273 mg/kg bw/d in females).
year of execution	:	2011	vehicle	:	none
test substance	:	BYI 02960-difluoroethyl aminofuranone, a metabolite of BYI 02960 (batch number NLL 8671-12-1: a white powder, 98.9% w/w purity)	GLP statement	:	Yes
Route	:	Dietary	guideline	:	OECD 407
Species	:	Crl:WI rat	acceptability	:	Yes
group size	:	10/sex/group	**NOAEL**	:	3000 ppm equating to approximately 243 mg/kg bw/day

Study design

BYI 02960-difluoroethyl aminofuranone, a metabolite of BYI 02960 (batch number NLL 8671-12-1: a white powder, 98.9% w/w purity) was administered continuously via the diet to groups of Wistar rats (10/sex/group) for 28 consecutive days at concentrations of 200, 800 and 3000 ppm (equating to approximately 17, 68 and 243 mg/kg body weight/day in males and 19, 76 and 273 mg/kg body weight/day in females). A similarly constituted group received untreated diet and served as a control. Animals were observed daily for mortality and clinical signs. Physical examinations were performed at least weekly. An evaluation for any potential neuro- or ophthalmo-toxicity was conducted on Day 26. Body weight and food consumption were measured at least weekly. Prior to necropsy, the oestrous cycle of all females was evaluated by examination of vaginal smears. Clinical pathology investigations (hematology, coagulation, clinical chemistry and urinalysis) were conducted at the completion of the treatment period, before necropsy on Day 28. At termination, necropsy with macroscopic examination was performed. Weights of selected organs were recorded and representative tissues/organs were sampled and preserved in appropriate fixatives. Full histopathology was performed on the selected list of tissues from the control and high dose animals and on any macroscopic abnormality from all animals.

Results

Table 6.9.2.3-3

Dose (ppm)	**0**		**200**		**800**		**3000**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Mortality	No unscheduled mortality during the study.							
Clinical signs	No treatment-related clinical signs noted during the study.							

Dose (ppm)	0		200		800		3000	
	m	f	m	f	m	f	m	f
Body weight	Slight but not statistically significant (on several occasions) lower body weight at 3000 ppm in males and females (5-6% below control at termination).							
Body weight gain (g)	148.9	64.8	168.7 (+13.30%)	52.4 (-19.14%)	162.7 (+9.27%)	61.4 (-5.25%)	130.6 (-12.29%)	56.2(-13.27%)
Food consumption	Mean food consumption was unaffected by treatment in either sex.							
Ophthalmology	No treatment-related changes at the ophthalmological examinations.							
Urinalysis	No statistically significant differences or toxicologically relevant effects							
FOB	There are no dose or gender consistent responses observed, only slightly lower landing foot splay mean values for forelimbs in the 800 and 3000 ppm males only, or slightly higher grip strength for hindlimbs in the 200 and 3000 ppm females only							
Haematology	No consistent dose or gender response. The individual and mean values remained within the physiological ranges							
Clin. Chemistry -total bilirubin (µmol/L)	4.740	7.460	4.070 (-14%)	7.960	4.670 (-1.5%)	7.460	3.480 (-27%)	8.240 (+10.5%)
- Glucose concentration (mmol/L)	6.497	7.216	7.403 (+14%)	7.686 (+6%)	5.583 (-14%)	6.900 (-4%)	5.846 (-10%)	5.671 (-11%)
- Urea concentration (mmol/L)	7.336	8.787	7.147 (-2.6%)	9.046 (+3%)	7.610 (+3.7%)	9.560 (+8.9%)	6.623 (-9.7%)	9.769 (+11%)
- Chol. (mmol/L)	1.703	1.941	1.572 (-7.7%)	1.996 (+2.8%)	1.882 (+10.5%)	2.026 (+4.4%)	1.900 (+11.6%)	2.170 (+11.8%)
- Alb. (g/L)	31.32	35.54	31.13 (-0.6%)	34.05 (-4.2%)	30.96 (-1.1%)	31.52* (-11.3%)	29.94** (-4.4%)	34.62 (2.6%)
- Tot. prot.	56.36	63.33	56.24 (-0.21%)	60.77 (-4%)	55.10 (-2.2)	58.62** (-7.4%)	54.41 (-3.5%)	58.75** (-7.2%)
- Bile aci	14.4	12.59	11.79 (-18%)	16.53 (+31%)	17.68* (+23%)	16.85 (+34%)	11.75 (-18%)	20.92** (+66%)

Dose (ppm)	0		200		800		3000	
	m	f	m	f	m	f	m	f
Organ weight	The values within the normal range and the variations with low magnitude showed no consistent response between genders, (Slightly higher kidney weight adjusted for the terminal body weight in the 3000 ppm male and female animals, but not when adjusted for the brain weight, slightly higher testes and seminal vehicles weights relative to the terminal body weight in the 3000 ppm males, or slightly higher testes, or liver (females) weight relative to the brain weight in the 800 ppm animals, but not in the other gender, and not at 3000 ppm)							
Pathology	No test item-related macroscopic or microscopic findings							

*p≤0.05 **p≤0.01, NC = no significant change

There was no unscheduled mortality during the study, clinical signs, signs of neurotoxicity or any test item related, or adverse effects on the landing foot splay or grip strength under the conditions of this study. Evaluation of the vaginal smears prior to necropsy showed the expected distribution of the oestrus cycle phases within the normal population of female Wistar rats.

There was a slight but not statistically significant trend towards lower body weights at 3000 ppm in both sexes (5 - 6% below control at termination), this body weight effect corresponds with findings in a preliminary study where body weights were 15 - 20% below control at 8000 ppm. There were statistically significantly lower weight gains in both sexes in the first week of the study but the overall weight gains for the study duration were not statistically different. Other statistically significant differences were not considered to be of biological significance.

There were no test item related adverse effects on the animal food consumption during the study. No test-item related adverse effects were noted on the food conversion efficiency FCE values (g/g, calculated as weekly body weight gain g/weekly food consumption g) at any of the dose levels tested and the mean values were similar with the ones recorded in the control group.

The variations in hematological parameter compared to controls were not considered toxicologically significant or related to treatment, in the absence of a consistent dose or gender response and as the individual and mean values remained within the physiological ranges.

No toxicologically significant, adverse effects were noted at evaluation of the clinical chemistry parameters evaluated at up to and including 3000 ppm. No statistically significant differences or toxicologically relevant effects were observed at urinalysis conducted at the completion of the treatment.

No test item-related macroscopic or microscopic findings were observed at the end of the study. There were no changes considered toxicologically significant in the absolute or relative organ weight values, relative to the body or brain weight, noted after BYI 02960-difluoroethyl aminofuranone administration in diet for 28 days, at up to and including 3000 ppm, evaluated immediately after completion of the treatment.

Acceptability

This study is considered acceptable.

Conclusion

Based on body weight effects, the NOAEL is considered to be 3000 ppm equating to approximately 243 mg/kg bw/day for the males and 273 mg/kg bw/day for the females.

B.6.9.3 BYI 02960-CHMP (6-chloropyridin-3-ylmethanol, IM-O, M21)

B.6.9.3.1 Acute toxicity BYI 02960-CHMP (6-chloropyridin-3-ylmethanol, IM-O, M21)

Study 1

Characteristics

reference	:	[redacted] 1993 amended in 1997	exposure	:	Gavage
type of study	:	Acute oral toxicity study	doses	:	M: 1000, 1500, 2000 and 3000 mg/kg bw/d F: 1000, 1300, 1500, 2000, 3000 mg/kg bw/d
year of execution	:	1993	vehicle	:	Ion-exchange water
test substance	:	IM-0((6-chloro-3-pyridyl)methanol, batch N° NK-2327'-6, 98.65% of purity)	GLP statement	:	Yes
route	:	Oral	guideline	:	According to OECD 401
species	:	Crj:CD (SD), SPF	acceptability	:	Acceptable
group size	:	5/sex/group	**LD50-cut off**	:	1,483 mg/kg bw/d

Study design

The study was performed in accordance with OECD 401.

The substance was tested at 1000, 1500, 2000 and 3000 mg/kg in five male and five female Sprague Dawley rats per group, plus an additional group of five females treated at 1300 mg/kg. The animals were randomly allocated to cages. Following an overnight fast, the animals received a single dose of IM-0 (6-chloropyridin-3-ylmethanol, BYI02960-CHMP) by gavage. The test substance was administered in ion-exchange at a volume of 10 mL/kg bw. Clinical signs and mortality rates were determined several times on the day of administration and subsequently at least once daily for an observation period of at least 14 days. Body weights were recorded prior to administration and on

days 1, 2, 3, 7 and 14 and at death except for animals that died on day 0. A gross necropsy was performed on all animals that died during the observation period and on all animals that survived until the termination of the experiment.

Results

Table 6.9.3.1-1 Mortality:

Dose mg/kg bw	Mortality Males	Mortality Females	Time of death
1000	-	-	
1300	Not tested	-	
1500	1/5	3/5	Within 1 day males and 2 days females
2000	3/5	5/5	Within 1 day
3000	5/5	5/5	Within 1 day

Clinical observations

The absence of righting reflex and a decrease in motor activity, hypotonea, prone position and ataxia were observed at all doses in both sexes. All signs disappeared on the next day of the application for the males and on day 2 for the females.

Body weight

No body weight effects were observed in males. A slight body weight loss was observed in 3/5 females treated at 1300 mg/kg and 1/3 females at 1500 mg/kg one day after the administration. The animals from these groups recovered thereafter.

Necropsy

Gastric haemorrhagies were observed in one male and one female treated at 1500 mg/kg.

Acceptability

This study is considered acceptable.

Conclusions

Under the experimental conditions of this study, the acute oral LD_{50} values for IM-0 are 1,842 mg/kg for males and 1,483 mg/kg for females.

B.6.9.3.2 Genotoxicity BYI 02960-CHMP (6-chloropyridin-3-ylmethanol, IM-O, M21)

Study 1

Study design and results

Type of study: Ames test, preincubation method

Indicator cells	Endpoint	Res. - act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
S. typh. TA 98 TA 100 TA 1535 TA 1537 E. coli WP2uvrA	 point mut. point mut. point mut. point mut. point mut.	 - - - - -	 - - - - -	rat liver	5,6-benzoflavone / phenobarbital	313-5000 µg/plate	Mochizuki, N. and Kanaguchi, Y. (1997)

Test substance: IM-0 (batch number NK-3120; purity 99.14%); tested in water
Precipitation: Precipitation of the test substance was observed at 5000 µg/plate.
Toxicity: No toxic effects were reported.
GLP statement: yes
According to OECD 471: yes
Adequate positive (+S9: 2-AA; -S9: ENNG, 2-NF, 9-AA) and negative (vehicle) controls were included.

Table 6.9.3.2-1 Pre-incubation assay with IM-0 - Mean number of revertants - first experiment

Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**WP2uvrA**	
Metabolic activation	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9
Vehicle control	29	29	135	132	9	10	7	14	30	32
IM-0										
313 µg/plate	21	37	124	140	15	8	5	7	25	40
625 µg/plate	34	36	139	117	14	9	6	15	28	34
1250 µg/plate	31	43	145	135	13	14	3	16	28	32
2500 µg/plate	30	33	140	146	9	15	9	17	26	28
5000 µg/plate	26	40	141	149	10	13	6	13	30	29
Pos. control	89	174	549	560	463	197	528	158	380	694

Table 6.9.3.2-2 Pre-incubation assay with IM-0 - Mean number of revertants - second experiment

Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**WP2uvrA**	
Metabolic activation	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9
Vehicle control	28	46	134	125	10	10	6	13	17	26
IM-0										
313 µg/plate	23	38	142	133	6	10	5	16	14	18
625 µg/plate	24	44	128	110	6	8	4	21	18	23
1250 µg/plate	22	44	131	115	8	14	7	15	19	21
2500 µg/plate	23	45	135	129	9	6	7	11	14	18
5000 µg/plate	19	46	136	144	11	7	5	18	18	14
Pos. control	87	113	517	563	489	203	615	144	301	574

In the first and second main experiment, no increase in the number of reverse mutant colonies was observed in any strain, either in the presence or absence of metabolic activation.

Acceptability

The study is considered acceptable.

Conclusions

IM-0 is not mutagenic in the Ames test.

B.6.9.3.3 Short-therm toxicity BYI 02960-CHMP (6-chloropyridin-3-ylmethanol, IM-O, M21)

Study 1

Characteristics

Reference	:	[redacted]; 1993 amended in 1997	exposure	:	90 days
type of study	:	Thirteen-week dietary subchronic toxicity study in rats	dose	:	0, 160, 800, 4000 and 20 000 ppm (corresponding to 0, 9.9, 48.9, 250.1 and 1246.6 mg/kg/day for males and 0, 11.1, 55.9, 275.9 and 1173.7 mg/kg/day for females)
year of execution	:	1993	vehicle	:	none
test substance	:	BYI 02960-CHMP (IM-0 or , 6-chloropyridin-3-ylmethanol) (batch number NK-3266 (Tox-563): a pale yellow crystal, 98.94% purity),	GLP statement	:	Yes
Route	:	diet	guideline	:	OECD 408
Species	:	Rat, Crj:CD^{TM} (SD)	acceptability	:	yes
group size	:	10/sex/dose	**NOAEL**	:	800 ppm (48.9 mg/kg/day)

Study design

BYI 02960-CHMP (IM-0 or 6-chloropyridin-3-ylmethanol) (batch number NK-3266 (Tox-563): a pale yellow crystal, 98.94% purity), a metabolite of the insecticide BYI 02960, was administered continuously via dietary administration to separate groups of Sprague Dawley rats (10/sex/group) at concentrations of 160, 800, 4000 and 20 000 ppm (corresponding to 9.9, 48.9, 250.1 and 1246.6 mg/kg/day for males and 11.1, 55.9, 275.9 and 1173.7 mg/kg/day for the females) for at least 90 days. A similarly constituted group of 10 males and 10 females received untreated diet and acted as a control.

Clinical signs were recorded daily and body weight and food consumption were measured weekly. A detailed physical examination was performed weekly throughout the study. Ophthalmological examinations were performed on the animals from the control group and the high dose group during

the acclimatization phase and during Week 12. Urine samples were collected overnight during the week before necropsy from all animals. Before necropsy a blood sample was collected from the carotid artery of each animal for haematology and clinical chemistry investigations. All animals were necropsied, selected organs weighed and a range of tissues were taken, fixed and examined microscopically.

Results

Table 6.9.3.3-1

Dose (ppm)	**0**		**160**		**800**		**4000**		**20000**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Mortality	No mortalities									
Clinical signs	Abnormal teeth, alopecia and excoriation in males, and alopecia, reddening in auricles, crust, lacrimation and abnormal teeth in females were sporadically seen in all groups, and the incidences were not treatment-related .									
Body weight	Statistically significant lower mean body weight at 20000 mg/kg/day throughout the study in males and females at study termination.									
Mean body weight (g) Relative ratios (%0 to control values	585.4	317.6	578.3 (99)	300.6 (95)	622.7 (106)	318.0 (100)	613.0 (105)	301.3 (95)	455.3 (78)**	243.3 (77)**
Food consump-tion	Mean food consumption was affected by treatment in either sex. Mean food consumption values (g/animal/day) of 20000 ppm males and females were decreased throughout the study when compared with the control, and attained statistical significance at weeks 1-4, 6, 9 and 13 in males and at every week in females. Mean food consumption values (g/kg/day) of 20000 ppm males were significantly decreased at the early phase of treatment (at weeks 7-8 and 12), while that of females in this group was significantly decreased at weeks 1, 2, 4, 8, 9 and 12.									
Ophthal-mology	At the examination performed prior to the study initiation, red areas in cornea were observed in two males, one from control and another from 20000 ppm group. At week 12 examinations, no effects of test compound treatment were evident in the males and females of 20000 ppm groups, compared to control.									
Urinalysis	No treatment related changes									
FOB	Not determined									
Haematolo gy	No treatment related changes.									
	0.56	0.75	0.58	0.78	0.60	0.72	0.66	0.85	0.57	0.77

Dose (ppm)	**0**		**160**		**800**		**4000**		**20000**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Clin. Chemistry -total bilirubin (mg/dL)										
- Glucose concentration (mg/dL)	188.5	147.9	200.2	150.5	185.7	148.2	208.4	149.5	157.0	132.3
- Creatinine concentration (mg/dL)	0.92	0.97	0.86	0.97	0.82	0.88	0.98	0.85	0.79	0.79
- Alanine aminotransferase (IU/l)	17	17	22	20	20	16	23*	17	17	13
- Alkaline phosphatise (IU/l)	114	54	109	66	102	55	112	63	113	91**
- Aspartate aminotransferase (IU/l)	49	52	56	59	52	51	57	54	46	50
- Potas-sium (neq/l)	4.83	4.41	5.03	5.11	5.00	5.09	5.18	4.94	5.11	5.00*
Organ weights										
- liver weight	14.26	6.86	14.55	6.70	15.76	7.18	15.52	6.92	11.39*	6.25
- liver weight / body weight	0.40	2.29	0.41	2.35	0.38	2.39	0.39	2.42	0.50**	2.68**
- brain	2.20	1.98	2.23	2.01	2.24	2.06	2.24	1.97	2.17	2.00
- brain /BW		0.67		0.71		0.69		0.70		0.86**
- lung	1.70	1.19	1.83	1.17	1.74	1.20	1.75	1.15	1.47*	1.14
- lung /BW		0.40		0.41		0.40		0.40		0.49*

Dose (ppm)	0		160		800		4000		20000	
	m	f	m	f	m	f	m	f	m	f
- kidney R/ L	1.64/ 1.60	0.98/ 0.97	1.78/ 1.75	0.98/ 0.96	1.83/ 1.80	0.99/ 0.96	1.77/ 1.80	0.97/ 0.96	1.54/ 1.54	0.92/ 0.93
-kidney L / body weight	0.29	0.33	0.32	0.34	0.31	0.33	0.31	0.34	0.35**	0.40**
-kidney R / body weight	0.30	0.30	0.33	0.34	0.31	0.33	0.31	0.34	0.35**	0.40**
- testis R/L -ovary R/L	1.69/ 1.69	0.04/ 0.04	1.69/ 1.70	0.04/ 0.04	1.72/ 1.72	0.04/ 0.04	1.69/ 1.68	0.03/ 0.03	1.62/ 1.65	0.03/ 0.04
- testis R/ BW	0.31		0.31		0.29		0.30		0.37*	
Pathology										
Macros-copy	No treatment related changes									
Microscopy	Test compound related lesions, consisted of eosinophilc intranuclear inclusions in the proximal tubular epithelium, were seen only in the kidney of males in the 4000 (70%) and 20000 (100%) ppm groups and in females in the 20000 (90%) ppm group.									

*p≤0.05 **p≤0.01

There were no signs of reaction to treatment in any treated group. All animals survived throughout the study.

Mean body weights for high dose (20 000 ppm) males and females were significantly less than respective control groups throughout the study. Mean body weights of the high dose groups were 78% of control values in males, and 77% in females. Mean body weight gains of the high dose groups were 67% of control values in males, and 57% in females.

Food consumption values of these groups were lower than control values at weeks 1-4, 6, 9, 13 (days 7 - 28, 42, 63, 91) examinations in males, and at all weeks in females. Food efficiency values of the 20 000 ppm animals were significantly decreased at weeks 1 and 10 (days 7 and 70) in males, and at week 1 (day 7) in females.

No treatment-related effects were observed at ophthalmological, haematological or urinalysis evaluations. A statistically significant increase in serum alkaline phosphatase activity was seen only in the 20000 ppm group females at study termination.

Statistically significant decreases in mean absolute weights of lung (male only) and liver (male only), and statistically significant increases in mean relative organ weight ratios of brain (both sexes), lung

(females only), liver (females only), kidney (both sexes) and testis (right side only) were seen only in 20000 ppm groups. However, these changes were attributed to the decreased body weights in these groups (reduction rates, 21% in males, 22% in females).
Necropsy revealed no compound-related lesions. Histologically, dose-related eosinophilic intranuclear inclusions were seen in the proximal tubular epithelium of kidneys for 20000 ppm males and females and 4000 ppm males. Other microscopic changes were occasionally seen in the control and treated groups, but they were considered unrelated to treatment with test compound.

Acceptability

This study is considered acceptable.

Conclusions

Based on the results mentioned above, the effects of the test item (IM-0), when administered in the diet to Sprague Dawley rats for 13 weeks, were decreased body weight gains, decreased food consumption values, increased serum alkaline phosphatase activity, and eosinophilic intranuclear inclusions in the proximal tubular epithelium of kidney. The NOAEL is 800 ppm (48.9 mg/kg/day) in males, and 4000 ppm (275.9 mg/kg/day) in females.

B.6.9.4 BYI 02960-6-CNA (6-chloronicotinic acid, IC-0, M27)

B.6.9.4.1 Acute toxicity BYI 02960-6-CNA (6-chloronicotinic acid, IC-0, M27)

<u>Study 1</u>

Characteristics

reference	:	[redacted]; 1993 amended in 1997	exposure	:	Gavage
type of study	:	Acute oral toxicity study	doses	:	Doses of 2000 and 5000 mg/kg
year of execution	:	1993	vehicle	:	Tween 80-ion-exchange water
test substance	:	IC-0 (6-chloronicotinic acid, batch Nr.5, 99.4% of purity)	GLP statement	:	Yes
route	:	Oral	guideline	:	According to OECD 401
species	:	Crj:CD (SD), SPF	acceptability	:	Acceptable
group size	:	5/sex/group	**LD50-cut off**	:	>5000 ppm

Study design

The study was performed in accordance with OECD 401.

The substance was tested at 2000 and 5000 mg/kg in five male and five female Sprague Dawley rats per group. The animals were randomly allocated to cages. Following an overnight fast, the animals received a single dose of IC-0 (6-chloronicotinic acid, BYI02960-6-CNA) by gavage. The test substance was administered in Tween 80 - ion-exchange water at a volume of 10 mL/kg bw. Clinical

signs and mortality rates were determined several times on the day of administration and subsequently at least once daily for an observation period of at least 14 days. Body weights were recorded prior to administration and on days 1, 2, 3, 7 and 14 and at death except on day 0. A gross necropsy was performed on all animals that died during the observation period and on all animals that survived until the termination of the experiment.

Results

Table 6.9.4.1-1 Mortality:

Dose mg/kg bw	Mortality Males	Mortality Females	Time of death
2000	-	-	
5000	-	-	

Clinical observations

No signs of toxicity were observed in any groups of either sex.

Body weight

No unusual changes in body weight were observed in 2,000 mg/kg in males. Body weights in 5,000 mg/kg males decreased on day 1 to day 2 after the administration, and recovered from day 3. Body weights in 2000 mg/kg females decreased on day 1 after the administration. Body weights in 5,000 mg/kg females decreased on days 1 to 3 after the administration and recovered thereafter.

Necropsy

No abnormality was observed in any rats at necropsy.

Acceptability

This study is considered acceptable.

Conclusions

Under the experimental conditions of this study, the acute oral LD_{50} value of IC-0 in rats is greater than 5000 mg/kg for both sexes.

B.6.9.4.2 Genotoxicity BYI 02960-6-CNA (6-chloronicotinic acid, IC-0, M27)

Study 1

Study design and results

Type of study: Ames test, preincubation method

Indicator cells	Endpoint	Res. - act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
S. typh. TA 98 TA 100 TA 1535 TA 1537 E. coli WP2uvrA	 point mut. point mut. point mut. point mut. point mut.	 - - - - -	 - - - - -	rat liver	5,6-benzoflavone / phenobarbital	313-5000 µg/plate	Mochizuki, N. and Kanaguchi, Y. (1997)

Test substance: IC-0 (batch number 5; purity 99.4%); tested in DMSO
Precipitation: Precipitation of the test substance was observed at 5000 µg/plate with metabolic activation.
Toxicity: Growth inhibition was observed at 5000 µg/plate without metabolic activation in TA98 and at 5000 µg/plate with metabolic activation in all the S. typh. strains.
GLP statement: yes
According to OECD 471: yes
Adequate positive (+S9: 2-AA; -S9: ENNG, 2-NF, 9-AA) and negative (vehicle) controls were included.

Table 6.9.4.2-1 Pre-incubation assay with IC-0 - Mean number of revertants - first experiment

Strain	TA 98		TA 100		TA 1535		TA 1537		WP2uvrA	
Metabolic activation	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9
Vehicle control	24	46	127	127	10	14	11	20	19	32
IC-0										
313 µg/plate	26	47	116	121	8	10	14	15	17	36
625 µg/plate	26	42	109	113	6	14	14	14	22	28
1250 µg/plate	25	38	135	129	8	11	19	13	20	31
2500 µg/plate	30	34	122	133	8	22	12	20	24	22
5000 µg/plate	10	13	134	90	4	9	10	10	18	28
Pos. control	88	154	557	592	496	184	548	164	445	663

Table 6.9.4.2-2 Pre-incubation assay with IC-0 - Mean number of revertants - second experiment

Strain	TA 98		TA 100		TA 1535		TA 1537		WP2uvrA	
Metabolic activation	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9
Vehicle control	24	35	127	120	12	15	14	12	24	27
IC-0										
313 µg/plate	27	43	125	124	9	17	11	19	22	24
625 µg/plate	35	45	132	107	11	16	10	14	27	21
1250 µg/plate	25	39	138	130	9	11	13	14	26	28
2500 µg/plate	31	39	134	114	15	15	14	17	20	23
5000 µg/plate	18	18	134	113	9	22	12	18	23	28
Pos. control	91	125	657	652	516	201	574	148	436	682

In the first and second main experiment, no increase in the number of reverse mutant colonies was observed in any strain, either in the presence or absence of metabolic activation.

Acceptability
The study is considered acceptable.

Conclusions
IC-0 is not mutagenic in the Ames test.

B.6.9.5 Summary of toxicity studies with the metabolites

Table 6.9.5-1 Summary of toxicity studies with the metabolites

Study	Species	Results
Difluoroacetic acid (DFA, BCS-AA56716, M44)		
Ames test Sokolowski, A. (2010)	*Salmonella Typh.*	Negative
In vitro HPRT Locus Gene Mutation Assay Hall, C. (2010)	Chinese hamster V79 lung cells	Negative
In vitro Chromosome Aberration Test Hall, C. (2010)	Chinese hamster V79 lung cells	Negative
Rat Acute Oral Study [redacted] 2010	Sprague Dawley Rat	300 mg/kg <LD_{50} < 2000 mg/kg
14-day range-finding study Kennel, P.2011	Wistar rat	NOAEL= 500 ppm (equating to 51 mg/kg/day) based on clinical chemistry changes
90-day dietary study [redacted].2012	Wistar rat	NOAEL = 200 ppm (12.7/15.6 mg/kg bw in M/F) Decreased BW and FC, decreased Hg conc., mean corpuscular volume, mean corpuscular Hg, hematocrit in females, decreased Glc, TBil, increased urea, higher ketones, focal glandular erosion/necrosis
BYI 02960-difluoroethyl-amino-furanone (BCS-CC98193, BYI 02960-DFEAF, M34)		
Ames test Sokolowski, A. (2011)	*Salmonella Typh.*	Negative
In vitro Chromosome Aberration Test Hall, C. (2011)	Chinese hamster V79 lung cells	Positive in absence of metabolic activation
In vitro HPRT Locus Gene Mutation Assay Wollney, H. (2011)	Chinese hamster V79 lung cells	Negative
In vivo tests	**Dose levels**	
Micronucleus Test in male mice - ip administration [redacted]. (2011)	125, 250 and 500 mg/kg	Negative
Unscheduled DNA synthesis_ oral administration [redacted]. (2011)	1000 and 2000 mg/kg	Negative
Rat Acute Oral Study [redacted] 2011	Sprague Dawley Rat	LD_{50} cut off ≥ 2 000 mg/kg/day
Range-finding dietary rat study [redacted]; 2012	Wistar Rat	Lower blood glucose concentration from 1280 ppm (equating to 135 mg/kg/day) in females
28-day dietary study [redacted]; 2012	Wistar Rat	NOAEL = 3000 ppm (243 and 273 mg/kg/day in males and females, respectively) based on body weight effects.
BYI 02960-CHMP (6-chloropyridin-3-ylmethanol, IM-O, M21)		
Ames test Mochizuki, N. and Kanaguchi, Y. (1997)	*Salmonella Typh.*	Negative

Rat Acute Oral Study [redacted]	Sprague Dawley Rat	LD_{50} in males = 1842 mg/kg/day LD_{50} in females = 1483 mg/kg/day
90-day dietary study [redacted]; 1993 amended in 1997	Sprague Dawley Rat	NOAEL= 800 ppm (48.9 mg/kg:/day) in males BWG and FC decreases, increase in alkaline phosphatase activity, eosinophilic intranuclear inclusions in proximal tubular epithelium of kidney
BYI 02960-6-CNA (6-chloronicotinic acid, IC-0, M27)		
Ames test Mochizuki, N. and Kanaguchi, Y. (1997)	*Salmonella Typh.*	Negative
Rat Acute Oral Study [redacted] 1993 amended in 1997	Sprague Dawley Rat	LD_{50} ≥5000 mg/kg/day in both males and females

B.6.9.6 Applicant's Position papers on metabolites

Position paper 1

Characteristics

Reference	:	Lasserre D.P; 2012	exposure	:	n.a.
type of study	:	Flupyradifurone (BYI 02960) and its major metabolite DFA (difluoroacetic acid) - Toxicological profile comparison	dose	:	n.a.
year of execution	:	2012	vehicle	:	n.a.
test substance	:	n.a.	GLP statement	:	n.a.
Route	:	n.a.	guideline	:	n.a.
Species	:	n.a.	acceptability	:	acceptable
group size	:	n.a.	**NOAEL**	:	n.a.

Summary

BYI 02960 is a new insecticide being developed by Bayer CropScience; a full toxicological package has been performed for registration purposes. Difluoroacetic acid is a plant and livestock metabolite of BYI 02960 and a major environment metabolite, having been found in soil metabolism studies at > 10% of applied dose. Additionally, DFA is a groundwater metabolite with a PECgw between 0.75 µg/L and 10 µg/L based on calculations according to EU models. DFA was detected in the rat ADME studies at approximately 6% of the administered dose. However since DFA was not found in the rat ADME studies above 10% of the applied dose, based upon EFSA guidance, select toxicology studies were conducted with DFA to compare its toxicological properties with those of parent compound BYI 02960 and to exclude that DFA is a relevant metabolite according to the EU Guidance document on the assessment of the relevance of metabolite in groundwater (Sanco/221/200 rev. 10). DFA has been tested in an acute oral rat study, *in vitro* genotoxicity studies and in a subchronic rat toxicity study. The purpose of this position paper is to demonstrate that the toxicological profile of DFA

is very similar to that of BYI 02960 and DFA should therefore be considered to be covered by the overall toxicological profile of the parent compound.

Acute toxicity studies

BYI 02960 (1) was administered to four groups of three Wistar female rats at 2000 mg/kg and 300 mg/kg(two groups of three animals for each concentration). In the groups treated at 2000 mg/kg, four of six animals died within 2 or 3 hours after administration. The following clinical signs were noted at this concentration: decreased motility, tremors, piloerection, labored breathing and clonical cramps. There was no effect on body weight and no necropsy findings in surviving animals. In the other animals, black liver or liver with black spots and hemorrhagic lung were observed.
No mortality was observed at 300 mg/kg. Clinical signs were limited to breathing sounds. No effects on body weight and no necropsy abnormalities were recorded.

Difluoroacetic acid (2) was administered to three groups of three Sprague-Dawley female rats at 2000 and 300 mg/kg (one group for 2000 mg/kg and two groups for 300 mg/kg). In the group treated at 2000 mg/kg, two of three animals died within 1 hour after administration, showing hypoactivity, dyspnea and/or lateral recumbency within 25 minutes prior to death. Sedation, then hypoactivity, lateral recumbency, dyspnea, piloerection and staggering gait were observed in the surviving animal from day 1 to day 9. Body weight loss of 8% was also observed at the end of the first week, but body weight gain returned to normal thereafter. No abnormality was recorded at necropsy.
No mortality was observed at 300 mg/kg. Clinical signs were limited to loud breathing in one female on day 3. Overall body weight gain was not affected and no abnormalities were recorded at necropsy.

From these two studies it can be concluded that BYI 02960 and difluoroacetic acid have a similar profile for acute toxicity. Both compounds are classified category IV according to the GHS classification system.

Genotoxicity studies

BYI 02960 was tested in four genotoxicity studies: three *in vitro* and one *in vivo* study. All of them were negative. BYI 02960 was tested at doses of up to 5000 g/plate on Salmonella typhimurium strains TA1535, TA100, TA1537, TA98 and TA102 (3), and at up to 10mM in the HPRT test (4) and the chromosome aberration test (5). In the *in vivo* micronucleus test on NMRI mice (6), three dose levels were tested: 10, 20 and 40 mg/kg (two injections at a 24-hour interval).

Difluoroacetic acid was tested in three *in vitro* studies. As all studies were negative, no further testing was done. Difluoroacetic acid was also tested at up to 5000 g/plate on Salmonella typhimurium strains TA1535, TA100, TA1537, TA98 and TA102 (7), and at up to 10mM in the HPRT test (8) and the chromosome aberration test (9).

Both compounds are not genotoxic.

Subchronic rat toxicity studies

BYI 02960 was administered to groups of 10 male and 10 female Wistar rats at 100, 500 and 2500 ppm over a period of at least 90 days. These dose levels equate to 6, 30 and 156 mg/kg/day in males and 7.6, 38 and 186 mg/kg/day in females. Effects are summarized below.

Table 6.9.6-01: Significant changes in the 90-day rat study with BYI 02960

	MALES			FEMALES		
Dose levels ppm	100	500	2500	100	500	2500
Conc mg/kg/day	6.0	30.2	156	7.6	38.3	186
Body weight and body weight gain			↓6% overall BW ↓12% overall BWG Good recovery		↓12% overall BWG	↓6% overall BW ↓15% overall BWG Good recovery
Food consumption			1st week ↓17% Several occ. ↓5%			↓9% to 29% from week 1 to 7
Haematology						↑15% platelet count, reversible
Clinical chemistry	↓15%TBIL NS, ↑16%Chol NS ↑21%Trigly NS	↓15%TBIL NS, ↓6%Glc NS, ↑10%Chol NS	↓38%TBIL **, ↓21%Glc **, ↑28%Chol NS ↑35%Trigly NS reversible	↓5%TBIL NS, ↓3%Glc NS, ↑8%Chol NS	↓10%TBIL NS, ↓7%Glc NS, ↑20%Chol NS ↑9%Trigly NS	↓45%TBIL **, ↓22%Glc **, ↑46%Chol ** ↑66%Tgly NS Partially reversible for TBIL ↓25%
Organ weights		Thyroid: ↑17 to 20%	Liver: ↑8 to 16% mainly due to ↓BW Thyroid: ↑20 to 26%			Liver: ↑8 to 15% mainly due to ↓BW
Macroscopy			Liver: enlargement: 4/10 Thyroid: dark 1/10			Liver: enlargement: 1/10
Histopathology			Liver: minimum to slight centrilobular hypertrophy 10/10 Thyroid: diffuse follicular cell hypertrophy 3/10			Liver: minimum centrilobular hypertrophy 3/10

** : p≤0.01
NS : Not statistically significant

Difluoroacetic acid was administered to groups of 10 male and 10 female Wistar rats at 200, 1000 and 6000 ppm. These dose levels equated to 12.7, 66 and 380 mg/kg/day in males and 15.6, 79 and 472 mg/kg/day in females. Effects are summarized below.

Table 6.9.6-2: Significant changes in the 90-day rat study with DFA

	MALES			FEMALES		
Dose levels ppm	200	1000	6000	200	1000	6000
Conc mg/kg/day	12.7	66.2	380	15.6	78.7	472
Body weight and body weight gain		↓13% overall BW ** ↓20% overall BWG **	↓7% overall BW ↓11% overall BWG		↓6% overall BW ↓9% overall BWG	↓9% overall BW ** ↓20% overall BWG **
Food consumption		↓5% overall	↓5% overall		↓4% overall	↓7% overall
Haematology					↓ 9% [Hg] ** ↓ 6% MCV ** ↓ 8% MCHg ** ↓ 7% Ht **	↓ 8% [Hg] ** ↓ 7% MCV ** ↓ 7% MCHg ** ↓ 6% Ht **
Clinical chemistry	↓27%TBIL * ↓29%Glc ** ↑15% Urea *	↓52%TBIL ** ↓45%Glc ** ↑26% Urea **	↓56%TBIL ** ↓45%Glc ** ↑18% Urea *	↓12%TBIL NS ↓27%Glc ** ↑14% Urea *	↓37%TBIL ** ↓49%Glc ** ↑23% Urea **	↓47%TBIL ** ↓53%Glc ** ↑12% Urea NS
Urinalysis : urine volume	↑ 112% NS	↑ 240% ***	↑ 266% ***	↑ 28% NS	↑ 105% NS	↑ 155% **
Urinalysis: Ketones	Grade 0:0 Grade 1:2 Grade 2:8 Grade 3:0 Grade 4:0	Grade 0:0 Grade 1:0 Grade 2:0 Grade 3:0 Grade 4:10	Grade 0:0 Grade 1:0 Grade 2:0 Grade 3:0 Grade 4:10	Grade 0:6 Grade 1:2 Grade 2:0 Grade 3:0 Grade 4:0	Grade 0:0 Grade 1:0 Grade 2:5 Grade 3:4 Grade 4:0	Grade 0:0 Grade 1:0 Grade 2:0 Grade 3:7 Grade 4:2
Macroscopic evaluation		Stomach: black foci 1/10	Stomach: black foci 3/10		Stomach: black foci 2/10	Stomach: black foci 2/10
Histopathology		Stomach: focal glandular erosion 1/9 (minimal)	Stomach: focal glandular erosion 2/10 (minimal & slight)		Stomach: focal glandular erosion 1/10 (moderate)	Stomach: focal glandular erosion 1/10 (slight)

* : $p \leq 0.05$
** : $p \leq 0.01$
***: $p \leq 0.001$

Conclusion

Although slightly different findings were observed in each of the 90-day rat studies, the overall toxicological profile is very similar. Therefore, we consider that difluoroacetic acid is covered by the toxicological endpoints obtained with the parent compound, BYI 02960. The compounds are of similar acute toxicity, although no clinical signs or neurotoxic effects were observed after difluoroacetic acid administration. The metabolic changes observed with difluoroacetic acid were also observed with BYI 02960 in a 28-day rat study (12) at 5000 ppm, where a marked decrease in total bilirubin (- 73%) and glucose (- 46%) and a significant increase in urea (+ 37%) and total cholesterol (+ 41%) compared to controls were observed at 5000 ppm. The most significant effect detected is the decrease in glucose, which however did not cause any change in the behavior of the animals nor did it cause any functional impairment. Furthermore, a decrease in glucose was also observed in the parent 90-day study after administration of BYI 02960 at 2500 ppm and this decrease in glucose was reversible as no significant change was observed at the end of a 28-day recovery period. Also, in the

2-year rat study, a decrease in glucose was observed in both males and females treated at 2000 ppm after 3 months of administration (- 23% in males and - 18% in females) and in males only after 6 months (- 16%) but no significant variations were observed thereafter (13). Therefore, the effect on glucose should be considered adaptative as demonstrated in the combined rat chronic toxicity and carcinogenicity study. The effects observed in the stomach after DFA administration are very minor lesions which could be due to stress and which are only seen at quite high dose levels and with very low incidence

In conclusion, no separate risk assessment for difluoroacetic acid is considered necessary. Risk assessment can be performed on the basis of total residue (as defined in the residue definition) and based upon parent ADI and ARfD.

Position paper 2

Characteristics

Reference	:	Spiegel, K; 2012	exposure	:	n.a.
type of study	:	Toxicological coverage of BYI 02960 plant and livestock metabolites	dose	:	n.a.
year of execution	:	2012	vehicle	:	n.a.
test substance	:	n.a.	GLP statement	:	n.a.
Route	:	n.a.	guideline	:	n.a.
Species	:	n.a.	acceptability	:	acceptable
group size	:	n.a.	**NOAEL**	:	n.a.

Background

The parent compound BYI 02960 shows a quite extensive metabolic behaviour in crops, and also in livestock, whereas metabolisation was less pronounced in the rat. Thus several plant and/or livestock metabolites were not detected as systemic metabolites in the rat ADME studies. To assess the possible health risk due to the dietary exposure to these metabolites, a new conservative risk assessment approach has been applied on basis of the scientific report “Impact of metabolic and degradation processes on the toxicological properties of residues of pesticides in food commodities” prepared by the Institute for Plant Protection Product Evaluation and Authorisation of the Austrian Agency (AGES, 2010) and the EFSA draft scientific opinion on “Exploring options for providing preliminary advice about possible human health risks based on the concept of Threshold of Toxiclogical Concern (TTC)”. These scientific reports refer to a decision tree which allows the evaluation of the human safety on basis of the exposure to a metabolite.
According to this approach no additional toxicity data are needed for a metabolite, if

(1) the dietary metabolite was detected as systemic metabolite in the rat ADME study and accounted for >10% of the administered dose,

(2) the metabolite shows structural similarities to the parent/precursor and if the metabolic pathway from parent/precursor to the questioned metabolite is simple and it is unlikely that the metabolisation step causes toxification (e.g. demethylation, hydroxylation of a ring system),

(3) the consumer is not exposed to this metabolite, i.e. that it accounts for <0.01 mg/kg in food items and <0.05 mg/kg in feed items, or

(4) the metabolite is considered not to be geno- or neurotoxic (based on DEREK) and the consumer exposure is assumed to be below the threshold of toxicological concern (TTC) for Cramer Class III compounds (<1.5 µg/kg bw/day, or 90 µg/person/day).

Evaluation of the toxicological coverage of BYI 02960 metabolites

(1) Dietary metabolites covered by the rat ADME studies

BYI 02960 was moderately metabolised in the rat. The parent compound represented the predominant part of the radioactivity in urine (the preferred route of excretion) of male and female rats. The main metabolite detected in urine and faeces was BYI 02960-OH, which accounted for 11% to 29% of the dose administered in excreta. Thus BYI 02960-OH was present in sufficient amount to contribute to the toxicological effects when testing the parent compound.
The two metabolites, 6-CNA (<1% to 6%) and BYI 02960-hippuric acid (1% to 11%) were also prominent in male but not in females rats. In sum the metabolites represented 10% to 16% of the administered dose in male rats. Since BYI 02960-hippuric acid can be formed from 6-CNA only, the proportion of BYI 02960-hippuric acid can be added to the 6-CNA proportion. Thus, when considering the two metabolites together, 6-CNA accounted for ≥10% of the dose administered dose in male rats. Since there is no sex difference in the toxicological profile of BYI 02960, it can be concluded that the proportion of 6-CNA in rat excreta is adequately addressed. Thus 6-CNA was present in a sufficient concentration to contribute to the toxicological effects when testing the parent compound.
All other metabolites detected in the rat ADME represented less than 5% of the administered dose.

(2) Metabolites showing structural similarity to parent/precursors and arising from metabolisation steps which will cause no toxification

Conjugation of metabolites is in general a detoxification step. Thus it can be considered that the toxicity of a conjugate is covered by its aglycon. Therefore, the toxicity of the conjugates of BYI 02960-OH (isomers of BYI 02960-OH-gluA, BYI 02960-OH-SA, BYI 02960-OH-glyc, isomers of BYI 02960-OH-glyc-SA) and of 6-CNA (6-CNA-glycerol-gluA) detected in plants and livestock can be considered as toxicologically covered.

(3) Metabolites showing no consumer exposure

For the exposure we would like to refere to the residue section. In summary, based on the residues levels detected in the livestock metabolism studies, it was assumed that most of the metabolites detected show no consumer exposure under real conditions. The highest residues in the goat matrices were estimated for parent compound, metabolite BYI 02960-OH and its glucuronic acid conjugates. BYI 02960-OH was detected in the rat ADME study, as well as two isomers of its glucuronic acid conjugate and thus a toxicological coverage is given.

In poultry none of the metabolites detected in the samples of the metabolism studies would exceed a residue level of 0.010 mg/kg under real conditions, except for the fatty acids which were detected in high concentrations in eggs, fat and liver. However since fatty acids as natural compounds do not exhibit any toxicological concern.

(4) Metabolites with a consumer exposure below the threshold of toxicological concern

The threshold of toxicological concern (TTC) is based on the concept that there are levels of exposure to chemicals that will result in no appreciable risk to health. The classification of chemicals according to their chemical structure is an important component of the current TTC approach. The classification scheme most widely used is that described by Cramer et al. (1978). Plant metabolites and degradates of pesticide active substances are assigned to Cramer Class III. The corresponding dietary exposure threshold is **1.5 µg/kg body weight per day** for substances with no structural alerts for genotoxicity and neurotoxicity. Neonicotinoids and pyrethroids are included in Cramer Class III according to the Scientific Report “Applicability of thresholds of toxicological concern in the dietary risk assessment of metabolites, degradation and reaction products of pesticides”, published by CRD in 2009. There is no reason to include them in the neurotoxic TTC grouping, with a threshold value of 0.3 µg/kg body weight per day.

In accordance with this concept, a two-step approach was applied for all BYI 02960 metabolites for which a consumer exposure was expected and no toxicological coverage was proven (by the rat ADME studies or additional toxicological testings).

1. Exclusion of genotoxicity:
   Each metabolite was tested for genotoxic alerts by DEREK. DEREK is a commonly used *in silico* expert system for the qualitative prediction of toxicity.
2. Estimation of the possible consumer exposure
   Based on the EFSA Pesticide Residue Intake Model (PRIMo), the possible consumer exposure of a metabolite or a group of metabolites was estimated. Conjugates and their aglycons were considered as a metabolite group and their concentrations (in mg a.s. equiv./kg) were summed up for a combined evaluation. Metabolites resulting from the same metabolic path were also considered as a metabolite group. For the estimation of the possible consumer exposure we would like te refere to the residue section. In conclusion: the consumer exposure to the metabolite group consisting of:

(1) BYI 02960-bromo/-chloro, BYI 02960-bromo-amino-furanone and BYI 02960-amino-furanone,
(2) BYI 02960-mercapto-lactic acid,
(3) BYI 02960-acetic acid and -acetic acid-glyc, and
(4) BYI 02960-glyoxylic acid, BYI 02960-AMCP-difluoroethanamine, BYI 02960-N-formyl- and BYI 02960-N-acetyl-AMCP-difluoroethanamine

are below the threshold of toxicological concern of 1.5 µg/kg bw/day.

(5) Metabolites with additional toxicity testing

Due to various considerations, additional toxicity studies were initiated for the metabolite difluoroacetic acid (a major soil metabolite with the potential, according to FOCUS groundwater modelling, to leach into shallow groundwater at concentrations of > 0.75 mg/L) and metabolite BYI 02960-difluoroethyl-amino-furanone (a proposed constituent of the residue definition for data collection in target and rotational crops; it was observed in the rat ADME study at levels less than 5% of the administered dose). Additional toxicity data are also available on metabolites BYI 02960-CHMP and 6-CNA. They are common metabolites with the active substance acetamiprid and several toxicological studies had been conducted for the submission of this insecticide. As described in detail in Section 3, KIIA 5.8, none of theses metabolites are acutely toxic or exhibit a genotoxic potential. The metabolites tested after subchronic (difluoroacetic acid, BYI 02960-CHMP) or subacute (BYI 02960-difluoroethyl-amino-furanone) administration to the rat were less toxic than BYI 02960 and are thus covered by the endpoints derived for the parent compound BYI 02960.

(6) Metabolites of no toxicological concern

For the natural compounds glucose, lactose and fatty acids no estimation of residue levels was considered necessary since they are of no toxicological concern.

Conclusions

A health risk due to the dietary exposure to plant and livestock metabolites of BYI 02960 can be excluded on basis of the data presented. All metabolites detected show either a consumer exposure below the agreed threshold level of toxicological concern (<1.5 µg/kg bw), or it has been shown that the metabolites are covered by the endpoints derived for the parent compound BYI 02960.

An overall evaluation of the plant and livestock metabolites is summarized in Table 5.11-13.

Table 6.9.6-3 Evaluation of plant and livestock metabolites

BYI 02960 metabolites of/with		
no toxicity concern	**no consumer exposure BYI02960-**	**exposure below TTC of 1.5 µg/kg bw BYI 02960-**
fatty acids	-acetyl-AMCP	-acetic acid
glucose	-acetyl-cysteinyl-nicotinic acid	-acetic acid-glyc

lactose	-AMCP-difluoroethanamine-SA	-amino-furanone
	-cysteinyl-nicotinic acid	-bromo-amino-furanone
	-des-difluoroethyl	-bromo/-chloro
	-des-difluoroethyl-OH-SA	-glyoxylic acid
	-difluoroethyl-OH-glyc	-N-acetyl-AMCP-difluoroethanamine
	-hippuric acid	-N-formyl-AMCP-difluoroethanamine
	-lactato-mercaptyl-nicotinic acid	-mercapto-lactic acid
	-methylthio-glyoxylic acid	
BYI 02960 metabolites		
covered by rat ADME **BYI 02960-**	**covered by aglycon** **BYI 02960-**	**with additional tox testings** **BYI 02960-**
-6-CNA	-6-CNA-glycerol-gluA	-CHMP
-OH	-OH-gluA	-difluoro acetic acid
	-OH-glyc	-difluoroethyl-amino-furanone
	-OH-SA	

B.6.10 MEDICAL DATA AND INFORMATION (ANNEX IIA 5.9)

B.6.10.1 Medical surveillance on manufacturing

The active ingredient has been produced in 2 campaigns in 2008 and 2010. Routine occupational medical examinations of the workers involved in production, not directly correlated to the campaigns, showed no abnormalities.

B.6.10.2 Clinical cases of poisoning incidents

As the active ingredient has not yet been marketed, no such experiences exist.

B.6.10.3 Observations on exposure of the general population

As the active ingredient has not yet been marketed, no such experiences exist.

B.6.10.4 Clinical signs and symptoms of poisoning and details of clinical tests

As no poisonings in human have occurred, no information is available

B.6.10.5 First aid measures and therapeutic regimes

- Remove patient from exposure/terminate exposure under self-protection
- Thorough skin decontamination with copious amounts water and soap, if available with polyethylenglykol 300 followed by water
  Note: Most formulations with this active ingredient can be decontaminated with water (and soap), so for formulations polythyleneglykol 300 is not required
- Flushing of the eyes with lukewarm water for 15 minutes
- Induction of vomiting should only be considered if a significant amount has been swallowed (more than a mouthful), if the ingestion was less than one hour ago, and if the patient is fully conscious. Induced vomiting can remove maximum 50% of the ingested substance. Note: Induction of vomiting is forbidden, if a formulation containing organic solvents has been ingested!

B.6.10.6 Expected effects of poisoning

As no poisonings in human have occurred, no information is available.

B.6.11 SUMMARY OF MAMMALIAN TOXICOLOGY AND PROPOSED ADI, AOEL, ARFD AND DRINKING WATER LIMIT (ANNEX IIA 5.10)

B.6.11.1 Toxicokinetics

Absorption, distribution, excretion and metabolism of flupyradifurone was investigated using three different labelling positions. The active substance was labelled with ^{14}C in the pyridinylmethylene bridge, in the 4-position of the furanone ring and in the 1-position of the ethyl side chain:

Pyridinylmethyl-^{14}C flupyradifurone | Furanone-4-^{14}C flupyradifurone | Ethyl-1-^{14}C flupyradifurone

The pyridinylmethyl-labelled compound was used in an ADME-study in which male and female rats were orally administered with a single low dose of 2 mg/kg bw or a high dose of 200 mg/kg bw. Due to the high water solubility of flupyradifurone, male rats were also given an intravenous dose of 2 mg/kg bw. In this study, the excretion via urine and faeces was investigated as well as the distribution in the plasma and the radioactivity concentration in organs and tissues at sacrifice. The metabolism was investigated in urine and faeces.

A quantitative whole body autoradiography study was conducted also using the pyridinylmethyl-labelled compound following a single oral dose of 5 mg/kg bw to male and female rats. In this study the excretion of radioactivity was determined in urine, faeces and expired air, as well as the distribution in the plasma and the radioactivity concentration in the organs and tissues at various time points.

The furanone-4-labelled compound was used in an ADME-study in which male and female rats were orally administered with a single dose of 2 mg/kg bw. In this study, the excretion via urine and faeces was investigated as well as the distribution in the plasma and the radioactivity concentration in the organs and tissues at sacrifice. The metabolism was investigated in urine and faeces.

A quantitative whole body autoradiography study was also conducted using the furanone-4-labelled compound following a single oral dose of 5 mg/kg bw to male and female rats. In this study the excretion of radioactivity was determined in urine, faeces and the expired air as well as the distribution in the plasma and the radioactivity concentration in the organs and tissues at various time points.

In an organ metabolism study, male and female rats were orally administered with a single dose of 3 mg/kg bw [furanone-4-^{14}C] flupyradifurone. Animals were sacrificed 6 hours after dosage and the metabolism was investigated in urine, plasma, and in extracts of liver, kidney, muscle and fat tissues.

The ethyl-1-labelled compound was used in an ADME-study in which male rats were orally administered with 2 mg/kg bw. In this study, the excretion via urine, faeces and expired air was investigated as well as the distribution in the plasma and the radioactivity concentration in the organs and tissues at sacrifice. The metabolism was investigated in urine and faeces.

In an organ metabolism study, male and female rats were orally administered with a single dose of 3 mg/kg bw [ethyl-1-^{14}C] flupyradifurone. The animals were sacrificed at 1, 6 or 24 hours after dosing. The total radioactivity was determined over the different time periods in urine, while in plasma, liver, kidney, muscle and fat tissue at different time points after sacrifice. The metabolism was investigated in urine, plasma, and in extracts of liver, kidney, muscle, and fat tissue.

Absorption

Following oral administration of a single low dose of flupyradifurone to male and female rats, the gastrointestinal absorption of radioactivity was high. It accounted for >80 % of the dose independent of the labelling position used. The value of >80% can be used for further toxicological risk assessment.

Distribution

The maximum plasma concentration was reached in most cases within 1 or 2 hours after administration of low doses. Only after administration of the high dose of 100 mg/kg bw the peak plasma concentration was observed between 2 and 4 hours after dosage. After reaching the peak concentration, the radioactivity levels in plasma declined steadily by several orders of magnitude in all studies independent of sex or labelling position of the test compound.
Quantitative whole body autoradiography revealed a fast absorption and distribution of the test compound with peak values observed already 1 hour after administration. At this time, the concentrations in liver and kidney were significantly higher than in blood, suggesting a preferred clearance from blood and distribution mainly to these organs which are mainly responsible for metabolism (liver) and excretion (kidney). Higher concentrations than in blood were also detected in the myocardium (heart) and in several glands. Very low levels were found in the brain, spinal cord and renal fat tissue. These results were similar in male and female rats, and independent of the labelling position of the test compound.

Excretion

Excretion was very fast, mainly renal and almost completed after 24 hours. No radioactivity was detected in the expired air after dosing of the pyridinylmethyl- and ethyl-1-labelled compounds, proving the stability of these labelling positions in the molecule. Only after administration of [furanone-4-^{14}C] flupyradifurone between 1% and 3% of the administered radioactivity was exhaled. This demonstrated that for a small portion of the dose (higher in male than in female rats) the furanone ring of the molecule obviously was opened and was subject of biotransformation to C-1 fragments.

A fast decline of radioactivity concentrations was observed for all organs and tissues in male and female rats during the entire test period. Concentrations fell for most organs and tissues below 5% of the maximum concentration after one day. After seven days, only very low concentrations were found in a few organs and tissues of rats dosed with the pyridinylmethyl-labelled test compound. In the study using the furanone-4-labelled compound, low radioactive residues were measured in almost all organs and tissues due to the incorporation of C1- or C2-fragments into the endogenous carbon pool. The residues in male rats were higher by a factor of 1.4 to 4.7 as compared to female rats. A similar ratio of approximately 3 (male/female) was also found for the formation of $^{14}CO_2$, and originates presumably from sex related differences in metabolism leading to more C1- and C2-fragments and also higher incorporation of these components into the endogenous carbon pool in male rats. In general males and female rats exhibited very similar absorption, distribution and excretion behaviour. The results of these studies demonstrate that there is no indication of any accumulation or significant retention of radioactivity in male and female rats. This observation is supported by the low P_{OW} of 1.2. Concentrations of radioactivity detected in tissues and organs at sacrifice were either very low or below the limit of detection

Metabolism

Flupyradifurone was intensively metabolised in the rat. Numerous metabolites were formed, most of them being minor ones. The parent compound represented the predominant part of the radioactivity in urine of male and female rats. In faeces of male rats, the metabolite BYI 02960-OH was more prominent than the parent compound. Two metabolites, BYI 02960-6-CNA and BYI 02960-hippuric acid were also prominent in male but not in females rats. The organ metabolism study using the ethyl-1-^{14}C label showed that in the 24 hours samples of plasma, and organs and tissues BYI 02960-DFA was by far the dominating metabolite accounting for more than 50% of the radioactivity. In general, the metabolic profiles in urine and faeces were very similar for both sexes but male rats showed a higher rate of metabolite formation as compared to female rats. 6-CNA is considered a major metabolite in male rat but not in females.

The principal metabolic reactions of flupyradifurone in rats were:

- Hydroxylation followed by conjugation with glucuronic acid or sulfate,
- cleavage of the difluoroethyl group forming BYI 02960-des-difluoroethyl, and difluoroacetic acid (BYI 02960-DFA),
- cleavage of the molecule at the pyridinylmethylene bridge forming BYI 02960-6-CNA, which was further conjugated with glycine to BYI 02960-hippuric acid and BYI 02960-difluoroethyl-amino-furanone.
- The figure below schematically shows the sites of the molecule, which are involved in the metabolic reactions:

Summarizing, the results of the metabolism studies conducted in the rat, a proposed metabolic pathway of flupyradifurone can be described as shown in this figure:

B.6.11.2 Toxicodynamics

Acute toxicity

Flupyradifurone (BYI 02960) needs to be classified as Acute oral toxic Cat. 4 H302 according to Regulation (EC) 1272/2008. Flupyradifurone does not need to be classified on the basis of its acute dermal, and inhalation toxicology in rats (Category 5/ Unclassified). Flupyradifurone is considered not irritating to skin and eyes (Category 5/ Unclassified). Flupyradifurone does not have sensitising properties in a LLNA (Category 5/ Unclassified).

Table 6.11.2-1 Classification

Type of study	Species	Results	Classification (proposed)
Oral route	Rat	Mortalities observed at 2000 mg/kg; none at 300 mg/kg	Category 4 H302 (LD_{50} cut off >300-2000 mg/kg)
Dermal route	Rat	LD_{50} > 2 000 mg/kg	Category 5 / Unclassified
Inhalation	Rat	LC_{50} at 4 hours > >4.7 mg/L (nose only) ~~4671 mg/m³~~	Category 5 / Unclassified
Primary skin irritation	Rabbit	Non irritating	Category 5 / Unclassified
Eye irritation	Rabbit	Slight redness of the conjunctivae, reversed within 48 hours	Category 5 / Unclassified
Skin sensitization	Mouse	Not sensitizing	Category 5 / Unclassified

Short-term toxicity

Oral

Four subacute oral range-finding studies were performed, with rat, mouse and dog.

In a 28-day study with rats exposed to 0, 75, 200 or 350 mg/kg bw/d via gavage, toxicologically relevant effects were clinical chemical findings, increased organ weights and pathological findings in the 200 mg/kg bw/d group. The NOAEL in this study is set at 75 mg/kg bw/d.

In a 28-day study with rats exposed to 0, 33.6 or 385 mg/kg bw/d via diet, toxicologically relevant effects were clinical chemical findings, increased liver and thyroid weights and associated pathological findings in the 385 mg/kg bw/d group. The NOAEL in this study is set at 33.6 mg/kg bw/d.

In a 28-day study with mouse exposed to 300, 600 and 1200 ppm (equating approximately to 50, 98 and 207 mg/kg bw/day in males and 59, 122, 240 mg/kg bw/day in females) via diet, no toxicologically relevant effects were observed. ~~Given the instability of BYI 02960 in rodent diet the actual concentration is considered to be in the region of 960 to 1080 ppm (166 to 186 mg/kg body weight/day for the males and 192 to 216 mg/kg body weight/day for the females, respectively).~~ The NOAEL in this study is set at 98 ~~166~~ ~~33.6~~ mg/kg bw/d.

In a 28-day study with dogs exposed to 0, 500, 2000 or 4000 ppm (equating approximately to 0, 16, 62, 118 mg/kg body weight/day in males and 0, 18, 77, 131 mg/kg body weight/day in females) via diet, toxicologically relevant effects were body weight loss and reduced body weight gain at the 4000 ppm group. The NOAEL in this study is set at 2000 ppm (62 mg/kg bw/d).

Four semichronic oral studies were performed, with rat, mouse and dog.

In a 90-day toxicity study with rat exposed to 0, 100, 500, 2500 ppm (equating to approximately 0, 6.0, 30.2, 156 mg/kg bw/day in males and 0, 7.6, 38.3, 186 mg/kg bw/day in females) via diet, toxicologically relevant effects were clinical chemical findings, increased relative liver and thyroid weight and associated pathological findings in the 2500 ppm group, and thyroid weight effects in the 500 ppm males. The NOAEL in this study is 100 ~~500~~ ppm (6.0 ~~30.2~~ mg/kg bw/d).

In a 90-day toxicity study with mouse exposed to 0, 100, 500, 2500 ppm (equating approximately to 0, 16, 81, 407 mg/kg bw/day in males and 0, 19, 98, 473 mg/kg bw/day in females) via diet, toxicologically relevant effects were clinical chemical findings, increased relative liver and decreased kidney weights and associated pathological findings in the 2500 ppm group. The NOAEL in this study is 500 ppm (81~~80.6~~ mg/kg bw/d).

In a 90-day toxicity study with dog exposed to 0, 400, 1200 or 3600/2400 ppm (equating approximately to 0, 12, 33 and 102/85 mg/kg bw/ day for males and 0, 12, 41 and 107/78 mg/kg bw/day for females) via diet, toxicologically relevant effects were reduced body weight, reduced body weight gain, clinical chemistry, significant increase in kidney weight and focal myofiber atrophy/degeneration in the 1200 ppm group. The NOAEL in this study is 400 ppm (12 mg/kg bw/d).

In a 1-year toxicity study with dog exposed to 0, 150, 300 and 1000 ppm (equating approximately to 0, 4.6, 7.8 and 28.1 mg/kg bwd/day for males and 0, 4.1, 7.8 and 28.2 mg/kg bw/day for females) via diet, toxicologically relevant effects were micropathology (degeneration of the skeletal muscle (*gastrocnemius* and *biceps femoris* (atrophy, necrosis and/or presence of inflammatory cells)) findings in the 1000 ppm group. The NOAEL in this study is 300 ppm (7.8 mg/kg bw/d).

Dermal

Male and female rats, 10 rats/sex/group (one control and three test substance-dosed groups) were administered BYI 02960 (50, 150, or 500 mg/kg/day) daily by dermal application for at least 28 consecutive days (minimum of 6 h/day) and euthanized one day following the last dose. Based on the lack of adverse findings observed in the study, the 28-day dermal exposure NOAEL in male and female rats dosed with BYI 02960 was 500 mg/kg bw/day.

Genotoxicity

BYI 02960 was not mutagenic in two Ames tests.

BYI 02960 did not induce structural chromosome aberrations in V79 cells when tested up to and including cytotoxic concentrations and showed no mutagenic potency in vitro in the CHO/HPRT assay.

BYI 02960 did not induce micronuclei in mouse bone marrow cells in two separate in vivo studies.

Overall it can be concluded that BYI 02960 has no mutagenic or clastogenic potential.

Long-term toxicity and carcinogenicity

In a two-year toxicity/carcinogenicity study with rats, effects in the liver and in a less extend in the thyroid and lung (females) were observed in the 2000 ppm dose group. The NOAEL for general toxicity was set at 400 ppm in diet, equal to the average daily intake of 15.8 mg/kg bw/d. No neoplastic lesions were observed in the study at any dose level.

In an 18-months carcinogenicity study with mice, effects in the liver and in de kidney (male) were observed in the 1500 ppm dose group. The NOAEL for general toxicity was set at 300 ppm equal to the average daily intake of 45 mg/kg bw/day. The NOAEL for carcinogenicity was set at the highest dose level of 1500 ppm, equal to the average daily intake of 224 and 263 mg/kg bw/day over 18 months for male and female mice, respectively.

Reproductive and developmental toxicity

In the rangefinder one generation rat reproduction study, BYI 02960 was administered continuously in the feed to Wistar rats (10 animals/dose/sex) at nominal dietary concentrations of 0, 200, 700, and 2000 ppm. The parental systemic LOAEL is 700 ppm (60.0 mg/kg bw/day females), based on decreased body weight, decreased body weight gain, alterations in food consumption during premating, and decreased spleen weights in females. The parental systemic NOAEL is 200 ppm (17.5 mg/kg bw/day).

The reproductive NOAEL is 147.5 mg/kg bw/day based on no reproductive findings observed at the highest dose tested.

The offspring LOAEL is 700 ppm (60.9 mg/kg bw/day). The LOAEL is based on maternal effects leading to secondarily-mediated effects on pup weight, pup weight gain, and organ weight changes (brain). The offspring NOAEL is 200 ppm (17.5 mg/kg bw/day).

A significant decrease in body weight, but <10% was observed in the mid-dose of the F1 females and a non significant decrease in body weight gain was found in the parental animals (21 % in females)

and the F1 animals (16 % in females). Therefore the parental systemic LOAEL is considered 500 ppm (32 mg/kg bw/d) based on decreased body weight gain in females. The parental systemic NOAEL is 100 ppm (6.4 ~~7.7~~ mg/kg bw/d). ~~Therefore the parental systemic LOAEL is considered 1800 ppm (117.4 mg BYI 02960/kg bw/day) based on increased liver and thyroid weights, and minimal centrilobular hypertrophy in males decreased body weight (>10%)(premating, gestation, and lactation), body weight gain (premating; P and F_1). The parental systemic NOAEL was 500 ppm (32.0 mg BYI 02960/kg bw/day).~~The reproductive NOAEL was 500 ppm (34.3 mg BYI 02960/kg bw/day females) based on decreased estrous cycle number, litter size, and the number of implants observed in the F_1 generation at the highest dietary level tested (LOAEL = 1800 ppm (134.0 mg BYI 02960/kg bw/day in females).
A significant decrease in body weight, but <10% was observed in the mid-dose of the F2 pups. The offspring LOAEL is therefore 1800 ppm (134.0 mg BYI 02960/kg bw/day) based on maternal effects leading to secondary effects on pup weight, and physical development. The offspring NOAEL is 500 ppm (34.3 mg BYI 02960/kg bw/day).

In a rat developmental study, BYI 02960 was administered daily by gavage to groups of 25 pregnant Sprague-Dawley female rats per dose-group at 15, 50 and 150 mg/kg/day from gestation day (GD) 6 to 20. Minimal maternal toxicity was observed at the high-dose level, manifested as decreased body weight gain and reduced food consumption. The mean absolute liver weight was increased in the high dose group.Based on these findings the Noael vor maternal toxicity was set at 50 mg/kg bw/day. The increased incidence of parietal incomplete ossification showed no dose response, the unossified cervical centrum and the incomplete ossification of the sternebra was within the historical background. A increase of a short costal cartilage was seen at the high-dose. Based on these findings, the NOAEL for developmental toxicity was set at 50 mg/kg bw/day.

In a complementary study, where groups of 23 sperm-positive female Sprague-Dawley rats were exposed to BYI 02960 by oral gavage from gestation day (GD) 6 to 20 at 20 and 30 mg/kg/day, no maternal toxicity was observed up to 30 mg/kg/day. Therefore, based on these two studies, it can be concluded that the NOEL for maternal toxicity was 30 mg/kg/day.

In a rabbit developmental study, groups of 23 time-mated pregnant female New Zealand White rabbits were administered BYI 02960 by oral gavage from gestation day (GD) 6 to 28 at 7.5, 15 and 40 mg/kg/day. The effects on (corrected) body weight gain and food consumption were slight and mainly transient, however considered adverse (>10%).The NOAEL for maternal toxicity and developmental toxicity was set at 15 mg/kg bw/day.~~There were no treatment-related findings for maternal and developmental toxicity therefore the NOAEL for maternal toxicity and developmental toxicity was set at 40 mg/kg bw/day.~~

Neurotoxicity

In an acute neurotoxicity study, BYI 02960 was administered by gavage in a single dose to non-fasted young adult Wistar rats at 0, 50, 200 and 800 mg/kg. Compound-related effects were observed at all dose concentrations in both sexes. The only treatment-related effects at 50 mg/kg were limited to higher incidences of piloerection in both sexes and dilated pupils in females only. A follow-up study was performed in order to establish a clear NOAEL for findings observed at all dose levels in the initial study. In this follow-up study, females only were used as they were equal or more sensitive than males at higher dose levels and were administered BYI 02960 at 20 or 35 mg/kg. No treatment-related effects were evident at either dose tested. The dose level of 35 mg/kg of BYI 02960 was considered to be the overall NOAEL for both sexes.

In a 90-day neurotoxicity study, through approximately 13 weeks of continuous dietary exposure to BYI 02960 at 100, 500 or 2500 ppm, there were no neurotoxic treatment-related findings apparent at any dietary level in either sex. Based on these findings, a NOAEL of 2500 ppm was established for the rat (equating to 143 mg BYI 02960/kg bw/day for males).). Body weight and food intake effects were seen at the highest dosage. The systemic NOAEL is therefore 29.4/34.8 mg/kg bw/d for males and females respectively.

In a developmental neurotoxicity study, dietary exposure to BYI 02960 from GD 6 through LD 21 to mated female Wistar rats resulted in treatment-related effects (maternal: decreased body weight gain. Offspring: decreased body weight gain, slightly increased in motor and locomotor activity, slightly increased auditory startle habituation) evident only at 1200 ppm (102 mg/kg bw/day) in maternal animals and offspring. ~~Therefore, the NOAEL for both maternal animals and offspring is 500 ppm (42.4 mg/kg bw/day) in this developmental neurotoxicity study.~~

The maternal NOAEL was 500 ppm (42.4 mg/kg bw/d) based on decreased body weight and body weight gain. The developmental NOAEL was 500 ppm (42.4 mg/kg bw/d) based on decreased offspring body weight and body weight gain. The developmental neurotoxicity NOAEL was 500 ppm (42.4 mg/kg bw/d) based on increased startle amplitude in females only on PND 60 and increased motor and locomotor activity on PND 13 in males only.

Further toxicological studies

Biokinetic study

Plasma concentration of BYI 02960 was assessed at three time points after continuous dietary administration for seven days. Groups of 5 male and 5 female Wistar rats received the diet admixed with the test substance at a concentration of 400 ppm (equating approximately to 22.6 mg/kg body weight/day in males and 32.4 mg/kg body weight/day in females).

During the first 15 days of acclimatization, the daily light/dark rhythm was 7 a.m. - 7 p.m. Rats being known to preferably eat during the dark period, the daily light/dark rhythm of rats was changed from November 25, 2009 (pre-study Day 16, *i.e.* 14 days prior to first day of treatment) onwards, *i.e.* dark period was from 2 a.m. to 2 p.m. and light period was from 2 p.m. to 2 a.m., in order to allow the blood sampling during the working day. Clinical signs were observed daily. Body weights were measured

just prior to the start of admixed food administration (Study Day 1) and on Study Day 8. Food consumption was recorded on Study Days 1 - 4, 4 - 7 and 7 - 8.
Whole blood was collected on heparin for plasma preparation from treated animals at three time points on Study Day 8 (8 a.m., 2 p.m. and 5 p.m.). The day after the end of the treatment period, animals were sacrificed without necropsy. Dietary administration of BYI 02960 induced no mortalities or treatment-related clinical signs.
The results obtained indicated a small sex difference in the plasma concentrations of BYI 02960, values being slightly higher for females than for males.
In male rats, a Cmax of 8.3 mg/L was measured at the time of collection of 8 a.m. In female rats, a Cmax of 9.4 mg/L was measured at the time collection of 2 p.m. However, in view of the inter-individual variability, the concentrations of BYI 02960 in plasma were considered similar between the three times of blood collection for both sexes (between 7.8 and 8.3 mg/L for males and between 8.8 and 9.4 mg/L for females). In conclusion, in the environmental conditions of the long-term studies (light: 7 a.m. to 7 p.m. & dark period: 7 p.m. to 7 a.m.) and assuming that animals are starting eating food when the light is switched off, the blood collection can be performed between 1 a.m. and 10 a.m. in order to measure the maximum concentration of BYI 02960.

28-Day immunotoxicity study
BYI 02960, was administered continuously via dietary administration to separate groups of female Wistar rats (10/group) at concentrations of 125, 600 and 3000 ppm (equating approximately to 10, 50, 230 mg/kg body weight/day) for at least 28 days. BYI 02960 was considered not to have any immunotoxic potential in female Wistar rats when given in the diet at dose levels up to 3000 ppm (corresponding to approximately 230 mg/kg/day) for at least 28 days.

Toxicity of metabolites
Difluoroacetic acid (DFA, BCS-AA56716) is a major soil, water and plant metabolite of BYI 02960; in the rat ADME study, it was found in urine at around 6% of the administered dose. This metabolite is devoid of genotoxic potential; the acute oral LD50 is between 300 and 2000 mg/kg, similar to parent compound. In a 14 day repeat dietary administration range finding study in the rat, the most significant findings were decreased mean glucose concentration in both sexes and an increase in urea concentration was observed in females only. In a 90-day rat study, DFA was administered in the diet to Wistar rats (10/sex/group) at concentrations of 200, 1000 and 6000 ppm. Lower mean glucose concentrations, lower total bilirubin and slightly higher mean urea concentrations were observed in both sexes at all doses. At 6000 and 1000 ppm dose levels, mean body weight, overall body weight gain and food consumption were reduced in both sexes. Lower hemoglobin concentration and lower mean corpuscular volume were observed in females, together with lower mean corpuscular hemoglobin and lower hematocrit, and higher ketone levels were noted in both sexes. A few black foci were also noted in the glandular part of the stomach in both sexes (including one control female), in correlation with a few cases of focal glandular erosion/necrosis observed at the microscopic examination. The minor changes noted in the clinical chemistry determination at the low dose are

considered not to be adverse effects of the test substance as they do not represent any functional impairment in the test organism. Therefore, the dose level of 200 ppm (equating to 12.7 and 15.6 mg/kg body weight/day in males and females, respectively) was considered to be a No Observed Adverse Effect Level (NOAEL) in the male and female Wistar rats. When the NOAEL is expressed in BYI 02960 equivalents, it equates to 38 and 47 mg/kg/day in males and females, respectively. Therefore, Difluoroacetic acid was not more toxic than BYI 02960 after subchronic administration to the rat. The metabolic changes observed with DFA are also observed with BYI 02960. The decrease in glucose was reversible and appeared to be adaptative as it was no longer significant during the second part of the rat carcinogenicity study.

BYI 02960-difluoroethyl-amino-furanone (BCS-CC98193, BYI 02960-DFEAF) is a minor plant metabolite of BYI 02960 and was also observed in the rat ADME study. It accounted for less than 10% of the administered dose in the rat urine. Since the confined rotational crop study with [furanone-4-14C]BYI 02960 indicated rather high residue levels of BYI 02960-difluoroethyl-amino-furanone in leafy crops and the absence of other suitable markers, BCS decided to include this metabolite in the residue definition for the data collection method for target and rotational crops (see residue section). The subsequent residue studies revealed that BYI 02960-difluoroethyl-amino-furanone is only a minor plant metabolite. However, based on the results of the confined rotational crop study, additional tox studies (acute toxicity testing, genotoxicity testing and subacute rat study) were conducted to show that the tox profile of the metabolite is covered by the endpoints derived from the parent compound. In the *in vitro* genotoxicity package, the Ames and HPRT tests were negative and the chromosome aberration test was positive. Therefore, two *in vivo* studies (*in vivo* micronucleus test and *in vivo* unscheduled DNA synthesis) were conducted, both of which were negative. Based upon the overall findings, BYI 02960-difluoroethyl-amino-furanone can be considered not genotoxic. The acute oral LD_{50} in rats was higher than 2000 mg/kg. The NOAEL of the 28-day rat study was 3000 ppm equating to 243 and 273 mg/kg/day in males and females, respectively based on body weight effects.

Several toxicology studies exist for two plant metabolites BYI 02960-CHMP ((6-chloro-3-pyridyl) methanol) and BYI 02960-6-CAN (6-chloronicotinic acid), which are metabolites common to other pesticides. For BYI 02960-CHMP, the Ames test was negative. The acute oral rat LD_{50} was 1842 mg/kg in males and 1483 mg/kg in females. In a 90-day rat study, BYI 02960-CHMP administered continuously via dietary administration to Sprague Dawley rats (10/sex/group) at concentrations of 160, 800, 4000 and 20 000 ppm resulted in decreases in mean body weights and mean food consumption in both sexes and a statistically significant increases in serum alkaline phosphatase activity in the 20000 ppm group females at study termination only. Histologically, dose-related eosinophilic intranuclear inclusions were seen in the proximal tubular epithelium of kidneys for 20000 ppm males and females and 4000 ppm males. The no observed effects level (NOEL) was 800 ppm (48.9 mg/kg/day) in males, and 4000 ppm (275.9 mg/kg/day) in females. When the NOEL is expressed in BYI 02960 equivalents, it equates to 97.8 and 551.8 mg/kg/day. Therefore, BYI 02960-CHMP was less toxic than BYI 02960 after subchronic administration to the rat.

For BYI 02960-6-CAN (6-chloronicotinic acid) an acute oral rat toxicity study and an Ames test were performed for the registration of acetamiprid. BYI 02960-6-CAN was not genotoxic and not acutely toxic.

The reference values of the parent flupyradifurone are applicable to both metabolites DFA and DFEAF; the experts considered that there was sufficient evidence to conclude that DFEAF is unlikely to have genotoxic potential.

B.6.11.3 ADI

The calculation of the ADI is based on the highest dose at which no adverse effect is observed in the most appropriate study in the most sensitive species. BYI 02960 was tested in several short-term studies in dogs, rats, and mice, in long-term toxicity studies in rats and mice, and in reproduction and developmental toxicity studies in rats and rabbits, providing the basis for the establishment of the ADI. ~~The lowest NOAEL (7.8 mg/kg/day) was obtained from a 1-year study in dog. Similar critical effects (myofiber degeneration, similar number of effected animals) were seen in the 90-day study with dog. It is therefore suggested to use both the 1-year dog study and the 90-day dog study to set the NOAEL of 12 mg/kg/d used as a starting point for the establishment of the ADI, also taking into account the sufficient dose spacing between the 90-day dog NOAEL and the 1-year LOAEL. Application of a safety factor for inter- and intraspecies differences of 100 results in an ADI of 0.12 mg/kg bw/day. The lowest NOAEL (7.7 mg/kg bw/d) was obtained from the 2-generation study in rat based on body weight effects in the parent animals. Application of a safety factor for inter- and intraspecies differences of 100 results in an ADI of 0.08 mg/kg bw/day (rounded from 0.077 mg/kg bw/day).~~

Considering the parental and offspring NOAEL in the multigeneration study, the experts agreed to set the ADI at 0.064 mg/kg bw per day, applying an uncertainty factor of 100.

Table 6.11.3-1 Studies considered for the establishment of the ADI

Study	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Reference
Semichronic toxicity studies			
90-d oral rat	6.0 ~~30.2~~	30.2 ~~156~~	[redacted] 2009
90-d oral mouse	81	407	[redacted] 2009
90-d oral dog	12	33	[redacted]. 2008
1-yr oral dog	7.8	28.1	[redacted] 2012
Chronic toxicity studies			
24-m oral chronic/carcino rat	15.8	80.9	[redacted] 2012
18-m oral carcino mouse	43	224	[redacted] 2012
Reproduction and teratogenicity studies			

1-generation rat - Parental - Developmental - Reproduction 2-generation rat - Parental - Developmental - Reproduction	 17.5 147.5 17.5 **6.4** ~~7.7~~ ~~32~~ 34 34	 60 - 61 32 ~~117~~ 134 134	[redacted], 2010 [redacted], 2011

B.6.11.4 ARfD (acute reference dose)

The calculation of the acute reference dose is based on the highest dose at which no adverse effect is observed in the most appropriate acute toxicity study in the most sensitive species. The maternal NOAEL of 15 mg/kg bw/d in the developmental toxicity study in rabbit is based on a reduced body weight of 12% during the first days of treatment which is an applicable effect to set the ARfD. Application of a safety factor for inter- and intraspecies differences of 100 results in an ARfD of 0.15 mg/kg bw/d.

~~Only slight body weight gain effects were observed at the top dose in the developmental toxicity studies in both rats and rabbits. Acutely toxic effects were seen only in the acute neurotoxixity study, where typical signs of nicotinergic insecticides have been observed. Therefore, it seems appropriate to set up the ARfD using the NOAEL of the acute neurotoxicity study. Application of a safety factor for inter- and intraspecies differences of 100 results in an ARfD of 0.35 mg/kg bw.~~

Table 6.11.4-1 Studies considered for the establishment of the ARfD

Study	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Reference
Reproduction and teratogenicity studies			
Teratogenicity study rat - Maternal - Developmental	50 50	150 150	[redacted], 2010
Maternal tolerability study rat - Maternal	30	-	[redacted], 2012
Teratogenicity study rabbit - Maternal - Developmental	**15** ~~40~~ 40	40 - -	[redacted] 2012
Neurotoxicity studies			
Acute neurotoxicity study in the rat	35	50	[redacted] 2011
90-day Neurotoxicity study in the rat - Systemic - Neurotoxicity	 29.4 143	 143 -	[redacted] 2011

B.6.11.5 AOEL

The calculation of the AOEL is based on the highest dose at which no adverse effect is observed in the most appropriate study in the most sensitive species. BYI 02960 was tested in several short-term studies in dogs, rats, and mice, in long-term toxicity studies in rats and mice, in reproduction and developmental toxicity studies in rats and rabbits, and neuro and immuno toxicity studies with rats providing the basis for the establishment of the short-term and long-term AOEL.

The lowest NOAEL (6.4 ~~7.7~~ mg/kg bw/d) was obtained from the 2-generation studie in rat based on body weight effects in the parent animals. A correction factor for oral absorption is not necessary since the oral absorption is >80%. Application of a safety factor for inter- and intraspecies differences of 100 results in an ADI of 0.08 mg/kg bw/day (rounded from 0.077 mg/kg bw/day).

~~The lowest NOAEL (7.8 mg/kg/day) was obtained from a 1-year study in dog. Similar critical effects (myofiber degeneration, similar number of effected animals) were seen in the 90-day study with dog. It is therefore suggested to use both the 1-year dog study and the 90-day dog study to set the NOAEL of 12 mg/kg/d used as a starting point for the establishment of the AOEL, also taking into account the sufficient dose spacing between the 90-day dog NOAEL and the 1-year LOAEL. A correction factor for oral absorption is not necessary since the oral absorption is >80%. Application of a safety factor for inter- and intraspecies differences of 100 results in an AOEL of 0.12 mg/kg bw/day for both short-therm and long-therm.~~

Table 6.11.5-1 Studies considered for the establishment of the AOEL

Study	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Reference
Semichronic toxicity studies			
90-d oral rat	6.0 ~~30.2~~	30.2 ~~156~~	[redacted] 2009
90-d oral mouse	81	407	[redacted] 2009
90-d oral dog	12	33	[redacted] 2008
1-yr oral dog	7.8	28.1	[redacted] 2012
Chronic toxicity studies			
24-m oral chronic/carcino rat	15.8	80.9	[redacted] 2012
18-m oral carcino mouse	43	224	[redacted] 2012
Reproduction and teratogenicity studies			
1-generation rat			[redacted] 2010
- Parental	17.5	60	
- Developmental	147.5	-	
- Reproduction	17.5	61	
2-generation rat			[redacted] 2011
- Parental	**6.4 ~~7.7~~** ~~32~~	32 ~~117~~	
- Developmental	34	134	
- Reproduction	34	134	
Neurotoxicity studies			
Acute neurotoxicity study in the rat	35	50	[redacted] 2011

90-day Neurotoxicity study in the rat - Systemic - Neurotoxicity	 29.4 143	 143 -	[redacted], 2011
Immunotoxicity studies			
28-day immunotoxicity in the rat - Systemic - Immunotoxic	 50 230	 230 -	[redacted] 2011

B.6.11.6 Drinking water limit

According to Council Directive 97/57/EC, exposure to BYI 02960 through drinking water should account for not more than 10% of the ADI. If it is assumed that the average daily consumption of water amounts to 2 litre per person of 60 kilogram, a potential drinking water limit of ((60x0.064 ~~0.08~~ ~~0.12~~)/10)/2 mg/l, i.e.0.192 ~~0.24~~ ~~0.36~~ mg/l can be established.

According to Document 8064/VI/79 of the European Commission, the EU drinking water limit for pesticides of 0.1 µg/l is applicable for BYI 02960.

B.6.12 ACUTE TOXICITY INCLUDING SKIN SENSITISATION OF PREPARATIONS (ANNEX IIIA 7.1)

B.6.12.1 Acute toxicity

STUDY 1

Characteristics

reference	:	[redacted]. 2010	exposure	:	gavage
type of study	:	Acute oral toxicity study	doses	:	2000 mg/kg bw
year of execution	:	2010	vehicle	:	Tap water
test substance	:	BYI 02960 SL 200 g/L (batch 2009-001253)	GLP statement	:	yes
route	:	oral	guideline	:	OECD 423
species	:	Rat, Wistar HsdCpb:Wu	acceptability	:	Acceptable
group size	:	3 females/step	**LD50**	:	>2000 mg/kg bw

Study design

The study was performed in accordance with OECD 423.

Groups of three female Wistar rat per step were orally exposed to BYI 02960 SL 200 g/L at a concentration of 2000 mg/kg bw. The test item was formulated in tap water with an administration volume of 10 ml/kg bw. Clinical signs and mortality rates were determined several times on the day of administration and subsequently at least once daily for an observation period of at least 14 days. The

weight gain of the animals was checked weekly. At the end of the study the animals were sacrificed, dissected and examined macroscopically.

Results

Mortality: No mortality was observed.

Symptoms of toxicity: Clinical signs consisted of decreased motility and temporary tremor.

Body weight: Body weight increased throughout the study period.

Pathology: No macroscopic pathologic abnormalities were observed

Acceptability

This study is considered acceptable.

Conclusion

The LD50 values of the formulation BYI 02960 SL 200 g/L was found to be greater than 2000 mg/kg bw.

STUDY 2

Characteristics

reference	:	[redacted]. 2010	exposure	:	24 hours on a 10% skin area (semi-occlusive)
type of study	:	Acute dermal toxicity	doses	:	2000 mg/kg bw
year of execution	:	2010	vehicle	:	None
test substance	:	BYI 02960 SL 200 g/L (batch 2009-001253)	GLP statement	:	yes
route	:	percutaneous	guideline	:	OECD 402
species	:	Rat, Wistar	acceptability	:	Acceptable
group size	:	5/sex	**LD_{50}**	:	>2000 mg/kg bw

Study design

The study was performed in accordance with OECD 402.

Groups of five male and five female Wistar rats received a single dermal dose of 2000 mg/kg bw of BYI 02960 SL 200 g/L (batch 2009-001253). The required amount of the pure liquid test substance was applied to an approximately 10% body surface area under semi-occlusive dressing for 24 hours. After 24 hours the dressings were removed and the area was rinsed. Clinical signs and mortality rates were determined several times on the day of application and subsequently at least once daily for an observation period for an observation period of at least 14 days. The weight gain of the animals was checked weekly until the end of the study.

Results

Mortality: No mortality was observed

Symptoms of toxicity: No treatment related findings were observed

Body weight: The mean body weights increased throughout the study period.

Pathology: No macroscopic pathologic abnormalities were observed.

Acceptability

The study is considered acceptable.

Conclusions

The acute dermal LD50 of BYI 02960 SL 200 g/L in male and female rats was found to be greater than 2000 mg/kg bw.

STUDY 3

Characteristics

reference	:	[redacted] 2010	exposure	:	4 hours; nose-only
type of study	:	Acute inhalation toxicity	doses	:	Nominal concentration 4236.1 and 9613.9 mg/m^3 Actual concentration: 1956.6 and 4483.0 mg/m^3 (MMAD 1.66-1.97 µm, GSD 1.69-1.78)
year of execution	:	2010	vehicle	:	none
test substance	:	BYI 02960 SL 200 g/L (batch 2009-001253)	GLP statement	:	yes
route	:	inhalation	guideline	:	OECD 403
species	:	Rat, Wistar HsD Cpb: Wu (SPF)	acceptability	:	Acceptable
group size	:	5/sex/dose	**LC_{50}**	:	3496 mg/m^3

Study design

The study was performed in accordance with OECD 403 (1981).

Groups of five male and female Wistar rats were nose-only exposed to BYI 02960 SL 200 g/L (batch 2009-001253) for 4 hours at a mean aerosol concentration of 1956.6 and 4483.0 mg/m^3. Body weights were measured before exposure, on day 1, 3, 7 and weekly thereafter. Clinical signs and mortality were examined several times on the day of exposure and at least once daily thereafter for a period of 14 days. At the end of the study period the surviving rats were sacrificed and were given a gross-pathological examination.

Results

Table 6.12.1-1 Mortality:

Mean aerosol concentration (mg/m^3)	Mortality	
	Males	Females
1956.6	0/5	0/5
4483.0	2/5	4/5

Symptoms of toxicity: In surviving animals in both doses clinical signs included, bradypnea, laboured breathing patterns, irregular breathing, piloerection, cyanosis, reduced motility, limp, gait high legged,

nasal discharge serous, nose with red encrustations, nose and muzzle with red encrustations, nostrils with red encrustations and hypothermia. On day 8 post exposure all rats were without clinical signs. In 4483.0 mg/m^3 exposed animals reflex measurements revealed reduced tonus and grip strength as well as an impaired righting response.

Body weight: In high dose males significantly reduced body weight compared to control.

Pathology: In surviving animals discoloration of the lung and/or kidney was observed. The rats that died showed findings such as a less collapsed lung, dark-red discoloration of the lung and a trachea filled with foamy viscous content. These finding were suggestive to lung oedema as cause of death. In addition light discoloration of the spleen, discoloration of the liver, reddish mucous content in the intestines were observed.

Acceptability

The study is considered acceptable.

Conclusion

LC50 value females 3496 mg/m^3, LC50 males > 4483 mg/m^3

B.6.12.2 Irritation and sensitisation

STUDY 1

Characteristics

reference	:	[redacted] 2010	exposure	:	4-hours semi-occlusive
type of study	:	Skin irritation study	doses	:	0.5 ml
year of execution	:	2010	vehicle	:	none
test substance	:	BYI 02960 SL 200 g/L (batch 2009-001253)	GLP statement	:	yes
route	:	dermal	guideline	:	OECD 404
species	:	Rabbits Crl:KBL(NZW)BR	acceptability	:	Acceptable
group size	:	Three females	**Effect**	:	Not skin irritating

Study design

The study was performed in accordance with OECD 404 (2002).

Three female New-Zealand white rabbits (Crl:KBL(NZW)BR) were dermally exposed to 0.5 ml of BYI 02960 SL 200 (batch 2009-001253) on a skin area of 2.5 x 2.5 cm^2 under semi-occlusive dressing. After 4 hours of exposure the patch was removed and the exposed area was washed with water. The dermal irritation was scored at approximately 1, 24, 48 and 72 hours after patch removal.

Results

The results are summarised below.

Table 6.12.2-1 Individual irritation scores

Scores observed after	1 hour	24 hours	48 hours	72 hours
Erythema	0/0/0	0/0/0	0/0/0	0/0/0
Oedema	0/0/0	0/0/0	0/0/0	0/0/0

B.6.12.2.1 Table 6.12.2-2 Mean value irritation scores

Animal	mean 24-72 hrs	
	erythema	oedema
1	0	0
2	0	0
3	0	0
mean	0	0

Acceptability

The study is considered acceptable.

Conclusion

The formulation BYI 02960 SL 200 is considering not irritating to skin.

STUDY 2

Characteristics

reference	:	[redacted]. 2010b	exposure	:	1 hour
type of study	:	Eye irritation	doses	:	0.1 ml
year of execution	:	2010	vehicle	:	none
test substance	:	BYI 02960 SL 200 g/L (batch 2009-001253)	GLP statement	:	yes
route	:	Ocular	guideline	:	OECD 405
species	:	Rabbits (Crl:KBL(NZW)BR)	acceptability	:	yes
group size	:	Three females	**Effect**	:	Not eye irritant

Study design

The study was performed in accordance with OECD 405 (2002).

Three female albino rabbits (Crl:KBL(NZW)BR) received 0.1 ml of BYI 02960 (batch 2009-001253) into the conjunctival sac of one eye. The lids were gently held together for about one second in order to prevent loss of the test compound. The eye, which remained untreated, served as control. The eye was rinsed approximately 24 hours following instillation. Eye irritations were scored and recorded approximately at 1, 24, 48 and 72 hours post application.

Results

The results are summarised below.

Table 6.12.2-3 Individual irritation scores

Scores observed after	1 hour	24 hours	48 hours	72 hours
Cornea/opacity	0/0/0	0/0/0	0/0/0	0/0/0
Iris	0/0/0	0/0/0	0/0/0	0/0/0
Conjunctiva redness	1/1/1	1/1/2	1/1/2	0/0/0
Conjunctiva chemosis	0/0/0	0/0/0	0/0/0	0/0/0

B.6.12.2.2 Table 6.12.2-4 Mean value irritation scores

Animal	mean 24-72 hrs			
	Corneal opacity	Iris	Conjunctiva redness	Conjunctiva chemosis
1	0	0	0.7	0
2	0	0	0.7	0
3	0	0	1.3	0
mean	0	0	0.9	0

Acceptability

The study is considered acceptable.

Conclusions

BYI 02960 is considered not irritating to eyes.

STUDY 3

Characteristics

reference	:	[redacted]. 2010	exposure	:	Epicutaneous, three consecutive days.
type of study	:	Skin sensitisation (LLNA)	doses	:	0, 25, 50,100%
year of execution	:	2010	vehicle	:	1% Pluronix Acid L92
test substance	:	BYI 02960 SL 200 g/L (batch 2009-001253)	GLP statement	:	yes
route	:	dermal	guideline	:	OECD 429
species	:	Mice, CBA/J	acceptability	:	yes
group size	:	5 females/group	**Effect**	:	Skin sensitising

Study design

The study was carried out according to OECD guideline 429. With the deviation that one animal of the control group and one animal at a concentration of 25% did not receive the injection of tritiated thymidine due to technical problems.

BYI 02960 SL 200 g/l (batch 2009-001253) was applied on the external surfaces of each ear (25 µl/ear) of groups of five female CBA/J mice for three consecutive days. On day 5, the cell proliferation

in the draining auricular lymph nodes was measured by incorporation of tritiated thymidine and the obtained values were used to calculate proliferation indices. The positive control group received 30% alpha-Hexylcinnamaldehyde (HCA).

Results

No mortality, signs of systemic toxicity or effects on body weight were observed. No cutaneous reactions were observed at the treated site.

Table 6.12.2-5 Stimulation index

Treatment	Cell count Stimulation Index
Vehicle	-
25%	1.3
50%	2.3
100%	3.0
HCA	6.4

Acceptability

The study is considered acceptable.

Conclusions

The formulation BYI 02960 SL 200 g/L was found to be sensitizing in the Local Lymph Node Assay at concentration of 100% (SI ≥ 3.0). According to EC classification criteria (2001/59/EC Directive), the formulation is labelled as R43, may cause sensitization by skin contact.

B.6.12.3 Summary

The results of the acute toxicity, irritation and sensitisation studies are presented in table 6.12.3-1, 6.12.3-2 and 6.12.3-3, respectively.

Table 6.12.3-1 Acute toxicity, LD_{50}/LC_{50} values

Test substance	LD_{50}/LC_{50}	Species	Route	Vehicle	Reference
BYI 02960 SL 200 g/L	>2000 mg/kg bw	Rat	Oral	Tap water	[redacted] 2012
BYI 02960 SL 200 g/L	>2000 mg/kg bw	Rat	Dermal	None	[redacted]. 2010
BYI 02960 SL 200 g/L	3496 mg/m^3	Rat	Inhalation	None	[redacted] 2010

Table 6.12.3-2 Skin and eye irritation studies

Test substance	Effect/Classification	Species	Route	Vehicle	Reference
BYI 02960 SL 200 g/L	Not skin irritating	Rabbit	Dermal	None	[redacted] 2010
BYI 02960 SL 200 g/L	Not eye irritating	Rabbit	Occular	None	[redacted] 2010b

Table 6.12.3-3 Sensitisation studies

Test substance	Effect/Classification	Species	Route	Vehicle	Reference
BYI 02960 SL 200 g/L	Skin sensitising (LLNA)	Mouse	Dermal	1% Pluronix Acid L92	[redacted] 2010

The formulation BYI 02960 SL 200 g/L does not need to be classified on the basis of its acute oral, dermal toxicology. According to the EC classification criteria (Directive 2001/59/EC) formulation BYI 02960 SL 200 g/L is classified with R20, Harmful by inhalation. BYI 02960 SL 200 g/L is considered not irritating to skin and eyes. BYI 02960 SL 200 g/L has sensitising properties in a LLNA and is classified with R43, may cause sensitization by skin contact.

B.6.13 DERMAL ABSORPTION (ANNEX IIIA 7.3)

B.6.13.1 Dermal absorption, *in vitro*

STUDY 1

Characteristics

reference	:	Odin-Feurtet, M. 2010	exposure	:	8 hours, unoccluded
type of study	:	*In vitro* rat and human dermal absorption study.	doses	:	200 g/L, 0.625 g/L and 0.1 g/L
year of execution	:	2010	vehicle	:	blank formulation BYI 02960 SL200
test substance	:	BYI 02960 Radiolabelled: [^{14}C]-BYI 02960, purity 99%, specific activity 4.37 MBq/mg Non-labelled: BYI 02960, purity 99.4%	GLP statement	:	yes
route	:	dermal	guideline	:	OECD 428
species	:	Human skin and rat (Wistar Rj: WI (IOPS HAN)) skin	acceptability	:	acceptable
group size	:	6/dose	**Result**	:	Human: 0.20%, 2.0%, 4.8% in high/mid/low dose Rat: 0.15%, 5.7%, 6.6% in high/mid/low dose

Study design

The *in vitro* percutaneous absorption of [^{14}C]-BYI02960 formulated as an SL200 formulation was studied following a single 8-hour application at three dose levels in excised human and rat skin. Three dose levels were used, the neat product (200 g BYI 02960/L formulation) and two spray dilutions (0.625 g BYI 02960/L formulation and a 0.1 g BYI 02960/L formulation). Due to high inter-individual variability in the groups of skin rat exposed to the low dose an additional six skin membranes were exposed to the low dose.

Dermatomed skin membranes were maintained in flow-through cells at approximately 32°C. The three liquid formulations were applied at a rate of 10 µL/cm2 to unoccluded skin samples mounted in flow-through diffusion cells. Receptor fluid samples were collected at hourly intervals during 24 hours. Eight hours post-application, the remaining dose material was washed off the skin with freshly prepared 1% v/v Tween 80 in PBS (phosphate buffered saline) using natural sponge swabs. At the end of the study (24 hours) the skin samples were swabbed again and were tape-stripped to remove residual surface dose and the stratum corneum. Tape strips were analyzed individually and the first two tape strips were considered non-absorbed. The skin samples were removed from the diffusion cells and taken for analysis.

Results

Some of the cells had to be excluded from analysis for the following reasons; technical problems at swabbing (R12, H15 and R19), high inter-individual variability in the groups of skin rat exposed to the low dose formulation (R13 to R18), different direct absorption compared to other cells in the group (H09, R08). The full results from the outliers were not included in the study.

Following a single application of BYI 02960 the total recovery ranged between 96.2% to 106.1%. High variability was observed in the skin compartment of up to 123% of the mean total % at dose site. At the high dose level, the mean total potentially absorbed dose was 0.20% and 0.15% of the applied dose for human skin and rat skin, respectively. Following application at the intermediate dose dermal absorption was 2.0 and 5.67% for human and rat skin, respectively. At the low dose 4.75% and 6.61% was potentially absorbed for human and rat skin, respectively.

Table 6.13.1-1: percentage of radioactivity after a single topical application

Dose levels	**Neat formulation; High dose**		**Spray dilution; Intermediated dose**		**Spray dilution; Low dose**	
Species	**Human (n=6)**	**Rat (n=6)**	**Human (n=5)**	**Rat (n=4)**	**Human (n=5)**	**Rat (n=5)**
Surface compartment						
Skin swabs (8 h)	105.61 ±4.37	105.10 ±1.41	97.28 ±1.72	85.50 ±6.25	89.40 ±3.42	85.48 ±8.40
Skin swabs (24h)[a]	0.09 ±0.15	0.02 ±0.01	0.44 ±0.31	0.63 ±0.66	0.97 ±0.89	5.66 ±3.19
Total skin swabs	105.70 ±4.40	105.11 ±1.42	97.73 ±1.77	86.12 ±6.03	90.37 ±3.61	91.13 ±6.57
Surface Dose (tape-strips 1&2)	0.13 ±0.13	0.13 ±0.21	0.49 ±0.26	2.14 ±1.17	0.99 ±0.52	5.56 ±3.50
Donor chamber	0.07 ±0.11	0.10 ±0.25	0.09 ±0.08	2.43 ±2.61	0.10 ±0.22	N.D
Total % non-absorbed	**105.90 ±1.50**	**105.35 ±1.52**	**98.30 ±1.67**	**90.69 ±3.99**	**91.46 ±3.45**	**96.99 ±4.24**

Skin compartment						
Skin[b]	0.09 ±0.09	0.02 ±0.02	0.47 ±0.32	1.74 ±1.86	1.11 ±0.91	1.09 ±0.56
Stratum Corneum[c]	0.10 ±0.12	0.06 ±0.08	1.15 ±0.53	2.85 ±3.83	3.00 ±1.83	4.40 ±2.89
Total % at dose site	**0.19 ±0.21**	**0.07 ±0.09**	**1.62 ±0.82**	**4.59 ±5.65**	**4.11 ±2.72**	**5.50 ±2.63**
Receptor compartment						
Receptor fluid (0-24h)	0.01 ±0.02	0.07 ±0.07	0.38 ±0.32	1.03 ±2.90	0.61 ±0.43	1.02 ±0.43
Receptor fluid terminal	0.002 ±0.005	N.D.	0.017 ±0.02	0.046 ±0.046	0.037 ±0.051	0.084 ±0.06
Receptor chamber	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Total % directly absorbed[d]	**0.02 ±0.03**	**0.07 ±0.07**	**0.39 ±0.34**	**1.08 ±0.33**	**0.65 ±0.48**	**1.11 ±0.44**
Total % potentially absorbable[e]	**0.20 ±0.22**	**0.15 ±0.09**	**2.00 ±1.07**	**5.67 ±5.96**	**4.75 ±2.96**	**6.60 ±2.80**
Total % recovery	**106.10 ±4.65**	**105.49 ±1.54**	**100.31 ±1.90**	**96.36 ±5.98**	**96.22 ±4.08**	**103.60 ±2.54**

[a] Sum of radioactivity found in swabs at termination and in surrounding swabs.
[b] sum of radioactivity found in skin after tape-stripping procedure and in surrounding skin.
[c] tape-strips excluding number 1&2 which are considered to be non-absorbed dose.
[d] sum of radioactivity found in receptor fluid (0-24h), receptor fluid terminal and receptor chamber.
[e] sum of radioactivity in Total % directly absorbed and Total % of dose site.
N.D. = not detected (below the limit of detection)
n = number of skin cells used for calculation

Acceptability

The study is considered acceptable.

Conclusion

At the low dose dermal absorption was 4.8% for human skin and 6.6% for rat skin. In the mid dose 2.0% of the applied dose was absorbed in human skin and 5.7% in rat skin. For the neat formulation 0.20% of the applied dose was potentially absorbed for human skin and 0.15% for rat skin.

B.6.13.2 Dermal absorption, *in vivo*

STUDY 1

Characteristics

reference	:	[redacted] 2010	exposure	:	8 hours, semi-occluded
type of study	:	*In vivo* rat dermal absorption study	doses	:	200, 0.625, 0.1 g/L
year of execution	:	2010	vehicle	:	blank formulation BYI 02960 200 SL
test substance	:	BYI 02960 Radiolabelled: [^{14}C]-BYI 02960, purity 99%, specific activity 4.37 MBq/mg Non-labelled: BYI 02960, purity 99.4%	GLP statement	:	yes
route	:	dermal	guideline	:	OECD 427

species	:	Rat, Wistar Rj: WI (IOPS HAN)	acceptability	:	Acceptable
group size	:	16/dose	**Result**	:	Concentrate: 21.8%, mid dose: 9.7% low dose: 20.6%

Study design

The study was conducted according to the OECD Guideline for the Testing of Chemicals, 427.

The percutaneous absorption of [^{14}C]-BYI02960 formulated as an SL200 formulation was studied following a single 8-hour application at three dose levels in male Wistar rats. The exposure levels were: the neat formulation containing 200 g BYI 02960/L, 0.625 g BYI 02960/L (1:320 dilution) and 0.1 g BYI 02960/L (1:2000 dilution). After dose application, the skin was semi-occluded with a perforated plastic cover held in place with surgical tape. Following the 8-hour exposure period, the remaining dose was washed off the skin with 1% v/v Tween 80 in phosphate buffered saline using sponge swabs. The swabs were retained for analysis. Group of four rats at each dose level was sacrificed at 8, 24, 72 and 168 hours post-application. Urine and faeces were collected separately into receivers at 0 to 8, 8 to 24 and at 24-hour intervals up to sacrifice. A tape stripping procedure was included in order to determine the distribution of the radioactivity through the skin. The following specimens were examined for residual radioactivity; urine, cage wash, faeces, whole blood, skin swabs, stratum corneum, tape strips, skin, surrounding skin, carcass.

Results

There were no treatment related clinical signs observed during the study.

<u>High dose</u>

After a single application of BYI 02960 SL200 at 200 g/L, the mean recovery rates were 113.4%, 102.4% 101.7% and 101.1% for the 8, 24, 72 and 168 hours groups, respectively. Total absorbed dose (skin compartment + systemic compartment) ranged between 17.0% to 21.8%.

Table 6.13.2-1: The mean distribution of radioactivity 8, 24, 72 and 168 hours after a single topical application of [14C]-BYI 02960 from a 200 g/L SL 200 formulation

Dose Group: 200 g/L (n= 4 rats/group)	**% of applied dose**							
	Hours post application							
	8		**24**		**72**		**168**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Skin swabs (8 hr& terminal)	83.36	8.01	74.06	5.56	70.84	7.71	66.75	17.42
Surface dose (tape strips 1 & 2)	0.33	0.08	0.77	0.71	0.29	0.14	0.43	0.28
Fur	0.06	0.08	n.s.	n.a.	1.10	1.05	0.58	0.10
Dressings	7.84	6.20	7.89	2.26	12.47	5.66	14.11	9.13

Total non-absorbed	**91.59**	**3.64**	**82.73**	**3.91**	**84.70**	**6.14**	**81.87**	**10.90**
Stratum corneum[a]	1.05	0.34	4.14	1.84	0.71	0.38	1.34	0.89
Treated skin[b]	3.31	1.66	2.41	1.70	1.32	0.90	2.00	1.13
Surrounding skin[c]	15.16	8.86	9.19	4.31	10.57	3.39	7.69	4.12
Total at dose site	**19.51**	**7.78**	**15.75**	**3.73**	**12.61**	**3.89**	**11.04**	**3.43**
Urine	0.01	0.01	0.21	0.12	0.78	0.43	2.18	0.54
Faeces	<LOQ	n.a.	0.07	0.03	0.16	0.08	0.94	0.62
Cage wash	0.02	0.02	0.08	0.05	0.22	0.11	2.04	2.25
Cardiac blood	0.01	0.003	0.01	0.004	0.004	0.006	0.013	0.01
Non-treated skin	1.31	1.47	2.68	2.12	2.71	1.53	2.30	2.02
Carcass	0.92	0.37	0.83	0.53	0.52	0.26	0.66	0.32
Total directly absorbed	**2.27**	**1.82**	**3.88**	**2.23**	**4.39**	**2.01**	**8.14**	**5.39**
Total potentially absorbed	**21.78**	**7.12**	**19.63**	**4.57**	**17.0**	**3.64**	**19.18**	**8.63**
Total Recovered	**113.4**	**7.89**	**102.4**	**4.63**	**101.7**	**5.08**	**101.1**	**2.40**

[a] = tape strips excluding surface dose strips 1 & 2,
[b] = skin at dose site after tape-stripping procedure,
[c] = skin immediately outside the dose application area,
SD = standard deviation, <LOQ = less than limit of quantification, n.a. = not applicable, n.s. = no sample.

Mid dose

After a single application of BYI 02960 SL200 at 0.625 g/L, the mean recovery rates were 99.8%, 99.4%, 92.3% and 95.6% for the 8, 24, 72 and 168 hours groups, respectively. Total absorbed dose (skin compartment + systemic compartment) ranged between 5.5 % to 9.66%.

Table 6.13.2-2: The mean distribution of radioactivity 8, 24, 72 and 168 hours after a single topical application of [14C]-BYI 02960 from a 0.625 g/L SL 200 formulation

Dose Group: 200 g/L (n= 4 rats/group)	**% of applied dose**							
	Hours post application							
	8		**24**		**72**		**168**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Skin swabs (8 hr& terminal)	89.26	4.68	88.71	6.26	76.93	6.30	85.88	6.50
Surface dose (tape strips 1 & 2)	1.26	0.43	2.15	1.90	4.14	3.06	1.61	0.67
Fur	n.s.	n.a.	n.s.	n.a.	1.17	1.38	1.03	2.07
Dressings	0.07	0.06	0.22	0.10	0.40	0.27	1.53	2.57
Total non-absorbed	**90.58**	**4.86**	**91.08**	**4.37**	**82.64**	**3.96**	**90.06**	**3.32**
Stratum corneum[a]	4.86	4.44	5.96	3.89	6.41	3.31	2.71	2.46
Treated skin[b]	1.88	0.71	0.67	0.16	0.49	0.33	0.43	0.31
Surrounding skin[c]	0.31	0.08	0.35	0.06	0.58	0.35	0.15	0.06
Total at dose site	**7.04**	**4.83**	**6.97**	**4.00**	**7.48**	**3.15**	**3.29**	**2.73**
Urine	0.26	0.23	0.34	0.17	0.84	0.28	1.10	0.07
Faeces	0.03	0.03	0.09	0.05	0.24	0.10	0.30	0.09
Cage wash	0.15	0.06	0.20	0.17	0.19	0.07	0.29	0.09
Cardiac blood	0.02	0.01	0.03	0.001	0.001	0.003	0.007	0.009
Non-treated skin	0.44	0.23	0.32	0.11	0.47	0.50	0.20	0.07

Carcass	1.33	0.26	0.34	0.13	0.44	0.12	0.353	0.09
Total directly absorbed	**2.21**	**0.52**	**1.31**	**0.16**	**2.18**	**0.31**	**2.24**	**0.06**
Total potentially absorbed	**9.26**	**4.98**	**8.28**	**4.03**	**9.66**	**3.08**	**5.53**	**2.70**
Total Recovered	**99.84**	**0.45**	**99.36**	**0.46**	**92.3**	**1.33**	**95.59**	**2.60**

[a] tape strips excluding surface dose strips 1&2.
[b] skin at dose site after tape-stripping procedure.
[c] skin immediately outside the dose application area
SD = standard deviation, n.s. = no sample, n.a. = not applicable.

Low dose

After a single application of BYI 02960 SL200 at 0.625 g/L, the mean recovery rates were 100.5%, 95.8%, 102.9% and 95.6% for the 8, 24, 72 and 168 hours groups, respectively.

Total absorbed dose (skin compartment + systemic compartment) ranged between 10.3% to 20.6%.

Table 6.13.2-3: The mean distribution of radioactivity 8, 24, 72 and 168 hours after a single topical application of [14C]-BYI 02960 from a 0.1 g/L SL 200 formulation

Dose Group: 200 g/L (n= 4 rats/group)	**% of applied dose**							
	Hours post application							
	8		**24**		**72**		**168**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Skin swabs (8 hr& terminal)	77.99	5.59	82.44	7.12	80.53	3.92	68.67	2.62
Surface dose (tape strips 1 & 2)	3.50	1.56	2.61	1.33	3.66	0.78	3.66	0.84
Fur	1.71	0.83	n.s.	n.a.	n.s.	n.a.	n.s.	n.a.
Dressings	0.64	0.87	0.41	0.28	0.49	0.25	2.65	1.91
Total non-absorbed	**83.33**	**3.58**	**85.47**	**7.45**	**84.68**	**3.64**	**74.98**	**4.04**
Stratum corneum[a]	8.74	4.50	6.26	3.03	11.69	3.14	10.37	2.68
Treated skin[b]	2.05	0.79	0.87	0.56	0.83	0.53	1.36	1.19
Surrounding skin[c]	1.53	0.16	0.41	0.03	0.40	0.26	0.73	0.34
Total at dose site	**12.32**	**3.93**	**7.54**	**3.58**	**12.92**	**2.89**	**12.46**	**2.87**
Urine	0.08	0.05	0.57	0.28	1.82	0.45	3.00	0.27
Faeces	<LOQ	n.a.	0.11	0.09	0.62	0.20	0.44	0.25
Cage wash	<LOQ	n.a.	0.12	0.15	0.34	0.39	0.70	0.44
Cardiac blood	0.07	0.01	0.06	0.08	0.11	0.09	0.09	0.06
Non-treated skin	1.55	0.38	0.53	0.11	0.76	0.12	0.79	0.05
Carcass	2.63	1.45	1.40	0.18	1.63	0.47	3.09	0.69
Total directly absorbed	**16.64**	**5.17**	**10.32**	**3.85**	**18.20**	**2.70**	**20.58**	**3.15**
Total potentially absorbed	**16.64**	**5.17**	**10.32**	**3.85**	**18.20**	**2.70**	**20.58**	**3.15**
Total Recovered	**100.5**	**3.49**	**95.79**	**3.27**	**102.9**	**1.03**	**95.56**	**1.99**

[a] tape strips excluding surface dose strips 1&2.
[b] skin at dose site after tape-stripping procedure.
[c] skin immediately outside the dose application area
SD = standard deviation, n.s. = no sample, n.a. = not applicable, <LOQ = less than limit of quantification.

Acceptability

The study is considered acceptable.

Conclusion

The amount of applied radiolabelled [14C]-BYI 02960 which can be considered as the maximum percentage that could be considered as potentially absorbable under the experimental conditions of this study was 21.8%, 9.7% and 20.6% for the high, intermediate and low dose formulations respectively.

B.6.13.3 Overall conclusion comparative dermal absorption

Table 6.13.3-1: Derivation of human dermal absorption of BYI 02960 from *in vivo* and *in vitro* dermal absorption data.

	Dermal absorption % of applied dose		
Test system	**Concentrate (200 g/L)**	**Intermediate (0.625 g/L)**	**Low (0.1 g/L)**
In vivo rat	21.8	9.7	20.6
In vitro rat	0.2	5.7	6.6
In vitro human	0.2	2.0	4.8
Ratio *in vitro* human / rat	1	0.35	0.73
Corrected human skin penetration	**22**	**3**	**15**

The predicted human dermal absorption value based on the *in vivo* data in rats corrected for the ratio of absorption between rats in humans *in vitro* is higher than the human *in vitro* data alone. The EFSA guidance on dermal absorption (EFSA Journal 2012; 10(4): 2655) states that in these cases it is justified to take the lower value from human *in vitro* data as rat data generally overpredict dermal absorption.

The dermal absorption for BYI02960 200SL in the human *in vitro* study was 0.20% (±0.22) for the concentrate and 2.0% (±1.07) and 4.75% (±2.96) for the intermediate dose (0.625 g/L) and low dose (0.1 g/L), respectively. There was significant variation between replicates with the standard deviation up to 110% of the mean absorption value. The EFSA guidance on dermal absorption recommends that if the variation between replicates is equal to or larger than 25% of the mean absorption consideration should be given to using a value other than the mean. The preferred method is the addition of a standard deviation to the mean values. This leads to a dermal absorption values for risk assessment of 0.4% for the concentrate, 3% for the intermediate dose (0.625 g/L) and 8% for the low dose of 0.1 g/L.

B.6.14 TOXICOLOGICAL DATA ON NON ACTIVE SUBSTANCES (ANNEX IIIA 7.4 AND POINT 4 OF THE INTRODUCTION)

MSDSs for the ingredients of BYI02960 200SL were submitted by the notifier.

Considering human toxicological endpoints (endpoints for which the formulation was tested are excluded):
None of the co-formulants is labelled.

B.6.15 DISLOGEABLE FOLIAR RESIDUES

Study 1

Characteristics

Reference	:	Freitag, T & Suke, S 2011	Application rate	:	2x 0.125 kg as/ha
Type of study	:	Dislogeable foliar residue study	Area	:	Northern Europe (Netherlands)
Year of execution	:	2010-2011	Plant	:	Lettuce
Test substance	:	BYI 02960 SL 200 G (Soluble liquid) 196.3 g/L flupyradifurone. (Batch no. 2010-000672)	Sampling	:	leaves
GLP statement	:	yes	Analysis	:	HPLC-MS/MS
Guideline	:	US EPA OPPTS 875.2100	Acceptability	:	Acceptable

Material and methods

The purpose of the study was to determine the magnitude of the dislodgeable foliar residues of flupyradifurone on lettuce leaf foliage after each of two spraying applications with BYI 02960 SL 200 (200 g flupyradifurone/L). The study was conducted in Northern Europe (The Netherlands) during the 2010 season. The actual application data are presented in the following table.

Table 6.15.1-1: Application parameters

Country	Application				
	Type	Number	Interval (days)	Growth stage (BBCH)	Rate (kg a.s./ha)
The Netherlands	Spraying	2	10	45 - 48	0.125

Samples were collected in a manner designed to obtain representative samples. They were taken, prepared in the field where necessary, transported and stored. Leaf punches were collected directly into a pre-labelled poly-propylene jar using a leaf punch sampler (Birkestrand Co; El Monte, CA). Each sample consisted of 40 disks cut with a leaf puncher with 2.523 cm diameter and a disk area of 5 cm^2. The leaf punches represented a total double-sided leaf surface area of 400 cm^2. A sample was collected from each of the three subplots to provide three replicate samplings at each sampling interval. Leaf punches were taken from the potential worker contact zone including upper, middle, and lower portions of the crop foliage and interior and exterior portions of the crop foliage. Control leaf punch samples were collected prior to the first application. Treated samples collected on the day of

application were taken after the spray had dried. After each sample was collected, the sampling jar was capped and kept cool for transport to the field site laboratory. Leaf punch samplers were cleaned after each sampling interval. The dislodging of the leaf samples was performed as soon as possible, but not later than 4 hours after collection. The samples were dislodged using a 0.01% Aerosol OT solution (i.e. docusate sodium salt which corresponds to a surfactant), and analysed by HPLC-MS/MS.

Results and discussion

The results are summarised in the following table.

Table 6.15.1-2: Amounts of dislodgeable foliar flupyradifurone residues on lettuce in the Netherlands [µg a.s./cm^2], two sided. Figures in bold indicate day of treatment

Sampling [DA1.T][#]	Dislodgeable foliar residues [µg a.s./cm^2]
-0	<0.01
0	**0.291**
3	<0.01
5	<0.01
7	<0.01
-10	<0.01
10	**<0.01***
11	<0.01
13	<0.01
15	<0.01
17	<0.01
20	<0.01

[#]:DA1.T: day after first treatment; " - " = before respective treatment; * for explanation see text

Already immediately after the treatment there is a clear decline of dislodgeable foliar residues, resulting in values <LOQ. The second application – 10 days after the first one – was performed as no rain was expected. However, 20 minutes after the application there was rainfall for about 20 minutes with one minute of hard rain. Obviously this has washed off any residues from the leaf surfaces.

Field fortification samples and lab fortification samples were spiked with 0.01, 0.1 and 1.0 µg/cm^2. The recovery values are presented below.

Table 6.15.1-3: Laboratory recovery data for flupyradifurone

Sample material	FL [µg/cm^2]	Single Values [%]	Mean Value	RSD [%]	LOQ [µg/cm^2]
Lettuce, leaf punch washings	0.01	88; 90; 92; 88; 92; 93	91	2.4	0.01
	0.10	91; 101; 94; 95; 114; 95	98	8.5	

1.0	94; 94; 92; 91; 97; 93	94	2.2
	Overall Recovery (n=18)	94	6.2

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantification
These recoveries were performed during the conduct of the DFR study.

Table6.15.1-4: Field Recovery data for flupyradifurone

Sample material	FL [µg/cm²]	Single Values [%]	Mean Value	RSD [%]	LOQ [µg/cm²]
Lettuce, leaf punch washings	0.01	69; 77; 66	71	8.0	0.01
	0.10	76; 66; 96	79	19.3	
	1.0	64; 56; 82	67	19.8	
		Overall Recovery (n=9)	72	16.3	

FL = Fortification Level, RSD = Relative Standard Deviation,
LOQ/D = Practical Limit of Quantification/Detection

The laboratory and procedural recoveries are within the acceptable range (70-110%, See Guidance for generating and reporting methods of analysis in support of pre-registration data requirements…(SANCO/3029/99 rev. 4) for 0.01 and 0.10 µg/cm². The field recovery for 1.0 µg/cm² is only 67%, however since the foliar dislogeable residues are maximally 0.291 µg/cm² the study is considered acceptable.

Conclusion

The DFR value at day 0 (i.e. shortly after application when the spray has dried) amounts to 0.29 µg/cm². This corresponds to 2.3 µg a.s./cm² per kg a.s./ha. Already three days after application the DFR is <LOQ (0.01 µg/cm²).

Due to the heavy rain shortly after the second application no results are available from this application. However, as the DFR values before the second application were already constantly <LOQ no other figures than the ones from the first application would have been expected for the second application: Three days after application the DFR values are <LOQ.

<u>Study 2</u>

Characteristics

Reference	:	Freitag, T. & Suke, S 2011	Application rate	:	2x 0.125 kg as/ha
Type of study	:	Dislogeable foliar residue study	Area	:	Southern Europe (Portugal)
Year of execution	:	2010-2011	Plant	:	Lettuce
Test substance	:	BYI 02960 SL 200 G (Soluble liquid) 196.3 g/L flupyradifurone. (Batch no. 2010-000672)	Sampling	:	leaves
GLP statement	:	yes	Analysis	:	HPLC-MS/MS
Guideline	:	US EPA OPPTS 875.2100	Acceptability	:	Acceptable

Material and methods

The purpose of the study was to determine the magnitude of the dislodgeable foliar residues of flupyradifurone on lettuce leaf foliage after each of two spraying applications with BYI 02960 SL 200 (200 g BYI 02960/L). The study was conducted in Southern Europe (Portugal) during the 2010 season. The actual application data are presented in the following table.

Table 6.15.1-5: Application parameters

Country	Application				
	Type	No	Interval (days)	Growth stage (BBCH)	Rate (kg a.s./ha)
Portugal	Spraying	2	10	43 - 47	0.125

Samples were collected in a manner designed to obtain representative samples. They were taken, prepared in the field where necessary, transported and stored. Leaf punches were collected directly into a pre-labelled poly-propylene jar using a leaf punch sampler (Birkestrand Co; El Monte, CA). Each sample consisted of 40 disks cut with a leaf puncher with 2.523 cm diameter and a disk area of 5 cm^2. The leaf punches represented a total double-sided leaf surface area of 400 cm^2. A sample was collected from each of the three subplots to provide three replicate samplings at each sampling interval. Leaf punches were taken from the potential worker contact zone including upper, middle, and lower portions of the crop foliage and interior and exterior portions of the crop foliage. Control leaf punch samples were collected prior to the first application. Treated samples collected on the day of application were taken after the spray had dried. After each sample was collected, the sampling jar was capped and kept cool for transport to the field site laboratory. Leaf punch samplers were cleaned after each sampling interval. The dislodging of the leaf samples was performed as soon as possible, but not later than 4 hours after collection. The samples were dislodged using a 0.01% Aerosol OT solution (i.e. docusate sodium salt which corresponds to a surfactant) and analysed by HPLC-MS/MS.

Results and discussion

The results are summarised in the following table.

Table 6.15.1-6: Amounts of dislodgeable foliar flupyradifurone residues on lettuce in Portugal [µg a.s./cm^2], two sided. Figures in bold indicate day of treatment

Sampling [DA1.T]#	Dislodgeable foliar residues [µg a.s./cm^2]
-0	<0.01
0	**0.110**
3	<0.01
5	<0.01
7	<0.01
-10	<0.01
10	**0.264**
11	0.012
13	<0.01

15	<0.01
17	<0.01
20	<0.01

#:DA1.T: day after first treatment; " - " = before respective treatment

After each treatment, there is an immediate decline of dislodgeable foliar residues resulting in values <LOQ, already 3 days after application.

Field fortification samples and lab fortification samples were spiked with 0.01, 0.1 and 1.0 µg/cm^2. The field recovery values are presented below (for the lab fortification samples see Study 1).

Table 6.15.1-7: Field recovery data for flupyradifurone

Sample material	FL [µg/cm^2]	Single Values [%]	Mean Value	RSD [%]	LOQ [µg/cm^2]
Lettuce, leaf punch washings	0.01	72; 87, 57	72	15	0.01
	0.10	79; 96; 89	88	9.7	
	1.0	97; 52; 69	72	23	
		Overall Recovery (n=9)	78	16	

FL = Fortification Level, RSD = Relative Standard Deviation,
LOQ/D = Practical Limit of Quantification/Detection

The laboratory and procedural recoveries are within the acceptable range (70-110%). The study report noted that the values 57 and 52% were unexpected low results and are probably due to inhomogenous sample solution in the used tubes.

Conclusion

The DFR value at day 0 (i.e. shortly after application when the spray has dried) amounts to 0.11 µg/cm². The second application results in a significant higher figure while the samples before the second application were already constantly <LOQ. Hence, there is no indication that residues from a former application could have accumulated. Most likely, there was just a lower target deposition at the first application.

The value of the second application (= 0.26 µg/cm²) corresponds to 2.1 µg a.s./cm^2 per kg a.s./ha, within three days after application the DFR values are <LOQ.

<u>Study 3 Foliar dislogeable residues</u>

Characteristics

Reference	:	Freitag, T. & Suke, S 2011	Application rate	:	2x 0.125 kg as/ha (1N for lettuce, 0.83N for hops)
Type of study	:	Dislogeable foliar residue study	Area	:	Greenhouse (The Netherlands)
Year of execution	:	2010-2011	Plant	:	Lettuce
Test substance	:	BYI 02960 SL 200 G (Soluble	Sampling	:	leaves

		liquid) 196.3 g/L flupyradifurone. (Batch no. 2010-000672)			
GLP statement	:	yes	Analysis	:	HPLC-MS/MS
Guideline	:	US EPA OPPTS 875.2100	Acceptability	:	Acceptable

Material and methodslettertype en grootte

The purpose of the study was to determine the magnitude of the dislodgeable foliar residues of flupyradifurone on lettuce leaf foliage after each of two spraying applications with BYI 02960 SL 200 (200 g flupyradifurone/L). The study was conducted in Northern Europe (The Netherlands) in the greenhouse during the 2010 season. The actual application data are presented in the following table.

Table 6.15.1-8: Application parameters

Country	Application				
	Type	Number of applications	Interval (days)	Growth stage (BBCH)	Rate (kg a.s./ha)
The Netherlands	Spraying	2	10	44 - 48	0.125

Samples were collected in a manner designed to obtain representative samples. They were taken, prepared in the field where necessary, transported and stored according to US EPA OPPTS 875.2100 Foliar Dislodgeable Residue Dissipation. Leaf punches were collected directly into a pre-labelled poly-propylene jar using a leaf punch sampler (Birkestrand Co; El Monte, CA). Each sample consisted of 40 disks cut with a leaf puncher with 2.523 cm diameter and a disk area of 5 cm^2. The leaf punches represented a total double-sided leaf surface area of 400 cm^2. A sample was collected from each of the three subplots to provide three replicate samplings at each sampling interval. Leaf punches were taken from the potential worker contact zone including upper, middle, and lower portions of the crop foliage and interior and exterior portions of the crop foliage. Control leaf punch samples were collected prior to the first application. Treated samples collected on the day of application were taken after the spray had dried. After each sample was collected, the sampling jar was capped and kept cool for transport to the field site laboratory. Leaf punch samplers were cleaned after each sampling interval. The dislodging of the leaf samples was performed as soon as possible, but not later than 4 hours after collection. The samples were dislodged using a 0.01% Aerosol OT solution (i.e. docusate sodium salt which corresponds to a surfactant) and analysed by HPLC-MS/MS.

Results and discussion

The results are summarised in the following table.

Table 6.15.1-9: Amounts of dislodgeable foliar flupyradifurone **residues on lettuce in the Netherlands [µg a.s./cm^2], two sided. Figures in bold indicate day of treatment**

Sampling [DA1.T][#]	Dislodgeable foliar residues [µg a.s./cm^2]
-0	<0.01
0	**0.293**
3	0.010
5	<0.01
7	<0.01
-10	<0.01
10	**0.316**
11	0.235
13	0.010
15	<0.01
17	<0.01
20	<0.01

[#]:DA1.T: day after first treatment; " - " = before respective treatment

After each treatment there is an immediate decline of dislodgeable foliar residues resulting in values at the LOQ already 3 days after application.

Field fortification samples and lab fortification samples were spiked with 0.01, 0.1 and 1.0 µg/cm^2. The field recovery values are presented below (for the lab fortification samples see Study 1).

Table 6.15.1-10: Field Recovery data for flupyradifurone

Sample material	FL [µg/cm^2]	Single Values [%]	Mean Value	RSD [%]	LOQ [µg/cm^2]
Lettuce, leaf punch washings	0.01	77; 78; 82	79	3.3	0.01
	0.10	82; 92; 81	85	7.2	
	1.0	1*; 87; 78	83	-	
		Overall Recovery (n=8)	82	6.2	

FL = Fortification Level, RSD = Relative Standard Deviation,
LOQ/D = Practical Limit of Quantification/Detection
*Unexpected low result. Either a wrong spiking solution was sent to the field or a mistake during the field phase occurred. This value is not considered.

The laboratory and procedural recoveries are within the acceptable range (70-110%).

Conclusion

The DFR value at day 0 (i.e. shortly after application when the spray has dried) amounts to 0.29 µg/cm². This corresponds to 2.3 µg a.s./cm^2 per kg a.s./ha. Then an immediate decline quickly leads to values <LOQ. The DFR value at day 10 – just after the second application when the spray

has dried – amounts to 0.32 µg/cm², corresponding to 2.5 µg a.s./cm² per kg a.s./ha. Again, three days after application the DFR values are at the LOQ.
On average, the DFR_0-values amount to 2.4 µg a.s./cm² per kg a.s./ha.

B.6.15.1 Overall conclusion on DFR

The DFR_0-values amount to similar values for field application in the Netherlands or Portugal or greenhouse applications in the Netherlands (respectively 2.3; 2.1 and 2.4 µg a.s./cm² per kg a.s./ha). Therefore, a DFR_0-value of 2.4 µg a.s./cm² per kg a.s./ha can be used in the human risk assessment.

B.6.16 EXPOSURE DATA (ANNEX IIIA 7.2)

Product information

Product	:	Sivanto
Purpose	:	Insecticide
Active substance (a.s.)	:	Flupyradifurone (BYI 02960) 200 g/L
Product type	:	Soluble liquid formulation SL
Package size	:	0.25, 0.5, 1, 3, 5 and 10 liter bottle (36-50-63 mm screw cap)

Internal operator exposure values without and with personal protective equipment (PPE) were calculated using the UK and the German model. For risk assessment purposes, the 75th percentile of the UK-model was used (UK-75th) and the geometric mean of the German model (DE-GM). For the greenhouse application, internal operator exposure values without and with PPE were calculated using the Dutch model. For risk assessment purposes, the 90th percentile of the Dutch model (NL-90th) was used.

For bystander exposure during manual or mechanical spraying outdoors, no formally approved models exist. As an estimate, the draft values proposed for the EUROPOEM II, 2002 model were used. These values represent the 90th percentile exposure values for bystanders. Since bystanding should as much as possible be prevented and will usually occur incidentally, it cannot be assumed that bystanders will be using any kind of personal protective equipment, therefore the use of this equipment is not considered in bystander risk assessment.
In greenhouses, no bystander exposure during application is considered, as Good Agricultural Practice requires absence of bystanders.

For worker exposure no formally approved models exist. As an estimate, the draft values proposed for the EUROPOEM II, 2002 model were used. These values represent the 90th percentile exposure values for workers.

For operator, worker and bystander risk assessment the semi-chronic systemic AOEL was used (see section B.6.10.5).
According to the GAP, the maximal dilution is 0.045 g/L. This is lower than the dose tested in the derma absorption studies (0.1 g/L), hence the value of the studies needs to be corrected. The *in vitro* human data also needed to be corrected for the large standard deviation (4.75% + 2.96% as described in section B.6.13.2). This results in a dermal absorption of (4.75+2.96) x (0.1/0.045) = 17% for the diluted formulation. It should be noted that the *pro rata* correction for the spray dilution is considered worst-case since in some scenario's the dilution is not as low as 0.045 g/L.

For exposure to undiluted formulation dermal absorption was estimated to be 0.4%, based on the same *in vitro* human data with a correction for the large standard deviation. In case only data for combined exposure to undiluted and diluted formulation were available, a dermal absorption of 17% was used as a worst case assumption. For inhalatory exposure a default value of 100% was used, *i.e.* internal exposure equals external exposure.

The basic assumptions, input data and calculations used in the risk assessment are further specified below.

B.6.16.1 Operator exposure (IIIA 7.2.1)

Mechanical upward spraying on hop (field)

Application technique	:	tractor mounted upwards high volume sprayers

Input data

Concentration a.s. in formulation	:	200 g a.s. /L
Spray volume	:	2000-3300 L/ha
Concentration in spray liquid	:	4.5-7.5 g a.s. /hL
Application rate	:	0.150 kg a.s./ha
		(0.75 L product/ha)

Mechanical downward spraying on lettuce (field)

Application technique	:	Tractor mounted boomsprayer

Input data

Concentration a.s. in formulation	:	200 g a.s. /L
Spray volume	:	500-1000 L/ha
Concentration in spray liquid	:	12.5-25 g a.s./hL
Application rate	:	0.125 kg a.s./ha

(0.625 L product/ha)

Manual downward spraying on lettuce (field)

Application technique	:	Knapsack

Input data

Concentration a.s. in formulation	:	200 g a.s. /L
Spray volume	:	500-1000 L/ha
Concentration in spray liquid	:	12.5-25 g a.s./hL
Application rate	:	0.125 kg a.s./ha
		(0.625 L product/ha)

Manual spraying on lettuce (greenhouse)

Application technique	:	Knapsack

Input data

Concentration a.s. in formulation	:	200 g a.s. /L
Spray volume	:	500-1000 L/ha
Concentration in spray liquid	:	12.5-25 g a.s./hL
Application rate	:	0.125 kg a.s./ha
		(0.625 L product/ha)

B.6.16.1.1 Exposure estimates with UK-POEM

The model is largely based on unpublished studies, carried out in the UK by industry and MAFF. 75th percentiles are calculated.

B.6.16.1.1.1 Mechanical upward spraying on hops without PPE

For the present purpose, the model for 10 L bottles with 63 mm closure is used, as this is considered a reasonable worst case.

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha			
Product	BYI 02960 200 SL		Active substance	BYI 02960
Formulation type	water-based		a.s. concentration	200 mg/ml
Dermal absorption from product	0.4	%	Dermal absorption from spray	17 %
Container	10 litres 63 mm closure			
PPE during mix/loading	None		PPE during application	None
Dose	0.75	l/ha	Work rate/day	15 ha
Application volume	2000	l/ha	Duration of spraying	6 h

EXPOSURE DURING MIXING AND LOADING

Container size	10	litres
Hand contamination/operation	0.05	ml
Application dose	0.75	litres product/ha
Work rate	15	ha/day
Number of operations	2	/day
Hand contamination	0.1	ml/day
Protective clothing	None	
Transmission to skin	100	%
Dermal exposure to formulation	0.1	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha			
Application volume	2000	spray/ha		
Volume of surface contamination	400	ml/h		
Distribution	Hands	Trunk	Legs	
	10%	65%	25%	
Clothing	None	Permeable	Permeable	
Penetration	100%	2%	5%	
Dermal exposure	10	5.2	5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	121.2	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.1	ml/day	121.2	ml/day
Concen. of a.s. product or spray	200	mg/ml	0.075	mg/ml
Dermal exposure to a.s.	20	mg/day	9.09	mg/day
Percent absorbed	0.4	%	17	%
Absorbed dose	0.08	mg/day	1.5453	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.05	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.075	mg/ml
Inhalation exposure to a.s.	0.0225	mg/day
Percent absorbed	100	%
Absorbed dose	0.0225	mg/day

PREDICTED EXPOSURE

Total absorbed dose	1.6478	mg/day
Operator body weight	60	kg
Operator exposure	0.027463333	mg/kg bw/day

B.6.16.1.1.2 Mechanical upward spraying on hops with PPE

For the present purpose, the model for 10 L bottles with 63 mm closure is used, as this is considered a reasonable worst case.

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha			
Product	BYI 02960 200 SL		Active substance	BYI 02960
Formulation type	water-based		a.s. concentration	200 mg/ml
Dermal absorption from product	0.4	%	Dermal absorption from spray	17 %
Container	10 litres 63 mm closure			
PPE during mix/loading	Gloves		PPE during application	Gloves
Dose	0.75	l/ha	Work rate/day	15 ha
Application volume	2000	l/ha	Duration of spraying	6 h

EXPOSURE DURING MIXING AND LOADING

Container size	10	litres
Hand contamination/operation	0.05	ml
Application dose	0.75	litres product/ha
Work rate	15	ha/day
Number of operations	2	/day
Hand contamination	0.1	ml/day
Protective clothing	Gloves	
Transmission to skin	5	%
Dermal exposure to formulation	0.005	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha			
Application volume	2000	spray/ha		
Volume of surface contamination	400	ml/h		
Distribution	Hands	Trunk	Legs	
	10%	65%	25%	
Clothing	Gloves	Permeable	Permeable	
Penetration	10%	2%	5%	
Dermal exposure	4	5.2	5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	85.2	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.005	ml/day	85.2	ml/day
Concen. of a.s. product or spray	200	mg/ml	0.075	mg/ml
Dermal exposure to a.s.	1	mg/day	6.39	mg/day
Percent absorbed	0.4	%	17	%
Absorbed dose	0.004	mg/day	1.0863	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.05	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.075	mg/ml
Inhalation exposure to a.s.	0.0225	mg/day
Percent absorbed	100	%
Absorbed dose	0.0225	mg/day

PREDICTED EXPOSURE

Total absorbed dose	1.1128	mg/day
Operator body weight	60	kg
Operator exposure	0.018546667	mg/kg bw/day

B.6.16.1.1.3 Mechanical downward spraying on lettuce (field) without PPE

For the present purpose, the model for 10 L bottles with 63 mm closure is used, as this is considered a reasonable worst case.

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Tractor-mounted/trailed boom sprayer: hydraulic nozzles			
Product	BYI 02960 200 SL		Active substance	BYI 02960
Formulation type	water-based		a.s. concentration	200 mg/ml
Dermal absorption from product	0.4	%	Dermal absorption from spray	17 %
Container	10 litres 63 mm closure			
PPE during mix/loading	None		PPE during application	None
Dose	0.625	l/ha	Work rate/day	50 ha
Application volume	500	l/ha	Duration of spraying	6 h

EXPOSURE DURING MIXING AND LOADING

Container size	10	litres
Hand contamination/operation	0.05	ml
Application dose	0.625	litres product/ha
Work rate	50	ha/day
Number of operations	4	/day
Hand contamination	0.2	ml/day
Protective clothing	None	
Transmission to skin	100	%
Dermal exposure to formulation	0.2	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed boom sprayer: hydraulic nozzles			
Application volume	500	spray/ha		
Volume of surface contamination	10	ml/h		
Distribution	Hands	Trunk	Legs	
	65%	10%	25%	
Clothing	None	Permeable	Permeable	
Penetration	100%	5%	15%	
Dermal exposure	6.5	0.05	0.375	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	41.55	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.2	ml/day	41.55	ml/day
Concen. of a.s. product or spray	200	mg/ml	0.25	mg/ml
Dermal exposure to a.s.	40	mg/day	10.3875	mg/day
Percent absorbed	0.4	%	17	%
Absorbed dose	0.16	mg/day	1.765875	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.01	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.25	mg/ml
Inhalation exposure to a.s.	0.015	mg/day
Percent absorbed	100	%
Absorbed dose	0.015	mg/day

PREDICTED EXPOSURE

Total absorbed dose	1.940875	mg/day
Operator body weight	60	kg
Operator exposure	0.032347917	mg/kg bw/day

B.6.16.1.1.4 Mechanical downward spraying on lettuce (field) with PPE

For the present purpose, the model for 10 L bottles with 63 mm closure is used, as this is considered a reasonable worst case.

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Tractor-mounted/trailed boom sprayer: hydraulic nozzles			
Product	BYI 02960 200 SL		Active substance	BYI 02960
Formulation type	water-based		a.s. concentration	200 mg/ml
Dermal absorption from product	0.4	%	Dermal absorption from spray	17 %
Container	10 litres 63 mm closure			
PPE during mix/loading	Gloves		PPE during application	Gloves
Dose	0.625	l/ha	Work rate/day	50 ha
Application volume	500	l/ha	Duration of spraying	6 h

EXPOSURE DURING MIXING AND LOADING

Container size	10	litres
Hand contamination/operation	0.05	ml
Application dose	0.625	litres product/ha
Work rate	50	ha/day
Number of operations	4	/day
Hand contamination	0.2	ml/day
Protective clothing	Gloves	
Transmission to skin	5	%
Dermal exposure to formulation	0.01	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed boom sprayer: hydraulic nozzles			
Application volume	500	spray/ha		
Volume of surface contamination	10	ml/h		
Distribution	Hands	Trunk	Legs	
	65%	10%	25%	
Clothing	Gloves	Permeable	Permeable	
Penetration	10%	5%	15%	
Dermal exposure	0.65	0.05	0.375	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	6.45	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.01	ml/day	6.45	ml/day
Concen. of a.s. product or spray	200	mg/ml	0.25	mg/ml
Dermal exposure to a.s.	2	mg/day	1.6125	mg/day
Percent absorbed	0.4	%	17	%
Absorbed dose	0.008	mg/day	0.274125	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.01	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.25	mg/ml
Inhalation exposure to a.s.	0.015	mg/day
Percent absorbed	100	%
Absorbed dose	0.015	mg/day

PREDICTED EXPOSURE

Total absorbed dose	0.297125	mg/day
Operator body weight	60	kg
Operator exposure	0.004952083	mg/kg bw/day

B.6.16.1.1.5 Manual downward spraying on lettuce (field) without PPE

For the present purpose, the model for 1 L bottles is used, as this is considered a reasonable worst case.

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target				
Product	BYI 02960 200 SL		Active substance	BYI 02960	
Formulation type	water-based		a.s. concentration	200	mg/ml
Dermal absorption from product	0.4	%	Dermal absorption from spray	17	%
Container	1 litre any closure				
PPE during mix/loading	None		PPE during application	None	
Dose	0.625	l/ha	Work rate/day	0.8	ha
Application volume	500	l/ha	Duration of spraying	6	h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0.01	ml
Application dose	0.625	litres product/ha
Work rate	0.8	ha/day
Number of operations	27	/day
Hand contamination	0.27	ml/day
Protective clothing	None	
Transmission to skin	100	%
Dermal exposure to formulation	0.27	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target			
Application volume	500	spray/ha		
Volume of surface contamination	50	ml/h		
Distribution	Hands	Trunk	Legs	
	25%	25%	50%	
Clothing	None	Permeable	Permeable	
Penetration	100%	20%	18%	
Dermal exposure	10	2.5	4.5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	102	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.27	ml/day	102	ml/day
Concen. of a.s. product or spray	200	mg/ml	0.25	mg/ml
Dermal exposure to a.s.	54	mg/day	25.5	mg/day
Percent absorbed	0.4	%	17	%
Absorbed dose	0.216	mg/day	4.335	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.02	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.25	mg/ml
Inhalation exposure to a.s.	0.03	mg/day
Percent absorbed	100	%
Absorbed dose	0.03	mg/day

PREDICTED EXPOSURE

Total absorbed dose	4.581	mg/day
Operator body weight	60	kg
Operator exposure	0.07635	mg/kg bw/day

B.6.16.1.1.5 Manual downward spraying on lettuce (field) with PPE

For the present purpose, the model for 1 L bottles is used, as this is considered a reasonable worst case.

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target				
Product	BYI 02960 200 SL		Active substance	BYI 02960	
Formulation type	water-based		a.s. concentration	200	mg/ml
Dermal absorption from product	0.4	%	Dermal absorption from spray	17	%
Container	1 litre any closure				
PPE during mix/loading	Gloves		PPE during application	Gloves and impermeable coveral	
Dose	0.625	l/ha	Work rate/day	0.8	ha
Application volume	500	l/ha	Duration of spraying	6	h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0.01	ml
Application dose	0.625	litres product/ha
Work rate	0.8	ha/day
Number of operations	27	/day
Hand contamination	0.27	ml/day
Protective clothing	Gloves	
Transmission to skin	5	%
Dermal exposure to formulation	0.0135	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target			
Application volume	500	spray/ha		
Volume of surface contamination	50	ml/h		
Distribution	Hands	Trunk	Legs	
	25%	25%	50%	
Clothing	Gloves	Impermeable	Impermeable	
Penetration	10%	5%	5%	
Dermal exposure	1.25	0.625	1.25	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	18.75	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.0135	ml/day	18.75	ml/day
Concen. of a.s. product or spray	200	mg/ml	0.25	mg/ml
Dermal exposure to a.s.	2.7	mg/day	4.6875	mg/day
Percent absorbed	0.4	%	17	%
Absorbed dose	0.0108	mg/day	0.796875	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.02	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.25	mg/ml
Inhalation exposure to a.s.	0.03	mg/day
Percent absorbed	100	%
Absorbed dose	0.03	mg/day

PREDICTED EXPOSURE

Total absorbed dose	0.837675	mg/day
Operator body weight	60	kg
Operator exposure	0.01396125	mg/kg bw/day

B.6.16.1.2 Exposure estimates with the German model

The German model is based on unpublished studies performed by industry and all carried out in Germany. The chosen statistic is the geometric mean (GM).

B.6.16.1.2.1 Mechanical upward spraying on hop (field)

= HIGH CROP TRACTOR MOUNTED =

	Treated area per day	**A =**	**8**	**ha/d**	at BBA = 8
	Use rate	**R =**	***0.15***	**kg a.i./ha**	

Mixing/loading of the product [mg/person per kg a.i.]				Appl. of the spray [mg/pers. per kg a.i.]	
	liquid	solid: WP	**solid: WG**	I*a = 0,018	D*a/c = 1,2
I*m	0.0006	0.07	0.008	D*a/h = 0,7	D*a/b = 9,6
D*m/h	2.4	6	2		

Estimated inhalation exposure:

Im = I*m x R x A	***0.0006***	0.15	8	0.00072	mg/pers. x d
Ia = I*a x R x A	0.018	0.15	8	0.0216	mg/pers. x d
			I, in total =	**0.02232**	**mg/pers. x d**

Estimated dermal exposure:

Dm/h = D*m/h x R x A	***2.4***	0.15	8	2.88	mg/pers. x d
Da/h = D*a/h x R x A	0.7	0.15	8	0.84	mg/pers. x d
Da/c = D*a/c x R x A	1.2	0.15	8	1.44	mg/pers. x d
Da/b = D*a/b x R x A	9.6	0.15	8	11.52	mg/pers. x d
			D, in total =	**16.68**	**mg/pers. x d**

Estimated inh. exp.		**PPE**	**factor**			
Im =	0.00072	-	1		0.00072	mg/pers. x d
Ia =	0.0216	-	1		0.0216	mg/pers. x d
					0.02232	**mg/pers. x d**

Estimated derm. exp.

Dm/h =	2.88	SS 110	0.01		0.0288	mg/pers. x d
Da/h =	0.84	SS 120	0.01		0.0084	mg/pers. x d
Da/c =	1.44		1		1.44	mg/pers. x d
Da/b =	11.52		1		11.52	mg/pers. x d
					12.9972	**mg/pers. x d**

		Estimated exposure		Systemic exposure	
	abs. rate	without PPE	with PPE	without PPE	with PPE
Inhalation: m/l	100%	0.00072	0.00072	0.00072	0.00072
Inhalation: appl.	100%	0.0216	0.0216	0.0216	0.0216
Dermal: m/l	0.4%	2.88	0.0288	0.01152	0.0001152
Dermal: appl.	17%	13.8	12.9684	2.346	2.204628
			mg/pers./d:	2.37984	2.2270632
kg bw:	70		mg/kg bw/d:	0.03399771	0.03181519
syst. AOEL:	0.12		**% of AOEL:**	**28.3314286**	**26.5126571**

Possible PPE: specific instructions	Abbr.	Red.-factor	to lower:
Particle filtering half mask (m/l)	ST 110	0.08	**Im**
Half mask with comb. filter (m/l)	ST 210	0.02	
Particle filtering half mask (appl.)	ST 120	0.08	**Ia**
Half mask with comb. filter (appl.)	ST 220	0.02	
Protective gloves (m/l)	**SS 110**	**0.01**	**Dm/h**
Protective gloves (appl.)	**SS 120**	**0.01**	**Da/h**
Half mask (appl.)	ST 120 / 220	0.8	**Da/c**
Broad-brimmed headgear (appl.: high crops)	**SS 420**	**0.5**	
Hood and visor (appl.: high crops)	SS 520	0.05	
Protective garment + sturdy footwear (appl.)	**SS 220**	**0.05**	**Da/b**

B.6.16.1.2.2 Mechanical downward spraying on lettuce (field)

= FIELD_CROP_TRACTOR_MOUNTED =

Treated area per day	**A =**	**20**	**ha/d**	at BBA = 20
Use rate	**R =**	***0.125***	**kg a.i./ha**	

Mixing/loading of the product [mg/person per kg a.i.]				Appl. of the spray [mg/pers. per kg a.i.]	
	liquid	solid: WP	**solid: WG**	I*a = 0,001	D*a/c = 0,06
I*m	0.0006	0.07	0.008	D*a/h = 0,38	D*a/b = 1,6
D*m/h	2.4	6	2		

Estimated inhalation exposure:

Im = I*m x R x A		***0.0006***	0.125	20	0.0015	mg/pers. x d
Ia = I*a x R x A		0.001	0.125	20	0.0025	mg/pers. x d
				I, in total =	**0.004**	**mg/pers. x d**

Estimated dermal exposure:

Dm/h = D*m/h x R x A		***2.4***	0.125	20	6	mg/pers. x d
Da/h = D*a/h x R x A		0.38	0.125	20	0.95	mg/pers. x d

Da/c = D*a/c x R x A		0.06	0.125	20	0.15	mg/pers. x d
Da/b = D*a/b x R x A		1.6	0.125	20	4	mg/pers. x d
				D, in total =	**11.1**	**mg/pers. x d**

Estimated inh. exp.		**PPE**	**factor**			
Im =	0.0015	-	1		0.0015	mg/pers. x d
Ia =	0.0025	-	1		0.0025	mg/pers. x d
					0.004	**mg/pers. x d**

Estimated derm. exp.						
Dm/h =	6	SS 110	0.01		0.06	mg/pers. x d
Da/h =	0.95	SS 120	0.01		0.0095	mg/pers. x d
Da/c =	0.15		1		0.15	mg/pers. x d
Da/b =	4		1		4	mg/pers. x d
					4.2195	**mg/pers. x d**

		Estimated exposure		**Systemic exposure**	
	abs. rate	**without PPE**	**with PPE**	**without PPE**	**with PPE**
Inhalation: m/l	100%	0.0015	0.0015	0.0015	0.0015
Inhalation: appl.	100%	0.0025	0.0025	0.0025	0.0025
Dermal: m/l	0.4%	6	0.06	0.024	0.00024
Dermal: appl.	17%	5.1	4.1595	0.867	0.707115
			mg/pers./d:	0.895	0.711355
kg bw:	70		mg/kg bw/d:	0.01278571	0.01016221
syst. AOEL:	0.12		**% of AOEL:**	**10.6547619**	**8.4685119**

Possible PPE: specific instructions	Abbr.	Red.-factor	to lower:
Particle filtering half mask (m/l)	ST 110	0.08	**Im**
Half mask with comb. filter (m/l)	ST 210	0.02	
Particle filtering half mask (appl.)	ST 120	0.08	**Ia**
Half mask with comb. filter (appl.)	ST 220	0.02	
Protective gloves (m/l)	**SS 110**	**0.01**	**Dm/h**
Protective gloves (appl.)	**SS 120**	**0.01**	**Da/h**
Half mask (appl.)	ST 120 / 220	0.8	**Da/c**
Broad-brimmed headgear (appl.: high crops)	**SS 420**	**0.5**	
Hood and visor (appl.: high crops)	SS 520	0.05	
Protective garment + sturdy footwear (appl.)	**SS 220**	**0.05**	**Da/b**

B.6.16.1.2.3 Manual downward spraying on lettuce (field)

No adequate module available in the German model.

B.6.16.1.3 Exposure estimates with the Dutch model

The model is based on studies published in the scientific literature and on studies performed in The Netherlands and indicative 90th percentiles are deduced from the exposure databases.

B.6.16.1.3.1 Manual spraying on lettuce (greenhouse)

OPERATOR EXPOSURE			DUTCH GREENHOUSE MODEL	
form	BYI 09260 200 SL		Application including mixing and loading	
a.s.	BYI 09260			
Parameter		**Value**	**Unit**	**References, comments**
MANUAL SPRAYING in greenhouses				
AR	Application rate	0.125	kg a.s./ha	summary of intended uses
A	Area treated	1	ha/ day	Dutch model
Inhalation Exposure				without PPE
SV	Surrogate Exposure Value	1	mg a.s./ kg a.s.	For dusting see note* (Dutch model)
	Inhalation Exposure (without PPE)	**0.125**	mg a.s./ day	IE = SV x AR x A
Inhalation Exposure (with PPE)				with PPE
	PPE-factor	1		Non-powered mask filtertype 2 (most conservative): 10; more advanced RPE: see note** (Dutch model)
	Inhalation Exposure (with PPE)	**0.125**	mg a.s./ day	IE(PPE) = (1/PPE factor) x IE
Dermal Exposure				without PPE
SV	Surrogate Exposure Value	200	mg a.s./ kg a.s.	For dusting see note* (Dutch model)
	Dermal Exposure	**25**	mg a.s./ day	DE = SV x AR x A
Dermal Exposure (with PPE)				with PPE
	PPE-factor	10		Gloves + coverall: 10 (Dutch model)
	Dermal Exposure (with PPE)	**2.5**	mg a.s./ day	DE(PPE) = (1/PPE-factor) x DE
Internal exposure				
IA	Inhalation Absorption	100	%	
DA	Dermal Absorption	17	%	
	AOEL	8.4	mg a.s./ day	based on 70 kg bw
		Without PPE	**With PPE**	
	Internal exposure	[mg a.s. / day]	[mg a.s. / day]	
	Inhalation	0.1250	0.1250	IE(int) = IE x (IA/100)
	Dermal	4.2500	0.4250	DE(int) = DE x (DA/100)
	Total	**4.3750**	**0.5500**	**sum**

	% AOEL			
	Inhalation	1	1	%AOEL = 100 x IE(int) / AOEL
	Dermal	51	5	%AOEL = 100 x DE(int) / AOEL
	Total	**52**	**7**	**sum**

* NOTE: The above mentioned model is for spraying in greenhouses. For dusting of carnations the surrogate values should be changed: inhalation should be 20 mg/kg instead of 1, and dermal should be 300 mg/kg instead of 200.

** Note: Only for gasforming/gaseous preparations and soil fumigation preparations: powered full-face filtering devices with filtertype 2 (factor 20), powered full-face filtering devices with filtertype 3 (factor 40)

B.6.16.1.4 Risk assessment for operators

Risk assessment was performed using the 75th percentile from the UK-model (UK-75th), the geometric mean from the German model (DE-GM) and the 90th percentile from the Dutch model (NL-90th).

Table 6.16.1.4-1 Operator internal exposure and risk assessment

Model	Route	Estimated internal exposure (mg a.s./day)		AOEL Systemic * (mg a.s/day)	% AOEL	
		without PPE	with PPE		without PPE	with PPE
Mechanical upward spraying on hop (field)						
UK- 75th	Respiratory	0.0225	0.0225	3.84	0.6	0.6
	Dermal	1.6253	1.0903	3.84	42.3	28.4
	Total	**1.6478**	**1.1128**	**3.84**	**42.9**	**29.0**
DE- GM	Respiratory	0.0288	0.0288	4.48	0.6	0.6
	Dermal	2.3575	2.2047	4.48	52.6	49.2
	Total	**2.3798**	**2.2271**	**4.48**	**53.1**	**49.7**
Mechanical downward spraying on lettuce (field)						
UK- 75th	Respiratory	0.015	0.015	3.84	0.4	0.4
	Dermal	1.9259	0.2821	3.84	50.2	7.3
	Total	**1.9409**	**0.2971**	**3.84**	**50.5**	**7.7**
DE- GM	Respiratory	0.004	0.004	4.48	0.1	0.1
	Dermal	0.891	0.77074	4.48	19.9	17.2
	Total	**0.895**	**0.7113**	**4.48**	**20.0**	**15.9**

Model	Route	Estimated internal exposure (mg a.s./day)		AOEL Systemic * (mg a.s/day)	% AOEL	
		without PPE	with PPE		without PPE	with PPE
*Manual downward spraying on lettuce (field) ** *						
UK- 75th	Respiratory	0.03	0.03	3.84	0.8	0.8
	Dermal	4.5510	0.8077	3.84	118.5	21.0
	Total	**4.5810**	**0.8377**	**3.84**	**119.3**	**21.8**
*Manual spraying on lettuce (indoors) *** *						
Dutch-90th	Respiratory	0.1250	0.1250	4.48	2.8	2.8
	Dermal	4.2500	0.4250	4.48	94.9	9.5
	Total	**4.3750**	**0.5500**	**4.48**	**97.7**	**12.3**

* Assuming a body weight of 60 kg for UK-POEM and 70 kg for other models
** No suitable module available in German modell
*** No suitable module available in UK-POEM and German model

B.6.16.2 Bystander exposure (IIIA 7.2.2)

Mechanical upward spraying on hop (field)

Input data

Application rate : 0.150 kg/ha

Spray volume : 2000 L/ha

Dermal exposure : 5% of the application rate (kg/ha) on an body surface of 2 m^2 and dermal absorption of 17%

Inhalation exposure : 0.06 mL spraying liquid per m^3, a duration of 1 hour, a ventilation rate of 1.25 m^3/hour, and inhalation absorption of 100%.

Downward spraying on lettuce (field)

Input data

Application rate : 0.125 kg a.s./ha

Spray volume : 500 L/ha

Dermal exposure : 0.5% of the application rate (kg/ha) on body surface of 2 m^2 and dermal absorption of 17%

Inhalation exposure : 0.03 mL spraying liquid per m^3, a duration of 1 hour, a ventilation rate of 1.25 m^3/hour, and inhalation absorption of 100%.

Manual spraying on lettuce indoors

The presence of bystanders should be prohibited.

For bystander exposure during manual or mechanical downward spraying outdoors, no formally approved models exist. As an estimate, the draft values proposed for the EUROPOEM II model will be used. Since bystanding should as much as possible be prevented and will usually occur incidentally, it cannot be assumed that bystanders will be using any kind of personal protective equipment. Therefore, the use of this equipment is not considered in bystander risk assessment.

In greenhouses, no bystander exposure during application is considered, as Good Agricultural Practice requires absence of bystanders.

B.6.16.2.1 Exposure estimates with EUROPOEM II

The values represent the 90 th percentile exposure values for bystanders.

B.6.16.2.1.1 Mechanical upward spraying on hop (field)

BYSTANDER EXPOSURE			EUROPOEM II MODEL	
form	BYI 02960 200 SL		Outdoor application	
as	BYI 02960			
Parameter		**Value**	**Unit**	**References, comments**
SPRAYING Process outdoor				
AR	Application rate	0.15	kg a.s. / ha	summary of intended uses
SV	Spray volume	2000	L / ha	summary of intended uses
Inhalation Exposure				without PPE
	Default value			
SE	Surrogate Exposure Value	0.06	mL / m3	downwards: 0.03; upwards: 0.06 (EUROPOEM II)
T	Time of exposure	1	h	most probable estimation*
RR	Respiratory rate	1.25	m3 / h	default
Inhalation Exposure		**0.0056**	mg a.s. / day	IE = (ARx1000/SV)xSExTxRR
Dermal Exposure				
	Default value			
SE	Surrogate Exposure Value	0.05		downwards: 0.005; upwards with leaves: 0.05; upward without leaves: 0.15 (EUROPOEM II)
SA	Surface area bystander	2	m2	EUROPOEM II
Dermal Exposure		**1.5**	mg a.s./ day	DE = SE xSA X (AR x 100)
Internal exposure				
IA	Inhalation Absorption	100	%	
DA	Dermal Absorption	17	%	
	AOEL	8.4	mg a.s./ day	based on 70 kg bw
		Without PPE		

Internal exposure	[mg a.s./ day]	
Inhalation	0.0056	IE(int) = IE x (IA/100)
Dermal	0.255	DE(int) = DE x (DA/100)
Total	**0.261**	**sum**
% AOEL		
Inhalation	0.1	%AOEL = 100 x IE(int) / AOEL
Dermal	3.0	%AOEL = 100 x DE(int) / AOEL
Total	**3**	**sum**

* One hour exposure is the default. It is referring to a potential 1 hour exposure, rather than the actual. It is based on the study design, and should not be lowered.

B.6.16.2.1.2 Downward spraying on lettuce (field)

BYSTANDER EXPOSURE			EUROPOEM II MODEL	
form	BYI 02960 200 SL		Outdoor application	
as	BYI 02960			
Parameter		**Value**	**Unit**	**References, comments**
SPRAYING Process outdoor				
AR	Application rate	0.125	kg a.s. / ha	summary of intended uses
SV	Spray volume	500	L / ha	summary of intended uses
Inhalation Exposure				without PPE
	Default value			
SE	Surrogate Exposure Value	0.03	mL / m3	downwards: 0.03; upwards: 0.06 (EUROPOEM II)
T	Time of exposure	1	h	most probable estimation*
RR	Respiratory rate	1.25	m3 / h	default
Inhalation Exposure		**0.0094**	mg a.s. / day	IE = (ARx1000/SV)xSExTxRR
Dermal Exposure				
	Default value			
SE	Surrogate Exposure Value	0.005		downwards: 0.005; upwards with leaves: 0.05; upward without leaves: 0.15 (EUROPOEM II)
SA	Surface area bystander	2	m2	EUROPOEM II
Dermal Exposure		**0.125**	mg a.s./ day	DE = SE xSA X (AR x 100)
Internal exposure				
IA	Inhalation Absorption	100	%	
DA	Dermal Absorption	17	%	
	AOEL	8.4	mg a.s./ day	based on 70 kg bw
		Without PPE		
	Internal exposure	[mg a.s./ day]		
	Inhalation	0.0094		IE(int) = IE x (IA/100)
	Dermal	0.021		DE(int) = DE x (DA/100)
	Total	**0.031**		**sum**
	% AOEL			
	Inhalation	0.1		%AOEL = 100 x IE(int) / AOEL
	Dermal	0.3		%AOEL = 100 x DE(int) / AOEL
	Total	**0**		**sum**
*	One hour exposure is the default. It is referring to a potential 1 hour exposure, rather than the actual. It is based on the study design, and should not be lowered.			

B.6.16.2.1.3 Manual spraying on lettuce indoors

The presence of bystanders should be kept at a minimum. This can easily be achieved in greenhouses, where no person should be allowed who is not involved in the spraying process.

B.6.16.2.2 Risk assessment for bystanders

Table 6.16.2.2-1 Bystander internal exposure and risk assessment

Route	Estimated internal exposure (mg a.s./day)	AOEL systemic * (mg a.s./day)	%AOEL
Exposure during mechanical upward spraying on hop (field)			
Respiratory	0.0056	4.48	0.1
Dermal	0.255	4.48	5.7
Total	**0.261**	**4.48**	**5.8**
Exposure during downward spraying on lettuce (field)			
Respiratory	0.0094	4.48	0.2
Dermal	0.021	4.48	0.5
Total	**0.031**	**4.48**	**0.7**

* Assuming a body weight of 70 kg

B.6.16.3 Resident exposure (IIIA 7.2.2)

Risk assessment was performed according to the German guideline published in 2008 (Martin et al., 2008).

Inputdata

Dermal Exposure (via deposits caused by spray drift):

SDE_R = (AR x D x TTR x TC x H x DA) / BW

Where: SDE_R = Systemic Exposure of Residents via the Dermal Route (mg/kg bw/day)

AR = Application Rate (mg/cm^2) x 1 (for no. of applications)

BYI 02960: 0.125 kg a.s./ha x 1 = 0.00125 mg/cm^2 (lettuce)

0.150 kg a.s./ha x 1 = 0.00150 mg/cm² (hops)

D = Drift (%): 0.29 (lettuce), 5.77 (hops)

TTR = Turf Transferable Residues (%): 5

TC = Transfer Coefficient (cm^2/hour): 7300 (adult), 2600 (child)

H = Exposure Duration (hours): 2

DA = Dermal Absorption (%):

BYI 02960: 17%

BW = Body Weight (kg/person): 60 (adult), 16.15 (child)

Inhalation Exposure (Vapour Drift):

SIE_R = (AC_V x IR x IA) / BW

Where: SIE_R = Systemic Exposure of Residents via Inhalation (mg/kg bw/day)

AC_V = Airborne Concentration of Vapour (mg/m^3): vapour pressure of BYI 02960 is very low i.e.: 9.1 x 10^{-7} Pa at 20°C; acc. to guideline this corresponds to a non-volatile substance (vapour pressure <1 x 10^5 Pa at 20°C). Thus, resident inhalation exposure can be estimated as negligible. (i.e. airborne conc. of 0 mg/m^3)

IR = Inhalation Rate (m^3/day): 16.57 (adult), 8.31 (child)

IA = Inhalation Absorption (%): 100

BW = Body Weight (kg/person): 60 (adult), 16.15 (child)

Child Oral Exposure

Children's hand-to-mouth exposure

SOE_H = (AR x D x TTR x SE x SA x Freq x H x OA) / BW

Where: SOE_H = Systemic Oral Exposure via the Hand to Mouth Route (mg/kg bw/day)

AR = Application Rate (mg/cm^2) x 1 (for no. of applications)

BYI 02960: 0.125 kg a.s./ha x 1 = 0.00125 mg/cm^2 (lettuce)

0.150 kg a.s./ha x 1 = 0.00150 mg/cm² (hops)

D = Drift (%): 0.29 (lettuce), 5.77 (hops)

TTR = Turf Transferable Residues (%): 5

SE = Saliva Extraction Factor (%): 50

SA = Surface Area of Hands (cm^2): 20

Freq = Frequency of Hand-to-Mouth (events/hour): 20

H = Exposure Duration (hours): 2

OA = Oral Absorption (%):

BYI 02960: 100

BW = Body Weight (kg/person): 16.15

Children's object-to-mouth exposure

SOE_O = (AR x D x DFR x IgR x OA) / BW

Where: SOE_O = Systemic Oral Exposure via the Object to Mouth Route (mg/kg bw/day)

AR = Application Rate (mg/cm^2) x 1 (for no. of applications)

BYI 02960: 0.125 kg a.s./ha x 1 = 0.00125 mg/cm^2 (lettuce)

0.150 kg a.s./ha x 1 = 0.00150 mg/cm² (hops)

D = Drift (%): 0.29 (lettuce), 5.77 (hops)

DFR = Dislodgeable Foliar Residues (%): 20

IgR = Ingestion Rate for Mouthing of Grass/Day (cm^2):25

OA = Oral Absorption (%):
BYI 02960: 100
BW = Body Weight (kg/person): 16.15

Total Systemic Exposure of Residents

Adults: $SE_R = SDE_R + SIE_R$ (mg/kg bw/day)
Children: $SE_R = SDE_R + SIE_R + SOE_H + SOE_O$ (mg/kg bw/day)

Where:
SE_R = Systemic Exposure of Residents (mg/kg bw/day)
SDE_R = Systemic Dermal Exposure of Residents (mg/kg bw/day)
SIE_R = Systemic Inhalation Exposure of Residents (mg/kg bw/day)
SOE_H = Systemic Oral Exposure via the Hand to Mouth Route (mg/kg bw/day)
SOE_O = Systemic Oral Exposure via the Object to Mouth Route (mg/kg bw/day)

Corresponding exposure calculations are presented in the following.

Resident exposure after application with field (Lettuce, tractor mounted spray boom)

Resident Exposure

Dermal Exposure = AR*D*TRR*TC*H*DA/BW

	Hops		Lettuce	
	Adults	**Child**	**Adults**	**Child**
AR	0.0015	0.0015	0.00125	0.00125
D	0.0577	0.0577	0.0029	0.0029
TTR	0.05	0.05	0.05	0.05
TC	7300	2600	7300	2600
H	2	2	2	2
DA	0.17	0.17	0.17	0.17
BW	60	16.15	60	16.15
DE	0.000179	0.000236874	7.49771E-06	9.92E-06

Inhalation exposure = Acv * IR * IA/BW

	Hops		Lettuce	
	Adults	**Child**	**Adults**	**Child**
Acv	0	0	0	0
IR	16.57	8.31	16.57	8.31
IA	1	1	1	1
BW	60	16.15	60	16.15
IE	0	0	0	0

Oral exposure (hand ot mouth) = AR * D*TTR*SE*SA*Freq*H*OA/BW

Hops **Lettuce**

	Child	Child
AR	0.0015	0.00125
D	0.0577	0.0029
TTR	0.05	0.05
SE	0.5	0.5
SA	20	20
Freq	20	20
H	2	2
OA	1	1
BW	16.15	16.15
OE(htm)	0.000107183	4.49E-06

Oral exposure (object to mouth) = AR * D * DFR* IgR * OA/BW

	Hops	Lettuce
	Child	Child
AR	0.0015	0.00125
D	0.0577	0.0029
DFR	0.2	0.2
IgR	25	25
OA	1	1
BW	16.15	16.15
OE (otm)	2.67957E-05	1.12E-06

Total Systemic Exposure of Residents mg/kg bw/d

Hops		Lettuce	
Adults	Child	Adults	Child
0.000179	0.000370852	7.49771E-06	1.55E-05

B.6.16.3.1 Risk assessment for residents

Table 6.16.3.1-1 Resident internal exposure and risk assessment

Route	Estimated internal exposure (mg/kg bw/day)	AEL systemic * (mg/kg bw/d)	%AEL
Resident exposure during mechanical upward spraying on hop (field)			
Adult			
Dermal	0.000179014	0.064	0.28
Respiratory	0	0.064	0
Total	0.000179014	**0.064**	**0.28**
Child			

Dermal	0.000236874	0.064	0.37
Respiratory	0	0.064	0
Oral (hand to mouth)	0.000107183	0.064	0.17
Oral (object to mouth)	2.67957E-05	0.064	0.04
Total	**0.000370852**	**0.064**	**0.58**
Resident exposure during mechanical downward spraying on lettuce (field)			
Adult			
Dermal	7.49771E-06	0.064	0.01
Respiratory	0	0.064	0
Total	**7.49771E-06**	**0.064**	**0.01**
Child			
Dermal	9.92105E-06	0.064	0.02
Respiratory	0	0.064	0
Oral (hand to mouth)	4.48916E-06	0.064	0.01
Oral (object to mouth)	1.12229E-06	0.064	<0.01
Total	**1.55325E-05**	**0.064**	**0.02**

B.6.16.4 Worker exposure (IIIA 7.2.3)

Re-entry activities in the cultivation of hops

Input data

Application rate : 0.15 kg a.s./ha

Duration of activities : 6 hours

Dermal exposure (transfer coefficient) : 0.45 m^2/hour

Re-entry activities in lettuce

Input data

Application rate : 0.125 kg a.s./ha

Dermal exposure (transfer coefficient) : 0.25 m^2/hour

Duration of activities : 6 hours

B.6.16.4.1 Exposure estimates with EUROPOEM II

B.6.16.4.1.1 Re-entry activities in hops outdoor, based on EUROPOEM II

WORKER EXPOSURE		EUROPOEM II MODEL		
form	BYI 02960 200 SL		Re-entry in the field	
a.s.	BYI 02960			
Parameter		**Value**	**Unit**	**References, comments**
Re-entry activities in the field				
AR	Application rate	0.15	kg a.s./ha	summary of intended uses
Worker				
Duration				
T		6	hours / day	default: 6 h (Europoem II)
Inhalation Exposure				without PPE
	no model available	-		
Dermal Exposure				
DFR	Dislodgeable foliar residue	30	mg a.s./m2/kg a.s./ha	default (Europoem II)
TC	Transfer coefficient	0.45	m2/ hour	vegetable (field): 0.25; ornamentals: 0.5; small fruit: 0.3; large fruit: 0.45 (Europoem II)
Dermal Exposure		**12.15**	mg a.s./ day	DE = DFR x AR x TC x T
Internal exposure				
DA	Dermal Absorption	17	%	
	PPE-factor dermal	5		gloves*
	AOEL	8.4	mg a.s./ day	based on 70 kg bw
		Without PPE	**With PPE**	
	Internal exposure	[mg a.s./ day]	[mg a.s./ day]	
	Inhalation	-	-	no model available
	Dermal	2.066	0.413	DE(int) = DE x (DA/100)
	Total	**2.066**	**0.413**	**sum**
	% AOEL			
	Inhalation	-	-	no model available
	Dermal	25	5	%AOEL = 100 x DE(int) / AOEL
	Total	**25**	**5**	**sum**

* It is assumed in the used TC values, that body exposure is already reduced by (protective) clothing. The use of gloves will result in an extra reduction factor of 5.

B.6.16.4.1.2 Re-entry activities in lettuce outdoor, based on EUROPOEM II

WORKER EXPOSURE		EUROPOEM II MODEL		
form	BYI 02960 200 SL		Re-entry in the field	
a.s.	BYI 02960			
Parameter		**Value**	**Unit**	**References, comments**
Re-entry activities in the field				
AR	Application rate	0.125	kg a.s./ha	summary of intended uses
Worker				

Duration				
T		6	hours / day	default: 6 h (Europoem II)
Inhalation Exposure				without PPE
	no model available	-		
Dermal Exposure				
DFR	Dislodgeable foliar residue	30	mg a.s./m2/kg a.s./ha	default (Europoem II)
TC	Transfer coefficient	0.25	m2/ hour	vegetable (field): 0.25; ornamentals: 0.5; small fruit: 0.3; large fruit: 0.45 (Europoem II)
Dermal Exposure		**5.625**	mg a.s./ day	DE = DFR x AR x TC x T
Internal exposure				
DA	Dermal Absorption	17	%	
	PPE-factor dermal	5		gloves*
	AOEL	8.4	mg a.s./ day	based on 70 kg bw
		Without PPE	**With PPE**	
	Internal exposure	[mg a.s./ day]	[mg a.s./ day]	
	Inhalation	-	-	no model available
	Dermal	0.956	0.191	DE(int) = DE x (DA/100)
	Total	**0.956**	**0.191**	**sum**
	% AOEL			
	Inhalation	-	-	no model available
	Dermal	11	2	%AOEL = 100 x DE(int) / AOEL
	Total	**11**	**2**	**sum**

* It is assumed in the used TC values, that body exposure is already reduced by (protective) clothing. The use of gloves will result in an extra reduction factor of 5.

B.6.16.4.1.2 Re-entry activities in lettuce indoor, based on EUROPOEM II & Dutch model

WORKER EXPOSURE				**EUROPOEM II & DUTCH MODEL**
form	BYI 02960 200 SL		**Re-entry in greenhouses**	
a.s.	BYI 02960			
Parameter		**Value**	**Unit**	**References, comments**
Re-entry activities in greenhouses				
AR	Application rate	0.125	kg a.s./ha	summary of intended uses
Worker				
Duration				
Tc	Cutting	3	hours / day	default: 3 h (Dutch model)
Tsb	Sorting/ bundling	3	hours / day	default: 3 h (Dutch model)
Tt	Total duration	6	hours / day	default: 6 h (Europoem II)
Inhalation Exposure				without PPE
Task Specific Factor				
	Surrogate value (indicative)			
TF	Cutting	0.1	(mg a.s./h)/ (kg/ha)	Dutch model
TF	Sorting/ bundling	0.01	(mg a.s./h)/ (kg/ha)	Dutch model

Inhalation Exposure				
	Cutting	0.0375	mg a.s./ day	IE = AR x Tc x TF
	Sorting/ bundling	0.00375	mg a.s./ day	IE = AR x Tsb x TF
	Total	**0.04125**	mg a.s./ day	sum
Dermal Exposure				
DFR	Dislodgeable foliar residue	30	mg a.s./m2/kg a.s./ha	default (Europoem II)
TC	Transfer coefficient	0.25	m2/ hour	vegetable (field): 0.25; ornamentals: 0.5; small fruit: 0.3; large fruit: 0.45 (Europoem II)
Dermal Exposure		**5.625**	mg a.s./ day	DE = DFR x AR x TC x Tt
Internal exposure				
IA	Inhalation Absorption	100	%	
DA	Dermal Absorption	17	%	
	PPE-factor inhalation	10		reduction factor*
	PPE-factor dermal	5		gloves**
	AOEL	8.4	mg a.s./ day	based on 70 kg bw
	Internal exposure	**Without PPE** [mg a.s./ day]	**With PPE** [mg a.s./ day]	
	Inhalation	0.041	0.004	IE(int) = IE x (IA100)
	Dermal	0.956	0.191	DE(int) = DE x (DA/100)
	Total	**0.998**	**0.195**	**sum**
	% AOEL			
	Inhalation	0	0	%AOEL = 100 x IE(int) / AOEL
	Dermal	11	2	%AOEL = 100 x DE(int) / AOEL
	Total	**12**	**2**	**sum**

* Breathing apparatus for workers can only be used in closed areas for a relatively short period of time.

** It is assumed in the used TC values, that body exposure is already reduced by (protective) clothing. The use of gloves will result in an extra reduction factor of 5.

B.6.16.4.2 Exposure estimated with a field study

No exposure study was submitted.

B.6.16.4.3 Risk assessment for workers

Table 6.16.4.3-1 Worker internal exposure and risk assessment

Model	Route	Estimated internal exposure (mg a.s./day)		AOEL Systemic * (mg a.s/day)	% AOEL	
		without PPE	with PPE		without PPE	with PPE

Model	Route	Estimated internal exposure (mg a.s./day)		AOEL Systemic * (mg a.s/day)	% AOEL	
		without PPE	with PPE		without PPE	with PPE
Re-entry exposure after mechanical upward spraying on hops						
EURO-POEM II	Respiratory	-	-	-	-	-
	Dermal	2.066	0.413	4.48	46.1	9.2
	Total	**2.066**	**0.413**	**4.48**	**46.1**	**9.2**
Re-entry exposure after downward spraying on lettuce outdoors						
EURO-POEM II	Respiratory	-	-	-	-	-
	Dermal	0.956	0.191	4.48	21.3	4.3
	Total	**0.956**	**0.191**	**4.48**	**21.3**	**4.3**
Re-entry exposure after spraying on lettuce indoors						
EURO-POEM II & Dutchs model	Respiratory	0.041	0.004	4.48	0.9	0.1
	Dermal	0.956	0.191	4.48	21.3	4.3
	Total	**0.998**	**0.195**	**4.48**	**22.3**	**4.4**

* Assuming a body weight of 70 kg
- The inhalation exposure after field application is not quantifiable with this method. However, it can be expected to be lower than respiratory exposure during application.

B.6.16.5 Conclusions on risk assessments for operators, bystanders and workers

- Safe use for operators was identified for mechanical upward spraying in hopspotato, mechanical downward spraying in lettuce (field use), manual downward spraying in lettuce (field use), without the use of PPE (UK-POEM and German and models (field) and Dutch greenhouse model). Safe use for operators was identified for the manual spraying in lettuce (greenhouse use) with the use of PPE (UK-POEM).
- Safe use for bystanders and residents was identified for mechanical upward spraying in hop, mechanical downward spraying on lettuce tomato (field use) using the EUROPOEM II 2002 model and the German guidline by Martin *et al*., respectively. No bystanders should be present in the greenhouses during application.
- Safe use for workers was identified for re-entry in hops (field), and in lettuce (field and greenhouses) without the use of PPE (EUROPOEM II 2002 model).

B.6.17 REFERENCES RELIED ON

Section B.6 Toxicology and metabolism (Annex IIA, point 5, Annex IIIa, point 7)

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIA 5.1.1 /01	[redacted]	2012	[Pyridinylmethyl-14C]BYI 02960 - Absorption, distribution, excretion, and metabolism in the rat [redacted] Report No.: MEF-11/747, Edition Number: M-422210-01-1 Date: 2012-01-12 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 5.1.1 /02	[redacted]	2011	Quantitative whole body autoradiography of [pyridinylmethyl-14C]BYI 02960 in male and female rats: Distribution of total radioactivity and elimination from blood, organs and tissues after single oral administration including determination of radioactivity in the excreta and exhaled 14CO2 [redacted] Report No.: MEF-11/276, Edition Number: M-409993-01-2 Date: 2011-05-30 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 5.1.2 /01	[redacted]	2011	[Furanone-4-14C]BYI 02960 - Absorption, distribution, excretion, and metabolism in the rat [redacted] Report No.: MEF-11/556, Edition Number: M-421499-01-1 Date: 2011-12-22 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 5.1.2 /02	[redacted]	2011	Quantitative whole body autoradiography of [furanone-4-14C]BYI 02960 in male and female rats: Distribution of total radioactivity and elimination from blood, organs and tissues after single oral administration including determination of radioactivity in the excreta and exhaled 14CO2 [redacted] Report No.: MEF-11/275, Edition Number: M-409859-01-2 Date: 2011-05-30 GLP/GEP: yes, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIA 5.1.2 /03	[redacted].	2011	[Furanone-4-14C]BYI 02960 - Metabolism in organs and tissues of male and female rats [redacted] Report No.: MEF-11/271, Edition Number: M-414034-02-1 Date: 2011-09-12 **...Amended: 2012-02-02** GLP/GEP: yes, unpublished	Yes	BCS
KIIA 5.1.3 /01	[redacted]	2011	[Ethyl-1-14C]BYI 02960 - Absorption, distribution, excretion, and metabolism in male rats [redacted] Report No.: MEF-11/555, Edition Number: M-415647-01-1 Date: 2011-10-10 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 5.1.3 /02	[redacted]	2011	[Ethyl-1-14C]BYI 02960 - Metabolism in organs and tissues of male and female rats (3 time-points) [redacted] Report No.: MEF-11/270, Edition Number: M-415416-02-1 Date: 2011-09-29 **...Amended: 2012-02-02** GLP/GEP: yes, unpublished	Yes	BCS
KIIA 5.2.1 /01	[redacted]	2009	BYI 02960 - Acute toxicity in the rat after oral administration [redacted] Bayer CropScience, Report No.: AT05287, Edition Number: M-349992-01-1 Date: 2009-06-08 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 5.2.2 /01	[redacted]	2009	BYI 02960 - Acute toxicity in the rat after dermal administration [redacted] Bayer CropScience, Report No.: AT05288, Edition Number: M-349995-01-1 Date: 2009-06-08 GLP/GEP: yes, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIA 5.2.3 /01	[redacted]	2010	BYI 02960 - Activity ID TXRVP033 - Acute inhalation toxicity in rats [redacted] Bayer CropScience, Report No.: AT05727, Edition Number: M-362791-01-1 Date: 2010-01-07 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 5.2.4 /01	[redacted]	2009	BYI 02960 - Acute skin irritation/corrosion on rabbits [redacted] Bayer CropScience, Report No.: AT05342, Edition Number: M-353761-01-1 Date: 2009-07-08 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 5.2.5 /01	[redacted]	2009	BYI 02960 - Acute eye irritation on rabbits [redacted] Bayer CropScience, Report No.: AT05341, Edition Number: M-361319-02-1 Date: 2009-07-08 **...Amended: 2009-10-29** GLP/GEP: yes, unpublished	Yes	BCS
KIIA 5.2.6 /01	Vohr, H. W.	2009	BYI 02960 - Local lymph node assay in mice (LLNA/IMDS) Bayer Schering Pharma AG, Wuppertal, Germany Bayer CropScience, Report No.: AT05334, Edition Number: M-353715-01-1 Date: 2009-06-29 GLP/GEP: yes, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIA 5.3.1 /01	[redacted]	2007	BYI 02960 - Exploratory 28-day toxicity study in the rat by gavage [redacted] Bayer CropScience, Report No.: SA 06075, Edition Number: M-283421-02-1 Date: 2007-02-02 **...Amended: 2009-02-24** GLP/GEP: no, unpublished	Yes	BCS
KIIA 5.3.1 /02	[redacted]	2008	BYI 02960 - Exploratory 28-day toxicity study in the rat by dietary administration [redacted] Bayer CropScience, Report No.: SA 07047, Edition Number: M-297120-01-1 Date: 2008-02-01 GLP/GEP: no, unpublished	Yes	BCS
KIIA 5.3.1 /03	[redacted]	2007	BYI 02960 : Preliminary 28-day toxicity study in the mouse by dietary administration [redacted] Bayer CropScience, Report No.: SA 07013, Edition Number: M-294820-01-1 Date: 2007-11-23 GLP/GEP: no, unpublished	Yes	BCS
KIIA 5.3.1 /04	[redacted]	2008	Preliminary 28-day toxicity study in the dog by dietary administration [redacted] Bayer CropScience, Report No.: SA07290, Edition Number: M-312461-01-1 Date: 2008-12-09 GLP/GEP: no, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIA 5.3.2 /01	[redacted]	2009	BYI 02960 - 90-day toxicity study in the rat by dietary administration - Amendment no.2 [redacted] Bayer CropScience, Report No.: SA 07294, Edition Number: M-329048-03-1 Date: 2009-02-10 **...Amended: 2012-03-21** GLP/GEP: yes, unpublished	Yes	BCS
KIIA 5.3.2 /02	[redacted]	2009	BYI 02960 - 90-day toxicity study in the mouse by dietary administration - Amendment no.1 [redacted] Bayer CropScience, Report No.: SA 07295, Edition Number: M-328668-03-1 Date: 2009-02-06 **...Amended: 2012-03-22** GLP/GEP: yes, unpublished	Yes	BCS
KIIA 5.3.3 /01	[redacted]	2010	A 90-day toxicity feeding study in the beagle dog with technical grade BYI 02960 [redacted] Bayer CropScience, Report No.: 09-S76-QQ, Edition Number: M-369978-01-1 Date: 2010-04-22 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 5.3.4 /01	[redacted].	2012	A chronic toxicity feeding study in the Beagle dog with technical grade BYI 02960 [redacted] Bayer CropScience, Report No.: 09-C76-RZ, Edition Number: M-425272-01-1 Date: 2012-02-17 GLP/GEP: yes, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIA 5.3.7 /01	[redacted].	2012	A subacute dermal toxicity study in rats with BYI 02960 [redacted] Bayer CropScience, Report No.: 11-S22-US, Edition Number: M-432336-01-1 EPA MRID No.: 48844115 Date: 2012-06-05 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 5.4.1 /01	[redacted].	2009	BYI 02960 (tested as BYI 02960 technical) (project: BYI 02960) - Salmonella/microsome test plate incorporation and preincubation method [redacted] Bayer CropScience, Report No.: AT05387, Edition Number: M-354173-01-1 Date: 2009-07-24 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 5.4.1 /02	Sokolowski, A.	2011	1st amendment to report Salmonella typhimurium reverse mutation assay with BYI 02960 Harlan Cytotest Cell Research GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 1425802, Edition Number: M-420539-02-1 Date: 2011-09-23 **...Amended: 2011-10-17** GLP/GEP: yes, unpublished	Yes	BCS
KIIA 5.4.2 /01	Thum, M.	2009	BYI 02960 (tested as BYI 02960 technical) - In vitro chromosome aberration test with chinese hamster V79 cells Bayer Schering Pharma AG, Wuppertal, Germany Bayer CropScience, Report No.: AT05626, Edition Number: M-359746-01-1 Date: 2009-11-11 GLP/GEP: yes, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIA 5.4.3 /01	Entian, G.	2009	BYI 02960 (tested as BYI 02960 technical) (project: BYI 02960) - V79/HPRT test in vitro for the detection of induced forward mutations Bayer Schering Pharma AG, Wuppertal, Germany Bayer CropScience, Report No.: AT05625, Edition Number: M-359743-01-1 Date: 2009-10-29 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 5.4.4 /01	Herbold, B.	2009	BYI 02960 - Micronucleus-test on the male mouse Bayer Schering Pharma AG, Wuppertal, Germany Bayer CropScience, Report No.: AT05350, Edition Number: M-353785-01-1 Date: 2009-07-09 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 5.4.4 /02	Wieland, J.	2011	Micronucleus assay in bone marrow cells of the mouse with BYI 02960-a.i. Harlan Cytotest Cell Research GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 1425801, Edition Number: M-420536-01-1 Date: 2011-11-10 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 5.5.2 /01	[redacted]	2012	BYI 02960 - Chronic toxicity and carcinogenicity study in the Wistar rat by dietary administration [redacted] Bayer CropScience, Report No.: M-428257-01-1, Edition Number: M-428257-01-1 Date: 2012-03-05 GLP/GEP: yes, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIA 5.5.3 /01	[redacted]	2012	BCS 02960 - Carcinogenicity study in the C57BL/6J mouse by dietary administration [redacted] Bayer CropScience, Report No.: SA 08338, Edition Number: M-425975-01-1 Date: 2012-02-24 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 5.6.1 /01	[redacted]	2010	Technical grade BYI 02960: A dose range-finding reproductive toxicity study in the Wistar rat [redacted] Bayer CropScience, Report No.: 09-P72-RB, Edition Number: M-394208-01-1 Date: 2010-11-01 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 5.6.1 /02	[redacted]	2009	Technical grade BYF 02960: A two-generation reproductive toxicity study in the Wistar rat [redacted] Bayer CropScience, Report No.: 09-R72-SA, Edition Number: M-417665-01-1 Date: 2009-10-05 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 5.6.10 /01	[redacted]	2010	BYI 02960: Developmental toxicity study in the rat by gavage [redacted] Bayer CropScience, Report No.: SA 08347, Edition Number: M-363938-01-1 Date: 2010-02-22 GLP/GEP: yes, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIA 5.6.10 /02	[redacted]	2012	BYI 02960 - Complementary maternal tolerability study in the pregnant Sprague-Dawley rat by gavage [redacted] Bayer CropScience, Report No.: SA 11140, Edition Number: M-425810-01-1 Date: 2012-02-21 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 5.6.11 /01	[redacted]	2012	BYI 02960 - Developmental toxicity study in the rabbit by gavage [redacted] Bayer CropScience, Report No.: SA 10314, Edition Number: M-423559-01-1 Date: 2012-01-26 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 5.7.1 /01	[redacted]	2011	BYI 02960 An acute neurotoxicity study in the rat by oral administration [redacted] Bayer CropScience, Report No.: SA 10096, Edition Number: M-415408-01-1 Date: 2011-09-30 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 5.7.4 /01	[redacted].	2011	BYI 02960 - 90-day neurotoxicity study in the rat by dietary administration [redacted] Bayer CropScience, Report No.: SA 09283, Edition Number: M-410022-01-1 Date: 2011-06-28 GLP/GEP: yes, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIA 5.7.5 /01	[redacted]	2012	A developmental neurotoxicity study with technical grade BYI 02960 in Wistar rats [redacted] Bayer CropScience, Report No.: 11-D72-UW, Edition Number: M-434203-01-1 EPA MRID No.: 48844140 Date: 2012-07-09 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 5.8 /01	Sokolowski, A.	2010	Salmonella typhimurium - Reverse mutation assay with BCS-AA56716 (metabolite of BYI 02960) Harlan CCR, Rossdorf, Germany Bayer CropScience, Report No.: 1351101, Edition Number: M-409724-01-1 Date: 2010-09-30 GLP/GEP: no, unpublished	Yes	BCS
KIIA 5.8 /02	Hall, C.	2010	BCS-AA56716 (metabolite of BYI 02960) - In vitro chromosome aberration test in Chinese hamster V79 cells Harlan CCR, Rossdorf, Germany Bayer CropScience, Report No.: 1351103, Edition Number: M-409726-01-1 Date: 2010-12-15 GLP/GEP: no, unpublished	Yes	BCS
KIIA 5.8 /03	Hall, C.	2010	BCS-AA56716 (metabolite of BYI 02960) - Gene mutation assay in Chinese hamster v79 cells in vitro (V79 / HPRT) Harlan CCR, Rossdorf, Germany Bayer CropScience, Report No.: 1351102, Edition Number: M-409727-01-1 Date: 2010-12-20 GLP/GEP: no, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIA 5.8 /04	[redacted]	2010	BCS-AA56716 - Acute oral toxicity in rats - Acute toxic class method [redacted] Bayer CropScience, Report No.: 37066 TAR, Edition Number: M-393372-01-1 Date: 2010-10-22 GLP/GEP: no, unpublished	Yes	BCS
KIIA 5.8 /05	[redacted]	2011	BCS-AA56716 (difluoroacetic acid): Preliminary 14-day toxicity study in the rat by dietary administration [redacted] Bayer CropScience, Report No.: SA 10323, Edition Number: M-414152-01-1 Date: 2011-09-19 GLP/GEP: no, unpublished	Yes	BCS
KIIA 5.8 /06	[redacted]	2012	BCS-AA56716 (Difluoroacetic acid) - 90-day toxicity study in the rat by dietary administration [redacted] Bayer CropScience, Report No.: SA 10324, Edition Number: M-424611-01-1 Date: 2012-02-02 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 5.8 /07	Sokolowski, A.	2011	Salmonella typhimurium reverse mutation assay with BYI 02960-difluoroethyl-amino-furanone (metabolite of byi-02960) Harlan CCR, Rossdorf, Germany Bayer CropScience, Report No.: 1399701, Edition Number: M-409728-01-1 Date: 2011-05-24 GLP/GEP: no, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIA 5.8 /08	Hall, C.	2010	BYI 02960-difluoroethyl-amino-furanone (metabolite of BYI 02960) - In vitro chromosome aberration test in Chinese hamster V79 cells Harlan CCR, Rossdorf, Germany Bayer CropScience, Report No.: 1399703, Edition Number: M-420108-01-1 Date: 2010-10-07 GLP/GEP: no, unpublished	Yes	BCS
KIIA 5.8 /09	Hall, C.	2010	BYI 0960-difluoroethyl-amino-furanone (metabolite of BYI 02960) - Gene mutation assay in Chinese hamster V79 cells in vitro (V79 / HPRT) Harlan CCR, Rossdorf, Germany Bayer CropScience, Report No.: 1399702, Edition Number: M-420095-01-1 Date: 2010-12-20 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 5.8 /10	Hall, C.	2011	Micronucleus assay in bone marrow cells of the mouse with BYI 02960-difluoroethyl-aminofuranone (metabolite of BYI 02960) Harlan Cytotest Cell Research GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 1421401, Edition Number: M-420540-01-1 Date: 2011-11-28 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 5.8 /11	[redacted]	2011	In vivo unscheduled DNA synthesis in rat hepatocytes with BYI 02960-difluoroethyl-amino-furanone (metabolite of BYI 02960) [redacted] Bayer CropScience, Report No.: 1421402, Edition Number: M-420111-01-1 Date: 2011-10-26 GLP/GEP: no, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIA 5.8 /12	[redacted]	2011	BYI-02960-difluoroethyl-amino-furanone acute oral toxicity in rats acute toxic class method [redacted] Bayer CropScience, Report No.: 37503 TAR, Edition Number: M-409674-01-1 Date: 2011-05-19 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 5.8 /13	[redacted].	2012	BYI 02960-difluoroethyl aminofuranone: A 14-day dose range finding toxicity/palatability study in rats [redacted] Bayer CropScience, Report No.: 11/116-100PE, Edition Number: M-426158-01-1 Date: 2012-02-29 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 5.8 /14	[redacted]	2012	BYI 02960-difluoroethyl aminofuranone: A 28-day dietary toxicity study in wistar rats [redacted] Bayer CropScience, Report No.: 11/116-100P, Edition Number: M-426136-01-1 Date: 2012-02-29 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 5.8 /15	Nobuo, M.; Yukihiro, K.	1997	Reverse mutation study on bacteria IM-0 Nippon Soda Co., Ltd., Odawara Reseach Center, Japan Nippon Soda Co., Ltd., JPN, Report No.: C007426, Report includes Trial Nos.: 9862 Edition Number: M-195904-01-1 Date: 1997-09-30 GLP/GEP: yes, unpublished	Yes	Nippon Soda Co., Ltd., JPN

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIA 5.8 /16	[redacted]	1997	Acute oral toxicity study in rats IM-0 [redacted] Nippon Soda Co., Ltd., JPN, Report No.: C007424, Report includes Trial Nos.: 3662 Edition Number: M-195899-01-1 Date: 1997-09-30 GLP/GEP: yes, unpublished	Yes	Nippon Soda Co., Ltd., JPN
KIIA 5.8 /17	[redacted]	1997	Thirteen-week dietary subchronic toxicity study in rats IM-0 [redacted] Nippon Soda Co., Ltd., JPN, Report No.: C007425, Report includes Trial Nos.: 0259 Edition Number: M-195901-01-1 Date: 1997-11-28 GLP/GEP: yes, unpublished	Yes	Nippon Soda Co., Ltd., JPN
KIIA 5.8 /18	Nobuo, M.; Yukihiro, K.	1997	Reverse mutation study on bacteria IC-0 Nippon Soda Co., Ltd., Odawara Reseach Center, Japan Nippon Soda Co., Ltd., JPN, Report No.: C007440, Report includes Trial Nos.: 9854 Edition Number: M-195932-01-1 Date: 1997-09-30 GLP/GEP: yes, unpublished	Yes	Nippon Soda Co., Ltd., JPN
KIIA 5.8 /19	[redacted]	1997	Acute oral toxicity study in rats IC-0 [redacted] Nippon Soda Co., Ltd., JPN, Report No.: C007439, Report includes Trial Nos.: 3686 Edition Number: M-195930-01-1 Date: 1997-09-30 GLP/GEP: yes, unpublished	Yes	Nippon Soda Co., Ltd., JPN

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIA 5.10 /01	[redacted]	2010	BYI 02960 - Biokinetic in the plasma of rats following 7 days exposure through the diet [redacted] Bayer CropScience, Report No.: SA 09334, Edition Number: M-385777-01-1 Date: 2010-07-08 GLP/GEP: no, unpublished	Yes	BCS
KIIA 5.10 /02	[redacted]	2011	BYI 02960: 28-day immunotoxicity study in the female wistar rat by dietary administration [redacted] Bayer CropScience, Report No.: SA 10353, Edition Number: M-414754-01-1 Date: 2011-09-22 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 5.10 /03	Lasserre, D.	2012	BYI 02960 and its major metabolite DFA (difluoroacetic acid) - Toxicological profile comparison Bayer S.A.S., Bayer CropScience, Sophia Antipolis, France Bayer CropScience, Report No.: M-428487-01-1, Edition Number: M-428487-01-1 Date: 2012-04-06 GLP/GEP: n.a., unpublished	Yes	BCS
KIIA 5.10 /04	Spiegel, K.	2012	Dietary assessment - Toxicological coverage of BYI 02960 plant and livestock metabolites Bayer CropScience, Report No.: M-428863-01-1, Edition Number: M-428863-01-1 Date: 2012-04-13 GLP/GEP: n.a., unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIIA1 7.1.1 /01	[redacted]	2010	BYI 02960 SL 200 g/L - Acute toxicity in the rat after oral administration [redacted] Bayer CropScience, Report No.: AT05943, Edition Number: M-385422-01-1 Date: 2010-06-17 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA1 7.1.2 /01	[redacted].	2010	BYI 02960 SL 200 g/L - Acute toxicity in the rat after dermal application [redacted] Bayer CropScience, Report No.: AT05944, Edition Number: M-385421-01-1 Date: 2010-06-17 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA1 7.1.3 /01	[redacted]	2010	BYI 02960 SL 200 g/L - Acute inhalation toxicity in rats [redacted] Bayer CropScience, Report No.: AT06016, Edition Number: M-392826-01-1 Date: 2010-10-18 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA1 7.1.4 /01	[redacted]	2010	BYI 02960 SL 200 g/L - Acute skin irritation/corrosion on rabbits [redacted] Bayer CropScience, Report No.: AT05908, Edition Number: M-370381-01-1 Date: 2010-05-06 GLP/GEP: yes, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIIA1 7.1.5 /01	[redacted]	2010	BYI 02960 SL 200 g/L - Acute eye irritation on rabbits [redacted] Bayer CropScience, Report No.: AT05812, Edition Number: M-364511-01-1 Date: 2010-02-25 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA1 7.1.6 /01	[redacted]	2010	BYI 02960 SL 200 g/L - Evaluation of potential skin sensitization in the local lymph node assay in the mouse [redacted] Bayer CropScience, Report No.: SA 10101, Edition Number: M-368808-01-1 Date: 2010-05-07 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA1 7.3.1 /01	Anon.	2010	Southern European greenhouse model overview European Crop Protection Association ECPA, Report No.: M-400719-01-1, Edition Number: M-400719-01-1 Date: 2010-10-31 GLP/GEP: n.a., unpublished	Yes	ECPA
KIIIA1 7.6.1 /01	[redacted]	2010	BYI 02960 (SL200) - In vivo dermal absorption study in the male rat [redacted] Bayer CropScience, Report No.: SA 09255, Edition Number: M-396841-01-1 Date: 2010-11-30 GLP/GEP: yes, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIIA1 7.6.2 /01	[redacted]	2010	BYI 02960 (SL200) - Comparative in vitro dermal absorption study using human and rat skin [redacted] Bayer CropScience, Report No.: SA 09254, Edition Number: M-391215-01-1 Date: 2010-09-28 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA1 7.7.1 /01	Freitag, T.; Stuke, S.	2011	Determination of the dislodgeable foliar residues (DFR) of BYI 02960 after spraying of BYI 02960 SL 200 on lettuce in the field in the Netherlands Bayer CropScience, Report No.: 10-2916, Report includes Trial Nos.: 10-2916-01 Edition Number: M-420640-01-1 Date: 2011-12-12 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA1 7.7.1 /02	Freitag, T.; Stuke, S.	2011	Determination of the dislodgeable foliar residues (DFR) of BYI 02960 after spraying of BYI 02960 SL 200 on lettuce in the field in Portugal Bayer CropScience, Report No.: 10-2917, Report includes Trial Nos.: 10-2917-01 Edition Number: M-420656-01-1 Date: 2011-12-15 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA1 7.7.1 /03	Freitag, T.; Stuke, S.	2011	Determination of the dislodgeable foliar residues (DFR) of BYI 02960 after spraying of BYI 02960 SL 200 on lettuce in the greenhouse in the Netherlands Bayer CropScience, Report No.: 10-2918, Report includes Trial Nos.: 10-2918-01 Edition Number: M-420641-01-1 Date: 2011-12-13 GLP/GEP: yes, unpublished	Yes	BCS

European Commission

FLUPYRADIFURONE

VOLUME 3

ANNEX B

B.7 Residues

Rapporteur Member State: The Netherlands

January 2014 November 2014 December 2014

Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible approval of flupyradifurone under Regulation (EC) 1107/2009

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B.7 Residue data

B.7.1 Metabolism, distribution and expression of residues in plants (IIA 6.2.1 and IIIA 8.2)

The metabolism of flupyradifurone has been investigated in the target plants apple, cotton, rice, tomato and potato, using different application techniques foliar and soil treatment, including tuber treatment, granular application and soil drench, and in confined rotational crops (CRC). The representative rotational crops were wheat, Swiss chard and turnips which were studied at three plant-back intervals after flupyradifurone was applied to the bare soil. The metabolism studies in primary crops were conducted at rates covering the anticipated worst case GAP, with flupyradiflurone formulated as 200 SL (for foliar and soil applications) and 480 FS (for seed treatment applications). In table 7.1-01 an overview of available metabolism studies is given.

Table 7.1.-01 Available metabolism studies

	Fruit / Fruiting vegetables	**Root and tuber vegetables**	**Pulses and oilseeds**	**Cereals / Rice**	**Leaf vegetables**
Foliar treatment	Apple 1) appl. at BBCH 69 2) appl. at BBCH 69 and at 14 days PHI	-	Cotton 1) appl. at BBCH 15-18 2) appl. at BBCH 15-18, and at 14 d. PHI	Paddy rice 1) appl. at BBCH 13-15 (1 day after transplantation), and at 30 days PHI	-
Soil/Seed treatment	Tomato 1) soil drench at BBCH 14-15 and 14 days later	Potato 1) Tuber treatment at BBCH 03 2) in-furrow spray at planting at BBCH 03	-	Paddy rice 2) Granular applications into the planting hole at transplantation	-
Rotational crops	-	Turnips plantback: 1, 4.5 and 10 months after soil appl.	-	Wheat plantback: 1, 4.5 and 10 months after soil appl.	Swiss chard plantback: 1, 4.5 and 10 months after soil appl.

As flupyradifurone contains separate ring systems, two different radiolabels were used in all plant and animal studies. These label positions are shown below:

* denotes position of radiolabel, labelled in the methyl group of the pyridinylmethyl moiety

[pyridinylmethyl-^{14}C]flupyradifurone

* denotes position of radiolabel, labelled in the 4-position of the furanone ring

[furanone-4-^{14}C]flupyradifurone

The notifier mentioned that during the investigation of the environmental fate of flupyradifurone, difluoroacetic acid (DFA) was found to be a major soil metabolite. Since this metabolite could not be detected by radioactivity in the ongoing plant metabolism (and CRC) studies with [furanone-4-^{14}C]- and [pyridinylmethyl- ^{14}C]flupyradifurone, all sample materials from the ongoing studies were analysed for DFA by LC-MS/MS to get an estimate of the residue levels. In addition, a target crop metabolism study with [ethyl- ^{14}C]flupyradifurone applied as a soil drench to tomato was conducted to address possible metabolites specific for this radiolabel and generate sample material with incurred residues for radiovalidation of extraction efficiency.

* denotes position of radiolabel, labelled in the ethyl group of the difluoroethyl amino moiety

[ethyl-1-^{14}C]flupyradifurone

Numerous metabolites were identified in the metabolism studies. The chemical structures and report names used in the summaries are given at the very end of this summary and in the List of Metabolites presented in Annex I.

All residue values given in mg/kg refer to parent compound equivalents if not indicated otherwise.

B.7.1.1 Primary crops

Study 1

Characteristics

Reference	:	Justus, K.; 2011a KIIA 6.2.1/01	Application rate	:	2 x 300 g a.s./ha; soil drench
Type of study	:	metabolism in plant	Total rate	:	600 g a.s./ha as soil drench
Year of execution	:	2009-2011	Area	:	greenhouse
Test substance	:	[furanone-4-^{14}C] Flupyradifurone; sample ID: KATH 6352; batch code BYI 02960-PU-01; purity 99.9% w/w	Plant	:	tomato
GLP statement	:	Yes	Sampling	:	fruit, flowers
Guideline	:	OECD 501, OPPTS 860.1300, PMRA Dir 98-02, Japanese MAFF, 12 Nousan 8147	Acceptability	:	acceptable

Study design

The metabolism of flupyradifurone in tomatoes was investigated corresponding the initially maximum envisaged use pattern in non-EU MSs as stated by the notifier, as part of the Global Joint Review of flupyradifurone, with currently a cumulative maximum of 0.45 kg as/ha. Four tomato plants (variety "Philona") were treated by soil drench application with [furanone-4-^{14}C]BYI 02960 (spec. radioactivity 35.5 µCi/mg; radiochemical purity >99%) formulated as SL 200. The first application was performed at BBCH 15 (5th leaf on main shoot unfolded) and a second application at BBCH 51 (1st inflorescence visible and first bud erect) which was 14 days later. Based on a planting density of 12,000 tomato plants/ha, the single application rate corresponded to 300 g a.s./ha; the total application rate was 600 g a.s./ha. Soil type was 85% white moor peat and 15% clay, enriched with water soluble nutrient salt mixture (ENS) and a pH of 5.8. Newly opened flowers were sampled 2-3 times a week starting at 6 days after the last application and continuing over a period of 30 days (until end of flowering). Fully ripe fruits were harvested 2-3 times a week from the remaining three plants, until no fruits were produced anymore (BBCH 89), which led to a harvest period (since tomato fruits ripen continuously) of 69 to 92 days after the last application.

An aliquot (500 g) of the harvested tomatoes (9.8 kg, total of 3 plants) and all collected flowers (4.1 g, from 1 plant) were homogenized and extracted four times with acetonitrile/water mixtures. The radioactivity in the extracts was determined by LSC and in the solids by combustion followed by LSC. After clean-up using a pre-conditioned SPE RP 18 cartridge, parent compound and metabolites in the extracts of tomato flowers and fruits were analysed by HPLC. Identification was performed by reverse phase HPLC and/or TLC co-chromatography with reference compounds as well as by comparison of HPLC profiles.

Results

Extraction released 85% and 94% of the TRR from the tomato fruits and flowers, respectively. Parent compound was the main component in both matrices and represented 36% of the TRR in fruits and 78% in flowers. Two major metabolites were detected in tomato fruits: glucose (or isomeric carbohydrates) (M45) represented 28% of the TRR, and BYI 02960-difluoroethyl-amino-furanone (DFEAF; M34) represented 10% of the TRR. In tomato flowers, DFEAF was detected as a minor

metabolite and represented 9.2% of the TRR; no radioactive glucose (or isomeric carbohydrates) was detected in flowers. The metabolite BYI-OH-glyc (M8) was a minor metabolite in tomato fruits and flowers and represented 5.5% and 6.6% of the TRR, respectively. One minor metabolite occurring in tomato fruits was not identified (4.3% of TRR and 0.004 mg/kg).

Table 7.1.1-1 Identification of radioactivity in tomato fruit and flowers after drench application with [furanone-4-^{14}C]flupyradifurone at a total rate of 600 g as/ha

	tomato fruits		tomato flowers	
TRR [mg/kg] =	0.096		0.72	
	% of TRR	mg/kg	% of TRR	mg/kg
Total extracted	**85**	**0.081**	**94**	**0.68**
parent compound	36	0.034	78	0.56
glucose/carbohydrates (M45)	28	0.026	---	---
DFEAF (M34)	10	0.010	9.2	0.066
BYI-OH-glyc (M8)	5.5	0.005	6.6	0.048
Total identified	**79**	**0.076**	**94**	**0.68**
unknown 1	4.3	0.004	---	---
Total unknown	**4.3**	**0.004**	**<0.1**	**<0.001**
Analysed extract(s)	84	0.080	94	0.68
Losses (not analysed)	1.3	0.001	---	---
Unextractable (PES*)	15	0.015	6.4	0.046
Accountability	**100**	**0.096**	**100**	**0.72**

* PES = post extraction solids

Conclusions

[Furanone-4-^{14}C]flupyradifurone was moderately metabolised in tomatoes. The following metabolic routes were observed:

- complete degradation of the furanone moiety and incorporation of carbon atoms into the natural compound pool, i.e. into glucose (or isomeric carbohydrates),
- cleavage of the pyridinylmethylamine bond and formation of BYI 02960-difluoroethylamino-furanone and
- hydroxylation of the methylene group of the furanone moiety followed by conjugation with glucose

On the basis of the results of this study, the following metabolic pathway is proposed (Figure 7.1.1-1).

Figure 7.1.1-1: Proposed metabolic pathway of [furanone-4-^{14}C]BYI 02960 in tomatoes

Guidelines & Limitations

The level of extraction, identification and characterisation of radioactivity in tomato is sufficient. The study results are acceptable.

STUDY 2

Characteristics

Reference	:	Justus, K.; 2011b KIIA 6.2.1/02	Application rate	:	2 x 300 g a.s./ha; soil drench
Type of study	:	metabolism in plant	Total rate	:	600 g a.s./ha as soil drench
Year of execution	:	2009-2011	Area	:	greenhouse
Test substance	:	[pyridinylmethyl-^{14}C] flupyradifurone; sample ID: KATH 6351; batch code BYI 02960-PU-01; purity 99.9% w/w	Plant	:	tomato
GLP statement	:	Yes	Sampling	:	fruit, flowers
Guideline	:	OECD 501, OPPTS 860.1300, PMRA Dir 98-02, Japanese MAFF, 12 Nousan 8147	Acceptability	:	acceptable

Study design

The study design is the same as described in study 1 (Justus 2011a), with the following changes:

- The radiolabel used is: [pyridinylmethyl-^{14}C]flupyradifurone with a specific radioactivity of 39.4 µCi/mg and radiochemical purity >98%.
- Sampling times were slightly different, for flowers: 3 to 36 days, and for fruits: 73-92 days after the last application.
- Weight of harvested tomato fruits was 9.1 kg (total of the 3 plants), weight of flowers was 7.6 g.

Results

Extraction released 99% and 97% of the TRR from the tomato fruits and flowers, respectively. Parent compound was a major component in both matrices and represented 24% of the TRR in tomato fruits and 66% in tomato flowers. In tomato fruits, the label-specific metabolite BYI 02960-CHMP-di-glyc (M23) was the main constituent (37% of the TRR), followed by parent compound and the label-specific metabolite 6-CNA (M27; 13% of the TRR). Another six metabolites were minor: BYI 02960-CHMP-glyc (M22; 5.1% of the TRR), BYI 02960-OH-glyc (M8; 3.4% of the TRR), BYI 02960-CHMP (M21; 3.3% of the TRR) and three unknown metabolites (<6% of TRR and <0.01 mg/kg). BYI 02960-CHMP is short for 6-chloro-3-hydroxymethanolpyridine.
In tomato flowers, parent compound represented the predominant portion besides four minor metabolites. The minor metabolites were identical with the metabolites identified in the tomato fruits, except for BYI 02960-CHMP, which was not present in flowers.

Table 7.1.1-2 Identification of radioactivity in tomato fruit and flowers after drench appl. with [pyridinylmethyl-^{14}C]flupyradifurone at a total rate of 600 g as/ha

	tomato fruits		**tomato flowers**	
TRR [mg/kg] =	0.13		1.3	
BYI 02960-	% TRR	mg/kg	% TRR	mg/kg
Total extracted	**99**	**0.13**	**97**	**1.21**
parent compound	24	0.031	66	0.829
6-CNA (M27)	13	0.017	7.0	0.087
CHMP-di-glyc (M23)	37	0.048	8.0	0.10
CHMP-glyc (M22)	5.1	0.007	9.5	0.12
CHMP (M21)	3.3	0.004	---	---
OH-glyc (M8)	3.4	0.004	5.9	0.073
Total identified	**86**	**0.11**	**97**	**1.2**
unknown 1	5.5	0.007	---	---
unknown 2	3.8	0.005	---	---
unknown 3	3.0	0.004	---	---
Total unknown	**12**	**0.016**	**<0.1**	**<0.001**
Analysed extract(s)	99	0.13	97	1.2
Losses (not analysed)	---	---	---	---
Unextractable (PES*)	1.5	0.002	3.5	0.044
Accountability	**100**	**0.13**	**100**	**1.3**

* PES = post extraction solids

Conclusions

[Pyridinylmethyl-^{14}C]flupyradifurone was moderately metabolised in tomatoes. The following metabolic routes were observed:

- cleavage of the pyridinylmethylamine bond followed by conjugation with carbohydrates or followed by oxidation of the methylene group to a carboxylic group, which were the major metabolic routes, and
- hydroxylation of the methylene group of the furanone moiety followed by conjugation with glucose, which was the minor metabolic route.

On the basis of the results of this study, the following metabolic pathway is proposed (Figure 7.1.1-2).

Figure 7.1.1-2: Proposed metabolic pathway of [pyridinylmethyl-^{14}C]BYI 02960 in tomatoes

Guidelines & Limitations

The level of extraction, identification and characterisation of radioactivity in tomato is sufficient. The study results are acceptable.

Study 3

Characteristics

Reference	:	Unold, M, Justus, K.; 2011 KIIA 6.2.1/03	Application rate	:	2 x 300 g a.s./ha; soil drench
Type of study	:	metabolism in plant	Total rate	:	600 g a.s./ha as soil drench
Year of execution	:	2010-2011	Area	:	greenhouse
Test substance	:	[ethyl-1-^{14}C] Flupyradifurone ; sample ID: KATH 6315; batch code BYI 02960-PU-01; purity 99.9% w/w	Plant	:	tomato
GLP statement	:	Yes	Sampling	:	fruit, flowers
Guideline	:	OECD 501, OPPTS 860.1300, PMRA Dir 98-02, Japanese MAFF, 12 Nousan 8147	Acceptability	:	acceptable

Study design

The study design is the same as described in study 1 (Justus 2011a), with the following changes:

- The radiolabel used is: [ethyl-1-^{14}C]flupyradifurone with a specific radioactivity of 35.5 µCi/mg and radiochemical purity >99%.
- Three in stead of four tomato plants were treated by soil drench application.

- Application and sampling times were slightly different. Soil drench was applied at BBCH 14-15 and than again 14 days later, as in study 1 and 2, but BBCH code was than 51-59. Flowers were sampled 1 to 32 days, and fruits 56 to 86 days after the last application, as tomatoes apparently ripened some more rapidly than in study 1 and 2.
- Weight of harvested tomato fruits was 6.1 kg (total from 2 plants) and 9.8 g of collected flowers. Aliquot of tomato sample for homogenization was 100 g, and of flowers 5.0 g.
- Samples were extracted three in stead of four times with acetonitrile/water mixtures.

Results

Extraction released more than 98% of the TRR from both tomato fruit and flower samples.
Besides parent compound, one major metabolite and two minor metabolites were identified.

The label-specific metabolite difluoroacetic acid (DFA; M44) was the main component detected in both matrices and represented 87% of the TRR in tomato fruits and 60% in tomato flowers. Since difluoroacetic acid was also detected as a major soil metabolite in the aerobic soil degradation studies, it can be expected that at least a part of the residues detected in tomato fruits and flowers originated from the uptake of the soil metabolite. Parent compound was the second major component in tomato fruits and flowers and represented 10% and 33% of the TRR, respectively. BYI 02960-OH-glyc (M8) and DFEAF (M34) were minor metabolites (<5% of the TRR) detected in fruits and flowers.

Table 7.1.1-3 Identification of radioactivity in tomato fruit and flowers after drench appl. with [ethyl-1-^{14}C]flupyradifurone at a total rate of 600 g as/ha

	tomato fruits		**tomato flowers**	
TRR [mg/kg] =	0.20		2.2	
BYI 02960-	% TRR	mg/kg	% TRR	mg/kg
Total extracted	**100**	**0.20**	**98**	**2.2**
Conventional extraction				
parent compound	10	0.020	33	0.74
DFA (M44)	87	0.17	60	1.3
DFEAF (M34)	2.2	0.004	3.1	0.068
OH-glyc (M8)	0.6	0.001	2.4	0.054
Total identified	**100**	**0.20**	**98**	**2.2**
Total unknown	**---**	**---**	**---**	**---**
Analysed extract(s)	100	0.20	98	2.2
Losses (not analysed)	**---**	**---**	**---**	**---**
Unextractable (PES*)	0.5	0.001	1.7	0.037
Accountability	**100**	**0.20**	**100**	**2.2**

* PES = post extraction solids

Conclusions

[Ethyl-1-^{14}C]flupyradifurone was metabolised to a significant extent in tomatoes. The following metabolic routes were observed:

- oxidative cleavage of the difluoroethylamine bond and formation of difluoroacetic acid (this degradation process occurs also in the soil and thus uptake of difluoroacetic acid from the soil is supposed as well)
- cleavage of the pyridinylmethylamine bond and formation of difluoroethylamino-furanone, and
- hydroxylation of the methylene group of the furanone moiety followed by conjugation with glucose

Since it is known that parent degrades to difluoroacetic acid in soil, the difluoroacetic acid residue is probably the result of both uptake of this metabolite from the soil and degradation of the parent compound BYI 02960 in the plant. On the basis of the results of this study, the following metabolic pathway is proposed (Figure 7.1.1-3).

Figure 7.1.1-3: Proposed metabolic pathway of [ethyl-1-^{14}C]BYI 02960 in tomatoes

Guidelines & Limitations

The level of extraction, identification and characterisation of radioactivity in tomato is sufficient. The study results are acceptable.

Overall conclusion metabolism in tomato (soil drench)

The metabolism of flupyradifurone was investigated in tomato fruits and flowers following two soil drench applications. Three studies were performed, each using flupyradifurone radioactively labeled at one of the three side groups of the central amine. Total radioactive residue was measured in fruits (70 to 106 days after the first, and 56 to 92 days after the last application) and flowers (15-50 days after

the first, and 1 to 36 days after the last application). Metabolites were identified or characterized as unknown, dependent on levels found.

At harvest, the total radioactive residue (TRR) in the tomato fruits was 0.10 mg/kg (furanone ring label), 0.13 mg/kg (pyridinylmethyl label) and 0.20 mg/kg (difluoroethyl label). Percentage of parent compound varied from 36% (furanone ring label) and 24% (pyridinylmethyl label) to 10% of TRR (difluoroethyl label).

One major metabolic route in the furanone ring label study was the complete degradation of the furanone moiety and incorporation of the carbon atoms into the natural compound pool, i.e. most probably into glucose (28% of TRR of furanone ring label). Another important route in the furanone ring label study was cleavage of the pyridinylmethylamine bond, forming DFEAF (10% of TRR). The cleavage of this bond was also detected in the studies conducted with the other 2 radiolabels, as DFEAF was also detected in the difluoroethyl label study (2.2% of TRR), and in the pyridinylmethyl label study, CHMP (whether or not conjugated to glycoside or diglycoside, together 45% of TRR) and 6-CNA (13%) were identified as corresponding counterparts to a total of 58% of TRR. 6-CNA is formed from CHMP by oxidation of the methylene group to a carboxylic group.

A minor metabolic route in all three studies was the hydroxylation of the methylene group of the furanone moiety, followed by conjugation with glucose. This metabolic route resulted in the non-label specific metabolite BYI 02960-OH-glyc, which accounted for 5.5%, 3.4% and 0.6% of TRR found in tomato fruit from studies with furanone ring, pyridinylmethyl, and difluoroethyl label, respectively.

In the study conducted with difluoroethyl label, the TRR (0.20 mg/kg) was significantly higher, and percentage parent compound (10%) significantly lower than in the metabolism studies conducted with the other two labels. The major metabolite found in the study with difluoroethyl label was difluoroacetic acid (DFA). Since it is known that flupyradifurone degrades to DFA in soil, the measured DFA residue is probably not only the result of degradation of the parent compound in the plant, but also from uptake of this metabolite from the soil. This uptake of radioactive DFA results in a higher amount of TRR, and consequently lower percentage of parent compound of TRR than in the other two metabolism studies, where the DFA taken up from the soil is not radioactively labeled.

The major metabolic route in the study with difluoroethyl label was oxidative cleavage of the difluoroethylamine bond, forming DFA (87% of TRR, partly taken up from soil). Minor routes were cleavage of the pyridinylmethylamine bond, forming DFEAF (2.2% of TRR) and hydroxylation of the methylene group of the furanone moiety, followed by conjugation with glucose, forming BYI 02960-OH-glyc (0.6% of TRR). Percentages of these two metabolites are lower than in the other studies partly because of higher TRR value due to the contributing radioactivity of DFA taken up from the soil.

When considering the results from all metabolism studies conducted on tomato, it can be concluded that flupyradifurone is rather extensively metabolised in this crop. A total of 6 major and 6 minor metabolites were found, and all major and 3 minor have been identified. The distribution of parent compound and metabolites in the edible commodity tomato fruits is summarized in Table 7.1.1-4.

Table 7.1.1-4: Parent compound and metabolites in tomato fruit after drench application of radiolabelled flupyradifurone at a total rate of 600 g a.s./ha

	tomato fruits					
Radiolabel position	[furanone-4-^{14}C]		[pyridinylmethyl-^{14}C]		[ethyl-1-^{14}C]	
TRR [mg/kg] =	0.096		0.13		0.20	
BYI 02960-	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
parent compound	36	0.034	24	0.031	10	0.020
DFA (M44)					*87*[a]	*0.17*
glucose/carbohydrates(M45)	*28*	*0.026*				
6-CNA (M27)			*13*	*0.017*		
CHMP-di-glyc (M23)			*37*	*0.048*		
CHMP-glyc (M22)			*5.1*	*0.007*		
CHMP (M21)			*3.3*	*0.004*		
DFEAF (M34)	*10*	*0.010*			*2.2*	*0.004*
OH-glyc (M8)	5.5	0.005	3.4	0.004	0.6	0.001
Total identified	**79**	**0.076**	**86**	**0.11**	**100**	**0.20**
Total unknown	**4.3**	**0.004**	**12**	**0.016**	**---**	**---**
Analysed extract(s)	84	0.080	99	0.13	100	0.20
Extract(s) not analysed	1.3	0.001	---	---	---	---
Total extracted	**85**	**0.081**	**99**	**0.13**	**100**	**0.20**
Unextractable (PES*)	15	0.015	2	0.002	0.5	0.001
Accountability	**100**	**0.096**	**100**	**0.13**	**100**	**0.20**

* post extraction solids
[a] probably partly taken up from the soil
Label specific metabolites are printed in italics

On basis of the metabolites identified, biotransformation of flupyradifurone in tomato proceeds by the following pathways:

- oxidative cleavage of the difluoroethylamine bond and formation of difluoroacetic acid (DFA)
- complete degradation of the furanone moiety and incorporation of carbon atoms into the natural compound pool, e.g. into glucose/carbohydrates
- cleavage of the pyridinylmethylamine bond and formation of BYI 02960-difluoroethylamino-furanone and its corresponding counterpart BYI 02960-CHMP, which was either conjugated with carbohydrates or oxidised to 6-chloronicotinic acid (6-CNA)
- hydroxylation of the furanone moiety followed by conjugation with glucose (formation of BYI 02960-OH-glyc)

The positions involved in the metabolic degradation are summarised in Figure 7.1.1-4.

Figure 7.1.1-4: Positions involved in metabolic degradation of BYI 02960 in tomato fruits and flowers

POTATOES

Study 4

Reference	:	Justus, K.; 2011c KIIA 6.2.1/04	Application rate	:	254 g a.s./ha (seed treatment), or 626 g a.s./ha (in furrow)
Type of study	:	metabolism in plant	Total rate	:	254 g a.s./ha (seed treatment) or 626 g a.s./ha (in furrow)
Year of execution	:	2009-2010	Area	:	glass-roofed vegetation area
Test substance	:	[furanone-4-^{14}C] Flupyradifurone; sample ID: KATH 6279; purity >99% w/w SL200: batch ID: 2009-000409 FS 480: batch ID: 2009-001351	Plant	:	potato
GLP statement	:	Yes	Sampling	:	tuber, leaves, remainders of seed potato
Guideline	:	OECD 501, OPPTS 860.1300, PMRA Dir 98-02, Japanese MAFF, 12 Nousan 8147	Acceptability	:	acceptable

Study design

The metabolism of flupyradifurone in potatoes was investigated in two experiments in potatoes (variety “Cilena”). In one experiment, three seed potato tubers were treated immediately prior to planting (BBCH 03) with [furanone-4-^{14}C]BYI 02960 (spec. radioactivity 106 µCi/mg; radiochemical purity >99%), formulated as FS 480, at an application rate of 10.0 g a.s./dt. Based on a seed density of 25 dt/ha (1 dt (deciton) is 100 kg), the application rate corresponded to 254 g a.s./ha. In the other experiment, the radioactive [furanone-4-^{14}C]BYI 02960 was formulated as an SL 200 and was sprayed in the furrow onto the soil at an application rate of 626 g a.s./ha (based on a seed density of 22 dt/ha) prior to planting of the seed potatoes (BBCH 03). According to the notifier, the target rates correspond to the initially anticipated maximum application rates for the two use types, which is currently maximally 450 g a.s./ha, as part of the Global Joint Review of flupyradifurone. Soil type used was sandy loam with a pH of 6.8. The potato tubers were harvested at maturity (BBCH 97; 3.3-3.5 kg), which was 97 days after the last application. Concurrently, the leaves and roots (0.23 kg) as well as the remainders of the seed potatoes (0.03-0.06 kg) were sampled to determine the TRR by combustion analysis. Potato tubers were cut into small cubes which were randomized by shuffling and stored frozen <-18°C.

Aliquots (300 g) of randomized potato cubes of each experiment were homogenized and extracted four times with acetonitrile/water mixtures. The remaining solids after conventional extraction of the potato tubers in the in-furrow treatment experiment were subjected two times to exhaustive extraction with acetonitrile/water (1:1, v/v) under microwave assistance (120°C for 20 min.). The radioactivity in the extracts was determined by LSC and in the solids by combustion followed by LSC. After clean-up using a pre-conditioned SPE RP 18 cartridge, parent compound and metabolites in the extracts of potato tubers were analysed by HPLC. Identification was performed by HPLC co-chromatography with reference compounds and/or by comparison of HPLC profiles.
In the tuber treatment experiment, the solids remaining after conventional extraction amounted to 0.026 mg/kg.. Since the distribution of the radioactivity in the extracts and solids after conventional extraction was nearly identical within the in-furrow and the tuber treatment experiments and the conventional extracts showed a very similar metabolite pattern, exhaustive extraction of the solids of the tuber treatment experiment was not considered necessary.

Results

At harvest, the total radioactive residues (TRR) in potato tubers were 0.078 mg a.s equiv./kg after tuber treatment and 0.17 mg a.s equiv./kg after in-furrow application. The TRR values were considerably higher in the remaining plant parts: leaves and roots: 7 mg a.s equiv./kg and remainders of the seed potatoes: 3.4 up to 36 mg/kg in seed treated potatoes. These plant parts were only sampled for optional analysis to support identification of metabolites, if needed.
Extraction of the homogenized potato tuber released 67% of TRR from in the tuber treatment experiment and 75% of the TRR in the in-furrow application experiments. Flupyradifurone was moderately metabolised in potatoes into six metabolites. Parent compound was the only major component (i.e. >10% of TRR) and represented 40% and 57% of the TRR in potato tubers, respectively. All metabolites were minor, each representing less than 10% of TRR and being below 0.01 mg/kg. Besides parent compound, two minor metabolites were identified: BYI 02960--OH-glyc (M8) and DFEAF (M34). The amounts of parent compound and metabolites are shown in Table 7.1.1-5.

Table 7.1.1-5: Distribution of radioactivity in potato tubers after tuber treatment or in-furrow application of [furanone-4-^{14}C]flupyradifurone

	potato tubers/ tuber treated		potato tubers/ in-furrow application	
TRR [mg a.s. equiv/kg] =	0.078		0.17	
BYI 02960-	**% of TRR**	**mg/kg**	**% of TRR**	**mg/kg**
Total extracted	**67.0**	**0.052**	**75.3**	**0.13**
Conventionally extraction				
parent compound	40	0.031	51	0.086
DFEAF (M34)	4.2	0.003	2.9	0.005
OH-glyc (M8)	6.6	0.005	4.4	0.007
Subtotal identified	**51**	**0.039**	**58**	**0.099**
unknown 1	7.2	0.006	5.5	0.009
unknown 2	1.7	0.001	---	---
unknown 3	2.4	0.002	1.9	0.003
unknown 4	1.1	0.001	---	---
subtotal unknown	**12**	**0.010**	**7.4**	**0.013**
Conventional extracts not analysed	3.9	0.003	3.8	0.006
Total conventional extraction	**67**	**0.052**	**69**	**0.12**
Microwave extraction				
parent compound	---	---	6.2	0.011
Subtotal identified			**6.2**	**0.011**
Microwave extracts not analysed	---	---	<0.1	<0.001
Total microwave extraction	---	---	**6.3**	**0.011**
Total identified	**51**	**0.039**	**64**	**0.11**
Total unknown	**12**	**0.010**	**7.4**	**0.013**
Analysed extract(s)	63	0.049	72	0.12
Losses (not analysed)	3.9	0.003	3.8	0.006
Unextractable (PES*)	33	0.026	25	0.042
Accountability	**100**	**0.078**	**100**	**0.17**

* post extraction solids

Conclusion

[Furanone-4-^{14}C]flupyradifurone was moderately metabolised in potatoes. The following metabolic routes were observed:

- hydroxylation of the methylene group of the furanone moiety followed by conjugation with glucose, and
- cleavage of the pyridinylmethylamine bond.

On the basis of the results, the following metabolic pathway is proposed (Figure 7.1.1-5).

Figure 7.1.1-5: Proposed metabolic pathway of [furanone-4-^{14}C]flupyradifurone in potato tubers

Guidelines & Limitations

The level of extraction, identification and characterisation of radioactivity in potato is sufficient. The study results are acceptable.

Study 5

Reference	:	Justus, K.; 2011d KIIA 6.2.1/05	Application rate	:	254 g a.s./ha (seed treatment), or 626 g a.s./ha (in furrow appl.)
Type of study	:	metabolism in plant	Total rate	:	254 g a.s./ha (seed treatment) or 626 g a.s./ha (in furrow appl.)
Year of execution	:	2009-2010	Area	:	glass-roofed vegetation area
Test substance	:	[pyridinylmethyl-^{14}C] flupyradifurone; sample ID: KATH 6280; purity >99% w/w SL200: batch ID: 2009-000409 FS 480: batch ID: 2009-001351	Plant	:	potato
GLP statement	:	Yes	Sampling	:	tuber, leaves and roots, remainders of seed potato
Guideline	:	OECD 501, OPPTS 860.1300, PMRA Dir 98-02, Japanese MAFF, 12 Nousan 8147	Acceptability	:	acceptable

Study design

The study design is the same as described in study 4 (Justus 2011c), with the following changes:

- The radiolabel used is [pyridinylmethyl-^{14}C]flupyradifurone with a specific radioactivity of 118 µCi/mg and radiochemical purity >99%.
- A slightly different application rate was used. The tuber treatment with FS 480 was applied at a rate of 10.0 g a.s./dt. Based on a seed density of 27 dt/ha, the application rate corresponded to 270 g a.s./ha. The SL 200 formulation was sprayed in the furrow onto the soil at an application rate of 626 g a.s./ha (based on a seed density of 28 dt/ha) prior to planting of the seed potatoes.

- No exhaustive extraction by use of microwave was performed
- Weight of harvested potatoes was 3.4 kg in both experiments. Weight of leaves and roots was 0.25-0.29 kg, and of remainders of the seed potato 0.007-0.016 kg.

Results

At harvest, the total radioactive residues (TRR) in potato tubers were 0.076 mg a.s equiv./kg after tuber treatment and 0.12 mg a.s equiv./kg after in-furrow application. The TRR values were considerably higher in the remaining plant parts: leaves and roots (appr. 100x) and remainders of the seed potatoes (7 to 33 mg a.s. equiv/kg). Extraction of the homogenized potato tuber released 93% and 90% of TRR from in the tuber and in-furrow treatment experiments, respectively. Flupyradifurone was moderately metabolised in potatoes into seven metabolites. Parent compound and 6-CNA (M27) were the only major components (i.e. >10% of TRR) and represented 40-44% and 18-22% of TRR (0.02 mg a.s. equiv./kg) in potato tubers, respectively. All other metabolites representing less than 10% of TRR and being below 0.01 mg/kg. Amounts of parent compound and metabolites are shown in Table 7.1.1-6.

Table 7.1.1-6 Identification of radioactivity in potato tuber after treatment with [pyridinylmethyl-^{14}C]flupyradifurone

	potato tubers / tuber treatment		**potato tubers/in-furrow application**	
TRR [mg a.s. equiv/kg] =	0.076		0.12	
BYI 02960-	% TRR	mg/kg	% TRR	mg/kg
Total extracted	**93**	**0.071**	**90**	**0.10**
parent compound	40	0.031	44	0.051
6-CNA (M27)	22	0.016	18	0.021
6-CNA-glycerol-gluA (M28)	---	---	2.3	0.003
CHMP-di-glyc (M23)	4.4	0.003	5.3	0.006
CHMP-glyc (M22)	3.7	0.003	2.4	0.003
CHMP (M21)	3.9	0.003	3.9	0.004
OH-glyc (M8)	6.7	0.005	4.7	0.005
Total identified	**81**	**0.061**	**81**	**0.093**
unknown 1	1.9	0.001	---	---
unknown 2	2.3	0.002	2.8	0.003
unknown 3	5.1	0.004	3.0	0.003
Total unknown	**9.3**	**0.007**	**5.8**	**0.007**
Analysed extract(s)	90	0.068	87	0.10
Losses (not analysed)	3.7	0.003	3.6	0.004
Unextractable (PES*)	6.6	0.005	9.6	0.011
Accountability	**100**	**0.076**	**100**	**0.12**

* post extraction solids

Conclusion

[Pyridinylmethyl-^{14}C]flupyradifurone was moderately metabolised in potatoes. The following metabolic routes were observed:

- cleavage of the pyridinylmethylamine bond followed by conjugation with carbohydrates or by oxidation of the methylene group to a carboxylic group and conjugation with carbohydrates or derivatives, which were the major metabolic routes, and
- hydroxylation of the methylene group of the furanone moiety followed by conjugation with glucose, which was the minor metabolic route

On the basis of the results of this study, the following metabolic pathway is proposed (Figure 7.1.1-6).

Figure 7.1.1-6: Proposed metabolic pathway of [pyridinylmethyl-^{14}C]flupyradifurone in potatoes

Guidelines & Limitations

The level of extraction, identification and characterisation of radioactivity in potato is sufficient. The study results are acceptable.

Overall conclusion on metabolism in potato (tuber treatment and in-furrow application)

The metabolism of flupyradifurone was investigated in potatoes in two studies following tuber or soil application with furanone and pyridinylmethyl label. The metabolite profiles of all tuber extracts were very similar, and at harvest, the predominant portion was always parent compound. However, subsequent analysis of the extracts on the non-radiolabelled metabolite difluoroacetic acid - which

cannot be detected with the radiolabels used - revealed even higher concentrations of this metabolite compared to the parent compound (see study 12).

In studies with furanone-labelled flupyradifurone, besides parent compound, six minor metabolites were detected, two of them were identified: BYI 02960-OH-glyc, a metabolite common to both radiolabels tested, and BYI 02960-difluoroethyl-aminofuranone, a metabolite specific to the furanone-label. The metabolites identified were also detected in tomato fruits after soil application and in the confined rotational crop studies. In studies with pyridinylmethy-labelled flupyradifurone, only one major metabolite (6-CNA) was detected besides parent compound. Additionally, eight minor metabolites were detected, five thereof were identified: BYI 02960-OH-glyc, a metabolite common to both radiolabels tested, and the label-specific metabolites BYI 02960CHMP, BYI 02960-CHMP-glyc, BYI 02960-CHMP-di-glyc and 6-CNA-glycerol-gluA. These metabolites or at least the aglycons were also detected in tomato fruits after soil application.

The major metabolic routes in this study were cleavage of the pyridinylmethylamine bond followed by conjugation with carbohydrates or by oxidation of the methylene group to a carboxylic group. A minor metabolic route was hydroxylation of the methylene group of the furanone moiety followed by conjugation with glucose. This metabolic route resulted in the non-label specific metabolite BYI 02960-OH-glyc, which was identified in nearly identical concentrations in both potato studies.
It can be concluded that BYI02960 is moderately metabolised in potato. A total of 2 major and 10 minor metabolites were found, and the 2 major and 6 of the 10 minor have been identified. The distribution of parent compound and metabolites in the edible commodity potato tuber is summarized in Table 7.1.1-7.

In order to gain information on the fate of the difluoroethane moiety of flupyradifurone, the tuber extracts obtained in the potato metabolism study with [pyridinylmethyl-^{14}C]BYI 02960 were additionally analysed for non-radiolabelled difluoroacetic acid by LC-MS/MS according to residue method 01304 (see study 12). High levels of this metabolite were found. After tuber treatment and in-furrow application, difluoroacetic acid accounted for 0.13 and 0.18 mg/kg in potato tubers, which, corrected for difference in molecular weight with a factor 3, is equal to 0.39 and 0.54 mg a.s. equivalent/kg. These concentrations are by a factor of approx. 10 higher compared to those of the parent compound indicating that difluoroacetic acid is the main residue in potato tubers. Since it is known that flupyradifurone degrades to difluoroacetic acid in soil, the high concentration of difluoroacetic acid is probably the result of both uptake of this metabolite from the soil and degradation of the parent compound in the plant.

Table 7.1.1-7: TRR values and distribution of parent compound and metabolites in potato tuber after tuber treatment and in-furrow application of flupyradifurone

	Potato tuber							
Radiolabel	[furanone-4-^{14}C]				[pyridinylmethyl-^{14}C]			
	tuber treatment		in-furrow appl.		tuber treatment		in-furrow appl.	
TRR [mg a.s. equiv/kg] =	0.078		0.17		0.076		0.12	
BYI 02960-	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
parent compound	40	0.031	57	0.097	40	0.031	44	0.051
6-CNA (M27)					*22*	*0.016*	*18*	*0.021*
6-CNA-glycerol-gluA (M28)					---	---	*2.3*	*0.003*
CHMP-di-glyc(M23)					*4.4*	*0.003*	*5.3*	*0.006*
CHMP-glyc(M22)					*3.7*	*0.003*	*2.4*	*0.003*
CHMP (M21)					*3.9*	*0.003*	*3.9*	*0.004*
DFEAF (M34)	*4.2*	*0.003*	*2.9*	*0.005*				
OH-glyc (M8)	6.6	0.005	4.4	0.007	6.7	0.005	4.7	0.005
Total identified	**51**	**0.039**	**64**	**0.11**	**81**	**0.061**	**81**	**0.093**
Total characterised	**12**	**0.01**	**7.4**	**0.013**	**9.3**	**0.007**	**5.8**	**0.007**
Analysed extract(s)	63	0.049	72	0.122	90	0.068	87	0.100
Extract(s) not analysed	**3.9**	**0.003**	**3.8**	**0.006**	**3.7**	**0.003**	**3.6**	**0.004**
Total extracted	67	0.052	75	0.129	93	0.071	90	0.104
Unextractable (PES*)	**33**	**0.026**	**25**	**0.042**	**6.6**	**0.005**	**9.6**	**0.011**
Accountability	**100**	**0.078**	**100**	**0.17**	**100**	**0.076**	**100**	**0.12**

* post extraction solids
Label specific metabolites are printed in italics.

These studies show that the metabolism of flupyradifurone in potato proceeds by the same pathways as the one presented for tomatoes (see Figure 7.1.1-4). The only difference is that the degradation step of the furanone moiety (incorporation of its carbon atoms into the natural compound pool) has not been demonstrated in the potato metabolism studies.

APPLES

Study 6

Reference	:	Justus, K.; 2011e KIIA 6.2.1/06	Application rate	:	foliar spray: 1x 86 g a.s./ha/mCH, or 2x 86 g a.s /ha/mCH
Type of study	:	metabolism in plant	Total rate	:	86 g a.s./ha/mCH, or 172 g a.s./ha/mCH
Year of execution	:	2009-2010	Area	:	glass-roofed vegetation area
Test substance	:	[furanone-4-^{14}C] Flupyradifurone ; sample ID: KATH 6279 (1st appl) KATH 6332 (2nd appl); batch code BYI 02960-PU-01; purity 99.9% w/w	Plant	:	apple tree
GLP statement	:	Yes	Sampling	:	apples, leaves
Guideline	:	OECD 501, OPPTS 860.1300, PMRA Dir 98-02, Japanese MAFF, 12 Nousan 8147	Acceptability	:	acceptable

CH = canopy height

Study design

The metabolism of flupyradifurone in apple was investigated in two experiments in apple trees (variety “James Grieve”) after foliar spray application. In one experiment (single foliar spray application), two

apple trees were treated with [furanone-4-^{14}C]BYI 02960 (spec. radioactivity 53 µCi/mg; radiochem. purity >99%), formulated as SL 200, at an application rate of 86 g a.s./ha/mCH (meter canopy height) at the end of flowering (BBCH 69, PHI = 98 days). In the other experiment (double foliar spray application), one apple tree was treated two times at a rate of 86 g a.s./ha/mCH per application. The application rates are calculated using a planting density of 3000 trees/ha. The first application was conducted at the end of flowering (BBCH 69) and the second at 14 days before harvest. The actual application rates of 86 g a.s./ha/mCH were slightly above the target rate of 75 g a.s./ha/mCH, which corresponds to the maximum application rates on apples of maximally 410 g/ha as stated by the notifier, as part of the Global Joint Review of flupyradifurone.
The apple trees used in the present study had a canopy height (CH) of 0.5 m and were cultivated in sandy loam soil with a pH of 6.8, in a planting container with a surface area of 0.091 m^2. The plants were grown in a glass-roofed vegetation area (natural temperature and light conditions). The apple fruits (3.1 and 1.4 kg respectively for single and double treated appletrees) were harvested at maturity (BBCH 87-89). Concurrently, leaves were sampled (0.30 and 0.13 kg, resp.) to determine the TRR and to support the analysis of metabolites. All apple fruits of single treated and one-third of double treated apples were cut in pieces and pieces were mixed, divided in aliquots and stored frozen at <-18°C. Remaining whole apple fruit was stored overnight at room temperature and than surface washed with dichloromethane.

Aliquots (300 g) of apple pieces of each experiment were homogenized and extracted four times with acetonitrile/water mixtures. The remaining solids after conventional extraction of the apple fruits were additionally subjected two times to exhaustive extraction with acetonitrile/water (1:1, v/v) under microwave assistance (120°C for 20 min.) and/or to enzymatic treatment with cellulase (3 days at room temperature). The radioactivity in the extracts and surface wash (dichloromethane) was determined by LSC and in the solids by combustion followed by LSC. After clean-up using a pre-conditioned SPE RP 18 cartridge, parent compound and metabolites in the surface wash and extracts of apple fruits and leaves, were analyses by reversed phase HPLC coupled to a radioactivity detector with a glass scintillator cell. Identification was performed by comparing the HPLC profiles with those obtained in the apple metabolism study with [pyridinylmethyl-^{14}C]BYI 02960, or by HPLC co-chromatography with authentic reference compounds.

Results

At harvest, the total radioactive residues (TRR) were 0.28 and 1.1-1.3 mg a.s eq./kg in apples treated once and twice by foliar application, respectively. In total, 87% to 97% of the TRR was extracted from the apple matrices. Of apples of the double application experiment, a portion of 7.5% of the TRR was removed by the surface wash, another portion of 81.9% was extracted conventionally and 3.9% of the TRR was extracted after treatment with cellulase. In leaves the TRR was 39 and 103 mg a.s. eq./kg. resp.

In total, 80% to 93% of the TRR was identified in apple fruits and leaves. The residues were dominated by parent compound in the double application experiment due to the late second application. However, irrespective of the amounts, the vast majority of metabolites was identical in the single and double application experiments. Flupyradifurone was metabolised in apples into a large number of metabolites. Parent compound was the major component in the profiles of the double application experiment and represented 71% and 74% of the TRR in the fruits and 58% of the TRR in the leaves. In the single application experiment, parent compound represented 7.4% of the TRR in the fruits and 26% of the TRR in the leaves. Glucose/carbohydrates (M45) was the only major metabolite (>10% of the TRR) in apple fruits, occurring at up to 72% of the TRR. The metabolite BYI 02960-OH-glyc (M8) was the only major metabolite in apple leaves, occurring at 36% and 17% of the TRR. All other metabolites were minor and occurred at < 7% of the TRR in the apple matrices. ~~DFEAF (M34),~~ BYI 02960-acetic acid-glyc (M16), BYI 02960-OH-glyc (M8), BYI 02960-acetic acid (M15) and BYI 02960-OH (M3) were detected in (washed) apple fruits and leaves, whereas DFEAF (M34) and BYI 02960-DFE-OH-glyc (M11) were only detected in apple leaves. Additional details about the amounts of parent compound and metabolites are shown in Tables 7.1.1-8 and Tables 7.1.1-9.

Conclusion

The residues were dominated by parent compound in the double application experiment, whereas the metabolite pattern was very similar in both experiments.

Four major metabolic routes of [furanone-4-^{14}C]BYI 02960 were observed in apples:

- hydroxylation of the furanone or the difluoroethyl moiety followed by conjugation with carbohydrates,
- complete degradation of the furanone moiety and incorporation of carbon atoms into the natural compound pool, i.e. into glucose/carbohydrates,
- oxidative degradation of the furanone moiety to an acetic acid group followed by conjugation with a carbohydrate, and
- cleavage of the pyridinylmethylamine bond.

On the basis of the results of this study it is concluded that the metabolism of [furanone-4-^{14}C]-BYI 02960 in apples, the metabolic pathway is proposed as shown in Figure 7.1.1-7.

Guidelines & Limitations

The level of extraction, identification and characterisation of radioactivity in apple is sufficient. The study results are acceptable.

Table 7.1.1-8: Distribution of radioactivity in apple fruit and leaves after a single foliar application of [furanone-4-^{14}C]flupyradifurone

	single application			
	apple fruits		apple leaves	
TRR [mg a.s. equiv/kg] =	0.28		57	
BYI 02960-	% TRR	mg/kg	% TRR	mg/kg
Conventional extraction				
parent compound	7.4	0.021	26	10
glucose/carbohydrates (M45)	50	0.14	2.5	0.99
DFEAF (M34)	---	---	---	---
acetic acid-glyc (M16)	0.3	0.001	6.4	2.5
OH-glyc (M8)	0.4	0.001	36.1	14
acetic acid (M15)	0.2	0.001	2.5	0.96
DFE-OH-glyc (M11)	---	---	5.8	2.3
OH (M3)	---	---	0.6	0.24
Subtotal identified	**59**	**0.16**	**80**	**31**
unknown 1	---	---	0.3	0.12
unknown 2, 4	---	---	---	---
unknown 3, 6, 7, 9	---	---	1.5 - 1.7	0.58 - 0.65
unknown 5	---	---	2.6	1.0
unknown 8	---	---	4.9	1.9
Subtotal characterised	**---**	**---**	14.0	5.5
Conventional extracts not analysed	1.1	0.003	0.3	0.12
Total conventional extraction	**60**	**0.17**	**94**	**37**
Microwave extraction				
glucose/carbohydrates (M45)	6.4	0.018		
DFEAF (M34)	1.0	0.003		
Subtotal identified	**7.5**	**0.021**		
unknown 2	2.0	0.005		
Subtotal characterised	2.0	0.005		
Microwave extracts not analysed	<0.1	<0.001		
Total microwave extraction	**9.5**	**0.027**	**---**	**---**
Cellulase digestion				
glucose/carbohydrates (M45)	15	0.042		
DFEAF (M34)	2.2	0.006		
Subtotal identified	**17**	**0.048**		
Subtotal characterised	---	---		
Total cellulase extraction	**17**	**0.048**	**---**	**---**
Total identified	**83**	**0.23**	**80**	**31**
Total characterised	**2.0**	**0.005**	**14**	**5.5**
Analysed extract(s)	85	0.24	94	37
Extracts not analysed	1.1	0.003	0.3	0.12
Total extracted	**87**	**0.24**	**94**	**37**
Unextractable (PES*)	14	0.038	5.7	2.2
Accountability	**100**	**0.28**	**100**	**39**

* post extraction solids

Table 7.1.1-9: Distribution of radioactivity in apple fruit and leaves after a double foliar application of [furanone-4-^{14}C]flupyradifurone

	double application					
	apples incl. wash		**apples not washed**		**apple leaves**	
TRR [mg a.s. equiv/kg] =	1.1		1.3		103	
BYI 02960-	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Surface wash						
parent compound	6.7	0.076				
glucose/carbohydrates (M45)	0.2	0.002				
OH-glyc (M8)	0.2	0.002				
acetic acid (M15)	0.1	0.001				
OH (M3)	0.1	0.001				
Subtotal identified	**7.2**	**0.082**				
unknown 8	<0.1	<0.001				
unknown 9	0.3	0.003				
Subtotal characterised	0.3	0.003				
Total surface wash	7.5	0.085				
Conventional extraction						
parent compound	65	0.73	74	0.95	58	60
glucose/carbohydrates (M45)	14	0.15	14	0.18	3.6	3.7
DFEAF (M34)	---	---	0.2	0.003	0.7	0.74
acetic acid-glyc (M16)	0.8	0.009	0.5	0.007	4.2	4.3
OH-glyc (M8)	1.0	0.012	1.1	0.014	17	18
acetic acid (M15)	0.6	0.007	0.7	0.009	1.2	1.2
DFE-OH-glyc (M11)	---	---	---	---	2.1	2.1
OH (M3)	0.9	0.010	0.8	0.010	0.6	0.63
Subtotal identified	**82**	**0.92**	**91**	**1.2**	**88**	**90**
unknown 2	---	---	---	---	--	--
unknown 1, 3, 4, 6, 7	---	---	---	---	0.5 - 0.8	0.47-0.85
unknown 5, 8	---	---	---	---	1.9 - 2.5	2.0 - 2.6
unknown 9	---	---	0.1	0.002	1.2	1.2
Subtotal characterised	---	---	0.1	0.002	8.5	8.8
Conv. extracts not analysed	0.3	0.003	0.7	0.009	0.4	0.44
Total conv. extraction	**82**	**0.93**	**92**	**1.2**	**97**	**99**
Cellulase digestion						
glucose/carbohydrates (M45)	3.3	0.037				
DFEAF (M34)	0.6	0.007				
Subtotal identified	**3.9**	**0.044**				
Subtotal characterised	---	---				
Total cellulase extraction	**3.9**	**0.044**	**---**	**---**	**---**	**---**
Total identified	**93**	**1.1**	**91**	**1.2**	**88**	**90**
Total characterised	**0.3**	**0.003**	**0.1**	**0.002**	**8.5**	**8.8**
Analysed extract(s)	93	1.1	91	1.2	96	99
Extracts not analysed	0.3	0.003	0.7	0.009	0.4	0.44
Total extracted	**93**	**1.1**	**92**	**1.2**	**97**	**99**
Unextractable (PES*)	6.7	0.076	8.1	0.10	3.5	3.6
Accountability	**100**	**1.1**	**100**	**1.3**	**100**	**103**

***** post-extraction solids

Figure 7.1.1-7: Proposed metabolic pathway of [furanone-4-^{14}C]BYI 02960 in apples

STUDY 7

Reference	:	Justus, K.; 2011f KIIA 6.2.1/07	Application rate	:	foliar spray: 1x 87 g a.s./ha/mCH, or 2x 85-87 g a.s./ha/mCH
Type of study	:	metabolism in plant	Total rate	:	87 g a.s./ha/mCH, or 172 g a.s./ha/mCH
Year of execution	:	2009-2010	Area	:	glass-roofed vegetation area
Test substance	:	[pyridinylmethyl-^{14}C]Flupyradifurone ; sample ID: KATH 6280 (1st appl) KATH 6333 (2nd appl); batch code BYI 02960-PU-01; purity 99.9% w/w	Plant	:	apple tree
GLP statement	:	Yes	Sampling	:	apples, leaves
Guideline	:	OECD 501, OPPTS 860.1300, PMRA Dir 98-02, Japanese MAFF, 12 Nousan 8147	Acceptability	:	acceptable

CH = canopy height

Study design

The study design is the same as described in study 6 (Justus 2011e), with the following changes:

- The radiolabel used is: [pyridinylmethyl-^{14}C]flupyradifurone with a specific radioactivity of 59 µCi/mg and radiochemical purity >98%.
- Application rates were slightly different, i.e. in stead of 86 g a.s./ha/mCH, the application rate was 87 g a.s./ha/mCH at the first treatment (both experiments), and 85 g a.s./ha/mCH at the second treatment (double application experiment only).
- Weight of harvested apple fruits was 4.3 and 1.7 kg, weight of leaves was 0.24 and 0.11 kg in single and double application experiments, respectively. Sample weights were 100 or 300 g for apple fruits and and 2 to 5 g for leaves.

Identification and characterisation:

Generally, major metabolites were identified by two independent chromatographic methods (reversed phase HPLC and normal phase TLC) or by LC-MS/MS after semi-preparative isolation of the compounds. Enzymatic treatment with ß-glucosidase was also used.

Since the profiles of the apple leaves showed higher levels of metabolites than the profiles of apple fruits (in terms of mg/kg), several metabolites were isolated with semi-preparative HPLC from the extract of apple leaves of the single treatment experiment. The isolated compounds were identified with spectroscopic methods, even if they were minor. The isolated and identified metabolites were furthermore used as reference compounds for co-chromatographic identification of metabolites in apple fruits. Other minor metabolites (<10% of the TRR and representing <0.01 mg/kg in edibles) were identified by HPLC co-chromatography with authentic reference compounds or by comparison of HPLC profiles.

Results

The TRR level in the apple fruits was 0.079 mg/kg in single application experiment and 1.9 (extraction including surface wash) and 0.55 mg/kg (extraction without surface wash) in double application experiment. The difference of the TRR values of double treated apples is presumably due to the low

amount of apples harvested and extracted and a partly inhomogeneous spray distribution. The TRR values of the apple leaves were 57 mg/kg in leaves of the single application experiment and 135 mg/kg in leaves of the double application experiment. Apple leaves were sampled to support the identification of metabolites.

In apple fruits and leaves the metabolites 6-CNA (M27), BYI 02960-CHMP-glyc (M22), BYI 02960-CHMP (M21), BYI 02960-acetic acid-glyc (M16), BYI 02960-OH-glyc (M8), BYI 02960-acetic acid (M15), BYI 02960-DFE-OH-glyc (M11), BYI 02960AMCP-difluoroethanamine (M36) and BYI 02960-OH (M3) were identified. The metabolite BYI 02960-CHMP-di-glyc was identified only in the leaves. In apple fruits all metabolites were minor (< 10% of the TRR). In apple leaves several minor and the following two major metabolites were detected and identified: BYI 02960-OH-glyc (19.9% and 15.4% of the TRR) and BYI 02960-CHMP-glyc (14.4% and 7.3% of the TRR). Additional details about the amounts of parent compound and metabolites are shown in Tables 7.1.1-10 and 7.1.1-11.

Table 7.1.1-10: TRR values and distribution in apple fruits and leaves after a single foliar treatment of [pyridinylmethyl-^{14}C]BYI 02960

	single application			
	apple fruits		**apple leaves**	
TRR [mg a.i. equiv./kg] =	0.079		57	
BYI 02960-	% TRR	mg/kg	% TRR	mg/kg
Conventional extraction				
parent compound	43	0.034	25	14
6-CNA (M27)	5.0	0.004	---	---
CHMP-di-glyc (M23)	---	---	0.6	0.3
CHMP-glyc (M22)	4.7	0.004	14	8.1
CHMP (M21)	4.0	0.003	1.3	0.73
acetic acid-glyc (M16)	3.5	0.003	5.1	2.9
OH-glyc (M8)	4.9	0.004	20	11
acetic acid (M15)	3.0	0.002	1.4	0.77
DFE-OH-glyc (M11)	1.4	0.001	6.4	3.6
AMCP-difluoroethanamine (M36)	8.4	0.007	0.4	0.26
OH (M3)	0.8	0.001	0.9	0.48
Total identified	**79**	**0.062**	**75**	**42**
unknown 1	2.7	0.002	---	---
unknown 2	6.6	0.005	---	---
unknown 3	3.5	0.003	1.8	1.0
unknown 4	2.0	0.002	3.6	2.1
unknown 20	0.6	<0.001	2.5	1.4
unknown 5, 8, 12, 13	---	---	---	---
unknown 7, 11, 14, 15, 17, 21	---	---	0.2 - 0.7	0.11 - 0.40
unknown 6, 10, 16, 19	---	---	1.1 - 1.9	0.63 -1.1
unknown 9, 18	---	---	2.4 - 2.6	1.4 - 1.5
Total characterised	**15**	**0.012**	**22**	**12**
Analysed extract(s)	94	0.074	97	55
Extracts not analysed	---	---	0.2	0.13
Total extracted	**94**	**0.074**	**97**	**55**
Unextractable (PES*)	5.8	0.005	3.3	1.9
Accountability	**100**	**0.079**	**100**	**57**

* post extraction solids

Table 7.1.1-11: TRR values and distribution in apple fruits and leaves after a double foliar treatment of [pyridinylmethyl-^{14}C]flupyradifurone

	double application experiment					
	apple fruits incl. surface wash		**apple fruits without surface wash**		**apple leaves**	
TRR [mg a.i. equiv./kg]	1.9		0.55		135	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Conventional extraction						
parent compound	88	1.7	86	0.47	48	65
6-CNA (M27)	0.5	0.009	1.5	0.008	0.3	0.44
CHMP-di-glyc (M23)	---	---	---	---	0.2	0.33
CHMP-glyc (M22)	0.5	0.010	0.9	0.005	7.3	9.8
CHMP (M21)	0.7	0.013	0.8	0.004	0.6	0.78
BYI-acetic acid-glyc (M16)	0.6	0.012	0.8	0.004	5.0	6.8
BYI-OH-glyc (M8)	1.3	0.024	1.7	0.009	15.4	21
BYI-acetic acid (M15)	0.8	0.015	1.1	0.006	1.0	1.4
BYI-DFE-OH-glyc (M11)	---	---	---	---	4.9	6.7
AMCP-difluoroethanamine (M36)	4.5	0.085	4.1	0.023	0.8	1.0
BYI-OH (M3)	1.0	0.020	1.0	0.005	0.7	0.94
Total identified	**99**	**1.8**	**98**	**0.53**	**85**	**114**
unknown 3	0.2	0.004	---	---	0.8	1.1
unknown 13	0.3	0.005	---	---	0.4	0.52
unknown 20	0.1	0.002	0.5	0.002	1.7	2.2
unknown 1, 2, 14, 15	---	---	---	---	---	---
unknown 5, 6, 7, 8, 12, 21	---	---	---	---	0.2 - 0.4	0.22 - 0.60
unknown 10, 11, 17, 19	---	---	---	---	0.5 -0.8	0.65 - 1.1
unknown 4, 9, 16, 18	---	---	---	---	1.0 - 2.0	1.4 - 2.7
Total characterised	**0.6**	**0.011**	**0.5**	**0.002**	**13**	**18**
Analysed extract(s)	99	1.9	98	0.53	98	132
Extracts not analysed	0.2	0.003	0.6	0.004	0.6	0.79
Total extracted	**99**	**1.9**	**99**	**0.54**	**98**	**133**
Unextractable (PES*)	0.8	0.015	1.3	0.007	1.6	2.2
Accountability	**100**	**1.9**	**100**	**0.55**	**100**	**135**

* post extraction solids

Conclusions

The residues were dominated by parent compound in the double application experiment, whereas the metabolite pattern was the same in both experiments. Three major metabolic routes of [pyridinylmethyl-^{14}C]BYI 02960 were observed in apples:

- hydroxylation followed by conjugation with carbohydrates,
- cleavage of the pyridinylmethylamine bond followed by conjugation with carbohydrates or by oxidation of the methylene group to a carboxylic group, and
- oxidative degradation of the furanone moiety to an acetic acid group followed either by conjugation with a carbohydrate or by further degradation of the moiety.

On the basis of the results of this study it is concluded that the metabolism of [pyridinylmethyl-^{14}C]-BYI 02960 in apples is well understood and the metabolic pathway is proposed in Figure 7.1.1-8.

Figure 7.1.1-8: Proposed metabolic pathway of [pyridinylmethyl-^{14}C]BYI 02960 in apples

Guidelines & Limitations

The level of extraction, identification and characterisation of radioactivity in apple is sufficient. The study results are acceptable.

Overall conclusions apple (foliar application)

The metabolism of flupyradifurone was investigated in apple fruits in two studies following foliar application with furanone- or pyridinylmethyl-labeled flupyradifurone. In order to gain information on the fate of the difluoroethane moiety of flupyradifurone, the extracts obtained in the apple metabolism study with [furanone-4-^{14}C]BYI 02960 were additionally analysed for non-radiolabelled difluoroacetic acid by LC-MS/MS according to residue method 01304 (see study 12). In both metabolism studies, single and double application experiments were done.

Furanone-labeled flupyradifurone

In the single application experiment a natural compound (glucose/carbohydrates) was by far the main compound in apple fruits, whereas parent compound was predominant in leaves. Nevertheless, the metabolite patterns were very similar in fruits and leaves in both experiments. Besides parent compound and glucose/carbohydrates, each of the other metabolites in apple fruits represented less than 4% of the TRR. In total, 5 and 6 metabolites were identified in the single and the double application experiment, respectively. In apple leaves, BYI -OH-glyc was identified as major metabolite, each of the other metabolites (6 to 7) represented less than 7% of the TRR. Overall, four major metabolic routes were detected: (1) hydroxylation of the parent compound (either the furanone moiety or the difluoroethyl moiety) followed by conjugation with carbohydrates, (2) oxidative degradation of the furanone moiety to an acetic acid group followed either by conjugation with a carbohydrate or by further degradation of the moiety, (3) complete degradation of the furanone moiety and incorporation of carbon atoms into the natural compound pool, i.e. into glucose/carbohydrates, and (4) cleavage of the pyridinylmethylamine bond.

The first two routes led to the non label-specific metabolites BYI -acetic acid and BYI -OH, their corresponding glycosides and to BYI -difluoroethyl-OH-glyc, which was detected in apple leaves only. These metabolites were also detected in the apple metabolism study with [pyridinylmethyl-^{14}C]BYI 02960. Molecule cleavage led to the label-specific metabolite BYI 02960-difluoroethyl-amino-furanone and degradation of the furanone moiety finally to glucose/carbohydrates. The corresponding counterparts CHMP and AMCP-difluoroethanamine were detected in the apple metabolism study with [pyridinylmethyl-^{14}C]BYI 02960. Thus, the results of the present metabolism study in apples are in very good conformity with the results of the corresponding study performed with [pyridinylmethyl-^{14}C]BYI 02960.

Pyridinylmethyl-labeled flupyradifurone

Parent compound was the main compound detected in both experiments in apple fruits at harvest. All metabolites represented less than 10% of the TRR. Nine metabolites were identified in fruits from which BYI 02960-AMCP-difluoroethanamine was the most prominent one, followed by 6-CNA (single application experiment) or BYI 02960-OH-glyc (double application experiment). Overall, three major metabolic routes were detected: (1) Oxidative degradation of the furanone moiety to an acetic acid group followed either by conjugation with a carbohydrate or by further degradation of the moiety; (2)

hydroxylation of the parent compound (either the furanone moiety or the difluoroethyl moiety) followed by conjugation with carbohydrates and (3) cleavage of the pyridinylmethylamine bond followed by conjugation with carbohydrates or by oxidation of the methylene group to a carboxylic group.
The first two routes led to the non label-specific metabolites BYI -acetic acid and BYI -OH, their corresponding glycosides and to BYI -difluoroethyl-OH-glyc. These metabolites were also detected in the apple metabolism study with the furanone label. Molecule cleavage led to the label-specific metabolites AMCP-difluoroethaneamine, 6-CNA, CHMP and its corresponding glycoside. The corresponding counterpart difluoroethyl-amino-furanone (DFEAF) was detected in the apple metabolism study with [furanone-4-^{14}C]BYI 02960. Thus, the results of the present metabolism study in apples are in good conformity with the results of the corresponding study performed with [furanone-4-^{14}C]BYI 02960.
When considering the results from both metabolism studies conducted on apple, it can be concluded that flupyradifurone is rather extensively metabolised in this crop. A total of 3 major (one in apple fruits and 2 in leaves) and more than 20 minor metabolites (5 thereof in fruits) were found. All major and 10 minor metabolites have been identified. The distribution of parent compound and metabolites in the edible commodity apple fruit is summarized in Table 7.1.1-12.

Table 7.1.1-12 TRR values and distribution of parent compound and metabolites in apple fruit after foliar application of flupyradifurone

	apple fruits							
Radiolabel	[furanone-4-^{14}C]				[pyridinylmethyl-^{14}C]			
	single appl.		double appl. w/o wash		single appl.		double appl. w/o wash	
TRR [mg a.i. /kg] =	0.28		1.3		0.079		0.55	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Total identified	**83**	**0.23**	**91**	**1.2**	**79**	**0.062**	**98**	**0.53**
parent compound	7.4	0.021	74	0.95	43	0.034	86	0.47
glucose/carbohydrates (M45)	*72*	*0.20*	*14*	*0.18*				
6-CNA (M27)					*5.0*	*0.004*	*1.5*	*0.008*
CHMP-glyc (M22)					*4.7*	*0.004*	*0.9*	*0.005*
CHMP(M21)					*4.0*	*0.003*	*0.8*	*0.004*
DFEAF (M34)	*3.2*	*0.009*	*0.2*	*0.003*				
BYI-acetic acid-glyc (M16)	0.3	0.001	0.5	0.007	3.5	0.003	0.8	0.004
BYI-OH-glyc (M8)	0.4	0.001	1.1	0.014	4.9	0.004	1.7	0.009
BYI-acetic acid (M15)	0.2	0.001	0.7	0.009	3.0	0.002	1.1	0.006
AMCP-difluoroethanamine (M36)					*8.4*	*0.007*	*4.1*	*0.023*
BYI-DFE-OH-glyc (M11)	---	---	---	---	1.4	0.001	---	---
BYI-OH (M3)	---	---	0.8	0.01	0.8	0.001	1.0	0.005
Total characterised	**2.0**	**0.005**	**0.1**	**0.002**	**15**	**0.012**	**0.5**	**0.002**
Analysed extract(s)	85	0.24	91	1.2	94	0.074	98	0.53
Extract(s) not analysed	1.2	0.003	0.7	0.009	---	---	0.6	0.004
Total extracted	**86**	**0.24**	**92**	**1.2**	**94**	**0.074**	**99**	**0.54**
Unextractable (PES*)	14	0.038	8.1	0.10	5.8	0.005	1.3	0.007
Accountability	**100**	**0.28**	**100**	**1.3**	**100**	**0.079**	**100**	**0.55**

* post extraction solids
Label specific metabolites are printed in italics.

Analysis of apple fruit and leave extracts on the non-radiolabelled metabolite difluoroacetic acid revealed that this metabolite represents a significant proportion of the residue. In apple fruits, difluoroacetic acid accounted for 0.23 mg/kg, corresponding to 0.69 mg a.s. equiv./kg in the single application experiment and represented by far the highest residue. In the double experiment difluoroacetic acid accounted for 0.04 mg/kg or 0.12 mg a.s. equiv./kg and was thus a main metabolite. Only parent compound and the natural compound glucose were detected in higher concentrations. In leaves quite high concentrations of difluoroacetic acid were found (1.9 mg a.s. equiv/kg in the single application experiment and 1.4 mg a.s. equiv./kg in the double application experiment, see study 12), however the proportions were low compared to the total radioactive residue detected.

On basis of the metabolites identified, biotransformation of BYI 02960 in apple proceeds by the following pathways:

- oxidative cleavage of the difluoroethylamine bond and formation of difluoroacetic acid

- oxidative degradation of the furanone moiety to an acetic acid group followed either by conjugation with a carbohydrate or by further degradation of the moiety
- complete degradation of the furanone moiety and incorporation of carbon atoms into the natural compound pool, e.g. into glucose/carbohydrates
- cleavage of the pyridinylmethylamine bond and formation of difluoroethylamino-furanone and its corresponding counterpart CHMP, which was either conjugated with carbohydrates or oxidised to 6-chloronicotinic acid (6-CNA)
- hydroxylation of the furanone or difluoroethane moiety followed by conjugation with glucose or another hexose

The positions involved in the metabolic degradation are summarised in Figure 7.1.1-8.

Figure 7.1.1-8: Positions involved in metabolic degradation of BYI 02960 in apple fruits and leaves

COTTON

Study 8

Reference	:	Schmeling, S. and Weber, E.; 2011a KIIA 6.2.1/08	Application rate	:	foliar application: 1x 209 g a.s./ha; or 2x (1x 209 and 1x 176 g a.s./ha)
Type of study	:	metabolism in plant	Total rate	:	209 g a.s./ha or 385 g a.s./ha
Year of execution	:	2009-2010	Area	:	greenhouse
Test substance	:	[furanone-4-^{14}C] Flupyradifurone ; sample ID: KATH 6269 (1st + 2nd appl); ; purity >99% w/w SL200: batch ID: 2009-000409	Plant	:	cotton
GLP statement	:	Yes	Sampling	:	whole immature plant, gin trash, lint, seeds
Guideline	:	OECD 501, OPPTS 860.1300, PMRA Dir 98-02, Japanese MAFF, 12 Nousan 8147	Acceptability	:	acceptable

Study design

The metabolism of flupyradifurone in cotton was investigated in cotton (*Gossypium hirsutum*, variety: Carmen), after foliar spray application. In one experiment (single application experiment), five cotton plants were treated with [furanone-4-^{14}C]BYI 02960 (specific radioactivity: 106 µCi/mg; radiochemical purity >98%) formulated as SL 200, at an actual application rate of 209 g a.s./ha when the sixth true leaf was unfolded (BBCH 16; PHI = 169 day), i.e. at an early growth stage. In another experiment, one cotton plant was treated two times, one time at an actual application rate of 209 g a.s./ha at BBCH 15 and a second time at an application rate of 176 g a.s./ha at 14 days before harvest of the cotton bolls (BBCH 95-97). The actual application rate of 385 g a.s./ha was slightly below the target rate of 400 g a.s./ha, which corresponds to the anticipated maximum application rate, which is maximally 410 g as/ha, as part of the Global Joint Review of flupyradifurone.. The cotton plants were cultivated by sowing cotton seeds in a soil “Einheitserde T” (85% white moor peat, 15% clay, pH=5.8) in planting pots with a surface area of 0.076 m^2 (1 plant/pot; i.e. approx.13 plants/m^2) and a volume of 15L. The plants were grown in a greenhouse.

Samples were taken at maturity of the seeds in both experiments from cotton seeds (which represent the edible RAC): 179 g (from 4 plants, single treatment) and 51 g (from 1 plant, double treatment); gin trash (1.1 and 0.19 kg, resp.) and lint (135 and 39 g, resp.). Additionally, one plant (31 g) of the single application experiment was sampled at an intermediate growth stage (DAT=28, at BBCH 22-24: two to four vegetative side shoots were visible) to support the identification of metabolites.

The radioactive residues were extracted three times with acetonitrile/water mixtures (8:2). In the case of seeds, the samples were extracted with heptane beforehand. For gin trash, additional exhaustive extraction steps were applied to the solids of the first extraction, comprising of two extraction steps with acetonitrile/water mixtures at 120 °C under microwave assistance. The radioactivity in the extracts was determined by LSC and in the solids by combustion followed by LSC. The intermediate and the gin trash extracts were subjected to an SPE clean-up using a pre-conditioned SPE RP 18 cartridge, whereas the seed and lint extracts were directly analyses by reversed phase HPLC, coupled to a radioactivity detector with a glass scintillator cell.The profiles of the extracts comprising a

reasonable amount of radioactivity were recorded by HPLC and all major and several minor components were identified. Identification of parent compound and metabolites common to both radiolabels was based on the metabolite profiles obtained in the cotton metabolism study with [pyridinylmethyl-^{14}C]flupyradifurone. In this study parent compound and the major metabolites were identified in at least one matrix either by LC-MS/MS after semi-preparative isolation of the compounds or by HPLC and TLC co-chromatography with authentic reference compounds. All assignments made in the present study were confirmed by HPLC co-chromatography in gin trash using authentic reference compounds.

The compounds BYI 02960-OH-glyc (M8) and BYI 02960-acetic acid (M15) co-eluted when using the neutral HPLC method for metabolite profiling. Therefore, the fraction was isolated from the gin trash extracts and was re-analysed with an acidic mobile phase. Both compounds were well separated using the acidic method and identification of both compounds became feasible by either HPLC co-chromatography with an authentic reference compound (BYI 02960-OH-glyc) or by LC-MS/MS (BYI 02960-acetic acid). The authentic reference compound used for co-chromatography was a metabolite isolated and identified in the apple metabolism study. The hexose in the conjugate was unambiguously assigned to D-glucose and the hydroxy group was located in the 5-position of the molecule. All assignments made were confirmed by HPLC co-chromatography in gin trash using authentic reference compounds. Assignment in the other matrices was performed by comparison of HPLC profiles since the metabolite profiles of all matrices were well comparable. No label-specific metabolite was identified.

Results

The TRR levels were 0.013 and 0.016 mg/kg in seeds, 0.19 and 2.8 mg/kg in gin trash, and <0.01 and 5.0 mg/kg in lint, in single and double application experiment respectively. The high residues were expected in gin trash and lint in the double application experiment, where the last treatment was performed 14 days before harvest when more than 90% of the cotton bolls were open. TRR in intermediate plant (single treatment) was 12 mg/kg. Due to the negligible residues, lint of the single application experiment was not subjected to extraction. The extracts of seeds (23% and 57% of 0.013 and 0.016 mg/kg for single and double application experiments respectively) were not further analyzed by HPLC due to the low radioactive residue levels (<0.01 mg/kg). Extraction efficiencies in lint, intermediate and gin trash ranged from 80% of the TRR for gin trash (single application experiment) to 97% for lint (double application experiment).

In total, 70 to 86% of the TRR in the matrices could be identified. Parent compound was the most prominent component, even in the single application experiment, and represented 38-70% of the TRR in all matrices analysed (lint, gin trash and intermediate). The sum of metabolites BYI 02960-OH-glyc (M8) and BYI 02960-acetic acid (M15) represented another major fraction in all matrices and accounted for 13-25% of the TRR; in gin trash BYI 02960-acetic acid represented 58% and 94% of the

combined M8 and M15 fraction in the single and double application experiment, respectively. Besides parent compound, M8 and M15, one additional major compound was detected only in gin trash: BYI 02960-OH (M3), representing 12% of the TRR in the single application experiment. All other metabolites detected in gin trash, lint and as well in the intermediate plant were minor or trace components (<10% of the TRR) in both single and double application experiments. Three of the minor metabolites were identified in gin trash: BYI 02960-glyoxylic acid (M17), BYI 02960-acetic acid-glyc (M16) and BYI 02960-bromo (M2) (probably co-eluting with small amounts of BYI 02960-chloro (M1)).

The TRR values of all plant matrices determined are shown in Table 7.1.1-13 and 7.1.1-14.

Table 7.1.1-13: TRR values and distribution of parent compound and metabolites in cotton matrices after a single foliar treatment of [furanone-4-^{14}C]flupyradifurone

	single application experiment			
	intermediate (DAT=28)		**gin trash (DAT=169)**	
TRR [mg/kg] =	12		0.19	
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg
Conventional extraction				
parent compound	42	5.2	38	0.072
OH-glyc/BYI-acetic acid (M8/M15)	25	3.1	13	0.024
OH (M3)	---	---	12	0.023
acetic acid-glyc (M16)	8.6	1.1	---	---
glyoxylic acid (M17)	---	---	0.9	0.002
bromo/BYI-chloro (M2/M1)	0.7	0.089	0.6	0.001
Subtotal identified	**77**	**9.5**	**64**	**0.12**
unknown 1	2.2	0.27	2.6	0.005
unknown 3, 11	1.0-1.1	0.12 - 0.14	---	---
unknown 5	6.6	0.82	---	---
unknown 7	2.5	0.31	---	---
unknown 4	---	---	0.6	0.001
unknown 2, 6, 8, 9, 10, 12	---	---	---	---
Subtotal characterised	**13**	**1.7**	**3.2**	**0.006**
Total conventional extraction	**90**	**11**	**67**	**0.13**
Microwave extraction				
parent compound			2.5	0.005
OH-glyc/ BYI-acetic acid (M8/M15)			2.9	0.006
OH (M3)			1.2	0.002
acetic acid-glyc (M16)			---	---
Subtotal identified			6.6	0.013
unknown 1			4.2	0.008
unknown 3, 5, 7			---	---
Subtotal characterised			**4.2**	**0.008**
Total microwave extraction	**---**	**---**	**11**	**0.021**
Total identified	**77**	**9.5**	**70**	**0.13**
Total characterised	**13**	**1.7**	**7.4**	**0.014**
Analysed extracts	90	11	78	0.15
Not analysed/losses#	0.4	0.053	2.6	0.005
Total extracted	**90**	**11**	**80**	**0.15**
Unextractable (PES*)	9.7	1.197	19.7	0.038
Accountability	**100**	**12**	**100**	**0.19**

* post extraction solid
\# losses during clean-up, concentration, etc

Table 7.1.1-14: TRR values and distribution of parent compound and metabolites in cotton matrices after two foliar treatments of [furanone-4-^{14}C]flupyradifurone

	double application experiment, PHI = 14 days			
	gin trash		**lint**	
TRR [mg/kg] =	2.8		5.0	
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg
Conventional extraction				
parent compound	53	1.5	70	3.5
OH-glyc/BYI-acetic acid (M8/M15)	20	0.55	14	0.69
OH (M3)	0.6	0.016	---	---
acetic acid-glyc (M16)	2.2	0.062	---	---
glyoxylic acid (M17)	1.6	0.044	0.2	0.009
bromo/BYI-chloro (M2/M1)	2.3	0.063	1.6	0.078
Subtotal identified	**80**	**2.2**	**86**	**4.3**
unknown 1	4.9	0.14	3.8	0.19
unknown 2, 3, 5, 7, 10	0.2-0.8	0.005-0.023	0.3-1.2	0.014-0.062
unknown 4, 6	0.5-2.0	0.013-0.056	---	---
unknown 8, 9, 12	---	---	0.3-2.7	0.016-0.14
unknown 11	---	---	---	---
Subtotal characterised	9.7	0.27	11	0.53
Total conventional extraction	**90**	**2.4**	**97**	**4.8**
Microwave extraction				
parent compound	1.1	0.029		
OH (M3)	---	---		
acetic acid-glyc (M16)	<0.1	0.001		
OH-glyc/BYI-acetic acid (M8/M15)	0.8	0.023		
Subtotal identified	**1.9**	**0.053**		
unknown 1	1.7	0.046		
unknown 3, 5, 7	0.1-0.1	0.001-0.002		
Subtotal characterised	**1.8**	**0.051**		
Total microwave extraction	**3.8**	**0.10**	**---**	**---**
Total identified	**82**	**2.3**	**86**	**4.3**
Total characterised	**12**	**0.32**	**11**	**0.53**
Analysed extracts	93	2.6	97	4.8
Not analysed/losses#	2.3	0.065	<0.1	<0.001
Total extracted	**96**	**2.7**	**97**	**4.8**
Unextractable (PES*)	4.2	0.12	3.4	0.17
Accountability	**100**	**2.8**	**100**	**5.0**

* post extraction solid
\# losses during clean-up, concentration, etc

Conclusions

Metabolism of [furanone-4-^{14}C]-flupyradifurone was moderate in cotton. The residues were dominated by parent compound in both the single and double application experiments. Cotton gin trash was the matrix showing the highest number of metabolites.

The following metabolic routes of [furanone-4-^{14}C]BYI 02960 were observed in cotton:

- hydroxylation of the methylene group of the furanone moiety followed by conjugation
- oxidative opening of the furanone moiety to an acetic acid group followed by further oxidation or conjugation with a carbohydrate (e.g. glycosylation), and
- halogenation (mostly bromination, to a minor extent chlorination) of the furanone moiety

Halogenation of the furanone moiety of the active substance probably occurred in the soil which was supported by the fact that small amounts of halogenated parent compound were identified in the aerobic soil degradation studies. Due to the early timing of the first application, a significant portion of the parent compound could have reached the soil and been subjected to soil metabolism processes. On the basis of these results, a metabolic pathway of [furanone-4-^{14}C]flupyradifurone in cotton can be proposed (Figure 7.1.1-9).

Figure 7.1.1-9 Proposed metabolic pathway of [furanone-4-^{14}C]BYI 02960 in cotton

Guidelines & Limitations

The level of extraction, identification and characterisation of radioactivity in cottton is sufficient. The study results are acceptable.

STUDY 9

Reference	:	Schmeling, S. and Weber, E.; 2011b KIIA 6.2.1/09	Application rate	:	foliar application: 1x 209 g a.s./ha; or 2x (1x 209 and 1x 176 g a.s./ha)
Type of study	:	metabolism in plant	Total rate	:	209 g a.s./ha or 385 g a.s./ha
Year of execution	:	2009-2010	Area	:	greenhouse
Test substance	:	[pyridinylmethyl- ^{14}C] Flupyradifurone ; sample ID: KATH 6270 (1st + 2nd appl); ; purity >99% w/w SL200: batch ID: 2009-000409	Plant	:	cotton
GLP statement	:	Yes	Sampling	:	whole immature plant, gin trash, lint, seeds
Guideline	:	OECD 501, OPPTS 860.1300, PMRA Dir 98-02, Japanese MAFF, 12 Nousan 8147	Acceptability	:	acceptable

Study design

The study design is the same as described in study 8 (Schmeling and Weber 2011a), with the following changes:

- The radiolabel used is: [pyridinylmethyl-^{14}C]flupyradifurone with a specific radioactivity of 118 µCi/mg and radiochemical purity >98%.
- Application rates were slightly different, i.e. the application rate was 206 g a.s./ha (in stead of 209 g a.s./ha), at the first treatment in both experiments (at BBCH=16), and 177 g a.s./ha (in stead of 176 g a.s./ha) at the second treatment (double application experiment only), 15 days before harvest.
- Weight of harvested intermediate was 41 g (1 plant, single application), weight of gin trash: 0.96 and 0.18 kg; of lint: 146 and 46 g; and of seeds: 213 and 60 g, from 4 and 1 plant(s) in single and double application experiments, respectively.

Parent compound and major metabolites were identified either by HPLC and TLC co-chromatography (two independent chromatographic systems) or by mass spectroscopic means (LC-MS/MS) in the gin trash extracts. Minor compounds were assigned by comparison with the corresponding profiles of the cotton metabolism study with [furanone-4-^{14}C]BYI 02960.

Results

The TRR levels were 0.045 and 0.068 mg/kg in seeds, 0.31 and 2.3 mg/kg in gin trash, and <0.01 and 8.8 mg/kg in lint, in single and double application experiments, respectively. TRR in intermediate plant (single treatment) was 14 mg/kg. Due to the negligible residues, lint of the single application experiment was not subjected to extraction. Extraction efficiencies ranged from 28% of the TRR for seeds (single application experiment) to 99% for lint (double application experiment).

In total, 16 to 90% of the TRR could be identified. Parent compound was the most prominent component in all matrices, except for seeds of the single application experiment, and accounted for 23% to 73% of the TRR. The labelspecific 6 CNA (M27) was the main (and only) compound identified in seeds of the single application experiment (16% of TRR), and was also found in all other cotton matrices. Additional major metabolites detected in cotton matrices were BYI 02960-OH (M3) (gin trash only), and the sum of co-eluting compounds BYI 02960-OH-glyc (M8) and BYI 02960-acetic acid (M15). Separation of the co-eluting compounds revealed that M15 represented approx. 67% of the fraction in gin trash in the single application experiment and approx. 94% of the fraction in the double application experiment. All other metabolites detected in cotton matrices represented minor or trace components (<10% of the TRR) in both single and double application experiments. Three of the minor metabolites were identified in gin trash: BYI 02960-glyoxylic acid (M17), BYI 02960-acetic acid-glyc (M16) and BYI 02960-bromo (M2) (probably co-eluting with small amounts of BYI 02960-chloro (M1)).

The TRR values of all plant matrices determined are shown in Table 7.1.1-15 and 7.1.1-16.

Tabel 7.1.1-15: TRR values and distribution of parent compound and metabolites in cotton matrices after a single foliar treatment of [pyridinylmethyl-^{14}C]flupyradifurone

	single application experiment					
	intermediate DAT =28		**gin trash PHI=169 days**		**seeds PHI=169 days**	
TRR [mg/kg] =	14		0.31		0.045	
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Conventional extraction						
parent compound	37	5.2	25	0.077	---	---
6-CNA (M27)	2.1	0.30	19	0.057	16	0.007
glyoxylic acid (M17)	1.5	0.21	2.1	0.007	---	---
acetic acid-glyc (M16)	6.4	0.90	---	---	---	---
OH-glyc /-acetic acid (M8/M15)	25	3.6	12	0.037	---	---
OH (M3)	1.2	0.17	13	0.042	---	---
bromo/BYI-chloro (M2/M1)	0.5	0.064	---	---	---	---
Subtotal identified	**74**	**10**	**71**	**0.22**	**16**	**0.007**
unknown 1	---	---	2.5	0.008	5.7	0.003
unknown 2	---	---	0.6	0.002	---	---
unknown 3, 7, 10	1.4-3.9	0.20-0.55	---	---	---	---
unknown 4, 5, 6, 11, 12	0.9-6.6	0.13-0.94	0.6-3.4	0.002-0.010	---	---
Subtotal characterised	**21**	**2.9**	**9.9**	**0.031**	**5.7**	**0.003**
Total conventional extraction[1]	**94**	**13**	**81**	**0.25**	**22**	**0.010**
Microwave extraction						
parent compound			1.6	0.005		
6-CNA (M27)			1.7	0.005		
OH-glyc /-acetic acid (M8/M15)			1.7	0.005		
OH (M3)			1.1	0.003		
Subtotal identified			**6.1**	**0.019**		
unknown 1			3.8	0.012		
unknown 11			0.5	0.002		
Subtotal characterised			4.3	0.013		
Total microwave extraction	**---**	**---**	**10**	**0.032**	**---**	**---**
Total identified	**74**	**10**	**77**	**0.24**	**16**	**0.007**
Total characterised	**21**	**2.9**	**14**	**0.044**	**5.7**	**0.003**
Analysed extracts	94	13	91	0.28	22	0.010
Not analysed/losses[2]	0.7	0.11	0.9	0.003	6.5	0.003
Total extracted	**95**	**13**	**92**	**0.29**	**28**	**0.013**
Unextractable (PES*)	5.0	0.71	8.0	0.025	72	0.032
Accountability	**100**	**14**	**100**	**0.31**	**100**	**0.045**

[1] analysed extracts only
[2] losses during clean-up, concentration, etc

Table 7.1.1-16: TRR values and distribution of parent compound and metabolites in cotton matrices after two foliar treatments of [pyridinylmethyl-^{14}C]BYI 02960

	double application experiment (PHI=14 days)					
(BYI- = BYI 02960)	**gin trash**		**lint**		**seeds**	
TRR [mg/kg] =	2.344		8.846		0.068	
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Conventional extraction						
parent compound	51	1.2	73	6.5	23	0.016
6-CNA (M27)	2.0	0.047	0.4	0.031	5.0	0.003
BYI-glyoxylic acid (M17)	1.5	0.035	0.2	0.015	---	---
BYI-acetic acid-glyc (M16)	3.6	0.085	---	---	---	---
BYI-OH-glyc /-acetic acid (M8/M15)	21	0.48	15	1.3	4.9	0.003
BYI-OH (M3)	1.3	0.030	---	---	---	---
BYI-bromo/BYI-chloro (M2/M1)	2.1	0.049	1.6	0.14	---	---
Subtotal identified	**83**	**1.9**	**90**	**7.9**	**33**	**0.023**
unknown 1, 2, 3, 4	0.1-2.2	0.004-0.052	---	---	---	---
unknown 5, 10	0.4-1.3	0.009-0.031	0.3-0.3	0.023-0.027	---	---
unknown 6, 7, 12	---	---	0.5 - 0.7	0.040-0.064	---	---
unknown 8, 9	--	---	1.5-4.9	0.13-0.43	---	---
unknown 11	1.4	0.033	0.8	0.070	5.2	0.003
Subtotal characterised	**6.1**	**0.14**	**9.5**	**0.84**	**5.2**	**0.003**
Total conventional extraction[1]	**89**	**2.1**	**99**	**8.8**	**39**	**0.026**
Microwave extraction						
parent compound	1.9	0.044				
6-CNA (M27)	0.2	0.005				
BYI-acetic acid-glyc (M16)	0.1	0.002				
BYI-OH-glyc /-acetic acid (M8/M15)	1.8	0.042				
Subtotal identified	**3.9**	**0.092**				
unknown 1, 2, 5, 6, 11	0.1-0.2	0.002 -0.006				
Subtotal characterised	0.8	0.018				
Total microwave extraction	**4.7**	**0.11**	**---**	**---**	**---**	**---**
Total identified	**87**	**2.0**	**90**	**7.9**	**33**	**0.023**
Total characterised	**6.9**	**0.16**	**9.5**	**0.84**	**5.2**	**0.003**
Analysed extracts	93	2.2	99	8.8	39	0.026
Not analysed/losses[2]	3.8	0.089	<0.1	<0.001	28	0.019
Total extracted	**97**	**2.3**	**99**	**8.8**	**66**	**0.045**
Unextractable (PES*)	2.8	0.065	0.8	0.073	34	0.023
Accountability	**100**	**2.3**	**100**	**8.8**	**100**	**0.068**

* post extraction solid
[1] analysed extracts only
[2] losses during clean-up, concentration, etc

Conclusions

Gin trash showed the highest number of metabolites along with the intermediate. It was shown that the metabolite profiles of all matrices were compatible with those obtained in the cotton metabolism study conducted with [furanone-4-^{14}C]flupyradifurone.

In seeds of the single application experiment, 6-CNA (M27) was identified as main compound. This finding corresponds with the known fact that weak acids tend to have good phloem mobility and are therefore transported selectively into the seeds as a phloem sink. Initial formation of 6-CNA took most probably place in the leaves, which is in line with the finding that 6-CNA is also the main metabolite detected in gin trash. 6-CNA was also detected in the seeds of the double application experiment,

however quite high amounts of parent compound and the co-eluting compounds BYI 02960-OH-glyc (M8) and BYI 02960-acetic acid (M15) superposed the 6-CNA amounts. Most probably the high amounts of these compounds were caused by contamination of the seeds by lint residues, as in the cotton bolls (capsules) the seeds are imbedded in the lint. As seeds are still covered by lint fibres, it is not uncommon to detect the major residues of lint as well in the seed extract. Since lint showed only high residues in the double application experiment, interfering residues were only detected in seeds of this experiment.

Figure 7.1.1-10 Proposed metabolic pathway of [pyridinylmethyl-^{14}C]BYI 02960 in cotton

Guidelines & Limitations

The level of extraction, identification and characterisation of radioactivity in cottton is sufficient. The study results are acceptable.

Overall Conclusions Cotton (foliar application)

The metabolism of flupyradifurone was investigated in the raw agricultural commodities cotton seeds and gin trash and as well in lint following two different spray application scenarios of furanone- or pyridinylmethyl-labelled flupyradifurone. In the single spray application experiment, cotton plants were treated once at PHI 169 day. In the double spray application experiment, one plant was treated at the early growth stage and additionally 14 days before harvest. The target rate per application in both

experiments was 200 g a.s./ha. In order to gain information on the fate of the difluoroethane moiety, the extracts obtained in the cotton metabolism study with pyridinylmethyl-labelled flupyradifurone were additionally analysed for non-radiolabelled difluoroacetic acid by LC-MS/MS according to residue method 01304 (see study 12).

As expected, the residues in the double spray application experiments were dominated by parent compound (at least 25% of TRR). However, even in the single spray applications, parent compound was the main residue (at least 23%) in all cotton matrices analysed, except for seeds (edible commodity). In seeds, 6-CNA (M27)was identified as the only prominent compound with pyridinylmethyl-labelled flupyradifurone; in furone-labelled flupyradifurone, residues in seeds were too low (<0.02 mg/kg) to analyse in both single and double treated cotton plants. Besides parent compound, BYI 02960-acetic acid (M15), BYI 02960-OH-glyc (M8), 6-CNA (M27) and BYI 02960-OH (M3, only gin trash and intermediate) were major metabolites detected in the different matrices. All other metabolites represented less than 10% of the TRR in all matrices.

Analysis of cotton seed and gin trash extracts on the non-radiolabelled metabolite difluoroacetic acid revealed that this metabolite represents a significant proportion of the residues in the single and double application experiments. Difluoroacetic acid accounted for 0.09 mg a.s. equiv./kg (0.03 mg/kg DFA) and 0.06 mg a.s. equiv./kg (0.02 mg/kg DFA) in cotton seeds in the single and the double application experiment and thus represents by far the main proportion of the residues. These findings support the assumption that difluoroacetic acid has a pronounced phloem mobility and is therefore transported selectively into the seeds as a phloem sink. In gin trash, difluoroacetic acid accounted for 0.12 mg a.s. equiv./kg (0.04 mg/kg DFA) in the single application experiment and exceeded the concentration of parent compound. In the double application experiment, parent compound and several metabolites showed higher concentrations than difluoroacetic acid due to the short PHI of the last application.

When considering the results from both metabolism studies conducted on cotton, it can be concluded that flupyradifurone is rather extensively metabolised in this crop. A total of 4 major and approx. 15 minor metabolites were found, and all major and 3 minor have been identified. The edible commodity cotton seed showed only very low radioactive residues and analysis of the extracts was only feasible in the study conducted with the pyridinylmethyl-label. The distribution of parent compound and metabolites in the edible commodity seeds is shown in Table 7.1.1-17; the distribution in the feed commodity gin trash is summarised in Table 7.1.1-18.

Table 7.1.1-17: TRR values and distribution of parent compound and metabolites in cotton seeds after foliar application of flupyradifurone

	cotton seed			
Radiolabel	**[pyridinylmethyl-^{14}C]**			
	single appl.		**double appl.**	
TRR [mg/kg] =	0.045		0.068	
(BYI- = BYI 02960)	% TRR	mg/kg	% TRR	mg/kg
parent compound	---	---	23	0.016
6-CNA (M27)	*16*	*0.007*	*5.0*	*0.003*
BYI-OH-glyc/BYI-acetic acid (M8/M15)	---	---	4.9	0.003
Total identified	**16**	**0.007**	**33**	**0.023**
Total characterised	**5.7**	**0.003**	**5.2**	**0.003**
Analysed extract(s)	22	0.010	39	0.026
Extract(s) not analysed	6.5	0.003	28	0.019
Total extracted	**28**	**0.013**	**66**	**0.045**
Unextractable (PES*)	72	0.032	34	0.023
Accountability	**100**	**0.045**	**100**	**0.068**

* post extraction solids
Label specific metabolites are printed in italics

Remark:

The metabolite profile of cotton seeds from the double application experiment shows most probably an interference from the lint profile since undelinted seeds were analysed.

Table 7.1.1-18: TRR values and distribution of parent compound and metabolites in gin trash after foliar application of flupyradifurone

	gin trash							
Radiolabel	[furanone-4-^{14}C]				[pyridinylmethyl-^{14}C]			
	single appl.		double appl.		single appl.		double appl.	
TRR [mg/kg] =	0.19		2.8		0.31		2.3	
(BYI- = BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
parent compound	40	0.076	54	1.5	26	0.082	53	1.2
6-CNA					*20*	*0.063*	*2.2*	*0.053*
BYI-glyoxylic acid (M17)	0.9	0.002	1.6	0.044	2.1	0.007	1.5	0.035
BYI-acetic acid-glyc (M16)	---	---	2.3	0.063	---	---	3.7	0.087
BYI-OH-glyc/-acetic acid (M8/M15)	16	0.030	21	0.58	14	0.043	22	0.53
BYI-OH (M3)	13	0.025	0.6	0.016	15	0.045	1.3	0.030
BYI-chloro/BYI-bromo (M2/M1)	0.6	0.001	2.3	0.063	---	---	2.1	0.049
Total identified	**70**	**0.134**	**82**	**2.3**	**77**	**0.24**	**87**	**2.0**
Total characterised	**7.4**	**0.014**	**12**	**0.32**	**14**	**0.044**	**6.9**	**0.16**
Analysed extract(s)	78	0.15	93	2.6	91	0.28	93	2.2
Extract(s) not analysed	2.6	0.005	2.3	0.065	0.9	0.003	3.8	0.089
Total extracted	**80**	**0.15**	**95.8**	**2.7**	**92**	**0.29**	**97**	**2.3**
Unextractable (PES*)	20	0.038	4.2	0.12	8.0	0.025	2.8	0.065
Accountability	**100**	**0.19**	**100.0**	**2.8**	**100**	**0.31**	**100**	**2.3**

* post extraction solids
Label specific metabolites are printed in italic.

On basis of the metabolites identified, biotransformation of flupyradifurone in cotton proceeds by the following pathways:

- oxidative cleavage of the difluoroethylamine bond and formation of difluoroacetic acid
- oxidative degradation of the furanone moiety to an acetic acid group followed either by conjugation with a carbohydrate or by further oxidation to BYI 02960-glyoxylic acid
- oxidative cleavage of the pyridinylmethylamine bond and formation of 6-CNA
- hydroxylation of the furanone moiety followed by conjugation with glucose
- halogenation (bromination and most probably chlorination, as well) of the furanone moiety

Halogenation of the furanone moiety of the active substance probably occurred in the soil which is supported by the fact that small amounts of halogenated parent compound were identified in the aerobic soil degradation studies. Due to the early timing of the first application, a significant portion of the parent compound could have reached the soil and been subjected to soil metabolism processes.

The positions involved in the metabolic degradation are summarised in Figure 7.1.1-10.

Figure 7.1.1-10: Positions involved in metabolic degradation of flupyradifurone in cotton matrices

PADDY RICE
Study 10

Reference	:	Schmeling, S. and Weber, E.; 2011c KIIA 6.2.1/10	Application rate	:	Granule application: 1x 409 g a.s./ha; or 2x spray application (1x 175 and 1x 240 g a.s./ha)
Type of study	:	metabolism in plant	Total rate	:	409 - 415 g a.s./ha
Year of execution	:	2009-2010	Area	:	greenhouse
Test substance	:	[furanone-4-^{14}C] Flupyradifurone ; sample ID: KATH 6302 (granule + 1st spray appl) KATH 6352 (2nd spray appl) ; purity >99% w/w SL200: batch ID: 2009-000409	Plant	:	paddy rice
GLP statement	:	Yes	Sampling	:	kernels, husks, straw
Guideline	:	OECD 501, OPPTS 860.1300, PMRA Dir 98-02, Japanese MAFF, 12 Nousan 8147	Acceptability	:	acceptable

Study design

The metabolism of flupyradifurone was investigated in paddy rice (*Oryza sativa L.* variety: Nihonbare) according to the maximum envisaged use pattern according to the notifier. Two different methods of application were covered in this study. One experiment was a granule treatment (GR) with [furanone-4-^{14}C]BYI 02960 (specific radioactivity: 106 µCi/mg; radiochemical purity >99%) applied during the transplanting of the rice seedlings at an application rate of 409 g a.s./ha. The granules were distributed equally in the planting holes directly before the seedlings (BBCH 13-15) were transplanted into the holes. Then the planting container was flooded with water. In the second experiment, the furanone-labelled flupyradifurone was formulated as an SL 200 and applied twice as spray treatment (SP) equally onto plants as well as the water surface; the first spray application took place directly after transplanting of the rice seedlings at a rate of 175 g a.s./ha and the second 29 days before harvest at a rate of 240 g a.s./ha.

The rice plants were cultivated in sandy loam soil with a pH of 6.8, in a planting container with a surface area of 0.5 m^2. The plants were cultivated in a climate chamber in a greenhouse. Rice was harvested at maturity (BBCH 89-92) and plantparts devided in kernels (31and 85 g), husks (21and 46 g) and straw (328 and 360 g, GR and SP respectively). All plant parts were dried at room temperature for five days. The homogenized samples of kernels, husks and straw were stored in a freezer (≤ -18°C) until analysis.

The radioactive residues were extracted three times with acetonitrile/water mixtures (8:2). The post-extraction solids (PES) of kernels and straw were subjected to an exhaustive extraction procedure. The solids were extracted with acetonitrile/water mixtures (8:2 and 1:1) at 120 °C for 15 min. under microwave assistance. Remaining solids of rice kernels were subjected to an additional third microwave extraction step with sodium chloride/water (1:99; w/v); kernels from the spray treatment were further subjected to a diastase treatment (pH 5, 26 °C, 20 h) The radioactivity in the extracts was determined by LSC and in the solids by combustion followed by LSC. The extracts of the sample materials husks and straw were subjected to a clean-up step using a pre-conditioned SPE RP 18 cartridge, whereas the kernel extracts were directly analyses by reversed phase HPLC, coupled to a

radioactivity detector with a glass scintillator cell.The profiles of the extracts comprising a reasonable amount of radioactivity were recorded by HPLC and all major and several minor components were identified. Identification of parent compound and metabolites common to both radiolabels was based on the metabolite profiles obtained in the rice metabolism study with [pyridinylmethyl-^{14}C]flupyra-difurone. In this study parent compound and all metabolites common to both radiolabels were identified by LC-MS/MS after semi-preparative isolation of the compounds or by HPLC co-chromatography with authentic reference compounds. Thus assignment of compounds in the present study was possible by comparing the metabolite profiles. Some of the assignments were confirmed by HPLC co-chromatography using authentic reference compounds. The only label-specific metabolite detected was identified by TLC co-chromatography with the polar fraction isolated and identified in the tomato metabolism study. Additionally, two non-polar metabolites were identified by LC-MS/MS after isolation of the compounds.

Results

The TRR levels were 0.14 and 0.66 mg/kg in kernels, 1.4 and 24 mg/kg in husks, and 2.9 and 20 mg/kg in straw, in single and double application experiment respectively. Extraction efficiencies ranged from 69% of the TRR for kernels (GR) to 94% for straw (SP) after exhaustive extraction. In total, 50 to 86% of the TRR in the matrices could be identified. Parent compound was the most prominent component, even in the single application experiment, and represented 23-75% of the TRR in all matrices. Glucose/carbohydrates (M45) was also detected as major metabolite in kernels (GR) and represented 27% of the TRR. Metabolite BYI 02960-chloro/bromo was detected as a major metabolite in straw after granular (GR) and spray treatment (SP) and accounted for approx. 11% of the TRR. Halogenation of the furanone moiety of the active substance probably occurred in the paddy soil. Soil contact with parent compound resulted in both experiments. All other detected metabolites were minor or trace components, including BYI 02960-acetic acid (M15), BYI 02960-glyoxylic acid (M17), and BYI 02960-OH (M3).

The TRR values of all plant matrices determined are shown in Table 7.1.1-19 and 7.1.1-20.

Table 7.1.1-19 TRR values and distribution of parent compound and metabolites in rice matrices after a single granule application of [furanone-4-^{14}C]flupyradifurone

	granule application experiment; PHI = 127 days					
	kernels		**husks**		**straw**	
TRR [mg/kg] =	0.14		1.4		2.9	
(BYI- = BYI 02960-)	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Conventional extraction						
parent compound	18	0.024	72	1.0	58	1.7
glucose/carbohydrates (M45)	1.7	0.002	1.0	0.014	3.1	0.088
BYI-glyoxylic acid (M17)	---	---	---	---	1.7	0.050
BYI-acetic acid (M15)	---	---	0.4	0.006	1.8	0.051
BYI-OH (M3)	---	---	---	---	0.8	0.024
BYI-bromo/BYI-chloro (M2/M1)	---	---	0.6	0.008	11	0.31
Subtotal identified	**19**	**0.027**	**74**	**1.0**	**76**	**2.2**
unknown 1, 5, 7	---	---	---	---	0.4 - 1.1	0.012-0.033
unknown 3	---	---	1.3	0.019	1.1	0.031
Subtotal characterised	**---**	**---**	**1.3**	**0.019**	**3.4**	**0.099**
Total extracted	**19**	**0.027**	**76**	**1.1**	**79**	**2.3**
Microwave extraction						
parent compound	5.6	0.008			6.4	0.18
glucose/carbohydrates (M45)	---	---			2.3	0.066
BYI-glyoxylic acid (M17)	---	---			0.2	0.007
BYI-acetic acid (M15)	---	---			0.2	0.007
BYI-OH (M3)	---	---			0.2	0.005
BYI-bromo/BYI-chloro (M2/M1)	---	---			0.8	0.022
Subtotal identified	**5.6**	**0.008**			**10**	**0.29**
unknown 3, 4, 5	---	---			0.2 - 0.4	0.005-0.011
Subtotal characterized	**---**	**---**			**0.8**	**0.023**
Total extracted	**5.6**	**0.008**	**---**	**---**	**11**	**0.31**
Exhaustive NaCl extraction						
organic phase	0.8	0.001				
aqueous phase	29	0.040				
glucose/carbohydrates (M45)	25	0.035				
Subtotal identified	**25**	**0.035**				
unknown 2	3.4	0.005				
Subtotal characterized	**4.2**	**0.006**				
Total extracted	**29**	**0.041**	**---**	**---**	**---**	**---**
Total identified	**50**	**0.070**	**74**	**1.0**	**86**	**2.5**
Total characterised	**4.2**	**0.006**	**1.3**	**0.019**	**4.2**	**0.12**
Analysed extract(s)	54	0.076	76	1.1	90	2.6
Extracts not analysed	14	0.020	n.q.	n.q.	1.3	0.037
Total extracted	**69**	**0.096**	**76**	**1.1**	**91**	**2.6**
Unextractable (PES*)	31	0.044	24	0.34	8.8	0.25
Accountability	**100**	**0.14**	**100**	**1.4**	**100**	**2.9**

* post extraction solids
n.q. not quantifiable (residues < LOQ)

Table 7.1.1-20 TRR values and distribution of parent compound and metabolites in rice matrices after two spray applications of [furanone-4-^{14}C]flupyradifurone

	spray application experiment; PHI = 29 days					
	kernels		**husks**		**straw**	
TRR [mg/kg] =	0.66		24		20	
(BYI- = BYI 02960-)	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Conventional extraction						
parent compound	53	0.35	75	18	53	11
glucose/carbohydrates (M45)	1.9	0.012	2.1	0.50	2.0	0.40
BYIglyoxylic acid (M17)	---	---	---	---	2.0	0.39
BYIacetic acid-glyc (M16)	0.4	0.003	0.2	0.054	1.7	0.33
BYI-acetic acid (M15)	5.9	0.039	7.0	1.7	7.2	1.4
BYIOH (M3)	---	---	---	---	0.4	0.081
BYIbromo/-chloro (M2/M1)	1.7	0.011	1.6	0.39	10	2.0
Subtotal identified	**63**	**0.42**	**86**	**21**	**76**	**15**
unknown 1, 4, 9	---	---	0.1-0.9	0.033-0.21	0.1-3.1	0.025-0.61
unknown 2, 5	---	---	---	---	0.3-0.9	0.053-0.17
unknown 3, 6, 7	0.8-0.9	0.005-0.006	0.4-2.5	0.10-0.61	0.2-1.2	0.044-0.23
Subtotal characterised	**2.7**	**0.017**	**4.9**	**1.2**	**6.9**	**1.4**
Total conventional extr.	**66**	**0.43**	**90**	**22**	**83**	**17**
Microwave extraction						
parent compound	3.3	0.021			3.6	0.72
glucose/carbohydrates (M45)	1.7	0.011			1.4	0.29
BYI-glyoxylic acid (M17)	---	---			0.1	0.023
BYI-acetic acid-glyc (M16)	---	---			0.7	0.13
BYI-acetic acid (M15)	0.3	0.002			0.8	0.15
BYI-OH (M3)	---	---			0.1	0.025
BYI-bromo/-chloro (M2/M1)	---	---			0.6	0.12
Subtotal identified	**5.2**	**0.034**			**7.3**	**1.5**
unknown 1, 3, 5, 8	---	---			0.1- 0.3	0.028-0.062
unknown 9	---	---			1.8	0.36
Subtotal characterised	**---**	**---**			**2.6**	**0.51**
Total microwave extr.	**5.2**	**0.034**	**---**	**---**	**9.9**	**2.0**
Exhaustive NaCl/diastase extraction						
organic phase	0.9	0.006				
aqueous phase	14	0.090				
Total exhaustive extr.	**15**	**0.096**	**---**	**---**	**---**	**---**
Total identified	**68**	**0.45**	**86**	**21**	**84**	**17**
Total characterised	**17**	**0.11**	**4.9**	**1.2**	**9.5**	**1.9**
Analysed extract(s)	86	0.56	90	22	93	19
Extracts not analysed	1.6	0.010	0.5	0.13	0.5	0.094
Total extracted	**87**	**0.57**	**91**	**22**	**94**	**19**
Unextractable (PES*)	13	0.085	9.1	2.2	6.4	1.3
Accountability	**100**	**0.66**	**100**	**24**	**100**	**20**

* post extraction solids

Conclusion

[Furanone-4-^{14}C]flupyradifurone was metabolised moderately in paddy rice. Parent compound was the most prominent component and represented approx. 23 – 75% of the TRR in all matrices.

The following metabolic routes of [Furanone-4-^{14}C]flupyradifurone were observed in paddy rice:

- halogenation (mostly bromination, to a minor extent chlorination) of the furanone moiety,
- hydroxylation of the methylene group of the furanone moiety,

- oxidative opening of the furanone moiety to an acetic acid group followed by further oxidation or conjugation with a carbohydrate (i.e. glycosylation), and
- complete degradation of the furanone moiety and incorporation of the carbon atoms into the natural compound pool, i.e. into glucose/carbohydrates

On the basis of the results of this study it is concluded that the metabolism of furanone-labelled flupyradifurone in rice is well understood and the following metabolic pathway is proposed.

Figure 7.1.1.-11 Proposed metabolic pathway of [furanone-4-^{14}C]flupyradifurone in rice

Guidelines & Limitations

None

Study 11

Reference	:	Schmeling, S. and Weber, E.; 2011d KIIA 6.2.1/11	Application rate	:	granular treatment: 1x 434 g a.s./ha; or 2x spray application (1x 178 and 1x 236 g a.s./ha)
Type of study	:	metabolism in plant	Total rate	:	434 and 414 g a.s./ha
Year of execution	:	2009-2010	Area	:	greenhouse
Test substance	:	[pyridinylmethyl-^{14}C] Flupyradifurone ; sample ID: KATH 6301 (granule + 1st spray appl) KATH 6351 (2nd spray appl) ; purity >99% w/w SL200: batch ID: 2009-000409	Plant	:	paddy rice
GLP statement	:	Yes	Sampling	:	kernels, husks, straw
Guideline	:	OECD 501, OPPTS 860.1300, PMRA Dir 98-02, Japanese MAFF, 12 Nousan 8147	Acceptability	:	acceptable

Study design

The study design is the same as described in study 10 (Schmeling, S. 2011c), with the following changes:

- The radiolabel used is: [pyridinylmethyl-^{14}C]flupyradifurone, with a specific radioactivity of 118 µCi/mg and radiochemical purity >98%.
- Application rates were slightly different, i.e. in stead of 409 g a.s./ha the application rate was 434 g a.s./ha at the granular treatment), and 178 and 236 g a.s./ha in the second treatment (double application experiment only).

Identification and characterisation

Identification of parent compound, the only major metabolite, and the next most prominent metabolite was performed by mass spectrometry after isolation of the compounds from the rice straw extract after spray application. Assignment of the compounds in other matrices was performed by HPLC co-chromatography or by comparison of the HPLC profiles. Additionally, four minor metabolites were identified in rice straw by HPLC co-chromatography using authentic reference compounds.

Results

Sample weight were 71 and 89 g for kernels, 41 and 43 g for husks and 386 and 344 g for straw, in single and double application experiment, respectively; TRR levels were 0.050 and 0.62 mg/kg in kernels, 1.6 and 24 mg/kg in husks, and 3.3 and 25 mg/kg in straw, respectively. Extraction efficiencies ranged from 74 to 97% after exhaustive extraction. In total, 74 to 89% of the TRR in the matrices could be identified. Parent compound was the most prominent component, even in the single application experiment, and represented 60-78% of the TRR in all matrices. One major metabolite (>10% of the TRR) was detected only in rice straw from the granular experiment. The metabolite was identified as BYI-bromo by LC-MS/MS. No other major metabolites were detected. MInor or trace components detected included BYI 02960-acetic acid (M15), 6-CNA (M27), BYI 02960-glyoxylic acid (M17), BYI 02960-acetic acid-glyc (M16), and BYI 02960-OH (M3). The TRR values of all plant matrices determined are shown in Table 7.1.1-21 and 7.1.1-22.

Table 7.1.1-21: TRR values and distribution of parent compound and metabolites in rice matrices after a single granule application of [pyridinylmethyl-^{14}C]BYI02960

	granule application experiment,PHI =127 DAYS					
	kernels		**husks**		**straw**	
TRR [mg/kg] =	0.050		1.6		3.3	
(BYI = BYI 02960)	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Conventional extraction						
parent compound	63	0.032	78	1.2	56	1.8
6-CNA (M27)	---	---	0.5	0.009	2.9	0.093
BYI-glyoxalic acid (M17)	---	---	---	---	0.2	0.007
BYI-acetic acid (M15)	---	---	---	---	1.8	0.058
BYI-OH (M3)	---	---	---	---	1.1	0.037
BYI-bromo/chloro (M2/M1)	---	---	0.6	0.010	12	0.39
Subtotal identified	**63**	**0.032**	**79**	**1.3**	**74**	**2.4**
unknown 3, 6, 7, 9, 11-14	---	---	---	---	0.3-1.4	0.011-0.047
unknown 4	---	---	0.8	0.012	0.7	0.022
Subtotal characterised	**---**	**---**	**0.8**	**0.012**	**6.9**	**0.23**
Total conventional extr.	**63**	**0.032**	**80**	**1.3**	**81**	**2.6**
Microwave extraction						
parent compound	6.8	0.003			3.7	0.12
6-CNA (M27)	4.7	0.002			1.0	0.031
BYI-acetic acid (M15)	---	---			0.2	0.006
BYI-OH (M3)	---	---			0.1	0.004
BYI-bromo/chloro (M2/M1)	---	---			0.5	0.018
Subtotal identified	**12**	**0.006**			**5.5**	**0.18**
unknown 2, 3, 5, 6, 12-14	---	---			0.1-0.8	0.003-0.025
Subtotal characterised	**---**	**---**			**1.7**	**0.056**
Total microwave extraction	**12**	**0.006**	**---**	**---**	**7.2**	**0.24**
Total identified	**74**	**0.037**	**79**	**1.3**	**79**	**2.6**
Total characterised	**---**	**---**	**0.8**	**0.012**	**8.6**	**0.28**
Analysed extract(s)	74	0.037	80	1.3	88	2.9
Losses/extracts not analysed	n.q.	n.q.	n.q.	n.q.	1.4	0.046
Total extracted	**74**	**0.037**	**80**	**1.3**	**89**	**2.9**
Unextractable (PES*)	26	0.013	20	0.33	11	0.35
Accountability	**100**	**0.050**	**100**	**1.6**	**100**	**3.3**

* post extraction solids
n.q. not quantified (= losses were < LOQ)

Table 7.1.1-22: TRR values and distribution of parent compound and metabolites in rice matrices after two spray applications of [pyridinylmethyl-^{14}C]flupyradifurone

	spray application experiment; PHI = 29 days					
	kernels		**husks**		**straw**	
TRR [mg/kg] =	0.62		24		25	
	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Conventional extraction						
parent compound	72	0.45	77	19	57	14
6-CNA (M27)	2.1	0.013	0.4	0.11	0.9	0.23
BYI-glyoxalic acid (M17)	0.4	0.003	---	---	2.0	0.51
BYI-acetic acid-glyc (M16)	0.6	0.003	0.2	0.048	1.2	0.31
BYI-acetic acid (M15)	7.8	0.048	6.5	1.5	6.6	1.6
BYI-OH (M3)	0.4	0.002	---	---	0.6	0.15
BYI-bromo/chloro (M2/M1)	1.5	0.009	1.2	0.30	7.9	2.0
Subtotal identified	**85**	**0.53**	**86**	**21**	**76**	**19**
unknown 1, 2, 3, 5, 7, 8, 11	---	---	---	---	0.1-0.3	0.022-0.079
unknown 4, 6, 12	---	---	0.2 -0.4	0.044-0.085	0.2-0.7	0.049-0.17
unknown 9, 10, 13, 14	0.5-1.2	0.003-0.008	0.4-1.7	0.090-0.40	0.3-2.9	0.068-0.72
Subtotal characterised	**3.6**	**0.022**	**4.1**	**0.99**	**7.5**	**1.9**
Total conventional extr.	**88**	**0.55**	**90**	**22**	**84**	**21**
Microwave extraction						
parent compound	3.2	0.020			3.9	0.96
6-CNA (M27)	0.9	0.006			0.3	0.073
BYI-glyoxalic acid (M17)	---	---			0.2	0.052
BYI-acetic acid-glyc (M16)	---	---			0.7	0.166
BYI-acetic acid (M15)	---	---			0.7	0.18
BYI-OH (M3)	---	---			0.2	0.043
BYI-bromo/chloro (M2/M1)	---	---			0.6	0.139
Subtotal identified	**4.1**	**0.026**			**6.5**	**1.6**
unknown 1, 2	---	---			<0.1-0.1	0.011-0.036
unknown 13	0.8	0.005			1.1	0.28
unknown 14	---	---			1.8	0.44
Subtotal characterized	**0.8**	**0.005**			**3.1**	**0.76**
Total microwave extraction	**4.9**	**0.031**	**---**	**---**	**9.6**	**2.4**
Exhaustive NaCl / diastase extraction						
organic phase	1.8	0.011				
aqueous phase	1.2	0.007				
Total NaCl / diastase extr.	**3.1**	**0.021**	**---**	**---**	**---**	**---**
Total identified	**89**	**0.55**	**86**	**21**	**83**	**20**
Total characterised	**7.4**	**0.045**	**4.1**	**0.99**	**10.6**	**2.6**
Analysed extract(s)	96	0.60	89.8	22	93.3	23
Extracts not analysed	0.9	0.007	0.5	0.11	0.3	0.075
Total extracted	**97**	**0.60**	**90.3**	**22**	**93.6**	**23**
Unextractable (PES*)	2.7	0.017	9.7	2.3	6.4	1.6
Accountability	**100**	**0.62**	**100**	**24**	**100**	**25**

* post extraction solids

Conclusions

[Pyridinylmethyl-^{14}C]BYI 02960 is metabolised moderately in rice. Residues were dominated by parent compound in the foliar application experiment, and parent compound was also the main compound in all matrices of rice after granule application. In total, seven different metabolites were identified in rice straw, which shows the most pronounced metabolite pattern. Only one of these metabolites, the halogenated parent compound, represented more than 10% of the TRR (approx. 12%).

Generally, four major metabolic routes of pyridinylmethyl-labelled flupyradifurone were observed in rice:

- hydroxylation of the methylene group of the furanone moiety,
- cleavage of the pyridinylmethylamine bond followed by oxidation of the methylene group to 6-CNA,
- oxidative degradation of the furanone moiety to an acetic acid group followed by either conjugation with a carbohydrate or further oxidation, and
- halogenation (mostly bromination, to a minor extent chlorination) of the furanone moiety,

It is considered likely that halogenation of the furanone moiety of the active substance occurred in the paddy soil/sediment. On the basis of the results of this study it is concluded that the metabolism of pyridinylmethyl-labelled flupyradifurone in rice is well understood and the following metabolic pathway is proposed.

Figure 7.1.1-12: Proposed metabolic pathway of [pyridinylmethyl-^{14}C]BYI 02960 in rice

Guidelines & Limitations

The level of extraction, identification and characterisation of radioactivity in paddy rice is sufficient. The study results are acceptable.

Overall Conclusions Rice (foliar and granule application)

The metabolism of the flupyradifurone was investigated in rice kernels, straw and husks following two different application scenarios of furanone- or pyridinylmethyl-labelled flupyradifurone. In a granule application experiment, rice was treated once at transplanting of the plants (BBCH 13-15), and in a spray application experiment, the plants were treated at transplanting and additionally 30 days before harvest. The total target application rate in both experiments was 400 g a.s./ha. Regardless of the application rate, the TRR values in all rice matrices were significant lower after the granule application compared to the foliar application. As expected, the residues in the spray application experiments were dominated by parent compound in both studies. But, even after the early granule application, parent compound was the main residue in rice husks and straw and, if not the main, a prominent residue in kernels.
Subsequent analysis of the extracts on the non-radiolabelled metabolite difluoroacetic acid – which cannot be detected with the radiolabels used – confirmed that parent compound represented always the highest proportion of the residue, except for rice kernels after granular application, where difluoroacetic acid was the main constituent (see study 12).

The lowest residue levels were detected in rice kernels, irrespective from the application scenario. After granule application in the furanone-label experiment, the main residue in rice kernels was represented by a natural compound (glucose/carbohydrates), indicating a quite intense degradation of the furanone moiety, whereas parent compound was the main compound after spray application, and in both experiments with the pyridinylmethyl-label.

The residue levels in straw and husks were significantly higher (by a factor of 10 to 66), but the metabolite patterns were very comparable for all matrices in both experiments. Besides parent compound or glucose, an additional major metabolite (>10% of the TRR) was detected with both labels but only in rice straw: BYI 02960-bromo, co-eluting with small amounts of BYI 02960-chloro. These halogenated metabolites were most likely not formed in the plants, but in the paddy soil/sediment and taken up by the plants.
In total, four additional minor metabolites were identified, but none of them exceeded 8% of the TRR. Overall, five major metabolic routes were detected: (1) Oxidative degradation of the furanone moiety to an acetic acid group followed by further oxidation or conjugation with a carbohydrate, (2) complete degradation of the furanone moiety and incorporation of carbon atoms into the natural compound pool, i.e. into glucose/carbohydrates; (3) cleavage of the pyridinylmethylamine bond followed by oxidation of the methylene group to 6-CNA; (4) hydroxylation of the furanone moiety of the parent compound and (5) halogenation (bromination, to a minor extent chlorination) of the furanone moiety, which probably occurred in the paddy soil/sediment. Only the second (only determined with furanone-label) and third route (only determined with pyridinylmethyl-label) led to a label-specific metabolite, which were each others counterpart. The concentrations of the metabolites common to both radiolabels tested

correspond very well when comparing the two metabolism studies conducted in rice.

When considering the results from both metabolism studies conducted in rice, it can be concluded that flupyradifurone is rather extensively metabolised in this crop. A total of 3 major and approx. 25 minor metabolites were found, and all major and 5 minor have been identified. The edible commodity rice kernel showed low radioactive residues, especially after granule application. The distribution of parent compound and metabolites in the edible commodity rice kernels is shown in Table 7.1.1-23.

Table 7.1.1-23: TRR values and distribution of parent compound and metabolites in rice kernels after granule and foliar application of flupyradifurone

	rice kernels							
Radiolabel	[furanone-4-^{14}C]				[pyridinylmethyl-^{14}C]			
	granule application		foliar application		granule application		foliar application	
TRR [mg/kg] =	0.14		0.66		0.050		0.62	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
parent compound	23	0.032	57	0.37	70	0.035	75	0.47
glucose/carbohydrates (M45)	*27*	*0.038*			*4.7*	*0.002*		
6-CNA (M27)			*3.6*	*0.023*			*3.1*	*0.019*
BYI-glyoxalic acid (M17)	---	---	---	---	---	---	0.4	0.003
BYI-acetic acid-glyc (M16)	---	---	0.4	0.003	---	---	0.6	0.003
BYI-acetic acid (M15)	---	---	6.1	0.040	---	---	7.8	0.048
BYI-OH (M3)	---	---	---	---	---	---	0.4	0.002
BYI-bromo/chloro (M2/M1)	---	---	1.7	0.011	---	---	1.5	0.009
Total identified	**50**	**0.070**	**68**	**0.45**	**74**	**0.037**	**89**	**0.55**
Total characterised	**4.2**	**0.006**	**17**	**0.11**	**---**	**---**	**7.4**	**0.045**
Analysed extract(s)	54	0.076	86	0.56	74	0.037	96	0.60
Extract(s) not analysed	14	0.020	1.6	0.010	---	---	0.9	0.007
Total extracted	**69**	**0.096**	**87**	**0.57**	**74**	**0.037**	**97**	**0.60**
Unextractable (PES*)	31	0.044	13	0.085	26	0.013	2.7	0.017
Accountability	**100**	**0.14**	**100**	**0.66**	**100**	**0.050**	**100**	**0.62**

* post extraction solids
Label specific metabolites are printed in italic.

Analysis of the extracts of rice kernels and the feed items husk and straw on the non-radiolabelled metabolite difluoroacetic acid revealed that this metabolite represents a significant proportion of the residues in rice kernels after granule and spray application and in husks and straw after granule application (see study 12). It is also detected in rice husks and straw after spray application, but only as minor metabolite. Difluoroacetic acid accounted for 0.06 and 0.24 mg a.s. equiv./kg in rice kernel after granule application and after spray application, respectively. These findings support the assumption that difluoroacetic acid has a pronounced phloem mobility and is therefore transported selectively into the seeds as a phloem sink. In rice straw and husks, the difluoroacetic acid concentrations ranged between 0.36 mg/kg and 1.4 mg/kg. Generally, higher concentrations of difluoroacetic acid were detected after spray application.

On basis of the metabolites identified, biotransformation of flupyradifurone in rice proceeds by the following pathways:

- oxidative cleavage of the difluoroethylamine bond and formation of difluoroacetic acid
- oxidative degradation of the furanone moiety to an acetic acid group followed either by conjugation with a carbohydrate or by further oxidation to BYI 02960-glyoxylic acid
- complete degradation of the furanone moiety and incorporation of carbon atoms into the natural compound pool, e.g. into glucose/carbohydrates
- oxidative cleavage of the pyridinylmethylamine bond and formation of 6-chloronicotinic acid (6-CNA)
- hydroxylation of the furanone moiety
- halogenation (bromination and most probably chlorination, as well) of the furanone moiety

It is considered likely that halogenation of the furanone moiety of the active substance occurred in the paddy soil/sediment. Uptake of the soil metabolite results in plant residues. The positions involved in the metabolic degradation are summarised in the following figure.

Figure 7.1.1-13: Positions involved in metabolic degradation of flupyradifurone in rice matrices

Study 12

Reference	:	Schoening, R. and Ruhl, S.; 2012 KIIA 6.2.1/12	Application rate	:	see B.7.1.1- study 5 to 11
Type of study	:	metabolism in plant	Total rate	:	see B.7.1.1- study 5 to 11
Year of execution	:	2011-2012	Area	:	greenhouse
Test substance	:	flupyradifurone	Plant	:	apple, potato, cotton and paddy rice
GLP statement	:	Yes	Sampling	:	
Guideline	:	OECD 501, OPPTS 860.1300, PMRA Dir 98-02, Japanese MAFF, 12 Nousan 8147	Acceptability	:	acceptable

Study design

Residues of non-radiolabelled difluoroacetic acid, the most plausible metabolite that can be formed from the difluoroethane moiety due to cleavage of BYI 02960, were determined in extracts of samples of apples, potatoes, cotton, rice, wheat, Swiss chard and turnips originating from plant metabolism and confined rotational crops studies after application of [pyridinylmethyl-^{14}C]BYI 02960 or [furanone-4-^{14}C]BYI 02960. To estimate the residue levels of difluoroacetic acid in these crop samples, non-radiolabelled difluoroacetic acid was analyzed according to the provisions of residue analytical method 01304.

Results

The residue levels determined in the samples from the different metabolism studies are summarized in the following tables. The residues are expressed as difluoroacetic acid equivalents.

Table 7.1.1-24: Summary of difluoroacetic acid residues in crop matrices after spray application of flupyradifurone

Metabolism study	Crop	Sample material	Use pattern	DFA residues [mg/kg]	Study reference number
Metabolism of [furanone-4-^{14}C]-BYI 02960 in apples	apple	fruits	one foliar spray application at BBCH 69, 86 g a.s./(ha x m CH)	0.23	study 6
		leaves		0.62	
		fruits	two foliar spray applications, at BBCH 69 and 14 days PHI, 2 x 86 g a.s./(ha x m CH)	0.04	
		leaves		0.45	
Metabolism of [pyridinylmethyl-^{14}C]-BYI 02960 in potatoes	potato	tuber	tuber treatment at planting (BBCH 03), 10.0 g a.s./dt	0.13	study 5
			in-furrow spray application at planting (BBCH 03), 626 g a.s./ha	0.18	
Metabolism of [pyridinylmethyl-^{14}C]-BYI 02960 in Cotton after Spray Application	cotton	gin trash	one spray application, 206 g a.s./ha (at BBCH 16)	0.04	study 9
		seeds		0.03	
		gin trash	two spray applications, 206 g a.s./ha (at BBCH 16), 177 g a.s./ha (at BBCH 95 - 97)	0.02	
		seeds		0.02	
Metabolism of [pyridinylmethyl-^{14}C]-BYI 02960 in paddy rice	rice	straw	two spray applications onto the plants at different growth stages, 178 g a.s./ha (at BBCH 13 - 15)	0.39	study 11
		husk		0.46	
		grains	236 g a.s./ha (at BBCH 87 - 89	0.08	
		straw	one granular application at the	0.12	

Metabolism study	Crop	Sample material	Use pattern	DFA residues [mg/kg]	Study reference number
		husk	time of transplanting, 434 g a.s./ha (at BBCH 13 - 15)	0.20	
		grains		0.02	

Table 7.1.1-25: Summary of difluoroacetic acid residues in rotational crop matrices after soil application of BYI 02960

Metabolism study	Rotation	Crop	Sample material	Residues [mg/kg]	Study reference number
Metabolism of [furanone-4-^{14}C]BYI 02960 in Confined Rotational Crops	1st rotation	wheat	forage	0.09	study B.7.1.2/1
			hay	0.32	
			straw	0.20	
			grain	1.15	
		Swiss chard	intermediate	0.08	
			mature	0.16	
		turnip	leaves	0.08	
			roots	0.02	
	2nd rotation	wheat	forage	0.02	
			hay	0.14	
			straw	0.06	
			grain	0.26	
		Swiss chard	intermediate	0.04	
			mature	0.05	
		turnip	leaves	0.03	
			roots	< 0.01	
	3rd rotation	wheat	forage	< 0.01	
			hay	0.01	
			straw	0.02	
			grain	0.05	
		Swiss chard	intermediate	< 0.01	
			mature	0.01	
		turnip	leaves	< 0.01	
			roots	< 0.01	

Conclusions

Significant levels of difluoroacetic acid (DFA) were detected in most of the primary and confined rotational crops under investigation irrespective of the application technique. High difluoroacetic acid concentrations after foliar spray application indicate that this metabolite is also formed in plants and not only in soil.

Guidelines & Limitations

The study is acceptable to address the residue levels of difluoroacetic acid in primary and rotational crops.

Study 13

Reference	:	Thielert, Schoening, 2012; KIIA 6.10/01	Application rate	:	-
Type of study	:	-	Total rate	:	-
Year of execution	:	2012	Area	:	-
Test substance	:	Flupyradifurone	Plant	:	Tomato, melon, cucumber
GLP statement	:	Yes	Sampling	:	-
Guideline	:	none	Acceptability	:	acceptable

Study design

Translocation experiments were performed by applying flupyradifurone parent labelled at the [pyridinylmethyl-^{14}C] and [ethyl-1-^{14}C] moiety of the molecule to fruiting vegetables grown in greenhouses. Plant parts were dried and analysed by a Fuji-Fujix BAS Cassette 2040 phosphor imager.

Application of [pyridinylmethyl-^{14}C]flupyradifurone to soil of tomato and melon plants

[pyridinylmethyl-^{14}C]flupyradifurone SL 200 was applied by droplet application on midribs of pinnate leaves, leaf lamina of 2 centre pinnate leaves and main stem above leaf 8 on tomato plants (variety Rentita).

Plant Samples were taken 1DAT and 4 DAT. Results are summarised in Table C7.1.1-25. An example autoradiograph is given in figure 7.1.1-14

Table 7.1.1-25: Results of the translocation experiments with [pyridinylmethyl-^{14}C]flupyradifurone in tomato plants

Dose	Location of application Spot	Translocation to	
		1DAT	4DAT
10 µl (0.33mg, 25kBq)	On Center midrib of leaf 7	Pinnate leaf adjacent to drop	Pinnate leaves adjacent and above drop
2x 5µl (0.16mg, x 1 kBq)	On either sides of midrib of pinnate leave of leaf 8	Pinnate leaf adjacent to drop	-
10 µl (0.33g, 25kBq)	On stem above leaf 8	In leaves 9 and 10	In leave 9 and 10 but not in newly formed top leaf 11
Conclusion from autoradiographs:	Flupyradifurone residues are found 'upstream' of the application spots and it is conclude that it is transported by xylem.		

Figure 7.1.1-14: **autoradiograph of potato plant 4 days after application of 0.33 mg (25kBq) [pyridinylmethyl-^{14}C]flupyradifurone above leave 8. After 4 days, radioactive residues were found in the upstream leaves present at the time of application but not in the newly formed top leaves.**

Application of [pyridinylmethyl-^{14}C]flupyradifurone to soil of tomato plants

Plants were drenched with 50 mL containing 24 mg flupyradifurone per plant labelled with 312kBq [pyridinylmethyl-^{14}C]flupyradifurone.

Figure 7.1.1-15: **Autoradiograph of tomato shoots 1, 3, 7, 14 and 24 days after application of 0.24 mg (312kBq) [pyridinylmethyl-^{14}C]-flupyradifurone per plant to the soil.**

Residues of flupyradifurone were rapidly taken up via the root system into tomato plants and translocated within 1-24 days after treatment in the direction of the transpiration stream into the shoot followed by a translaminar distribution into the leaves. Over time, radiolabelled compounds accumulated at the leaf margins while leaf laminae were gradually depleted. It is concluded that flupyradifurone and its metabolites are transported by xylem very well (Figure 7.1.1-15).

Application of [pyridinylmethyl-^{14}C]flupyradifurone to soil of melon plants

Plants were drenched with 50 mL containing 36 mg flupyradifurone per plant labelled with 322kBq [pyridinylmethyl-^{14}C]flupyradifurone.

Figure 7.1.1-16: **Autoradiograph of melon shoots 31 days after application of 0.36 mg (322kBq) [pyridinylmethyl-14C]flupyradifurone to the soil.**

White arrows: location of flowers

Radiolabelled flupyradifurone and its metabolites were taken up via the root system into cantaloupe plants and were translocated via the xylem in the direction of the transpiration stream into the shoot followed by a translaminar distribution into leaves and flowers. Over time (31DAT), radiolabelled compounds accumulated at the leaf margins while leaf laminae were gradually depleted (Figure 7.1.1-16).

Application of [ethyl-1-^{14}C]flupyradifurone to cucumber shoots

Radioactive residues of flupyradifurone (and metabolites) showed a rapid acropetal uptake of radiolabelled compounds into the plant and translocation within 1-14 days after treatment mainly via the xylem in direction of the transpiration stream and a translaminar distribution into adjacent plant cells, irrespective of the application point. Radioactivity accumulated at the leaf margins over time. Autoradiographs indicated also a translocation of radioactive compounds from treatment points on leaf 4 and leaf axil 4 via the phloem into plant parts below and above the application points, i.e. leaves, stems, petioles, tendrils, flowers and fruitlets.

Table 7.1.1-26: Results of the translocation experiments with [ethyl-1-^{14}C]flupyradifurone in cucumber plants.

Dose	Location of application Spot	1DAT	7DAT	14DAT
1 x 25kBq/6mg	Leaf axil of leaf 4	Main transport into leaf lamina 4. Limited transport to leaf 3 and leaf 5, 6.	Limited transport to leaf 3, 5-10. Transport into leaf lamina 4.	Limited transport to leaf 3, 7, especially the leaf margins. Moderate transport to leaf 5 including side shoot, flowers and fruits. Moderate transport to shoots of leaf 3 High transport from leaf lamina 4 to the leaf margins
2 x 25kBq/6mg	On either sides of midrib of pinnate leave of leave 4	Main transport into laminae of leaf 4. Limited transport to leaf 5,6,7,8	Main transport into margins of laminae of leaf 4. Limited transport to axil of leaf 4 and underlying leaf 3 and leaves 5, 6, 7, 8	Transport into leaf veins, margins of lamina and petiole of leaf 4. Limited transport to leaf 3, 5, 6, 7, 8, 9, 10, 11.
Conclusion from autoradiographs:		Flupyradifurone and/or metabolites are directly transported 'upstream' of the application spots and it is conclude that it is transported by xylem. Later on, flupyradifurone and/or metabolites are also found to be transported in leaves below the application spot and it is concluded that it can be transported by phloem as well.		

Determination of BYI02960, DFEAF, DFA and nictotinoic acid in cucumber shoots

Unlabelled flupuradifurone was applied to cucumber plants 2 times in a dose of 7.8 mg per plant per meter canopy height. Plants were divided in levels (from top to root, see figure at right):

- Leaf level 6-10 (untreated leaves 6-10)
- Leaf level 1-5 (treated leaves 1-5)
- Leaf level 0 (untreated leaves).

Application interval was 10 days, with the last application 3, 7, 14 and 28 days before harvest.

Residues of flupyradifurone parent (BYI 02960) and its metabolites difluoroacetic acid (DFA), BYI 02960-difluoroethyl-amino-furanone (DFEAF) and 6-chloronicotinic acid (6-CNA), each analyte expressed as parent, were determined by LC-MS/MS according to method 01304.

Residues have been determined in treated leaves (level 1-5), untreated leaves below (level 0) and untreated leaves and fruit above the application zone (level 6-10). Results are tabulated in Tables 7.1.1-27 to 7.1.1-33. Positive residues are marked in bold.

Table 7.1.1-27: Residues in cucumber fruits after 2 treatments of leaves in level 1-5, 3DAT.

Sample name	*Treated Zone (T) or Untreated Zone (U)*	*Residue in fruits in mg/kg parent equivalents*			
		BYI 02960	*DFEAF*	*DFA*	*6-CNA*
Level 1-5					
Plant 1, level 4	control	<0.01	<0.01	<0.02	<0.005
Plant 8, level 1	T	<0.01	<0.01	<0.02	<0.005
Plant 9, level 4	T	0.04	<0.01	<0.02	<0.005
Plant 14, level 4	T	<0.01	<0.01	<0.02	<0.005
Plant 16, level 5	T	<0.01	<0.01	<0.02	<0.005
Level 6-10					
Plant 8, level 6	U	<0.01	<0.01	<0.02	<0.005
Plant 9, level 6	U	<0.01	<0.01	<0.02	<0.005

Table 7.1.1-27: Residues in cucumber fruits after 2 treatments of leaves in level 1-5, 3DAT.

Sample name	*Treated Zone (T) or Untreated Zone (U)*	*Residue in fruits in mg/kg parent equivalents*			
		BYI 02960	*DFEAF*	*DFA*	*6-CNA*
Plant 14, level 6	U	<0.01	<0.01	<0.02	<0.005
Plant 16, level 6	U	<0.01	<0.01	<0.02	<0.005
Plant 14, level 7	U	<0.01	<0.01	<0.02	<0.005
Plant 16, level 7	U	<0.01	<0.01	<0.02	<0.005
Plant 18, level 8	U	<0.01	<0.01	<0.02	<0.005
Plant 9, level 10	U	<0.01	<0.01	<0.02	<0.005

Table 7.1.1-27: Residues in cucumber leaves after 2 treatments of leaves level 1-5, 3DAT

Sample name	*Treated Zone (T) or Untreated Zone (U)*	*Residue in leaves in mg/kg parent equivalents*			
		BYI 02960	*DFEAF*	*DFA*	*6-CNA*
Level 1-5					
Plant 1, level 4	control	<0.01	<0.01	**0.04**	<0.005
Plant 8, level 1	T	**39**	**0.25**	**9.2**	**2.3**
Plant 9, level 4	T	**37**	**0.29**	**9.7**	**2.0**
Plant 14, level 4	T	**42**	**0.20**	**9.3**	**2.3**
Plant 16, level 5	T	**8,4**	**0.08**	**2.1**	**0.33**

Table 7.1.1-28: Residues in cucumber fruits after 2 treatments of leaves level 1-5, 7DAT

Sample name	*Treated Zone (T) or Untreated Zone (U)*	*Residue in fruits in mg/kg parent equivalents*			
		BYI 02960	*DFEAF*	*DFA*	*6-CNA*
Level 1-5					
Plant 1, level 4	control	<0.01	<0.01	<0.02	<0.005
Plant 20, level 1	T	<0.01	<0.01	**0.21**	<0.005
Plant 6, level 3	T	**0.04**	<0.01	**0.09**	<0.005

Table 7.1.1-28:	**Residues in cucumber fruits after 2 treatments of leaves level 1-5, 7DAT**				
Sample name	*Treated Zone (T) or Untreated Zone (U)*	*Residue in fruits in mg/kg parent equivalents*			
		BYI 02960	*DFEAF*	*DFA*	*6-CNA*
Plant 3, level 4	T	**0.01**	<0.01	**0.13**	<0.005
Plant 20, level 4	T	**0.01**	<0.01	**0.28**	<0.005
Plant 22, level 5	T	<0.01	<0.01	**0.23**	<0.005
Level 0					
Plant 3, level 0	U	<0.01	<0.01	**0.07**	<0.005
Plant 6, level 0	U	<0.01	<0.01	**0.07**	<0.005
Plant 22, level 0	U	<0.01	<0.01	**0.16**	<0.005
Level 6-10					
Plant 20, level 6	U	<0.01	<0.01	**0.04**	<0.005
Plant 22, level 6	U	<0.01	<0.01	**0.04**	<0.005
Plant 6, level 7	U	<0.01	<0.01	<0.02	<0.005
Plant 3, level 8	U	<0.01	<0.01	**0.05**	<0.005
Plant 6, level 9	U	<0.01	<0.01	**0.05**	<0.005
Plant 20, level 9	U	<0.01	<0.01	**0.07**	<0.005
Plant 22, level 9	U	<0.01	<0.01	**0.04**	<0.005
Plant 3, level 10	U	<0.01	<0.01	**0.04**	<0.005

Table 7.1.1-28:	**Residues in cucumber leaves after 2 treatments of leaves level 1-5, 7DAT**				
Sample name	*Treated Zone (T) or Untreated Zone (U)*	*Residue in leaves in mg/kg parent equivalents*			
		BYI 02960	*DFEAF*	*DFA*	*6-CNA*
Level 1-5					
Plant 1, level 4	control	<0.01	<0.01	**0.05**	<0.005
Plant 8, level 1	T	**39**	**0.23**	**11**	**2.3**
Plant 9, level 4	T	**29**	**0.27**	**7.5**	**1.7**

Table 7.1.1-28: Residues in cucumber leaves after 2 treatments of leaves level 1-5, 7DAT					
Sample name	*Treated Zone (T) or Untreated Zone (U)*	*Residue in leaves in mg/kg parent equivalents*			
		BYI 02960	*DFEAF*	*DFA*	*6-CNA*
Plant 14, level 4	T	**22**	**0.25**	**9.0**	**1.5**
Plant 16, level 5	T	**30**	**0.31**	**11**	**2.4**

Table 7.1.1-29: Residues in cucumber fruits after 2 treatments of leaves level 1-5, 14DAT					
Sample name	*Treated Zone (T) or Untreated Zone (U)*	*Residue in fruits in mg/kg parent equivalents*			
		BYI 02960	*DFEAF*	*DFA*	*6-CNA*
Level 1-5					
Plant 1, level 3	control	<0.01	<0.01	<0.02	<0.005
Plant 17, level 3	T	<0.01	<0.01	**0.57**	**0.02**
Plant 19, level 3	T	**0.01**	<0.01	**0.48**	**0.02**
Plant 4, level 5	T	<0.01	<0.01	**0.56**	<0.005
Plant 21, level 5	T	<0.01	<0.01	**0.58**	**0.02**
Level 0					
Plant 4, level 0	U	<0.01	<0.01	**0.46**	<0.005
Plant 17, level 0	U	<0.01	<0.01	**0.31**	<0.005
Plant 19, level 0	U	<0.01	<0.01	**0.36**	<0.005
Plant 21, level 0	U	<0.01	<0.01	**0.30**	<0.005
Level 6-10					
Plant 17, level 6	U	<0.01	<0.01	**0.34**	**0.02**
Plant 21, level 6	U	0.01	<0.01	**0.07**	<0.005
Plant 4, level 7	U	<0.01	<0.01	**0.10**	<0.005
Plant 19, level 8	U	<0.01	<0.01	**0.08**	<0.005

Table 7.1.1-30: Residues in cucumber leaves after 2 treatments of leaves level 1-5, 14DAT					
Sample name	*Treated Zone (T) or Untreated Zone (U)*	*Residue in leaves in mg/kg parent equivalents*			
		BYI 02960	*DFEAF*	*DFA*	*6-CNA*
Level 1-5					
Plant 1, level 3	control	<0.01	<0.01	**0.07**	<0.005
Mix of: Plant 4, level 5 Plant 17, level 3 Plant 19, level 3 Plant 21, level 5	T	**22**	**0.30**	**11**	**1.9**

Table 7.1.1-31: Residues in cucumber fruits after 2 treatments of leaves level 1-5, 21DAT					
Sample name	*Treated Zone (T) or Untreated Zone (U)*	*Residue in fruits in mg/kg parent equivalents*			
		BYI 02960	*DFEAF*	*DFA*	*6-CNA*
Level 1-5					
Plant 1, level 1	control	<0.01	<0.01	<0.02	<0.005
Plant 12, level 3	T	0.02	<0.01	**0.33**	<0.005
Plant 18, level 3	T	0.03	<0.01	**0.22**	<0.005
Plant 15, level 4	T	<0.01	<0.01	**0.72**	<0.005
Plant 10, level 5	T	<0.01	<0.01	**0.56**	<0.005
Level 0					
Plant 10, level 0	U	<0.01	<0.01	**0.44**	<0.005
Plant 15, level 0	U	0.04	<0.01	**0.54**	<0.005
Plant 18, level 0	U	<0.01	<0.01	**0.31**	<0.005
Level 6-10					
Plant 15, level 6	U	<0.01	<0.01	**0.32**	<0.005
Plant 12, level 6	U	**<**0.01	<0.01	**0.13**	<0.005
Plant 10, level 7	U	<0.01	<0.01	**0.09**	<0.005
Plant 18, level 11	U	<0.01	<0.01	**0.11**	<0.005

Table 7.1.1-31:	**Residues in cucumber leaves after 2 treatments of leaves level 1-5, 14DAT**				
Sample name	*Treated Zone (T) or Untreated Zone (U)*	*Residue in leaves in mg/kg parent equivalents*			
		BYI 02960	*DFEAF*	*DFA*	*6-CNA*
Level 1-5					
Plant 1, level 6	control	<0.01	<0.01	<0.02	<0.005
Mix of: Plant 10, level 5 Plant 15, level 4 Plant 18, level 3 Plant 12, level 3	T	**8.8**	**0.12**	**4.4**	**0.71**

Table 7.1.1-32:	**Residues in cucumber fruits after 2 treatments of leaves level 1-5, 28DAT**				
Sample name	*Treated Zone (T) or Untreated Zone (U)*	*Residue in fruits in mg/kg parent equivalents*			
		BYI 02960	*DFEAF*	*DFA*	*6-CNA*
Level 6-10					
Plant 1, level 6	control	<0.01	<0.01	**<0.02**	<0.005
Plant 7, level 4	U	**<**0.01	<0.01	**0.58**	<0.005
Plant 7, level 5	U	0.01	<0.01	**0.80**	<0.005
Plant 7, level 10	U	<0.01	<0.01	**0.21**	<0.005

Table 7.1.1-33:	**Residues in cucumber leaves after 2 treatments of leaves level 1-5, 28DAT**				
Sample name	*Treated Zone (T) or Untreated Zone (U)*	*Residue in leaves in mg/kg parent equivalents*			
		BYI 02960	*DFEAF*	*DFA*	*6-CNA*
Level 6-10					
Plant 1, level 6	control	<0.01	<0.01	<0.02	<0.005
Level 1-5					
Plant 7, level 5	T	**8.8**	**0.16**	**6.7**	**1.36**

1. Flupyradifurone could almost not be found translocated out of treated leaves containing between 8.8-42 mg flupyradifurone/kg into untreated cucumbers within 3-28 days after application. Only in 3 out of 54 analyzed cucumbers from different plants minute amounts of flupyradifurone ranging between 0.01-0.04 mg/kg cucumber fresh weight were detected 3-28 days after application. Peak concentrations were measured in treated leaves 3 days after application. The low percentage of cucumbers (5.6% out of 54 cucumbers) containing amounts of parent compound close to the LOQ presumably due to a contamination during the application.
2. DFEAF could not be detected in any of the cucumbers analyzed although it was detected in treated leaves at concentrations ranging between 0.08-0.31 mg DFEAF/kg leaf fresh weight. Peak concentrations in treated leaves were detected 7 days after application.
3. DFA was formed in treated leaves at concentrations between 2.1-11 mg per kg leaf fresh weight and translocated via the phloem into cucumbers growing in adjacent leaf axils of treated leaves at a range of 0.06-0.80 mg DFA/kg cucumber fresh weight. Even in cucumbers growing in leaf axils of untreated leaf zones between 0.03-0.54 mg DFA/kg cucumber fresh weight were found. Peak concentrations were reached 21 and 28 days after application, respectively.
4. 6-CNA was found only in 4 out of 54 cucumbers in low amounts (0.02 mg/kg fresh weight) 14 days after application close to the LOQ (0.01 mg/kg fresh weight). In treated leaves, however, 6-CNA reached concentrations between 0.33-2.4 mg/kg leaf fresh weight, peaking 7 days after application.

Conclusions on systemicity

The authors refer to Bromilow et al. (1991) and Rocher et al. (2006) who described a model for xylem and phloem transport based on the physico-chemical properties (water solubility and ionization state of acids, pKa and fat solubility, log Kow) of substances. The systemic behaviour of [pyridinylmethyl-^{14}C]flupyradifurone, [ethyl-1-^{14}C]flupyradifurone and non-labelled flupyradifurone, DFA, DFEAF and 6-CNA after foliar and soil drench application in tomato, melon and cucumber plants correspond well with this model.

	Flupyradifurone ★	6-CNA ★	DFA ★	DFEAF ★
pKa	neutral at 2-12	3.24	1.1	neutral at 2-12
log Kow	1.2	1.15	-3.1/-3.5	-0.2

Flupyradifurone (neutral, log Kow = 1.2) was mainly translocated via the xylem apart from a few cucumbers where minute concentrations slightly above the LOQ were detected.

DFA (pKa = 1.1, Log Kow ~ -3.5) and 6-CNA (pKa = 3.24 and Log Kow = 1.15) can be retained in the phloem and translocated into basipetal and acropteal directions within a plant from a treated source to a growing sink.
Data suggest a distinct phloem transport of DFA which can lead to increasing concentrations into growing cucumbers and leaves. Theoretically, a higher proportion of 6-CNA was expected to be translocated via the phloem due to its more favourable physico-chemical properties regarding the model.

DFEAF (neutral, log Kow = -0.2) was translocated via the xylem only.

Guidelines & Limitations

The specially designed residue study to confirm the model predictions of Bromilow et al. on the basis of the physicochemical properties (log Kow and pKa confirmed the translocation behaviour and the systemicity) of the parent compound and its major metabolites.

B.7.1.2 Succeeding and rotational crops (IIA 6.6.2)

STUDY 1
Characteristics

Reference	:	Klempner, A.; 2011 KIIA 6.6.2/01	Application rate	:	436 g a.s./ha (1.75N)
Type of study	:	Metabolism in Rotational Crops	Area	:	planting container with a surface area of 1 m^2
Year of execution	:	2008-2011	Plant	:	wheat (cereal), Swiss chard (leafy vegetable) and turnips (root vegetable)
Test substance	:	[furanone-4-^{14}C] Flupyradifurone ; sample ID: KATH 6104; ; purity >99% w/w; batch code BYI 02960-PU-01; purity 99.9% w/w	Plant back intervals	:	29, 135 and 296 days
GLP statement	:	Yes	Acceptability	:	Acceptable

Guidelines:

- OECD 502 Metabolism in Rotational Crops
- US EPA Residue Chemistry Test Guideline OPPTS 860.1850: Confined Accumulation in Rotational Crops
- European Parliament and Council Regulation (EC) No 1107/2009

Description

Test material

Chemical structure	* position of the radiolabel
Radiolabelled test material	[furanone-4-^{14}C]BYI 02960
Specific radioactivity	3.97 MBq/mg (106.46 µCi/mg)
Chemical Purity	> 99% (HPLC)
Radiochemical purity	> 99% (HPLC and TLC)

Experimental conditions

The metabolism of flupyradifurone was investigated in the representative rotational crops wheat (cereal), Swiss chard (leafy vegetable) and turnips (root vegetable) from three consecutive rotations. [Furanone-4-^{14}C]BYI 02960 was formulated as an SL 300 and sprayed onto the bare soil of a planting container (approx. 1 m^2). The actual application rate corresponded to 436 g a.s./ha, above the anticipated maximum seasonal field rate of 250 g a.s./ha for EU uses (1.75N). The crops were each sown at 29, 135 and 296 days after the soil application, representing the first, second and third rotation.

Sampling

Intermediate raw agricultural commodities (RACs) investigated were Swiss chard immature (BBCH 44-46), wheat forage (at BBCH growth stage 29-31) and wheat hay (sampled at BBCH 79-83 and dried for five days). All other RACs (wheat straw, wheat grain, Swiss chard, turnip leaves and turnip roots) were harvested at maturity.

Extraction and quantification

An aliquot of each homogenized RAC was extracted conventionally three to four times with ACN/water (8:2, v/v). The extracts were combined, purified using a pre-conditioned SPE RP 18 cartridge, concentrated and analysed by HPLC. The radioactivity in the extracts was determined by LSC, in the solids by combustion followed by LSC. The actual TRR values of the samples were determined by summing up the radioactivity measured in the extracts and in the remaining solids.

Post-extraction solids of wheat straw from the 1st and 2nd rotation were subjected to a sequential extraction procedure after one exhaustive extraction step with ACN/water (8:2, v/v) at increased temperature (60 °C) under microwave conditions. The solids of the microwave extraction step were subjected to a treatment with sodium chloride solution (2 h at 100 °C under microwave assistance), a diastase incubation (approx. 20 h at 26 °C), a treatment with EDTA solution (3 h at 100 °C under microwave assistance) and a cellulase treatment (approx. 20 h at 40 °C) at adjusted temperatures to break down the plant cell walls and liberate residues bound to cell walls or in the cells. The remaining solids of the sequential extraction were extracted finally in two steps with a 5N HCl and a 5N NaOH solution (each 2 h at 100 °C). Extracts of the first three exhaustive extraction steps were concentrated and analysed separately by HPLC. Extracts of the EDTA, the cellulase and the HCl treatment were combined and adjusted to pH 7. The combined extract was concentrated, centrifuged and analysed by HPLC. The NaOH extract was not submitted to HPLC due to its high viscosity.

Post-extraction solids of grains from the 1st and the 2nd rotation were subjected to three subsequent exhaustive extraction steps: Microwave extraction with ACN/water at 100 °C followed by a digestion step with diastase (20 h at 26 °C) and subsequent microwave extraction with a sodium chloride solution (2 h at 100 °C). Only the first exhaustive extract was analysed by HPLC, the two following extracts were highly viscous due to a high matrix load and could not injected in the HPLC system. Therefore a further aliquot of the post extraction solids of grains from the 1st rotation was subjected to digestion with diastase over 14 days at ambient temperature. The residues in the diastase digestion solution were further characterized by partitioning against dichloromethane.

Identification and characterisation

Identification was achieved by spectroscopic evidence (LC-MS/MS) after isolation and purification of the respective compounds from the conventional extracts of wheat straw or Swiss chard of the 1st rotation. HPLC co-chromatography with the isolated and identified radiolabelled metabolites allowed

the assignment of the compounds in other extracts. The polar fraction detected in wheat grains was identified by TLC co-chromatography using the natural compound (glucose/carbohydrates) identified in the tomato metabolism study conducted with [furanone-4-^{14}C]BYI 02960, as reference compound. Other polar metabolites/fractions were also analysed by normal phase TLC.

Storage stability

All samples were conventionally extracted and analysed by HPLC within a few days after sampling. Quantitative ^{14}C-HPLC analysis of the extracts was performed with the profiling method BYI02960_NEUTR within one week after harvest. Only for wheat forage and hay, Swiss chard and turnips from the 1st rotation, the metabolic profile was measured with the preliminary profiling method BYI02960_CRC directly after extraction. For better comparison of metabolic pattern over all RACs of all rotations, the profiles of wheat forage and hay, Swiss chard and turnips of the 1st rotation were re-analysed with method BYI02960_NEUTR about 1 – 2 months after extract preparation. These new metabolic profiles were then considered as basis for quantification. Extract stability has been demonstrated for this time period.

Exhaustive extractions were started for wheat straw and wheat grain within approx. two to seven months after harvest. Since the compounds identified in the exhaustive extracts were comparable with the compounds of the conventional extracts, it was concluded that the residues in the solids were stable and reflected the released residues at the time of exhaustive extraction.

Thus it can be concluded that the residues in all matrices were sufficiently stable during the experimental period of the study and that the first quantified profiles represented the metabolic pattern in the samples at harvest. Nevertheless, storage stability of BYI 02960 residues in frozen sample material was additionally demonstrated in wheat straw (1st and 2nd rotation) and in Swiss chard (1st rotation) for a storage period of about 18 to 24 months by qualitatively and quantitatively comparing raw chromatography data . The stored sample material was subjected to a second extraction. Analysis of the new extracts showed the same metabolite patterns as the first extracts and confirmed the storage stability of the residues in the stored RACs.

Extract stability has been demonstrated for the time of the sample preparation and the identification process of metabolites, as well: The metabolite patterns of stored extracts remained stable when re-analysing extracts, e.g. for isolation of metabolites or identification purposes. Neither significant degradation nor transformation of metabolites in extracts was observed.

Hence, it was concluded that the results of the present study were not impacted by the storage of the samples and that no further storage stability investigations are required.

Results

The TRR values of all RACs declined significantly (by a factor of 5 to 13) from the first to the third rotation. Highest residues were detected in the non-edible commodities wheat straw and wheat hay, as shown in Table 7.1.2-1.

Table 7.1.2-1 TRR values in the different RACs of the three rotations after soil application of [furanone-4-^{14}C]BYI 02960 (Plant Back intervals of 29, 135 and 296 days)

TRR [mg/kg]	Wheat				Swiss chard		Turnips	
	forage	hay	straw	grain	imm.	mature	leaves	roots
1st rotation	0.78	2.0	6.3	0.48	0.85	0.87	0.68	0.074
2nd rotation	0.19	1.1	1.5	0.10	0.31	0.26	0.16	0.014
3rd rotation	0.11	0.25	0.46	0.047	0.18	0.15	0.090	0.008

Except for wheat grain, extraction efficiencies ranged from 70% to 97% for all commodities of all rotations after conventional extraction. Subsequent exhaustive extraction steps (microwave and/or digestive extraction steps), applied to the post extraction solids in wheat grain and wheat straw, increased the extraction efficiencies to a range of 83% to 98% of the TRR.

Parent compound BYI 02960 and about 30 metabolites were detected in the conventional and exhaustive extracts of the various samples of the three rotations. Of these the active substance and 14 metabolites were identified by LC-MS/MS. The other metabolites, none of them exceeding 5% of the TRR or 0.05 mg/kg, were characterised by their extraction behaviour and their retention in radio-HPLC or TLC. The amounts of active substance and metabolites in all RACs are summarized in Table 7.1.2-2 to Table 7.1.2-9 for the three rotations.

Parent compound was by far the main component detected in all matrices of all rotations, except for wheat grain. Parent accounted for 34% to 64% of the TRR in the commodities of the 1st rotation, for 28% to 68% in the 2nd rotation and covered 18% to 72% in the 3rd rotation, not considering grains. In wheat grains, only trace amounts of parent compound were detected (<1% and 2% of the TRR). The highest proportion of parent compound was always detected in turnip leaves.

Six of the identified metabolites were specific to the radiolabel used: BYI 02960-difluoroethyl-amino-furanone, its conjugate BYI 02960-mercapto-lactic acid, BYI 02960-difluoroethyl-amino-furanone-OH-glyc, BYI 02960-amino-furanone, BYI 02960-bromo-amino-furanone, and the natural compound glucose (or probably an isomeric carbohydrate). The natural compound was identified as the main component in wheat grain after subjecting the post extraction solids of the conventional extraction (1st rotation) to a diastase digestion step. The released radioactivity in the aqueous digestion solution was partitioned against dichloromethane. The majority of the radioactivity remained in the aqueous phase indicating that the metabolite was probably a polar natural compound. Isolation of the polar fraction by semi-preparative HPLC and following analysis by normal phase TLC showed one spot indicating that the fraction is represented most probably by one compound. TLC co-chromatography with the natural

compound isolated and identified in the tomato metabolism study with [furanone-4-^{14}C]BYI 02960 confirmed the correspondance of the metabolites. TLC analysis of the isolated polar fractions detected in the conventional and exhaustive extracts of wheat straw and in the conventional extracts of Swiss chard and turnip roots revealed that these fractions were represented by several compound (up to eight). Only minor percentages of the fractions appear identical with glucose. Based upon TLC, for turnip roots, a trace amount was assigned to the natural compound.

Label unspecific metabolites (= metabolites common to both radiolabels tested) were BYI 02960-OH and its conjugates, BYI 02960-acetic acid, its conjugate BYI 02960-acetic acid-glyc and its successor molecule BYI 02960-glyoxylic acid and the chlorinated/brominated parent compound. These metabolites were also detected in the CRC study conducted with [pyridinylmethyl-^{14}C]BYI 02960. The metabolites BYI 02960-acetic acid and BYI 02960-OH-glyc were not separated by the HPLC profiling method and co-eluted in one peak or one peak zone. Separation and sub-quantification of the metabolites was shown for wheat straw (1st rotation), Swiss chard (1st rotation) and turnip leaves (1st rotation) as representative crops. Therefore the corresponding peak zones were isolated by semi-preparative HPLC, concentrated and re-analysed with an acidic HPLC method which separated the two metabolites. The ratios of BYI 02960-acetic acid and BYI 02960-OH-glyc obtained for wheat straw were assigned to all wheat samples, the ratio of Swiss chard to immature and mature Swiss chard, and the ratio of turnip leaves to turnip roots of all rotations. BYI 02960-OH-glyc was the prominent compound in all samples of all rotations.

Overall, identification rates were high for all samples of all rotations. For some RACs (e.g., wheat straw and wheat grain), high identification rates were only shown for the 1st and 2nd rotation (or in the case of grains for the 1st rotation only) since exhaustive extraction and following analysis of the exhaustive extracts was only conducted exemplarily for these samples. Thus, lower identification rates in this study were generally linked to higher bound residues (wheat matrices) and no subsequent exhaustive extraction step. In the case of turnip roots of the 2nd rotation, the identification rate was also quite low (53%) since one unknown compound represented a high percentage of the TRR, but at a very low residue level (0.004 mg/kg).

Table 7.1.2-2 Distribution of parent compound and metabolites in wheat matrices after a 29 day plant back interval (1st rotation, [furanone-4-^{14}C]BYI 02960)

1st Rotation	wheat forage		wheat hay		wheat straw		wheat grains	
(BYI- = BYI 02960)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Conventional extraction								
parent compound	47	0.37	34	0.67	34	2.1	0.4	0.002
glucose/carbohydrates (M45)	---	---	---	---	---	---	---	---
amino-furanone (M42)	1.9	0.015	3.8	0.076	1.9	0.12	0.5	0.002
DFEAF-mercapto-lactic acid (M41)	1.6	0.013	0.4	0.008	1.1	0.068	---	---
bromo-amino-furanone (M43)	2.0	0.016	1.6	0.033	2.6	0.16	---	---
DFEAF-OH-glyc (M35)	3.2	0.025	5.8	0.12	3.5	0.22	---	---
DFEAF (M34)	9.9	0.077	10.2	0.21	5.3	0.33	---	---
BYI-glyoxylic acid (M17)	16	0.12	11.3	0.23	15	0.93	5.1	0.024
BYI-acetic acid-glyc (M16)	---	---	1.2	0.024	0.7	0.043	---	---
BYI-acetic acid (M15)	1.7	0.013	1.6	0.031	1.7	0.11	0.6	0.003
BYI-OH-glyc (M8)	3.6	0.028	3.4	0.067	3.6	0.23	1.4	0.007
BYI-OH (M3)	1.3	0.010	1.9	0.038	2.3	0.15	2.3	0.011
BYI-bromo / chloro (M2/M1)	0.3	0.003	---	---	0.2	0.011	---	---
Subtotal identified	**88**	**0.69**	**75**	**1.5**	**71**	**4.5**	**10**	**0.049**
unknown 1a[1]	1.5	0.012	3.7	0.075	3.6	0.23	2.3	0.011
unknown 2, 3, 4, 5, 6, 16	---	---	---	---	0.1-0.3	0.008-0.022	---	---
unknown 7	---	---	0.3	0.007	0.5	0.032	---	---
unknown 8, 10, 12, 13, 14, 15	---	---	---	---	---	---	---	---
unknown 9	---	---	---	---	---	---	0.7-1.2	0.003-0.006
unknown 17	---	---	2.0	0.041	---	---	---	---
Subtotal characterised	**1.5**	**0.012**	**6.1**	**0.12**	**5.5**	**0.35**	**4.2**	**0.020**
Total conventional extr.	**90**	**0.70**	**81**	**1.6**	**77**	**4.8**	**15**	**0.069**
Additional extractions								
glucose/carbohydrates(M45)					**---**	**---**	71	0.34
other compounds[2]					8.9	0.57	**---**	**---**
Subtotal identified					**8.9**	**0.57**	**71**	**0.34**
Subtotal characterised					**7.2**	**0.46**	**---**	**---**
Total identified	**88**	**0.69**	**75**	**1.5**	**80**	**5.1**	**81**	**0.39**
Total characterised	**1.5**	**0.012**	**6.1**	**0.12**	**13**	**0.80**	**4.2**	**0.020**
Total extractable	**90**	**0.70**	**82**	**1.6**	**98**	**6.2**	**88**	**0.42**
Not analysed/losses	0.3	0.002	0.7	0.015	0.7	0.042	3.0	0.014
Unextractable (PES*)	10	0.081	18	0.37	1.6	0.099	12	0.058
Accountability	**100**	**0.78**	**100**	**2.0**	**100**	**6.3**	**100**	**0.48**

* post extraction solids

BYI- = BYI 02960 = flupyradifurone-

[1] polar unkown peak 1a in the conventional extract of wheat straw of the 1st rotation consisted of 4 different metabolites, all of them were minor according to TLC subquantification

[2] See Tabel 7.1.2-3 for specifications

Table 7.1.2-3 Residues in additional extracts of wheat straw after a 29 day plant back interval (1st rotation, [furanone-4-^{14}C]BYI 02960) measured after additional extractions

Additional extractions wheat straw, 1nd rotation								
extraction method	***ACN/water*, 60°C**		***1% NaCl, 100*°C**		**diastase**		**EDTA+cellul+5NHCl**	
(BYI- = BYI 02960)	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**
parent compound	1.6	0.10	2.0	0.12	0.7	0.042	0.9	0.057
amino-furanone (M42)	0.2	0.013	0.7	0.044	0.3	0.016	0.5	0.032
bromo-amino-furanone (M43)	0.2	0.011	---	---	---	---	---	---
DFEAF-OH-glyc (M35)	0.2	0.012	---	---	---	---	---	---
DFEAF (M34)	0.2	0.013	0.3	0.019	0.1	0.009	---	---
BYI-glyoxylic acid (M17)	0.5	0.029	0.1	0.008	0.1	0.003	---	---
BYI-acetic acid (M15)	0.1	0.003	<0.1	0.003	---	---	---	---
BYI-OH-glyc (M8)	0.1	0.007	0.1	0.006	---	---	---	---
BYI-OH (M3)	0.1	0.007	0.1	0.006	<0.1	0.001	---	---
Subtotal identified	**3.1**	**0.20**	**3.3**	**0.21**	**1.1**	**0.072**	**1.4**	**0.089**
unknown 1a[1]	1.4	0.085	1.4	0.086	0.8	0.050	3.6	0.23
Subtotal characterised	**1.4**	**0.085**	**1.4**	**0.086**	**0.8**	**0.050**	**3.6**	**0.23**
Total	**4.5**	**0.28**	**4.7**	**0.30**	**1.9**	**0.12**	**5.1**	**0.32**

BYI- = BYI 02960 = flupyradifurone-

Table 7.1.2-4 Distribution of parent compound and metabolites in wheat matrices after a 135 day plant back interval (2nd rotation, [furanone-4-^{14}C]BYI 02960)

2nd Rotation	wheat forage		wheat hay		wheat straw		wheat grains	
(BYI- = BYI 02960)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Conventional extraction								
parent compound	64	0.12	29	0.31	29	0.43	0.5	0.001
glucose/carbohydrates (M45)	---	---	---	---	---	---	---	---
amino-furanone (M42)	2.0	0.004	6.2	0.067	4.1	0.062	1.3	0.001
DFEAF-mercapto-lactic acid (M41)	1.6	0.003	3.5	0.037	4.4	0.067	---	---
bromo-amino-furanone (M43)	3.3	0.006	9.9	0.107	6.1	0.093	---	---
DFEAF-OH-glyc (M35)	1.3	0.003	7.3	0.078	4.2	0.064	---	---
DFEAF (M34)	8.2	0.016	6.9	0.075	5.3	0.081	---	---
BYI-glyoxylic acid (M17)	0.3	<0.001	1.9	0.020	1.2	0.018	0.8	0.001
BYI-acetic acid-glyc (M16)	---	---	0.5	0.005	0.9	0.013	---	---
BYI-acetic acid (M15)	1.6	0.003	1.6	0.017	2.3	0.035	1.0	0.001
BYI-OH-glyc (M8)	3.4	0.007	3.4	0.037	5.0	0.076	2.1	0.002
BYI-OH (M3)	1.8	0.003	1.9	0.021	2.7	0.042	3.4	0.003
BYI-bromo / chloro (M2/M1)	---	---	---	---	---	---	---	---
Subtotal identified	**87**	**0.17**	**72**	**0.78**	**65**	**0.98**	**9.2**	**0.009**
unknown 1a[1]	1.3	0.002	4.3	0.047	5.1	0.078	2.5	0.003
unknown 4	0.4	0.001	0.6	0.006	0.6	0.009	---	---
unknown 7	0.2	<0.001	0.5	0.006	---	---	---	---
unknown 9, 11	---	---	---	---	---	---	0.7-0.9	0.001
unknown 12	0.3	0.001	---	---	---	---	---	---
unknown 2, 3, 5, 6, 8, 10, 13-17	---	---	---	---	---	---	---	---
Subtotal characterised	**2.2**	**0.004**	**5.4**	**0.059**	**5.8**	**0.087**	**4.2**	**0.004**
Total conventional extr.	**90**	**0.17**	**78**	**0.84**	**70**	**1.1**	**13**	**0.014**
Additional extractions								
Subtotal identified	**---**	**---**	**---**	**---**	**[2]12**	**0.18**	**---**	**---**
Subtotal characterised	**---**	**---**	**---**	**---**	**[2]6.7**	**0.10**	**---**	**---**
Subtotal extracted							**[3]67**	**0.069**
5N NaOH extraction								
Subtotal extracted	**---**	**---**	**---**	**---**	**8.7**	**0.13**	**---**	**---**
Total identified	**87**	**0.17**	**72**	**0.78**	**76**	**1.2**	**9.2**	**0.009**
Total characterised	**2.2**	**0.004**	**5.4**	**0.059**	**13**	**0.19**	**4.2**	**0.004**
Total extractable	**90**	**0.17**	**78**	**0.84**	**98**	**1.5**	**83**	**0.085**
Not analysed/losses	---	---	0.5	0.006	0.3	0.005	69	0.071
Unextractable (PES*)	10	0.020	22	0.24	2.2	0.033	17	0.018
Accountability	**100**	**0.19**	**100**	**1.1**	**100**	**1.4**	**100**	**0.10**

* post extraction solids
[1] polar unkown peak 1a in the conventional extract of wheat straw of the 1st rotation consisted of 4 different metabolites, all of them were minor according to TLC subquantification
[2] See Tabel 7.1.2-5 for specifications
[3] no analysis performed, but presumably glucose/carbohydrates as identified in grains of 1st rotation; 2,5% was extracted with ACN//water at 60°C; 42% with 1% NaCl at 100°C, and 24% after digestion with diastase.

Table 7.1.2-5 Residues in additional extracts of wheat straw after a 135 day plant back interval (2st rotation, [furanone-4-14C]BYI 02960) measured after additional extractions

wheat straw, 2nd rotation								
extractions	***ACN/water*, 60°C**		***1% NaCl*, 100°C**		**diastase dig.**		**EDTA+cellul+5NHCl**	
(BYI- = BYI 02960)	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**
parent compound	2.0	0.031	2.3	0.035	1.0	0.015	1.6	0.024
amino-furanone (M42)	0.7	0.011	0.8	0.012	0.4	0.006	1.3	0.019
DFEAF-OH-glyc (M35)	0.1	0.002	---	---	---	---	---	---
DFEAF (M34)	0.3	0.005	0.3	0.004	---	---	---	---
BYI-glyoxylic acid (M17)	0.1	0.001	---	---	---	---	---	---
BYI-acetic acid (M15)	0.1	0.001	<0.1	0.001	---	---	---	---
BYI-OH-glyc (M8)	0.2	0.003	0.1	0.001	---	---	---	---
BYI-OH (M3)	0.2	0.004	0.1	0.002	---	---	---	---
Subtotal identified	**3.8**	**0.057**	**3.6**	**0.055**	**1.4**	**0.021**	**2.9**	**0.043**
unknown 1a[1]	1.0	0.015	1.5	0.023	0.9	0.014	3.3	0.050
Subtotal characterised	**1.0**	**0.015**	**1.5**	**0.023**	**0.9**	**0.014**	**3.3**	**0.050**
Total	**4.8**	**0.073**	**5.2**	**0.078**	**2.3**	**0.035**	**6.2**	**0.094**

BYI- = BYI 02960 (= flupyradifurone-)

Table 7.1.2-6 Distribution of parent compound and metabolites in wheat matrices after a 296 day plant back interval (3rd rotation, [furanone-4-14C]BYI 02960)

3rd Rotation	**wheat forage**		**wheat hay**		**wheat straw**		**wheat grains**	
(BYI- = BYI 02960)	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**
Conventional extraction								
parent compound	43.0	0.048	18.3	0.047	20.7	0.096	2.0	0.001
glucose/carbohydrates (M45)	---	---	---	---	---	---	---	---
amino-furanone (M42)	3.6	0.004	7.4	0.019	4.9	0.023	---	---
DFEAF-mercapto-lactic acid (M41)	2.6	0.003	5.1	0.013	1.5	0.007	---	---
bromo-amino-furanone (M43)	5.7	0.006	10.7	0.027	7.4	0.034	---	---
DFEAF-OH-glyc (M35)	5.5	0.006	8.6	0.022	8.7	0.040	---	---
DFEAF (M34)	12.1	0.013	8.3	0.021	5.2	0.024	---	---
BYI-glyoxylic acid (M17)	---	---	0.5	0.001	---	---	---	---
BYI-acetic acid (M15)	1.2	0.001	1.1	0.003	1.6	0.007	0.8	<0.001
BYI-OH-glyc (M8)	2.5	0.003	2.3	0.006	3.4	0.016	1.7	0.001
BYI-OH (M3)	0.8	0.001	1.6	0.004	2.1	0.010	2.8	0.001
BYI-bromo / chloro (M2/M1)	---	---	---	---	---	---	---	---
Total identified	**77**	**0.085**	**64**	**0.16**	**56**	**0.26**	**7.4**	**0.003**
unknown 1a[1]	2.4	0.003	4.7	0.012	12	0.055	3.2	0.001
unknown 4	0.7	0.001	0.8	0.002	0.9	0.004	---	---
unknown 9	---	---	---	---	---	---	1.1	0.001
Total characterised	**3.1**	**0.003**	**5.5**	**0.014**	**13**	**0.060**	**4.3**	**0.002**
Total extractable	**80.2**	**0.089**	**70**	**0.18**	**70**	**0.33**	**20**	**0.010**
Not analysed/losses	---	---	---	---	1.8	0.008	8.8	0.004
Unextractable (PES*)	20	0.022	31	0.077	30	0.14	[2]80	[2]0.037
Accountability	**100**	**0.11**	**100**	**0.25**	**100**	**0.46**	**100**	**0.047**

* post extraction solids
[1] polar unkown peak 1a in the conventional extract of wheat straw of the 1st rotation consisted of 4 different metabolites, all of them were minor according to TLC subquantification
[2] no analysis performed, but presumably glucose/carbohydrates as identified in grains of 1st rotation

Table 7.1.2-7 Distribution of parent compound and metabolites in Swiss chard and turnip matrices after a 29 day plant back interval (1st rotation, [furanone-4-^{14}C]BYI 02960)

1st Rotation	Swiss chard immature		Swiss chard mature		turnip leaves		turnip roots	
(BYI- = BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Conventional extraction								
parent compound	54	0.46	43	0.37	64	0.44	56	0.041
glucose/carbohydrates (M45)	---	---	---	---	---	---	3.4	0.003
amino-furanone (M42)	1.8	0.015	3.1	0.027	0.9	0.006	4.1	0.003
DFEAF-OH-glyc (M35)	---	---	1.2	0.010	---	---	---	---
DFEAF (M34)	17	0.14	17	0.15	1.0	0.007	---	---
BYI-glyoxylic acid (M17)	3.7	0.031	4.7	0.041	6.6	0.045	12	0.009
BYI-OH-glyc-SA, isomer 1 (M12)	1.2	0.010	2.5	0.022	---	---	---	---
BYI-acetic acid-glyc (M16)	0.5	0.004	1.6	0.014	3.5	0.024	---	---
BYI-OH-glyc-SA, isomer 2 (M13)	3.3	0.028	3.2	0.028	---	---	---	---
BYI-acetic acid (M15)	0.4	0.003	0.6	0.005	1.2	0.008	0.2	<0.001
BYI-OH-glyc (M8)	8.5	0.072	14	0.119	11.2	0.076	2.1	<0.001
BYI-OH (M3)	2.0	0.017	1.9	0.017	1.7	0.012	0.4	<0.001
BYI-bromo / chloro (M2/M1)	0.6	0.005	0.4	0.003	1.1	0.007	1.3	0.001
Total identified	**93**	**0.79**	**92**	**0.80**	**92**	**0.62**	**80**	**0.058**
unknown 1a[1]	2.5	0.021	3.5	0.031	1.4	0.010	---	---
unknown 1b	---	---	---	---	---	---	6.0	0.004
unknown 6, 7, 8, 10, 11	---	---	---	---	0.2-0.8	0.002-0.006	---	---
unknown 14	---	---	0.3	0.002	0.8	0.006	---	---
unknown 17	---	---	---	---	---	---	2.4	0.002
Total characterised	**2.5**	**0.021**	**[1]3.8**	**0.033**	**5.1**	**0.035**	**8.4**	**0.006**
Total extractable	**96**	**0.81**	**96**	**0.84**	**97**	**0.66**	**88**	**0.065**
Not analysed/losses	0.4	0.003	0.3	0.002	---	---	---	---
Unextractable (PES*)	4.3	0.037	3.9	0.034	3.4	0.023	11.9	0.009
Accountability	**100**	**0.85**	**100**	**0.87**	**100**	**0.68**	**100**	**0.073**

* post extraction solids
[1] polar unkown peak 1a in Swiss chard consisted of 8 different metabolites, all of them were minor according to TLC subquantification

Table 7.1.2-8 Distribution of parent compound and metabolites in Swiss chard and turnip matrices after a 135 day plant back interval (2nd rotation, [furanone-4-^{14}C]BYI 02960)

2nd Rotation	Swiss chard immature		Swiss chard mature		turnip leaves		turnip roots	
(BYI- = BYI 02960)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Conventional extraction								
parent compound	55	0.17	28	0.072	68	0.11	31	0.004
glucose/carbohydrates (M45)	---	---	---	---	---	---	16.0	0.002
amino-furanone (M42)	8.8	0.027	8.8	0.023	0.6	0.001	---	---
DFEAF-OH-glyc (M35)	---	---	---	---	---	---	---	---
DFEAF (M34)	10	0.032	17	0.046	1.0	0.002	---	---
BYI-glyoxylic acid (M17)	---	---	---	---	1.5	0.002	1.8	<0.001
BYI-OH-glyc-SA, isomer 1 (M12)	2.9	0.009	3.5	0.009	---	---	---	---
BYI-acetic acid-glyc (M16)	1.8	0.006	1.1	0.003	0.8	0.001	---	---
BYI-OH-glyc-SA, isomer 2 (M13)	2.0	0.006	3.2	0.008	---	---	---	---
BYI-acetic acid (M15)	0.5	0.002	0.8	0.002	1.3	0.002	0.2	<0.001
BYI-OH-glyc (M8)	12	0.036	18	0.047	13	0.020	2.3	<0.001
BYI-OH (M3)	2.0	0.006	2.4	0.006	1.1	0.002	---	---
BYI-bromo / chloro (M2/M1)	0.5	0.001	---	---	1.3	0.002	1.6	<0.001
Total identified	**96**	**0.30**	**83**	**0.22**	**89**	**0.14**	**53**	**0.007**
unknown 1a[1]	0.9	0.003	9.3	0.025	1.4	0.002	---	---
unknown 1b	---	---	---	---	---	---	28.0	0.004
unknown 6, 7, 8	---	---	---	---	0.5-0.8	0.001	---	---
unknown 14	0.4	0.001	---	---	2.8	0.004	1.3	<0.001
unknown 15	0.4	0.001	---	---	---	---	---	---
Total characterised	**1.6**	**0.005**	**9.3**	**0.025**	**6.0**	**0.010**	**29**	**0.004**
Total extractable	**97**	**0.30**	**93**	**0.24**	**95**	**0.15**	**82**	**0.012**
Not analysed/losses	---	---	0.5	0.001	---	---	---	---
Unextractable (PES*)	2.9	0.009	8	0.020	5.5	0.009	17.7	0.003
Accountability	**100**	**0.31**	**100**	**0.26**	**100**	**0.16**	**100**	**0.014**

* post extraction solids
[1] polar unkown peak 1a in Swiss chard consisted of 8 different metabolites, all of them were minor according to TLC subquantification

Table 7.1.2-9 Distribution of parent compound and metabolites in Swiss chard and turnip matrices after a 296 day plant back interval (3rd rotation, [furanone-4-^{14}C]BYI 02960)

3rd Rotation	Swiss chard immature		Swiss chard mature		turnip leaves		turnip roots	
(BYI- = BYI 02960)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Conventional extraction								
parent compound	37	0.066	33	0.051	72	0.065	70	0.006
glucose/carbohydrates (M45)	---	---	---	---	---	---	5.3	<0.001
amino-furanone (M42)	1.3	0.002	1.8	0.003	0.5	<0.001	5.0	<0.001
DFEAF-OH-glyc (M35)	2.1	0.004	1.9	0.003	---	---	---	---
DFEAF (M34)	14	0.025	15.6	0.024	1.6	0.001	---	---
BYI-glyoxylic acid (M17)	0.2	<0.001	---	---	1.7	0.002	---	---
BYI-OH-glyc-SA, isomer 1 (M12)	1.7	0.003	2.4	0.004	---	---	---	---
BYI-acetic acid-glyc (M16)	0.2	<0.001	0.2	<0.001	0.5	<0.001	---	---
BYI-OH-glyc-SA, isomer 2 (M13)	4.2	0.008	2.4	0.004	---	---	---	---
BYI-acetic acid (M15)	0.9	0.002	0.9	0.001	1.1	0.001	0.3	<0.001
BYI-OH-glyc (M8)	22	0.040	21.9	0.033	10.2	0.009	3.1	<0.001
BYI-OH (M3)	3.6	0.007	3.1	0.005	0.6	0.001	---	---
BYI-bromo / chloro (M2/M1)	0.5	0.001	0.6	0.001	1.8	0.002	2.5	<0.001
Total identified	**87**	**0.16**	**84**	**0.13**	**90**	**0.081**	**86**	**0.007**
unknown 1a[1]	3.4	0.006	1.8	0.003	1.1	0.001	---	---
unknown 1b	---	---	---	---	---	---	9.2	0.001
unknown 3, 4, 5, 11, 15	0.3-0.8	≤0.001	0.2-0.8	≤0.001	---	---	---	---
unknown 6	---	---	---	---	1.1	0.001	---	---
unknown 13	---	---	0.6	0.001	---	---	---	---
unknown 14	0.2	<0.001	---	---	---	---	---	---
Total characterised	**6.5**	**0.012**	**4.9**	**0.008**	**2.3**	**0.002**	**9.2**	**0.001**
Total extractable	**93.8**	**0.169**	**89.1**	**0.135**	**92.5**	**0.083**	**95.5**	**0.008**
Not analysed/losses	**---**	**---**	**---**	**---**	**---**	**---**	**---**	**---**
Unextractable (PES*)	6.2	0.011	10.9	0.017	7.5	0.007	4.5	<0.001
Accountability	**100**	**0.180**	**100**	**0.152**	**100**	**0.090**	**100**	**0.008**

* post extraction solids
[1] polar unkown peak 1a in Swiss chard of 1st rotation consisted of 8 different metabolites, all of them were minor according to TLC subquantification

Conclusions

Parent compound was the most prominent compound in all RACs of all rotations, except in wheat grains, where it was detected as a minor compound only. The main compound detected in grains accounted for approx. 70% of the TRR in the sample of the 1st rotation and was identified as a natural compound (glucose/carbohydrates). Major metabolites in other wheat RACs, Swiss chard and turnips were BYI 02960-OH-glyc, -glyoxylic acid, -difluoroethyl-amino-furanone, -difluorethyl-amino-furanone-OH-glyc and BYI 02960-bromo-amino-furanone which were detected in a range of approx. 10% - 22% of the TRR in numerous samples. All other identified metabolites were detected at minor or trace levels. In total, fourteen metabolites were identified; six of them were specific to the radiolabel tested.

[Furanone-4-^{14}C]BYI 02960 was rather extensively metabolised in confined rotational crops. The following metabolic routes were observed:

- cleavage of the pyridinylmethylamine bond and formation of metabolites based on difluoroethyl-amino-furanone and amino-furanone structures,
- BYI 02960-difluorethyl-amino-furanone conjugation with either mercapto-lactic acid or conjugation with carbohydrates after hydroxylation,
- hydroxylation of the methylene group of the furanone moiety followed by conjugation with carbohydrates and sulphate,
- oxidative degradation of the furanone moiety to BYI 02960-acetic acid, which was either subjected to conjugation with glucose or to a further oxidation step
- complete degradation of the furanone moiety and incorporation of carbon atoms into the natural compound pool, i.e. into glucose/carbohydrates, and
- halogenation of the furanone ring with bromine or chlorine.

Halogenation of the furanone moiety of the active substance probably occurred in the soil which is supported by the fact that small amounts of halogenated parent compound have been identified in the aerobic soil degradation studies. Metabolite BYI 02960-glyoxylic acid, the oxidation product of BYI 02960-acetic acid, was probably a transient soil metabolite that was taken up by the plants, since it was only prominent in the samples of the 1st rotation.

On the basis of the results of this study it is concluded that the metabolism of [furanone-4-^{14}C]BYI 02960 in confined rotational crops is well understood and the following metabolic pathway is proposed:

Figure 7.1.2-1 Proposed metabolic pathway of [furanone-4-^{14}C]BYI 02960 in confined rotational crops

Study 2

Characteristics

Reference	:	Breuer-Rehm, M.; 2011 KIIA 6.6.2/02	Application rate	:	433 g a.s./ha (1.75N)
Type of study	:	Metabolism in Rotational Crops	Area	:	planting container with a surface area of 1 m^2
Year of execution	:	2008-2011	Plant	:	wheat (cereal), Swiss chard (leafy vegetable) and turnips (root vegetable)
Test substance	:	[pyridinylmethyl-^{14}C] Flupyradifurone ; sample ID: KATH 6105; ; purity >99% w/w; batch ID 2008-0.02556	Plant back intervals	:	29, 135 and 296 days
GLP statement	:	Yes	Acceptability	:	Acceptable

Guidelines:

- OECD 502 Metabolism in Rotational Crops
- US EPA Residue Chemistry Test Guideline OPPTS 860.1850: Confined Accumulation in Rotational Crops
- European Parliament and Council Regulation (EC) No 1107/2009

Description

Test material

Chemical structure	* position of the radiolabel
Radiolabelled test material	[pyridinylmethyl-^{14}C]BYI 02960
Specific radioactivity (before radiodilution)	4.37 MBq/mg (118.08 µCi/mg)
Chemical Purity	> 99% (HPLC)
Radiochemical purity	> 99% (HPLC and TLC)

Experimental conditions

The metabolism of flupyradifurone was investigated in the representative rotational crops wheat (cereal), Swiss chard (leafy vegetable) and turnips (root vegetable) from three consecutive rotations. [Pyridinylmethyl-^{14}C]BYI 02960 was formulated as an SL 300 and used for one spray application onto the soil of a planting container (approx. 1 m^2). The actual application rate corresponded to 433 g a.s./ha, above the anticipated maximum seasonal field rate of 250 g a.s./ha (1.75N) for EU uses and 400 g a.s./ha for US uses. The crops were each sown at 29, 135 and 296 days after the soil application, representing the first, second and third rotation.

Sampling

Intermediate raw agricultural commodities (RACs) investigated were Swiss chard immature (BBCH 44-46), wheat forage (at BBCH growth stage 29-31) and wheat hay (sampled at BBCH 79-83 and dried

for five days). All other RACs (wheat straw, wheat grain, Swiss chard, turnip leaves and turnip roots) were harvested at maturity.

Extraction and quantification

An aliquot of each homogenized RAC was extracted conventionally three to four times with ACN/water (8:2, v/v). The extracts were combined, purified using a pre-conditioned SPE RP 18 cartridge, concentrated and analysed by HPLC. The radioactivity in the extracts was determined by LSC, in the solids by combustion followed by LSC. The actual TRR values of the samples were determined by summing up the radioactivity measured in the extracts and in the remaining solids.

Post-extraction solids of forage and hay (1st rotation) were extracted two times with an ACN/water mixture under microwave assistance at increased temperature.

Post-extraction solids of wheat straw (1st rotation) were subjected to a sequential extraction procedure after one exhaustive extraction step with ACN/water (8:2, v/v) at increased temperature (60 °C) under microwave conditions. The solids of the microwave extraction step were subjected to a treatment with sodium chloride solution (2 h at 100 °C under microwave assistance), a diastase incubation (approx. 6 h at 26°C), a treatment with EDTA solution (1 h at 100 °C under microwave assistance) and a cellulase treatment (approx. 12 h at 40 °C) at adjusted temperatures to break down the plant cell walls and liberate residues bound to cell walls or in the cells. The remaining solids of the sequential extraction were extracted finally in two steps with a 5N HCl and a 0.1N NaOH solution. Extracts of the EDTA, the cellulase and the HCl treatment were combined and adjusted to pH 7. The extract was concentrated, centrifuged and analysed by HPLC. The NaOH extract was not submitted to HPLC due to its high viscosity.

Post-extraction solids of grains (1st rotation) were subjected to four subsequent exhaustive extraction steps under microwave conditions at increased temperature. Extraction was performed with ACN/water mixtures, 0.1N HCL and 0.1N NaOH.The ACN/water extracts were combined, concentrated and analysed by HPLC. The post-extraction solids of grains of the 2nd rotation were subjected to one exhaustive extraction step with ACN/water (8:2, v/v) at increased temperature under microwave assistance, followed by a diastase digestion (20 h at 26 °C) and a treatment with sodium chloride solution at 100 °C. HPLC analysis of the extracts was not performed due to the low radioactivity levels in the extracts.

Identification an characterisation

Identification was achieved by spectroscopic evidence (LC-MS/MS) after isolation and purification of the respective compounds from the conventional extracts of wheat straw or Swiss chard of the 1st rotation. HPLC co-chromatography with the isolated and identified radiolabelled metabolites and HPLC comparison of metabolic profiles of the different extracts allowed the assignment of the

compounds in all other extracts.

Storage stability

All conventional extraction experiments and the first HPLC analyses were performed within 9 days after harvest of the plants, with the exception for wheat grain of the second rotation which was extracted within 7 days after harvest, but analysed only 154 days after harvest. Therefore, the extract stability was investigated by re-analysing the grain extract after a storage time of more than 21 months in the freezer at ≤ -18 °C. No change in the metabolite pattern was detected indicating that the residues in the extracts are stability for at least 21 months.

Quantitative ^{14}C-HPLC analysis of the extracts was performed with the profiling method BYI02960_NEUTR. Only for wheat forage and hay, Swiss chard and turnips from the 1st rotation, the metabolic profile was measured with the preliminary profiling method BYI02960_CRC directly after extraction. The preliminary method was based on the same type of column and the same eluents but had a slightly shorter gradient. The metabolite profiles obtained from the two methods were comparable, however profiling method BYI02960_NEUTR showed an improved separation for some metabolites. For better comparison of metabolic pattern over all RACs of all rotations, the profiles of wheat forage and hay, Swiss chard and turnips of the 1st rotation were re-analysed with method BYI02960_NEUTR about 1 – 2 months after extract preparation. These new metabolic profiles were then considered as basis for quantification. Extract stability has been demonstrated for this time period.

Exhaustive extractions were started within approx. four to seven months after harvest. Since the compounds identified in the exhaustive extracts were identical with the compounds of the conventional extracts, it was concluded that the residues in the solids were stable and reflected the released residues at the time of exhaustive extraction.

Thus it can be concluded that the residues in all matrices were sufficiently stable during the experimental period of the study and that the first quantified profiles represented the metabolic pattern in the samples at harvest. Nevertheless, storage stability of BYI 02960 residues in frozen sample material was additionally demonstrated for Swiss chard and wheat straw for at least 20 or 18 months, respectively. Swiss chard and wheat straw samples were extracted and analysed for a second time after storage of the samples at ≤ -18 °C for at least 18 months. Comparison of the HPLC profiles showed no changes in the metabolite patterns.

As well, extract stability was proven for selected matrices (wheat forage, wheat grain, Swiss chard, turnip leaves and turnip roots) by re-analysis of stored extracts. All extracts were stable for at least 4 months (period tested for turnip roots). For wheat grain an extract stability of at least 21 months was demonstrated, as mentioned above.

Results

The TRR values of all RACs declined significantly (by a factor of 7 to 18) from the first to the third rotation. Highest residues were detected in the non-edible commodities of wheat and in the edible commodities of Swiss chard, as shown in Table 7.1.2-10.

Table 7.1.2-10 TRR values in the different RACs of the three rotations after soil application of [pyridinylmethyl-^{14}C]BYI 02960 (Plant Back intervals of 29, 135 and 296 days)

TRR [mg/kg]	Wheat				Swiss chard		Turnips	
	forage	hay	straw	grain	imm.	mature	leaves	roots
1st rotation	1.4	2.4	9.0	0.18	1.4	1.5	0.82	0.072
2nd rotation	0.301	1.0	2.1	0.057	0.33	0.44	0.23	0.022
3rd rotation	0.12	0.32	0.49	0.017	0.14	0.13	0.083	0.008

The radioactive residues were efficiently extracted from all commodities of all rotations with acetonitrile/water mixtures, except for wheat grain where only 35.4% to 52.2% of the TRR was detected after conventional extraction. For all wheat matrices additional exhaustive extraction steps were applied to the solids after conventional extraction. Exhaustive extraction comprised one to two extraction steps with acetonitrile/water mixtures at increased temperature (60 °C) under microwave assistance and in the case of wheat straw (1st and 2nd rotation) and wheat grain (2nd rotation) additional sequential extraction steps including enzymatic digestion steps. For wheat grain, exhaustive extraction released additional high amounts of radioactivity from the post-extraction solids. The extracted portions increased from 52.2% to 83.8% of the TRR (1st rotation) and from 35.4% to 89.4% of the TRR (2nd rotation). For the other wheat matrices, the additionally extracted portions ranged between 3.5% and 20.5% of the TRR depending on the exhaustive extraction steps applied. Thus, extraction efficiencies were increased significantly by exhaustive extraction steps, if applied – and extraction efficiencies above 80% resulted, even for wheat grain.

Parent compound BYI 02960 and about 50 metabolites were detected in the conventional and exhaustive extracts of the various samples of the three rotations. Of these the active substance and 17 metabolites were identified by LC-MS/MS. The other metabolites, none exceeding 5% of the TRR or 0.05 mg/kg, were characterised by their extraction behaviour and their retention time in radio-HPLC. The amounts of active substance and metabolites in all RACs are summarized in Table 7.1.2-11 to Table 7.1.2-18 for the three rotations.

Except in wheat grain, parent compound was by far the main component detected in all matrices of all rotations, accounting for 8% to 62% of the TRR in the commodities of the 1st rotation, 1% to 67% in the 2nd rotation and 14% to 69% in the 3rd rotation. The lowest percentage was always detected in wheat grain and the highest in turnip leaves.

Eight of the identified metabolites were specific to the radiolabel used: BYI 02960-CHMP, three

different conjugates of this metabolite, the corresponding oxidation product 6-CNA and its glycerol-glucuronic acid conjugates (3 isomers) and two oxidation products of the postulated cleavage product BYI 02960-AMCP-difluoroethanamine. The isomers of 6-CNA-glycerol-gluA were detected as major metabolites in all matrices of wheat. In wheat grains they represented the highest proportion of the TRR. It is probable that the weak acid 6-CNA with its pronounced phloem mobility was transported into the seeds as a phloem sink, with conjugation occurring after transport. The 6-CNA conjugates were not identified in the matrices of Swiss chard or turnips.

Identified metabolites common to both radiolabels were BYI 02960-OH and its conjugates, BYI 02960-acetic acid, its conjugates and its successor molecule BYI 02960-glyoxylic acid and the chlorinated/brominated parent compound.

Overall, identification rates were high and ranged from 68% - 87% of the TRR in all RACs apart from wheat grains. In grains the identification rate was lower (29% - 58% of the TRR), but at least additional significant portions of the TRR were characterized by the extraction behaviour of the residues. The solids remaining after the conventional extraction of the grains of the 2nd rotation were subjected in a first step to microwave extraction with ACN/water (8:2, v/v) at increased temperature (15 min at 100 °C). An additional portion of approx. 5% of the TRR was released. Analysis of this extract showed the known major compounds of the conventional extract besides a high amount of a polar unknown compound. Enzymatic treatment of the post-extraction solids of the microwave extraction with diastase (approx. 20 h at 26 °C) solubilised a significant higher portion of the TRR (approx. 21% of the TRR), indicating that a quite polar residue was released. Subsequent extraction of the remaining solids with a sodium chloride solution (2 h at 100 °C) released another significant portion of the TRR (approx. 28% of the TRR). These extraction steps indicate that a major part of the residues in grain is of polar nature – and can be most probably assigned to natural compounds.

Table 7.1.2-11 Distribution of parent compound and metabolites in wheat matrices after a 29 day plant back interval (1st rotation, [pyridinylmethyl-^{14}C]BYI 02960)

1st Rotation	wheat forage		wheat hay		wheat straw		wheat grains	
(BYI_ = BYI 02960)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Conventional extraction								
parent compound	44	0.62	25	0.60	31	2.8	1.8	0.003
6-CNA (M27)	2.0	0.029	3.0	0.072	2.8	0.25	3.8	0.007
6-CNA-glycerol-gluA (1) (M28)	3.2	0.045	3.4	0.083	2.1	0.19	2.3	0.004
6-CNA-glycerol-gluA (2+3) (M29)	14	0.20	24	0.57	19	1.7	16	0.028
BYI-CHMP-glyc (M22)	1.7	0.024	5.0	0.120	2.4	0.21	---	---
BYI-CHMP (M21)	1.2	0.016	1.5	0.036	1.2	0.11	---	---
BYI-glyoxylic acid (M17)	12	0.17	7.3	0.176	5.6	0.51	6.0	0.011
BYI-acetic acid-glyc (M16)	0.6	0.009	---	---	0.8	0.072	---	---
BYI-OH-glyc (M8)	3.4	0.048	4.0	0.095	3.2	0.28	5.0	0.009
BYI-acetic acid (M15)	1.2	0.017	1.4	0.033	1.1	0.10	1.7	0.003
BYI-OH (M3)	1.4	0.020	2.2	0.052	2.4	0.21	6.8	0.012
BYI-bromo / chloro (M2/M1)	---	---	0.9	0.021	0.4	0.036	---	---
Subtotal identified	**85**	**1.2**	**77**	**1.9**	**73**	**6.5**	**43**	**0.077**
unknown 1	0.8	0.011	---	---	---	---	---	---
unknown 2, 7, 9, 17, 18, 24, 26	---	---	---	---	0.2-0.6	0.02-0.06	---	---
unknown 3, 6	---	---	0.7-1.1	0.01-0.03	---	---	---	---
unknown 5, 16, 27	---	---	0.5-1.0	0.01-0.03	0.2-0.7	0.02-0.06	---	---
unknown 10	---	---	---	---	0.6	0.050	2.2	0.004
unknown 14	0.7	0.010	---	---	0.5	0.042	---	---
unknown 21	---	---	0.9	0.022	---	---	4.1	0.007
unknown 25	0.7	0.010	0.8	0.018	0.3	0.023	2.6	0.005
unknown 28, 29	1.0-1.8	0.01-0.03	0.6-0.8	0.01-0.02	0.6-0.9	0.05-0.08	---	---
unknown 33	---	---	---	---	1.4	0.12	---	---
Subtotal characterised	**5.0**	**0.071**	**7.3**	**0.176**	**8.4**	**0.75**	**8.9**	**0.016**
Total conventional extr.	**90**	**1.3**	**85**	**2.0**	**81**	**7.3**	**52**	**0.093**
Microwave extraction I (ACN/water)								
parent compound	1.6	0.023	3.1	0.075	2.4	0.214	6.5	0.012
6-CNA (M27)	---	---	---	---	0.1	0.012	---	---
6-CNA-glycerol-gluA (2+3) (M29)	---	---	---	---	1.4	0.12	4.4	0.008
BYI-glyoxylic acid (M17)	---	---	---	---	0.6	0.058	---	---
BYI-OH-glyc (M8)	---	---	1.6	0.040	0.1	0.012	---	---
BYI-acetic acid (M15)	---	---	0.6	0.014	0.1	0.004	---	---
BYI-OH (M3)	---	---	---	---	0.2	0.017	3.6	0.006
Subtotal identified	**1.6**	**0.023**	**5.3**	**0.13**	**4.9**	**0.44**	**15**	**0.026**
unknown 1	0.5	0.007	2.7	0.065	0.4	0.036	4.9	0.009
unknown 29	1.3	0.019	---	---	0.1	0.011	---	---
Subtotal characterised	**1.8**	**0.026**	**2.7**	**0.065**	**0.5**	**0.047**	**4.9**	**0.009**
Total microwave extr. I	**3.5**	**0.049**	**8.0**	**0.19**	**5.4**	**0.49**	**19**	**0.034**
Additional extractions								
Subtotal identified					**[1]5.8**	**0.52**		
Subtotal characterised					**[1]3.0**	**0.26**		
Subtotal extracted							**[2]12.2**	**0.022**
Total identified	**87**	**1.2**	**83**	**2.0**	**83**	**7.5**	**58**	**0.10**
Total characterised	**6.9**	**0.097**	**10**	**0.24**	**12**	**1.1**	**14**	**0.025**
Total extractable	**94**	**1.3**	**93**	**2.2**	**95**	**8.6**	**84**	**0.15**
Not analysed/losses	0.3	0.004	---	---	4.5	0.40	12	0.022
Unextractable (PES)	6.2	0.087	7.5	0.18	0.4	0.035	16	0.029
Accountability	**100**	**1.4**	**100**	**2.4**	**100**	**9.0**	**100**	**0.18**

(1); (2 + 3) isomer 1; isomer 2 and/or isomer 3
[1] See Table 7.1.2-12 for specifications
[2] no analysis was feasible; 2.3% was extracted with 0.1N HCl, 9.9% with 5N NaOH

Table 7.1.2-12 Residues in additional extracts of wheat straw after a 29 day plant back interval (1st rotation, [pyridinylmethyl-^{14}C]BYI 02960) measured after additional extractions

wheat straw 1st rotation	***1% NaCl, 100°C***		**diastase digest.**		**EDTA+cellul+5NHCl**	
parent compound	1.0	0.087	0.7	0.064	0.7	0.062
6-CNA (M27)	0.8	0.076	0.6	0.055	0.7	0.066
6-CNA-glycerol-gluA (1) (M28)	0.2	0.016	---	---	---	---
6-CNA-glycerol-gluA (2+3) (M29)	0.2	0.020	0.1	0.005	0.2	0.019
BYI-glyoxylic acid (M17)	---	---	0.2	0.020	0.3	0.028
BYI-OH (M3)	0.1	0.006	<0.1	0.004	---	---
Subtotal identified	**2.3**	**0.21**	**1.6**	**0.15**	**1.9**	**0.18**
unknown 1	0.4	0.040	0.3	0.024	0.8	0.071
unknown 5	---	---	0.1	0.006	---	---
unknown 9	---	---	0.1	0.008	---	---
unknown 29	0.2	0.015	0.1	0.013	1.0	0.088
Subtotal characterised	**0.6**	**0.055**	**0.6**	**0.051**	**1.8**	**0.16**
Total	**2.9**	**0.26**	**2.2**	**0.199**	**3.7**	**0.34**

BYI- BYI 02960 = flupyradifurone-

Table 7.1.2-13 Distribution of parent compound and metabolites in wheat matrices after a 135 day plant back interval (2nd rotation, [pyridinylmethyl-^{14}C]BYI 02960)

2nd Rotation	wheat forage		wheat hay		wheat straw		wheat grains	
(BYI- = BYI 02960)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Conventional extraction								
parent compound	60	0.18	28	0.28	32	0.68	1.1	0.001
6-CNA (M27)	1.6	0.005	3.2	0.032	4.0	0.086	---	---
6-CNA-glycerol-gluA (1) (M28)	4.4	0.014	2.4	0.024	2.4	0.051	2.5	0.001
6-CNA-glycerol-gluA (2+3) (M29)	14	0.044	24	0.24	22	0.47	12	0.007
BYI-CHMP-glyc (M22)	0.7	0.002	0.8	0.008	2.2	0.048	---	---
BYI-CHMP (M21)	---	---	0.5	0.005	---	---	---	---
BYI-glyoxylic acid (M17)	---	---	1.0	0.010	1.7	0.036	0.9	0.001
BYI-acetic acid-glyc (M16)	0.7	0.002	---	---	---	---	---	---
BYI-OH-glyc (M8)	2.6	0.008	4.5	0.045	5.3	0.11	4.6	0.003
BYI-acetic acid (M15)	0.9	0.003	1.6	0.016	1.8	0.039	1.6	0.001
BYI-OH (M3)	1.7	0.005	1.9	0.019	3.4	0.072	6.4	0.004
Subtotal identified	**87**	**0.27**	**68**	**0.69**	**75**	**1.6**	**29**	**0.017**
unknown 1	0.3	0.001	0.4	0.004	1.3	0.028	---	---
unknown 2	1.2	0.004	---	---	---	---	---	---
unknown 3, 5, 7, 17, 18, 20	---	---	0.4-1.2	0.004-0.012	---	---	---	---
unknown 10	---	---	0.5	0.005	---	---	1.1	0.001
unknown 14	---	---	2.3	0.023	1.1	0.024	---	---
unknown 21	---	---	---	---	---	---	2.1	0.001
unknown 24, 27, 28, 29	0.3-0.6	0.001-0.002	0.2-0.8	0.002-0.008	---	---	---	---
unknown 25	---	---	---	---	1.8	0.039	2.3	0.001
Subtotal characterised	**3.3**	**0.010**	**9.8**	**0.099**	**4.2**	**0.091**	**5.6**	**0.003**
Total conventional extr.	**90**	**0.28**	**78**	**0.78**	**79**	**1.7**	**35**	**0.020**
Additional extractions								
Subtotal identified					[1]**9.2**	**0.19**		
Subtotal characterised					[1]**3.8**	**0.080**		
Total identified	**87**	**0.27**	**68**	**0.69**	**84**	**1.8**	**29**	**0.017**
Total characterised	**3.3**	**0.010**	**9.8**	**0.099**	**8.0**	**0.17**	**5.6**	**0.003**
Total extractable	**90**	**0.28**	**78**	**0.78**	**92**	**2.0**	**35**	**0.020**
Not analysed/losses	---	---	---	---	7.9	0.17	55	0.032
Unextractable (PES*)	10	0.031	22	0.23	0.3	0.007	11	0.006
Accountability	**100**	**0.31**	**100**	**1.0**	**100**	**2.1**	**100**	**0.057**

(1); (2+3) isomer 1; isomer 2 and/or isomer 3
[1] see table 7.1.2-14 for specification

Table 7.1.2-14 Residues in wheat straw after a 135 day plant back interval (2nd rotation, [pyridinylmethyl-^{14}C]BYI 02960) measured after additional extractions

wheat straw, 2nd rotation								
extraction step	***ACN/water*, 60°C**		***1% NaCl*, 60°C**		**diastase digestion**		**EDTA+cellul+5NHCl**	
(BYI- = BYI 02960)	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**
parent compound	3.4	0.072	0.9	0.020	0.6	0.013	0.7	0.013
6-CNA (M27)	1.5	0.032	0.9	0.019	0.5	0.011	0.5	0.011
BYI-OH (M3)	0.3	0.007	--	--	--	--	--	--
Subtotal identified	**5.2**	**0.11**	**1.8**	**0.040**	**1.1**	**0.024**	**1.1**	**0.024**
unknown 1	1.6	0.034	0.3	0.006	0.3	0.006	0.7	0.014
unknown 29	--	--	--	--	0.1	0.002	0.8	0.017
Subtotal characterised	**1.6**	**0.034**	**0.3**	**0.006**	**0.4**	**0.008**	**1.5**	**0.032**
Total	**6.7**	**0.15**	**2.1**	**0.046**	**1.5**	**0.032**	**2.6**	**0.056**

Table 7.1.2-15 Distribution of parent compound and metabolites in wheat matrices after a 296 day plant back interval (3rd rotation, [pyridinylmethyl-^{14}C]BYI 02960)

3rd Rotation	**wheat forage**		**wheat hay**		**wheat straw**		**wheat grains**	
(BYI- = BYI 02960)	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**
Conventional extraction								
parent compound	45	0.053	20	0.063	26	0.13	14	0.002
BYI-6-CNA (M27)	2.7	0.003	4.3	0.014	4.8	0.024	---	---
BYI-6-CNA-glycerol-gluA (1) (M28)	3.1	0.004	3.6	0.011	4.5	0.022	---	---
BYI-6-CNA-glycerol-gluA (2+3) (M29)	19	0.022	30	0.095	28	0.14	11	0.002
BYI-CHMP-glyc (M22)	3.1	0.004	4.9	0.016	2.9	0.014	---	---
BYI-CHMP (M21)	1.4	0.002	2.9	0.009	---	---	---	---
BYI-glyoxylic acid (M17)	0.8	0.001	---	---	2.6	0.013	---	---
BYIOH -glyc (M8)	3.2	0.004	3.0	0.010	2.9	0.014	5.2	0.001
BYI-acetic acid-glyc (M16)	1.1	0.001	1.1	0.003	1.0	0.005	1.8	<0.001
BYI-OH (M3)	1.0	0.001	1.7	0.005	2.1	0.010	5.5	0.001
Total identified	**81**	**0.094**	**71**	**0.23**	**75**	**0.37**	**38**	**0.006**
unknown 1, 7, 10, 16, 27	---	---	0.5-1.3	0.002-	---	---	---	---
unknown 21, 25, 28	---	---	---	0.004 ---	---	---	1.8- 2.8	<0.001- 0.001
unknown 24	1.5	0.002	---	---	---	---	---	---
Total characterised	**1.5**	**0.002**	**4.2**	**0.014**	**---**	**---**	**7.1**	**0.001**
Total extractable	**82.2**	**0.096**	**75.0**	**0.241**	**75.4**	**0.370**	**44.8**	**0.007**
Not analysed/losses	---	---	---	---	1.2	0.006	---	---
Unextractable (PES*)	17.8	0.021	25.0	0.080	23.4	0.115	55.2	0.009
Accountability	**100**	**0.117**	**100**	**0.321**	**100**	**0.491**	**100**	**0.017**

(1), (2 + 3) isomer 1; isomer 2 and/or isomer 3

Table 7.1.2-16 Distribution of parent compound and metabolites in Swiss chard and turnip matrices after a 29 day plant back interval (1st rotation, [pyridinylmethyl-^{14}C]BYI 02960)

1st Rotation	Swiss chard immature		Swiss chard mature		turnip leaves		turnip roots	
(BYI- = BYI 02960)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Conventional extraction								
parent compound	57	0.78	46	0.687	62	0.51	58	0.042
BYI-CHMP-glyc-tri-SA (M25)	1.8	0.025	2.3	0.034	---	---	---	---
6-CNA (M27)	7.3	0.099	7.0	0.103	1.0	0.008	8.5	0.006
BYI-CHMP-glyc-di-SA (M24)	2.5	0.035	3.0	0.045	---	---	---	---
BYI-CHMP-glyc (M22)	5.5	0.075	5.4	0.080	4.5	0.037	---	---
BYI-CHMP (M21)	---	---	---	---	---	---	4.1	0.003
BYI-glyoxylic acid (M17)	1.5	0.021	2.6	0.039	2.6	0.021	8.6	0.006
BYI-acetic acid-glyc (M16)	1.6	0.022	---	---	0.4	0.003	---	---
BYI-acetic acid-glyc/OH-glyc-SA (isomer 2) (M16 / M13)	---	---	2.5	0.037	---	---	---	---
BYI-OH-glyc (M8)	7.4	0.101	10.9	0.162	9.4	0.076	2.5	0.002
BYI-OH-glyc-SA (1) (M12)	1.1	0.015	1.7	0.025	0.6	0.005	---	---
BYI-OH-glyc-SA (2) (M13)	0.5	0.006	---	---	1.7	0.014	---	---
BYI-N-formyl-/N-acetyl-AMCP-difluoroethanamine (M38/M39)	4.0	0.055	2.9	0.043	3.6	0.030	3.5	0.003
BYI-OH (M3)	1.8	0.024	1.6	0.023	1.4	0.011	0.5	<0.001
BYI-bromo / chloro (M2/M1)	0.7	0.009	0.5	0.008	1.3	0.011	1.4	0.001
Total identified	**93**	**1.3**	**87**	**1.3**	**89**	**0.72**	**87**	**0.063**
unknown 1	---	---	0.3	0.005	0.4	0.004	2.2	0.002
unknown 2, 14, 31, 32	---	---	0.2-0.5	0.003-0.007	---	---	---	---
unknown 6, 18, 25, 26, 28	---	---	---	---	0.4-1.3	0.004-0.011	---	---
unknown 7	---	---	---	---	---	---	1.1	0.001
unknown 11, 15	0.7-0.8	0.010-0.011	0.3-1.1	0.004-0.016	---	---	---	---
unknown 12, 16, 30	---	---	0.3-1.2	0.004-0.018	0.6-2.0	0.005-0.016	---	---
unknown 13	3.0	0.041	5.0	0.075	---	---	---	---
unknown 17, 29	---	---	---	---	---	---	---	---
unknown 27	---	---	---	---	<0.1	<0.001	---	---
Total characterised	**4.5**	**0.062**	**9.9**	**0.15**	**8.8**	**0.071**	**3.2**	**0.002**
Total extractable	**98**	**1.3**	**97**	**1.4**	**98**	**0.80**	**90**	**0.065**
Not analysed/losses	0.3	0.004	0.5	0.008	---	---	---	---
Unextractable (PES*)	2.1	0.028	2.9	0.043	2.3	0.019	10	0.007
Accountability	**100**	**1.4**	**100**	**1.5**	**100**	**0.82**	**100**	**0.072**

(1); (2) isomer 1; isomer 2
* post extraction solids

Table 7.1.2-17 Distribution of residues in Swiss chard and turnip matrices after a 135 day plant back interval (2nd rotation, [pyridinylmethyl-^{14}C]BYI 02960)

2nd Rotation	Swiss chard immature		Swiss chard mature		turnip leaves		turnip roots	
(BYI- = BYI 02960)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Conventional extraction								
parent compound	51	0.17	25	0.11	67	0.15	48	0.011
BYI-CHMP-glyc-tri-SA (M25)	2.8	0.009	2.0	0.009	---	---	---	---
6-CNA (M27)	2.9	0.010	2.9	0.013	0.8	0.002	7.7	0.002
BYI-CHMP-glyc-di-SA (M24)	1.8	0.006	2.4	0.010	---	---	---	---
BYI-CHMP-glyc (M22)	5.1	0.017	1.3	0.006	4.9	0.011	2.0	<0.001
BYI-CHMP (M21)	---	---	---	---	---	---	1.1	<0.001
BYI-acetic acid-glyc (M16)	---	---	3.0	0.013	---	---	---	---
BYI-OH-glyc (M8)	17	0.058	25	0.11	11	0.025	2.4	0.001
BYI-OH-glyc-SA (1) (M12)	1.6	0.005	2.9	0.013	---	---	---	---
BYI-OH-glyc-SA (2) (M13)	2.5	0.008	6.3	0.028	---	---	---	---
BYI-N-formyl-/N-acetyl-AMCP-difluoroethanamine (M38/M39)	1.6	0.005	---	---	1.2	0.003	---	---
BYI-OH (M3)	3.9	0.013	3.6	0.016	1.6	0.004	---	---
BYI-bromo / chloro (M2/M1)	0.5	0.002	0.2	0.001	1.6	0.004	2.0	<0.001
Total identified	**91**	**0.30**	**75**	**0.33**	**88**	**0.20**	**64**	**0.013**
unknown 1	1.4	0.005	6.8	0.030	---	---	5.6	0.001
unknown 5	---	---	---	---	---	---	1.1	<0.001
unknown 11, 31	---	---	0.6-1.5	0.002-0.006	---	---	---	---
unknown 13	3.6	0.012	4.3	0.019	---	---	0.6	<0.001
unknown 14	---	---	1.2	0.005	0.7	0.002	---	---
unknown 15	0.9	0.003	0.7	0.003	---	---	---	---
unknown 16, 19	---	---	1.1-4.8	0.005-0.021	---	---	---	---
unknown 18, 25	---	---	---	---	1.7-1.9	0.004	---	---
unknown 30	0.8	0.003	0.6	0.003	5.7	0.013	6.5	0.001
Total characterised	**6.7**	**0.022**	**15**	**0.064**	**10**	**0.023**	**14**	**0.003**
Total extractable	**98**	**0.33**	**96**	**0.42**	**98**	**0.23**	**78**	**0.015**
Not analysed/losses	---	---	0.7	0.003	---	---	0.6	<0.001
Unextractable (PES*)	1.9	0.006	3.2	0.014	1.9	0.004	22	0.005
Accountability	**100**	**0.33**	**100**	**0.44**	**100**	**0.23**	**100**	**0.022**

(1); (2) isomer 1; isomer 2

Table 7.1.2-38 Distribution of parent compound and metabolites in Swiss chard and turnip matrices after a 296 day plant back interval (3rd rotation, [pyridinylmethyl-^{14}C]BYI 02960)

3rd Rotation	Swiss chard immature		Swiss chard mature		turnip leaves		turnip roots	
(BYI- = BYI 02960)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Conventional extraction								
parent compound	32	0.042	27.4	0.036	69.2	0.058	64.8	0.005
CHMP-glyc-tri-SA (M25)	2.5	0.003	0.4	0.001	---	---	---	---
6-CNA (M27)	5.3	0.007	5.1	0.007	0.8	0.001	4.7	<0.001
CHMP-glyc-di-SA (M24)	2.6	0.003	2.8	0.004	---	---	---	---
CHMP-glyc (M22)	5.1	0.007	6.2	0.008	5.9	0.005	---	---
CHMP (M21)	---	---	---	---	---	---	1.4	<0.001
BYI-glyoxylic acid (M17)	2.1	0.003	1.0	0.001	---	---	---	---
BYI-acetic acid-glyc/-OH-glyc-SA (isomer 2) (M16 / M13)	---	---	2.8	0.004	0.9	0.001	---	---
BYI-OH-glyc (M8)	24.6	0.033	28.1	0.036	9.6	0.008	3.8	<0.001
BYI-OH-glyc-SA (1) (M12)	5.3	0.007	2.9	0.004	0.5	<0.001	---	---
BYI-OH-glyc-SA (2) (M13)	0.5	0.001	---	---	---	---	---	---
N-formyl-/N-acetyl-AMCP-difluoroethanamine (M38/M39)	0.8	0.001	1.8	0.002	2.2	0.002	---	---
BYI-OH (M3)	3.8	0.005	3.4	0.004	---	---	---	---
BYI-bromo / chloro (M2/M1)	0.7	0.001	0.4	0.001	1.6	0.001	2.5	<0.001
Total identified	**84.9**	**0.114**	**82.4**	**0.107**	**90.7**	**0.075**	**77.1**	**0.005**
unknown 1	---	---	0.8	0.001	0.4	<0.001	8.4	0.001
unknown 2, 12, 18	---	---	---	---	0.2-1.8	≤0.001	---	---
unknown 4	0.4	0.001	---	---	---	---	---	---
unknown 5, 6, 11, 14, 16	0.2-1.0	≤0.001	0.5-5.7	0.001-0.007	---	---	---	---
unknown 7	---	---	0.5	0.001	0.3	<0.001	---	---
unknown 13, 30	1.1-4.4	0.001-0.006	---	---	---	---	1.1-3.2	<0.001
unknown 15	0.5	0.001	---	---	0.4	<0.001	---	---
unknown 32	---	---	1.2	0.002	---	---	---	---
Total characterised	**9.0**	**0.012**	**11.6**	**0.016**	**4.2**	**0.002**	**12.7**	**0.001**
Total extractable	**93.9**	**0.126**	**94.0**	**0.123**	**94.9**	**0.078**	**89.8**	**0.006**
Not analysed/losses	---	---	---	---	---	---	3.3	<0.001
Unextractable (PES*)	6.1	0.008	6.0	0.008	5.1	0.004	6.9	0.001
Accountability	**100**	**0.135**	**100**	**0.130**	**100**	**0.083**	**100**	**0.008**

(1); (2) isomer 1; isomer 2
BYI- flupyradifurone-

Conclusions

[Pyridinylmethyl-^{14}C]BYI 02960 was rather extensively metabolised in confined rotational crops. However, parent compound was detected as main component in all RACs of all rotations, except in wheat grain of the first and the second rotation. Considering all samples, BYI 02960-6-CNA-glycerol-gluA (a mixture of two isomers) was the main metabolite in the wheat matrices and BYI 02960-OH-glyc was the main metabolite detected in Swiss chard and turnips. The following main metabolic routes were observed:

- cleavage of the pyridinylmethylamine bond followed by conjugation with carbohydrates and sulphate or by oxidation of the methylene group to a carboxylic group,
- hydroxylation of the methylene group of the furanone moiety followed by conjugation with carbohydrates and sulphate,
- cleavage or complete degradation of the furanone moiety followed by several oxidation and conjugation reactions, and
- halogenation of the furanone moiety with bromine or chlorine.

Halogenation of the furanone moiety of the active substance probably occurred in the soil which is supported by the fact that small amounts of halogenated parent compound were identified in the aerobic soil degradation studies.

On the basis of the results of this study it is concluded that the metabolism of [pyridinylmethyl-^{14}C]BYI 02960 in confined rotational crops is well understood and the following metabolic pathway is proposed:

Guidelines & Limitations

The level of extraction, identification and characterisation in plant samples is sufficient. The study results are reliable and acceptable.

Figure 7.1.2-2 Proposed metabolic pathway of [pyridinylmethyl-^{14}C]BYI 02960 in confined rotational crops

Overall conclusion for confined rotational crops

The metabolism of the insecticide BYI 02960 was investigated in the rotational crops wheat, Swiss chard and turnips from three consecutive rotations in two separate experiments with (1) [furanone-4-^{14}C]BYI 02960 or (2) [pyridinylmethyl-^{14}C]BYI 02960. In each study, parent compound was applied to the bare soil 29 days before sowing of the crops of the first rotation. The plant back intervals were 29, 135 and 296 days after the soil application, representing the first, second and third rotation. Sample materials under investigation were the immature raw agricultural commodities wheat forage, wheat hay and Swiss chard at an intermediate growth stage and the mature raw agricultural commodities wheat straw and wheat grain, Swiss chard and turnip leaves and roots.

(1) Label 1: [furanone-4-^{14}C]BYI 02960

The total radioactive residues (TRR) in all RACs declined significantly from the first to the third rotation. Parent compound was the main component detected in all RACs in all rotations (except for wheat grain), indicating that no major metabolites were formed in the soil and taken up by the plants. These results are in line with the aerobic soil degradation studies which show a quite high mineralization rate of BYI 02960, with only two major soil metabolites detected, both of them being label-specific and not possible to detect in the present study. The formation of BYI 02960-chloro and BYI 02960-bromo in soil was detected to a minor extent. Subsequent uptake of these soil metabolites is most probably the explanation for the trace levels detected in the different RACs. Further degradation of these halogenated compounds in the plant was probably the cause of metabolites such as BYI 02960-bromo-amino-furanone.

The main metabolite in wheat grains was assigned to a natural compound. Diastase digestion of the post extraction solids of the conventional extraction led to one polar metabolite which was identified as glucose by TLC co-chromatography. Radiolabelled glucose was also found in trace levels in turnip roots. Other label-specific metabolites identified were BYI 02960-difluoroethyl-amino-furanone, its conjugates BYI 02960-mercapto-lactic acid and BYI 02960-difluoroethyl-amino-furanone-OH-glyc, as well as the metabolites BYI 02960-amino-furanone and BYI 02960-bromo-amino-furanone. All other identified metabolites were not label specific and were detected at comparable residue levels in the CRC study performed with [pyridinylmethyl-^{14}C]BYI 02960.

On basis of the metabolites identified, the metabolic pathway was deduced. One major metabolic route was the cleavage of the pyridinylmethylamine bond and the formation of BYI 02960-difluoroethylamino-furanone and BYI 02960-amino-furanone after loss of the difluoroethyl moiety. The cleavage of the molecule was also detected in the CRC study conducted with [pyridinylmethyl-^{14}C]BYI 02960. BYI 02960-CHMP, 6-CNA and conjugates of these metabolites were identified as the corresponding counterparts. Oxidative degradation of the furanone moiety was also an important degradation path resulting in the metabolites BYI 02960-acetic acid, BYI 02960-glyoxylic and

corresponding conjugates. Hydroxylation of the furanone moiety and subsequent conjugation with carbohydrates was an additional metabolic route resulting in metabolites common to both radiolabels tested. Complete degradation of the furanone moiety and incorporation of the carbon atoms into the natural compound pool, i.e. most probably into glucose was also observed. Halogenation of the furanone moiety of the active substance was most probably a process which occurred in the soil.

The results of the present metabolism study in confined rotation crops are in good conformity with the results of the corresponding study performed with [pyridinylmethyl-^{14}C]BYI 02960.

(2) Label 2: [pyridinylmethyl-^{14}C]BYI 02960

The total radioactive residues (TRR) in all RACs declined significantly from the first to the third rotation. Parent compound was the main component detected in most of the RACs in all rotation (not in wheat grain of the 1st rotation, not in wheat grain and mature Swiss chard in the 2nd rotation, and not in wheat hay, wheat straw and mature Swiss chard of the 3rd rotation). Other major metabolites were the glucuronic acid conjugates of 6-CNA-glycerol (which were detected in quite high amounts in all wheat matrices) and BYI 02960-OH-glyc (which were detected in significant amounts in Swiss chard). These results are in line with the aerobic soil degradation studies which show a quite high mineralization rate of BYI 02960, with only two major soil metabolites detected, one of them being label-specific and not possible to detect in the present study, and the other 6-CNA, which would be expected to be taken up by the plants and further metabolized (conjugated). The formation of 6-CNA is expected to also occur in the plant, supported by the fact that the preceding metabolite BYI 02960-CHMP - or conjugates of it - were also identified in several plant matrices. Metabolite 6-CNA and its preciding metabolite BYI 02960-CHMP, as well as their corresponding conjugates were the only label specific metabolites detected. All other metabolites identified were not label specific and were detected at comparable residue levels in the CRC study performed with [furanone-4-^{14}C]BYI 02960.

On basis of the metabolites identified, the metabolic pathway was deduced. Overall, three major metabolic routes were detected: (1) Cleavage of the pyridinylmethylamine bond followed by conjugation with carbohydrates and sulphate or by oxidation of the methylene group to a carboxylic group and further conjugation, (2) hydroxylation of the methylene group of the furanone moiety followed by conjugation with carbohydrates and sulphate, and (3) cleavage or complete degradation of the furanone moiety followed by several oxidation and conjugation reactions. Halogenation of the furanone moiety of the active substance was most probably a process which occurred in the soil. Most probably the metabolites were taken up by the roots and were not formed in the plants. This is in line with the finding of the aerobic soil degradation studies summarized in KAII 7.1.

The cleavage of the molecule was also detected in the CRC study conducted with [furanone-4-^{14}C]BYI 02960. BYI 02960-difluoroethyl-amino-furanone and BYI 02960-amino-furanone and conjugates of these metabolites were identified as the corresponding counterparts to BYI 02960-

CHMP and 6-CNA or their conjugates. Thus, the results of the present metabolism study in confined rotation crops are in good conformity with the results of the corresponding study performed with [furanone-4-^{14}C]BYI 02960.

When considering the results from both confined rotational crop studies conducted, it can be concluded that BYI02960 is rather extensively metabolised in rotational crops. A total of 6 major and approx. 40 minor metabolites were found, and all major and 14 minor have been identified. The distribution of parent compound and metabolites in the edible matrices of confined rotational crops are shown in Table 7.1.2-19 to Table 7.1.2-25.

In order to gain information on the fate of the difluoroethane moiety of BYI 02960, the extracts obtained in the CRC study with [furanone-4-^{14}C]BYI 02960 were additionally analysed for non-radiolabelled difluoroacetic acid by LC-MS/MS according to residue method 01304. Significant levels of difluoroacetic acid were detected in all plant matrices of the first and second rotation. DFA represented the main proportion of the residues in the edible crops wheat grain, Swiss chard and turnip roots and as well as in the feed item wheat hay. In wheat forage and straw and turnip leaves, DFA was less prominent than parent, but it was still a major compound.

In corresponding crop samples from different plant back intervals, the DFA levels generally decreased significantly from the first to the third rotation, showing DFA levels slightly above or below the limit of quantification in all crops of the third rotation. The highest DFA concentrations were detected in wheat grains ranging from 4.45 mg a.s. equiv./kg in the first rotation to 0.15 mg a.s. equiv./kg in the third rotation.

On basis of the metabolites identified, biotransformation of BYI 02960 in confined rotational crops proceeds by the following main pathways:

- oxidative cleavage of the difluoroethylamine bond and formation of difluoroacetic acid
- cleavage of the pyridinylmethylamine bond and formation of BYI 02960-difluoroethyl-amino-furanone and the corresponding counterparts BYI 02960-CHMP and 6-CNA followed by several conjugation reactions
- cleavage of the furanone moiety followed by several oxidation and conjugation reactions
- complete degradation of the furanone moiety and incorporation of carbon atoms into the natural compound pool, e.g. into glucose/carbohydrates
- hydroxylation of the methylene group of the furanone moiety followed by conjugation with carbohydrates and sulphate
- halogenation (bromination and most probably chlorination, as well) of the furanone moiety

Halogenation of the furanone moiety of the active substance probably occurred in the soil which is supported by the fact that small amounts of halogenated parent compound were identified in the aerobic soil degradation studies.

The positions involved in the metabolic degradation are summarised in the following figure.

Table 7.1.2-19 TRR values and distribution of parent compound and metabolites in the edible matrix Swiss chard (rotational crop; 1st rotation)

	Confined rotational crops							
	Swiss chard, immature				Swiss chard, mature			
	[furanone-4-^{14}C]		[pyridinylmethyl-^{14}C]		[furanone-4-^{14}C]		[pyridinylmethyl-^{14}C]	
TRR [mg/kg] =		0.85		1.4		0.87		1.48
(BYI- = BYI 02960)	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**
parent compound	54	0.46	57	0.78	43	0.37	46	0.69
glucose/carbohydrates (M45)	---	---			---	---		
BYI-amino-furanone (M42)	*1.8*	*0.015*			*3.1*	*0.027*		
BYI-CHMP-glyc-tri-SA (M25)			*1.8*	*0.025*			*2.3*	*0.034*
6-CNA (M27)			*7.3*	*0.099*			*7.0*	*0.10*
BYI-6-CNA-glycerol-gluA (1) (M28)			---	---			---	---
BYI-6-CNA-glycerol-gluA (2+3) (M29)			---	---			---	---
BYI-CHMP-glyc-di-SA (M24)			*2.5*	*0.035*			*3.0*	*0.045*
BYI-CHMP-glyc (M22)			*5.5*	*0.075*			*5.4*	*0.080*
BYI-CHMP (M21)			---	---			---	---
BYI-DFEAF-OH-glyc (M35)	---	---			*1.2*	*0.010*		
DFEAF (M34)	*17*	*0.14*			*17*	*0.15*		
BYI-glyoxylic acid (M17)	3.7	0.031	1.5	0.021	4.7	0.041	2.6	0.039
BYI-OH-glyc-SA (1) (M12)	1.2	0.010	1.1	0.015	2.5	0.022	1.7	0.025
BYI-acetic acid-glyc (M16)	0.5	0.004	1.6	0.022	1.6	0.014	---	---
BYI-OH-glyc-SA (2) (M13)	3.3	0.028	0.5	0.006	3.2	0.028	[1]2.5	[1]0.037
BYI-OH-glyc (M8)	8.5	0.072	7.4	0.10	14	0.12	11	0.16
BYI-acetic acid (M15)	0.4	0.003	---	---	0.6	0.005	---	---
BYI-N-formyl-/N-acetyl-AMCP-difluoroethanamine (M38/M39)			*4.0*	*0.055*			*2.9*	*0.043*
BYI-OH (M3)	2.0	0.017	1.8	0.024	1.9	0.017	1.6	0.023
BYI-bromo / chloro (M2/M1)	0.6	0.005	0.7	0.009	0.4	0.003	0.5	0.008
Total identified	**93**	**0.79**	**93.2**	**1.3**	**92**	**0.80**	**87**	**1.3**
Total characterised	**2.5**	**0.021**	**4.5**	**0.062**	**3.8**	**0.033**	**9.9**	**0.15**
Analysed extract(s)	95	0.81	97.7	1.3	96	0.84	97	1.4
Extract(s) not analysed	0.4	0.003	0.3	0.004	0.3	0.002	0.5	0.008
Total extracted	96	0.81	97.7	1.3	96	0.84	97	1.4
Unextractable (PES*)	4.3	0.037	2.1	0.028	3.9	0.034	2.9	0.043
Accountability	**100**	**0.85**	**100**	**1.4**	**100**	**0.87**	**100**	**1.5**

[1] co-elution with BYI -acetic acid-glyc(M16)
(1) isomer 1; (2) isomer 2; (2 + 3) isomer 2 and/or isomer 3
Label specific metabolites are printed in italic

Table 7.1.2-20 TRR values and distribution of parent compound and metabolites in the edible matrix Swiss chard (rotational crop; 2nd rotation)

	Confined rotational crops							
	Swiss chard, immature				Swiss chard, mature			
	[furanone-4-^{14}C]		[pyridinyl-methyl-^{14}C]		[furanone-4-^{14}C]		[pyridinyl-methyl-^{14}C]	
TRR [mg/kg] =		0.31		0.33		0.26		0.44
(BYI- = BYI 02960)	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**
parent compound	55	0.17	51	0.17	28	0.072	25	0.11
glucose/carbohydrates (M45)	---	---			---	---		
BYI-amino-furanone (M42)	*8.8*	*0.027*			*8.8*	*0.023*		
BYI-CHMP-glyc-tri-SA (M25)			*2.8*	*0.009*			*2.0*	*0.009*
6-CNA (M27)			*2.9*	*0.010*			*2.9*	*0.013*
BYI-6-CNA-glycerol-gluA (1) (M28)			---	---			---	---
BYI-6-CNA-glycerol-gluA (2+3) (M29)			---	---			---	---
BYI-CHMP-glyc-di-SA (M24)			*1.8*	*0.006*			*2.4*	*0.010*
BYI-CHMP-glyc (M22)			*5.1*	*0.017*			*1.3*	*0.006*
BYI-CHMP (M21)			---	---			---	---
BYI-DFEAF-OH-glyc (M35)	---	---			---	---		
BYI-DFEAF (M34)	*10*	*0.032*			*17*	*0.046*		
BYI-glyoxylic acid (M17)	---	---	---	---	---	---	---	---
BYI-OH-glyc-SA (1) (M12)	2.9	0.009	1.6	0.005	3.5	0.009	2.9	0.013
BYI-acetic acid-glyc (M16)	1.8	0.006	---	---	1.1	0.003	3.0	0.013
BYI-OH-glyc-SA (2) (M13)	2.0	0.006	2.5	0.008	3.2	0.008	6.3	0.028
BYI-OH-glyc (M8)	12	0.036	17.4	0.058	18.0	0.047	25.3	0.111
BYI-acetic acid (M15)	0.5	0.002	---	---	0.8	0.002	---	---
BYI-N-formyl-/N-acetyl-AMCP-difluoroethanamine (M38/M39)			*1.6*	*0.005*			---	---
BYI-OH (M3)	2.0	0.006	3.9	0.013	2.4	0.006	3.6	0.016
BYI-bromo / chloro (M2/M1)	0.5	0.001	0.5	0.002	---	---	0.2	0.001
Total identified	**96**	**0.30**	**91**	**0.30**	**83**	**0.22**	**75**	**0.33**
Total characterised	**1.6**	**0.005**	**6.7**	**0.022**	**9.3**	**0.025**	**15**	**0.064**
Analysed extract(s)	97	0.30	91	0.30	92.1	0.24	96.1	0.42
Extract(s) not analysed	---	---	---	---	0.5	0.001	0.7	0.003
Total extracted	97	0.30	98	0.33	93	0.24	96	0.42
Unextractable (PES*)	2.9	0.009	1.9	0.006	7.5	0.02	3.2	0.014
Accountability	**100**	**0.31**	**100**	**0.33**	**100**	**0.26**	**100**	**0.44**

(1) isomer 1; (2) isomer 2; (2 + 3) isomer 2 and/or isomer 3
Label specific metabolites are printed in italic.

Table 7.1.2-21 TRR values and distribution of parent compound and metabolites in the edible matrix Swiss chard (rotational crop; 3rd rotation)

	Confined rotational crops							
	Swiss chard, immature				Swiss chard, mature			
	[furanone-4-^{14}C]		[pyridinyl-methyl-^{14}C]		[furanone-4-^{14}C]		[pyridinyl-methyl-^{14}C]	
TRR [mg/kg] =	0.18		0.14		0.15		0.13	
(BYI- = BYI 02960-)	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**
parent compound	37	0.066	32	0.042	33	0.051	27	0.036
glucose/carbohydrates (M45)	---	---			---	---		
BYI-amino-furanone (M42)	*1.3*	*0.002*			*1.8*	*0.003*		
BYI-CHMP-glyc-tri-SA (M25)			*2.5*	*0.003*			*0.4*	*0.001*
6-CNA (M27)			*5.3*	*0.007*			*5.1*	*0.007*
BYI-6-CNA-glycerol-gluA (1) (M28)			---	---			---	---
BYI-6-CNA-glycerol-gluA (2+3) (M29)			---	---			---	---
BYI-CHMP-glyc-di-SA (M24)			*2.6*	*0.003*			*2.8*	*0.004*
BYI-CHMP-glyc (M22)			*5.1*	*0.007*			*6.2*	*0.008*
BYI-CHMP (M21)			---	---			---	---
BYI-DFEAF-OH-glyc (M35)	*2.1*	*0.004*			*1.9*	*0.003*		
BYI-DFEAF (M34)	*14*	*0.025*			*16*	*0.024*		
BYI-glyoxylic acid (M17)	0.2	<0.001	2.1	0.003	---	---	1.0	0.001
BYI-OH-glyc-SA (1) (M12)	1.7	0.003	5.3	0.007	2.4	0.004	2.9	0.004
BYI-acetic acid-glyc (M16)	0.2	<0.001	---	---	0.2	<0.001	---	---
BYI-OH-glyc-SA (2) (M13)	4.2	0.008	0.5	0.001	2.4	0.004	[1]2.8	[1]0.004
BYI-OH-glyc (M8)	22	0.04	25	0.033	22	0.033	28	0.036
BYI-acetic acid (M15)	0.9	0.002	---	---	0.9	0.001	---	---
BYI-N-formyl-/N-acetyl-AMCP-difluoroethanamine (M38/M39)			*0.8*	*0.001*			*1.8*	*0.002*
BYI-OH (M3)	3.6	0.007	3.8	0.005	3.1	0.005	3.4	0.004
BYI-bromo / chloro (M2/M1)	0.5	0.001	0.7	0.001	0.6	0.001	0.4	0.001
Total identified	**87**	**0.16**	**85**	**0.114**	**84**	**0.128**	**82**	**0.11**
Total characterised	**6.5**	**0.012**	**9.0**	**0.012**	**4.9**	**0.008**	**12**	**0.016**
Analysed extract(s)	94	0.17	85	0.114	89	0.14	82.4	0.11
Extract(s) not analysed	---	---	---	---	---	---	---	---
Total extracted	94	0.17	94	0.126	89	0.14	94	0.12
Unextractable (PES*)	6.2	0.011	6.1	0.008	11	0.017	6.0	0.008
Accountability	**100**	**0.18**	**100**	**0.134**	**100**	**0.152**	**100**	**0.13**

[1] co-elution with BYI -acetic acid-glyc (M16)
(1) isomer 1; (2) isomer 2; (2 + 3) isomer 2 and/or isomer 3
Label specific metabolites are printed in italic.

Table 7.1.2-22 TRR values and distribution of parent compound and metabolites in edible matrices wheat grains and turnip roots (rotational crops; 1st rotation)

	Confined rotational crops							
	wheat grains				turnip roots			
	[furanone-4-^{14}C]		[pyridinyl-methyl-^{14}C]		[furanone-4-^{14}C]		[pyridinyl-methyl-^{14}C]	
TRR [mg/kg] =	0.48		0.18		0.074		0.072	
(BYI- = BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
parent compound	0.4	0.002	8.3	0.015	56	0.041	58	0.042
glucose/carbohydrates (M45)	*71*	*0.34*			3.4	*0.003*		
BYI-amino-furanone (M42)	*0.5*	*0.002*			4.1	*0.003*		
6-CNA (M27)			*3.8*	*0.007*			*8.5*	*0.006*
BYI-6-CNA-glycerol-gluA (1) (M28)			*2.3*	*0.004*			---	---
BYI-6-CNA-glycerol-gluA (2+3) (M29)			*20*	*0.036*			---	---
BYI-CHMP (M21)			---	---			*4.1*	*0.003*
BYI-glyoxylic acid (M17)	5.1	0.024	6.0	0.011	12	0.009	8.6	0.006
BYI-OH-glyc (M8)	1.4	0.007	5.0	0.009	2.1	<0.001	2.5	0.002
BYI-acetic acid (M15)	0.6	0.003	1.7	0.003	0.2	<0.001	---	---
BYI-N-formyl-/N-acetyl-AMCP-difluoroethanamine (M38/M39)			---	---			3.5	0.003
BYI-OH (M3)	2.3	0.011	10	0.019	0.4	<0.001	0.5	<0.001
BYI-bromo / chloro (M2/M1)	---	---	---	---	1.3	0.001	1.4	0.001
Total identified	**81**	**0.39**	**58**	**0.10**	**80**	**0.058**	**87**	**0.063**
Total characterised	**4.2**	**0.020**	**14**	**0.025**	**8.4**	**0.006**	**3.2**	**0.002**
Analysed extract(s)	85	0.41	72	0.13	88	0.065	90	0.065
Extract(s) not analysed	3.0	0.014	12	0.022	---	---	---	---
Total extracted	88	0.42	84	0.15	88	0.065	90	0.065
Unextractable (PES*)	12	0.058	16	0.029	12	0.009	10	0.007
Accountability	**100**	**0.48**	**100**	**0.18**	**100**	**0.074**	**100**	**0.072**

(1) isomer 1; (2) isomer 2; (2 + 3) isomer 2 and/or isomer 3
Label specific metabolites are printed in italic.

Table 7.1.2-23 TRR values and distribution of parent compound and metabolites in edible matrices wheat grains and turnip roots (rotational crops; 2nd rotation)

	Confined rotational crops							
	wheat grains				turnip roots			
(BYI- = BYI 02960-)	[furanone-4-^{14}C]		[pyridinyl-methyl-^{14}C]		[furanone-4-^{14}C]		[pyridinyl-methyl-^{14}C]	
TRR [mg/kg] =		0.103		0.057		0.014		0.022
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
parent compound	0.5	0.001	1.1	0.001	31	0.004	48	0.011
glucose/carbohydrates (M45)	*---*	*---*			*16*	*0.002*		
BYI-amino-furanone (M42)	*1.3*	*0.001*			*---*	*---*		
6-CNA (M27)			*---*	*---*			*7.7*	*0.002*
BYI-6-CNA-glycerol-gluA (1) (M28)			*2.5*	*0.001*			*---*	*---*
BYI-6-CNA-glycerol-gluA (2+3) (M29)			*12*	*0.007*			*---*	*---*
BYI-CHMP-glyc (M22)			*---*	*---*			*2.0*	*<0.001*
BYI-CHMP (M21)			*---*	*---*			*1.1*	*<0.001*
BYI-glyoxylic acid (M17)	0.8	0.001	0.9	0.001	1.8	<0.001	---	---
BYI-OH-glyc (M8)	2.1	0.002	4.6	0.003	2.3	<0.001	2.4	0.001
BYI-acetic acid (M15)	1.0	0.001	1.6	0.001	0.2	<0.001	---	---
BYI-OH (M3)	3.4	0.003	6.4	0.004	---	---	---	---
BYI-bromo / chloro (M2/M1)	---	---	---	---	1.6	<0.001	2.0	<0.001
Total identified	**9.2**	**0.009**	**29**	**0.017**	**53**	**0.007**	**64**	**0.013**
Total characterised	**4.2**	**0.004**	**5.6**	**0.003**	**29**	**0.004**	**14**	**0.003**
Analysed extract(s)	13	0.014	35	0.02	82	0.012	78	0.015
Extract(s) not analysed	[1]69	[1]0.071	55	0.032	---	---	0.6	<0.001
Total extracted	83	0.085	35	0.020	82	0.012	78	0.015
Unextractable (PES*)	17	0.018	11	0.006	18	0.003	22	0.005
Accountability	**100**	**0.10**	**100**	**0.057**	**100**	**0.014**	**100**	**0.022**

[1] no analysis performed, but presumably glucose/carbohydrates as identified in grains of 1st rotation
(1) isomer 1; (2) isomer 2; (2 + 3) isomer 2 and/or isomer 3
Label specific metabolites are printed in italic.

Table 7.1.2-24 TRR values and distribution of parent compound and metabolites in edible matrices wheat grains and turnip roots (rotational crops; 3rd rotation)

	Confined rotational crops							
	wheat grains				turnip roots			
	[furanone-^{14}C]		[pyridinyl-^{14}C]		[furanone-4-^{14}C]		[pyridinyl-^{14}C]	
TRR [mg/kg] =		0.047		0.017		0.008		0.008
(BYI- = BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
parent compound	2.0	0.001	14	0.002	70	0.006	65	0.005
glucose/carbohydrates (M45)	---	---			*5.3*	*<0.001*		
BYI-amino-furanone (M42)	---	---			*5.0*	*<0.001*		
6-CNA (M27)			---	---			*4.7*	*<0.001*
BYI-6-CNA-glycerol-gluA (2+3) (M29)			*11*	*0.002*			---	---
BYI-CHMP (M21)			---	---			*1.4*	*<0.001*
BYI-OH-glyc (M8)	1.7	0.001	5.2	0.001	3.1	<0.001	3.8	<0.001
BYI-acetic acid (M15)	0.8	<0.001	1.8	<0.001	0.3	<0.001	---	---
BYI-OH (M3)	2.8	0.001	5.5	0.001	---	---	---	---
BYI-bromo / chloro (M2/M1)	---	---	---	---	2.5	<0.001	2.5	<0.001
Total identified	**7.4**	**0.003**	**38**	**0.006**	**86**	**0.007**	**77**	**0.005**
Total characterised	**4.3**	**0.002**	**7.1**	**0.001**	**9.2**	**0.001**	**13**	**0.001**
Analysed extract(s)	12	0.005	45	0.007	96	0.007	90	0.006
Extract(s) not analysed	8.8	0.004	---	---	---	---	3.3	<0.001
Total extracted	20	0.01	45	0.007	96	0.008	90	0.006
Unextractable (PES*)	[1]80	[1]0.037	55	0.009	4.5	<0.001	6.9	0.001
Accountability	**100**	**0.047**	**100**	**0.017**	**100**	**0.008**	**100**	**0.008**

[1] no analysis performed, but presumably glucose/carbohydrates as identified in grains of 1st rotation
(1) isomer 1; (2) isomer 2; (2 + 3) isomer 2 and/or isomer 3
Label specific metabolites are printed in italic.

B.7.2 Metabolism, distribution and expression of residues in livestock (IIA 6.2.2, IIA 6.2.3, IIA 6.2.4 and IIIA 8.2)

Both crops, i.e. lettuce and hops, in the intended use for the European evaluation of flupyradifurone are not used as livestock feed. Nevertheless, the applicant submitted livestock metabolism and livestock feeding studies, anticipating future uses on feeding crops in EU and with regard to residues that can occur in crops grown in rotation (see B.7.9). ~~Furthermore, use on several crops that can be used as livestock feed is authorised in Australia, Brazil, Canada, Mexico and US (Partners in the Joint Global Review), i.e. potatoes and other root and tuber vegeatbles, kale, beans, pome fruits, cereal grains and corn, clover seed forage, soy bean and cotton seed. An MRL dossier was already received by the RMS and has been evaluated. However, it is assumed that imported crops are generally not used as livestock feed.~~

Livestock diet calculations were conducted using the results from the rotational crops and EU uses on feeding crops supported in the MRL dossier.

For the dietary burden calculator, the ~~separate~~ total residues of flupyradifurone and DFA were used as input, since residue levels of flupyradifurone are at or below LOQ in rotational crops and DFA is the major contributor to the total residue level (levels of DFEAF at or below LOQ). The metabolism and feeding studies were carried out with flupyradifurone only. The residues are separated into BYI 02960 and DFA residues in the worst-case diet to estimate the expected worst-case residue levels in products of anilmal origin of the two compounds in animal matrices on the basis of the different transfer factors determined in the feeding studies (See B.7.8 and B.7.12).

The input values for the dietary burden calculator are:

Table 7.2-1 Input values for flupyradifurone for livestock dietary burden calculations

Commodity	BYI 02960		DFA as flupyradifurone		Total (sum BYI 09260 and DFA)		Comment
	STMR	HR	STMR	HR	STMR	HR	
grass	0.01	0.04	0.08	0.37	0.09	0.39	Rotational crop, barley field study green material
Rape forage	0.01	0.04	0.08	0.37	0.09	0.39	
Silage (alfalfa, clover, grass and maize)	0.01	0.04	0.08	0.37	0.09	0.39	
Cabbage, kale	0.03	0.08	0.05	0.12	0.07	0.20	Rotational crop, lettuce
Beet leaves (sugar and fodder)	0.03	0.08	0.05	0.12	0.07	0.20	
Hay (clover, alfalfa, grass)	0.02	0.04	0.09	0.34	0.11	0.38	Rotational crop, barley field study straw
Cereals straws	0.02	0.04	0.09	0.34	0.11	0.38	

Commodity	BYI 02960		DFA as flupyradifurone		Total (sum BYI 09260 and DFA)		Comment
	STMR	HR	STMR	HR	STMR	HR	
Cereal grain	0.01	0.01	0.33	0.63	0.34 STMR-P 2.21	0.64	Rotational crop, barley field study grain Whiteflour bran: PF 6.50
Pulses	0.01	0.01	1.55	2.3	1.56	2.31	Rotational crop, field peas
Roots and tubers	0.01	0.01	0.11	0.25	0.12	0.26	Rotational crop, potatoes
Oilseeds	0.01	0.01	0.08	0.15	0.09 STMR-P 0.09	0.16	Rotational crop, winter rape Oilseeds meal: PF 1.0
Apple **pomace, wet**	0.07 STMR-P 0.10	0.32	0.02 STMR-P 0.02	0.07	0.09 STMR-P 0.12	0.36	Residue trial P-factor of 1.49 for BYI 02960 and 0.75 for DFA

~~**Table 7.2-1 Input values for flupyradifurone for livestock dietary burden calculations**~~

~~Commodity~~	~~Median dietary burden~~		~~Maximum dietary burden~~	
	~~Input value (mg/kg)~~	~~Comment~~	~~Input value (mg/kg)~~	~~Comment~~
~~BYI 02960~~				
~~grass~~	~~0.01~~	~~Rotational crop, barley field study green material~~	~~0.02~~	~~Rotational crop, barley field study green material~~
~~Rape forage~~	~~0.01~~		~~0.02~~	
~~Silage (alfalfa, clover, grass and maize)~~	~~0.01~~		~~0.02~~	
~~Cabbage, kale~~	~~0.03~~	~~Rotational crop, lettuce~~	~~0.08~~	~~Rotational crop, lettuce~~
~~Beet leaves (sugar and fodder)~~	~~0.03~~		~~0.08~~	
~~Hay (clover, alfalfa, grass)~~	~~0.02~~	~~Rotational crop, barley field study straw~~	~~0.04~~	~~Rotational crop, barley field study straw~~
~~Cereals straws~~	~~0.02~~		~~0.04~~	
~~Cereal grain~~	~~0.01~~	~~Rotational crop, barley field study grain~~ ~~Bran: PF 3.46~~	~~0.01~~	~~Rotational crop, barley field study grain~~
~~Pulses~~	~~0.01~~	~~Rotational crop, field peas~~	~~0.01~~	~~Rotational crop, field peas~~
~~Roots and tubers~~	~~0.01~~	~~Rotational crop, potatoes~~	~~0.01~~	~~Rotational crop, potatoes~~

Commodity	Median dietary burden		Maximum dietary burden	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
Oilseeds	0.01	Rotational crop, winter rape Oilseeds meal: PF 0.14	0.01	Rotational crop, winter rape
Apple pomace, wet*	0.10	Residue trial	-	Residue trial

* Based on STMR (0.067 mg/kg) and HR (0.32 mg/kg) values from the supervised residue trials (dataset NEU and SEU together) and a mean P factor of 1.49 (2 studies from NEU, 2 studies from SEU) for BYI 02960.

Table 7.2-2 Input values for DFA for livestock dietary burden calculations

Commodity	Median dietary burden		Maximum dietary burden	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
DFA				
grass	0.08	Rotational crop, barley field study green material	0.37	Rotational crop, barley field study green material
Rape forage	0.08		0.37	
Silage (alfalfa, clover, grass and maize)	0.08		0.37	
Cabbage, kale	0.05	Rotational crop, lettcue	0.12	Rotational crop, lettuce
Beet leaves (sugar and fodder)	0.05		0.12	
Hay (clover, alfalfa, grass)	0.09	Rotational crop, barley field study straw	0.34	Rotational crop, barley field study straw
Cereals straws	0.09		0.34	
Cereal grain	0.33	Rotational crop, barley field study grain Bran: PF 1.1	0.63	Rotational crop, barley field study grain
Pulses	1.5	Rotational crop, field peas	2.3	Rotational crop, field peas
Roots and tubers	0.11	Rotational crop, potatoes	0.25	Rotational crop, potatoes

~~Commodity~~	~~Median dietary burden~~		~~Maximum dietary burden~~	
	~~Input value (mg/kg)~~	~~Comment~~	~~Input value (mg/kg)~~	~~Comment~~
~~Oilseeds~~	~~0.08~~	~~Rotational crop, winter rape~~ ~~Oilseeds meal: PF 0.13~~	~~0.15~~	~~Rotational crop, winter rape~~
~~Apple pomace, wet*~~	~~0.015~~	~~Residue trial~~	~~-~~	~~Residue trial~~

~~* Based on STMR (0.02 mg/kg) and HR (0.069 mg/kg) from the supervised residue trials (dataset NEU and SEU together) and a mean P-factor of 0.75 for DFA.~~

The results in Table 7.2-3 show that the theoretical intake of ~~total~~ flupyradifurone ~~(~~parent and DFA~~)~~, separately, residues from rotational crops and pome fruit is more than 0.1 mg/kg diet (DM) for cattle, poultry and pigs. Therefore, metabolism studies in these species are required. The N rate in the studies was not calculated ~~for the total flupyradifurone residues~~, since ~~whilst~~ the animals were dosed with parent only. ~~Hence, the N rate is provisional.~~

Calculation of dietary burden

The estimated dietary burden of ~~total~~ BYI 02960 and DFA residues in livestock commodities, based on EU crop residue data and the European dietary burden calculator, are presented for poultry, cattle and pigs below in table 7.2-3.

Table 7.2-2 Theoretical dietary intake calculations for livestock for total flypyradifurone residues

	Maximum dietary burden mg/kg bw/d (mg/kg dry feed)	Median dietary burden mg/kg bw/d (mg/kg dry feed)	Highest contributing commodity	Dietary burden triggered ?
BYI 02960				
Dairy ruminant	0.007 (0.20)	0.004 (0.11)	Grasses	Y
Meat ruminant	0.009 (0.20)	0.008 (0.19)	Grasses	Y
Pig	0.007 (0.19)	0.004 (0.11)	Sugar beet tops	Y
poultry	0.004 (0.06)	0.008 (0.19)	Cabbage	Y

	Maximum dietary burden mg/kg bw/d (mg/kg dry feed)	Median dietary burden mg/kg bw/d (mg/kg dry feed)	Highest contributing commodity	Dietary burden triggered ?
DFA as flupyradifurone				
Dairy ruminant	0.074 (2.05)	0.033 (0.90)	Grasses	Y
Meat ruminant	0.103 (2.40)	0.049 (1.15)	Grasses	Y
Pig	0.094 (2.36)	0.056 (1.40)	Swedes	Y
poultry	0.080 (1.27)	0.061 (0.97)	Peas (dry)	Y

	~~Maximum dietary burden mg/kg bw/d (mg/kg dry feed)~~	~~Median dietary burden mg/kg bw/d (mg/kg dry feed)~~	~~Highest contributing commodity~~	~~Dietary burden triggered ?~~
~~***Total BYI 02960 + DFA***~~				
~~Dairy ruminant~~	~~0.0819 (2.252)~~	~~0.0498 (1.369)~~	~~Wheat bran~~	~~Y~~
~~Meat ruminant~~	~~0.1120 (2.614)~~	~~0.0677 (1.579)~~	~~Turnips~~	~~Y~~
~~Pig~~	~~0.1026 (2.565)~~	~~0.0681 (1.702)~~	~~Peas (dry)~~	~~Y~~
~~poultry~~	~~0.0959 (1.519)~~	~~0.0753 (1.192)~~	~~Peas (dry)~~	~~Y~~

~~***BYI 02960***~~				
~~Dairy ruminant~~	~~0.0089 (0.245)~~	~~0.0042 (0.116)~~	~~Cabbage~~	~~Y~~
~~Meat ruminant~~	~~0.0112 (0.261)~~	~~0.0083 (0.193)~~	~~Apple pomace~~	~~Y~~

~~Pig~~	~~0.0076~~ ~~(0.191)~~	~~0.0045~~ ~~(0.113)~~	~~Turnip~~	~~Y~~
~~poultry~~	~~0.0036~~ ~~(0.057)~~	~~0.0025~~ ~~(0.039)~~	~~Turnip~~	~~Y~~
~~***DFA***~~				
~~Dairy ruminant~~	~~0.0744~~ ~~(2.045)~~	~~0.0320~~ ~~(0.879)~~	~~Peas (dry)~~	~~Y~~
~~Meat ruminant~~	~~0.1077~~ ~~(2.512)~~	~~0.0481~~ ~~(1.123)~~	~~Turnips~~	~~Y~~
~~Pig~~	~~0.0933~~ ~~(2.332)~~	~~0.0543~~ ~~(1.358)~~	~~Peas (dry)~~	~~Y~~
~~poultry~~	~~0.0782~~ ~~(1.239)~~	~~0.0591~~ ~~(0.935)~~	~~Peas (dry)~~	~~Y~~
~~***BYI 02960 + DFA***~~				
~~Dairy ruminant~~	~~0.0816~~ ~~(2.245)~~	~~0.0363~~ ~~(0.998)~~	~~Turnips~~	~~Y~~
~~Meat ruminant~~	~~0.1171~~ ~~(2.732)~~	~~0.0553~~ ~~(1.290)~~	~~Turnips~~	~~Y~~
~~Pig~~	~~0.0998~~ ~~(2.495)~~	~~0.0598~~ ~~(1.496)~~	~~Turnips~~	~~Y~~
~~poultry~~	~~0.0832~~ ~~(1.318)~~	~~0.0632~~ ~~(1.001)~~	~~Peas (dry)~~	~~Y~~

B.7.2.1 Summary of studies in ruminants and poultry

Poultry

STUDY 1

Characteristics

Reference	:	[redacted] 2012a KIIA 6.2.2/01	GLP statement	:	Yes
Type of study	:	Metabolism in laying hen	Guideline	:	OECD-Guideline for the Testing of Chemicals No. 503: Metabolism in Livestock US-EPA, Residue Chemistry OPPTS 860.1300;
Year of execution	:	2010-2011	Dose	:	14 consecutive days with a 1.02 mg/kg body weight
Test substance	:	(1) [Pyridinyl,ethyl-^{14}C]BYI02960, radiochemical purity > 99 % by radio-HPLC and > 98 % by radio-TLC (2) Nonlabeled testsubstance Batch BYI 02960-PU-02, chemical purity 99,4%	Vehicle	:	0.5 % aqueous Tragacanth® suspension
			Concentration in feed	:	16.18 mg a.s. /kg dry feed/day 1.02 mg/kg bw/d
			Sampling	:	Eggs, excreta, liver, gall bladder, kidneys, leg and breast muscle, skin without subcutaneous fat, subcutaneous fat and eggs from the ovary and oviduct
Animal	:	Laying hen (*Gallus gallus domesticus*) White Leghorn	Acceptability	:	acceptable

Study design

The metabolism and excretion of [pyridinylmethyl-^{14}C]BYI 02960 (common name: flupyradifurone) were investigated in laying hens (White Leghorn). Six hens (mean body weight 1.64 kg) were orally dosed once daily in the morning for 14 consecutive days with an aqueous 0.5% Tragacanth® suspension of 1.02 mg/kg body weight (3.50 MBq/mg after radiodilution) which corresponded to 16.18 mg a.s./kg dry feed/day ~~(12N–11N)~~. The animals were sacrificed six hours after the last administration. Total radioactive residues (TRR) were determined daily in the eggs and excreta, and at sacrifice in the dissected organs and tissues (muscle, fat, liver, kidney, skin and eggs from ovary/oviduct). Eggs, muscle, fat, liver and excreta were extracted and analysed for parent compound and metabolites.

Results

For analysis of parent compound and metabolites, eggs, muscle, fat, liver and excreta from day 13 were pooled from all six animals. Eggs, muscle and liver as well as excreta were conventionally extracted with mixtures of acetonitrile/water and pure acetonitrile. Fat was extracted with acetonitrile and n-heptane followed by a partition procedure yielding an acetonitrile and n-heptane phase. Post-extraction solids of liver were exhaustively extracted using microwave assistance. The resulting extracts of eggs, muscle and liver represented over 92% of the total radioactive residue (TRR). Approx. 80% of the TRR was extractable in fat. Residues in solids of fat were very low and amounted to 0.004 mg/kg, only.

After purification and concentration, the extracts were analysed using HPLC with radiometric detection. The HPLC profiling method was based on the use of a reversed phase column and a neutral water/acetonitrile gradient.

The metabolite pattern of the extracts of the current hen study and the hen study with the ^{14}C-label in the furanone ring of the molecule were comparable, except the label specific metabolites (see study 2 below).

The metabolic profile of excreta was similar to profiles from eggs, organs and tissues.

Therefore parent compound and metabolites were isolated from excreta and identified by LC-MS/MS or NMR. The identied metabolites were used as reference compounds for the assignment in extracts of eggs, muscle, fat and liver by HPLC cochromatography.

The overall recovery rate was 96.11% of the total dose. The remaining radioactivity was expected to still be present in the gastro-intestinal tract at sacrifice, due to the short period between the last administration and sacrifice. On average, only 0.24% of the total dose was measured in the eggs. At sacrifice, the radioactive residues in the organs and tissues were calculated or estimated to be about 0.37% of the total dose, half of which was detected in the skeletal muscle (0.19%). Until sacrifice, the excretion of radioactivity accounted for 95.51% of the total dose.

The concentration of radioactivity in eggs ranged from 0.016 mg/kg at day one to 0.119 mg/kg at sacrifice. Following a rather linear increase, a plateau level of approx. 0.08 mg/kg was reached six days after the first administration.

In the organs and tissues, the highest radioactivity concentrations were determined in kidney (1.073 mg/kg) and liver (0.435 mg/kg) indicating the significance of these organs for excretion and metabolism. These values corresponded to 0.05% and 0.08% of the total dose, respectively. The residue level of the eggs collected from the ovary and oviduct at sacrifice (0.147 mg/kg) was insignificantly higher than the levels of the laid eggs collected at sacrifice (0.119 mg/kg). This indicated that the egg yolk was not a preferential site for secretion. The residue levels of liver and kidney were followed in decreasing order by those found in the skin (0.094 mg/kg), muscle (0.070 mg/kg) and subcutaneous fat (0.021 mg/kg). The residue level of the total muscle corresponded to about 0.19% of the total dose assuming a value of 40% of the body weight for this tissue. Assuming values of 12% and 4% of the body weight for fat and skin, the residues in both tissues accounted for about 0.04% of the total dose.

Approx. 86% of TRR for eggs, 84% of TRR for muscle, 78% of TRR for fat and 59 % of TRR for liver were identified. All other residues were characterised by extraction and chromatographic behaviour. Generally, the amounts of parent compound and metabolites were very low.

The parent compound was the major compound in eggs, muscle and fat (approx. 10 to 20% of TRR) and amounted on a low level of ≤0.017 mg/kg, only. Major metabolites were BYI 02960-acetyl-AMCP in eggs, muscle and fat and BYI 02960-OH-SA in fat and liver. Other prominent metabolites were BYI 02960-lactato-mercaptyl-nicotinic acid, BYI 02960-6-CNA, BYI 02960-des-difluoroethyl and BYI 02960-OH. Minor residues were identified as BYI 02960-acetyl-cysteinyl-nicotinic acid, BYI 02960-des-difluoroethyl-OHSA and BYI 02960-AMCP-difluoroethanamine-SA. In addition, BYI 02960-acetic acid was detected in excreta. The results are shown in the Tables below.

Table 7.2.1-01 Distribution of residues in eggs, muscle, fat, liver and kidney of laying hens following oral administration of 14 daily doses of [pyridinylmethyl-^{14}C] BYI02960 at a dose rate of 1.02 mg/kg (mean of six hens)

Sample	Percent of total dose administered	Concentration of total radioactivity [mg/kg]
Liver	0.08	0.435
Kidney	0.05	1.073
Eggs from ovary/oviduct	0.01	0.147
Muscle, total	0.19	0.070
Skin, total	0.02	0.094
Fat, total	0.02	0.021
Organs/tissues, total	0.37	-----
Eggs	0.24	0.081
Excreta, total	95.51	-----
Total Recovery	96.11	-----

Table 7.2.1-02 Time course of total radioactive residues in eggs

Time after the first admin. [d]	Admin. no.	TRR [mg/kg]
0	1	-
1	2	0.016
2	3	0.044
3	4	0.061
4	5	0.068
5	6	0.075
6	7	0.080
7	8	0.083
8	9	0.083
9	10	0.087
10	11	0.090
11	12	0.087
12	13	0.085
13	14	0.088
13.25	-	0.119

The concentration of the total radioactivity (expressed as mg a.s. equiv. /kg), as well as the distribution of the parent compound and metabolites and the identification rates in eggs, liver, muscle and fat are summarised in the following table:

Table 7.2.1-03 Recovery of radioactivity in hen eggs, tissues and organs after 14 daily oral doses of 1.02 mg/kg body weight (equivalent to 16.18 mg a.s. /kg dry feed/day mg/kg feed as received)

		Eggs (day 3 to 13.25)		Muscle		Fat		Liver	
TRR [mg/kg]		0.084		0.070		0.021		0.435	
Sample/Report name BYI 02960-		% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Conventional extraction		96.1	0.081	92.6	0.064	79.7	0.017	74.6	0.324
lactato-mercaptyl-nicotinic acid	M33	4.0	0.003	3.6	0.002	---	---	15.5	0.068
acetyl-cysteinyl-nicotinic acid	M32	---	---	---	---	---	---	0.3	0.001
6-CNA	M27	7.2	0.006	8.8	0.006	1.8	<0.001	6.4	0.028
des-difluoroethyl-OH-SA	M20	---	---	2.1	0.001	5.6	0.001	3.1	0.014
acetyl-AMCP	M40	23.1	0.019	40.2	0.028	28.5	0.006	6.3	0.027
des-difluoroethyl	M19	8.9	0.007	9.9	0.007	5.0	0.001	1.8	0.008
AMCP-difluoroethanamine-SA	M37	---	---	---	---	---	---	0.3	0.001
OH-SA	M09	5.1	0.004	1.8	0.001	16.2	0.003	22.5	0.098
OH	M03	18.0	0.015	8.1	0.006	5.5	0.001	1.5	0.007
parent compound		19.8	0.017	9.8	0.007	15.3	0.003	0.9	0.004
Identified in conventional extract		86.2	0.072	84.2	0.059	77.9	0.016	58.6	0.255
Characterised in conventional extract		9.9	0.008	8.4	0.006	1.8	<0.001	16.0	0.070
Exhaustive extraction		n.a.		n.a.		n.a.		19.8	0.086
Total identified		**86.2**	**0.072**	**84.2**	**0.059**	**77.9**	**0.016**	**58.6**	**0.255**
Total characterised		**9.9**	**0.008**	**8.4**	**0.006**	**1.8**	**<0.001**	**35.8**	**0.156**
Total extracted		96.1	0.081	92.6	0.064	79.7	0.017	94.5	0.411
Solids		3.9	0.003	7.4	0.005	20.3	0.004	5.5	0.024
Accountability		**100.0**	**0.084**	**100.0**	**0.070**	**100.0**	**0.021**	**100.0**	**0.435**

The main metabolic reactions of [pyridinylmethyl-14C-]BYI 02960 in the laying hen were:

- hydroxylation in position 5 of the furanone ring forming BYI 02960-hydroxy followed by conjugation with sulfuric acid to BYI 02960-OH-SA
- oxidative cleavage of the pyridinylmethyl moiety forming BYI 02960-6-CNA
- substitution of the chloro group of BYI 02960-6-CNA with glutathione followed by degradation resulting into two conjugates BYI 02960-acetyl-cysteinyl-nicotinic acid and BYI 02960-lactato-mercaptyl-nicotinic acid
- cleavage of the difluoroethyl group forming BYI 02960-des-difluoroethyl followed by hydroxylation and conjugation with sulfuric acid to BYI 02960-des-difluoroethyl- OH-SA
- cleavage of the furanone ring and conjugation with sulfonic acid forming BYI 02960-AMCP-difluoroethanamine-SA
- oxidative degradation of the furanone ring forming BYI 02960-acetic acid
- cleavage of the pyridinylmethyl moiety forming an alcohol conjugated with serine (BYI 02960-CHMP-serinate)
- cleavage of the furanone ring and the difluoroethyl group forming an amine followed by acetylation (BYI 02960-acetyl-AMCP)

Conclusions

Metabolites in Eggs

Besides the parent compound (0.017 mg/kg; 19.8%), major metabolites in eggs were BYI 02960-acetyl-AMCP (0.019 mg/kg; 23.1%) and BYI 02960-OH (0.015 mg/kg, 18.0%). Further metabolites were BYI 02960-lactato-mercaptyl-nicotinic acid, BYI 02960-6-CNA, BYI 02960-des-difluoroethyl, BYI 02960-OH-SA and two unknown metabolites. They were detected in low amounts ≤0.007 mg/kg (≤ 8.5%TRR).

Metabolites in Muscle

The main metabolite in muscle was BYI 02960-acetyl-AMCP, which amounted to 0.028 mg/kg (40.2%). Parent compound, BYI 02960-6-CNA, BYI 02960-des-difluoroethyl and BYI 02960-OH were quantified in amounts between 0.006 to 0.007 mg/kg (approx. 9%). Minor identified metabolites were BYI 02960-lactato-mercaptyl-nicotinic acid, BYI 02960-des-difluoroethyl-OH-SA and BYI 02960-OH-SA (each ≤0.002 mg/kg). Two unknown metabolites were identified, which were detected in low amounts ≤0.007 mg/kg (≤ 8.5%TRR).

Metabolites in Fat

No residues were detected in the n-heptane phase. The main metabolites in the polar phase of fat were parent compound (0.003 mg/kg, 15.3%), BYI 02960-acetyl-AMCP (0.006 mg/kg, 28.5%) and BYI02960-OH-SA (0.003 mg/kg, 16.2%). All other metabolites were detected in amounts ≤0.001 mg/kg, including one unknown identified metabolite.

Metabolites in Liver

The majority of the radioactive residue (approx. 75%) was extracted with acetonitrile/water. The main metabolites in this extract were BYI 02960-OH-SA (0.098 mg/kg, 22.5%), BYI 02960-lactato-mercaptyl-nicotinic acid (0.068 mg/kg, 15.5%), BYI 02960-6-CNA (0.028 mg/kg, 6.4%) and BYI 02960-acetyl-AMCP (0.027 mg/kg, 6.3%). The parent compound was only detected in negligible amounts of 0.004 mg/kg. All other metabolites amounted to ≤0.014 mg/kg, except one unknown metabolite (0.027 mg/kg, 6.3%). A total of ten unknown metabolites were identified.

Guidelines & Limitations

The study results are reliable and acceptable.

STUDY 2

Characteristics

Reference	:	[redacted] 2012b KIIA 6.2.2/02	GLP statement	:	Yes, according to Japan MAFF GLP standard 11 Nousan 6283; US EPA – FIFRA GLP (40CFR Part 160); Principles of GLP, German Chemical Law, current version of Annex 1
Type of study	:	Metabolism in laying hen	Guideline	:	OECD-Guideline for the Testing of Chemicals No. 503: Metabolism in Livestock US-EPA, Residue Chemistry OPPTS 860.1300;
Year of execution	:	2010	Dose	:	14 consecutive days with an 1.05 mg/kg body weight
Test substance	:	(1) [Furanone-4-^{14}C]BYI02960, radiochemical purity > 98 % by HPLC and > 99 % by TLC (2) Nonlabeled testsubstance Batch BYI 02960-PU-02, chemical purity 99,4%	Vehicle	:	0.5 % aqueous Tragacanth® suspension
			Concentration in feed	:	17.13 mg a.s. /kg dry feed/day
			Sampling	:	Eggs, excreta, liver, gall bladder, kidneys, leg and breast muscle, skin without subcutaneous fat, subcutaneous fat and eggs from the ovary and oviduct
Animal	:	Laying hen (*Gallus gallus domesticus*) White Leghorn	Acceptability	:	acceptable

Study design

The metabolism and excretion of [furanone-4-^{14}C]BYI 02960 (common name: flupyradifurone) were investigated in laying hens (White leghorn). Six hens (mean bodyweight 1.56 kg) were orally dosed once daily in the morning for 14 consecutive days with an aqueous 0.5% Tragacanth® suspension of 1.05 mg of the active substance per kg body weight (3.50 MBq/mg after radiodilution) which corresponded to 17.13 mg a.s./kg dry feed/day ~~(13N–11N)~~. The animals were sacrificed six hours after the last administration. Total radioactive residues were determined daily in the eggs and excreta, and at sacrifice in the dissected organs and tissues (muscle, fat, liver, kidney, skin and eggs from ovary/oviduct). Eggs, muscle, fat, liver and excreta were extracted and analysed for parent compound and metabolites.

Results

For metabolism investigations, eggs of day 2 to 7 and 8 to 13.25, muscle, fat, liver and excreta from day 13 were pooled from all six animals. Eggs, muscle, fat and liver were conventionally extracted with acetonitrile and n-heptane followed by a partition procedure. In case of eggs, muscle and liver the extraction procedure was continued with a mixture of acetonitrile/water. Excreta were conventionally extracted with acetonitrile/water followed by pure acetonitrile.

Post-extraction solids of eggs, muscle and liver were exhaustively extracted with mixtures of acetonitrile/water followed by acetonitrile/water/formic acid using microwave assistance. The extraction efficiencies amounted to greater than 81% of TRR in all samples. Residues in the remaining solids were low and amounted between 0.006 (eggs day 2 to 7) and 0.036 mg/kg (liver).

The overall recovery rate was 82.16% of the total dose. A part of the remaining radioactivity was expected to still be present in the gastro-intestinal tract at sacrifice, due to the short period between

the last administration and sacrifice. Another part is probably exhaled as $^{14}CO^{2}$. The formation of carbon dioxide was also shown in a rat quantitative whole-body autoradiography study using the test compound with the same labelling position (B.6.1, study 4). An average amount of 2.35% of the total dose was determined in the eggs. At sacrifice, the residues in the organs and tissues were calculated or estimated to be about 1.8% of the total dose from which about 28% was detected in the skeletal muscle (0.5%). Until sacrifice, the excretion products accounted for 78.01% of the total dose.
The concentration of radioactivity in eggs ranged from 0.024 mg/kg at day one to 1.198 mg/kg at sacrifice. Following a linear increase a plateau level of 1.035 mg/kg was reached nine days after the first administration.

In the organs and tissues, the highest radioactivity concentrations were determined in liver (2.178 mg/kg) and kidney (1.083 mg/kg) indicating the significance of these organs for metabolism and excretion. These values corresponded to 0.37% and 0.05% of the total dose, respectively. The residue level of the eggs collected from the ovary and oviduct at sacrifice (2.774 mg/kg) was by a factor of 2.3 higher than the levels of the laid eggs collected at sacrifice (1.198 mg/kg). This indicated that the egg yolk was a preferential site for secretion of the radioactivity. The residue levels of liver and kidney were followed in decreasing order by those found in the subcutaneous fat (0.427 mg/kg), skin (0.257 mg/kg) and muscle (0.183 mg/kg). The residue level of the total muscle corresponded to about 0.50% of the total dose assuming a value of 40% of the body weight for this tissue. Assuming values of 12% and 4% of the body weight for fat and skin, the residues in both tissues accounted for about 0.41% of the total dose.
The major part of residues was detected in the n-heptane phases of eggs (days 2 to 7: approx. 52% of TRR and days 8 to 13.25: approx. 58% of TRR), fat (approx. 96% of TRR) and liver (approx. 52% of TRR). The residues in the n-heptane phase of muscle amounted to approx. 8% of TRR, only.
Residues in the conventional acetonitrile/water extract of eggs, muscle, fat and liver were detected in approx. the same residue level as analysed in the laying hen study with the pyridinylmethyl label. The residues amounted between approx. 0.011 mg/kg (2.6% of TRR) for fat and 0.450 mg/kg (20.7% of TRR) for liver.
A significant part of residues was exhaustively extracted using microwave assistance. Between approx. 4 and 10% of TRR were detected in the neutral exhaustive extracts and between 8 and 40% of TRR in the acidic exhaustive extracts of eggs, muscle and liver.
Losses of radioactivity during the extraction procedure of eggs, muscle and liver amounted between 5 and 18% of TRR. No significant radioactivity was detected in distillates. The radioactive residues in the n-heptane phases of eggs, muscle, fat and liver showed the same chromatographic behaviour (TLC) and were identified as fatty acids after saponification. These residues in the n-heptane phases were specific for the furanone label and were caused by the cleavage and subsequent total degradation of the furanone ring to smaller carbon units (C1- or C2-fragments), which entered into the carbon pool of endogenous compounds.

Residues, which were dissolved in the conventional acetonitrile/water extracts and exhaustive extracts were analysed by HPLC with radiometric detection. The metabolite patterns of the current hen study and the corresponding extracts of the hen study with the ^{14}C-label in the pyridinylmethyl ring(see above) were comparable, except the label specific metabolites.

Parent compound, BYI 02960-OH, BYI 02960-OH-SA, BYI 02960-des-difluoroethyl, BYI 02960-des-difluoroethyl-OH-SA were identified in the conventional acetonitrile/water extracts by comparing the retention times and the metabolite pattern from the current study with those from the laying hen study with the pyridinylmethyl label. The metabolites were quantified in nearly the same low amounts as detected in the conventional extracts of the study with the pyridinylmethyl label. In addition BYI 02960-acetic acid was detected in excreta.

The TRRs (expressed as mg a.s. equiv. /kg) and percent of TRR-values, distribution of the parent compound and metabolites and the identification rates in all edible samples are summarised in the table 7.2.2-01

Table 7.2.2-01 Distribution of residues in eggs, muscle, fat, liver and kidney of laying hens following oral administration of 14 daily doses of [furanone-4-^{14}C] BYI02960 pyridinylmethyl-^{14}C] BYI02960 at a dose rate of 1.05 mg/kg

Sample	Percent of total dose administered	Concentration of total radioactivity [mg/kg]
Liver	0.37	2.178
Kidney	0.05	1.083
Eggs from ovary/oviduct	0.46	2.774
Muscle, total	0.50	0.183
Skin, total	0.07	0.257
Fat, total	0.34	0.427
Organs/tissues, total	1.80	-----
Eggs	2.35	0.757
Excreta, total	78.01	-----
Total Recovery	82.16	-----

Table 7.2.2-02 Time course of total radioactive residues in eggs

Time after the first admin. [d]	Admin. no.	TRR [mg/kg]
0	1	-
1	2	0.024
2	3	0.146
3	4	0.284
4	5	0.438
5	6	0.647
6	7	0.789
7	8	0.923
8	9	1.002
9	10	1.035

Time after the first admin. [d]	Admin. no.	TRR [mg/kg]
10	11	1.035
11	12	1.100
12	13	1.036
13	14	1.005
13.25	-	1.198

The concentration of the total radioactivity (expressed as mg a.s. equiv. /kg) as well as the distribution of the parent compound and metabolites and the identification rates in all edible samples are summarised in the following table:

Table 7.2.2-03 Recovery of radioactivity in hen eggs, tissues and organs after 14 daily oral doses of 1.05 mg/kg body weight (equivalent to 17.13 mg a.s. /kg dry feed/day mg/kg feed as received)

		Eggs (day 2 to 7)		Eggs (day 8 to 13.25)		Muscle		Fat		Liver	
TRR [mg/kg]		0.540		1.048		0.183		0.427		2.178	
Sample/Report name BYI 02960-		% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Conventional extraction		65.0	0.351	66.8	0.701	38.6	0.071	98.5	0.421	72.1	1.571
n-heptane phase (identified as fatty acids)		52.0	0.281	58.3	0.611	8.1	0.015	95.9	0.410	51.5	1.121
des-difluoroethyl-OH-SA	M20	0.1	0.001	n.d.	n.d.	0.5	0.001			0.2	0.004
des-difluoroethyl	M19	1.2	0.006	0.6	0.007	2.6	0.005			0.8	0.017
OH-SA	M09	0.6	0.003	0.5	0.005	n.d.	n.d.			5.1	0.112
OH	M03	2.3	0.013	1.6	0.016	2.4	0.004			0.8	0.018
Parent compound		2.3	0.013	1.6	0.016	2.9	0.005			0.5	0.010
identified in the conventional extract		58.5	0.316	62.5	0.656	16.5	0.030	95.9	0.410	58.9	1.282
characterised in the conventional extract		6.4	0.035	4.3	0.045	22.0	0.040	2.6	0.011	13.2	0.288
losses during the conventional extraction		10.7	0.058	10.7	0.112	10.3	0.019	n.d.		n.d.	
Exhaustive extraction											
- neutral ACN/water extract		7.0	0.038	3.6	0.038	8.1	0.015	n.a.		9.6	0.209
- 1st acidic ACN/water extract		8.3	0.045	10.0	0.105	39.5	0.072	n.a.		12.1	0.264
- 2nd acidic ACN/water extract		1.1	0.006	3.3	0.035	see **		n.a.		n.a.	
characterised in the exhaustive extracts		16.5	0.089	16.9	0.177	47.7	0.087	n.a.		21.7	0.473
Total identified		58.5	0.316	62.5	0.656	16.5	0.030	95.9	0.410	58.9	1.282
Total characterised		**22.9**	**0.124**	**21.2**	**0.223**	**69.7**	**0.128**	**2.6**	**0.011**	**34.9**	**0.761**
Total extracted		**81.5**	**0.440**	**83.8**	**0.878**	**86.2**	**0.158**	**98.5**	**0.421**	**93.8**	**2.044**
Total losses		17.5	0.094	14.7	0.155	10.3	0.019	n.d.	n.d.	4.5	0.098
Solids		1.1	0.006	1.5	0.016	3.5	0.006	1.5	0.006	1.7	0.036
Accountability		**100.0**	**0.540**	**100.0**	**1.048**	**100.0**	**0.183**	**100.0**	**0.427**	**100.0**	**2.178**

** Both acidic ACN/water extracts of muscle were combined before concentration.

The main metabolic reactions of [furanone-4-^{14}C-]BYI 02960 in the laying hen were:

- cleavage and subsequent total degradation of the furanone ring forming smaller carbon units (C1- or C2-fragments), which entered into the carbon pool of endogenous compounds and were used for example for the biosynthesis of fatty acids
- hydroxylation in position 5 of the furanone ring forming BYI 02960-hydroxy followed by conjugation with sulphuric acid to BYI 02960-OH-SA
- cleavage of the difluoroethyl group forming BYI 02960-des-difluoroethyl followed by hydroxylation and conjugation with sulfuric acid to BYI 02960-des-difluoroethyl-OH-SA
- oxidative degradation of the furanone ring forming BYI 02960-acetic acid

Conclusions

Metabolites in Eggs

The main part of the radioactivity in eggs (days 2 to 7 0.281 mg/kg, 52.0%; days 8 to sacrifice 0.611 mg/kg, 58.3%) was detected in the n-heptane phase and identified as natural fats. The parent compound, BYI 02960-OH, BYI 02960-OH-SA, BYI 02960-des-difluoroethyl and BYI 02960-des-difluoroethyl-OH-SA were identified in low amounts (≤ 0.016 mg/kg, ≤1.6%). Polar metabolites were not further quantified due to the low concentration (≤ 0.010 mg/kg).

Metabolites in Muscle

Residues in the n-heptane phase of muscle were identified as natural fats and amounted to 0.015 mg/kg (8.1%), only. Very low amounts (≤ 0.005 mg/kg) of parent compound, BYI 02960-OH, BYI 02960-des-difluoroethyl and BYI 02960-des-difluoroethyl-OH-SA were identified in the acetonitrile/water extract. Metabolites in the polar region R1 of the acetonitrile/water extract were further analysed and subquantified by thin layer chromatography. The concentrations ranged from 0.001 to 0.012 mg/kg (0.4-6.6%). The commonality of these metabolites with polar metabolites, which were detected in the urine of rats and in the urine, liver and kidney of the goat, was demonstrated by comparison of the thin layer chromatograms.

Metabolites in the acidic exhaustive extract represented the major portion of residues in the muscle sample (0.072 mg/kg, 39.5%). They were characterised by their extraction behaviour. An HPLC analysis could not be performed, due to the high matrix burden in the extract.

Metabolites in Fat

The dominating part of the radioactive residues in fat (0.410 mg/kg. 95.9%) was identified as fatty acids after saponification. The fatty acids were formed from smaller carbon units (C-1- or C-2-fragments), which entered the carbon pool of endogenous compounds after cleavage and subsequent degradation of the furanone ring. A small part (0.011 mg/kg, 2.6%) was detected in the acetonitrile phase and was not further investigated.

Metabolites in Liver

More than half of the residues in liver (1.121 mg/kg, 51.5%) was identified as natural fats. Parent compound, BYI 02960-OH, BYI 02960-OH-SA, BYI 02960-des-difluoroethyl and BYI 02960-des-difluoroethyl-OH-SA were identified in the acetonitrile/water extract. The most prominent metabolite was BYI 02960-OH-SA which amounted to 0.112 mg/kg (5.1%). All other identified metabolites were detected in low amounts between 0.004 and 0.050 mg/kg (0.2-2.3%).

Guidelines & Limitations

The study results are reliable and acceptable.

Ruminants

STUDY 1

Characteristics

Reference	:	[redacted] 2011a KIIA 6.2.3/01	GLP statement	:	Yes
Type of study	:	Metabolism in lactating goat	Guideline	:	OECD-Guideline for the Testing of Chemicals No. 503: Metabolism in Livestock US-EPA, Residue Chemistry OPPTS 860.1300; European Parliament and Council Regulation (EC) No 1107/2009
Year of execution	:	2010-2011	Dose	:	5 consecutive days with at 0.92 mg/kg bw/d
Test substance	:	(1) [Pyridinyl,ethyl-^{14}C]BYI02960, radiochemical purity > 99 % by radio-HPLC (2) Non-labelled tes tsubstance Batch BYI 02960-PU-02, chemical purity 99,4%	Vehicle Concentration in feed Sampling	: : :	60.41 mg as/day by capsule 24.4 mg a.s. /kg dry feed/day Milk, edible organs and tissues and urine
Animal	:	Lactating goat (*Capra hircus*) 'Weiße Deutsche Edelzeige'	Acceptability	:	acceptable

Study design

The metabolism and excretion of [pyridinylmethyl-^{14}C]BYI 02960 were investigated in the lactating goat. The goat (66 kg body weight) was orally dosed once daily for five consecutive days in the morning after milking with 1.0 mg of the active substance per kg body weight (3.425 MBq/mg after radio dilution) which corresponded to 24.4 mg a.s. /kg dry feed/day ~~(9N)~~. The animal was sacrificed about six hours after the last administration. Total radioactive residues (TRR) were determined in milk and excreta at various sampling intervals, and in muscle, fat, kidney and liver at sacrifice. Milk, edible organs and tissues as well as urine were analysed for the unchanged parent compound and metabolites.

Results

The overall recovery amounted to 88.75% of the total dose. Much of the remaining radioactivity (ca. 11%) was expected to be still present in the gastro-intestinal tract at sacrifice, due to the short period between the last administration and sacrifice. An amount of 0.78% of the total dose was secreted with

the milk. At sacrifice, the residues in the organs and tissues were calculated or estimated to be 2.94% of the total dose from which about 71% was detected in the skeletal muscle.

Until sacrifice, the excretion of radioactivity accounted for 85.02% of the total dose. A portion of 71.74% was found in the urine and 13.28% in the faeces. The urinary and faecal excretion started immediately after the first administration.
The concentration of radioactivity in milk samples ranged from 0.053 mg/kg at 96 hours to 1.345 mg/kg at sacrifice, 102 hours after the first administration. The time course in the evening and morning milk pool samples showed a pronounced diurnal pattern. The radioactive residues increased significantly during the eight hour period after each administration followed by a decrease to a very low level of about 0.05 mg/kg measured prior to the next dosing. A stable plateau level of about 0.3 mg/kg was reached already at 8 hours after the first administration.

In the organs and tissues, the highest concentrations were determined in kidney (1.869 mg/kg) and liver (1.215 mg/kg) indicating the significance of these organs for excretion and metabolism. These values corresponded to 0.10% and 0.50% of the total dose, respectively. For muscle and fat 0.356 mg/kg and 0.106 mg/kg, respectively, were determined. The radioactivity concentration of the muscle corresponded to 2.10% and that of fat to 0.25% of the total dose assuming a value of 30% and 12% of the body weight for these tissues.
For analysis of parent compound and metabolites, milk, muscle, kidney and liver were extracted with mixtures of acetonitrile/water. For milk an additional extraction step with acetone was performed. Fat was extracted with mixtures of n-heptane and acetonitrile/water followed by a solvent partitioning step yielding an n-heptane and an acetonitrile/water phase. No residues were found in the n-heptane phase of fat. The resulting extracts of milk, muscle, fat, kidney and liver represented between 92.9% and 99.8% of the total radioactive residue. Unextractable residues in milk, muscle and fat were below 0.002 mg/kg. For kidney and liver they were 0.021 mg/kg and 0.086 mg/kg, respectively.
After purification and concentration, the extracts were analysed by HPLC with radiometric detection. The HPLC profiling method was based on the use of a reversed phase column and a neutral water/acetonitrile gradient. The metabolite pattern of the extracts of the current goat study and the goat study with the furanone label (study 2, see below) were comparable with the exception of the label specific metabolites.
All prominent metabolites of the extracts of milk, muscle, fat, kidney and liver were also detected in urine. Therefore, the isolation and identification of metabolites were performed using the urine samples. Isolated metabolites were identified by LC-MS/MS and used as radiolabelled reference compounds for the identification in milk, muscle, fat, kidney and liver by HPLC co-chromatography. The identification of the minor metabolite BYI 02960-AMCP-difluoroethanamine was performed in the liver by HPLC co-chromatography with the non-radiolabelled reference compound.
Approximately 99% of the total radioactivity in milk, muscle, fat and kidney and ca. 93% in liver was identified. All other residues were characterised by their extraction behaviour. The unchanged parent

compound was by far the major constituent of the residue in milk, organs and tissues and amounted to 0.165 mg/kg (88.8%) for milk, 0.349 mg/kg (98.0%) for muscle, 0.105 mg/kg (99.2%) for fat, 0.650 mg/kg (34.8%) for kidney and 1.028 mg/kg (84.6%) for liver.

Extensive metabolism was detected in the kidney, where the main portion of metabolites was found. The major metabolite in the kidney was BYI 02960-OH, with a concentration of 0.299 mg/kg (16.0%). Four prominent glucuronic acid conjugates of the hydroxylated parent compound were detected in the kidney as well as in the urine. The four isomers were named BYI 02960-OH-gluA (isomer 1 to isomer 4); their concentration ranged from 0.112 mg/kg (6.0%) to 0.175 mg/kg (9.3%). BYI 02960-OH-gluA (isomer 2) and BYI 02960-OH-gluA (isomer 3) are diastereomers, which are hydroxylated and conjugated in the 5-position of the furanone ring. BYI 02960-OH-gluA (isomer 4) is hydroxylated and conjugated in the difluoroethyl side chain of the molecule and BYI 02960-OH-gluA (isomer 1) in an unknown position.

A further metabolite was BYI 02960-hippuric acid, which was detected in milk (0.017 mg/kg, 9.1%) and kidney (0.178 mg/kg, 9.5%). Traces of BYI 02960-hippuric acid were also found in the liver (0.010 mg/kg, 0.8%). BYI 02960-cysteinyl-nicotinic acid was identified in the kidney (0.114 mg/kg, 6.1%) and in the liver (0.058 mg/kg, 4.8%). Minor identified metabolites were BYI 02960-methylthio-glyoxylic acid in milk and muscle and BYI 02960-AMCP-difluoroethanamine in kidney and liver. The concentration of each was below 0.020 mg/kg.

The concentration of the total radioactivty (expressed as mg a.s. equiv. /kg) as well as the distribution of the parent compound and metabolites and the identification rates in milk and edible tissues are summarised in the table 7.2.3-03

Table 7.2.3-01 Distribution of residues in milk, muscle, fat, liver and kidney of lactating goats following oral administration of 5 daily doses of [pyridinylmethyl-^{14}C] BYI02960 at a dose rate of 0.92 mg/kg

Sample	Percent of total dose administered	Concentration of total radioactivity [mg/kg]
Liver	0.50	1.215
Kidney	0.10	1.869
Body muscle, total	2.10	0.356
Body Fat, total	0.25	0.106
Organs/tissues, total	2.94	-----
Milk, 0-102h	0.78	0.177
Excreta, total	85.02	-----
Total Recovery	88.75	-----

Table 7.2.3-02 Time course of total radioactive residues in milk

Dose nr.	Time after the first admin. [h]	Net weight [g]	Secretion per period [% of total dose admin.]	TRR [mg/kg]	µg a.s. equivalents
1	0	-	-	-	-
	8	1,226.42	0.12	0.292	357.62
	24 (PA)	1,965.00	0.04	0.060	117.96
2	32	1,318.20	0.15	0.336	442.39
	48 (PA)	1,743.77	0.03	0.055	96.33
3	56	1,292.06	0.14	0.318	411.26
	72 (PA)	1,715.47	0.03	0.055	94.57
4	80	1,306.57	0.14	0.318	415.62
	96 (PA)	1,791.55	0.03	0.053	94.27
5	102	916.37	0.10	1.345	316.42
Total 0 - 102 h		13,2275.41			2,346.45
Mean TRR 0 - 102 h [mg/kg]				0.177	

Table 7.2.3-03 Recovery of radioactivity in goat milk, tissues and organs after 6 daily oral doses of 0.92 mg/kg body weight (equivalent to 24.4 mg a.s. /kg dry feed/day mg/kg feed as received)

		Milk (24 to 102 h)		Muscle		Fat		Kidney		Liver	
TRR [mg/kg]		0.186		0.356		0.106		1.869		1.215	
Sample/ Report name BYI 02960-		% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
ACN/water extract		99.3	0.184	99.4	0.353	99.2	0.105	98.8	1.847	92.8	1.128
cysteinyl-nicotinic acid	M31	---	---	---	---	---	---	6.1	0.114	4.8	0.058
hippuric acid	M30	9.1	0.017	---	---	---	---	9.5	0.178	0.8	0.010
methylthio-glyoxylic acid	M18	1.5	0.003	1.3	0.005	---	---	---	---	---	---
OH-gluA (isomer 1)	M04	---	---	---	---	---	---	6.0	0.112	---	---
OH-gluA (isomer 2)	M05	---	---	---	---	---	---	9.3	0.175	1.4	0.016
OH-gluA (isomer 3)	M06	---	---	---	---	---	---	8.4	0.158	---	---
OH-gluA (isomer 4)	M07	---	---	---	---	---	---	7.5	0.141	---	---
AMCP-difluoroethanamine	M36	---	---	---	---	---	---	1.1	0.020	1.2	0.015
OH	M03	---	---	---	---	---	---	16.0	0.299	---	---
Parent compound		88.8	0.165	98.0	0.349	99.2	0.105	34.8	0.650	84.6	1.028
Total identified		**99.3**	**0.184**	**99.4**	**0.353**	**99.2**	**0.105**	**98.8**	**1.847**	**92.8**	**1.128**
Total extracted		**99.5**	**0.185**	**99.5**	**0.354**	**99.8**	**0.106**	**98.9**	**1.848**	**92.9**	**1.129**
Solids		0.5	0.001	0.5	0.002	0.2	<0.001	1.1	0.021	7.1	0.086
Accountability		**100.0**	**0.186**	**100.0**	**0.356**	**100.0**	**0.106**	**100.0**	**1.869**	**100.0**	**1.215**

The metabolic reactions of [pyridinylmethyl-14C-]BYI 02960 in the lactating goat are:

- Hydroxylation followed by conjugation with glucuronic acid forming two diastereomeric conjugates of BYI 02960-OH (BYI 02960-OH-gluA, isomer 2 and 3), the hydroxylation and conjugation being in the 5-position of the furanone ring. One isomer (BYI 02960-OH-gluA, isomer 4) with hydroxylation and conjugation in the difluoroethyl side chain and one isomer (BYI 02960-OH-gluA, isomer 1) with hydroxylation and conjugation in an unknown position
- Oxidative cleavage of the pyridinylmethyl bridge forming BYI 02960-6-CNA followed by conjugation with glycine to form BYI 02960-hippuric acid
- Substitution of the chlorine atom of BYI 02960 with glutathione followed by degradation resulting in the conjugate BYI 02960-cysteine
- Oxidative cleavage of the pyridinylmethyl bridge of BYI 02960-cysteine forming BYI 02960-cysteinyl-nicotinic acid
- Further degradation of BYI 02960-cysteine in the cysteine group and the furanone ring forming BYI 02960-methylthio-glyoxylic acid
- Cleavage of the furanone ring forming BYI 02960-AMCP-difluoroethanamine
- Cleavage of the difluoroethyl group forming BYI 02960-des-difluoroethyl

Study 2

Characteristics

Reference	:	[redacted], 2011b KIIA 6.2.3/02	GLP statement	:	Yes
Type of study	:	Metabolism in lactating goat	Guideline	:	OECD-Guideline for the Testing of Chemicals No. 503: Metabolism in Livestock US-EPA, Residue Chemistry OPPTS 860.1300; European Parliament and Council Regulation (EC) No 1107/2009
Year of execution	:	2010-2011	Dose	:	5 consecutive days with at 1.0 mg/kg bw/d
Test substance	:	(1) [Furanone-4-^{14}C]BYI02960, radiochemical purity > 99 % by radio-HPLC (2) Non-labelled tes tsubstance Batch BYI 02960-PU-02, chemical purity 99,4%	Vehicle Concentration in feed Sampling	: : :	47.01 mg as/day by capsule 28.82 mg a.s. /kg dry feed/day Milk, edible organs and tissues and urine
Animal	:	Lactating goat (*Capra hircus*) 'Weiße Deutsche Edelzeige'	Acceptability	:	acceptable

Study design

The metabolism and excretion of [furanone-4-^{14}C]BYI 02960 (common name: flupyradifurone) were investigated in the lactating goat. The goat (bodyweight 55 kg) was orally dosed once daily for five consecutive days in the morning after milking with 1.0 mg of the active substance per kg body weight (3.50 MBq/mg after radiodilution) which corresponded to 28.82 mg a.s. /kg dry feed/day ~~(11N)~~. The animal was sacrificed about six hours after the last administration. Total radioactive residues (TRR) were determined in milk and excreta at various sampling intervals, and in muscle, fat, kidney and liver

at sacrifice. Milk, edible organs and tissues and urine were analysed for the unchanged parent compound and metabolites.

Results

The overall recovery amounted to 78.94% of the total dose. Much of the remaining radioactivity (ca. 21%) was expected to still be present in the gastro-intestinal tract at sacrifice, due to the short period between the last administration and sacrifice. A significant proportion is probably exhaled as $^{14}CO^2$ since the formation of carbon dioxide had been clearly shown in a rat quantitative whole body autoradiography study using the test compound labelled at the same position (see B.6.1, study 4). An amount of 2.58% of the total dose was secreted with the milk. At sacrifice, the residues in the organs and tissues were calculated or estimated to be 4.22% of the total dose from which about 69% was detected in the skeletal muscle.

Until sacrifice, the excretion of radioactivity accounted for 72.14% of the total dose. A portion of 69.15% was found in the urine and 3.0% in the faeces. The urinary and faecal excretion started immediately after the first administration.

Total Radioactive Residues in Milk, Organs and Tissues

The concentration of radioactivity in milk samples ranged from 0.755 mg/kg at 24 hours to 1.213 mg/kg at 56 hours after the first administration. At sacrifice, the residue concentration was 1.165 mg/kg. The time course in the evening and morning milk pool samples showed a pronounced diurnal pattern. The residues increased significantly during the eight hour period after the second and the fifth administration followed by a decrease measured prior to the delivery of the next dose. A plateau level of about 1.1 mg/kg was reached ca. 50 hours after the first administration.

In the organs and tissues, the highest concentrations were determined in liver (1.746 mg/kg) and kidney (1.472 mg/kg) indicating the significance of these organs for excretion and metabolism. These values corresponded to 0.65% and 0.09% of the total dose, respectively. For muscle and fat 0.539 mg/kg and 0.265 mg/kg, respectively, were determined. The radioactivity concentration of the muscle corresponded to 2.91% and of fat to 0.57% of the total dose assuming a value of 30% and 12% of the body weight for these tissues.

For analysis of parent compound and metabolites, milk, muscle, kidney and liver were extracted with mixtures of acetonitrile/water. Fat was extracted with mixtures of n-heptane and acetonitrile/water followed by a solvent partition yielding an n-heptane and acetonitrile/water phase. Residues in the n-heptane phase of fat amounted to only 0.014 mg/kg (5.4%). The resulting acetonitrile/water extracts of milk, muscle, fat, kidney and liver represented between 73.3% and 95.0% of the total radioactive residue. Solids of kidney and liver were exhaustively extracted with mixtures of acetonitrile/water, 0.1 N HCl and 0.1 N NaOH using microwave assistance. The exhaustive extracts were not further investigated, due to their low amount of residues and/or their high matrix content. Unextractable residues in milk, muscle and fat were low and amounted to ≤0.090 mg/kg. The extraction residues of kidney and liver were completely solubilised after the exhaustive extraction with sodium hydroxide.

After purification and concentration, the conventional acetonitrile/water extracts were analysed by HPLC with radiometric detection. The HPLC profiling method was based on the use of a reversed phase column and a neutral water/acetonitrile gradient. The metabolite pattern of the extracts of the current goat study and the goat study with the pyridinylmethyl label (study 1, see above) were comparable with the exception of the label specific metabolites.

All prominent metabolites of the extracts of milk, muscle, fat, kidney and liver were also detected in urine, except lactose in the milk. Therefore, the assignment of metabolites in the profiles of the extracts was performed based on the comparison with the urine profiles of the current study and the study with the pyridinylmethyl label. Four isomers of BYI 02960-OH-gluA were isolated and identified by comparison of the LC-MS/MS data. Further identification of the BYI 02960-OH-gluA isomers was achieved by comparison of the degradation rates of isomers with the increasing rates of their aglycones and decomposition product, based on the observed decomposition of the isomers in two urine samples. The radioactive lactose in the milk was identified after isolation and acetylation by HPLC co-chromatography with the radiolabelled reference compound.

Approximately 91% of the total residue in milk, ca. 90% in muscle, 83% in fat, 79% in kidney and ca. 60% in liver was identified. All other residues were characterised by their extraction behaviour. The unchanged parent compound was the main constituent of the residue in organs and tissues and amounted to 0.475 mg/kg (88.1%) for muscle, 0.213 mg/kg (80.5%) for fat, 0.744 mg/kg (50.5%) for kidney and 1.045 mg/kg (59.8%) for liver. Lower amounts of parent compound (0.250 mg/kg, 23.9%) were found in the milk. The major part of residues in the milk was radioactive lactose and amounted to 0.698 mg/kg (66.8%). The lactose was specific for the furanone label and was formed by cleavage and subsequent degradation of the furanone ring to smaller carbon units (C-1- or C-2-fragments), which entered the carbon pool of endogenous compounds.

Extensive metabolism was detected in the kidney, where the main portion of metabolites was found. The main metabolite in kidney was BYI 02960-OH (hydroxylation in the 5-position of the furanone ring), which amounted to 0.215 mg/kg (14.6%). Four prominent glucuronic acid conjugates of hydroxylated parent compound were detected in the kidney and in the urine. The four isomers were named BYI 02960-OH-gluA (isomer 1 to isomer 4). Their concentrations were between 0.032 mg/kg (2.2%) and 0.069 mg/kg (4.7%) in the kidney. BYI 02960-OH-gluA (isomer 2) and BYI 02960-OH-gluA (isomer 3) were diastereomers, which were hydroxylated and conjugated in 5-position of the furanone ring. BYI 02960-OH-gluA (isomer 4) was hydroxylated and conjugated in the difluoroethyl side chain and BYI 02960-OH-gluA (isomer 1) in an unknown position. A minor metabolite was BYI 02960-des-difluoroethyl which amounted to 0.019 mg/kg (1.3%) in the kidney.

Metabolites in the polar region of the acetonitrile/water extracts (≤ 5 min) were specific for the furanone label. Their concentrations ranged from 0.021 to 0.037 mg/kg for kidney and from 0.019 to 0.059 mg/kg for liver. These polar metabolites showed the same thin layer chromatographic behaviour as the metabolites, which were detected in the corresponding polar region of rat urine (see B.6.1, study 4) and the extracts of muscle and liver of laying hen (see B.7.2, hen study 2). The polar region of muscle and fat was not further analysed, due to the low amount of radioactivity (≤ 0.024 mg/kg).

The formation of radioactive lactose and polar metabolites was mainly caused by the partial instability of the radiolabel used. For a small portion of the total dose the furanone ring of the molecule obviously underwent extensive biotransformation to C-1- and C-2-fragments resulting in an accumulation of radioactivity in biomolecules during the dosing for five days. A part of these fragments was probably also converted to the terminal product $^{14}CO_2$, which might explain the lower overall recovery as compared to the study with the metabolically stable pyridinylmethyl label.

The concentration of the total radioactivty (expressed as mg a.s. equiv. /kg) as well as the distribution of the parent compound and metabolites and the identification rates in milk and edible tissues are summarised in table 7.2.4-02.

Table 7.2.4-01 Distribution of residues in milk, muscle, fat, liver and kidney of lactating goats following oral administration of 5 daily doses of [furanone-4-^{14}C]BYI 02960 at a dose rate of 0.85 mg/kg

Sample	Percent of total dose administered	Concentration of total radioactivity [mg/kg]
Liver	0.65	1.746
Kidney	0.09	1.472
Body muscle, total	2.91	0.539
Body Fat, total	0.57	0.265
Organs/tissues, total	4.22	-----
Milk, 0-102h	2.58	0.961
Excreta, total	72.14	-----
Total Recovery	78.94	-----

Table 7.2.4-02 Time course of total radioactive residues in milk

Dose nr.	Time after the first admin. [h]	Net weight [g]	Secretion per period [% of total dose admin.]	TRR [mg/kg]	µg a.s. equivalents
1	0	-	-	-	-
	8	861.13	0.30	0.820	706.13
	24 (PA)	1,180.19	0.38	0.755	890.45
2	32	871.11	0.42	1.130	984.35
	48 (PA)	873.22	0.30	0.814	710.63
3	56	528.64	0.27	1.213	641.24
	72 (PA)	558.54	0.24	0.997	556.92
4	80	448.99	0.23	1.205	541.03
	96 (PA)	714.86	0.30	0.992	709.43
5	102	288.03	0.14	1.165	335.55
Total 0 - 102 h					6,075.74
Mean TRR 0 - 102 h [mg/kg]				0.961	

Table 7.2.4-03 Recovery of radioactivity in goat milk, tissues and organs after 6 daily oral doses of 0.85 mg/kg body weight (equivalent to 28.8 mg a.s. /kg dry feed/day mg/kg feed as received)

		Milk (24 to 102 h)		Muscle		Fat		Kidney		Liver	
TRR [mg/kg]		1.046		0.539		0.265		1.472		1.746	
Sample/ Report name BYI 02960-		% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
ACN/water extract		90.7	0.948	95.0	0.512	88.4	0.234	89.1	1.311	73.3	1.280
Lactose	M46	66.8	0.698	---	---	---	---	---	---	---	---
Polar metabolites		---	---	4.4	0.024	5.0	0.013	10.0	0.148	12.1	0.211
OH-gluA (isomer 1)	M04	---	---	---	---	---	---	2.2	0.032	---	---
OH-gluA (isomer 2)	M05	---	---	---	---	---	---	2.2	0.032	---	---
OH-gluA (isomer 3)	M06	---	---	---	---	---	---	4.7	0.069	---	---
OH-gluA (isomer 4)	M07	---	---	---	---	---	---	3.5	0.052	---	---
Des-difluoroethyl	M19	---	---	---	---	---	---	1.3	0.019	---	---
OH	M03	---	---	1.8	0.010	2.9	0.008	14.6	0.215	---	---
Parent compound		23.9	0.250	88.1	0.475	80.5	0.213	50.5	0.744	59.8	1.045
Total identified		**90.7**	**0.948**	**89.9**	**0.484**	**83.4**	**0.221**	**79.0**	**1.163**	**59.8**	**1.045**
Total extracted		**91.4**	**0.956**	**95.2**	**0.513**	**94.1**	**0.249**	**100.0**	**1.472**	**100.0**	**1.746**
Solids		8.6	0.090	4.8	0.026	5.9	0.016	n.d.	n.d.	n.d.	n.d.
Accountability		**100.0**	**1.046**	**100.0**	**0.539**	**100.0**	**0.265**	**100.0**	**1.472**	**100.0**	**1.746**

The metabolic and excretion behaviour of [furanone-4-14C]BYI 02960 in the lactating goat can be characterised by the following observations:

- The concentration of radioactivity in milk and edible tissues were relatively high compared to the dose level and the dosing period of five days. This was mainly caused by the partial instability of the radiolabel used. For a small portion of the total dose the furanone ring obviously underwent extensive biotransformation to C1- and C2-fragments that resulted in an accumulation of radioactivity in biomolecules during dosing for five days.
  A part of these fragments was probably also converted to the terminal product $14CO_2$ as can be derived from the lower overall recovery compared to the study with the metabolically stable pyridinylmethyl label.
- However, the fact should be considered that an exaggerated dose level of 28.82 mg/kg feed/day was administered. Furthermore, the significant amount of radioactivity detected in urine and faeces and the relatively high radioactivity in liver and kidney at sacrifice indicate that the residues are further metabolised and finally eliminated.
- The total radioactive residues in milk showed a diurnal pattern after the second administration as they declined significantly prior to the delivery of the next dose. A residue plateau level was reached after the third administration.
- The radioactive residues were efficiently extracted from milk as well as from edible organs and tissues; extraction rates were between 91.4% and 100.0%.

- Parent compound was the main constituent of the residues in muscle (ca. 88%), fat (ca. 81%), kidney (ca. 51%) and liver (ca. 60%). Approximately 24% of the total radioactivity in the milk was identified as parent compound.
- The main residue in the milk was radioactive lactose (approx. 67%)
- Extensive metabolism was detected in the kidney. BYI 02960-OH, four glucuronic acid conjugates of hydroxylated BYI 02960 and BYI 02960-des-difluoroethyl were identified.
    - The main metabolic reactions in the lactating goat are:
    - Cleavage and subsequent degradation of the furanone ring forming small carbon units (C-1- or C-2-fragments), entering the carbon pool of endogenous compounds and finally being used for example for the biosynthesis of lactose
    - Hydroxylation followed by conjugation with glucuronic acid forming two diastereomeric conjugates of BYI 02960-OH (BYI 02960-OH-gluA, isomer 2 and 3) with hydroxylation and conjugation in the 5-position of the furanone ring, one isomer (BYI 02960-OH-gluA, isomer 4) with the hydroxylation and conjugation in the difluoroethyl side chain and one isomer (BYI 02960-OH-gluA, isomer 1) with the hydroxylation and conjugation in an unknown position
    - Cleavage of the difluoroethyl group forming BYI 02960-des-difluoroethyl

B.7.2.2 List of identified compounds

For a full list of metabolites, see Annex I at the end of this chapter.

B.7.2.3 Metabolic pathway

Figure 7.2.3-1: Proposed metabolic pathway of [pyridinylmethyl-14C] BYI02960 in laying hens

Figure 7.2.3-2: Proposed metabolic pathway of [furanone-4-14C] BYI02960 in laying hens

Figure7.2.3-3: Proposed metabolic pathway of [pyridinylmethyl-14C] BYI02960 in the lactating goat

Figure 7.2.3-4: Proposed metabolic pathway of [furanone-4-14C] BYI02960 in the lactating goat

Pigs

The dominating constituent of the residue in the milk and the edible organs and tissues of the goat is the unchanged parent compound. As the main metabolite, the hydroxylated derivative (BYI 02960-OH) was identified mainly in the kidney. Further metabolites in the kidney and partly also in the liver were determined as glucuronide conjugates of BYI 02960-OH and as BYI 02960 hippuric acid which is the glycine conjugate of 6-chloronicotinic acid. All these metabolites also do occur in the rat in significant amounts (KIIA 5.1). A few minor metabolites were found exclusively in the goat. These are BYI 02960 methylthio-glyoxylic acid which occurred in milk and muscle at concentrations below 0.005 mg/kg and BYI 02960 AMCP-difluoroethanamine which was found in kindey and liver also at low concentrations (< 0.020 mg/kg).

The only major goat metabolite which was not found in the rat is BYI 02960 cysteinyl-nicotinic acid. This was found in the kidney (0.114 mg/kg) and the liver (0.058 mg/kg). This compound is formed as a product of a typical detoxification reaction by conjugation of 6-chloronicotinic acid with cysteine under loss of the chlorine atom. The compound is very polar and easily excretable via urine which is supported by the fact that it was only found in kidney and liver but not in the peripheral compartments of muscle or fat. In this context it should also be noted that the lactating goat was dosed at an exaggerated dose level of ca. 24 mg/kg dry feed which is 5 times higher as the 1X dose level in the cattle feeding study.

Taking all these results into consideration, it can be concluded that the ruminant and rat metabolism follow the same metabolic pathway and thus are neither different in extent nor in nature. Furthermore, no sub-structures of known potential toxicological concern became apparent. Thus a pig metabolism study was rendered unnecessary.

B.7.3 Definition of the residue (IIA 6.7.1; IIIA 8.7.1)

B.7.3.1 Definition of the residue in plant material

Metabolism studies have been conducted in five diverse crops (tomato, apple, potato, cotton, and rice) for foliar and soil applications and in confined rotational crops (Swiss chard, turnip, and wheat) after application of flupyradifurone onto bare soil and cultivating of succeeding crops at three plant-back intervals. The plant metabolism studies have shown a reasonably consistent metabolic profile across both foliar and soil application.

The only residues of flupyradifurone that were consistently observed at significant levels across all primary and succeeding crops were the parent compound and DFA, both of which are specific to flupyradifurone use. DFA has currently not been attributed to other active substance than flupyradifurone. If it is demonstrated in future that other sources lead to DFA, setting separate residue definitions will facilitate handling this metabolite. In primary crops, all other major metabolites were detected in individual RACs only, and generally at low concentrations. Only the natural class of

compounds glucose/ carbohydrates, which was formed from the degradation of the furanone moiety and incorporation of ^{14}C into natural plant constituents, was detected in relatively high concentrations.

In confined rotational crops, nine major metabolites were detected besides the parent compound and DFA. However, all of them were detected in individual RACs only, mainly in feed commodities. The major metabolites detected in food commodities were generally at low concentrations, except for glucose/carbohydrates, which are natural compounds, and the metabolites BYI 02960-OH and BYI 02960-OH-glyc, which were also observed in the rat. The metabolite difluoroethyl-amino-furanone (=DFEAF) was also a major metabolite found in Swiss chard RACs in all rotations. Since it was not observed in the rat and the metabolism studies indicated that no other appropriate marker compound was present, BCS included this metabolite in the residue definition for the data collection method.

BYI 02960-difluoroethyl-amino-furanone appeared to be a suitable marker compound for estimating residue levels of other metabolites, if deemed necessary. For the sake of consistency, BCS analysed the samples of all supervised residue trials on target plants and rotational crops for these three compounds. Thus the residue definition for risk assessment for target plants and rotational crops has three constituents: BYI 02960 (parent compound), DFA, and BYI 02960-difluoroethyl-amino-furanone (DFEAF). However, since DFEAF was only present in 11% of analysed samples and in low amounts only, it was not deemd necessary to include this metabolite in the residue definition for risk assessment.

No other metabolite need be included in the residue definition for risk assessment, since it has been shown that none of the metabolites present a risk for the consumer (see B.6.9.6). All metabolites identified in the metabolism studies show either no consumer exposure under realistic conditions or a consumer exposure below the agreed Threshold level of Toxicological Concern (TTC; <1.5 µg/kg bw), or it has been shown that the metabolites are covered by the endpoints derived for the parent compound flupyradifurone (either via additional toxicity testing or their presence in the rat ADME studies).

Residue definition for monitoring:

Results from supervised residue trials show that flupyradifurone and difluoroacitic acid (DFA) are, by far, the most prominent components of the residues in target plants and rotational crops. The residues of metabolite difluoroethyl-amino-furanone (DFEAF) were below the LOQ of 0.01 mg/kg in virtually all samples. Considering these results as well as those from the metabolism studies, the proposed residue definition for monitoring for target plants and rotational crops is:

~~**The sum of parent compound and DFA, expressed in flupyradifurone equivalents.**~~

1) flupyradifurone

2) DFA, expressed as DFA

Separatly

Residues in supervised residue trials and feeding studies were expressed as flupyradifurone. To convert the residue levels found to expressed as DFA, the residue level is corrected using the molecular weight of flupyradifurone (289 g/mol) andDFA (96 g/mol), resulting in a correction factor of 0.33.

Residue definition risk assessment:

For flupyradifurone residues in target plants (primary crops) and rotational crops (succeeding crops), the proposed residue definition for data collection and risk assessment is:

The sum of parent compound flupyradifurone and its metabolites DFA ~~and difluoroethyl-amino-furanone (DFEAF)~~, expressed in flupyradifurone equivalents. A conversion factor is not required to cover the difference between the residue definition for monitoring and risk assessment as both DFA and flupyradifurone are set as ~~part of~~ the residue definition for monitoring ~~and as DFEAF is found in levels below <0.01 mg/kg in supervised residue trials and in rotational crops. In the unprotected lettuce supervised residue trials, DFEAF was <0.01 mg/kg in the majority of the trials (seven in each region), with a maximum of 0.021 mg/kg in one SEU trial and 0.04 mg/kg in one NEU trial. The ratio of flupyradifurone+DFA to DFEAF in mg/kg is 0.01-0.06. In the protected lettuce trials, DFEAF was detected in six trials, in the range of 0.01-0.02 mg/kg. The ratio of flupyradifurone+DFA to DFEAF in mg/kg is 0.003-0.011. Hence, the conversion factor for lettuce is also proposed to be 1.~~

B.7.3.2 Definition of the residue in animal products

The results of the feeding studies conducted in poultry and cattle with flupyradifurone only, show that parent flupyradifurone and metabolite DFA were by far the predominant compounds detected. Since livestock will in practice mainly be exposed to DFA through feed, the peer review meeting TC 107 conclude that a feeding study in poultry and cattle conducted with DFA are required. The residues of the metabolite BYI 02960-acetyl-AMCP were well below the LOQ of 0.01 mg/kg in all animal matrices at the corresponding 1X dose levels; the metabolite BYI 02960-OH was detected in cattle liver and kidney, only (0.011-0.019 mg/kg). However, this metabolite is also a major rat metabolite and therefore its toxicity is covered by the endpoints derived for the parent compound.

Considering these results in combination with those from livestock metabolism conducted with flupyradifurone only, BYI 02960 parent compound and its metabolite DFA (expressed as their sum, in BYI 02960 equivalents) are the proposed constituents for the residue definitions for risk assessment and enforcement in animal matrices.

No other metabolite need be included in the residue definition for risk assessment, since it has been shown that none of the metabolites pose a risk for the consumer (see B.6.9.6). All metabolites identified in the metabolism studies show either no consumer exposure under realistic conditions or a consumer exposure below the agreed Threshold level of Toxicological Concern (TTC; <1.5 µg/kg bw), or it has been shown that the metabolites are covered by the endpoints derived for the parent

compound BYI 02960 (either via additional toxicity testing or their presence in the rat ADME studies).

Residue definition for monitoring:
~~**The sum of parent compound and DFA, expressed in flupyradifurone equivalents.**~~

1) flupyradifurone

2) DFA, expressed as DFA

separatly

This is contrary to the conclusion of Peer Review Neeting TC 107, where it was concluded to maintain the residue definition as it was proposed in the first draft of the DAR. However, for consistency, the residue definitions for monitoring for plants and animal products are attuned.

Residue definition risk assessment:
The sum of Flupyradifurone and metabolite DFA, expressed in flupyradifurone equivalents.

B.7.4 Use pattern

BYI 02960 (common name: flupyradifurone) is intended to be registered in Europe for use in lettuce (protected and unprotected) and in northern Europe for outdoor use in hops. Use pattern (GAP) information is summarized in Table 7.4-01 and Tabel 7.4.-02.

Table 7.4-1 Identification of GAP of intended uses

Crop and / or situation (a)	Country and/or region	Product name	F G or I (b)	Pest or group of pests controlled (c)	Formulation		Application			Application rate per treatment			PHI (days) (l)	Remarks: (m)
					Type (d - f)	Conc. of as (i)	method kind (f - h)	growth stage & season (j)	number min max (k)	kg as/hL min max	water L/ha min max	kg as/ha min max		
Hops	N-EU (residue zone)	Sivanto	F	Phorodon humuli, aphids	SL	200	spray	BBCH 31-75 May June.	1	0.0045-0.0075	2000-3300	0.150	21	
Lettuce	N/S-EU (residue zone)	Sivanto	F	Nasonovia ribisnigri aphids	SL	200	spray	BBCH 12-49 May--Oct	1	0.0125-0.0250	500-1000	0.125	10	Max 1 application ~~use~~ per 24 months
Lettuce	N/S-EU (residue zone)	Sivanto	F	Nasonovia ribisnigr aphids	SL	200	spray	BBCH 41-49 May--Oct	1	0.0125-0.0250	500-1000	0.125	3	Max 1 application ~~use~~ per 12 months
Lettuce (soil bound)	N-EU S-EU (residue zone)	Sivanto	G	Nasonovia ribisnigri, aphids	SL	200	spray	BBCH 12-49	2	0.0125-0.0250	500-1000	0.125	3	Interval 10d; Max 2 applications ~~uses~~ per 12 months

Remarks:
(a) For crops, Codex (or other, e.g. EU) classifications should be used; where relevant, the use situation should be described (e.g. fumigation of a structure)
(b) Outdoor or field use (F), glasshouse application (G) or indoor application (I)
(c) e.g. biting and suckling insects, soil born insects, foliar fungi, weeds
(d) e.g. wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(e) GCPF Codes - GIFAP Technical Monograph No 2, 1989
(f) All abbreviations used must be explained
(g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated
(i) g/kg or g/l
(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(k) The minimum and maximum number of application possible under practical conditions of use must be provided
(l) PHI - minimum pre-harvest interval
(m) Remarks may include: Extent of use/economic importance/restrictions

B.7.5 Identification of critical GAPs

A summary of the cGAPs for Northern and Southern European regions is given in Table 7.5-1.

Table 7.5-1 Identification of the cGAP of Northern and Southern European regions

Crop and / or situation (a)	Country and/or region	Product name	F G or I (b)	Pest or group of pests controlled (c)	Formulation		Application			Application rate per treatment			PHI (days) (l)	Remarks: (m)
					Type (d - f)	Conc. of as (i)	method kind (f - h)	growth stage & season (j)	number min max (k)	kg as/hL max	water L/ha min	kg as/ha max		
Agricultural uses														
Hops	N-EU (residue zone)	Sivanto	F	*Phorodon humuli,* aphids	SL	200	spray	BBCH 31-75 May June.	1	0.0075	2000	0.150	21	
Lettuce	N/S-EU (residue zone)	Sivanto	F	*Nasonovia ribisnigri* aphids	SL	200	spray	BBCH 12-49 May-Oct	1	0.0250	500	0.125	10	Max 1 application ~~use~~ per 24 months
Lettuce	N/S-EU (residue zone)	Sivanto	F	*Nasonovia ribisnigri* aphids	SL	200	spray	BBCH 41-49 May-Oct	1	0.0250	500	0.125	3	Max 1 application ~~use~~ per 12 months
Lettuce	N-/S-EU (residue zone)	Sivanto	G	*Nasonovia ribisnigri,* aphids	SL	200	spray	BBCH 12-49	2	0.0250	500	0.125	3	Interval 10d; Max 2 applications ~~uses~~ per 12 months

Remarks:
(a) For crops, Codex (or other, e.g. EU) classifications should be used; where relevant, the use situation should be described (e.g. fumigation of a structure)
(b) Outdoor or field use (F), glasshouse application (G) or indoor application (I)
(c) e.g. biting and suckling insects, soil born insects, foliar fungi, weeds
(d) e.g. wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(e) GCPF Codes - GIFAP Technical Monograph No 2, 1989
(f) All abbreviations used must be explained
(g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated
(i) g/kg or g/l
(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(k) The minimum and maximum number of application possible under practical conditions of use must be provided
(l) PHI - minimum pre-harvest interval
(m) Remarks may include: Extent of use/economic importance/restrictions

B.7.6 Residues resulting from supervised trials (IIA 6.3; IIIA 8.3)

B.7.6.1 Methods of analysis applied in the supervised trials

Flupyradifurone residues in lettuce and hops were determined using method 01304 (2010 trials lettuce and hops and 2011 trials hops) or method 01212 (2011 trials lettuce). The methods were validated for the determination of parent BYI 02960 and its metabolites difluoroacetic acid (DFA) and BYI 02960-difluoroethyl-aminofuranone (DFEAF) in crops with high water content (see Volume 3 B.5.2). The respective LOQs for BYI 02960 and its metabolites DFA and DFEAF in lettuce were 0.01, 0.02, and 0.01 mg/kg (all in parent equivalents) yielding a calculated total-residue LOQ of 0.04 mg/kg.. The respective LOQs for the three analytes in hops were 0.1, 0.2, and 0.1 mg/kg (all in parent equivalents), yielding a calculated total-residue LOQ of 0.40 mg/kg.

Lettuce

Concurrent recoveries were run together with the analysis of the samples of lettuce with method 01304. Details of recovery data are shown in table 7.6.1-1. All values were within acceptable ranges.

Table 7.6.1-1: Recovery data for the determination of BYI 02960 and its metabolites difluoroacetic acid (DFA) and BYI 02960-difluoroethyl-aminofuranone (DFEAF) in lettuce with method 01304

Study No. Trial No.	Crop	Portion analysed	a.s./ metabolite	n	Fortification level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
10-2223 10-2223-01 to 10-2223-05 GLP: yes 2010	lettuce, head	head	BYI 02960	15	0.01	79;87;102; 106; 107;109;110; 116;92;97;107; 108;114;116; 117	79	117	104	10.7
				5	0.10	88;90;90;92;93	88	93	91	2.2
10-2213 10-2213-01 to 10-2213-05 GLP: yes 2010				2	0.50	103;106	103	106	105	
				2	1.0	92;94	92	94	93	
				2	5.0	90;98	90	98	94	
				26	overall		79	117	100	10.5
			difluoroacetic acid	12	0.02	90;93;94;95;97 ;112;112;116; 86;89;93;95	86	116	98	10.2
10-2212 10-2212-01 to				3	0.05	90;94;98	90	98	94	4.3
				2	0.20	92;94	92	94	93	
				5	0.50	93;101;90;91; 92	90	101	93	4.7

Study No. Trial No.	Crop	Portion analysed	a.s./ metabolite	n	Fortifi-cation level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
10-2212-05 GLP: yes 2010				2	1.0	90;92	90	92	91	
				2	5.0	90;89	89	90	90	
				26	overall		86	116	95	7.9
			BYI 02960-difluoroethyl-aminofuranone	15	0.01	87;93;95;100;100;104;105;107;83;83;86;88;90;92;96	83	107	94	8.4
				5	0.10	85;98;97;97;99	85	99	95	6.1
				2	0.50	97;109	97	109	103	
				2	1.0	86;101	86	101	94	
				2	5.0	97;96	96	97	97	
				26	overall		83	109	95	7.7
10-2223 10-2223-01 to 10-2223-05 GLP: yes 2010		leaf, outer	BYI 02960	1	0.01	115	115	115	115	
				1	10	94	94	94	94	
				2	overall		94	115	105	
			difluoroacetic acid	1	0.02	94	94	94	94	
				1	10	90	90	90	90	
				2	overall		90	94	92	
			BYI 02960-difluoroethyl-aminofuranone	1	0.01	90	90	90	90	
				1	10	95	95	95	95	
				2	overall		90	95	93	
		washings	BYI 02960	5	0.01	99;102;106;109;117	99	117	107	6.5
				3	0.50	95;97;111	95	111	101	8.6
				8	overall		95	117	105	7.3
			difluoroacetic acid	5	0.02	95;96;97;97;103	95	103	98	3.2
				3	0.50	91;94;97	91	97	94	3.2
				8	overall		91	103	96	3.5
			BYI 02960-difluoroethyl-aminofuranone	5	0.01	95;99;100;100;107	95	107	100	4.3
				3	0.50	86;88;96	86	96	90	5.9
				8	overall		86	107	96	7.1
		head, inner	BYI 02960	1	0.01	114	114	114	114	
				1	overall		114	114	114	

Study No. Trial No.	Crop	Portion analysed	a.s./ metabolite	n	Fortifi-cation level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
		parts	difluoroacetic acid	1	0.02	93	93	93	93	
				1	overall		93	93	93	
			BYI 02960-difluoroethyl-aminofuranone	1	0.01	93	93	93	93	
				1	overall		93	93	93	

Concurrent recoveries were run together with the analysis of the samples of lettuce with method 01212. Details of recovery data are shown in table 7.6.1.-2. The recoveries are relatively high, but the overall mean for each analyte is within the acceptable range, except for BYI 02960-difluoroethyl-aminofuranone (113%). Nonetheless, the method is considered acceptable.

Table 7.6.1-2: Recovery data for the determination of BYI 02960 and its metabolites difluoroacetic acid (DFA) and BYI 02960-difluoroethyl-aminofuranone (DFEA) in lettuce with method 01212

Study No. Trial No.	Crop	Portion analysed	a.s./ metabolite		Fortifi-cation level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
11-2082	lettuce	head	BYI 02960	2	0.01	106;111	106	111	109	
				2	0.10	104;105	104	105	105	
11-2082-01 to 11-2082-04				1	2.0	119	119	119	119	
				1	8.0	114	114	114	114	
GLP: yes 2011				6	overall		104	119	110	5.4
11-2071			difluoroacetic acid	2	0.02	89;109	89	109	99	
				2	0.20	98;106	98	106	102	
11-2071-01 to 11-2071-04				1	4.0	102	102	102	102	
				1	16	102	102	102	102	
GLP: yes 2011				6	overall		89	109	101	6.9
11-2070			BYI 02960-difluoroethyl-aminofuranone	2	0.01	114;123	114	123	119	
				2	0.10	108;109	108	109	109	
11-2070-01 to				1	2.0	107	107	107	107	
				1	8.0	116	116	116	116	

Study No. Trial No.	Crop	Portion analysed	a.s./ metabolite		Fortifi-cation level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
11-2070-04 GLP: yes 2011				6	overall		107	123	113	5.4

Hops

During the conduct of the 2010 studies, both validation and concurrent recoveries of BYI 02960 and its metabolites DFA and DFEAF were obtained from samples of hop cones (green and dried). The validation work was done due to the fact that hops are considered "difficult to analyze" but were not included in the original validation set for method 01304. In 2011, samples were analyzed for concurrent recoveries.

Concurrent recoveries were run together with the analysis of the samples of lettuce with method 01304. Details of recovery data are shown in table 7.6.1-3. Values were within acceptable ranges.

Table 7.6.1-3 Recovery data for the determination of BYI 02960 and its metabolites difluoroacetic acid (DFA) and BYI 02960-difluoroethyl-aminofuranone (DFEA) in hops with method 01304.

Study No. Trial No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortifi-cation level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
10-2225	hop	cone, green	BYI 02960	6	0.10	89;89;91;94; 95;107	89	107	94	7.2
10-2225-01 to 10-2225-04				5	1.0	85;86;87;92;98	85	98	90	6.0
				1	5.0	87	87	87	87	
GLP: yes 2010				12	overall		85	107	92	6.8
			difluoroacetic acid	6	0.20	91;92;95;99; 100;115	91	115	99	8.9
				5	1.0	76;79;83;84;94	76	94	83	8.2
				1	5.0	86	86	86	86	
				12	overall		76	115	91	11.7
			BYI 02960-difluoroethyl-aminofuranone	6	0.10	68;73;79;85; 95;96	68	96	83	13.9
				5	1.0	76;77;78;84;91	76	91	81	7.8
				1	5.0	80	80	80	80	
				12	overall		68	96	82	10.6
		cone, kiln-dried	BYI 02960	6	0.10	102;103;103; 104;105;106	102	106	104	1.4
				5	1.0	107;108;111; 114;115	107	115	111	3.2

Study No. Trial No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
				1	5.0	112	112	112	112	
				12	overall		102	115	108	4.2
			difluoroacetic acid	6	0.20	82;96;97;103;103;106	82	106	98	8.9
				5	1.0	101;105;106;107;110	101	110	106	3.1
				1	5.0	98	98	98	98	
				12	overall		82	110	101	7.3
			BYI 02960-difluoroethyl-aminofuranone	6	0.10	89;100;106;107;107;108	89	108	103	7.2
				5	1.0	108;109;110;112;114	108	114	111	2.2
				1	5.0	112	112	112	112	
				12	overall		89	114	107	6.2
11-2076 11-2076-01 to 11-2076-04 GLP: yes 2011	hop	cone, green	BYI 02960	1	0.10	90	90	90	90	
				3	1.0	84;85;85	84	85	85	0.7
				4	overall		84	90	86	3.1
			difluoroacetic acid	1	0.20	88	88	88	88	
				3	1.0	77;79;82	77	82	79	3.2
				4	overall		77	88	82	5.9
			BYI 02960-difluoroethyl-aminofuranone	1	0.10	91	91	91	91	
				3	1.0	87;90;91	87	91	89	2.3
				4	overall		87	91	90	2.1
11-2076 11-2076-01 to 11-2076-04 GLP: yes 2011	hop	cone, kiln-dried	BYI 02960	1	0.10	90	90	90	90	
				1	1.0	87	87	87	87	
				2	overall		87	90	89	
			difluoroacetic acid	1	0.20	73	73	73	73	
				1	1.0	70	70	70	70	
				2	overall		70	73	72	
			BYI 02960-difluoroethyl-aminofuranone	1	0.10	79	79	79	79	
				1	1.0	91	91	91	91	
				2	overall		79	91	85	

B.7.6.2 Supervised residue trials in lettuce

Study 1

Characteristics

reference	:	Schulte, G. 2012a KIIA 6.3.1.1/05	GLP statement	:	yes (certified laboratory); Deviations: none
Type of study	:	Supervised residue trial (Greenhouse Europe)	Guideline	:	EEC 7029/VI/95 rev. 5, appendix B (1997)
Year of execution	:	2010	Dose	:	0.125 kg as/ha
test substance	:	BYI 02960 SL 200 formulation Batch no: 2010-000672	LOQ	:	BYI 02960 : 0.01 mg/kg DFA: 0.02 mg/kg DFEAF : 0.01 mg/kg
Species	:	Lettuce *(Lactuca sativa)*	storage Acceptability	: :	≤ 286 d at -18°C Y

Study design

Five trials in lettuce were performed in the 2010 growing season in the greenhouse in France, Germany, the Netherlands, and Italy. The lettuce varieties used were either head-forming (3 trials) or open leaf (2) varieties. An SL formulation containing 200 g/L BYI 02960 was applied to the crop with two applications at intervals of 10 days (11 days in one trial) at a nominal rate of 0.625 L/ha, corresponding to 125 g/ha BYI 02960 a.s.; the water rate was 400-600 L/ha.

Samples of lettuce heads were taken immediately prior and subsequent to the final application, and at several intervals thereafter (up to 7 days after treatment). The envisaged PHI was 3 days.

Samples were investigated for BYI 02960 and its metabolites DFA and DFEAF using method 01304. The respective LOQs for the 3 analytes were 0.01, 0.02, and 0.01 mg/kg (all in parent equivalents).

Results

On day 0, immediately following the final treatment, residue levels of flupyradifurone in lettuce heads were between 1.5 and 6.7 mg/kg and <0.02 mg/kg for DFA, expressed as DFA. By day 3 — the envisaged PHI — the levels had declined to 1.4-3.5 mg/kg ~~(n=9)~~ for flupyradifurone and to 0.007-<0.02 mg/kg for DFA, expressed as DFA. Residues continued to decrease until day 7, the final sampling event, when levels ranged from ~~0.78-1.8~~ 0.74-1.7 mg/kg flupyradifurone and to 0.007-<0.02 mg/kg for DFA, expressed as DFA.

Details of the trials are presented in table 7.6.2-~~3~~2.

Conclusion

In study 10-2212 five acceptable residue trials in lettuce performed in accordance with the cGAP for greenhouse use have been provided. Relevant residue data for risk assessment, sum of flupyradifurone and DFA, expressed as flupyradifurone for the critical GAP are: 1.42; 1.82; 2.02; 2.53;

3.52 mg/kg; Relevant residue data for MRL setting are: 1.4, 1.8, 2.0, 2.5, 3.5 mg/kg for flupyradifurone and 0.01-<0.02 mg/kg for DFA, expressed as DFA.

Study 2

Characteristics

reference	:	Uceda, L. 2012c KIIA 6.3.1.1/06	GLP statement	:	yes (certified laboratory); Deviations: none
Type of study	:	Supervised residue trial (Greenhouse Europe)	Guideline	:	EEC 7029/VI/95 rev. 5, appendix B (1997)
Year of execution	:	2011	Dose	:	0.125 kg as/ha
test substance	:	BYI 02960 SL 200 formulation Batch no: 2010-007173	LOQ	:	BYI 02960 : 0.01 mg/kg DFA: 0.02 mg/kg DFEAF : 0.01 mg/kg
Species	:	Lettuce (*Lactuca sativa*)	storage Acceptability	: :	≤228 d at -18°C Y

Study design

Four trials in lettuce were performed in the 2011 growing season in the greenhouse in France, Spain, Germany, and Italy. An SL formulation containing 200 g/L BYI 02960 was applied to the crop with two applications at intervals of 10 days (interval in one trial: 9 day); at a nominal rate of 0.625 L/ha, corresponding to 125 g/ha BYI 02960 a.s.; the water rate was 400-600 L/ha. All lettuce varieties used were leafy (open-head) varieties. The volumes of water ranged from 400-600 L/ha.

Samples of lettuce heads were taken immediately prior and subsequent to the final application, and at several intervals thereafter (up 14 days after treatment). The envisaged PHI was 3 days.

Samples were investigated for BYI 02960 and its metabolites DFA and DFEAF using method 01212. The respective LOQs for the 3 analytes were 0.01, 0.02, and 0.01 mg/kg (all in parent equivalents).

Results

On day 0, immediately following the final treatment, residue levels of flupyradifurone in lettuce heads were between 3.0 and 7.~~67~~ mg/kg and between 0.009-0.043 mg/kg for DFA, expressed as DFA. By day 3 — the envisaged PHI — the levels had declined to ~~0.52~~ 0.73-6.0 mg/kg for flupyradifurone and to 0.007-0.02 mg/kg for DFA, expressed as DFA. For flupyradifurone, residues continued to decrease until day 14, the final sampling event, when levels ranged from 0.13-2.6 ~~0.21-2.7~~ mg/kg, residues increased to 0.009-0.043 mg/kg for DFA, expressed as DFA.

Details of the trials are presented in table 7.6.2-~~3~~2.

Conclusion

In study 11-2070 four acceptable residue trials in lettuce performed in accordance with the cGAP for greenhouse use have been provided. Relevant residue data for risk assessment, sum of flupyradifurone and DFA, expressed as flupyradifurone for the critical GAP are : 0.80, 2.2, 2.7 and 6.0

mg/kg. Relevant residue data for MRL setting are: 0.73; 2.1; 2.6; 6.0 mg/kg for flupyradifurone and 0.009; 0.018; 0.023; 0.043 mg/kg for DFA, expressed as DFA.

Study 3

Characteristics

reference	:	Noss & Bauer, 2012 KIIA 6.3.1.1/01	GLP statement	:	yes (certified laboratory); Deviations: none
Type of study	:	Supervised residue trial (Northern Europe)	Guideline	:	EEC 7029/VI/95 rev. 5, appendix B (1997)
Year of execution	:	2010	Dose	:	0.125 kg as/ha
test substance	:	BYI 02960 SL 200 formulation Batch no: 2010-000672	LOQ	:	BYI 02960 : 0.01 mg/kg DFA: 0.02 mg/kg DFEAF : 0.01 mg/kg
Species	:	Lettuce (*Lactuca sativa*)	storage Acceptability	: :	≤ 202 d at -18°C Y

Study design

Five trials in lettuce were performed in the 2010 growing season in Northern Europe (Netherlands, Belgium, France, and Germany). The lettuce varieties used were either closed-head (3 trials) or leafy (2) varieties. An SL formulation containing 200 g/L BYI 02960 was applied to the crop with two applications at intervals of 10 days (9 days in one trial) at a nominal rate of 0.625 L/ha, corresponding to 125 g/ha BYI 02960 a.s.; the water rate was 300-600 L/ha.

Samples of lettuce heads were taken immediately prior and subsequent to the final application, and at several intervals thereafter (up to 7 days after treatment in 2010).

Samples were investigated for BYI 02960 and its metabolites DFA and DFEAF using method 01304. The respective LOQs for the 3 analytes were 0.01, 0.02, and 0.01 mg/kg (all in parent equivalents).

Results

Lettuce heads were taken 9-10 days after the first treatment (before the final treatment) in order to represent a 1-application use with a 10-day PHI, as is envisaged for general agricultural use in northern European fields. Total residue levels of flupyradifurone and DFA were: 0.10, 0.15, 0.22, 0.39 and 0.82 ~~0.11, 0.16, 0.23, 0.40 and 0.83~~ mg/kg.

On day 0, immediately following the 2nd and final treatment, residue levels of flupyradifurone in lettuce heads were between 1.5 and 4.1 mg/kg and <0.02 mg/kg for DFA, expressed as DFA. By day 3 the levels had declined to 0.37-1.0 ~~0.40, 0.61, 0.71, 0.87 and 1.0~~ mg/kg kg for flupyradifurone and to <0.02 mg/kg for DFA, expressed as DFA. For flupyradifurone, residues continued to decrease until day 7, the final sampling event, when levels ranged from 0.21-0.66 ~~0.25-0.69~~ mg/kg, residues increased to 0.01-<0.02 mg/kg for DFA, expressed as DFA.

Details of the trials are presented in table 7.6.2-43.

Conclusion

In study 10-2223 five acceptable residue trials performed in accordance with the cGAP for outdoor use of Northern Europe with a 10-day PHI have been provided with lettuce. Relevant residue data for risk assessment, sum of flupyradifurone and DFA, expressed as flupyradifurone are: 0.10, 0.15, 0.22, 0.39 and 0.82 ~~0.11, 0.16, 0.23, 0.40 and 0.83~~ mg/kg. Relevant residue data for MRL setting are: 0.08, 0.13, 0.20, 0.37 and 0.80 mg/kg for flupyradifurone and <0.02 mg/kg for DFA, expressed as DFA.

The residue trials were not in accordance with the agricultural cGAP for outdoor use in Northern Europe with a 3-day PHI. No samples were taken 3 days after the first application. In case the residue levels before the second treatment would have declined below the LOQ, the residue levels at 3 days after the 2nd treatment could be considered in order to represent a 1-application use with a 3-day PHI. This is however not the case. The trial results at 3 days after the 2nd treatment represent a more critical use, since the first application additionally contributes to the residue levels.

The results from the trials performed with outdoor lettuce in Northern Europe at 3 days after the 2nd treatment, are however valid to determine whether indoor or outdoor uses can be identified as the worst case residue situation. Relevant residue data for risk assessment, sum of flupyradifurone and DFA, expressed as flupyradifurone for the critical GAP of two applications of 0.125 kg as/ha and PHI 3 days are: 0.39, 0.60, 0.70, 0.85 and 1.02 ~~0.40, 0.61, 0.71, 0.87 and 1.0~~ mg/kg.

Moreover, the tested GAP with two applications and a pre-harvest interval of 3 days is the "home and garden" use, which was submitted in parallel and is envisaged for registration shortly after the agricultural use (see the Evaluation Report for Flupyradifurone as an appendix to Vol. 3, B7). Since this field use is more critical than the agricultural field use, it was agreed with the RMS to conduct all field residue trials according to this GAP to identify the most critical consumer exposure and to avoid re-evaluation of MRLs due to this use. Hence, the trials in the DAR are not in accordance with the cGAP in the DAR, but with the cGAP supported in EU for MRL setting.

Study 4

Characteristics

reference	:	Uceda, L. 2012a KIIA 6.3.1.1/02	GLP statement	:	yes (certified laboratory); Deviations: none
Type of study	:	Supervised residue trial (Northern Europe)	Guideline	:	EEC 7029/VI/95 rev. 5, appendix B (1997)
Year of execution	:	2010	Dose	:	0.125 kg as/ha
test substance	:	BYI 02960 SL 200 formulation Batch no: 2010-007173	LOQ	:	BYI 02960 : 0.01 mg/kg DFA: 0.02 mg/kg DFEAF : 0.01 mg/kg
Species	:	Lettuce (*Lactuca sativa*)	storage Acceptability	: :	≤ 150 d at -18°C Y

Study design

Four trials in lettuce were performed in the 2011 growing season in Northern Europe (France, Belgium, and Germany (2)). An SL formulation containing 200 g/L BYI 02960 was applied to the crop

with two applications at intervals of 10 days at a nominal rate of 0.625 L/ha, corresponding to 125 g/ha BYI 02960 a.s.; the water rate was 500-750 L/ha. All lettuce varieties used were leafy (open-head) varieties.

In two trials samples of lettuce heads were taken immediately prior and subsequent to the second application, and at several intervals thereafter. In two other trials samples of lettuce heads were taken immediately prior and several intervals after the first after the second application. The envisaged PHI is 3 days after one application.

Samples were investigated for BYI 02960 and its metabolites DFA and DFEAF using method 01212. The respective LOQs for the 3 analytes were 0.01, 0.02, and 0.01 mg/kg (all in parent equivalents).

Results

Lettuce heads were taken 9-10 days after the first treatment (before the final treatment) in order to represent a 1-application use with a 10-day PHI. On day 0, immediately following the first treatment, total residue levels in lettuce heads were 2.9 and 5.6 mg/kg (measured in two trials). 9-10 days after the first treatment (before the final treatment) total residue levels of flupyradifurone and DFA were 0.13, 0.21, 0.28 and 0.62 ~~, 0.14, 0.22, 0.28 and 0.65~~ mg/kg.

Only in two trials lettuce heads were taken 2 days after the first treatment (8 days before the final treatment) in order to represent a 1-application use with a 3-day PHI. On day 0, immediately following the first treatment, total residue levels of flupyradifurone and DF in lettuce heads were 2.9 and 5.6 mg/kg. Two days after the first treatment (8 days before the final treatment) total residue levels were 2.0 and 2.6 ~~2.7~~ mg/kg.

~~Ten days after the first treatment (before the final treatment) total residue levels of flupyradifurone and DF were 0.14, 0.22, 0.28 and 0.65 mg/kg.~~

On day 0, immediately following the 2nd and final treatment, residue levels of flupyradifurone in lettuce heads were between 2.6 and 3.8 ~~3.9~~ mg/kg and between 0.007 and <0.02 mg/kg for DFA, expressed as DFA. By day 3 the levels had declined to 0.11, 0.43, 1.5 and 2.9 ~~0.14, 0.47, 1.6 and 3.0~~ mg/kg for flupyradifurone and increased to 0.008-0.032 mg/kg for DFA, expressed as DFA. For flupyradifurone, residues continued to decrease until day 14, the final sampling event, when levels ranged from 0.04-0.95 ~~0.047-1.0~~ mg/kg, residues increased to 0.008-0.066 mg/kg for DFA, expressed as DFA.

Details of the trials are presented in table 7.6.2-~~4~~ 3.

Conclusion

In study 11-2082 four acceptable residue trials performed in accordance with the cGAP for outdoor use of Northern Europe with a 10-day PHI have been provided with lettuce. Relevant residue data for

risk assessment, sum of flupyradifurone and DFA, expressed as flupyradifurone are: 0.13, 0.21, 0.28 and 0.62 ~~, 0.14, 0.22, 0.28 and 0.65~~ mg/kg. Relevant residue data for MRL setting are: 0.11, 0.19, 0.25 and 0.57 mg/kg for flupyradifurone and <0.02, <0.02, 0.009 and 0.016 mg/kg for DFA, expressed as DFA.

The residue trials were not in accordance with the agricultural cGAP for outdoor use in Northern Europe with a 3-day PHI. No samples were taken 3 days after the first application. In two trial samples were taken two days after the first treatment. Total residue levels were 2.0 and 2.6 ~~2.7~~ mg/kg. The trial results at 3 days after the 2nd treatment represent a more critical use, since the first application additionally contributes to the residue levels.

The results from the trials performed with outdoor lettuce in Northern Europe at 3 days after the 2nd treatment, are however valid to determine whether indoor or outdoor uses can be identified as the worst case residue situation. Relevant residue data for the critical GAP of two applications of 0.125 kg as/ha and PHI 3 days are: 0.13, 0.46, 1.52 ~~0.14, 0.47, 1.6~~ and 3.0 mg/kg.

Moreover, the tested GAP with two applications and a pre-harvest interval of 3 days is the "home and garden" use, which was submitted in parallel and is envisaged for registration shortly after the agricultural use (see the Evaluation Report for Flupyradifurone as an appendix to Vol. 3, B7). Since this field use is more critical than the agricultural field use, it was agreed with the RMS to conduct all field residue trials according to this GAP to identify the most critical consumer exposure and to avoid re-evaluation of MRLs due to this use. Hence, the trials in the DAR are not in accordance with the cGAP in the DAR, but with the cGAP supported in EU for MRL setting.

Study 5

Characteristics

reference	:	Schoening, R.; Bauer, J. 2011 KIIA 6.3.1.1/03	GLP statement	:	yes (certified laboratory); Deviations: none
Type of study	:	Supervised residue trial (~~Northern~~ Sourthern Europe)	Guideline	:	EEC 7029/VI/95 rev. 5, appendix B (1997)
Year of execution	:	2010	Dose	:	0.125 kg as/ha
test substance	:	BYI 02960 SL 200 formulation Batch no: 2010-000672	LOQ	:	BYI 02960 : 0.01 mg/kg DFA: 0.02 mg/kg DFEAF : 0.01 mg/kg
Species	:	Lettuce (*Lactuca sativa*)	storage Acceptability	: :	≤ 151 d at -18°C Y

Study design

Five trials in lettuce were performed in the 2010 growing season in Southern Europe (France (South), Spain and Italy). The lettuce varieties used were either closed-head (3 trials) or leafy (2) varieties. An SL formulation containing 200 g/L BYI 02960 was applied to the crop with two applications at intervals of 10 days (11 days in one trial) at a nominal rate of 0.625 L/ha, corresponding to 125 g/ha BYI 02960 a.s.; the water rate was 500-700 L/ha.

Samples of lettuce heads were taken immediately prior and subsequent to the second application, and at several intervals thereafter (up to 7 days after treatment).

Samples were investigated for BYI 02960 and its metabolites DFA and DFEAF using method 01304. The respective LOQs for the 3 analytes were 0.01, 0.02, and 0.01 mg/kg (all in parent equivalents).

Results

Lettuce heads were taken 10-11 days after the first treatment (before the final treatment) in order to represent a 1-application use with a 10-day PHI. Total residue levels of flupyradifurone and DFA were: 2x0.06, 0.08, 0.41 and 0.82 ~~2x0.07, 0.09, 0.41 and 0.83~~ mg/kg.

On day 0, immediately following the 2nd and final treatment, residue levels of flupyradifurone in lettuce heads were between 2.6 ~~2.7~~ and 3.8 mg/kg and 0.007-<0.02 mg/kg for DFA, expressed as DFA. By day 3 the levels had declined to levels between 0.40, 0.48, 1.1, 2.1 ~~0.43, 0.53, 1.2, 2.2~~ and 2.7 mg/kg for flupyradifurone and to 0.007-<0.02 mg/kg for DFA, expressed as DFA. For flupyradifurone, residues continued to decrease until day 7, the final sampling event, when levels ranged from ~~0.15-1.4~~ 0.09-1.3 mg/kg, residues increased to 0.007-0.017 mg/kg for DFA, expressed as DFA.

Details of the trials are presented in table 7.6.2-~~5~~4.

Conclusion

In study 10-2213 five acceptable residue trials performed in accordance with the cGAP for outdoor use of Southern Europe with a 10d PHI have been provided with lettuce. Relevant residue data for risk assessment, sum of flupyradifurone and DFA, expressed as flupyradifurone are: 2x0.06, 0.08, 0.41 and 0.82 ~~2x0.07, 0.09, 0.41 and 0.83~~ mg/kg. Relevant residue data for MRL setting are: 2x0.04, 0.05, 0.38 and 0.80 mg/kg for flupyradifurone and 0.007, 2x 0.01 and 2x <0.02 mg/kg for DFA, expressed as DFA.

The residue trials were not in accordance with the agricultural cGAP for outdoor use in Southern Europe with a 3d PHI. No samples were taken 3 days after the first application. As residues did not decline to a level below LOQ 10d after the first application, the residue levels at 3 days after the 2nd treatment cannot be considered as representative of the cGAP with 1 application and PHI 3d. The results at 3 days after the 2nd treatment represents a more critical use, since the first application additionally contributes to the residue levels.

The results from the trials performed with outdoor lettuce in Southern Europe at 3 days after the 2nd treatment, are however valid to determine whether indoor or outdoor uses can be identified as the worst case residue situation. Relevant residue data for risk assessment, sum of flupyradifurone and

DFA, expressed as flupyradifurone for the critical GAP of two applications of 0.125 kg as/ha and PHI 3 days are: 0.42 ~~0.43~~, 0.53, 1.12 ~~1.2~~, 2.2 and 2.7 mg/kg.

Moreover, the tested GAP with two applications and a pre-harvest interval of 3 days is the "home and garden" use, which was submitted in parallel and is envisaged for registration shortly after the agricultural use (see the Evaluation Report for Flupyradifurone as an appendix to Vol. 3, B7). Since this field use is more critical than the agricultural field use, it was agreed with the RMS to conduct all field residue trials according to this GAP to identify the most critical consumer exposure and to avoid re-evaluation of MRLs due to this use. Hence, the trials in the DAR are not in accordance with the cGAP in the DAR, but with the cGAP supported in EU for MRL setting.

Study 6

Characteristics

reference	:	Uceda, L. 2012b KIIA 6.3.1.1/04	GLP statement	:	yes (certified laboratory); Deviations: none
Type of study	:	Supervised residue trial (~~Northern~~ Sourthern Europe)	Guideline	:	EEC 7029/VI/95 rev. 5, appendix B (1997)
Year of execution	:	2010	Dose	:	0.125 kg as/ha
test substance	:	BYI 02960 SL 200 formulation Batch no: 2010-007173	LOQ	:	BYI 02960 : 0.01 mg/kg DFA: 0.02 mg/kg DFEAF : 0.01 mg/kg
Species	:	Lettuce (*Lactuca sativa*)	storage	:	≤ 183 d at -18°C
			Acceptability	:	Y – for determination of worst case (indoor or outdoor)

Study design

Four trials in lettuce were performed in the 2011 growing season in Southern Europe (France, Spain, Portugal, and Italy). An SL formulation containing 200 g/L BYI 02960 was applied to the crop with two applications at intervals of 10 days at a nominal rate of 0.625 L/ha, corresponding to 125 g/ha BYI 02960 a.s.; the water rate was 500-750 L/ha. All lettuce varieties used were leafy (open-head) varieties

Samples of lettuce heads were taken immediately prior and subsequent to the final application, and at several intervals thereafter (up 14 days after treatment). The envisaged PHI was 3 days.

Samples were investigated for BYI 02960 and its metabolites DFA and DFEAF using method 01212. The respective LOQs for the 3 analytes were 0.01, 0.02, and 0.01 mg/kg (all in parent equivalents).

Results

Lettuce heads were taken 9-10 days after the first treatment (before the final treatment) in order to represent a single application use with a 10d PHI, as is envisaged for general agricultural use in northern European fields. On day 0, immediately following the first treatment, total residue levels of flupyradifurone and DFA in lettuce heads were 5.3 ~~5.4~~ mg/kg (measured in one trial) and 1.8 to 3.4 ~~3.5~~ mg/kg two days after the first treatment (measured in two trials). 9-10 Days after the first treatment

(before the final treatment) total residue levels were 0.14, 0.31, 0.48 and 0.60 ~~0.15, 0.32, 0.49 and 0.61~~ mg/kg.

On day 0, immediately following the 2nd and final treatment, residue levels of flupyradifurone in lettuce heads were between 2.6 ~~2.7~~ and 7.3 ~~7.4~~ mg/kg and 0.007-0.021 mg/kg for DFA, expressed as DFA. By day 3 the levels had declined to 0.35, 0.72, 1.5 and 3.1 ~~0.39, 0.78, 1.6 and 3.2~~ mg/kg for flupyradifurone and increased to 0.01-0.028 mg/kg for DFA, expressed as DFA. For flupyradifurone, residues continued to decrease until day 14, the final sampling event, when levels ranged from 0.033-0.17 ~~0.094-0.3~~ mg/kg, residues increased to 0.013-0.050 mg/kg for DFA, expressed as DFA.

Details of the trials are presented in table 7.6.2-~~5~~4.

Conclusion

In study 11-2071 four acceptable residue trials performed in accordance with the cGAP for outdoor use of Southern Europe with a 10-day PHI have been provided with lettuce. Relevant residue data for risk assessment, sum of flupyradifurone and DFA, expressed as flupyradifurone are: 0.14, 0.31 ~~0.22~~, ~~0.28~~ 0.48 and 0.60 ~~0.65~~ mg/kg. Relevant residue data for MRL setting are: 0.11, 0.29, 0.43 and 0.55 mg/kg for flupyradifurone and 0.010, 0.017 and 2x <0.02 mg/kg for DFA, expressed as DFA.

The residue trials were not in accordance with the cGAP for outdoor use in Southern Europe with a 3d PHI. No samples were taken 3 days after the first application. In two trial samples were taken two days after the first treatment. Total residue levels for risk assessment, sum of flupyradifurone and DFA, expressed as flupyradifurone were 1.8 ~~2.0~~ and 3.4 ~~2.7~~ mg/kg. The trial results at 3 days after the 2nd treatment represent a more critical use, since the first application cumulatively contributes to the residue levels.

The results from the trials performed with outdoor lettuce in Southern Europe at 3 days after the 2nd treatment are however valid to determine whether indoor or outdoor uses with can be identified as the worst case residue situation. Relevant residue data for risk assessment, sum of flupyradifurone and DFA, expressed as flupyradifurone for the critical GAP of two applications of 0.125 kg as/ha and PHI 3 days are: 0.38, 0.76, 1.58 and 3.18 ~~0.39, 0.78, 1.6 and 3.2~~ mg/kg.

Overall conclusion lettuce

Sets of GLP trials were conducted in 2010 and 2011 in Northern and Southern European fields and in greenhouses. Flupyradifurone was applied twice as an SL formulation (BYI 02960 SL 200, containing 200 g/L BYI 02960 a.s.), with 10-day intervals.

Nine acceptable residue trials performed in accordance with the cGAP for greenhouse use have been provided.

For the envisaged outdoor use in Northern and Southern Europe with a 10d PHI the required number of trials are available (nine acceptable trials for each region).

For the envisaged outdoor use in Northern and Southern Europe with a 3d PHI, the residue trials performed with lettuce in Northern and Southern Europe do not represent the agricultural cGAP, but the 'home & garden use", which has a more critical GAP, except for two trials with sampling 2 days after the 1st treatment. No samples were taken 3 days after the first application. However, the results from the trials performed with outdoor lettuce in Northern and Southern Europe at 3 days after the 2nd treatment are valid to determine whether indoor or outdoor uses can be identified as the worst case residue situation.
Since the the home & garden use is the critcal field use that will determine the MRL, the trials represent the most critical EU use, see the Evaluation Report for setting MRLs for flupyradifurone, as an appendix to vol 3, B7.

Based on a comparison of the residue values from field and greenhouse testing and using the same use pattern, it is evident that the greenhouse use yielded higher total residues in lettuce than did the field uses. In the greenhouse trials a highest total residue value (HR) of 6.04 mg/kg was determined. The STMR in these trials was also the highest for any set, at 2.12 mg/kg. See table 7.6.2-1~~2~~.

A comparison of the residue values from field and greenhouse testing using the most critical use pattern (2x125 g as/ha, int. 10d, PHI 3d) is presented in table 7.6.2-1 ~~and an overview of residue data in lettuce in compliance with envisaged uses is presented in table 7.6.2-2.~~

Details of the trials are presented in tables 7.6.2-2~~3~~ to 7.6.2-4~~5~~. Representative total residue levels for agricultural use in the greenhouse after two applications at the PHI of 3 days are underlined.
The minimum required number of trials for crops is 8 for each region where the crop is considered major. All intended crops in this dossier are considered major in their intended use regions. For protected crops (lettuce) the required 8 trials are available. Although for the open field lettuce in Northern and Southern Europe no trails in compliance with the envisaged uses are available, sufficient trials were performed providing evidence that the envisaged greenhouse can be identified as the worst case residue situation. Therefore no further trials are required.

Table 7.6.2-1 Comparison of the residue values from field and greenhouse testing using the most critical use pattern (2x125 g as/ha, int. 10d, PHI 3d).

Commodity	Residue region	Outdoor/ indoor	Enforcement		Risk assessment			MRL proposal (mg/kg)		Median CF	Comments
			BYI 02960	DFA	(Sum of BYI 02960 (parent compound) and DFA, expressed as BYI 02960)	Median residue (mg/kg)	Highest residue (mg/kg)	BYI 02960	DFA		
Lettuce	NEU/ SEU	indoor	0.73; 1.4; 1.8; 2.0; 2.1; 2.5; 2.6; 3.5; 6.0	0.009; 0.01; 0.013; 0.018; <0.02; <0.02; <0.02; 0.023; 0.043;	0.79; 1.42; 1.82; 2.02; 2.12; 2.53; 2.64; 3.52; 6.04;	2.12	6.04	9	0.06	1	Agricultural use (2 x 0.125 g a.s./ha, interval = 10 days, PHI = 3 days Unrounded MRL BYI 02960: 8.597 DFA: 0.060 Greenhouse use identified as the worst case residue situation
Lettuce	NEU	outdoor	0.08; 0.11; 0.13; 0.19 0.20; 0.25; 0.37; 0.57; 0.80;	0.009; 0.016; <0.02; <0.02; <0.02; <0.02; <0.02; <0.02; <0.02;	0.10; 0.13; 0.15; 0.21; 0.22; 0.28; 0.39; 0.62; 0.82	0.22	0.82	1.5	0.04	1	Agricultural use (1 x 0.125 g a.s./ha, PHI = 10 days Unrounded MRL BYI 02960: 1.265 DFA: 0.036 No trials compliant with the cGAP with PHI 3d (1x125 g as/ha, int. 10d, PHI 3d). Identified as less critical than Greenhouse use
Lettuce	SEU	outdoor	0.04; 0.04; 0.05; 0.11; 0.29; 0.38; 0.43; 0.55; 0.80	<0.02; <0.02; <0.02; 0.02; 0.029; 0.03; 0.03; 0.046; 0.051	0.06; 0.06; 0.08; 0.14; 0.31; 0.41; 0.48; 0.60; 0.82	0.31	0.82	1.5	0.08	1	Agricultural use (1 x 0.125 g a.s./ha, PHI = 10 days Unrounded MRL BYI 02960: 1.366 DFA: 0.076 No trials compliant with the cGAP with PHI 3d (1x125 g as/ha, int. 10d, PHI 3d). Identified as less critical than Greenhouse use
Lettuce	NEU	outdoor	0.11; 0.37; 0.43; 0.58; 0.68; 0.83; 1.0; 1.5;	0.009; 0.009; 0.010; <0.02;	0.13; 0.39; 0.46; 0.60; 0.70; 0.83; 1.02; 1.52; 3.00	0.60	3.00	5.0	0.1	1	Home and garden use (2 x 0.125 g a.s./ha, interval = 10 days, PHI = 3 days) Unrounded MRL

Commodity	Residue region	Outdoor/ indoor	Enforcement		Risk assessment			MRL proposal (mg/kg)		Median CF	Comments
			BYI 02960	DFA	(Sum of BYI 02960 (parent compound) and DFA, expressed as BYI 02960)	Median residue (mg/kg)	Highest residue (mg/kg)	BYI 02960	DFA		
			2.9	<0.02; <0.02; <0.02; <0.02; 0.066							BYI 02960: 4.430 DFA: 0.091
Lettuce	SEU	outdoor	0.35; 0.40; 0.48; 0.72; 1.1; 1.5; 2.1; 2.7; 3.1	0.007; 0.010; 0.013; 0.016; 0.017; 0.017; <0.02; 0.043; 0.05;	0.38; 0.42; 0.53; 0.76; 1.12; 1.58; 2.15; 2.73; 3.18	1.12	3.18	6	0.08	1	Home and garden use (2 x 0.125 g a.s./ha, interval = 10 days, PHI = 3 days) Unrounded MRL BYI 02960: 5.526 DFA: 0.081

~~Commodity~~	~~Residue region~~	~~Outdoor/ indoor~~	~~Enforcement (Sum of BYI 02960 (parent compound) and DFA) expressed as BYI 02960)~~	~~Risk assessment (Sum of BYI 02960 (parent compound), DFA, and BYI 02960-difluoroethyl-amino-furanone (DFEAF), expressed as BYI 02960)~~	~~Median residue (mg/kg)~~	~~Highest residue (mg/kg)~~	~~MRL proposal (mg/kg)~~	~~Median CF~~	~~Comments~~
~~Lettuce~~	~~NEU/ SEU~~	~~indoor~~	~~0.73, 1.2, 1.3, 2.0, 2.0, 2.1, 2.6, 3.5 , 6.0~~	~~0.80, 1.4, 1.8, 2.0, 2.2, 2.5, 2.7, 3.5 6.0~~	~~2.2~~	~~6.0~~	~~9~~	~~1*~~	~~Agricultural use (2 x 0.125 g a.s./ha, interval = 10 days, PHI = 3 days MRL OECD unrounded: 8.714 mg/kg Greenhouse use identified as the worst case residue situation~~
~~Lettuce~~	~~NEU~~	~~outdoor~~	~~0.10, 0.13, 0.15, 0.21, 0.22, 0.28, 0.39, 0.62, 0.82~~	~~0.11, 0.14, 0.16, 0.22, 0.23, 0.28, 0.40, 0.65, 0.83~~	~~0.22~~	~~0.82~~		~~1*~~	~~MRL OECD unrounded: 1.306 mg/kg~~
~~Lettuce~~	~~SEU~~	~~outdoor~~	~~0.06, 0.06, 0.08, 0.14, 0.31,~~	~~0.07, 0.07, 0.09, 0.15, 0.32, 0.41, 0.49,~~	~~0.31~~	~~0.82~~		~~1*~~	~~MRL OECD unrounded: 1.412~~

			0.41, 0.48, 0.60, 0.82	0.61, 0.83,					mg/kg
Lettuce	NEU	outdoor	0.13; 0.39; 0.46; 0.60; 0.70; 0.85; 1.0; 1.5; 3.0	0.14; 0.40; 0.47; 0.61; 0.71; 0.87; 1.0; 1.6; 3.0	0.7	3.0		1*	Home and garden use (2 x 0.125 g a.s./ha, interval = 10 days, PHI = 3 days) MRL OECD unrounded: 4.402 mg/kg
Lettuce	SEU	outdoor	0.38; 0.42; 0.53; 0.76; 1.1; 1.6; 2.2; 2.7; 3.2	0.39; 0.43; 0.53; 0.78; 1.2; 1.6; 2.2; 2.7; 3.2	1.1	3.2		1*	Home and garden use (2 x 0.125 g a.s./ha, interval = 10 days, PHI = 3 days) MRL OECD unrounded: 5.641 mg/kg

* Since the metabolite BYI 02960-difluoroethyl-amino-furanone (DFEAF) was detected at levels below the LOQ of 0.01 mg/kg, or at levels representing less than 5% of the total residue, a Conversion factor (monitoring to risk assessment) of 1 is proposed.

Table 7.6.2-2 Overview of the available residue trials data in lettuce in compliance with envisaged uses.

Commodity	Residue region	Outdoor/ indoor	Enforcement (Sum of BYI 02960 (parent compound) and DFA) expressed as BYI 02960)	Risk assessment (Sum of BYI 02960 (parent compound), DFA, and BYI 02960-difluoroethyl-amino-furanone (DFEAF), expressed as BYI 02960)	Median residue (mg/kg)	Highest residue (mg/kg)	MRL proposal (mg/kg)	Median CF	Comments
Lettuce	NEU /SEU	indoor	0.73, 1.2, 1.3, 2.0, 2.0, 2.1, 2.6, 3.5 , 6.0 mg//kg	0.80, 1.4, 1.8, 2.0, 2.2, 2.5, 2.7, 3.5 6.0 mg//kg	2.2	6.0	9	1*	9 trials compliant with the cGAP for protected lettuce (2x125 g as/ha, int. 10d, PHI 3d). Mean + 4SD: 8.714 CF * 3 Mean: 77.143
Lettuce	NEU	outdoor	0.10, 0.13, 0.15, 0.21, 0.22, 0.28, 0.39, 0.62, 0.82 mg//kg	0.11, 0.14, 0.16, 0.22, 0.23, 0.28, 0.40, 0.65, 0.83 mg//kg	0.22	0.82		1*	9 trials compliant with the cGAP with PHI 10d (1x125 g as/ha, PHI 10d) Mean + 4SD: 1.306 CF * 3 Mean: 0.973
Lettuce	NEU	outdoor	-	-	-	-			No trials compliant with the cGAP with PHI 3d (1x125 g as/ha, int. 10d, PHI 3d). Identified as less critical than protected use (see table 7.6.2-1)
Lettuce	SEU	outdoor	0.06, 0.06, 0.08, 0.14, 0.31, 0.41, 0.48, 0.60, 0.82 mg//kg	0.07, 0.07, 0.09, 0.15, 0.32, 0.41, 0.49, 0.61, 0.83 mg//kg	0.31	0.82		1*	9 trials compliant with the cGAP with PHI 10d (1x125 g as/ha, PHI 10d) Mean + 4SD: 1.412 CF * 3 Mean: 0.987
Lettuce	SEU	outdoor	-	-	-	-			No trials compliant with the cGAP with PHI 3d (1x125 g as/ha, int. 10d, PHI 3d). Identified as less critical than Greenhouse use (see table 7.6.2-1)
Lettuce	NEU	outdoor	0.13; 0.39; 0.46; 0.60; 0.70; 0.85; 1.0; 1.5; 3.0	0.14; 0.40; 0.47; 0.61; 0.71; 0.87; 1.0; 1.6; 3.0	0.7	3.0		1*	Home and garden use (2 x 0.125 g a.s./ha, interval = 10 days, PHI = 3 days). Included in MRL application MRL OECD unrounded: 4.402 mg/kg
Lettuce	SEU	outdoor	0.38; 0.42; 0.53; 0.76; 1.1; 1.6; 2.2; 2.7; 3.2	0.39; 0.43; 0.53; 0.78; 1.2; 1.6; 2.2; 2.7; 3.2	1.1	3.2		1*	Home and garden use (2 x 0.125 g a.s./ha, interval = 10 days, PHI = 3 days). Included in MRL application MRL OECD unrounded: 5.641 mg/kg

* Since the metabolite BYI 02960-difluoroethyl-amino-furanone (DFEAF) was detected at levels below the LOQ of 0.01 mg/kg, or at levels less representing less than 5% of the total residue, a Conversion factor (monitoring to risk assessment) of 1 is proposed.

Table 7.6.2-~~3~~ 2: Summary of supervised residue trials with lettuce in the greenhouse in Northern and Southern Europe

Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) expressed as BYI 02960					DALT (days)	Remarks
			kg a.s./ha	Water l/ha	kg a.s./hl				BYI 02960	DFA as BYI 02960	DFE AF	DFA as DFA	Total BYI 02960 + DFA, as BYI 02960		
10-2212-01; France; 95000 Cergy; 2010	lettuce Kitonia, leafy variety	1. 15/03/2010 2. -- 3. 06/05/2010 to 12/05/2010	0.125 0.125	600 600	0.0208 0.0208	27/04/2010 07/05/2010 .	BBCH 49	head	0.23 1.5 1.3 1.4 1.2 1.4	<0.02 <0.02 <0.02 <0.02 0.02 0.02	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.02 <0.02 <0.02 <0.02 0.007 0.007	0.25 1.52 1.32 1.42 1.22 1.42	0* 0 1 3 5 7	Reference: KIIA 6.3.1.1 /05 Study: 10-2212 Formulation: BYI 02960 SL 200 Method: 01304 BYI 02960 LOQ = 0.01 mg/kg DFA LOQ = 0.02 mg/kg DFEAF LOQ = 0.01 mg/kg CF DFA as BYI 02960 to DFA as DFA: 0.33
10-2212-02; Germany; 42799 Leichlingen; 2010	lettuce Antoni, leafy variety	1. 27/01/2010 2. -- 3. 08/04/2010 to 15/04/2010	0.125 0.125	600 600	0.0208 0.0208	26/03/2010 06/04/2010	BBCH 48	head	2.7 6.7 2.3 2.0 1.5 1.3	<0.02 <0.02 <0.02 <0.02 <0.02 <0.02	0.01 0.02 <0.01 0.01 <0.01 <0.01	<0.02 <0.02 <0.02 <0.02 <0.02 <0.02	2.72 6.72 2.32 2.02 1.52 1.32	0* 0 1 3 5 7	
10-2212-03; Germany; 53332 Bornheim; 2010	lettuce Torpedo, butterhead variety	1. 15/03/2010 2. -- 3. 23/04/2010 to 30/04/2010	0.125 0.125	400 400	0.0313 0.0313	13/04/2010 23/04/2010	BBCH 48	head	0.13 5.2 4.6 3.5 2.4 1.7	<0.02 <0.02 <0.02 <0.02 0.02 0.02	<0.01 0.01 0.01 0.01 0.01 0.01	<0.02 <0.02 <0.02 <0.02 0.007 0.007	0.15 5.22 4.62 3.52 2.42 1.72	0* 0 1 3 5 7	
10-2212-04; Netherlands; 2988 DA	lettuce Gardia, butterhead	1. 01/08/2010 2. -- 3. 25/09/2010 to	0.125 0.125	600 600	0.0208 0.0208	14/09/2010 24/09/2010	BBCH 45	head	0.29 3.1	0.02 0.02	0.01 0.02	0.007 0.007	0.31 3.12	0* 0	

Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) expressed as BYI 02960					DALT (days)	Remarks
			kg a.s./ha	Water l/ha	kg a.s./hl				BYI 02960	DFA as BYI 02960	DFE AF	DFA as DFA	Total BYI 02960 + DFA, as BYI 02960		
Ridderkerk ; 2010	variety	30/09/2010							2.5	0.02	0.02	0.007	2.52	1	
									2.5	0.03	0.02	0.01	2.53	3	
									2.0	0.03	0.03	0.01	2.03	5	
									0.99	0.04	0.02	0.013	1.03	7	
10-2212-05; Italy; 97019 Vittoria; 2010	lettuce Cappuc-cina, butterhead variety	1. 10/03/2010 2. -- 3. 12/04/2010 to 25/04/2010	0.125 0.125	500 500	0.0250 0.0250	02/04/2010 12/04/2010	BBCH 45	head	0.65	<0.02	0.01	<0.02	0.67	0*	
									3.8	<0.02	0.02	<0.02	3.82	0	
									2.7	<0.02	0.02	<0.02	2.72	1	
									1.8	0.02	0.02	0.007	1.82	3	
									1.3	0.02	0.01	0.007	1.32	5	
									0.74	0.03	0.01	0.01	0.77	7	
11-2070-01; France; 95000 Cergy ; 2011	lettuce Quenty, open leaf variety	1. 16/03/2011 2. -- 3. 02/05/2011 to 12/05/2011	0.125 0.125	600 600	0.0208 0.0208	22/04/2011 02/05/2011	BBCH 48	head	3.0	<0.02	<0.01	<0.02	3.02	0	Reference: KIIA 6.3.1.1 /06 Study: 11-2070 Formulation: BYI 02960 SL 200 Method: 01212 BYI 02960 LOQ = 0.01 mg/kg DFA LOQ = 0.02 mg/kg DFEAF LOQ = 0.01 mg/kg
									2.1	0.022	0.011	0.007	2.12	3	
									0.58	0.026	<0.01	0.009	0.61	7	
									0.39	0.028	<0.01	0.009	0.42	10	
									0.18	0.026	<0.01	0.009	0.21	14	
11-2070-02; Italy; 76011 Bisceglie; 2011	lettuce Expedition RZ, Green incised-leaf variety	1. 12/02/2011 2. -- 3. 01/04/2011 to 13/04/2011	0.125 0.125	600 600	0.0208 0.0208	22/03/2011 01/04/2011	BBCH 47	head	4.1	0.040	<0.01	0.013	4.14	0	
									0.73	0.059	<0.01	0.02	0.79	3	
									0.43	0.073	<0.01	0.024	0.50	7	
									0.32	0.098	<0.01	0.033	0.42	10	
									0.13	0.13	<0.01	0.043	0.26	14	
11-2070-03 ; Spain ; 08415	Lettuce, Oak Leaf, leaf variety	1.17/01/2011 2. -- 3. 30/03/2011 to	0.125 0.125	500 400	0.0250 0.0313	02/03/2011 11/03/2011	BBCH 47	head	7.6	0.029	0.024	0.01	7.63	0	
									6.0	0.035	0.027	0.012	6.04	3	

Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) expressed as BYI 02960					DALT (days)	Remarks
			kg a.s./ha	Water l/ha	kg a.s./hl				BYI 02960	DFA as BYI 02960	DFEAF	DFA as DFA	Total BYI 02960 + DFA, as BYI 02960		
Bigues i Riells ; 2011		13/04/2011							4.6	0.055	0.037	0.018	4.66	7	CF DFA as BYI 02960 to DFA as DFA: 0.33
									2.9	0.061	0.038	0.02	2.96	11	
									2.6	0.069	0.049	0.023	2.67	13	
11-2070-04; Germany; 69121 HD-Handschuhsheim; 2011	lettuce Judita, head lettuce	1. 21/01/2011 2. -- 3. 04/04/2011 to 18/04/2012	0.125 0.125	600 600	0.0208 0.0208	25/03/2011 04/04/2011	BBCH 49	head	3.7	0.027	0.015	0.009	3.73	0	
									2.6	0.036	0.014	0.012	2.64	3	
									0.87	0.043	0.010	0.014	0.91	7	
									0.46	0.053	<0.01	0.018	0.51	10	
									0.23	0.041	<0.01	0.014	0.27	14	

DFA = Difluoroacetic acid
DFEAF = Difluoroethyl-amino-furanone
Total residu of BYI 02960 + DFA + DFEAF
0* DALT: residue levels in samples taken immediately before the second treatment (= ten days after the first treatment)

Table 7.6.2-4 3 Summary of supervised residue trials with lettuce (outdoor) in Northern Europe

Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) expressed as BYI 02960					DALT (days)	Remarks
			kg a.s./ha	Water l/ha	kg a.s./hl				BYI 02960	DFA as BYI 02960	DFE AF	DFA as DFA	Total BYI 02960 + DFA, as BYI 02960		
10-2223-01 Netherlands 1681 ND Zwaagdijk-Oost 2010	lettuce Gisela, Butterhead variety	1. 01/07/2010 2. -- 3. 04/08/2010 to 10/08/2010	0.125 0.125	300 300	0.0417 0.0417	23/07/2010 02/08/2010 .	BBCH 48	head	0.20 1.9 1.8 0.58 0.34 0.22	<0.02 <0.02 <0.02 <0.02 <0.02 <0.02	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.02 <0.02 <0.02 <0.02 <0.02 <0.02	0.22 1.92 1.82 0.60 0.36 0.24	0* 0 1 3 5 7	Reference: KIIA 6.3.1.1 /01 Study: 10-2223 Formulation: BYI 02960 SL 200 Method: 01304 BYI 02960 LOQ = 0.01 mg/kg DFA LOQ = 0.02 mg/kg DFEAF LOQ = 0.01 mg/kg CF DFA as BYI 02960 to DFA as DFA: 0.33
10-2223-02 Belgium 6210 Villers-Perwin 2010	lettuce Lucan, Butterhead variety	1. 13/04/2010 2. -- 3. 17/06/2010 to 23/06/2010	0.125 0.125	500 500	0.0250 0.0250	04/06/2010 14/06/2010	BBCH 48	head	0.08 1.7 0.43 0.37 0.34 0.21	<0.02 <0.02 <0.02 <0.02 <0.02 <0.02	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.02 <0.02 <0.02 <0.02 <0.02 <0.02	0.10 1.72 0.45 0.39 0.36 0.23	0* 0 1 3 5 7	
10-2223-03 France 95000 Cergy 2010	lettuce Abago, Butterhead variety	1. 24/03/2010 2. -- 3. 29/05/2010 to 08/06/2010	0.125 0.125	600 600	0.0208 0.0208	18/05/2010 28/05/2010	BBCH 48	head	0.13 1.5 1.3 0.68 0.52 0.46	<0.02 <0.02 <0.02 <0.02 0.02 0.03	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.02 <0.02 <0.02 <0.02 0.007 0.010	0.15 1.52 1.32 0.70 0.54 0.49	0* 0 1 3 5 7	
10-2223-04 Germany 40764	lettuce Cavernet Lollo rosso,	1. 28/04/2010 2. -- 3. 14/06/2010 to	0.125 0.125	300 300	0.0417 0.0417	01/06/2010 11/06/2010	BBCH 48	head	0.37 1.7	<0.02 <0.02	<0.01 <0.01	<0.02 <0.02	0.39 1.72	0* 0	

Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) expressed as BYI 02960					DALT (days)	Remarks
			kg a.s./ha	Water l/ha	kg a.s./hl				BYI 02960	DFA as BYI 02960	DFE AF	DFA as DFA	Total BYI 02960 + DFA, as BYI 02960		
Langenfeld-Reusrath 2010	loose leaf variety	20/07/2010							1.1	<0.02	<0.01	<0.02	1.12	1	
									1.0	<0.02	0.01	<0.02	1.02	3	
									0.87	<0.02	<0.01	<0.02	0.89	5	
									0.66	<0.02	<0.01	<0.02	0.68	7	
10-2223-05 Germany 67125 Schauernheim 2010	lettuce Chloe Lollo rosso, loose leaf variety	1. 07/09/2010 2. -- 3. 16/10/2010 to 22/10/2010	0.125 0.125	400 400	0.0313 0.0313	06/10/2010 15/10/2010	BBCH 48	head	0.80	<0.02	0.01	<0.02	0.82	0*	
									4.1	<0.02	0.02	<0.02	4.12	0	
									1.0	<0.02	0.01	<0.02	1.02	1	
									0.83	<0.02	0.01	<0.02	0.85	3	
									0.83	<0.02	0.01	<0.02	0.85	5	
									0.65	<0.02	<0.01	<0.02	0.67	7	
11-2082-01 Germany 40764 Langenfeld 2011	lettuce Aleppo Lollo bionda, loose leaf variety	1. 26/04/2011 2. -- 3. 30/05/2011 to 10/06/2011	0.125 0.125	600 600	0.0208 0.0208	17/05/2011 27/05/2011	BBCH 48	head	0.19	<0.02	<0.01	<0.02	0.21	0*	Reference: KIIA 6.3.1.1 /02 Study: 11-2082 Formulation: BYI 02960 SL 200 Method: 01212 BYI 02960 LOQ = 0.01 mg/kg DFA LOQ = 0.02 mg/kg
									2.7	0.020	<0.01	0.007	2.72	0	
									1.5	0.023	0.017	0.008	1.52	3	
									0.52	0.028	<0.01	0.009	0.55	7	
									0.13	0.020	<0.01	0.007	0.15	10	
									0.073	0.024	<0.01	0.008	0.10	14	
11-2082-02 Germany 59457 Werl-Westönnen 2011	lettuce Kitara Lollo bionda, loose leaf variety	1. 28/04/2011 2. -- 3. 26/05/2011 to 15/06/2011	0.125 0.125	500 500	0.0250 0.0250	21/05/2011 31/05/2011	BBCH 48	head	0.11	<0.02	<0.01	<0.02	0.13	0*	
									2.6	<0.02	<0.01	<0.02	2.62	0	
									0.11	<0.02	<0.01	<0.02	0.13	3	
									0.033	0.022	<0.01	0.007	0.06	7	
									0.023	0.027	<0.01	0.009	0.05	10	
									0.011	0.027	<0.01	0.009	0.04	14	

Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) expressed as BYI 02960					DALT (days)	Remarks
			kg a.s./ha	Water l/ha	kg a.s./hl				BYI 02960	DFA as BYI 02960	DFEAF	DFA as DFA	Total BYI 02960 + DFA, as BYI 02960		
11-2082-03 France 37230 Fondettes 2011	lettuce Quenty Feuille de chene (oak leaf lettuce)	1.05/05/2011 2. -- 3. 20/06/2011 to 15/07/2011	0.125 0.125	600 600	0.0208 0.0208	14/06/2011 24/06/2011	BBCH 48	head	2.9 2.6 1.4 0.76 0.57 3.8 2.9 1.6 0.9 0.75	<0.02 <0.02 0.027 0.037 0.049 0.048 0.096 0.11 0.14 0.20	<0.01 0.028 0.049 0.044 0.040 0.053 0.093 0.074 0.062 0.082	<0.02 <0.02 0.009 0.012 0.016 0.016 0.032 0.037 0.047 0.066	2.92 2.62 1.43 0.80 0.62 3.85 3.00 1.71 1.04 0.95	-10 -8 -5 -2 0* 0 3 7 10 14	DFEAF LOQ = 0.01 mg/kg CF DFA as BYI 02960 to DFA as DFA: 0.33
11-2082-04 Belgium 6210 Villers-Perwin 2011	lettuce Funnas, leafy variety curly	1. 22/04/2011 2. -- 3. 30/05/2011 to 20/06/2011	0.125 0.125	750 750	0.0167 0.0167	17/05/2011 27/05/2011	BBCH 48	head	5.6 2.0 0.63 0.35 0.25 2.8 0.43 0.22 0.12 0.058	<0.02 <0.02 0.028 0.030 0.026 0.022 0.028 0.030 0.026 0.025	0.011 0.015 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.02 <0.02 0.009 0.010 0.009 0.007 0.009 0.010 0.009 0.008	5.62 2.02 0.66 0.38 0.28 2.82 0.46 0.25 0.15 0.08	-10 -8 -5 -2 0* 0 3 7 10 14	

DFA = Difluoroacetic acid
DFEAF Difluoroethyl-amino-furanone
Total residu of BYI 02960 + DFA + DFEAF

0* DALT: residue levels in samples taken immediately before the second treatment (= ten days after the first treatment)

Table 7.6.2-4 3: Summary of supervised residue trials with lettuce (outdoor) in Southern Europe

Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) expressed as BYI 02960					DALT (days)	Remarks
			kg a.s./ha	Water l/ha	kg a.s./hl				BYI 02960	DFA as BYI 02960	DFEAF	DFA as DFA	Total BYI 02960 + DFA, as BYI 02960		
10-2213-01 France (south) 86380 Marigny brizay 2010	lettuce Madita Head	1. 14/05/2010 2. -- 3. 14/06/2010 to 24/06/2010	0.125 0.125	500 500	0.0250 0.0250	01/06/2010 11/06/2010 .	BBCH 48	head	0.04 2.6 0.57 0.40 0.29 0.15	<0.02 <0.02 <0.02 0.02 0.02 0.02	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.02 <0.02 <0.02 0.007 0.007 0.007	0.06 2.62 0.59 0.42 0.31 0.17	0* 0 1 3 5 7	Reference: KIIA 6.3.1.1 /03 Study: 10-2213 Formulation: BYI 02960 SL 200 Method: 01304 BYI 02960 LOQ = 0.01 mg/kg DFA LOQ = 0.02 mg/kg DFEAF LOQ = 0.01 mg/kg CF DFA as BYI 02960 to DFA as DFA: 0.33
10-2213-02 Spain 46440 Almussafes 2010	lettuce Dauair Trocadero	1. 20/09/2010 2. -- 3. 15/11/2010 to 15/12/2010	0.125 0.125	600 600	0.0208 0.0208	12/11/2010 22/11/2010	BBCH 49	head	0.80 3.8 3.5 2.7 2.3 1.3	0.02 0.02 0.03 0.03 0.03 0.03	<0.01 0.02 0.02 0.02 0.02 0.01	0.007 0.007 0.010 0.010 0.010 0.010	0.82 3.82 3.53 2.73 2.33 1.33	0* 0 1 3 4 7	
10-2213-03 Italy 45020 Lusia Rovigo 2010	lettuce Ballerina butterhead	1. 11/05/2010 2. -- 3. 01/06/2010 to 30/06/2010	0.125 0.125	600 600	0.0208 0.0208	01/06/2010 11/06/2010	BBCH 46	head	0.05 2.7 2.2 0.48 0.21 0.09	0.03 0.04 0.04 0.05 0.05 0.05	<0.01 0.01 0.02 <0.01 <0.01 <0.01	0.010 0.013 0.013 0.017 0.017 0.017	0.08 2.74 2.24 0.53 0.26 0.14	0* 0 1 3 5 7	
10-2213-04	lettuce	1. 19/05/2010	0.125	600	0.0208	21/06/2010		head	0.38	0.03	<0.01	0.010	0.41	0*	

Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) expressed as BYI 02960					DALT (days)	Remarks
			kg a.s./ha	Water l/ha	kg a.s./hl				BYI 02960	DFA as BYI 02960	DFE AF	DFA as DFA	Total BYI 02960 + DFA, as BYI 02960		
Spain 08850 Gava 2010	Murai Lollo Rosso, loose leaf variety	2. -- 3. 26/06/2010 to 10/07/2010	0.125	600	0.0208	01/07/2010	BBCH 49		3.6 3.0 2.1 2.0 1.3	0.03 0.04 0.05 0.05 0.05	0.02 0.02 0.02 0.02 0.02	0.010 0.013 0.017 0.017 0.017	3.63 3.04 2.15 2.05 1.35	0 1 4 5 7	
10-2213-05 Spain 70031 Andria 2010	lettuce Bergamo Blond lollo, loose leaf variety	1. 01/09/2010 2. -- 3. 15/10/2010 to 31/10/2010	0.125 0.125	700 700	0.0179 0.0179	04/10/2010 15/10/2010	BBCH 49	head	0.04 2.9 1.9 1.1 0.21 0.17	<0.02 <0.02 <0.02 <0.02 <0.02 0.02	<0.01 <0.01 0.01 0.02 <0.01 <0.01	<0.02 <0.02 <0.02 <0.02 <0.02 0.007	0.06 2.92 1.92 1.12 0.23 0.19	0* 0 1 3 5 7	
11-2071-01 Spain 46230 Alginet 2011	lettuce Livigna RZ, loose leaf variety	1. 11/05/2011 2. -- 3. 20/06/2011 to 05/07/2011	0.125 0.125	500 500	0.0250 0.0250	14/06/2011 24/06/2011	BBCH 49	head	0.43 2.6 1.5 0.77 0.57 0.033	0.046 0.062 0.077 0.097 0.11 0.15	0.021 0.031 0.046 0.034 0.024 <0.01	0.015 0.021 0.026 0.032 0.037 0.050	0.48 2.66 1.58 0.87 0.68 0.18	0* 0 3 7 10 14	Reference: KIIA 6.3.1.1 /04 Study: 11-2071 Formulation: BYI 02960 SL 200 Method: 01212 BYI 02960 LOQ = 0.01 mg/kg DFA LOQ = 0.02 mg/kg
11-2071-02 Italy 95100 Catania 2011	lettuce Lollo Rosso, loose leaf variety	1. 10/06/2011 2. -- 3. 10/07/2011 to 25/07/2011	0.125 0.125	700 700	0.0179 0.0179	28/06/2011 08/07/2011	BBCH 46	head	0.55 7.3 3.1	0.051 0.039 0.083/ 0.032 **	0.011 0.023 0.045	0.017 0.013 0.028 / 0.011	0.60 7.34 3.18	0* 0 3	

Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) expressed as BYI 02960					DALT (days)	Remarks
			kg a.s./ha	Water l/ha	kg a.s./hl				BYI 02960	DFA as BYI 02960	DFE AF	DFA as DFA	Total BYI 02960 + DFA, as BYI 02960		
									1.4	0.13	0.025	0.043	1.53	7	DFEAF LOQ = 0.01 mg/kg
									0.24	0.11	<0.01	0.037	0.35	10	
									0.17	0.12	<0.01	0.040	0.29	14	CF DFA as BYI 02960 to DFA as DFA: 0.33
11-2071-03 France (south) 31200 Toulouse - Croix daurade 2011	lettuce Pitice, loose leaf variety	1.18/04/2011 2. -- 3. 25/05/2011 to 10/06/2011	0.125 0.125	800 800	0.0156 0.0156	18/05/2011 27/05/2011	BBCH 48	head	5.3	<0.02	0.015	<0.02	5.32	-9	
									3.4	<0.02	0.024	<0.02	3.42	-7	
									0.69	<0.02	0.014	<0.02	0.71	-4	
									0.39	<0.02	<0.01	<0.02	0.41	-1	In trial 11-2071-04, the first sampling was taken before first application instead of after first application
									0.29	<0.02	<0.01	<0.02	0.31	0*	
									3.5	0.020	0.022	0.007	3.52	0	
									0.72	0.035	0.020	0.012	0.76	3	
									0.25	0.032	0.011	0.011	0.28	7	
									0.20	0.047	0.012	0.016	0.25	10	
									0.094	0.046	<0.01	0.015	0.14	14	
11-2071-04 Portugal 2715-252 Almargem do Bispo 2011	lettuce Caypira, loose leaf variety	1. 30/04/2011 2. -- 3. 30/04/2011 to ….	0.125 0.125	800 800	0.0156 0.0156	10/05/2011 20/05/2011	BBCH 49	head	<0.01	<0.02	<0.01	<0.02	0.03	-10	
									1.8	0.022	0.021	0.007	1.82	-8	
									0.92	0.031	0.015	0.010	0.95	-5	
									0.27	0.027	<0.01	0.009	0.30	-2	
									0.11	0.029	<0.01	0.010	0.14	0*	
									1.9	0.026	<0.01	0.009	1.93	0	
									0.35	0.030	<0.01	0.010	0.38	3	
									0.16	0.031	<0.01	0.010	0.19	7	
									0.067	0.034	<0.01	0.011	0.10	10	

Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) expressed as BYI 02960					DALT (days)	Remarks
			kg a.s./ha	Water l/ha	kg a.s./hl				BYI 02960	DFA as BYI 02960	DFEAF	DFA as DFA	Total BYI 02960 + DFA, as BYI 02960		
									0.046	0.038	<0.01	0.013	0.08	14	

** residues in control samples.
DFA = Difluoroacetic acid
DFEAF Difluoroethyl-amino-furanone
Total residu of BYI 02960 + DFA + DFEAF
0* DALT: residue levels in samples taken immediately before the second treatment (= ten days after the first treatment

B.7.6.3 Supervised residue trials in hops

Study 1

Characteristics

reference	:	Noss, G.; Ballmann, A. 2012a KIIA 6.3.1.2/01	GLP statement	:	yes (certified laboratory); Deviations: none
Type of study	:	Supervised residue trial (Northern Europe)	Guideline	:	EEC 7029/VI/95 rev. 5, appendix B (1997)
Year of execution	:	2010	Dose	:	0.120 kg as/ha
test substance	:	BYI 02960 SL 200 formulation Batch no: 2010-000672	LOQ	:	BYI 02960 : 0.1 mg/kg DFA: 0.2 mg/kg DFEAF : 0.1 mg/kg
Species	:	Hops *(Humulus lupulus)*	storage Acceptability	: :	≤ 222 d at -18°C Y

Study design

Four trials in hops were performed in the 2010 growing season in Northern Europe (all in Germany). An SL formulation containing 200 g/L BYI 02960 was applied once to the crop at a nominal rate of 0.6 L/ha, corresponding to 120 g/ha BYI 02960 a.s.; the water rate was 2000-3000 L/ha.

Samples of green hop cones were taken immediately after the final application and at several intervals thereafter (up to 28 days after treatment). The envisaged PHI was 21 days. (In one trial the PHI samples were taken on day 20). In addition to the green cone samples, additional cones were taken at the later sampling intervals (nominally days 14, 21, and 28) and dried according to standard practice, as dry cones are the primary traded commodity from the grower to the market.

Samples were investigated for BYI 02960 and its metabolites DFA and DFEAF using method 01304. The respective LOQs for the 3 analytes were 0.1, 0.2, and 0.1 mg/kg (all in parent equivalents), yielding a calculated total-residue LOQ of 0.40 mg/kg.

Results

The apparent residues in the control samples used for fortification experiments, were below 30% of the LOQ, with the exception for BYI 02960 for the sample material kiln-dried cone (LOQ-level). In this case, recovery was corrected for apparent residue in the control sample.

Analyses of green cones showed that total residue levels generally declined with time. On day 0, immediately following treatment, residue levels in green hop cones were between 0.79 and 1.7 mg/kg By day 21 — the envisaged PHI (samples were taken on day 20 in one trial — the levels had declined to <0.40-0.82 mg/kg. Residues continued to decrease until day 27-28, the final sampling event, when levels ranged from <0.40-0.46 mg/kg.

The residue behaviour was somewhat less predictable in dried cones. Residue levels on day 21 (20 in one trial) ranged from 0.78-1.2 mg/kg. In one of the trials, the residue level at the final sampling interval (28 days) was higher than at the PHI (day 21): 1.6 mg/kg. In the other trials on day 28 (day 27

in one trial), they were lower, at <0.40-0.79 mg/kg. Taking the highest residues at relevant sampling intervals into consideration (either day 21 or 28), residues ranged from 0.78-1.6 mg/kg.

Details of the trials are presented in table 7.6.2-9.

Conclusion

In study 10-2225 four outdoor residue trials in Northern Europe in hops were provided. The dose rate of 0.120 kg as/ha was less than the envisaged use of 0.150 kg as/ha. The deviation from the envisaged use (< 25%) is considered acceptable. Taking the highest residues at relevant sampling intervals into consideration residue data for dried cones (RAC) for the critical GAP are : 0.78, 1.1, 1.2 and 1.6 mg/kg.

Study 2

Characteristics

reference	:	Noss, G.; Ballmann, A. 2012b KIIA 6.3.1.2/02	GLP statement	:	yes (certified laboratory); Deviations: none
Type of study	:	Supervised residue trial (Northern Europe)	Guideline	:	EEC 7029/VI/95 rev. 5, appendix B (1997)
Year of execution	:	2011	Dose	:	0.120 kg as/ha
test substance	:	BYI 02960 SL 200 formulation Batch no: 2010-007173	LOQ	:	BYI 02960 : 0.1 mg/kg DFA: 0.2 mg/kg DFEAF : 0.1 mg/kg
Species	:	Hops *(Humulus lupulus)*	storage Acceptability	: :	≤ 117 d at -18°C Y

Study design

Four trials in hops were performed in the 2011 growing season in Northern Europe (all in Germany). An SL formulation containing 200 g/L BYI 02960 was applied once to the crop at a nominal rate of 0.75 L/ha, corresponding to 150 g/ha BYI 02960 a.s.; the water rate was 2000-3000 L/ha.

Samples of green hop cones were taken immediately after the final application and at several intervals thereafter (up to 28 days after treatment). The envisaged PHI was 21 days. (In one trial the PHI samples were taken on day 20; in one other, on day 22.) In addition to the green cone samples, additional cones were taken at the later sampling intervals (nominally days 14, 21, and 28) and dried according to standard practice, as dry cones are the primary traded commodity from the grower to the market.

Samples were investigated for BYI 02960 and its metabolites DFA and DFEAF using method 01304. The respective LOQs for the 3 analytes were 0.1, 0.2, and 0.1 mg/kg (all in parent equivalents), yielding a calculated total-residue LOQ of 0.40 mg/kg.

Results

Analyses of green cones showed that total residue levels generally declined with time. On day 0, immediately following treatment, residue levels in green hop cones were between 0.85 and 2.7 mg/kg.

By day 21 — the envisaged PHI (samples were taken on day 20 in one trial and day 22 in one further trial) — the levels had declined to <0.40-0.87 mg/kg. At day 26-28, the final sampling event, levels ranged from 0.40-0.69 mg/kg.

In dried cones in two out of four trials, residue levels at the final sampling interval (28 days) were higher than at the PHI (day 21). Residue levels on day 21 (20 in one trial, 22 in one other) ranged from 0.56-2.4 mg/kg. On day 28 (day 26 in one trial) residue levels ranged from 0.59-2.3 mg/kg. Taking the highest residues at relevant sampling intervals into consideration (either day 21 or 28), residues ranged from 0.61-2.4 mg/kg.

Details of the trials are presented in table 7.6.3-2.

Conclusion

In study 11-2076 four acceptable residue trials performed in accordance with the cGAP for outdoor use of Northern Europe have been provided with hops. Taking the highest residues at relevant sampling intervals into consideration residue data for dried cones (RAC) for the critical GAP are : 0.61, 0.73, 2.3 and 2.4 mg/kg.

Overall conclusion hops

In hops, eight valid trials were conducted in the northern European residue region in the years 2010-2011. Flupyradifurone was applied once as an SL 200 formulation at an active substance rate of 120 - 150 g/ha. All applications were at acceptable rates, and all trials were conducted according to GLP.

To evaluate this use, samples of both green and dried hop cones were taken at several intervals after the final application, including the envisaged PHI of 21 days. Samples were analyzed for the relevant residues of BYI 02960, comprising the parent compound and its metabolites DFA and DFEAF. The residues of all three analytes were summed to yield a calculated "total residue of BYI 02960". The results of the trials presented above demonstrate that:

- total residues of BYI 02960 dissipated rapidly in green hop cones, from levels of 0.79-2.7 mg/kg on day 0 after the treatment to <0.40-0.87 mg/kg on day 21 (envisaged PHI). The respective median values were 1.3 and 0.47 mg/kg;
- in dried cones, residue levels also tended to decline with time. When evaluating the highest residues at relevant sampling intervals (either day 21 or, in three trials, day 28), residues ranged from 0.61-2.4 mg/kg, with a median of 1.15 mg/kg.

An overview of residue data in hops is presented in table 7.6.3-1. Details of the trials are presented in table 7.6.3-2.

Compared to the residue definition for monitoring the metabolite BYI 02960-difluoroethyl-amino-furanon (DFEAF) is included in the residue definition for risk assessment. Since DFEAF was not detected at levels at or above the LOQ of 0.1 mg/kg a conversion factor (monitoring to risk assessment) of 1 is proposed.

Tabel 7.6.3-1 Overview of the available residue trials data in hops.

Commodity	Residue region	Outdoor/ indoor	Enforcement		Risk assessment			MRL proposal (mg/kg)		Median CF	Comments
			BYI 02960	DFA	(Sum of BYI 02960 (parent compound) and DFA, expressed as BYI 02960)	Median residue (mg/kg)	Highest residue (mg/kg)	BYI 02960	DFA		
Hop cone, kiln-dried	NEU	outdoor	0.14, 0.31, 0.43, 0.48, 0.77, 0.90, 1.1, 1.8, 2.0	0.09, 0.09, 0.13, 0.17, <0.2, <0.2, <0.2, <0.2, <0.2	0.34, 0.51, 0.63, 0.68, 1.05, 1.11, 1.5, 2.27, 2.3	1.05	2.3	4	0.4	1	8 trials on hops compliant with the cGAP (1x120 g as/ha, PHI 21d), cone, kiln-dried Unrounded MRL BYI 02960: 3.488 DFA: 0.357

~~Commodity~~	~~Residue region~~	~~Outdoor/ indoor~~	~~Enforcement (Sum of BYI 02960 (parent compound) and DFA) expressed as BYI 02960)~~	~~Risk assessment (Sum of BYI 02960 (parent compound), DFA, and BYI 02960-difluoroethyl-amino-furanone (DFEAF), expressed as BYI 02960)~~	~~Median residue (mg/kg)~~	~~Highest residue (mg/kg)~~	~~MRL proposal (mg/kg)~~	~~Median CF~~	~~Comments~~
~~Hop cone, kiln-dried~~	~~NEU~~	~~outdoor~~	~~0.51, 0.63, 0.68, 1.0, 1.1, 1.5, 2.2, 2.3 mg/kg~~	~~0.61, 0.73, 0.78, 1.1, 1.2, 1.6, 2.3 2.4 mg/kg~~	~~1.15~~	~~2.4~~	~~5~~	~~1*~~	~~8 trials on hops compliant with the cGAP (1x120 g as/ha, PHI 21d), cone, kiln-dried Mean + 4SD: 4.031 CF * 3 Mean: 3.720~~

* Since the metabolite BYI 02960-difluoroethyl-amino-furanone (DFEAF) was not detected at levels above the LOQ of 0.1 mg/kg a conversion factor of 1 is proposed.

Table 7.6.3-2: Summary of supervised residue trials with hops (outdoor) in Northern Europe

Report-No. Location	Commodity/ Variety	Date of 1) Sowing or	Application rate per treatment	Dates of treatments	Growth stage	Portion analysed	Residues (mg/kg) expressed as BYI 02960	DALT (days)	Remarks

incl. Postal code and date		planting 2) Flowering 3) Harvest	kg a.s./ha	Water l/ha	kg a.s./hl	or no. of treatments and last date	at last treatment or date		BYI 02960	DFA as BYI 02960	DFEAF	DFA as DFA	Total BYI 02960 + DFA, as BYI 02960		
10-2225-01; Germany; 91792 Ellingen; 2010	hop Hallertauer Gold	1. 1992 2. 20/07/2010 to 22/08/2010 3. 02/09/2010 to 11/09/2010	0.12	3000	0.004	13/08/2010	BBCH 73-74	cone, green	1.3	<0.2	<0.1	<0.2	1.5	0	Reference: KIIA 6.3.1.2 /01 Study: 10-2225 Formulation: BYI 02960 SL 200 Method: 01304 BYI 02960 LOQ = 0.1 mg/kg DFA LOQ = 0.2 mg/kg DFEAF LOQ = 0.1 mg/kg CF DFA as BYI 02960 to DFA as DFA: 0.33
									0.62	<0.2	<0.1	<0.2	0.82	7	
									0.29	<0.2	<0.1	<0.2	0.49	14	
									0.52	<0.2	<0.1	<0.2	0.72	21	
									0.16	<0.2	<0.1	<0.2	0.36	28	
								cone, kiln-dried	1.5	0.27	<0.1	0.09	1.77	14	
									0.81	0.20	<0.1	0.07	1.01	21	
									1.1	0.40	<0.1	0.13	1.5	28	
10-2225-02; Germany; 99955 Luetzensoemmer; 2010	hop Magnum	1. 1993 2. 21/07/2010 to 24/08/2010 3. 01/10/2010 to 03/10/2010	0.12	2200	0.0055	20/08/2010	BBCH 75	cone, green	0.49	<0.2	<0.1	<0.2	0.69	0	
									0.27	<0.2	<0.1	<0.2	0.47	8	
									0.19	<0.2	<0.1	<0.2	0.39	13	
									<0.1	<0.2	<0.1	<0.2	0.3	20	
									<0.1	<0.2	<0.1	<0.2	0.3	27	
								cone, kiln-dried	0.54	<0.2	<0.1	<0.2	0.74	13	
									0.48	<0.2	<0.1	<0.2	0.68	20	
									<0.1	<0.2	<0.1	<0.2	0.3	27	
10-2225-03; Germany; 04769 Muegeln; 2010	hop Hallertauer Magnum	1. 1994 2. 09/07/2010 to 19/08/2010 3. 10/09/2010 to 26/09/2010	0.12	2200	0.0055	23/08/2010	BBCH 71-75	cone, green	1.4	<0.2	<0.1	<0.2	1.6	0	
									0.54	<0.2	<0.1	<0.2	0.74	7	
									0.36	<0.2	<0.1	<0.2	0.56	14	
									0.20	<0.2	<0.1	<0.2	0.4	21	
									<0.1	<0.2	<0.1	<0.2	0.3	28	
								cone, kiln-dried	1.4	0.25	<0.1	0.08	1.65	14	
									0.77	0.28	<0.1	0.09	1.05	21	

Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) expressed as BYI 02960					DALT (days)	Remarks
			kg a.s./ha	Water l/ha	kg a.s./hl				BYI 02960	DFA as BYI 02960	DFE AF	DFA as DFA	Total BYI 02960 + DFA, as BYI 02960		
									0.32	<0.2	<0.1	<0.2	0.52	28	
0-2225-04; Germany; 88069 Tettnang; 2010	hop Hallertauer Tradition	1. 2005 2. 12/07/2010 to 28/07/2010 3. 13/09/2010	0.12	2200	0.0055	21/08/2010	BBCH 85	cone, green	0.56	<0.2	<0.1	<0.2	0.76	0	
									0.27	<0.2	<0.1	<0.2	0.47	8	
									0.17	<0.2	<0.1	<0.2	0.37	14	
									0.14	<0.2	<0.1	<0.2	0.34	21	
									<0.1	<0.2	<0.1	<0.2	0.3	28	
								cone, kiln-dried	0.54	<0.2	<0.1	<0.2	0.74	14	
									0.90	0.21	<0.1	<0.2	1.11	21	
									0.49	<0.2	<0.1	<0.2	0.69	28	
11-2076-01; Germany; 91792 Ellingen; 2011	hop Hallertauer mittelfrüh	1. 1964 2. 25/06/2011 to 26/07/2011 3. 02/09/2011 to 03/09/2011	0.15	2500	0.006	04/08/2011	BBCH 75	cone, green	2.4	<0.2	<0.1	<0.2	2.6	0	Reference: KIIA 6.3.1.2 /02 Study: 11-2076 Formulation: BYI 02960 SL 200 Method: 01304 BYI 02960 LOQ = 0.1 mg/kg DFA LOQ = 0.2 mg/kg DFEAF LOQ = 0.1 mg/kg CF DFA as BYI 02960
									0.47	<0.2	<0.1	<0.2	0.67	14	
									0.51	<0.2	<0.1	<0.2	0.71	21	
									0.39	<0.2	<0.1	<0.2	0.59	28	
								cone, kiln-dried	1.0	0.36	<0.1	0.12	1.36	21	
									1.8	0.5	<0.1	0.17	2.3	28	
11-2076-02; Germany; 99955 Luetzensoemmern; 2011	hop Magnum	1. 1993 2. 20/07/2011 to 04/08/2011 3. 21/09/2011 to 24/09/2011	0.15	2500	0.006	23/08/2011	BBCH 73	cone, green	0.55	<0.2	<0.1	<0.2	0.75	0	
									0.21	<0.2	<0.1	<0.2	0.41	14	
									<0.1	<0.2	<0.1	<0.2	0.3	21	
									0.10	<0.2	<0.1	<0.2	0.3	28	
								cone, kiln-dried	0.26	<0.2	<0.1	<0.2	0.46	21	
									0.31	<0.2	<0.1	<0.2	0.51	28	
11-2076-03;	hop	1. 1994	0.15	2500	0.006	17/08/2011	BBCH 84-	cone, green	2.1	<0.2	<0.1	<0.2	2.3	0	

Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) expressed as BYI 02960					DALT (days)	Remarks
			kg a.s./ha	Water l/ha	kg a.s./hl				BYI 02960	DFA as BYI 02960	DFEAF	DFA as DFA	Total BYI 02960 + DFA, as BYI 02960		
Germany; 04703 Meinitz; 2011	Hallertauer Tradition	2. 12/06/2011 to 17/07/2011 3. 05/09/2011 to 13/09/2011					86		0.78	<0.2	<0.1	<0.2	0.98	13	to DFA as DFA: 0.33
									0.57	<0.2	<0.1	<0.2	0.77	20	
									0.23	<0.2	<0.1	<0.2	0.43	26	
								cone, kiln-dried	2.0	0.27	<0.1	0.09	2.27	20	
									0.49	<0.2	<0.1	<0.2	0.69	26	
11-2076-04; Germany; 88069 Tettnang; 2011	hop Tettnanger	1. 1995 2. june 2011 to august 2011 3. 25/08/2011 to 15/09/2011	0.15	2000	0.0075	16/08/2011	BBCH 75-78	cone, green	0.61	<0.2	<0.1	<0.2	0.81	0	
									0.11	<0.2	<0.1	<0.2	0.31	13	
									<0.1	<0.2	<0.1	<0.2	0.3	22	
									0.11	<0.2	<0.1	<0.2	0.31	28	
								cone, kiln-dried	0.43	<0.2	<0.1	<0.2	0.63	22	
									0.29	<0.2	<0.1	<0.2	0.49	28	

DFA = Difluoroacetic acid
DFEAF = Difluoroethyl-amino-furanone
Total residu of BYI 02960 + DFA + DFEAF
Representative total residue levels for agricultural use in the greenhouse after two applications at the PHI of 3 days are underlined.

B.7.6.4 Storage stability (IIA 6.1)

Plant matrices

The longest periods of frozen storage of samples from plant residue studies (field residue, processing, or rotational crop trials) are shown in table 7.6.4-1 below:

Table 7.6.4-1: Periods of frozen storage (approx. -18°C) of plant-based samples (between sampling and analysis) for matrices relevant to this dossier

Sample material		Longest storage duration (d)	Study	
Crop	Matrix		Report no.	Reference no
lettuce	head	300	10-2503	6.6.3/01
	outer leaves inner head parts inner leaves washed inner leaves	224	10-3223	6.5.4/01
	washing water	225	10-3223	6.5.4/01
hops	green cone	219	10-3407	6.5.4/02
	dried cone	223	10-2225	6.3.2/01
	beer hops draff brewer's yeast	220	10-3407	6.5.4/02
barley	grain straw	480	10-2503	6.6.3/01
	green material	540	10-2503	6.6.3/01
carrot	root	257	10-2503	6.6.3/01
turnip	root (body)	180	10-2503	6.6.3/01
	leaf	177	10-2503	6.6.3/01

Storage stability data was required and submitted for the relevant residues of flupyradifurone in plant matrices.

STUDY 1
Characteristics

Reference	:	Netzband, Timberlake, Harbin, 2012; KIIA 6.1.1 /01	GLP statement	:	Y
Type of study	:	Storage stability	Guidelines	:	EPA Ref.: OPPTS 860.1380, Storage Stability Data OECD Guideline for the Testing of Chemicals No. 506, Stability of Pesticide Residues in Stored Commodities, Oct. 2007. PMRA Residue Chemistry Guidelines, Regulatory Directive 98-02, Section 5, Storage Stability Data, June 1998
Year of execution	:	2011-2012			
Storage duration	:	18 months	Matrix	:	High acid, high oil, high protein, high starch, high water, specialty matrix (coffee)
Test substance	:	Flupyradifurone, DFA, DFEAF	Acceptability	:	Acceptable

Study design

To determine the freezer storage stability of the relevant residues of flupyradifurone in plant materials, individual 5-g control samples of orange fruit (high acid content), spinach leaves and tomato fruit (high

water content), wheat grain (high starch content), bean seed (high protein content), coffee bean (specific matrix), soybean seed (high oil content), and sugar cane were separately spiked with 5.0 µg of either flupyradifurone parent compound, DFA, or DFEAF. Except for the day-0 analysis, samples were stored in glass containers in a freezer at an average temperature of -23°C for later use. For day-0 analysis, three treated samples of each material were chosen, as well as one control sample of each. Samples were then also analysed after nominal intervals of 1, 2½, 5-6, 12 and 18months. At each of these intervals, two treated samples of each material were removed from storage and analyzed, as well as a control sample and two samples for concurrent recovery. Samples used for concurrent recoveries were prepared at the same time and stored in the same fashion as the control samples, and spiked on the day of analysis.

Flupyradifurone and its metabolites were analytically determined using analytical method 01304 (see B.5.2), which was validated prior to and parallel to the residue analysis of the samples. The LOQ was 0.01 mg/kg for parent and DFEAF and 0.02 mg/kg for DFA in matrices with high water and high acid content and 0.05 mg/kg in all other matrices, expressed in BYI 02960 equivalents. The mean values of the concurrent recovery rates per compound, sample material, and spiking level were in the range of 82-106%, with RSDs in the range of 3-14%.

Results

At day 0, average residue recoveries of BYI 02960 ranged from 91-111% of nominal; of DFEAF ranged from 101-110% of nominal; and of DFA ranged from 79-101% of nominal. In samples analysed after approximately 18 months of frozen storage (556-560 days), the apparent (uncorrected) storage stability recoveries ranged from 89-100% ~~92-113%~~ for BYI 02960, 87-147% ~~111-147%~~ for DFA, and from 80-112% ~~85-104%~~ for DFEAF. The concurrent recoveries (= procedural recovery for freshly spiked control samples) after approx. 18 months ranged from 80-102% for BYI 02960, from 79-107% for DFA and from 83-114% for DFEAF. At all sampling dates and in all sample materials, the relevant components of the residue of BYI 02960 were above 70%. Even in the case of the lower values in the given ranges, there was no evidence of any continued degradation of any of the analytes in any of the sample materials. Thus, all analytes can be considered stable in all relevant plant matrix types for a period of at least 18 months (556 to 560 days).

All storage stability results are summarised below in tables 7.6.3-1 to 7.6.1-3.

Table 7.6.4-2 Summary of stability data for deep-frozen samples fortified with BYI 02960 – samples fortified at 1 mg/kg

Matrix	Storage period (d)	Concurrent recovery (%)	Recovery in stored samples (%)		
			apparent	normalized to day 0	corrected for recovery
sugar cane	0	(100)	100	100	100
	29	101	92	92	91
	77	91	85	85	93
	149	98	94	94	96

Matrix	Storage period (d)	Concurrent recovery (%)	Recovery in stored samples (%)		
			apparent	normalized to day 0	corrected for recovery
	372	87	93	93	107
	559	92	92	89	104
coffee bean (green)	0	(100)	94	100	100
	33	97	93	99	96
	81	81	81	85	99
	152	85	104	110	122
	370	93	83	88	89
	560	94	100	91	104
orange fruit	0	(100)	96	100	100
	28	89	84	88	95
	77	86	95	98	111
	148	104	84	87	81
	365	102	80	83	79
	556	90	94	80	113
soybean seed	0	(100)	93	100	100
	28	93	93	101	100
	75	93	96	104	104
	148	91	97	105	106
	371	96	98	106	103
	558	89	96	95	93
navy bean	0	(100)	111	100	100
	26	91	107	96	117
	75	94	110	99	117
	148	100	123	111	123
	364	113	127	114	112
	558	94	84	100	94
tomato fruit	0	(100)	95	100	100
	28	90	92	97	103
	76	86	89	94	102
	148	107	105	111	98
	370	89	95	101	107
	558	94	99	102	92
spinach	0	(100)	91	100	100
	26	96	97	106	101
	75	90	85	93	94
	147	94	104	114	110
	364	119	97	106	81
	557	100	110	98	103
wheat grain	0	(100)	94	100	100
	27	93	90	95	97
	76	89	82	87	93
	186	97	109	116	113
	362	92	77	81	83
	557	91	97	97	94

Table 7.6.4-3 Summary of stability data for deep-frozen samples fortified with DFA – samples fortified at 1 mg/kg

Matrix	Storage period (d)	Concurrent recovery (%)	Recovery in stored samples (%)		
			apparent	normalized to day 0	corrected for recovery
sugar cane	0	(100)	98	100	100
	29	97	96	99	100
	77	97	92	94	94
	149	86	100	102	116
	372	97	99	101	102
	559	137	140	93	147
coffee bean (green)	0	(100)	90	100	100
	33	90	89	98	99
	81	77	76	84	99
	152	80	94	104	118
	370	73	79	87	108
	560	108	120	85	128
orange fruit	0	(100)	95	100	100
	28	98	100	106	102
	77	89	99	105	112
	148	87	93	98	107
	365	94	98	103	104
	556	119	126	92	131
soybean seed	0	(100)	79	100	100
	28	89	71	89	79
	75	93	82	103	88
	148	75	98	124	131
	371	73	79	99	107
	558	87	110	79	111
navy bean	0	(100)	96	100	100
	26	96	99	104	104
	75	98	108	113	110
	148	79	100	104	127
	364	80	78	82	98
	558	128	134	99	130
tomato fruit	0	(100)	101	100	100
	28	99	96	95	97
	76	94	94	93	100
	148	84	98	96	117
	370	98	105	104	108
	558	134	133	102	132
spinach	0	(100)	100	100	100
	26	98	97	97	99
	75	90	87	87	97
	147	85	100	99	117
	364	70	74	74	106
	557	147	146	107	138
wheat grain	0	(100)	92	100	100
	27	97	98	107	102
	76	88	94	102	107
	186	78	98	107	126
	362	72	71	78	100

Matrix	Storage period (d)	Concurrent recovery (%)	Recovery in stored samples (%)		
			apparent	normalized to day 0	corrected for recovery
	557	116	126	88	132

Table 7.6.4-4: Summary of stability data for deep-frozen samples fortified with BYI 02960-difluoroethylaminofuranone – samples fortified at 1 mg/kg

Matrix	Storage period (d)	Concurrent recovery (%)	Recovery in stored samples (%)		
			apparent	normalized to day 0	corrected for recovery
sugar cane	0	(100)	110	100	100
	29	89	81	74	91
	77	93	88	80	95
	149	117	112	102	96
	372	87	93	85	108
	559	94	85	83	112
coffee bean (green)	0	(100)	101	100	100
	33	100	94	93	94
	81	89	88	87	99
	152	118	100	99	85
	370	90	86	85	95
	560	89	88	88	101
orange fruit	0	(100)	104	100	100
	28	100	109	105	109
	77	98	94	90	96
	148	119	110	105	92
	365	97	92	88	95
	556	85	81	97	87
soybean seed	0	(100)	107	100	100
	28	99	106	99	107
	75	103	94	87	91
	148	117	117	108	100
	371	84	85	79	101
	558	87	81	97	89
navy bean	0	(100)	102	100	100
	26	109	102	100	93
	75	107	93	91	87
	148	116	103	101	89
	364	93	88	86	94
	558	104	102	114	90
tomato fruit	0	(100)	110	100	100
	28	96	116	105	121
	76	103	100	91	98
	148	118	101	92	86
	370	97	84	76	87
	558	89	81	111	80
spinach	0	(100)	106	100	100
	26	107	109	103	102
	75	101	96	91	96
	147	114	109	102	95
	364	105	95	90	91
	557	95	89	102	93

Matrix	Storage period (d)	Concurrent recovery (%)	Recovery in stored samples (%)		
			apparent	normalized to day 0	corrected for recovery
wheat grain	0	(100)	103	100	100
	27	101	106	103	105
	76	96	91	88	94
	186	104	99	96	95
	362	92	90	88	98
	557	87	84	100	87

Conclusions

During a storage period of 18 months under deep-freezer conditions, the components of the relevant residues of flupyradifurone (including parent compound, DFEAF, and DFA) were stable in orange fruit (high acid content), spinach leaves and tomato fruit (high water content), wheat grain (high starch content), bean seed (high protein content), coffee bean (specific matrix), soybean seed (high oil content), and sugar cane (high water content), representing a wide array of plant-based sample materials. These results validate the residue values reported in supervised field trials and processing studies with respect to storage stability of samples frozen prior to analysis.

Guidelines & Limitations

Some samples (barley green material, straw and grain) in the field rotational crop studies were stored for periods of up to 16-18 months; these periods are covered by the storage stability study.

Animal matrices

The longest storage periods for samples from animal residue studies (feeding studies) are shown in table 7.4.4-5 below:

Table 7.4.4-5: Periods of frozen storage (approx. -15 to -20°C) of animal-based samples (between sampling and analysis) for matrices relevant to this dossier

Sample material		Analyte group*	Longest storage duration (d)	Study	
Animal	Matrix			Report no.	Reference no
ruminant	milk	A	16	RARVP050	6.4.2/01
		B	25		
	cream	A & B	11		
	whey	A	5		
		B	7		
	fat	A	14		
		B	40		
	kidney	A	22		
		B	41		
	liver	A	21		
		B	37		
	muscle	A	19		
		B	41		
	urine	A & B	33		
poultry	eggs	A & B	12	RARVP041	6.4.1/01

Sample material		Analyte group*	Longest storage duration (d)	Study	
Animal	Matrix			Report no.	Reference no
	fat	A & B	11		
	liver	A & B	11		
	muscle	A & B	11		
	excreta	A & B	18		

* analyte group A comprises BYI 02960, BYI 02960-acetyl-AMCP, and BYI 02960-OH
analyte group B comprises DFA

Based on the information presented here, no storage stability data was required for the relevant residues of BYI 02960 in most animal matrices. Only samples with DFA in bovine fat, kidney, liver, muscle, and urine; as well as with BYI 02960, BYI 02960-acetyl-AMCP, and BYI 02960-OH in bovine urine were stored for periods of >1 month.

STUDY 1
Characteristics

Reference	:	Moore, S.M., & Harbin, A.M.; 2012 KIIA 6.1.1 /02	GLP statement	:	Yes
Type of study	:	Storage stability	Guidelines	:	APVMA Residue Guideline No. 23 OECD Guideline 505 OPPTS 860.1480 – Meat/Milk/Poultry/Eggs DACO 7.5. – Meat/Milk/Poultry/Eggs
Year of execution	:	2011-2012			
Storage duration	:	43d	Matrix	:	Bovine fat, kidney, liver and muscle
Test substance	:	DFA Batch no: 2009-000240, purity 99.3% w/w	Acceptability	:	Acceptable

Study design

To determine the freezer storage stability of the relevant residues of flupyradifurone in animal materials, individual control samples of bovine fat, kidney, liver, and muscle were separately spiked with DFA, at a nominal concentration of 0.20 mg/kg. Samples were stored in a freezer at a temperature of -15°C. For day-0 analysis, three treated samples of each material were chosen, as well as one control sample of each. Samples were then analysed after an interval of 43 days, in order to cover the longest period of storage for these matrices in the feeding study. At this interval, two treated samples of each material were removed from storage and analyzed, as well as a control sample and two samples for concurrent recovery. Samples used for concurrent recoveries were prepared at the same time and stored in the same fashion as the control samples, and spiked on the day of analysis.

Urine samples were stored for 33 days between sampling and analysis, this is marginally longer than one month. As the primary purpose of the urine residue values in the feeding study was to elucidate transfer factors for the two main components of the residue [BYI 02960 and DFA], storage stability data is not considered necessary.

DFA was analytically determined using analytical method 01304 (see B.5.2), which was validated prior to and parallel to the residue analysis of the samples. The LOQ of the method was 0.02 mg/kg for DFA in milk, cream, fat, kidney, liver, muscle and urine, expressed in BYI 02960 equivalents. Only for whey the LOQ was 0.05 mg/kg, expressed in BYI 02960 equivalents.
The mean values of the concurrent recovery rates per compound, sample material, and spiking level were in the range of 60-88%, with standard deviations < 10%.

Results

At day 0, average residue recoveries of DFA ranged from 60-86% of nominal. In samples analysed after 43 days of frozen storage, storage stability recoveries, normalized to day 0, ranged from 100-109%. When corrected for concurrent recovery, they ranged from 97-100% There was no evidence of any continued degradation of DFA in any of the sample materials. Thus, it can be considered stable in all relevant animal matrix types over the tested period of 43 days.

All storage stability results are summarised below in table 6.1.1-7.

Table 7.6.4-2: Summary of stability data for deep-frozen samples fortified with DFA – samples fortified at 0.20 mg/kg

Matrix	Storage period (d)	Concurrent recovery (%)	Recovery in stored samples (%)		
			apparent	normalized to day 0	corrected for recovery
bovine fat	0	-	85.8	100	100
	43	87.8	86.0	100.2	98.0
bovine kidney	0	-	68.7	100	100
	43	72.5	72.5	105.5	100
bovine muscle	0	-	65.3	100	100
	43	69.0	69.0	105.6	100
bovine liver	0	-	60.3	100	100
	43	68.0	65.8	109.1	96.7

Conclusions

During a storage period of 43 days under deep-freezer conditions, DFA was stable in bovine fat, kidney, liver, and muscle. These results validate the residue values reported in the cattle feeding study with respect to storage stability of samples frozen prior to analysis.

Guidelines & Limitations

The study is considered acceptable.

B.7.6.5 Summary of storage stability studies

Flupyradifurone DFEAF, and DFA are stable in orange fruit (high acid content), spinach leaves and tomato fruit (high water content), wheat grain (high starch content), bean seed (high protein content), coffee bean (specific matrix), soybean seed (high oil content), and sugar cane (high water content) for at least 18 months when stored frozen.
DFA is stable in animal fat, kidney, liver, and muscle for at least 43 days, when stored frozen.

B.7.7 Effects of industrial processing and/or household preparation (IIA 6.5; IIIA 8.5)

B.7.7.1 Nature of the residue

STUDY 1
Characteristics

Reference	:	Weber, E.; 2011 KIIA 6.5.1 /01	GLP	:	yes
Type of study	:	Hydrolysis study	Guideline	:	European Council Directive 91/414/EEC as amended by Commission Directive 96/68/EC Section 6.5, Subsection 6.5.1. OECD Guideline for the Testing of Chemicals 507 Nature of the Pesticide Residues in Processed Commodities - High Temperature Hydrolysis, adopted 2007-10-16
Year of execution	:	2010	Acceptability	:	Acceptable
Test substance	:	[pyridinylmethyl-^{14}C] flupyradifurone; sample ID: KATH 6301; purity >99% w/w			

Study design

The behaviour of flupyradifurone was studied in buffered drinking water under conditions of processing. The radiolabelled test compound [pyridinylmethyl-^{14}C]BYI02960 was used for the hydrolysis investigations.

One concentration (approx. 1.0 mg/L) of the analyte was prepared in sterilized buffered drinking water and incubated under three representative sets of hydrolysis conditions:

Pasteurisation:	90°C at pH 4 for 20 min
Baking, brewing, boiling:	100°C at pH 5 for 60 min
Sterilisation:	120°C at pH 6 for 20 min

The tests at 90°C and 100°C were carried out by using a dry block heater. The test at 120°C was performed in an autoclave. After termination of each test and cooling to room temperature, the pH value was measured. Three aliquots were again taken from each test solution for the determination of the radioactivity content by Liquid Scintillation Counting (LSC). The identity of the test compound was confirmed by LC-MS/MS. In the test solutions, parent compound was identified by HPLC comparison. The pH values of the test solutions were adjusted to pH 4, pH 5, and pH 6 (each ± 0.1) and remained as required. The temperatures were in the ranges of 90 ± 5°C, 100 ± 5°C and 120 ± 5°C during the test periods.

The applied radioactivity was defined as the amount of radioactivity measured in the samples taken at the beginning of the incubation period.

Results

All material balances were in the range between 97.3 to 104.9%. The material balances demonstrate that no radioactivity dissipated from the test systems.

Chromatograms were identical before and after hydrolysis. The test compound was found in amounts of 100.0% in any test solution after hydrolysis. The identity of the test compound was proven after hydrolysis by HPLC cochromatography with the non radiolabelled reference compound

The test compound BYI 02960 was stable under all conditions of high temperature hydrolysis for simulation of food processing. The following conditions were tested:

- pH 4 / 90°C (20 min) - pasteurisation
- pH 5 / 100°C (60 min) - baking, brewing, and boiling
- pH 6 / 120°C (20 min) - sterilisation

Conclusions

Flupyradifurone stable under all conditions of high temperature hydrolysis for simulation of food processing. The following conditions were tested:

- pH 4 / 90°C (20 min) - pasteurisation
- pH 5 / 100°C (60 min) - baking, brewing, and boiling
- pH 6 / 120°C (20 min) - sterilisation

No hydrolysis products of flupyradifurone were detected above an estimated LOD of 0.5%.

Guidelines & Limitations

The nature of DFA and DFEAF residues was not studied, even though DFA is the predominant residue in plant products apart from parent flupyradifurone. DFA is also the predeominant residue in animal products, apart from flupyradifurone.

The applicant argues that the chemical structure of the metabolite DFEAF is a substructure of the parent compound. All functional groups, chemical moieties and their constitution of DFEAF are also present in the chemical structure of the parent compound. Therefore the chemical stability DFEAF is covered by studies with the parent compound. As parent compound was proved to be stable under the processing conditions, it is concluded that this is also true for DFEAF. In addition, in almost all uses worldwide – and certainly in all EU-relevant uses – DFEAF does not play any significant role in the field residues, generally comprising <5% of the total residue.

Due to the similarity of the chemical structures, the properties of DFA (= difluoroacetic acid) can be interpolated from the properties of TFA (= trifluoroacetic acid) and HOAc (acetic acid). The latter compounds are very common in organic chemistry, have often been used in chemical synthesis and many properties have been investigated. It can be deduced from literature data that these two compounds are stable under the conditions to be tested in a processing study. By interpolation of these properties, it is concluded that DFA is also stable under the processing conditions.

RMS agrees with this and no nature of the residue study is required for DFEAF and DFA.

B.7.7.2 Effects on residue levels

STUDY 1
Characteristics

Reference	:	Schulte, G. & Bauer, J.; 2012a KIIA 6.5.4 /01	GLP	:	Yes
Type of study	:	Follow-up study	Guideline	:	EU Council Directive 91/414/EEC amended by Commission Directive 96/68/EC EU Guidance Working Document 7035/VI/95 rev. 5 OECD Guideline for the Testing of Chemicals No. 508, Magnitude of the Pesticide Residues in Processed Commodities EPA Ref. OPPTS 860.1500, Crop Field Trials
Test substance	:	BYI 02960 SL 200 formulation Batch no: 2010-000672			
Year of execution	:	2010	Acceptability	:	Acceptable

Study design

Four trials were conducted in the northern European residue region, in Germany (2), the Netherlands, and Belgium, in order to determine the total residues of BYI 02960 in unprocessed lettuce heads and then in washed and unwashed commodities including external and internal leaves

The field trials were conducted as part of study 10-2223 (see table 7.6.2-4). Two of the trials were conducted on open-headed lettuces, two on closed-head varieties. BYI 02960 SL 200 was sprayed twice at an application rate of approx. 125 g a.s./ha and a water volume of 300-600 L/ha. The final application was conducted at a pre-harvest interval of 3 days.
After processing, residue analysis was performed according to method 01304 as used in the RAC trials themselves (see B.5.2.). The limits of quantitation were 0.01 mg/kg (BYI 02960 and DFEAF) and 0.02 mg/kg (DFA), expressed in parent compound equivalents, yielding a calculated total-residue LOQ of 0.04 mg/kg, in all matrices. Prior and parallel to the residue analysis, the method was validated by recovery experiments (see table 7.6.1-1), which was considered acceptable.
Lettuce "processing" was designed to simulate typical household kitchen practices in the use and preparation of lettuce for salads.

The complete outer layer of leaves were removed from the heads, yielding outer leaves and inner head parts. From the inner head parts, leaves were separated from the stalk (inner leaves), then washed in standing water (washed inner leaves and washing water).

Results

The total residues of BYI 02960 (parent compound plus DFA and DFEAF) in the harvested heads at day 3 ranged from 0.40-1.0 mg/kg, as summarized previously (see B.7.6.2, study 3). These values were used for the calculation of "processing" factors.

<u>Outer leaves and inner head parts</u>:
The outer leaves were analyzed separately after their removal; the residue levels were from 0.71-

1.8 mg/kg. In the remaining inner head parts, residue levels were considerably lower, at 0.30-0.74 mg/kg.

Based on these values, it is evident that a large proportion of the residues result from surface deposits. The measured residue levels lead to mean "processing" factors of 1.8 for outer leaves and 0.73 for the inner head parts.

Inner leaves and washed inner leaves:

After separation from the stalks, inner leaves samples prior and subsequent to washing. Residue values ranged from 0.08-0.83 mg/kg and 0.29-0.52 mg/kg in the unwashed and washed inner leaves, respectively. In the intermediate product washing water, they were very low, ranging from <0.04-0.05 mg/kg.

These findings indicate that the remainder of the residues are absorbed to a large extent. Washing does not affect them; indeed, the residues are virtually the same in both washed and unwashed leaves (and also essentially as in the inner head parts above). Based on these values, mean "processing" factors of 0.76 and 0.67 can be calculated from lettuce head to unwashed and washed inner leaves, respectively.

The trial data and transfer factors for the total residues of BYI 02960 are summarized below in tables 7.7.2-1 and 7.7.2-2.

Table 7.7.2-1: Results of residue processing trials conducted in/on lettuce after spraying with BYI 02960 SL 200 in European fields

Study No. (Trial No.) Country GLP	Portion analyzed	DALT (days)	Residues (mg/kg) expressed as BYI 02960				Transfer factors		
			BYI 02960	DFA	DFEAF	total residue of BYI 02960 calc.		BYI 02960	DFA
10-2223 (10-2223-01) Netherlands GLP: yes	head	3	0.58	<0.02	<0.01	0.61	-	-	-
	head, inner parts	3	0.46	0.02	<0.01	0.49	~~0.80~~	0,79	n.a.
	leaf, outer	3	1.3	0.02	0.02	1.3	~~2.1~~	2,24	n.a.
	leaf, inner	3	0.45	0.02	<0.01	0.48	~~0.79~~	0,78	n.a.
	leaf, inner, washed	3	0.48	<0.02	<0.01	0.51	~~0.84~~	0,83	n.a.
	washings	3	<0.01	<0.02	<0.01	<0.04	~~<0.07~~	n.a.	n.a.
10-2223 (10-2223-	head	3	0.37	<0.02	<0.01	0.40	-	-	-
	head, inner parts	3	0.27	<0.02	<0.01	0.30	~~0.75~~	0,73	

Study No. (Trial No.) Country GLP	Portion analyzed	DALT (days)	Residues (mg/kg) expressed as BYI 02960				Transfer factors		
			BYI 02960	DFA	DFEAF	total residue of BYI 02960 calc.		BYI 02960	DFA
02) Belgium	leaf, outer	3	0.68	<0.02	0.01	0.71	~~1.8~~	1,84	n.a.
	leaf, inner	3	0.43	<0.02	<0.01	0.46	~~1.2~~	1,16	n.a.
GLP: yes	leaf, inner, washed	3	0.26	<0.02	<0.01	0.29	~~0.73~~	0,70	n.a.
	washings	3	0.02	<0.02	<0.01	0.05	~~0.13~~	0,05	n.a.
10-2223 (10-2223-04) Germany	head	3	1.0	<0.02	0.01	1.0	~~-~~	-	-
	head, inner parts	3	0.71	<0.02	<0.01	0.74	~~0.74~~	0,71	n.a.
	leaf, outer	3	1.8	0.02	0.01	1.8	~~1.8~~	1,80	n.a.
	leaf, inner	3	0.05	<0.02	<0.01	0.08	~~0.08~~	0,05	n.a.
GLP: yes	leaf, inner, washed	3	0.47	<0.02	<0.01	0.50	~~0.20~~	0,47	n.a.
	washings	3	0.02	<0.02	<0.01	0.05	~~0.05~~	0,02	n.a.
10-2223 (10-2223-05) Germany	head	3	0.83	<0.02	0.01	0.87	~~-~~	-	-
	head, inner parts	3	0.52	<0.02	<0.01	0.55	~~0.63~~	0,63	n.a.
	leaf, outer	3	1.3	0.02	0.01	1.4	~~1.6~~	1,57	n.a.
	leaf, inner	3	0.80	<0.02	0.01	0.83	~~0.95~~	0,96	n.a.
GLP: yes	leaf, inner, washed	3	0.49	<0.02	<0.01	0.52	~~0.60~~	0,59	n.a.
	washings	3	0.02	<0.02	<0.01	0.05	~~0.06~~	0,02	n.a.

Table 7.7.2-2: Mean processing factors from lettuce processing studies

Compound	outer leaves	inner head parts	inner leaves	washed inner leaves	washing water
BYI 02960	1.8	0.72	0.87	0.65	0.02
DFA	n.a.	n.a.	n.a.	n.a.	n.a.

Conclusions

The mean value of total residue "processing" factors for the outer leaves was 1.8, and 0.72 ~~0.73~~ to the inner head parts. Further washing is of little consequence, with mean "processing" factors for unwashed or washed inner leaves of 0.87 ~~0.76~~ and 0.65 ~~0.67~~, respectively. Typical household preparation steps, e.g. for salad preparation, will result in lower total residues of BYI 02960 than in the RAC itself, as the main portion of the residues is in/on the outer leaves.

Guidelines & Limitations

None.

STUDY 2

Characteristics

Reference	:	Schulte, G. & Bauer, J.; 2012b KIIA 6.5.4 /02	GLP	:	Yes
Type of study	:	Follow-up study	Guideline	:	EU Council Directive 91/414/EEC amended by Commission Directive 96/68/EC
Test substance	:	BYI 02960 SL 200 formulation Batch no: 2010-000672			EU Guidance Working Document 7035/VI/95 rev. 5 OECD Guideline for the Testing of Chemicals No. 508, Magnitude of the Pesticide Residues in Processed Commodities EPA Ref. OPPTS 860.1500, Crop Field Trials
Year of execution	:	20102011	Acceptability	:	Acceptable

Study design

Two trials were conducted in Germany in order to determine the total residues of flupyradifurone in green and dried hop cones and then, after processing, in beer as well as the additional processing intermediates hops draff and brewer's yeast.

The field trials were conducted at 3N the normal application rate for hops, in an attempt to ensure that quantifiable residues will be present in the RAC samples. BYI 02960 SL 200 was sprayed once at an application rate of approx. 360 g a.s./ha and a water volume of 3000 L/ha. The application was conducted at a pre-harvest interval of 21 days.

After processing (described below), residue analysis was performed according to method 01304 as used in the RAC trials themselves (see table 7.6.1-2 and B.5.2). The limits of quantitation were 0.1 mg/kg (BYI 02960 and DFEAF) and 0.2 mg/kg (DFA), expressed in parent compound equivalents, yielding a calculated total-residue LOQ of 0.4 mg/kg, in all matrices except beer, in which the LOQs were 10 times lower (0.01 and 0.02 mg/kg; total residue: 0.04 mg/kg).

Preparation of beer:

Beer processing simulated industrial brewing practices. Prior to the addition of hops to the wort, ground malted barley and water were mixed in a mash tun ("mashing"), then the wort was separated from the insoluble malt components ("lautering").

Green hop cones were dried in hop driers, according to standard industrial procedures, then ground and pelletted. Hop pellets were added to the wort, which was then boiled. Subsequently, the flocs were separated via a whirlpool in which the sludge (hops draff) deposited at the bottom of the cone-shaped vessel. After addition of yeast, the mixture was allowed to ferment in bottom-fermentation containers ("primary fermentation", temp. 9°C). As soon as the extract content of the young beer was sufficient, storing began. Before maturation, however, the young beer was cooled.

During the main fermentation, the yeast deposits on the bottom of the tank; this material is sampled (brewer's yeast). The beer is stored under pressure at approx. 2°C for 3-4 weeks. All further sludge-

like materials settle to the bottom. The matured liquid is filtered and then the final product, beer, is sampled.

Limited validation sets and concurrent recoveries of BYI 02960 and its metabolites DFA and DFEAF were obtained from samples of hop matrices (green and dried cones, hops draff, brewer's yeast, and beer). In all matrices except for beer, recovery samples for parent compound and DFEAF were spiked at levels of 0.10 mg/kg and 1.0 mg/kg, as well as 5.0 mg/kg for hop cones (expressed in BYI 02960 equivalents); in beer, spiking levels were 0.01 and 0.10 mg/kg. Mean recoveries in the larger validations sets (n > 2) for all matrices were 81-111%, with RSDs of 1.4-13.9%; n=3-6. Even in the case of values over 110%, these values were considered to be acceptable because they were only marginally higher and the RSD values were very low; also, in the cases of the exceptions, the overall means of all recovery analyses for the given matrices with each individual analyte were 107-108%, with overall RSDs of 4.2-7.1%.

For DFA, concurrent recovery samples for all matrices except beer were spiked at levels of 0.20 mg/kg and 1.0 mg/kg, as well as 5.0 mg/kg for hop cones (expressed in BYI 02960 equivalents); fortification levels in beer were 0.02 and 0.20 mg/kg. Mean recoveries in the larger validations sets (n > 2) in all matrices were 83-109%, with RSDs of 2.6-14.1%; n=3-6. All values were within acceptable ranges.

Results

Beer:

The total residues of BYI 02960 (parent compound plus DFA and DFEAF) in the harvested green cones at day 21 were 0.73 and 1.5 mg/kg. After drying, the values were 3.0 and 5.0 mg/kg. As the dried cone is generally considered to be the "RAC" in the case of hops, all processing factors are based on the residues in the dried commodity.

Residue values in beer were 0.04 and 0.05 mg/kg. Based on these values, a mean transfer factor of 0.01 to beer can be calculated from dried hop cones (0.04 from green cones). Residue levels in all sampled intermediate products (hops draff and brewer's yeast) were below the LOQ of 0.4 mg/kg. All relevant data

All trial data are summarised further below in table 7.7.2-2. The transfer factors for the total residues of BYI 02960 are summarized below in table 7.7.2-3.

Table 7.7.2-2: Summary of supervised residue trials with hops (outdoor) in Northern Europe

Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) expressed as BYI 02960					PHI (days)	Remarks
			kg a.s./ha	Water l/ha	kg a.s./hl				BYI 02960	DFA, as BYI 02960	DFE AF	DFA, as DFA	Total BYI 02960 and DFA		
10-3407-01 Germany 04685 Golzern 2010	hop Nugget	1. 1994 2. 06/07/2010 to 28/07/2010 3. 13/09/2010	0.36	3000	0.012	23/08/2010	BBCH 71	cone, green	0.43	<0.2	<0.1	<0.2	~~0.73~~ 0.63	21	Study: 10-3407 Formulation: BYI 02960 SL 200 Method: 01304 BYI 02960 LOQ = 0.1 mg/kg DFA LOQ = 0.2 mg/kg DFEAF LOQ = 0.1 mg/kg
10-3407-02) Germany 99706 Hohene-bra 2010	hop Nordischer Brauer	1. 1987 2. 05/07/2010 to 02/08/2010 3. 03/09/2010	0.36	3000	0.012	13/08/2010	BBCH 75	cone, green	1.1	0.37	<0.1	0.12	1.5	21	

Table 7.7.2-3: Results of residue processing trials conducted in/on hops

Study No. (Trial No.) Country GLP	Portion analyzed	DALT (days)	Residues (mg/kg) expressed as BYI 02960				Transfer factors		
			BYI 02960	DFA	DFEAF	total residue of BYI 02960 calc.		BYI 02960	DFA
10-3407	cone, green	21	0.43	<0.2	<0.1	0.73	-		
(10-3407-01)	cone, kiln-dried	21	2.2	0.72	<0.1	3.0	-	5.12	n.a.
Germany	hops draff	21	<0.1	<0.2	<0.1	<0.4	~~<0.1~~ 3	n.a.	n.a.
GLP: yes	brewer's yeast	21	<0.1	<0.2	<0.1	<0.4	~~<0.1~~ 3	n.a.	n.a.
	beer	21	0.01	<0.02	<0.01	0.04	~~0.01~~	0.02	n.a.
10-3407	cone, green	21	1.1	0.37	<0.1	1.5	-		
(10-3407-02)	cone, kiln-dried	21	4.2	0.76	<0.1	5.0	-	3.82	2.05
Germany	hops draff	21	<0.1	<0.2	<0.1	<0.4	~~<0.0~~ 8	n.a.	n.a.
GLP: yes	brewer's yeast	21	<0.1	<0.2	<0.1	<0.4	~~<0.0~~ 8	n.a.	n.a.
	beer	21	0.02	<0.02	<0.01	0.05	~~0.01~~	0.02	n.a.

DALT = days after last treatment

Conclusions

The mean value of total residue transfer factors for beer was 0.01. In the intermediates, the average processing factor was <0.1. Thus, for the total residues of BYI 02960, processing to beer will not result in any concentration of the residues.

Guidelines & Limitations

None.

B.7.7.3 Summary of the processing studies

The nature of flupyradifurone residues was studied, revealing that flupyradifurone is stable under all conditions of high temperature hydrolysis.

The nature of DFA and DFEAF residues was not studied, eventhough DFA is the predominant residue in plant products apart from parent flupyradifurone, e.g. DFA was detected up to 0.4 mg/kg in hops. DFEAF was detected up to 0.093 mg/kg in lettuce, however generally DFEAF residues represented <5% of the total residue. DFA is also the predeominant residue in animal products, apart from flupyradifurone. However, a scientificand acceptable argumentation was provided to waive the processing hydrolysis studies for the two metabolites.

Household preparation of lettuce heads, i.e. removing outer leaves and washing the remaning head (separated in inner and outer head) resulted in processing factors of 1.8 for outer leaves, 0.72 ~~0.73~~ for inner head parts and 0.87 ~~0.76~~ for inner leaves.

Using hops in the production of beer, the transfer factors was determined to be 0.01. In the intermediates in the production of beer, the average processing factor was <0.1.

B.7.8 Livestock feeding studies (IIA 6.4; III 8.5)

The test substance used in the study should be representative of the residue in the feedstuffs. In the case of this new insecticide flupyradifurone, by far the major part of the residue in plants is formed by parent flupyradifurone and its metabolite DFA, in varying ratios. Animal metabolism studies show that these two components are also the major contributors to the relevant residue in animals. To cover the needs of all involved countries and regions, several concepts for feeding studies might be applied. After discussion with RMS the Netherlands, it was decided to feed parent flupyradifurone and derive separate transfer factors for the total residue of flupyradifurone + DFA, as well as separately for DFA alone.

Conducting the feeding studies with a mixture of both the parent compound and its primary food/feed-relevant metabolite, DFA, seems attractive when viewed from a 1-region "EU-only" perspective, the feeding studies for the flupyradifurone project (Joint OECD dossier) should be designed to be used in all relevant regions (NAFTA, EU, Brazil and Australia). It is in this point that weaknesses become evident: different crops, different residue levels and compositions, different "feeding tables" used for their evaluation and finally, difficulties in evaluating the results in the long term make this strategy seem less attractive.

Performing separate feeding studies would effectively alleviate the above-mentioned concerns. Dose calculation, result evaluation, and robustness in the face of parameter change are all facilitated by this concept. While it is the most straightforward, precise approach, it would require the use of many more animals.
Seen in this light, the concept of feeding BYI 02960 parent alone and calculating transfer factors for DFA was considered as an acceptable option. While effectively yielding similarly robust data to the two-study solution, only one study for each animal type would be necessary, thus saving animal lives.

While the nature of the calculations/evaluations for the total residue and for parent compound itself are relatively straightforward, using the study data to evaluate the metabolite DFA required careful consideration. As agreed with the RMS, separate transfer factors for DFA were estimated in both the poultry and cattle feeding studies on the basis of the available data after dosage of the active substance flupyradifurone to laying hens and cattle. In order to accomplish this, a theoretical dose of DFA must be estimated in each study. For the estimation of the theoretical dose, the amounts of DFA in all organs/tissues and particularly in the urine (ruminant) or excreta (poultry) must be considered. These absolute residues are representative of the minimum systemic exposure to DFA during the studies and therefore provide the basis for the calculation of a theoretical dose of DFA in the feed.

This, in turn, allows the calculation of transfer factors and, thus, the contribution of DFA to MRLs in animal matrices.

For dietary burden calculations, see table 7.2-2.

B.7.8.1 Ruminants and poultry

STUDY 1

Characteristics

Reference	:	[redacted]; 2012 KIIA 6.4.1 /01	Animal products	:	Eggs, liver, muscle (thigh and breast), and fat (abdominal and subcutaneous), excreta
Type of study	:	Magnitude of residues in animal tissues and animal products (livestock feeding)	GLP statement	:	yes
Year of execution	:	2011	Guideline	:	OPPTS 860.1480 – meat/milk/poultry/eggs OECD Guideline 505 APVMA Residue Guideline No. 23 DACO 7.5 – Meat/milk/poultry/eggs
Test substance	:	Flupyradifurone; batch no 2009-000240, purity 99.3% w/w	Dose levels	:	0 - 1.5 – 6.5 – 19.4 – 65.1 mg/kg feed/day
Type of animal	:	Laying hen (*Gallus gallus domesticus*) White Leghorn	Acceptability	:	acceptable

Study design

Eighty-four mature laying hens (*Gallus gallus domesticus*) were dosed orally, via capsule, for 29 consecutive days with flupyradifurone at dose rates of 0 mg/kg feed/day (control; 24 hens, 6 subgroups), 1.5 mg/kg feed/day (1.2N group; 0.10 mg as/kg bw/d; 12 hens, 3 subgroups), 6.5 mg/kg feed/day (5.4N ~~4.3N~~ ~~4.9N~~ group; 0.45 mg as/kg bw/d; 12 hens, 3 subgroups; which is approximated a 1X NAFTA dose), 19.4 mg/kg feed/day (16N ~~13N~~ ~~14.7N~~ group; 1.31 mg as/kg bw/d; 12 hens, 3 subgroups; which is approximated a 3X NAFTA dose), and 65.1 mg/kg feed/day (54N ~~43N~~ ~~49N~~ group; 4.54 mg as/kg bw/d; 24 hens, 6 subgroups; which is approximated a 11X NAFTA dose).

The dose rates were adjusted weekly, based on the actual weekly feed consumption by the hens in each dose group during the previous week, resulting in an increase of the fed dose when expressed in mg/kg bodyweight, see table

Table 7.8.1-1 Dosing regime used in the poultry feeding study

Dose Group	Timing	Dose rate* (mg/kg feed)	Feed intake fresh (g/bird/d)	Feed intake dry (g/bird/d)	Administered dose (mg)	Average body weight** (kg)	Average BYI 02960 a.s. (mg/kg bw)
Control	4 Weeks		127.8		0	1.47	0
1.2N (US 0.3X)	Week 1	1.5	110.6	95.0	0.14	1.49	0.09
	Week 2	1.5	131.3	112.8	0.16	1.50	0.10
	Week 3	1.4	127.9	109.9	0.14	1.51	0.11
	Week 4	1.6	140.4	120.6	0.17	1.52	0.13
Overall Average		1.5	134.1	109.6	0.15	1.50	0.10
5.4N ~~4.3N~~ ~~4.9N~~	Week 1	6.6	108.4	93.6	0.62	1.41	0.43

Dose Group	Timing	Dose rate* (mg/kg feed)	Feed intake fresh (g/bird/d)	Feed intake dry (g/bird/d)	Administered dose (mg)	Average body weight** (kg)	Average BYI 02960 a.s. (mg/kg bw)
	Week 2	6.4	121.9	106.8	0.67	1.47	0.46
	Week 3	6.5	116.5	102.5	0.65	1.47	0.45
	Week 4	6.4	134.2	116.6	0.74	1.45	0.51
Overall Average		6.5	125.4	104.9	0.67	1.45	0.46
16N ~~13N~~ ~~14.7N~~ (US 3X)	Week 1	19.6	108.3	93.1	1.83	1.44	1.27
	Week 2	19.8	119.0	103.4	2.03	1.48	1.37
	Week 3	19.7	116.2	100.5	1.96	1.49	1.32
	Week 4	18.3	137.1	117.5	2.16	1.49	1.45
Overall Average		19.4	126.6	103.6	1.99	1.47	1.35
54N ~~43N~~ ~~49N~~ (US 11X)	Week 1	64.6	109.2	93.8	6.05	1.45	4.18
	Week 2	67.4	120.4	103.4	6.97	1.48	4.72
	Week 3	65.6	117.1	100.6	6.6	1.49	4.42
	Week 4	62.9	134.8	115.7	7.28	1.50	4.84
Overall Average		65.1	125.9	103.4	6.72	1.48	4.54

* dose rate in feed calculated on a dry weight basis
** these weights reflect those determined at the end of the given study week

Eggs were collected twice daily (afternoon and morning prior to the day's dosing). The eggs collected in the afternoon from each sub-group were combined with the eggs collected the following morning from the same sub-group. The egg contents were combined (shells discarded) by sub-group into a labeled container, weighed, and thoroughly mixed by vigorous shaking. Composite egg samples collected on days 0, 2, 4, 7, 10, 14, 17, 21, 24, 28, 35, 42 and 49 were shipped for analysis for the 54N ~~49N~~ dose group; for all other doses, only the egg samples from days 24 and 28 were analyzed.

On day 29 of the study, twelve hens from the control and 54N ~~43N~~ ~~49N~~ dose groups and all hens in the 1.2N, 5.4N ~~4.3N~~ ~~4.9N~~ dose, and the 16N ~~13N~~ ~~4.9N~~ dose groups were sacrificed by CO_2 asphyxiation within 24 hours of the administration of the final dose. Liver (entire), muscle (thigh and breast), and fat (abdominal and subcutaneous) were collected and homogenized in the presence of dry ice.

Twenty four hens (12 from the control group and 12 from the 54N ~~43N~~ ~~49N~~ group) entered into a 21-day depuration phase, (as required for Australia), following the administration of the final dose. Egg and tissue samples were collected on study days 35, 42, and 49 for analysis.

To estimate the extent of exposure of laying hens to DFA following the oral administration of BYI 02960, excreta was also collected daily from all the subgroups of the 5.4N ~~4.3N~~ ~~4.9N~~ and from three of six subgroups of the 54N ~~49N~~ group during the entire dosing period and composited by sub-

group. The entire sample from each sub-group was mixed at the end of the dosing period, and a subsample was homogenized for analysis.

BYI 02960 and its metabolites were analytically determined using analytical method RV-004-A11-04 (see B.5.2.), which was validated prior to and parallel to the residue analysis of the samples. The LOQ was 0.01 mg/kg for all analytes (parent compound, DFA, BYI 02960-acetyl-AMCP, and BYI 02960-OH), expressed in BYI 02960 equivalents.

Table 7.8.1-2: Concurrent recovery data for the relevant residues of BYI 02960 in poultry matrices

Study No.	Matrix	a.s./metabolite	*n*	Spike level (mg/kg)	Recovery (%)				
					Individual recoveries	Min	Max	Mean	RSD
RARVP041	eggs	BYI 02960	17	0.01	113,115,118,103, 109,94,98,87,84,77, 93, 76, 115, 94, 84, 94, 84	76	118	96	14.2
			3	4.0	100, 81, 95	81	100	92	10.7
		DFA	17	0.01	100,112,109,78, 87,74,81,76,78, 68,77,70,83,73, 79, 76, 71	68	112	82	15.9
			3	4.0	75, 70, 71	70	75	72	3.7
		BYI 02960-acetyl-AMCP	16	0.01	84,97,89,120,92, 103,100,76,86, 100, 70, 89, 99, 104, 91, 90	70	120	93	12.7
			3	4.0	93, 81, 90	81	93	88	7.1
		BYI 02960-OH	17	0.01	102,109,101,89, 99, 90,96, 83, 90, 72, 95, 89, 113, 107, 73, 96, 99	72	113	94	12.0
			3	4.0	102, 89, 96	89	102	96	6.8
	fat	BYI 02960	12	0.01	87, 88, 77, 85, 95, 103, 96, 94, 88, 93, 75, 105	75	105	91	9.5
			3	4.0	110, 104, 106	104	110	107	2.6
		DFA	12	0.01	97, 91, 94, 97, 84, 80, 88, 96, 83, 85, 75, 74	74	97	87	9.4
			3	4.0	100, 96, 92	92	100	96	4.2
		BYI 02960-acetyl-AMCP	12	0.01	119,81,96,85, 99, 77, 100, 92, 95, 107, 95, 87	77	119	94	12.2
			3	4.0	109, 111, 108	108	111	109	1.4
		BYI 02960-OH	12	0.01	95, 106, 100, 87, 100, 88, 90, 93, 87, 86, 90, 102	86	106	94	7.2
			3	4.0	117, 113, 118	113	118	116	2.1
	liver	BYI 02960	12	0.01	93,104,121,114, 102, 114,90,94, 110, 95, 84, 92	84	121	101	11.4

Study No.	Matrix	a.s./metabolite	*n*	Spike level (mg/kg)	Recovery (%)				
					Individual recoveries	Min	Max	Mean	RSD
			3	4.0	112, 102, 100	100	112	104	6.2
		DFA	12	0.01	75, 90, 83, 97, 89, 93, 81, 103, 84, 77, 78, 82	75	103	86	10.2
			3	4.0	91, 85, 82	82	91	86	5.2
		BYI 02960-acetyl-AMCP	12	0.01	108,112,116,117, 96, 116, 96, 114, 115, 89, 96, 94	89	117	106	10.1
			3	4.0	115, 100, 99	99	115	105	8.4
		BYI 02960-OH	12	0.01	84, 85, 120, 83, 74, 88, 89, 97, 101, 72, 91, 71	71	120	88	15.6
			3	4.0	108, 106, 107	106	108	107	0.7
RARVP041	muscle	BYI 02960	12	0.01	117,106,115,119, 96, 119, 82, 81, 116, 94, 115, 98	81	119	105	13.5
			3	4.0	108, 112, 108	108	112	109	2.1
		DFA	12	0.01	91, 95, 101, 85, 82, 92, 88, 118, 98, 87, 86, 104	82	118	95	10.4
			3	4.0	86, 83, 80	80	86	83	3.6
		BYI 02960-acetyl-AMCP	12	0.01	114,116,117,135, 103,119,118,116, 110, 101, 92, 88	88	135	111	11.6
			3	4.0	108, 107, 101	101	108	105	3.5
		BYI 02960-OH	12	0.01	100,112,114,97, 105,93,120,119, 111, 107, 107, 81	81	120	106	10.6
			3	4.0	108, 112, 110	108	112	110	1.8
	excreta	BYI 02960	8	0.01	87, 110, 104, 91, 112, 109, 88, 111	87	112	102	10.8
			3	4.0	98, 96, 93	93	98	95	2.6
		DFA	8	0.01	118,110,103,114, 112, 121, 99, 92	92	121	109	9.1
			3	4.0	86, 86, 88	86	88	87	1.3
		BYI 02960-acetyl-AMCP	8	0.01	112,106, 98,101, 99, 108, 109, 109	98	112	105	5.0
			3	4.0	98, 91, 91	91	98	93	4.3
		BYI 02960-OH	8	0.01	102,104,100,110, 102,105,101,91	91	105	102	5.3
			3	4.0	97, 99, 91	91	99	95	4.4

All concentrations are expressed in parent compound equivalents.

Results

The proposed residue definition for both enforcement and risk assessment includes parent flupyradifurone plus DFA, which are by far the two major components of the residue. At sacrifice the following values were determined for the nominal worst-case of 1N for the combined residue of the two components (calculated in the "traditional" manner, i.e. if one component is <LOQ, it is calculated as being *at* the LOQ): 0.093 mg/kg in muscle, 0.039 mg/kg in fat, and 0.11 mg/kg in liver, respectively. In eggs taken on days 24 and 28, total residues in the 1.2N group were 0.061 and 0.057 mg/kg, respectively.
The residues found in the eggs, tissues, and excreta collected from the laying hens during dosing, at the end of the dosing period, and during the depuration phase are presented in tables 7.8.1-3 and 7.8.1-4.

With respect to eggs, the highest total BYI 02960 residues were found in the day-28 eggs from the 54N ~~43N 49N~~ dose group. However, as stated previously, dose rates calculated with respect to mg a.s./kg bw increased during the dosing period (cf. table 7.8.1-2). The residue data from the 54N ~~43N 49N~~ egg samples, when evaluated against the dose rate increases calculated as mg a.s./kg bw, suggest that BYI 02960 residues actually reached a plateau between day 4 and day 7 (see table 7.8.1-3); increases in residue levels appear only to be due to increased dose rates. This estimation is in line with the results of the poultry metabolism studies, in which the plateau level in whole eggs was reached at day 6 (pyridinylmethyl label, B.7.2.1-02) or day 9 (furanone label, B.7.2.2-02).

In the depuration phase, total BYI 02960 residues in eggs, fat, liver, and muscle from the 54N ~~43N 49N~~ dose group hens declined from 1.722, 1.230, 3.480, and 2.410 mg/kg, respectively, to <LOQ at 14 days after cessation of dose administration (=day 42 of the study). The residue data provided in this study are suitable for regulatory purposes. (Depuration data are also presented in tables 7.8.1-3 and 7.8.1-4.)

The levels of DFA residues found in the excreta are summarized in table 7.8.1-6. DFA residues represented 65% to 74% of the total residues of BYI 02960 in the excreta samples, suggesting significant exposure of the laying hens to DFA following the daily oral administration of BYI 02960 to laying hens over 29 days.

Table 7.8.1-3: Levels of the relevant residues of BYI 02960 in eggs

group	sampling day	Residue levels of individual analytes (mg/kg)				Total residue levels (mg/kg)	
		BYI 02960 LOD = 0.004 LOQ = 0.01	DFA LOD = 0.003 LOQ = 0.01	-AMCP* LOD = 0.003 LOQ = 0.01	-OH LOD = 0.003 LOQ = 0.01	sum of 4†	BYI 02960 + DFA‡
1.2N	24	<LOD/LOQ	0.051	<LOD/LOQ	<LOD/LOQ	0.057	0.061
	28	<LOD/LOQ	0.047	<LOD/LOQ	<LOD/LOQ	0.053	0.057
5.4N	24	<LOD/LOQ	0.155	<LOD/LOQ	<LOD/LOQ	0.166	0.165

group	sampling day	Residue levels of individual analytes (mg/kg)				Total residue levels (mg/kg)	
		BYI 02960 LOD = 0.004 LOQ = 0.01	DFA LOD = 0.003 LOQ = 0.01	-AMCP* LOD = 0.003 LOQ = 0.01	-OH LOD = 0.003 LOQ = 0.01	sum of 4†	BYI 02960 + DFA‡
~~4.3N~~ ~~4.9N~~							
	28	<LOD/LOQ	0.163	<LOD/LOQ	<LOD/LOQ	0.183	0.173
16N ~~13N~~ ~~14.7N~~	24	0.019	0.497	0.017	0.014	0.545	0.516
	28	0.023	0.508	0.015	0.018	0.565	0.532
54N ~~43N~~ ~~49N~~ ⅄	0	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	<0.02
	2	0.048	0.334	0.019	0.024	0.424	0.382
	4	0.068	0.898	0.045	0.027	1.037	0.966
	7	0.054	1.022	0.042	0.026	1.143	1.076
	10	0.065	1.211	0.052	0.041	1.368	1.276
	14	0.063	0.972	0.038	0.038	1.110	1.035
	17	0.080	1.170	0.043	0.055	1.347	1.250
	21	0.071	1.202	0.043	0.050	1.366	1.273
	24	0.082	1.486	0.059	0.050	1.676	1.568
	28	0.173	1.414	0.051	0.084	1.722	1.587
	35**	<LOD/LOQ	0.130	<LOD/LOQ	<LOD/LOQ	0.140	0.140
	42**	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	0.013	<0.02
	49**	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	<0.02

All metabolite residues expressed in parent compound equivalents

* AMCP = BYI 02960-acetyl-AMCP
** depuration phase, no dosing (sampling days 35-49)
† this value, as shown in the study report, includes values below the LOQ calculated *at the apparent residue value(above LOD, below LOQ)*
‡ this value reflects the proposed residue definition, and as such calculates each component at or above the respective LOQ
⅄ day 0-28 values shown for the high-dose group reflect an average of *all* animals, including those designated for depuration

Table 7.8.1-4: Levels of the relevant residues of BYI 02960 in poultry tissues

group	sampling day	Residue levels of individual analytes (mg/kg)				Total residue levels (mg/kg)	
		BYI 02960	DFA	-AMCP*	-OH	sum of 4†	BYI 02960 + DFA‡
POULTRY ***FAT***							
1.2N	29	<LOD/LOQ	0.029	<LOD/LOQ	<LOD/LOQ	0.033	0.039
5.4N ~~4.3N~~ ~~4.9N~~	29	<LOD/LOQ	0.117	<LOD/LOQ	<LOD/LOQ	0.121	0.127
16N ~~13N~~ ~~14.7N~~	29	<LOD/LOQ	0.272	<LOD/LOQ	<LOD/LOQ	0.281	0.282
54N ~~43N~~ ~~49N~~ ⅄	29	0.192	1.006	0.021	0.010	1.230	1.198
	35**	<LOD/LOQ	0.041	<LOD/LOQ	<LOD/LOQ	0.056	0.051

group	sampling day	Residue levels of individual analytes (mg/kg)				Total residue levels (mg/kg)	
		BYI 02960	DFA	-AMCP*	-OH	sum of 4†	BYI 02960 + DFA‡
	42**	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	0.009	<0.02
	49**	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	0.013	<0.02
POULTRY ***LIVER***							
1.2N	29	<LOD/LOQ	0.104	<LOD/LOQ	<LOD/LOQ	0.108	0.114
5.4N ~~4.3N~~ ~~4.9N~~	29	0.006/0.011	0.413	0.011	0.006/0.011	0.444	0.423
16N ~~13N~~ ~~14.7N~~	29	>LOD/<LOQ	1.012	0.025	>LOD/<LOQ	1.043	1.022
54N ~~43N~~ ~~49N~~ ⅄	29	0.032	3.313	0.083	0.051	3.480	3.345
	35**	<LOD/LOQ	0.085	<LOD/LOQ	<LOD/LOQ	0.089	0.088
	42**	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	0.012	<0.02
	49**	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	0.006	<0.02
POULTRY ***MUSCLE***							
1.2N	29	<LOD/LOQ	0.083	<LOD/LOQ	<LOD/LOQ	0.0883	0.093
5.4N ~~4.3N~~ ~~4.9N~~	29	>LOD/<LOQ	0.290	0.010	>LOD/<LOQ	0.304	0.300
16N ~~13N~~ ~~14.7N~~	29	>LOD/<LOQ	0.719	0.024	>LOD/<LOQ	0.750	0.729
54N ~~43N~~ ~~49N~~ ⅄	29	0.039	2.27	0.069	0.032	2.410	2.309
	35**	<LOD/LOQ	0.051	<LOD/LOQ	<LOD/LOQ	0.055	0.061
	42**	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	0.011	<0.02
	49**	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	0.007	<0.02

All metabolite residues expressed in parent compound equivalents

* AMCP = BYI 02960-acetyl-AMCP
** depuration phase, no dosing (sampling days 35-49)
† this value, as shown in the study report, includes values below the LOQ calculated *at the apparent residue value(above LOD, below LOQ)*
‡ this value reflects the proposed residue definition, and as such calculates each component at or above the respective LOQ
⅄ values shown for the high-dose group reflect an average of *all* animals, including those designated for depuration

Table 7.8.1-5: Weekly dose rates versus total residues of BYI 02960 residues in eggs from 54N ~~43N~~ ~~49N~~ dose group

Dose group	Dose week	Sampling interval	Dose administered (mg)	Average body weight (kg)	Dose rate (mg a.s./kg bw)	Total residues* of BYI 02960 (mg/kg)
54N ~~43N~~ ~~49N~~	1	day Zero	6.05	1.45	4.17	<LOD
		day 2				0.424
		day 4				1.037
	2	day 7	6.97	1.48	4.71	1.143
		day 10				1.368
	3	day 14	6.60	1.49	4.43	1.110

		day 17				1.347
	4	day 21	7.28	1.50	4.85	1.366
		day 24				1.676
		day 28				1.722

* total residues here are the sum of BYI 02960, DFA, BYI 02960-acetyl-AMCP, and BYI 02960-OH, expressed as parent cpd.

Table 7.8.1-6: Proportion of DFA in the total residues of BYI 02960 in excreta

Matrix	Dose group	Composite sample*	Total BYI 02960 residue** (mg/kg)	DFA residues† (mg/kg)	Proportion of DFA in total residue (%)
excreta	5.4N ~~4.3N~~ ~~4.9N~~	Subgroup A	1.628	1.097	67
		Subgroup B	1.587	1.077	68
		Subgroup C	1.824	1.247	68
	54N ~~43N~~ ~~49N~~	Subgroup A	12.647	8.253	65
		Subgroup B	14.445	9.493	66
		Subgroup C	15.374	11.300	74

* Composite sample from entire 29-day dosing period per subgroup

** "total BYI 02960 residue" is the sum of BYI 02960, BYI 02960-OH, BYI 02960-acetyl-AMCP, and DFA residues, expressed in BYI 02960 equivalents

† expressed in BYI 02960 equivalents

Conclusion

BYI 02960 was administered orally (via capsule) to laying hens for 29 consecutive days at average dose rates of 1.5 mg/kg feed (1.2N) 6.5 mg/kg feed (5.4N ~~4.3N~~ ~~4.9N~~; which approximated a 1X NAFTA dose), 19.4 mg/kg feed (16N ~~13N~~ ~~14.7N~~), and 65.1 mg/kg feed (54N ~~43N~~ ~~49N~~). Feed consumption, body weights, and egg production were not adversely affected by compound administration.

After the final dose, the animals were sacrificed and the key edible tissues were analyzed for the relevant residues of BYI 02960. While data were generated for four analytes in the study itself, only two – BYI 02960 and DFA – are proposed for the residue definitions (enforcement and risk assessment) for BYI 02960. The combined residues of BYI 02960 + DFA in poultry tissues at sacrifice in the 1N dosing group were 0.093 mg/kg in muscle, 0.039 mg/kg in fat, and 0.11 mg/kg in liver, expressed in parent compound equivalents. Prior to sacrifice, residues in eggs were measured at various intervals in the high-dose group, and on days 24 and 28 in the three lower dose groups. In the 1Ndose group, residues (BYI 02960 + DFA) in eggs amounted to 0.061 and 0.057 mg/kg, respectively.

Residue analysis of the high-dose eggs showed that levels reached a plateau in eggs. Though residues were highest at day 28, residue data from the 54N ~~43N~~ ~~49N~~ egg samples, when evaluated against the dose rate increases calculated as mg a.s./kg bw, suggest that BYI 02960 residues actually reached a plateau between day 4 and day 7; increases in residue levels were only due to increased dose rates. This estimation is in line with the results of the poultry metabolism studies, in which the plateau level in whole eggs was reached at day 6 (pyridinylmethyl label) or day 9 (furanone label).

Depuration occurred quickly. Total BYI 02960 residues in eggs, fat, liver, and muscle from the 54N ~~43N~~ ~~49N~~ dose level hens declined from 1.722, 1.230, 3.480, and 2.410 mg/kg, respectively, to <LOQ at 14 days after cessation of dose administration (=day 42 of the study). The residue data provided in this study are suitable for regulatory purposes.

DFA residues represented 65% to 74% of the total BYI 02960 residues in the excreta samples, suggesting significant exposure of the laying hens to DFA residues following the administration of BYI 02960 for 29 days.

Transfer factors calculated for DFA and the total residue

As agreed with the notifier, separate transfer factors for DFA (difluoroacetic acid) were estimated from the poultry feeding study on the basis of the data available after dosage of the active substance BYI 02960 to laying hens. In order to accomplish this, a theoretical dose of DFA must be estimated. For this estimation, the amounts of DFA in all organs/tissues and particularly in excreta must be considered. The absolute amount of DFA formed in the animals, and which has thus been systemically available, can then be equated with a minimum dose theoretically fed to the animals.

The estimation of the dose of DFA theoretically fed to the animals was conducted on the basis of the data collected for the highest dose group (54N ~~43N~~ ~~49N~~ in EU), using the animals of the subgroups A to C (actual mean dose of BYI 02960 = 65.1 mg/kg feed), see tables 7.8.1-5 and 7.8.1-6.

According to the balance established, the minimum amount of DFA systemically available was 746.2 mg a.s. equiv. for twelve animals (animals of subgroups A, B & C), corresponding to **62.2 mg a.s. equiv. per animal**.

Calculation of transfer factors:

The total amount of BYI 02960 administered in the feeding study accounted for 193.5 mg per animal (4.54 mg/kg bw/day × 1.47 kg bw × 29 days). Thus the total amount of systemically available DFA represented 32.1% of the amount administered as BYI 02960 (62.2 mg/193.5 mg = 32.1%).

Expressing the dose of BYI 02960 in [mg/kg bw/d], the mean dose level in the 54N ~~41N~~ ~~49N~~ dose group accounted for 4.54 mg a.s./kg bw/d. The theoretical dose of DFA can thus be calculated to be **1.46 mg a.s. equiv/kg bw/d** (4.54 mg a.s./kg bw/d × 32.1% = 1.46 mg a.s. equiv/kg bw/d) equal to 20.9 mg a.s./kg dry feed.

On the basis of the DFA residues in eggs and organs/tissues and the theoretical dose of DFA, transfer factors have been calculated, according to the following equation:

$$\textbf{TF} = \frac{\textbf{residue level in edible commodity}}{\textbf{residue level in the diet}}$$

The following tables summarise the residue values determined in the animal matrices and the corresponding transfer factors derived from them.

DFA transfer:

Assuming that the theoretical dose of DFA is reflected by the same proportion (32.1% of the parent dose) for all dose levels, transfer factors for DFA can be estimated at all levels.

It has to be mentioned that, except for the highest dose level, no composite samples of eggs were collected which covered the whole administration phase. Thus for the 1N, 5.4N ~~4.3N~~ and 16N ~~13N~~ ~~12N~~ dose levels, only egg samples from day 28 (the plateau level had been reached by that time) were considered when calculating the transfer.

Table 7.8.1-7: Transfer factors calculated for DFA

Animal matrix	DFA residue [mg a.s equiv./kg]	Transfer factor based on dietary burden in [mg/kg bw/d]	Transfer factor based on dietary burden in [mg/kg dry feed]
Theoretical dose administered: 1.46 mg DFA/kg bw/d = 20.9 mg DFA/kg dry feed (expressed in parent equivalents)			
egg composite* (days 1 to 29)	1.130	0.77	0.054
muscle*	2.270	1.55	0.108
fat*	1.006	0.69	0.048
liver*	3.313	2.27	0.158
Theoretical dose administered: 0.42 mg DFA/kg bw/d = 6.2 mg DFA/kg dry feed (expressed in parent equivalents)			
egg (day 28)	0.508	1.21	0.082
muscle	0.719	1.71	0.115
fat	0.272	0.65	0.044
liver	1.012	2.40	0.163
Theoretical dose administered: 0.15 mg DFA/kg bw/d = 2.1 mg DFA/kg dry feed (expressed in parent equivalents)			
egg (day 28)	0.163	1.12	0.078
muscle	0.290	2.00	0.139
fat	0.117	0.81	0.056
liver	0.413	2.85	0.198
Theoretical dose administered: 0.03 mg DFA/kg bw/d = 0.5 mg DFA/kg dry feed (expressed in parent equivalents)			
egg (day 28)	0.047	1.47	0.097
muscle	0.083	2.59	0.172
fat	0.029	0.91	0.060
liver	0.104	3.25	0.216

* animals of subgroups A to C were used to calculate the average/weighted residues (*without* "depuration" animals)

Comparing the transfer factors estimated for the animal matrices at different dose levels, it is evident that the transfer of DFA into animal matrices is less pronounced at higher doses. The residue levels in the animal matrices do not increase linearly with the dose levels. The residues detected in eggs and organs/tissues are slightly higher at lower dose levels indicating even more pronounced excretion of DFA at higher dose levels.

Transfer of BYI 02960 residues:

The transfer of the relevant residues of BYI 02960 into edible matrices was also calculated on the basis of the dose administered to the animals in [mg/kg dry feed] or [mg/kg bw/d] and the total residues of BYI 02960 detected in the animal matrices in [mg/kg].

Since the total residue for data collection is different to the relevant residue for enforcement and risk assessment, separate transfer factors were calculated for all animal matrices in all dose groups tested. The total residue as defined for data collection comprises the compounds BYI 02960, BYI 02960-OH,

BYI 02960-acetyl-AMCP, and DFA, wheras only parent compound BYI 02960 and DFA were proposed as relevant residue for enforcement and risk assessment (see B.7.3.2).

The following tables refer to calculations on the basis of the total residue for data collection (BYI 02960, BYI 02960-OH, BYI 02960-acetyl-AMCP and DFA):

Table 7.8.1-8: Transfer factors calculated for the total residue as defined in the data collection method (parent compound BYI 02960, BYI 0296-acetyl-AMCP, BYI 02960-OH and DFA)

Animal matrix	Total residue [mg a.s equiv./kg]	Transfer factor based on dietary burden in [mg/kg bw/d]	Transfer factor based on dietary burden in [mg/kg dry feed]
Average dose administered: 4.54 mg a.s./kg bw/d = 65.1 mg a.s./kg dry feed (54N ~~43N~~ ~~49N~~)			
egg composite* (days 1 to 29)	1.308	0.29	0.020
muscle*	2.409	0.53	0.037
fat*	1.229	0.27	0.019
liver*	3.480	0.77	0.053
Average dose administered: 1.31 mg a.s./kg bw/d = 19.4 mg a.s./kg dry feed (16N ~~13N~~ ~~15N~~)			
egg (day 28)	0.565	0.43	0.029
muscle	0.759	0.58	0.039
fat	0.302	0.23	0.016
liver	1.054	0.80	0.055
Average dose administered: 0.45 mg a.s./kg bw/d = 6.5 mg a.s./kg dry feed (5.4N ~~4.3N~~ ~~5N~~)			
egg (day 28)	0.193	0.43	0.030
muscle	0.320	0.71	0.049
fat	0.147	0.33	0.023
liver	0.444	0.99	0.068
Average dose administered: 0.10 mg a.s./kg bw/d = 1.5 mg a.s./kg dry feed (1.2N)			
egg (day 28)	0.077	0.77	0.051
muscle	0.113	1.13	0.075
fat	0.059	0.59	0.039
liver	0.134	1.34	0.089

* animals of subgroups A to C were used to calculate the average/weighted residues (*without* "depuration" animals)

In the subsequent table, the transfer factors were elucidated for the combined residues of BYI 02960 + DFA (*without* the other analytes), as these two compounds have been proposed as the relevant residues of BYI 02960 in poulty matrices for enforcement and risk assessment (see B.7.3.2).

Table 7.8.1-9: Transfer factors calculated for the compounds relevant for enforcement/risk assessment (parent compound BYI 02960 and DFA)

Animal matrix	Total residue [mg a.s equiv./kg]	Transfer factor based on dietary burden in [mg/kg bw/d]	Transfer factor based on dietary burden in [mg/kg dry feed]
Average dose administered: : 4.54 mg a.s./kg bw/d = 65.1 mg a.s./kg dry feed (54N ~~43N~~ ~~49N~~)			
egg composite* (days 1 to 29)	1.212	0.27	0.019
muscle*	2.309	0.51	0.035
fat*	1.199	0.26	0.018
liver*	3.346	0.74	0.051
Average dose administered: 1.31 mg a.s./kg bw/d = 19.4 mg a.s./kg dry feed (16N ~~13N~~ ~~15N~~)			
egg (day 28)	0.532	0.41	0.027
muscle	0.729	0.56	0.038
fat	0.282	0.22	0.015
liver	1.022	0.78	0.053
Average dose administered: 0.45 mg a.s./kg bw/d = 6.5 mg a.s./kg dry feed (5.4N ~~4.3N~~ ~~5N~~)			
egg (day 28)	0.173	0.38	0.027
muscle	0.300	0.67	0.046
fat	0.127	0.28	0.020
liver	0.423	0.94	0.065
Average dose administered: 0.10 mg a.s./kg bw/d = 1.5 mg a.s./kg dry feed (1.2N)			
egg (day 28)	0.057	0.57	0.038
muscle	0.093	0.93	0.062
fat	0.039	0.39	0.026
liver	0.114	1.14	0.076

* animals of subgroups A to C were used to calculate the average/weighted residues (*without* "depuration" animals)

As was the case for the transfer factors calculated for DFA, the transfer factors for the total residue (either as defined for data collection or for enforcement/risk assessment) are not independent of the dose fed. Higher doses result in a lower transfer of the compounds into the animal matrices. This is in line with the findings that DFA represents the predominant component of the BYI 02960 residues in poultry matrices. Moreover, the "total residue" transfer factors calculated for the lower doses overestimate the transfer of the residues into the animal matrices since all/most components, except for DFA, are well below the LOD in all/most matrices. Although the compounds were not detectable, they were considered to be *at* the LOQ level of 0.01 mg/kg when calculating the total residues. Therefore it is more appropriate to use the transfer factors calculated for parent compound + DFA (relevant residues of BYI 02960 for risk assessment and enforcement) rather than using the transfer factors calculated for the total residue as defined for data collection.

Table 7.8.1-10: Total residues calculated for animal matrices after dosing of BYI 02960 to laying hens

Dose level [mg/kg feed]	Residues [mg a.s. equiv./kg]		
	Animal matrix	**total residue for data collection[1]**	**total residue for enforcement / risk assessment[2]**
1.2N (1.5 mg/kg dry feed)	eggs	0.077	0.057
	fat	0.059	0.039
	liver	0.134	0.114
	muscle	0.113	0.093
5.4N ~~4.3N~~ ~~5N~~ (6.5 mg/kg dry feed)	eggs	0.193	0.173
	fat	0.147	0.127
	liver	0.444	0.423
	muscle	0.320	0.300
16N ~~13N~~ ~~15N~~ (19.4 mg/kg dry feed)	eggs	0.565	0.532
	fat	0.302	0.282
	liver	1.054	1.022
	muscle	0.759	0.729
54N ~~43N~~ ~~49N~~ (65.1 mg/kg dry feed)	eggs	1.308	1.212
	fat	1.229	1.199
	liver	3.480	3.346
	muscle	2.409	2.309

[1] total residue for data collection: BYI 02960, DFA, BYI 02960-acetyl-AMCP and BYI 02960-hydroxy
[2] total residue for enforcement / risk assessment: BYI 02960 and DFA

Remark: All values <LOQ of 0.01 mg/kg were considered as being *at* 0.01 mg/kg to calculate the total residue

It is evident that parent compound BYI 02960 was metabolized rather quickly to DFA after feeding of BYI 02960 to poultry, and quite high amounts of DFA were systemically available. As expected, the highest DFA concentration – and therefore the highest transfer factor – was detected in the metabolizing organ liver, followed by muscle and eggs. However, the transfer of total BYI 02960 residues into all edible matrices is rather low at all dose levels tested.

Feeding of DFA only would thus result in approx. threefold higher transfer factors, as demonstrated above (cf. Table 7.8.1-8). The highest transfer was also determined for liver, followed by muscle and eggs. The lowest transfer factor was determined for fat.

Using these transfer factors derived for the combined residue of BYI 02960 + DFA as well as for DFA alone, estimations of the residue levels reasonably expected to be determined in poultry tissues and eggs after feeding of a mixture of BYI 02960 and DFA (as would realistically be anticipated in the feed crops) can be calculated.

Lactating ruminants

STUDY 1

Characteristics

Reference	:	[redacted]; 2012b KIIA 6.4.2/01	Animal products	:	Muscle, liver, kidney, subcutaneous, omental and renal fat, milk (whole milk, cream, skim milk, whey)
Type of study	:	Magnitude of residues in animal tissues and animal products (livestock feeding)	GLP statement	:	yes
Year of execution	:	2011-2012	Guideline	:	OPPTS 860.1480 – meat/milk/poultry/eggs OECD Guideline 505 APVMA Residue Guideline No. 23 DACO 7.5 – Meat/milk/poultry/eggs
Test substance	:	Flupyradifurone Batch/Lot Number: 2009-000240, Purity: 99.3%	Dose levels	:	0 – 4.8 – 23 – 50 – 135 mg/kg feed/d
Type of animal	:	Lactating cow (*Bos Taurus*) Holstein dairy cows	Acceptability	:	acceptable

Study design

Twenty Holstein dairy cows (*Bos Taurus*, approximately 2.5 to 3.5 years of age) purchased from a local dairy were dosed orally, via capsule, for 29 consecutive days with flupyradifurone at dose rates of 0 mg/kg feed/day (control; 2 cows), 4.8 mg/kg feed/day (1.6N ~~1.8N~~ ~~2N~~ group; 0.18 mg as/kg bw/d; 4 cows;), 23 mg/kg feed/day (8N ~~9N~~ ~~8N~~ group; 0.90 mg as/kg bw/d; 3 cows; which is approximated a 1X NAFTA dose), 50 mg/kg feed/day (17N ~~19N~~ ~~18N~~ group; 1.84 mg as/kg bw/d; three cows), and 135 mg/kg feed/day (44N ~~52N~~ ~~49N~~ group; 4.90 mg as/kg bw/d; 7 cows).
The animals were dosed orally once per day each morning after milking and feeding.

Milk was collected twice daily (afternoon and morning), and the milk was weighed after each milking. On study days 0, 2, 4, 7, 10, 14, 17, 19, 25, 28, 29, 30, 31, 35, 38, and 42, a subsample of the evening milk was retained (refrigerated) and then composited with the morning milk proportional to the amount collected at each milking. Samples from all of these sampling events were analyzed for the 44N ~~52N~~ group; only day-28 samples were analyzed from the other dose groups. Additional 25-day milk was collected for processing into whey (skim milk) and cream (milk fat) from the one control cow and three of the 44N ~~52N~~ ~~50N~~ dose group cows.

On study day 29, all animals except the four depuration cows were humanely sacrificed at least three hours following the last dose. The depuration cows (44N ~~52N~~ ~~49N~~) were sacrificed on study days 32, 36, and 43 (a control cow was also sacrificed on day 43).

Representative samples of liver (each lobe), kidney (center and ends), fat (approximately equal composite of omental, renal, and subcutaneous), and muscle (composite of loin, round, and flank) were collected. The tissue samples were homogenized in the presence of dry ice and the homogenized samples were placed in a freezer prior to shipping.

To estimate the levels of exposure of dairy cows to DFA residues following administration of BYI 02960, urine was collected from two cows (one each from 1.6N ~~1.8N~~ ~~2N~~ and 44N ~~52N~~ ~~49N~~ dose groups). Starting on study day 1, prior to morning dosing, the 24-hour collected sample from each cow was weighed and a 50-g aliquot was subsampled. (Unfortunately, these samples were not always composited in a manner proportional to the amounts collected. However, since a 24-hour period was always covered, the "inaccuracy" will be negligible.) These subsamples were pooled by week, resulting in one sample per week for four weeks. The samples were frozen prior to shipping and shipped frozen for analysis.

BYI 02960 and its metabolites were analytically determined using analytical method RV-004-A11-04 (see B.5.2), which was validated prior to and parallel to the residue analysis of the samples. The LOQ was 0.01 mg/kg for all analytes (parent compound, BYI 02960-acetyl-AMCP, and BYI 02960-OH) except DFA, for which it was 0.02 mg/kg (0.05 mg/kg in whey). All values are expressed in BYI 02960 equivalents.

Table 7.8.1-11: Concurrent recovery data for the relevant residues of BYI 02960 in bovine matrices

Study no.	Matrix	a.s./metabolite	n	Spike Level (mg/kg)	Recovery[a]				
					Individual recoveries	Min	Max	Mean	RSD
RARVP050	milk	BYI 02960	7	0.010	109,109,108, 110,108,107,102	102	110	108	2
			1	0.0250	107	107	107	-	-
			5	0.050	10,102,97, 105, 105	97	105	102	3
			3	0.10	103, 109 ,104	103	109	105	3
			1	0.250	103	103	103	-	-
			3	2.0	10, 102 ,103	10	103	102	2
		difluoroacetic acid	7	0.020	85,88,80,88,92, 76, 84	76	88	85	6
			7	0.050	81,77,78,89,91, 93, 93	77	93	86	8
			3	0.20	91, 92 ,94	91	94	92	2
			3	0.40	85, 85 ,93	85	93	88	5
		BYI 02960-acetyl-AMCP	7	0.010	103,105,104,104, 109 ,106, 101	101	109	105	2
			1	0.0250	112	112	112	-	-
			5	0.050	103, 102 ,103, 109, 107	102	109	105	3
			3	0.10	108, 112 ,107	107	112	109	2
			1	0.250	103	103	103	-	-
			3	2.0	98, 99 ,99	98	99	99	1
		BYI 02960-OH	7	0.010	115,110,111,105, 113 ,110, 108	105	115	110	3
			1	0.0250	106	106	106	-	-
			5	0.050	101, 104 ,10, 106, 106	10	106	103	3
			3	0.10	110, 108 ,105	105	110	108	2
			1	0.250	105	105	105	-	-
			3	2.0	101, 105 ,101	101	105	102	2
	cream	BYI 02960	7	0.010	109,112,107,118, 105 ,110, 106	105	118	110	4
			3	0.10	109, 107 ,113	107	113	110	3
			3	1.0	111, 109 ,111	109	111	110	1
		difluoroacetic acid	7	0.020	103, 95 ,89, 93, 85 ,103, 96	85	103	95	7
			3	0.20	101, 102 ,106	101	106	103	3
		BYI 02960-acetyl-AMCP	7	0.010	106, 108 ,102, 113, 93 ,98, 93	93	113	102	8
			3	0.10	111, 111 ,114	111	114	112	2
			3	1.0	114, 109 ,115	109	115	113	3
		BYI 02960-OH	7	0.010	116,115,113,124, 107 ,109, 111	107	124	114	5
			3	0.10	110, 111 ,113	110	113	111	1
			3	1.0	114, 111 ,114	111	114	113	2
RARVP050	whey	BYI 02960	7	0.010	98, 99 ,10, 98, 105 ,101, 104	98	105	101	3
			7	0.0250	104,106,102,102, 101 ,102, 106	101	106	103	2

Study no.	Matrix	a.s./metabolite	n	Spike Level (mg/kg)	Recovery[a]				
					Individual recoveries	Min	Max	Mean	RSD
			3	0.10	101, 102 ,102	101	102	102	1
			3	0.250	105, 103 ,104	103	105	104	1
			3	1.0	102, 10 ,104	10	104	102	2
		difluoroacetic acid	7	0.050	96,94,95,95, 87,96,89	87	96	93	4
			3	0.50	102, 94 ,99	94	102	98	4
		BYI 02960-acetyl-AMCP	7	0.010	99,10,102,103, 104 ,103, 106	99	106	102	2
			3	0.10	102, 102 ,103	102	103	102	1
		BYI 02960-OH	7	0.010	104,104,104,104, 102 ,10, 103	10	104	103	1
			7	0.0250	107,112,104,109, 107 ,108, 107	104	112	108	2
			3	0.10	101, 10 ,102	10	102	101	1
			3	0.250	106, 112 ,107	106	112	108	3
	fat	BYI 02960	7	0.010	104,101,105,96, 107 ,106, 103	96	107	103	4
			2	0.050	93, 10	93	10	97	
			3	0.10	97, 10 ,101	97	101	99	2
			3	1.50	92, 95 ,99	92	99	95	4
		difluoroacetic acid	7	0.020	81, 96 ,88, 95, 92,86, 87	81	96	89	6
			2	0.050	89, 93	89	93	91	-
			3	0.60	88, 89 ,90	88	90	89	1
		BYI 02960-acetyl-AMCP	7	0.010	94, 97 ,10, 98, 99 ,97, 96	94	10	97	2
			2	0.050	97, 98	97	98	98	-
			3	0.10	96, 98 ,101	96	101	98	3
			3	1.50	101, 102 ,106	101	106	103	3
		BYI 02960-OH	7	0.010	106,107,109,101, 108 ,107, 105	101	109	106	2
			2	0.050	98, 105	98	105	102	-
			3	0.10	97, 101 ,106	97	106	101	4
			3	1.50	94, 97 ,98	94	98	96	2
RARVP050	kidney	BYI 02960	7	0.010	98,99,95,91,94, 98, 95	91	99	96	3
			3	0.050	93, 87 ,98	87	98	93	6
			3	0.10	96, 96 ,94	94	96	95	1
			3	6.0	90, 92 ,97	90	97	93	4
		difluoroacetic acid	7	0.020	74,73,74,79,66, 69, 66	66	79	72	7
			2	0.050	69, 72	69	72	71	-
			3	0.80	84, 79 ,84	79	84	82	4
		BYI 02960-acetyl-AMCP	7	0.010	102, 103 ,105, 99, 99 ,99, 96	96	105	10	3
			3	0.050	10, 94 ,90	90	10	95	5
			3	0.10	98, 99 ,97	97	99	98	1
			3	6.0	91, 93 ,95	91	95	93	2

Study no.	Matrix	a.s./metabolite	n	Spike Level (mg/kg)	Recovery[a]				
					Individual recoveries	Min	Max	Mean	RSD
		BYI 02960-OH	7	0.010	101, 90 ,99, 94, 90 ,105, 98	90	105	97	6
			3	0.050	97, 93 ,95	93	97	95	2
			3	0.10	98, 98 ,98	98	98	98	0
			3	6.0	95, 94 ,10	94	10	96	3
	liver	BYI 02960	7	0.010	98, 90 ,89, 93, 91,85, 84	84	98	90	5
			3	0.050	96, 98 ,96	96	98	97	1
			3	0.10	87, 88 ,91	87	91	89	2
			3	4.0	90, 93 ,91	90	93	91	2
		difluoroacetic acid	7	0.020	87, 75 ,97, 68, 73,73, 82	68	97	79	13
			2	0.050	64, 72	64	72	68	-
			3	0.60	79, 80 ,89	79	89	83	7
		BYI 02960-acetyl-AMCP	7	0.010	99, 85 ,104, 93, 98 ,86, 80	80	104	92	9
			3	0.050	103, 109 ,95	95	109	102	7
			3	0.10	93, 92 ,98	92	98	94	3
			3	4.0	93, 94 ,94	93	94	94	1
		BYI 02960-OH	7	0.010	93, 88 ,88, 90, 95,90, 87	87	95	90	3
			3	0.050	98, 10 ,93	93	10	97	4
			3	0.10	91, 91 ,92	91	92	91	1
			3	4.0	97, 98 ,95	95	98	97	2
RARVP050	muscle	BYI 02960	7	0.010	92, 97 ,92, 10, 96 ,98, 10	92	10	96	3
			2	0.050	95, 99	95	99	97	-
			3	0.10	93, 98 ,93	93	98	95	3
			3	2.0	92, 90 ,89	89	92	90	2
		difluoroacetic acid	7	0.020	66, 71 ,76, 76, 77,78, 73	66	78	74	6
			3	0.050	71, 78 ,70	70	78	73	6
			3	0.50	67, 65 ,73	65	73	68	6
		BYI 02960-acetyl-AMCP	7	0.010	89, 101 ,92, 98, 98 ,101, 99	89	101	97	5
			2	0.050	95, 103	95	103	99	-
			3	0.10	93, 103 ,91	91	103	96	7
			3	2.0	96, 96 ,95	95	96	96	1
		BYI 02960-OH	7	0.010	93, 10 ,106, 95, 101 ,101, 99	93	106	99	4
			2	0.050	117, 96	96	117	107	-
			3	0.10	96, 101 ,94	94	101	97	4
			3	2.0	92, 92 ,92	92	92	92	0
	urine	BYI 02960	7	0.010	102,104,103,103, 104 ,103, 101	101	104	103	1
			3	0.10	102, 102 ,102	102	102	102	0
			3	40.0	109, 103 ,104	103	109	105	3
		difluoroacetic acid	7	0.020	78, 85 ,67, 73,	67	85	75	8

Study no.	Matrix	a.s./metabolite	n	Spike Level (mg/kg)	Recovery[a]				
					Individual recoveries	Min	Max	Mean	RSD
					79,70, 76				
			3	0.20	92, 91 ,92	91	92	92	1
			3	5.0	106, 102 ,101	101	106	103	3
		BYI 02960-acetyl-AMCP	7	0.010	112,119,106,109, 113 ,111, 110	106	119	111	4
			3	0.10	108, 109 ,107	107	109	108	1
			3	40.0	109, 105 ,107	105	109	107	2
		BYI 02960-OH	7	0.010	109,116,110,108, 113 ,113, 112	108	116	112	2
			3	0.10	107, 105 ,105	105	107	106	1
			3	40.0	110, 106 ,105	105	110	107	2

All concentrations are expressed in parent compound equivalents.

Results

The mean values of the concurrent recovery rates per compound, sample material, and spiking level were generally in the range of 70-110%, with very few (and minor) exceptions. As the relative standard deviations were always low – in the range of ~~0~~ 1-13% – all concurrent recoveries were considered to be acceptable. Details of recovery data are shown in table 7.8.1-11.

Feed consumption and milk production were not adversely affected by treatment with BYI 02960. Although some animals gained weight while others lost weight, average body weights decreased slightly in all groups, including the control, but somewhat more in the highest dose group. Thus it appears that 44N ~~52N~~ ~~49N~~ dosing may have had an effect on body weight. One cow in the 44N ~~52N~~ ~~49N~~ group suffered health complications (chronic mastitis, metritis) for which she was treated, but which could not be resolved during the study period. Her condition affected both her feed consumption and body weight, although no effect was evident with respect to milk production.

As the proposed residue definition for both enforcement and risk assessment will include only parent BYI 02960 + DFA, which are by far the two major components of the residue, the critical values for this study may not reflect the total of all components as listed above. In the nominal worst-case dietary burden groups of 1.6N ~~1.8N~~ ~~2N~~, the combined residues of the two components (calculated in the "traditional" manner, i.e. if one component is <LOQ, it is calculated as being *at* the LOQ) were as follows: 0.063 mg/kg in muscle, 0.041 mg/kg in fat, 0.165 mg/kg in liver, and ~~0.180~~ 0.176 mg/kg in kidney, respectively. In milk taken on day 28, total residues in the 1.6N ~~1.8N~~ ~~2N~~ group were 0.043 mg/kg, respectively.

In addition to the general testing, day-25 milk samples from the 44N ~~52N~~ ~~50N~~ dose group were separated into cream and whey, in order to determine if the residues preferentially collect in more aqueous or fatty compartments. The processing/transfer factors (PF) for the total residue (four components) indicated a very slight preference for more aqueous conditions, with a PF of 1.15 to whey and 0.79 to cream. These factors were also representative for the 2 analytes of primary concern, BYI 02960 parent compound and DFA.

The residues found in the milk, tissues, and excreta collected from the dairy cows during dosing, at the end of the dosing period, and during the depuration phase are presented in tables 7.8.1-12 and 7.8.1-13.

With respect to milk, the highest total BYI 02960 residues were found in the day-17 sample from the 44N ~~52N~~ ~~49N~~ dose group. Thus, BYI 02960 residues reached a plateau between at that time and remained at that approximate level for the remainder of the dosing period. The highest total residues in milk at any time in the study in this group were 0.979 mg/kg (total of 4 components); when evaluating only the two analytes of importance, BYI 02960 and DFA, the level was 0.974 mg/kg. This was only slightly higher than 0.886 and 0.806 mg/kg, the values determined in the 44N ~~52N~~ ~~49N~~ group on days 28 and 29, respectively.

In the depuration phase, total BYI 02960 residues in milk, fat, liver, kidney, and muscle from the 50N dose group cows declined from 0.81, 1.40, 1.59, 5.39, and 1.91 mg/kg, respectively, to <LOQ at 6-7 days after cessation of dose administration (=day 35-36 of the study). The residue data provided in this study are suitable for regulatory purposes. Depuration data are also presented in tables 7.8.1-12 and 7.8.1-13.

The levels of DFA residues found in urine are summarized in table table 7.8.1-14. DFA residues represented 6% to 8% of the total residues of BYI 02960 in the urine samples, suggesting low but significant exposure of the dairy cattle to DFA following the daily oral administration of BYI 02960 over 29 days.

Table 7.8.1-12: Levels of the relevant residues of BYI 02960 in milk

group	sampling day	Residue levels of individual analytes (mg/kg)				Total residue levels (mg/kg)	
		BYI 02960	DFA	-AMCP*	-OH	sum of 4**	BYI 02960 + DFA
1.6N ~~1.8N~~ ~~2N~~	28	0.023	<0.02	<0.01	<0.01	0.028	0.043
8N ~~9N~~ ~~8N~~	28	0.108	0.021	<0.01	<0.01	0.125	0.129
17N	28	0.267	0.041	<0.01	<0.01	0.310	0.308

group	sampling day	Residue levels of individual analytes (mg/kg)				Total residue levels (mg/kg)	
		BYI 02960	DFA	-AMCP*	-OH	sum of 4**	BYI 02960 + DFA
19N ~~18N~~							
44N ~~52N~~ ~~49N~~⅄	2	0.759	0.081	<0.01	<0.01	0.831	0.840
	4	0.869	0.105	<0.01	<0.01	0.978	0.973
	7	0.688	0.138	<0.01	<0.01	0.830	0.826
	10	0.763	0.137	<0.01	<0.01	0.906	0.900
	14	0.783	0.151	<0.01	<0.01	0.940	0.935
	17	0.831	0.143	<0.01	<0.01	0.979	0.974
	19	0.825	0.130	<0.01	<0.01	0.960	0.955
	25	0.651	0.115	<0.01	<0.01	0.770	0.765
	25†	0.553	0.050	<0.01	<0.01	0.607	0.603
	25‡	0.758	0.123	<0.01	<0.01	0.887	0.881
	28	0.748	0.138	<0.01	<0.01	0.892	0.886
	29***	0.667	0.140	<0.01	<0.01	0.811	0.806
	30***	0.059	0.078	<0.01	<0.01	0.138	0.137
	31***	<0.01	0.043	<0.01	<0.01	0.050	0.053
	35***	<0.01	<0.02	<0.01	<0.01	<0.05	<0.03

* AMCP = BYI 02960-acetyl-AMCP
** this value, as shown in the study report, includes values below the LOQ calculated *at the apparent residue value*
*** depuration phase, no dosing (sampling days 35-49)
† values for cream
‡ values for whey
⅄ day-28 values for the 2N and 49N groups include samples from all animals (including "urine" and "depuration" animals)

Table 7.8.1-13: Levels of the relevant residues of BYI 02960 in bovine tissues

group	sampling day	Residue levels of individual analytes (mg/kg)				Total residue levels (mg/kg)	
		BYI 02960	DFA	-AMCP*	-OH	sum of 4‡	BYI 02960 + DFA
	BOVINE ***FAT***						
1.6N ~~1.8N~~ ~~2N~~†	29	0.021	<0.02	<0.01	<0.01	0.032	0.041
8N ~~9N~~ ~~8N~~	29	0.109	0.038	<0.01	<0.01	0.151	0.147
17N ~~19N~~ ~~18N~~	29	0.285	0.099	<0.01	<0.01	0.388	0.384
44N ~~52N~~ ~~49N~~†	29	0.977	0.392	<0.01	0.020	1.390	1.369
	32**	<0.01	0.100	<0.01	<0.01	0.111	0.110
	36**	<0.01	<0.02	<0.01	<0.01	0.019	<0.03
	43**	<0.01	<0.02	<0.01	<0.01	<LOD	<0.03
	BOVINE ***KIDNEY***						
1.6N ~~1.8N~~	29	0.159	0.017	<0.01	0.019	0.193	0.176

group	sampling day	Residue levels of individual analytes (mg/kg)				Total residue levels (mg/kg)	
		BYI 02960	DFA	-AMCP*	-OH	sum of 4‡	BYI 02960 + DFA
2N†							
8N 9N 8N	29	0.786	0.081	<0.01	0.026	0.893	0.867
17N 19N 18N	29	1.789	0.203	<0.01	0.045	2.037	1.992
44N 52N 49N†	29	4.720	0.558	<0.01	0.103	5.380	5.279
	32**	0.045	0.141	<0.01	<0.01	0.187	0.186
	36**	<0.02	<0.01	<0.01	<0.01	0.010	<0.03
	43**	<0.02	<0.01	<0.01	<0.01	<LOD	<0.03
BOVINE ***LIVER***							
1.6N 1.8N 2N†	29	0.145	<0.02	<0.01	0.011	0.159	0.165
8N 9N 8N	29	0.755	0.057	<0.01	0.011	0.824	0.812
17N 19N 18N	29	1.680	0.132	<0.01	0.020	1.842	1.812
44N 52N 49N†	29	3.451	0.390	<0.01	0.035	3.890	3.841
	32**	0.033	0.106	<0.01	<0.01	0.140	0.139
	36**	<0.02	<0.01	<0.01	<0.01	0.012	<0.03
	43**	<0.02	<0.01	<0.01	<0.01	<LOD	<0.03
BOVINE ***MUSCLE***							
1.6N 1.8N 2N†	29	0.043	<0.02	<0.01	<0.01	0.057	0.063
8N 9N 8N	29	0.250	0.054	<0.01	<0.01	0.307	0.304
17N 19N 18N	29	0.597	0.136	<0.01	<0.01	0.739	0.733
44N 52N 49N†	29	1.505	0.385	<0.01	0.014	1.901	1.890
	32**	0.017	0.095	<0.01	<0.01	0.114	0.112
	36**	<0.02	<0.01	<0.01	<0.01	0.018	<0.03
	43**	<0.02	<0.01	<0.01	<0.01	<LOD	<0.03

* AMCP = BYI 02960-acetyl-AMCP
** depuration phase, no dosing (sampling days 35-49)
† day-29 values for the 2N and 49N groups include samples from all animals (including "urine" and "depuration" animals)
‡ this value, as shown in the study report, includes values below the LOQ calculated *at the apparent residue value*

Table 7.8.1-124: Proportion of DFA in the total residues of BYI 02960 in urine

Matrix	Dose group	Sampling week	Urine collected (kg)*	Total BYI 02960 residue** (mg/kg)	DFA residues† (mg/kg)	Proportion of DFA in total residue (%)
urine	1.6N ~~1.8N~~ ~~2N~~	week 1	146	1.85	0.112	6
		week 2	122	2.18	0.185	8
		week 3	125	2.24	0.184	8
		week 4	142	1.81	0.142	8
	44N ~~52N~~ ~~49N~~	week 1	226	29.1	1.92	7
		week 2	218	30.8	2.33	8
		week 3	159	46.0	3.71	8
		week 4	152	40.9	3.33	8

* Daily average (determined for each of the four study weeks) was multiplied by 7 days to determine the weekly urine output. Both the 2Nand 50N animals experienced some hose blockages; the urine lost during these events was estimated by the in-life phase facility but is not included

** "total BYI 02960 residue" is the sum of BYI 02960, BYI 02960-OH, BYI 02960-acetyl-AMCP, and DFA residues, expressed in BYI 02960 equivalents

† expressed in BYI 02960 equivalents

Conclusion

BYI 02960 was administered orally (via capsule) to dairy cows for 29 consecutive days at average *actual* dose rates of 4.8 mg/kg feed (1.6N ~~1.8N~~ ~~2N~~ dose), 23 mg/kg feed (8N ~~9N~~ ~~8N~~, which approximated a nominal NAFTA 1N dose), 50 mg/kg feed (17N ~~19N~~ ~~18N~~), and 135 mg/kg feed (44N ~~52N~~ ~~49N~~). Feed consumption, body weights, and milk production were not adversely affected by compound administration.

After the final dose, the animals were sacrificed and the key edible tissues were analyzed for the relevant residues of BYI 02960. While data were generated for four analytes in the study itself, only two – BYI 02960 and DFA – are proposed for the residue definitions (enforcement and risk assessment) for BYI 02960. The combined residues of BYI 02960 + DFA in bovine tissues at sacrifice in the 1.6N ~~1.8N~~ ~~2N~~ dosing group were 0.063 mg/kg in muscle, 0.041 mg/kg in fat, 0.18 mg/kg in kidney, and 0.17 mg/kg in liver, expressed in parent compound equivalents. Prior to sacrifice, residues in milk were measured at various intervals in the high-dose group, and on day 28 in the three lower dose groups. In the 1.6N ~~1.8N~~ ~~2N~~ dose group, the residues (BYI 02960 + DFA) amounted to 0.043 mg/kg.

Residue levels reached a plateau in milk. Highest residue were determined at day 17 in the 44N ~~52N~~ ~~50N~~ milk samples, remaining at similar levels for the remainder of the study.

Depuration occurred quickly. Total BYI 02960 residues in milk, fat, liver, kidney, and muscle from the 44N ~~52N~~ ~~49N~~ dose level cows declined from 0.81, 1.40, 1.59, 5.39, and 1.91 mg/kg, respectively, to <LOQ at 6-7 days after cessation of dose administration (=day 35-36 of the study). The residue data provided in this study are suitable for regulatory purposes.

DFA residues represented 6% to 8% of the total BYI 02960 residues in the urine samples, suggesting low but significant exposure of the dairy cows to DFA residues following the administration of BYI 02960 for 29 days.

Transfer factors calculated for DFA and the total residue

As agreed with the notifier, separate transfer factors for DFA were estimated from the cattle feeding study on the basis of the data available after dosage of the active substance BYI 02960 to lactating cows. In order to accomplish this, a theoretical dose of DFA must be estimated. For this estimation, the amounts of difluoroacetic in all organs/tissues and particularly in urine must be considered. In the rat ADME study, the major amount of the total radioactivity, i.e. > 80% of the dose administered, was excreted via the urine. DFA is a polar compound with a low molecular weight, so it can be assumed that is excreted mainly via the urine. Thus the role of excretion via faeces will be minimal, and this matrix was not considered in the dairy cow balance. The absolute amount of DFA formed in the cows and which was thus systemically available can then be equated with a minimum dose theoretically fed to the animals.

The estimation of the dose of DFA theoretically fed to the animals was conducted on the basis of the data collected for the 44N ~~52N~~ ~~50N~~ dose group, using three animals (non-depuration), and the one animal in this dose group for collection of urine (actual mean dose of BYI 02960 = 135 mg/kg feed), and additionally on the basis of the data collected for the 1.6N ~~1.8N~~ ~~2N~~ dose group animals, and one additional animal dosed at 1.6N ~~1.8N~~ ~~2N~~ for collection of urine (actual mean dose of BYI 02960 = 4.8 mg/kg feed).

Minimum dose theoretically fed to lactating cows:

The residue levels in urine, milk, tissues and organs and the weights of the animal matrices (average weight for one animal) used for the calculation of the minimum theoretical dose are summarized in Table 7.8.1-13 (44N ~~52N~~ ~~50N~~ and 1.6N ~~1.8N~~ ~~2N~~ dose).

Table 7.8.1-135: Calculation of absolute DFA amounts in cattle matrices on the basis of the sample weights and the residues in the samples

Animal matrix	Weight [kg]	DFA residue* [mg a.s. equiv./kg]	Absolute DFA amounts [mg a.s. equiv.]
Actual dose administered: 4.90 mg/kg bw/d = 135 mg/kg dry feed (44N ~~52N~~ ~~49N~~)			
milk composite (day 1-28)	874	0.118[5]	103.13
muscle	162[1]	0.381	61.72
fat	64.9[2]	0.335	21.74
liver	8.1[3]	0.380	3.078
kidneys	1.4[4]	0.512	0.717
urine	754	2.70[5]	2035.8
total (one animal)			**2226.2**
Actual dose administered: 0.18 mg/kg bw/d = 4.8 mg/kg dry feed (1.6N ~~1.8N~~ ~~2N~~)			
milk (day 28)	845	0.004	3.50
muscle	165[1]	0.013	2.09
fat	65.9[2]	0.011	0.75
liver	8.2[3]	0.012	0.10
kidneys	1.4[4]	0.018	0.02
urine	535	0.153[5]	82.10
total (one animal)			**88.56**

* milk and organs/tissues:
average/weighted residues were calculated for the "residue animals" using samples from 44N ~~52N~~ ~~49N~~ dose group (non-depuration) and from animals for the 1.6N ~~1.8N~~ ~~2N~~ dose group
urine:
residues were calculated from samples of the "urine collection" animals " in dose groups 44N ~~52N~~ ~~49N~~ and 1.8N ~~2N~~

[1] total muscle weight was calculated assuming a value of 30%of the mean body weight for this tissue
[2] total fat weight was calculated assuming a value of 12%of the mean body weight for this tissue
[3] total liver weight was calculated assuming a value of 1.5% of the mean body weight for this tissue
[4] total kidney weight was calculated assuming a value of 0.25% of the mean body weight for this tissue
[5] weighted DFA residue value are available in original study report.

According to the balance established, the minimum amount of DFA systemically available was **2226.2 mg a.s. equiv. per animal** in the 44N ~~52N~~ ~~49N~~ dose experiment and **88.6 mg a.s. equiv. per animal** in the 1.6N ~~1.8N~~ ~~2N~~ dose experiment.

Calculation of transfer factors:

44N ~~52N~~ ~~49N~~ dose:

The total amount of BYI 02960 administered in the 44N ~~52N~~ ~~49N~~ dose experiment of the feeding study accounted for 76876 mg per animal (4.9 mg/kg bw/day × 541 kg bw × 29 days). Thus the total amount of DFA being systemically available represented 2.9% of the amount administered as BYI 02960.

The theoretical dose of DFA can thus be calculated as being **0.14 mg a.s. equiv./kg bw/d** (4.90 mg a.s./kg feed × 2.9% = 0.14 mg a.s. equiv./kg bw/d) equal to 3.9 mg a.s. equiv./kg dry feed.

1.6N ~~1.8N~~ ~~2N~~ dose:

The total amount of BYI 02960 administered in the 2Ndose experiment of the feeding study accounted for 2929.5 mg per animal (0.184 mg/kg bw/day × 549 kg bw × 29 days). Thus the total amount of DFA being systemically available represented 3.0% of the amount administered as BYI 02960. This proportion fits very well with the percentage calculated for the 44N ~~52N~~ ~~50N~~ dose (2.9% of the BYI 02960 dose administered).

The theoretical dose of DFA can thus be calculated as being **0.005 mg a.s. equiv./kg bw/d** (0.184 mg/kg bw/day × 3.0% = 0.005 mg a.s. equiv./ kg bw/day) equal to 0.14 mg a.s. equiv./kg dry feed.

On the basis of the DFA residues in milk and organs/tissues and the theoretical dose of DFA, transfer factors have been calculated, according to the following equation:

$$\textbf{TF} = \frac{\textbf{residue level in edible commodity}}{\textbf{residue level in the diet}}$$

The following tables summarise the residue values determined in the animal matrices and the corresponding transfer factors derived from them.

DFA transfer:

Assuming that the theoretical dose of DFA is reflected by the same proportion for all dose levels (as shown for the 44N ~~52N~~ ~~49N~~ and the 1.6N ~~1.8N~~ ~~2N~~ dose), transfer factors for DFA were estimated at all levels. In contrast to the 44N ~~52N~~ ~~49N~~ dose experiment (where weighted residues in milk have been calculated), residues in milk were estimated in all other dose groups on the basis of the 28-day sample, i.e. at a point in time at which the plateau had clearly been reached.

Table 7.8.1-146: Transfer factors calculated for DFA

Animal matrix	DFA residue* [mg a.s equiv./kg]	Transfer factor based on dietary burden in [mg/kg bw/d]	Transfer factor based on dietary burden in [mg/kg dry feed]
Theoretical DFA dose administered: 0.14 mg a.s. equiv./kg bw/d = 3.9 mg/kg dry feed; expressed in parent equivalents (44N ~~52N~~ ~~49N~~)			
milk composite (days 1 to 28)	0.118	0.83	0.030
muscle	0.381	2.68	0.097
fat	0.335	2.36	0.086
liver	0.380	2.67	0.097
kidney	0.512	3.60	0.131
Theoretical DFA dose administered: 0.06 mg a.s. equiv./kg bw/d = 1.45 mg/kg dry feed; expressed in parent equivalents (17N ~~19N~~ ~~18N~~)			
milk (day 28)	0.041	0.74	0.028
muscle	0.136	2.46	0.094
fat	0.099	1.79	0.068
liver	0.132	2.39	0.091
kidney	0.203	3.68	0.140
Theoretical dose administered: 0.03 mg a.s. equiv./kg bw/d = 0.67 mg/kg dry feed; expressed in parent equivalents (8N ~~9N~~ ~~8N~~)			
milk (day 28)	0.016	0.61	0.024
muscle	0.054	2.07	0.081
fat	0.038	1.46	0.057
liver	0.057	2.18	0.085
kidney	0.081	3.10	0.121
Theoretical dose administered: 0.005 mg a.s. equiv./ kg bw/day = 0.14 mg/kg dry feed; expressed in parent equivalents (1.6N ~~1.8N~~ ~~2N~~)			
milk (day 28)	0.004	0.77	0.028
muscle	0.013	2.49	0.088
fat	0.011	2.11	0.079
liver	0.012	2.30	0.086
kidney	0.017	3.26	0.125

* average/weighted residues were calculated using samples from "residue" animals for the 44N ~~52N~~ ~~49N~~ dose group and from animals for the 1.6N ~~1.8N~~ ~~2N~~ dose group, in agreement with calculation of residues in the samples of all other dose groups which consisted of "residue" animals, only. Samples of the "urine collection" (44N ~~52N~~ ~~49N~~ and 1.6N ~~1.8N~~ ~~2N~~) and "depuration" animals (44N ~~52N~~ ~~49N~~) were not included.

Theoretical DFA dose was calculated using the percentage of 2.9%

Comparing the transfer factors estimated for the animal matrices at different dose levels, it is obvious that the transfer factors correspond very well. The transfer factors for all matrices were in a very narrow range, e.g. the transfer factor (based on dietary burden in mg/kg dry feed) for milk ranged from 0.024 to 0.030 and the one for muscle from 0.081 to 0.097. The residue levels in the animal matrices increase linearly with increasing dose levels.

Transfer of BYI 02960 residues:

The transfer of BYI 02960 residues into edible matrices was also calculated on the basis of the dose administered to the animals in [mg/kg bw/d] and [mg/kg dry feed] and the total residue of BYI 02960 detected in the animal matrices in [mg/kg].

Since the total residue for data collection is different from the relevant residue for enforcement and risk assessment, separate transfer factors were calculated for all animal matrices in all dose groups tested. The total residue as defined for data collection comprises the compounds BYI 02960, BYI 02960-OH, BYI 02960-acetyl-AMCP, and DFA, wheras only parent compound BYI 02960 + DFA were proposed as relevant residue for enforcement and risk assessment (see B.7.3.2).

The following tables refer to calculations on the basis of the total residue for data collection (BYI 02960, BYI 02960-OH, BYI 02960-acetyl-AMCP and DFA):

Table 7.8.1-157: Transfer factors calculated for the total residue consisting of parent compound BYI 02960, BYI 0296-acetyl-AMCP, BYI 02960-OH and DFA

Animal matrix	Total residue* [mg a.s equiv./kg]	Transfer factor based on dietary burden in [mg/kg bw/d]	Transfer factor based on dietary burden in [mg/kg dry feed]
Average dose administered: 4.90 mg/kg bw/d = 135 mg/kg dry feed (44N 52N 49N)			
milk composite (days 1 to 28)	0.942	0.20	0.007
muscle	1.784	0.39	0.013
fat	1.206	0.28	0.009
liver	3.903	0.79	0.029
kidney	5.027	1.10	0.037
Average dose administered: 1.84 mg/kg bw/d = 50 mg/kg dry feed (17N 19N 18N)			
milk (day 28)	0.328	0.17	0.007
muscle	0.753	0.40	0.015
fat	0.404	0.21	0.008
liver	1.841	1.00	0.037
kidney	2.046	1.11	0.041
Average dose administered: 0.90 mg/kg bw/d = 23 mg/kg dry feed (8N 9N 8N)			
milk (day 28)	0.148	0.14	0.006
muscle	0.324	0.34	0.014
fat	0.167	0.17	0.007
liver	0.833 0	0.92	0.036
kidney	0.903	0.99	0.039
Average dose administered: 0.18 mg/kg bw/d = 4.8 mg/kg dry feed (1.6N 1.8N 2N)			
milk (day 28)	0.065	0.16	0.013
muscle	0.085	0.32	0.018
fat	0.062	0.18	0.013
liver	0.195	0.88	0.041
kidney	0.209	1.07	0.043

* average/weighted residues were calculated using samples from "residue" animals for the 44N 52N 49N dose group and from animals for the 1.6N 1.8N 2N dose group, in agreement with calculation of residues in the samples of all other dose groups which consisted of "residue" animals, only. Samples of the "urine collection" (44N 52N 49N

and 1.8N ~~2N~~) and "depuration" animals (44N ~~52N~~ ~~49N~~) were not included.

Remark
All values below the LOQ of 0.01 mg/kg (BYI 02960, BYI 02960-acetyl-AMCP and BYI 02960-OH) or below the LOQ of 0.02 mg/kg (DFA) were considered as being *at* 0.01 or 0.02 mg/kg, respectively, to calculate the total residue.

In the subsequent table, the transfer factors were elucidated for the combined residues of BYI 02960 + DFA (*without* the other analytes), as these two compounds have been proposed as the relevant residues of BYI 02960 in poulty matrices for enforcement and risk assessment (see B.7.3.2).

Table 7.8.1-1~~6~~8: Transfer factors calculated for the total residue consisting of parent compound BYI 02960 + DFA

Animal matrix	Total residue [mg a.s equiv./kg]	Transfer factor based on dietary burden in [mg/kg bw/d]	Transfer factor based on dietary burden in [mg/kg dry feed]
Average dose administered: 4.90 mg/kg bw/d = 135 mg/kg dry feed (44N ~~52N~~ ~~49N~~)			
milk composite (days 1 to 28)	0.922	0.20	0.007
muscle	1.760	0.39	0.013
fat	1.182	0.28	0.009
liver	3.851	0.78	0.029
kidney	4.919	1.08	0.036
Average dose administered: 1.84 mg/kg bw/d = 50 mg/kg dry feed (17N ~~19N~~ ~~18N~~)			
milk (day 28)	0.308	0.17	0.006
muscle	0.733	0.40	0.015
fat	0.384	0.21	0.008
liver	1.811	0.98	0.036
kidney	1.992	1.08	0.040
Average dose administered: 0.90 mg/kg bw/d = 23 mg/kg dry feed (8N ~~9N~~ ~~8N~~)			
milk (day28)	0.128	0.14	0.006
muscle	0.304	0.34	0.013
fat	0.147	0.16	0.006
liver	0.812	0.90	0.035
kidney	0.867	0.96	0.038
Average dose administered: 0.18 mg/kg bw/d = 4.8 mg/kg dry feed (1.6N ~~1.8N~~ ~~2N~~)			
milk (day 28)	0.045	0.24	0.009
muscle	0.065	0.35	0.013
fat	0.042	0.23	0.009
liver	0.174	0.92	0.036
kidney	0.179	0.98	0.037

* average/weighted residues were calculated using samples from "residue" animals for the 44N ~~52N~~ ~~49N~~ dose group and from animals for the 1.6N ~~1.8N~~ ~~2N~~ dose group, in agreement with calculation of residues in the samples of all other dose groups which consisted of "residue" animals, only. Samples of the "urine collection" (44N ~~52N~~ ~~49N~~ and 1.6N ~~1.8N~~ ~~2N~~) and "depuration" animals (44N ~~52N~~ ~~49N~~) were not included.

Remark:
All values below the LOQ of 0.01 mg/kg (BYI 02960) or below the LOQ of 0.02 mg/kg (DFA) were considered as being *at* 0.01 or 0.02 mg/kg, respectively, to calculate the total residue.

The predominant component of the residues in milk, edible tissues, and organs was parent compound BYI 02960, followed by DFA. The metabolite BYI 02960-acetyl-AMCP was below the LOD in all dose groups, and BYI 02960-OH was detected in the high dose groups only. When calculating the total residue, all constituents of the residue definition are considered. Compounds detected below the LOQ were estimated to be at the LOQ level. Thus the total residue overestimates the proportion of BYI 02960-acetyl-AMCP and BYI 02960-OH, especially at a 1X dose where neither of the two metabolites were detected. Therefore, calculation of transfer factors should be restricted to parent compound + DFA, the constituents of the residue definition for enforcement and risk assessment.

In milk, DFA concentrations were lower than those for parent by a factor of approx. 6 to 7, as soon as the plateau was reached in milk. DFA concentrations in muscle and fat were lower by a factor of approx. 2 to 4, and by a factor of approx. 9 in the metabolizing organs liver and kidney. Clearly, parent compound BYI 02960 was metabolized quite slowly to DFA after feeding BYI 02960 to lactating cows. As soon as DFA was formed, it was distributed equally within the body. Transfer into milk was rather low.

Feeding of DFA only would result in approx. three to tenfold higher transfer factors, as demonstrated above (see Table 7.8.1-14). The highest transfer was also detected into the metabolizing organs liver and kidney, followed by muscle and fat. The lowest transfer factor was determined for milk.

Using these transfer factors derived for the combined residue of BYI 02960 + DFA as well as for DFA alone, estimations of the residue levels reasonably expected to be determined in ruminant tissues and milk after feeding of a mixture of BYI 02960 and DFA (as would realistically be anticipated in the feed crops) can be calculated.

B.7.9 Residues in succeeding or rotational crops (IIA 6.6; III 8.6)

Residue levels reported in the summary texts of this paragraph are reported in accordance with the initially proposed residue definition for risk assessment and monitoring, i.e. the sum of flupyradifurone, DFA and DFEAF and the sym of flupyradifurone and DFA, respectively. Results in the residue trials summary tables were amended to represent the revised residue definitions of DFA, expressedas DFA and flupyraduifurone for monitoring and the sum of flupyradifurone and DFA< expressed as flupyradifurone for risk assessment.

STUDY 1

reference	:	Schoening, R.; Bauer, J. 2012 KIIA 6.6.3/01	GLP statement	:	yes
Type of study	:	Rotational crop - Field trial	Guideline	:	EU Council Directive 91/414/EEC amended by Commission Directive 96/68/EC EU Guidance Working Document 7029/VI/95 rev. 5 OECD Guideline for the Testing of Chemicals No. 504, Residues in Rotational Crops
Year of execution	:	2010-2011	Dose	:	0.200 kg as/ha
test substance	:	BYI 02960 SL 200 formulation Batch no: 2010-000672	LOQ	:	BYI 02960 : 0.01 mg/kg DFA: 0.02 mg/kg DFEAF : 0.01 mg/kg
Species	:	barley, carrot, lettuce *(Lactuca sativa),* and turnip	storage	:	≤ 12 months at -18°C, with the exception of barley samples from trial 10-2503-01 (480 - 540 days)
			Acceptability	:	Yes, with the exception of barley samples from trial 10-2503-01

Study design

Multi-crop, multi-plot/plantback field rotational crop residue trials were conducted in Europe, two in the northern and two in the southern residue region, as follows:

In 2010-11, four trials (one each in Germany, the Netherlands, Spain, and southern France) were conducted to support the use of BYI 02960 SL 200 in field-grown, non-perennial crops. A single application was made at a nominal rate of 1 L/ha, corresponding to 200 g/ha BYI 02960 a.s., which reflected the projected rate for soil treatment with BYI 02960 (via irrigation) in arable, non-perennial crops, such as lettuce. Water rates were 300-400 L/ha. Applications were either made to bare soil or to lettuce ("target crop"); in the latter case, the crop was then harvested from the field before further crops were planted. All treatments were made at the scheduled rates. PBIs were calculated as time between application to soil or target crop and the sowing/planting of the succeeding crop.

At various intervals, crops were planted back onto the test area in order to simulate a crop failure ("rotation 1", plant-back interval [PBI] 25-33 days), a second use of the plot in the same year ("rotation 2", PBI 60-200 days), or use of the same plot in the succeeding year ("rotation 3", PBI 260-330 days).

In each rotation, 3 different crops representing different botanical groups were planted: a root crop (carrots or turnips), a leafy crop (lettuce), or cereal crop (barley).

Samples of the rotational crops were taken at their respective harvest times, as well as at one earlier interval (immature RACs for lettuce and root crops, or fodder ["green material"] for barley).

Studies conducted to assess the storage stability of frozen samples demonstrated that the relevant residues of BYI 02960 (including parent compound, BYI 02960-difluoroethyl-amino-furanone, and DFA) were stable during a storage period of 18 months under deep-freezer conditions. Samples of the rotational crops were stored for a period of less than 18 months and are therefore covered by the available storage stability investigations.
Samples were investigated for BYI 02960 and its metabolites DFA and DFEAF using method 01304. The respective LOQs for the 3 analytes were 0.01, 0.02, and 0.01 mg/kg (all in parent equivalents), yielding a calculated total-residue LOQ of 0.04 mg/kg.
In addition to the analysis of BYI 02960, DFA and DFEAF (all expressed as BYI 02960 and summarised up to the total residue of BYI 02960 calc.), residues of 6-chloronicotinic acid were determined as supplemental information, with an LOQ of 0.005 mg/kg expressed as 6-chloronicotinic acid (equivalent to 0.01 mg/kg expressed as BYI 02960).

Results

Concurrent recoveries of BYI 02960 and its metabolites DFA and DFEAF were obtained from samples of carrots, turnips, lettuce, and barley. Recovery data for BYI 02960 in carrots, lettuce, turnips and barley are presented in table 7.9-1. (Validation recoveries were conducted separately.

Root crops:
Concurrent recovery samples for parent compound and DFEAF were spiked at levels of 0.01, 0.10, 0.50, and 1.0 mg/kg (expressed in BYI 02960 equivalents). Mean recoveries in root crop samples (carrot or turnip roots, turnip leaves) were 74-108%, with RSDs of the larger validations sets (n > 2, at the LOQ) of 7.1-16.0%; n = 1-6. All values were within acceptable ranges.

For DFA, concurrent recovery samples were spiked at levels of 0.02, 0.20, 0.50, and 1.0 mg/kg (expressed in BYI 02960 equivalents). Mean recoveries were 84-109%, with RSDs (of the larger validations sets [n > 2]) of 7.4-17.1%; n = 1-6.

Leafy crops:
Concurrent recovery samples for parent compound and DFEAF were spiked at levels of 0.01, 0.10 , 0.50, 1.0 and 5.0 mg/kg (expressed in BYI 02960 equivalents). Mean recoveries in root crop samples (lettuce heads) were 91-105%, with RSDs of the larger validations sets (n > 2) of 2.2-10.7%; n = 2-15.

For DFA, concurrent recovery samples were spiked at levels of 0.02-5.0 mg/kg (expressed in BYI 02960 equivalents). Mean recoveries were 90-98%, with RSDs (of the larger validations sets [n > 2]) of 4.3-10.2%; n = 2-12.

Cereal crops:
Concurrent recovery samples for parent compound and DFEAF were spiked at levels of 0.01, 0.10 and 1.0 mg/kg (expressed in BYI 02960 equivalents). Mean recoveries in cereal crop samples (green material, grain, and straw) were 76-110%, with RSDs of the larger validations sets (n > 2, at the LOQ) of 5.6-9.2%; n = 1-4. All values were within acceptable ranges.

For DFA, concurrent recovery samples were spiked at levels of 0.02, 0.05, 0.20, 0.50, and 1.0 mg/kg (expressed in BYI 02960 equivalents). Mean recoveries were 71-108%, with RSDs (of the larger validations sets [n > 2]) of 3.0-7.1%; n = 1-3.

For 6-CNA, concurrent recovery samples were spiked at levels of 0.005, 0.05 and 0.30 mg/kg for turnips and carrots and 0.005, 0.05 and 0.50 mg/kg for cereal metraices and 0.005, 0.05, 0.30, 0.50 and 2.7 mg/kg for lettuce (expressed in BYI 02960 equivalents). Mean recoveries were 76-109%, with RSDs (of the larger validations sets [n > 2]) of 4.4-16.1%; n = 1-16.

Table 7.9-1: Concurrent recoveries for BYI 02960 residues in rotational crop matrices (carrots, turnips and barley)

Study No. Trial No. / GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
10-2503 (10-2503-01), (10-2503-02) and (10-2503-04) Plots T-1A to T-3A GLP: yes 2010	carrot	root	BYI 02960	6	0.01	90;99;110;111;120;120	90	120	108	11.0
				1	0.10	87	87	87	87	
				2	0.50	99;107	99	107	103	
				1	1.0	80	80	80	80	
				10	overall				102	13.4
			DFA	6	0.02	91;99;109;114;114;114	91	114	107	9.1
				1	0.20	86	86	86	86	
				2	0.50	97;97	97	97	97	
				1	1.0	84	84	84	84	
				10	overall				101	11.6
			DFEAF	6	0.01	90;92;106;113;118;119	90	119	106	12.0
				1	0.10	95	95	95	95	
				2	0.50	101;105	101	105	103	
				1	1.0	91	91	91	91	
				10	overall				103	10.7

Study No. Trial No. / GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
			6-CNA	6	0.005	84; 89; 98; 106; 108; 110	84	110	99	11
				1	0.05	92	-	-	-	-
				2	0.30	106; 111	106	111	109	-
				1	0.50	82	-	-	-	-
				10	overall				99	11.2
10-2503 (10-2503-03) Plots T-1A to T-3A GLP: yes 2010	turnip, edible	body	BYI 02960	3	0.01	77;95;100	77	100	91	13.3
				1	0.10	74	74	74	74	
				1	0.50	92	92	92	92	
				5	overall				88	13.1
			DFA	3	0.02	83;112;115	83	115	103	17.1
				1	0.20	85	85	85	85	
				1	0.50	86	86	86	86	
				5	overall				96	16.5
			DFEAF	3	0.01	84;105;116	84	116	102	16.0
				1	0.10	97	97	97	97	
				1	0.50	99	99	99	99	
				5	overall				100	11.7
			6-CNA	3	0.005	83; 111; 112	83	112	102	16.1
				1	0.05	95	-	-	-	-
				1	0.30	93	-	-	-	-
				4	overall				99	12.6
		leaf	BYI 02960	3	0.01	94;104;108	94	108	102	7.1
				1	0.10	81	81	81	81	
				1	0.50	103	103	103	103	
				5	overall				98	11.0
			DFA	3	0.02	100;113;115	100	115	109	7.4
				1	0.20	87	87	87	87	
				1	0.50	88	88	88	88	
				5	overall				101	13.2
			DFEAF	3	0.01	90;99;108	90	108	99	9.1
				1	0.10	92	92	92	92	
				1	0.50	99	99	99	99	
				5	overall				98	7.3
			6-CNA	6	0.005	88; 98; 102	88	102	96	7.5
				1	0.05	93	-	-	-	-
				2	0.30	96	-	-	-	-
				1	overall				95	5.5
10-2503 (10-2503-01) to (10-2503-04)	barley	green material	BYI 02960	2	0.01	92;104	92	104	98	
				1	0.10	88	88	88	88	
				1	1.0	85	85	85	85	
				4	overall				92	9.0

Study No. Trial No. / GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
Plots T-1C to T-3C GLP: yes 2010			DFA	2	0.02	88;97	88	97	93	
				1	0.20	87	87	87	87	
				1	1.0	82	82	82	82	
				4	overall				89	7.1
			DFEAF	2	0.01	91;93	91	93	92	
				1	0.10	99	99	99	99	
				1	1.0	95	95	95	95	
				4	overall				95	3.6
			6-CNA	2	0.005	79; 88	79	88	84	
				1	0.05	96	-	-	-	-
				1	0.50	90	-	-	-	-
				1	overall				88	8.0
		grain	BYI 02960	4	0.01	86;91;98;89	86	98	91	5.6
				2	0.10	90;88	88	90	89	
				1	1.0	105	105	105	105	
				7	overall				92	7.3
			DFA	3	0.02	94;99;99	94	99	97	3.0
				1	0.05	82	82	82	82	
				1	0.20	72	72	72	72	
				1	0.50	74	74	74	74	
				1	1.0	71	71	71	71	
				7	overall				84	15.0
			DFEAF	4	0.01	92;95;95;82	82	95	91	6.8
				2	0.10	92;90	90	92	91	
				1	1.0	110	110	110	110	
				7	overall				94	9.0
			6-CNA	4	0.005	81; 85; 89; 90	81	90	86	4.8
				2	0.05	91; 89	89	92	90	-
				1	0.50	82	-	-	-	-
				7	overall				87	4.6
		straw	BYI 02960	4	0.01	95;100;116;98	95	116	102	9.2
				2	0.10	65;92	65	92	79	
				1	1.0	76	76	76	76	
				7	overall				92	18.2
			DFA	3	0.02	101;106;116	101	116	108	7.1
				1	0.05	95	95	95	95	
				1	0.20	66	66	66	66	
				1	0.50	95	95	95	95	
				1	1.0	71	71	71	71	
				7	overall				93	19.6
			DFEAF	4	0.01	81;83;96;80	80	96	85	8.8
				2	0.10	71;95	71	95	83	

Study No. Trial No. / GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
				1	1.0	82	82	82	82	
				7	overall				84	10.5
			6-CNA	4	0.005	69; 73; 83; 78	69	83	73	8.0
				2	0.05	64; 93	64	93	79	-
				1	0.50	77	-	-	-	-
				6	overall				77	12.4
10-2503 (10-2503-01) to (10-2503-04) Plots T-1B to T-3B GLP: yes 2010	lettuce	head	BYI 02960	15	0.01	79;87;102;106; 107109;110;116;92; 97;107;108;114; 116;117	79	117	104	10.7
				5	0.10	88;90;90;92;93	88	93	91	2.2
				2	0.50	103;106	103	106	105	
				2	1.0	92;94	92	94	93	
				2	5.0	90;98	90	98	94	
				26	overall				100	10.5
			DFA	12	0.02	90;93;94;95;97; 112;112;116;86; 89;93;95	86	116	98	10.2
				3	0.05	90;94;98	90	98	94	4.3
				2	0.20	92;94	92	94	93	
				5	0.50	93;101;90;91;92	90	101	93	4.7
				2	1.0	90;92	90	92	91	
				2	5.0	90;89	89	90	90	
				26	overall				95	7.9
			DFEAF	15	0.01	87;93;95;100;100;104;105;107;83;8386;88;90;92;96	83	107	94	8.4
				5	0.10	85;98;97;97;99	85	99	95	6.1
				2	0.50	97;109	97	109	103	
				2	1.0	86;101	86	101	94	
				2	5.0	97;96	96	97	97	
				26	overall				95	7.7
			6-CNA	15	0.005	65; 81; 91; 92; 101; 104; 104; 107; 86; 89; 91; 92; 94; 103; 104	65	107	94	11.8
				5	0.05	98; 104; 93; 95; 95	93	104	97	4.4
				2	0.30	102; 108	102	108	105	-
				2	0.50	91; 109	91	109	100	-
				2	2.7	100; 103	100	103	102	-
				26	overall				96	10.0

Root crops:

All trial data are summarised in table 7.9-2.

The total residues of BYI 02960 (parent compound plus DFA and DFEAF) in the harvested roots of carrots or turnips were generally highest in the first rotation, i.e. after the shortest plant-back interval (PBI) of 25-30 days, when they ranged at an "intermediate" growth stage (including "early harvest", BBCH 47-49) from 0.05-0.14 mg/kg, and at typical harvest ripeness (BBCH 49) from 0.05-0.12 mg/kg. In one trial, however, the residues in the 3rd and final rotation (PBI 284 days) were slightly higher than in the first rotation, at 0.06 mg/kg compared to 0.05 mg/kg. Thus, highest residues in roots in the entire study ranged from 0.06-0.14 mg/kg (median 0.08 mg/kg).
Residues of 6-CNA were <0.005 mg/kg in all treated samples.

By the third rotation, residues in harvestable roots ranged from <0.04-0.06 mg/kg, with a median value of 0.05 mg/kg.

In turnip tops (=leaves; one trial only), highest residue levels were seen in the first rotation (0.24 mg/kg), but these were not significantly different from those seen in the third rotation (0.21 mg/kg).

Leafy crops:
All trial data are summarised in table 7.9-3.

The total residues of BYI 02960 (parent compound plus DFA and DFEAF) in the harvested heads of lettuce were highest in the first rotation, i.e. after the shortest plant-back interval (PBI) of 25-30 days, when they ranged at an "intermediate" growth stage (from BBCH 41-47, including "early harvest", BBCH 46-47) from 0.06-0.21 mg/kg, and at typical harvest ripeness (BBCH 49) from <0.04-0.16 mg/kg. Thus, highest residues in "marketable" heads in the entire study ranged from <0.04-0.16 mg/kg, with a median value of 0.08 mg/kg.
Residues of 6-CNA were <0.005 mg/kg in all treated samples.

By the third rotation, total residues in lettuce heads ranged from <0.04-0.10 mg/kg (median <0.04 mg/kg).

Cereal crops:
All trial data are summarised in table 7.9-4.

The total residues of BYI 02960 (parent compound plus DFA and DFEAF) in the harvested barley grain were highest in the first rotation, i.e. after the shortest plant-back interval (PBI) of 25-33 days, when they ranged from 0.11-0.65 mg/kg (median 0.35 mg/kg). In one trial, however, no grain samples could be taken due to damage by geese (trial 10-2503-02 1st rotation, Netherlands). Based on the

data from straw, it is clear that the missing trial would have yielded residues well below the highest residues and probably below the current median. Residues in straw from the first rotation ranged from <0.07-0.39 mg/kg, with a median value of 0.12 mg/kg. (The level of residues in straw in the Dutch trial were the lowest of any trial.)
Residues of 6-CNA were <0.005 mg/kg in all grain and green material samples. In the straw samples from the SEU trials, residues declined from 0.01 mg/kg at 218 DALT to 0.006 mg/kg at 419 DALT in the French trial and from 0.02 mg/kg at 233 DALT to <0.005 mg/kg at 363 DALT.

By the third rotation, residues in grain ranged from 0.08-0.39 mg/kg, with a median value of 0.12 mg/kg. In straw, they had dropped to <0.07-0.19 mg/kg (median <0.07 mg/kg).
Early in the respective growing periods of each rotation, samples were also taken of green material (forage). Again, residues were highest in the first rotation, in which they ranged from 0.05-0.41 mg/kg, with a median of 0.10 mg/kg. (Residue levels in green material in the Dutch trial were 0.06 mg/kg, below the median and the 2nd-lowest value in this study.)

Conclusion

In order to support the use in the EU of BYI 02960 in non-perennial crops, four multi-plantback multi-crop rotational crop trials were conducted in Europe (2 each in the northern and southern residue regions) in the years 2010-2011. BYI 02960 was applied once as an SL 200 formulation either to bare soil or to a target crop (lettuce) at an active substance rate of 200 g/ha, the target crop was then harvested, and crops representing 3 different botanical groups (roots, leafy veg., cereals) were planted on the plots at 3 intervals thereafter. All applications were at the required rates, and all trials were conducted according to GLP.

To evaluate the potential residues in following crops, samples of the rotated crops were taken at an intermediate stage and at usual full harvest ripeness. Samples were analyzed for the relevant residues of BYI 02960, comprising the parent compound and its metabolites DFA and DFEAF. The residues of all three analytes were summed to yield a calculated "total residue of BYI 02960". The results of the trials presented above demonstrate that:

- total residues of BYI 02960 in all rotational crops tended to be highest in the first, earliest rotation, i.e. grown after a plant-back interval (PBI) of 25-33 days.
- highest total residues of BYI 02960 in rotational root crops (here: carrot and turnip roots) ranged from 0.05-0.14 mg/kg (median 0.06 mg/kg; n=4). In one trial, residues were also determined in the leaves; the highest measured value was in the first rotation, at 0.24 mg/kg.
- highest total residues of BYI 02960 in marketable rotational leafy crops (here: lettuce) ranged from <0.04-0.16 mg/kg, with a median value of 0.07 mg/kg (n=4).
- Residues of 6-CNA were <0.005 mg/kg in all treated samples, except for barley straw grown in Southern Europe, with a maximum residue level of 0.02 mg/kg in the first rotation.

– in rotated cereals (here: barley), the highest total residues of BYI 02960 in grain ranged from 0.11-0.65 mg/kg. The median value here was 0.35 mg/kg, but only 3 trials could be evaluated as geese ate the grain in the 4th trial. Samples were also taken of the fodder-relevant commodities green material and straw. Residues in straw at harvest were <0.07-0.39 mg/kg (median 0.12 mg/kg; n=4), and in green material taken earlier in the rotation they ranged from 0.05-0.41 mg/kg (median 0.10 mg/kg; n=4).

Table 7.9-2: Summary of field rotational crop trials with root crops

Report-No. Location incl. Postal code and date	Soil or target Crop /Variety	Application rate per treatment			Date of treatment	Growth stage at last treatment or date	Rotational Crop, Variety rotation information	Growth stage at sampling	Portion analysed	Residues (mg/kg) expressed as BYI 02960					DALT (days)	Remarks
		kg a.s./ha	Water l/ha	kg a.s./hl						BYI 02960	DFA as BYI 02960	DFEAF	DFA as DFA	Total BYI 02960 + DFA, as BYI 02960		
10-2503-01-T-1A Germany 51399 Burscheid EU-N 2010	soil	0.20	300	0.067	12/04/2010		Carrot, Cestas F1 (Rotation 1) PBI 25 days	47 49	root	<0.01 <0.01	0.03 0.03	<0.01 <0.01	0.010 0.010	0.04 0.04	95 109	
10-2503-01-T-2A Germany 51399 Burscheid EU-N 2010	lettuce Argentines, Butterhead variety	0.20	300	0.067	28/05/2010	19	Carrot, Cestas F1 (Rotation 2) PBI 70 days	48 49	root	<0.01 <0.01	<0.02 <0.02	<0.01 <0.01	<0.02 <0.02	<0.03 <0.03	150 164	
10-2503-01-T-3A Germany 51399 Burscheid EU-N 2010	lettuce Argentines, Butterhead variety	0.20	300	0.067	28/05/2010	19	Carrot, Cestas (Rotation 3) PBI 284 days	48 49	root	<0.01 <0.01	0.03 0.04	<0.01 <0.01	0.010 0.013	0.04 0.05	396 410	

Report-No. Location incl. Postal code and date	Soil or target Crop /Variety	Application rate per treatment			Date of treatment	Growth stage at last treatment or date	Rotational Crop, Variety rotation information	Growth stage at sampling	Portion analysed	Residues (mg/kg) expressed as BYI 02960					DALT (days)	Remarks
		kg a.s./ha	Water l/ha	kg a.s./hl						BYI 02960	DFA as BYI 02960	DFEAF	DFA as DFA	Total BYI 02960 + DFA, as BYI 02960		
10-2503-02-T-1A Netherlands 1681 ND Zwaagdijk EU-N 2010	soil	0.20	400	0.050	10/05/2010		Carrot, Nerja (Rotation 1) PBI 25 days	48 49	root	<0.01 <0.01	0.03 0.05	<0.01 <0.01	0.010 0.017	0.04 0.06	92 106	
10-2503-02-T-2A Netherlands 1681 ND Zwaagdijk EU-N 2010	lettuce Gisela, Butterhead variety	0.20	400	0.050	27/05/2010	19	Carrot, Nerja (Rotation 2) PBI 61 days	48 49	root	<0.01 <0.01	0.03 0.03	<0.01 <0.01	0.010 0.010	0.04 0.04	151 165	
10-2503-02-T-3A Netherlands 1681 ND Zwaagdijk EU-N 2010	lettuce Gisela, Butterhead variety	0.20	400	0.050	27/05/2010	19	Carrot, Nerja (Rotation 3) PBI 328 days	47 49	root	<0.01 <0.01	<0.02 <0.02	<0.01 <0.01	<0.02 <0.02	<0.03 <0.03	406 420	

Report-No. Location incl. Postal code and date	Soil or target Crop /Variety	Application rate per treatment			Date of treatment	Growth stage at last treatment or date	Rotational Crop, Variety rotation information	Growth stage at sampling	Portion analysed	Residues (mg/kg) expressed as BYI 02960					DALT (days)	Remarks
		kg a.s./ha	Water l/ha	kg a.s./hl						BYI 02960	DFA as BYI 02960	DFEAF	DFA as DFA	Total BYI 02960 + DFA, as BYI 02960		
10-2503-03-T-1A France 31200 Toulouse EU-S 2010	soil	0.20	400	0.050	23/07/2010		Turnip, edible Aramis (Rotation 1) PBI 25 days	49 49 49 49	body leaf	<0.01 <0.01 0.03 0.02	0.12 0.10 0.20 0.07	<0.01 <0.01 <0.01 <0.01	0.040 0.033 0.066 0.023	0.13 0.11 0.023 0.09	82 96 82 96	
10-2503-03-T-2A France 31200 Toulouse EU-S 2010	lettuce Pitice, Loose leaf	0.20	400	0.050	08/06/2010	19	Turnip, edible Aramis (Rotation 2) PBI 70 days	49 49 49 49	body leaf	<0.01 <0.01 0.02 <0.01	0.05 0.04 0.08 0.03	<0.01 <0.01 <0.01 <0.01	0.017 0.013 0.027 0.010	0.06 0.05 0.10 0.04	127 141 127 141	
10-2503-03-T-3A France 31200 Toulouse EU-S 2010	lettuce Pitice, Loose leaf	0.20	400	0.050	08/06/2010	19	Carrot, Turnip, edible Aramis (Rotation 3) PBI 314 days	47 47 49 49	body leaf	<0.01 <0.01 0.03 0.04	0.04 0.03 0.11 0.16	<0.01 <0.01 <0.01 <0.01	0.013 0.010 0.037 0.053	0.05 0.04 0.14 0.20	380 394 380 394	

Report-No. Location incl. Postal code and date	Soil or target Crop /Variety	Application rate per treatment			Date of treatment	Growth stage at last treatment or date	Rotational Crop, Variety rotation information	Growth stage at sampling	Portion analysed	Residues (mg/kg) expressed as BYI 02960					DALT (days)	Remarks
		kg a.s./ha	Water l/ha	kg a.s./hl						BYI 02960	DFA as BYI 02960	DFEAF	DFA as DFA	Total BYI 02960 + DFA, as BYI 02960		
10-2503-04-T-1A Spain 08520 Llerona EU-S 2010	soil	0.20	300	0.067	08/03/2010		Carrot, Coral Nantesa (Rotation 1) PBI 30 days	47 49	root	<0.01 <0.01	0.05 0.06	<0.01 <0.01	0.017 0.020	0.06 0.07	100 114	
10-2503-04-T-2A Spain 08520 Llerona EU-S 2010	lettuce Maravilla, Butterroot	0.212	318	0.067	02/07/2010	19	Carrot, Coral Nantesa (Rotation 2) PBI 145 days	49 49	root	<0.01 <0.01	0.02 0.03	<0.01 <0.01	0.007 0.010	0.03 0.04	314 328	
10-2503-04-T-3A Spain 08520 Llerona EU-S 2010	lettuce Maravilla, Butterroot	0.20	300	0.067	02/07/2010	19	Carrot, Coral Nantesa (Rotation 3) PBI 279 days	47 49	root	<0.01 <0.01	<0.02 0.03	<0.01 <0.01	<0.02 0.01	<0.03 <0.04	349 363	

DFA = Difluoroacetic acid
DFEAF Difluoroethyl-amino-furanone
Total residu of BYI 02960 Calc.

Table 7.9-3: Summary of field rotational crop trials with leafy crops

Report-No. Location incl. Postal code and date	Soil or target Crop /Variety	Application rate per treatment			Date of treatment	Growth stage at last treatment or date	Rotational Crop, Variety rotation information	Growth stage at sampling	Portion analysed	Residues (mg/kg) expressed as BYI 02960					DALT (days)	Remarks
		kg a.s./ha	Water l/ha	kg a.s./hl						BYI 02960	DFA as BYI 02960	DFEAF	DFA as DFA	Total BYI 02960 + DFA, as BYI 02960		
10-2503-01-T-1B Germany 51399 Burscheid EU-N 2010	soil	0.20	300	0.067	12/04/2012		Lettuce, Argentines, Butterhead variety (Rotation 1) PBI 25 days	46 49	head	0.01 <0.01	0.06 0.05	<0.01 <0.01	0.02 0.017	0.07 0.06	63 77	
10-2503-01-T-2B Germany 51399 Burscheid EU-N 2010	lettuce Argentines, Butterhead variety	0.20	300	0.067	28/05/2012	19	Lettuce, Argentines, Butterhead variety (Rotation 2) PBI 77 days	46 49	head	<0.01 <0.01	<0.02 <0.02	<0.01 <0.01	<0.02 <0.02	<0.03 <0.03	124 138	
10-2503-01-T-3B Germany 51399 Burscheid EU-N 2010	lettuce Argentines, Butterhead variety	0.20	300	0.067	28/05/2012	19	Lettuce, Aleppo, Loose leaf variety (Rotation 3) PBI 320 days	46 49	head	<0.01 <0.01	<0.02 <0.02	<0.01 <0.01	<0.02 <0.02	<0.03 <0.03	354 368	

Report-No. Location incl. Postal code and date	Soil or target Crop /Variety	Application rate per treatment			Date of treatment	Growth stage at last treatment or date	Rotational Crop, Variety rotation information	Growth stage at sampling	Portion analysed	Residues (mg/kg) expressed as BYI 02960					DALT (days)	Remarks
		kg a.s./ha	Water l/ha	kg a.s./hl						BYI 02960	DFA as BYI 02960	DFEAF	DFA as DFA	Total BYI 02960 + DFA, as BYI 02960		
10-2503-02-T-1B Netherlands 1681 ND Zwaagdijk EU-N 2010	soil	0.20	400	0.050	07/06/2010		Lettuce, Lucan, Butterhead variety (Rotation 1) PBI 25 days	41 49	head	0.03 <0.01	<0.02 <0.02	<0.01 <0.01	<0.02 <0.02	0.05 <0.03	46 60	
10-2503-02-T-2B Netherlands 1681 ND Zwaagdijk EU-N 2010	lettuce Gisela, Butterhead variety	0.20	400	0.050	27/05/2010	19	Lettuce, Lucan, Butterhead variety (Rotation 2) PBI 64 days	46 49	head	<0.01 <0.01	<0.02 <0.02	<0.01 <0.01	<0.02 <0.02	<0.03 <0.03	95 109	
10-2503-02-T-3B Netherlands 1681 ND Zwaagdijk EU-N 2010	lettuce Gisela, Butterhead variety	0.20	400	0.050	27/05/2010	19	Lettuce, Lucan, Butterhead variety (Rotation 3) PBI 329 days	45 49	head	<0.01 <0.01	<0.02 <0.02	<0.01 <0.01	<0.02 <0.02	<0.03 <0.03	358 372	

Report-No. Location incl. Postal code and date	Soil or target Crop /Variety	Application rate per treatment			Date of treatment	Growth stage at last treatment or date	Rotational Crop, Variety rotation information	Growth stage at sampling	Portion analysed	Residues (mg/kg) expressed as BYI 02960					DALT (days)	Remarks
		kg a.s./ha	Water l/ha	kg a.s./hl						BYI 02960	DFA as BYI 02960	DFEAF	DFA as DFA	Total BYI 02960 + DFA, as BYI 02960		
10-2503-03-T-1B France 31200 Toulouse EU-S 2010	soil	0.20	400	0.050	14/04/2010		Lettuce, Pitice, Loose leaf (Rotation 1) PBI 28 days	41 49	head	0.08 0.03	0.12 0.11	<0.01 <0.01	0.040 0.037	0.20 0.14	55 69	
10-2503-03-T-2B France 31200 Toulouse EU-S 2010	lettuce Pitice, Loose leaf	0.20	400	0.050	08/06/2010	19	Lettuce, Pitice, Loose leaf (Rotation 2) PBI 70 days	46 49	head	0.03 0.01	0.08 0.09	<0.01 <0.01	0.027 0.030	0.11 0.10	107 121	
10-2503-03-T-3B France 31200 Toulouse EU-S 2010	lettuce Pitice, Loose leaf	0.20	400	0.050	08/06/2010	19	Lettuce, Pitice, Loose leaf (Rotation 3) PBI 314 days	46 49	head	0.02 <0.01	0.08 0.05	<0.01 <0.01	0.027 0.017	0.10 0.06	357 371	

Report-No. Location incl. Postal code and date	Soil or target Crop /Variety	Application rate per treatment			Date of treatment	Growth stage at last treatment or date	Rotational Crop, Variety rotation information	Growth stage at sampling	Portion analysed	Residues (mg/kg) expressed as BYI 02960					DALT (days)	Remarks
		kg a.s./ha	Water l/ha	kg a.s./hl						BYI 02960	DFA as BYI 02960	DFEAF	DFA as DFA	Total BYI 02960 + DFA, as BYI 02960		
10-2503-04-T-1B Spain 08520 Llerona EU-S 2010	soil	0.20	300	0.067	08/03/2010		Lettuce, Murai, (leafy variety) (Rotation 1) PBI 30 days	47 49	head	0.03 0.01	0.03 0.03	<0.01 <0.01	0.010 0.010	0.06 0.04	72 85	
10-2503-04-T-2B Spain 08520 Llerona EU-S 2010	lettuce Maravilla, Butterroot	0.212	318	0.067	02/07/2010	19	Lettuce, Pelican, Butterhead (Rotation 2) PBI 145 days	48 49	head	<0.01 <0.01	<0.02 <0.02	<0.01 <0.01	<0.02 <0.02	<0.03 <0.03	291 305	
10-2503-04-T-3B Spain 08520 Llerona EU-S 2010	lettuce Maravilla, Butterroot	0.20	300	0.067	02/07/2010	19	Lettuce, Murai (leafy variety) (Rotation 3) PBI 279 days	47 49	head	<0.01 <0.01	<0.02 <0.02	<0.01 <0.01	<0.02 <0.02	<0.03 <0.03	321 334	

DFA = Difluoroacetic acid
DFEAF Difluoroethyl-amino-furanone
Total residu of BYI 02960 Calc.

Table 7.9-4: Summary of field rotational crop trials with cereal crops

Report-No. Location incl. Postal code and date	Soil or target Crop /Variety	Application rate per treatment			Date of treatment	Growth stage at last treatment or date	Rotational Crop, Variety rotation information	Growth stage at sampling	Portion analysed	Residues (mg/kg) expressed as BYI 02960					DALT (days)	Remarks
		kg a.s./ha	Water l/ha	kg a.s./hl						BYI 02960	DFA as BYI 02960	DFEAF	DFA as DFA	Total BYI 02960 + DFA, as BYI 02960		
10-2503-01-T-1C Germany 51399 Burscheid EU-N 2010	soil	0.20	300	0.067	12/04/2012		Barley, Simba (Rotation 1) PBI 25 days	29	green material	0.01	0.11	<0.01	0.037	0.12	56	
								89	grain	<0.01	0.33	<0.01	0.110	0.34	116	
								89	straw	0.02	0.13	<0.01	0.043	0.15	116	
10-2503-01-T-2C Germany 51399 Burscheid EU-N 2010	lettuce Argentines, Butterhead variety	0.20	300	0.067	28/05/2012	19	Barley, Leibnitz (Rotation 2) PBI 116 days	29	green material	<0.01	0.03	<0.01	0.010	0.04	305	
								89	grain	<0.01	0.10	<0.01	0.033	0.11	406	
								89	straw	<0.01	<0.05	<0.01	<0.05	<0.06	406	
10-2503-01-T-3C Germany 51399 Burscheid EU-N 2010	lettuce Argentines, Butterhead variety	0.20	300	0.067	28/05/2012	19	Barley, Simba (Rotation 3) PBI 284 days	29	green material	<0.01	<0.02	<0.01	<0.02	<0.03	333	
								89	grain	<0.01	0.10	<0.01	0.033	0.11	420	
								89	straw	<0.01	<0.05	<0.01	<0.05	<0.06	420	
10-2503-02-T-1C Netherlands	soil	0.20	400	0.050	08/09/2010		Barley, Cervoise	30	green material	<0.01	0.04	<0.01	0.013	0.06	226	
								89	straw	<0.01	<0.05	<0.01	<0.05	<0.06	329	

Report-No. Location incl. Postal code and date	Soil or target Crop /Variety	Application rate per treatment			Date of treatment	Growth stage at last treatment or date	Rotational Crop, Variety rotation information	Growth stage at sampling	Portion analysed	Residues (mg/kg) expressed as BYI 02960					DALT (days)	Remarks
		kg a.s./ha	Water l/ha	kg a.s./hl						BYI 02960	DFA as BYI 02960	DFEAF	DFA as DFA	Total BYI 02960 + DFA, as BYI 02960		
1681 ND Zwaagdijk EU-N 2010							(Rotation 1) PBI 33 days									
10-2503-02-T-2C Netherlands 1681 ND Zwaagdijk EU-N 2010	lettuce Gisela, Butterhead variety	0.20	400	0.050	27/05/2010	19	Barley, Cervoise (Rotation 2) PBI 137 days	30	green material	<0.01	0.03	<0.01	0.010	0.04	330	
								89	grain	<0.01	0.04	<0.01	0.013	0.05	446	
								89	straw	<0.01	<0.05	<0.01	<0.05	<0.06	446	
10-2503-02-T-3C Netherlands 1681 ND Zwaagdijk EU-N 2010	lettuce Gisela, Butterhead variety	0.20	400	0.050	27/05/2010	19	Barley, Tripple (Rotation 3) PBI 295 days	30	green material	<0.01	0.02	<0.01	0.007	0.03	350	
								89	grain	<0.01	0.06	<0.01	0.020	0.07	459	
								89	straw	<0.01	<0.05	<0.01	<0.05	<0.06	459	
10-2503-03-T-1C France 31200 Toulouse EU-S 2010	soil	0.20	300	0.067	23/11/2010		Barley, Attraction (Rotation 1) PBI 27 days	30	green material	0.02	0.37	<0.01	0.123	0.39	126	
								89	grain	<0.01	0.63	<0.01	0.209	0.64	218	
								89	straw	0.04	0.34	<0.01	0.113	0.38	218	

Report-No. Location incl. Postal code and date	Soil or target Crop /Variety	Application rate per treatment			Date of treatment	Growth stage at last treatment or date	Rotational Crop, Variety rotation information	Growth stage at sampling	Portion analysed	Residues (mg/kg) expressed as BYI 02960					DALT (days)	Remarks
		kg a.s./ha	Water l/ha	kg a.s./hl						BYI 02960	DFA as BYI 02960	DFEAF	DFA as DFA	Total BYI 02960 + DFA, as BYI 02960		
10-2503-03-T-2C France 31200 Toulouse EU-S 2010	lettuce Pitice, Loose leaf	0.20	300	0.067	08/06/2010	19	Barley, Attraction (Rotation 2) PBI 195 days	30	green material	0.01	0.10	<0.01	0.033	0.11	294	
								89	grain	<0.01	0.26	<0.01	0.086	0.27	386	
								89	straw	0.02	0.10	<0.01	0.033	0.12	386	
10-2503-03-T-3C France 31200 Toulouse EU-S 2010	lettuce Pitice, Loose leaf	0.20	300	0.067	08/06/2010	19	Barley, Attraction (Rotation 3) PBI 290 days	29	green material	0.03	0.15	<0.01	0.050	0.18	324	rainfall within 6h after application (total of 17 mm within 24 h)
								89	grain	<0.01	0.37	<0.01	0.123	0.38	419	
								89	straw	<0.01	0.17	<0.01	0.056	0.18	419	
10-2503-04-T-1C Spain 08520 Llerona EU-S 2010	soil	0.20	300	0.067	26/10/2010		Barley, Graphic (Rotation 1) PBI 27 days	32	green material	<0.01	0.03	<0.01	0.010	0.04	133	
								89	grain	<0.01	0.09	<0.01	0.030	0.10	233	
								89	straw	0.01	0.05	<0.01	0.017	0.06	233	
10-2503-04-T-2C Spain 08520 Llerona EU-S 2010	lettuce Maravilla, Butterroot	0.212	318	0.067	02/07/2010	19	Barley, Graphic (Rotation 2) PBI 143 days	32	green material	<0.01	0.03	<0.01	0.01	0.04	249	
								89	grain	<0.01	0.09	<0.01	0.03	0.10	349	
								89	straw	<0.01	<0.05	<0.01	<0.05	<0.06	349	

Report-No. Location incl. Postal code and date	Soil or target Crop /Variety	Application rate per treatment			Date of treatment	Growth stage at last treatment or date	Rotational Crop, Variety rotation information	Growth stage at sampling	Portion analysed	Residues (mg/kg) expressed as BYI 02960					DALT (days)	Remarks
		kg a.s./ha	Water l/ha	kg a.s./hl						BYI 02960	DFA as BYI 02960	DFEAF	DFA as DFA	Total BYI 02960 + DFA, as BYI 02960		
10-2503-04-T-3C Spain 08520 Llerona EU-S 2010	lettuce Maravilla, Butterroot	0.20	300	0.067	02/07/2010	19	Barley, Graphic (Rotation 3) PBI 266 days	31	green material	<0.01	0.02	<0.01	0.007	0.03	306	
								89	grain	<0.01	0.09	<0.01	0.03	0.10	363	
								89	straw	<0.01	<0.05	<0.01	<0.05	<0.06	363	

DFA = Difluoroacetic acid
DFEAF Difluoroethyl-amino-furanone
Total residu of BYI 02960 Calc.

STUDY 2

reference	:	Schulte, G. 2012b KIIA 6.6.3.1.2/01	GLP statement	:	yes
Type of study	:	Rotational crop - Field trial	Guideline	:	EU Council Directive 91/414/EEC amended by Commission Directive 96/68/EC EU Guidance Working Document 7029/VI/95 rev. 5 OECD Guideline for the Testing of Chemicals No. 504, Residues in Rotational Crops
Year of execution	:	2011	Dose	:	0.125 kg as/ha
test substance	:	BYI 02960 SL 200 formulation Batch no: 2010-007173	LOQ	:	BYI 02960 : 0.01 mg/kg DFA: 0.02 mg/kg DFEAF : 0.01 mg/kg
Species	:	potato	storage Acceptability	: :	81-200d months at -18°C Yes

Study design

A rotational crop program was run for potatoes at 4 locations in 2011 (2 in Northern and 2 in Southern Europe). BYI 02960 was applied 2 times at a level of 125g ai/ha to bare soil, with interval 9-10d. Potatoes were planted ~~30~~ 25-33 DAT and harvested 98-137 DAT. Samples were stored frozen until analysis during 81-200d at temperatures ≤ -18°C.

Residues of BYI 02960 and its metabolites DFA (difluoroacetic acid) and BYI 02960-difluoroethyl-aminofuranone (DFEAF) were determined according to method 01304. Residues of BYI 02960 and its metabolites difluoroacetic acid and BYI 02960-difluoroethylaminofuranone were extracted from the sample materials (approx. 5 g) twice using a mixture of acetonitrile/water (4/1, v/v) with 2.2 mL/L formic acid. The crude extract was cleaned up using a Varian Bond Elute C18 solid phase extraction. After elution of the residues, the extract was mixed with reconstitution solution and an isotopically labelled internal standard solution was added. The residues were quantified by HPLC-MS/MS.

Afterwards the total residue of BYI 02960 was calculated by summing up the individual residues of BYI 02960, difluoroacetic acid and BYI 02960 difluoroethylaminofuranone (each analyte expressed as parent).

Conclusion

Concurrent recoveries have been determined along with the analysis of the processing samples (Table 7.9-3). Recoveries have been determined in tubers and in have been found to be all in the required range of 70-110% ± 20%.

The LOQ of analytical Method 01304 for BYI 02960 parent and BYI 02960-difluoroethylaminofuranone (DFEAF) was 0.01 mg/kg and 0.02 mg/kg for DFA in potato tuber.

Residues have been determined for BYI 02960 parent, DFA and BYI 02960-difluoroethyl-aminofuranone in tubers at harvest. Residues are reported in Table 7.9-4. Residues in control

samples were all < LOQ (results not shown). Residues of BYI 02960 parent and BYI 02960-difluoroethylaminofuranone were always <0.01 mg/kg. Instead, only residues of DFA were found at levels of 0.028-0.25 mg/kg (parent equivalent). Thus, in rotational potatoes sampled at harvest, the total residues of BYI 02960 in tubers ranged from 0.048-0.27 mg/kg, with a median value of 0.13 mg/kg (n=4).

STUDY 3

reference	:	Schulte, G. 2012c KIIA 6.6.3.1.3/01	GLP statement	:	yes
Type of study	:	Rotational crop - Field trial	Guideline	:	EU Council Directive 91/414/EEC amended by Commission Directive 96/68/EC EU Guidance Working Document 7029/VI/95 rev. 5 OECD Guideline for the Testing of Chemicals No. 504, Residues in Rotational Crops
Year of execution	:	2011	Dose	:	0.125 kg as/ha
test substance	:	BYI 02960 SL 200 formulation Batch no: 2010-007173	LOQ	:	BYI 02960 : 0.01 mg/kg DFA: 0.02 mg/kg DFEAF : 0.01 mg/kg
Species	:	leek	storage Acceptability	: :	61-145d at -18°C Yes

Study design

A rotational crop program was run for leek at 4 locations in 2011 (2 in Northern and 2 in Southern Europe). BYI 02960 was applied 2 times at a level of 125g ai/ha to bare soil, with interval 10-13d. Leek was planted ~~26-33~~ 97-130 DAT and harvested 97-112 DAT (BBCH49). Samples were stored frozen until analysis during 61-145d at temperatures ≤ -18°C.

Residues of BYI 02960 and its metabolites DFA (difluoroacetic acid) and BYI 02960-difluoroethyl-aminofuranone were determined according to method 01304 (also called Method RV-001-P10-02). Residues of BYI 02960 and its metabolites difluoroacetic acid and BYI 02960-difluoroethylaminofuranone were extracted from the sample materials (approx. 5 g) twice using a mixture of acetonitrile/water (4/1, v/v) with 2.2 mL/L formic acid. The crude extract was cleaned up using a Varian Bond Elute C18 solid phase extraction. After elution of the residues, the extract was mixed with reconstitution solution and an isotopically labelled internal standard solution was added. The residues were quantified by HPLC-MS/MS.

Afterwards the total residue of BYI 02960 was calculated by summing up the individual residues of BYI 02960, DFA and BYI 02960 difluoroethylaminofuranone (each analyte expressed as parent).

Conclusion

Concurrent recoveries have been determined along with the analysis of the processing samples (Table 7.9-3). Recoveries have been determined in leek shafts (whole plant without root) and have been found to be all in the required range of 70-110% ± 20%.

The LOQ of analytical Method 01304 for BYI 02960 parent and BYI 02960-difluoroethylaminofuranone was 0.01 mg/kg and 0.02 mg/kg for DFA in leek.

Residues have been determined for BYI 02960 parent, DFA and BYI 02960-difluoroethyl-aminofuranone in leek at harvest. Residues are reported in Table 7.9-4. Residues in control samples were all < LOQ (results not shown). Residues of BYI 02960 parent and BYI 02960-difluoroethyl-aminofuranone were always <0.01 mg/kg. Instead, only residues of DFA were found at levels of 0.020-0.23 mg/kg (parent equivalent). Thus, the total residues of BYI 02960 in leek (whole plant without root) ranged from 0.040-0.25 mg/kg, with a median value of 0.085 mg/kg (n=4).

STUDY 4

reference	:	Schulte, G. 2012d KIIA 6.6.3.1.4/01	GLP statement	:	yes
Type of study	:	Rotational crop - Field trial	Guideline	:	EU Council Directive 91/414/EEC amended by Commission Directive 96/68/EC EU Guidance Working Document 7029/VI/95 rev. 5 OECD Guideline for the Testing of Chemicals No. 504, Residues in Rotational Crops
Year of execution	:	2011	Dose	:	0.125 kg as/ha
test substance	:	BYI 02960 SL 200 formulation Batch no: 2010-007173	LOQ	:	BYI 02960 : 0.01 mg/kg DFA: 0.02 mg/kg DFEAF : 0.01 mg/kg
Species	:	cucumber	storage Acceptability	: :	89-117d at -18°C Yes

Study design

A rotational crop program was run for cucumber at 4 locations in 2011 (2 in Northern and 2 in Southern Europe). BYI 02960 was applied 2 times at a level of 125g ai/ha to bare soil, with interval 10d. Cucumbers were planted ~~29-36~~ 25-30 DAT and harvested 69-83 DAT (BBCH 69-83). Samples were stored frozen until analysis during 89-117d at temperatures ≤ -18°C.

Residues of BYI 02960 and its metabolites DFA (difluoroacetic acid) and BYI 02960-difluoroethyl-aminofuranone were determined according to method 01304. Residues of BYI 02960 and its metabolites difluoroacetic acid and BYI 02960-difluoroethylaminofuranone were extracted from the sample materials (approx. 5 g) twice using a mixture of acetonitrile/water (4/1, v/v) with 2.2 mL/L formic acid. The crude extract was cleaned up using a Varian Bond Elute C18 solid phase extraction. After elution of the residues, the extract was mixed with reconstitution solution and an isotopically labelled internal standard solution was added. The residues were quantified by HPLC-MS/MS.
Afterwards the total residue of BYI 02960 was calculated by summing up the individual residues of BYI 02960, difluoroacetic acid and BYI 02960 difluoroethylaminofuranone (each analyte expressed as parent).

Conclusion

Concurrent recoveries have been determined along with the analysis of the processing samples (Table 7.9-3). Recoveries have been determined in cucumber fruit and in have been found to be all in the required range of 70-110% ± 20%.

The LOQ of analytical Method 01304 for BYI 02960 parent and BYI 02960-difluoroethylaminofuranone was 0.01 mg/kg and 0.02 mg/kg for DFA in cucumber.

Residues have been determined for BYI 02960 parent, DFA and BYI 02960-difluoroethyl-aminofuranone in cucumber at harvest. Residues are reported in Table 7.9-4. Residues in control samples were all < LOQ (results not shown). Residues of BYI 02960 parent and BYI 02960-difluoroethylaminofuranone were always <0.01 mg/kg. Instead, only residues of DFA were found at levels of 0.047-0.41 mg/kg (parent equivalent). Thus, The total residues of BYI 02960 in cucumber fruit ranged from 0.067-0.43 mg/kg, with a median value of 0.32 mg/kg (n=4).

STUDY 5

reference	:	Schulte, G. 2012e KIIA 6.6.3.1.5/01	GLP statement	:	yes
Type of study	:	Rotational crop - Field trial	Guideline	:	EU Council Directive 91/414/EEC amended by Commission Directive 96/68/EC EU Guidance Working Document 7029/VI/95 rev. 5 OECD Guideline for the Testing of Chemicals No. 504, Residues in Rotational Crops
Year of execution	:	2011	Dose	:	0.125 kg as/ha
test substance	:	BYI 02960 SL 200 formulation Batch no: 2010-007173	LOQ	:	BYI 02960 : 0.01 mg/kg DFA: 0.02 mg/kg DFEAF : 0.01 mg/kg
Species	:	onion	storage	:	49-94d at -18°C
			Acceptability	:	Yes

Study design

A rotational crop program was run for onions at 4 locations in 2011 (2 in Northern and 2 in Southern Europe). BYI 02960 was applied 2 times at a level of 125g ai/ha to bare soil, with interval 10-11d. Onion was sown elsewhere and planted on the plot (Southern trials) or sown directly on the plot (Northern trials) 25-~~32~~ 33 DAT and harvested 116-175DAT (BBCH 47-49). Samples were stored frozen until analysis during 49-94d at temperatures ≤ -18°C.

Residues of BYI 02960 and its metabolites difluoroacetic acid and BYI 02960-difluoroethyl-aminofuranone were determined according to method 01304. Residues of BYI 02960 and its metabolites difluoroacetic acid and BYI 02960-difluoroethylaminofuranone were extracted from the sample materials (approx. 5 g) twice using a mixture of acetonitrile/water (4/1, v/v) with 2.2 mL/L formic acid. The crude extract was cleaned up using a Varian Bond Elute C18 solid phase extraction.

After elution of the residues, the extract was mixed with reconstitution solution and an isotopically labelled internal standard solution was added. The residues were quantified by HPLC-MS/MS.

Afterwards the total residue of BYI 02960 was calculated by summing up the individual residues of BYI 02960, DFA (difluoroacetic acid) and BYI 02960-difluoroethylaminofuranone (each analyte expressed as parent).

Conclusion

Concurrent recoveries have been determined along with the analysis of the bulb samples (Table 7.9-3). Recoveries have been determined in onion bulb and in have been found to be all in the required range of 70-110% ± 20%.

The LOQ of analytical Method 01304 for BYI 02960 parent and BYI 02960-difluoroethylaminofuranone was 0.01 mg/kg and 0.02 mg/kg for DFA in onion.

Residues have been determined for BYI 02960 parent, DFA and BYI 02960-difluoroethyl-aminofuranone in cucumber at harvest. Residues are reported in Table 7.9-4. Residues in control samples were all < LOQ (results not shown). Residues of BYI 02960 parent and BYI 02960-difluoroethylaminofuranone were always <0.01 mg/kg. Instead, only residues of DFA were found at levels of <0.02-0.16 mg/kg (parent equivalent). Thus, the total residues of BYI 02960 in onion bulb ranged from <0.04-0.18 mg/kg, with a median value of 0.085 mg/kg (n=4).

STUDY 6

reference	:	Schulte, G. 2012f KIIA 6.6.3.1.6/01	GLP statement	:	yes
Type of study	:	Rotational crop - Field trial	Guideline	:	EU Council Directive 91/414/EEC amended by Commission Directive 96/68/EC EU Guidance Working Document 7029/VI/95 rev. 5 OECD Guideline for the Testing of Chemicals No. 504, Residues in Rotational Crops
Year of execution	:	2011	Dose	:	0.125 kg as/ha
test substance	:	BYI 02960 SL 200 formulation Batch no: 2010-007173	LOQ	:	BYI 02960 : 0.01 mg/kg DFA: 0.02 mg/kg DFEAF : 0.01 mg/kg
Species	:	French bean	storage Acceptability	: :	76-114d at -18°C Yes

Study design

A rotational crop program was run for french beans at 4 locations in 2011 (2 in Northern and 2 in Southern Europe). BYI 02960 was applied 2 times at a level of 125g ai/ha to bare soil, with interval 10-12d. Beans were sown 25-30 DAT and harvested 85-105 DAT (BBCH 73-79). Samples were stored frozen until analysis during 76-114d at temperatures ≤ -18°C.

Residues of BYI 02960 and its metabolites DFA (difluoroacetic acid) and BYI 02960-difluoroethyl-aminofuranone were determined according to method 01304. Residues of BYI 02960 and its metabolites DFA and BYI 02960-difluoroethylaminofuranone were extracted from the sample materials (approx. 5 g) twice using a mixture of acetonitrile/water (4/1, v/v) with 2.2 mL/L formic acid. The crude extract was cleaned up using a Varian Bond Elute C18 solid phase extraction. After elution of the residues, the extract was mixed with reconstitution solution and an isotopically labelled internal standard solution was added. The residues were quantified by HPLC-MS/MS.

Afterwards the total residue of BYI 02960 was calculated by summing up the individual residues of BYI 02960, DFA and BYI 02960 difluoroethylaminofuranone (each analyte expressed as parent).

Conclusion

Concurrent recoveries have been determined along with the analysis of the bulb samples (Table 7.9-3). Recoveries have been determined in beans with pods and have been found to be all in the required range of 70-110% ± 20%.

The LOQ of analytical Method 01304 for BYI 02960 parent and BYI 02960-difluoroethylaminofuranone was 0.01 mg/kg and 0.02 mg/kg for DFA in bean.

Residues have been determined for BYI 02960 parent, DFA and BYI 02960-difluoroethyl-aminofuranone in beans at harvest. Residues are reported in Table 7.9-4. Residues in control samples were all < LOQ (results not shown). Residues of BYI 02960 parent and BYI 02960-difluoroethyl-aminofuranone were always <0.01 mg/kg. Residues of DFA ranged between 0.26 and 1.1 mg/kg. Thus, the total residues of BYI 02960 in bean pods ranged from 0.28-1.1 mg/kg, with a median value of 0.50 mg/kg (n=4).

STUDY 7

reference	:	Schulte, G. 2012g KIIA 6.6.3.1.7/01	GLP statement	:	yes
Type of study	:	Rotational crop - Field trial	Guideline	:	EU Council Directive 91/414/EEC amended by Commission Directive 96/68/EC EU Guidance Working Document 7029/VI/95 rev. 5 OECD Guideline for the Testing of Chemicals No. 504, Residues in Rotational Crops
Year of execution	:	2011	Dose	:	0.125 kg as/ha
test substance	:	BYI 02960 SL 200 formulation Batch no: 2010-007173	LOQ	:	BYI 02960 : 0.01 mg/kg DFA: 0.02 mg/kg DFEAF : 0.01 mg/kg
Species	:	Field pea	storage	:	91-126d at -18°C
			Acceptability	:	Yes

Study design

A rotational crop program was run for dry peas at 4 locations in 2011 (2 in Northern and 2 in Southern Europe). BYI 02960 was applied 2 times at a level of 125g ai/ha to bare soil, with interval 10-12d. Peas were sown 25-35 DAT and harvested 100-144 DAT (BBCH 89). Samples were stored frozen until analysis during 91-126d at temperatures ≤ -18°C.

Residues of BYI 02960 and its metabolites DFA (difluoroacetic acid) and BYI 02960-difluoroethyl-aminofuranone were determined according to method 01304. Residues of BYI 02960 and its metabolites DFA and BYI 02960-difluoroethylaminofuranone were extracted from the sample materials (approx. 5 g) twice using a mixture of acetonitrile/water (4/1, v/v) with 2.2 mL/L formic acid. The crude extract was cleaned up using a Varian Bond Elute C18 solid phase extraction. After elution of the residues, the extract was mixed with reconstitution solution and an isotopically labelled internal standard solution was added. The residues were quantified by HPLC-MS/MS.

Afterwards the total residue of BYI 02960 was calculated by summing up the individual residues of BYI 02960, DFA and BYI 02960 difluoroethylaminofuranone (each analyte expressed as parent).

Conclusion

Concurrent recoveries have been determined along with the analysis of the bulb samples (Table 7.9-3). Recoveries have been determined in dry peas and have been found to be all in the required range of 70-110% ± 20%.

The LOQ of analytical Method 01304 for BYI 02960 parent and BYI 02960-difluoroethylaminofuranone was 0.01 mg/kg and 0.02 mg/kg for DFA in dry peas.

Residues have been determined for BYI 02960 parent, DFA and BYI 02960-difluoroethyl-aminofuranone in peas at harvest. Residues are reported in Table 7.9-4. Residues in control samples were in the range of 0.031-0.11 mg/kg. Residues of BYI 02960 parent and BYI 02960-difluoroethyl-aminofuranone were always <0.01 mg/kg. Residues of DFA ranged from 0.65 to 2.3 mg/kg (parent equivalents). Thus, the total residues of BYI 02960 in dry pea seeds ranged from 0.67–2.3 mg/kg, with a median value of 1.55 mg/kg (n=4).

STUDY 8

reference	:	Schulte, G. 2012h KIIA 6.6.3.1.8/01	GLP statement	:	yes
Type of study	:	Rotational crop - Field trial	Guideline	:	EU Council Directive 91/414/EEC amended by Commission Directive 96/68/EC EU Guidance Working Document 7029/VI/95 rev. 5 OECD Guideline for the Testing of

					Chemicals No. 504, Residues in Rotational Crops
Year of execution	:	2011	Dose	:	0.125 kg as/ha
test substance	:	BYI 02960 SL 200 formulation Batch no: 2010-007173	LOQ	:	BYI 02960 : 0.01 mg/kg DFA: 0.02 mg/kg DFEAF : 0.01 mg/kg
Species	:	winter rape	storage	:	80-119d at -18°C
			Acceptability	:	Yes

Study design

A rotational crop program was run for oilseed rape at 4 locations in 2011 (2 in Northern and 2 in Southern Europe). BYI 02960 was applied 2 times at a level of 125g ai/ha to bare soil, with interval 9-10d. Winter rape was sown 27-41 DAT and harvested 290-369 DAT (BBCH 89). Samples were stored frozen until analysis during 91-126d at temperatures ≤ -18°C.

Residues of BYI 02960 and its metabolites DFA (difluoroacetic acid) and BYI 02960-difluoroethyl-aminofuranone were determined according to method 01304. Residues of BYI 02960 and its metabolites DFA and BYI 02960-difluoroethylaminofuranone were extracted from the sample materials (approx. 5 g) twice using a mixture of acetonitrile/water (4/1, v/v) with 2.2 mL/L formic acid. The crude extract was cleaned up using a Varian Bond Elute C18 solid phase extraction. After elution of the residues, the extract was mixed with reconstitution solution and an isotopically labelled internal standard solution was added. The residues were quantified by HPLC-MS/MS.

Afterwards the total residue of BYI 02960 was calculated by summing up the individual residues of BYI 02960, DFA and BYI 02960 difluoroethylaminofuranone (each analyte expressed as parent).

Conclusion

Concurrent recoveries have been determined along with the analysis of the bulb samples (Table 7.9-3). Recoveries have been determined in dry peas and have been found to be all in the required range of 70-110% ± 20%.

The LOQ of analytical Method 01304 for BYI 02960 parent and BYI 02960-difluoroethylaminofuranone was 0.01 mg/kg and 0.02 mg/kg for DFA in oilseed rape.

Residues have been determined for BYI 02960 parent, DFA and BYI 02960-difluoroethyl-aminofuranone in rape seeds at harvest. Residues are reported in Table 7.9-4. Residues in control samples were all < LOQ (results not shown). Residues of BYI 02960 parent and BYI 02960-difluoroethylaminofuranone were always <0.01 mg/kg. Residues of DFA ranged from 0.05 to 0.15 mg/kg (parent equivalents). Thus, the residues of the total residue of BYI 02960 in rape seeds ranged from 0.070–0.17 mg/kg, with a median value of 0.101 mg/kg (n=4).

Table 7.9-3: Concurrent recoveries for BYI 02960 in rotational crop samples (potato, leek, cucumber, onion, French bean, field pea and winter rape)

Study No. Trial No.	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%)	
						Mean recovery	RSD
11-2550	Potato	Tuber	a.s.	7	0.01	96	6
				4	0.10	92	7
				1	1.0	94	-
			overall	12		94	6
			DFEAF	7	0.01	94	6
				4	0.10	96	6
				1	1.0	101	-
			overall	12		95	6
			DFA	7	0.02	100	13
				4	0.20	92	5
				1	1.0	88	-
			overall	12		97	11
11-2551	Leek	shoot without root	a.s.	5	0.01	87	10
				3	0.10	87	4
				8		87	8
			DFEAF	5	0.01	86	10
				3	0.10	89	3
			overall	8		87	8
			DFA	5	0.02	88	13
				3	0.20	85	3
			overall	8		87	10
11-2552	Cucumber	fruits	a.s.	5	0.01	102	6
				4	0.10	94	1
				2	1.0	90	-
			overall	11		97	7
			DFEAF	5	0.01	97	3
				4	0.10	95	4
				2	1.0	96	-
			overall	11		96	4
			DFA	5	0.01	96	12
				4	0.10	89	7
				2	1.0	87	-
			overall	11		92	10
11-2553	Onion	bulb	a.s.	5	0.01	92	3
				3	0.10	97	4
			overall	8		94	4
			DFEAF	5	0.01	88	7
				3	0.10	94	3
			overall	8		91	6
			DFA	5	0.02	80	4
				3	0.20	79	2

Study No. Trial No.	Crop	Portion analysed	a.s./metabolite	n	Fortifi-cation level (mg/kg)	Recovery (%)	
						Mean recovery	RSD
			overall	8		80	3
11-2555	French bean	Beans with pods	a.s	6	0.01	91	2
				4	0.10	88	3
				2	5.0	84	-
			overall	12		89	4
			DFEAF	6	0.01	89	6
				4	0.10	94	2
				2	5.0	104	-
			overall			93	7
			DFA	6	0.02	94	5
				4	0.20	82	3
				2	5.0	83	-
			overall	12		88	8
11-2556	Field pea	dry pea seed	a.s.	7	0.01	93	3
				3	0.10	87	3
				2	1.0	85	-
				2	5.0	96	-
			overall	14		91	5
			DFEAF	7	0.01	92	3
				3	0.10	96	2
				2	1.0	95	-
				2	5.0	101	-
			overall	14		94	4
			DFA	5	0.02	92	8
				3	0.20	68	5
				2	0.1	66	-
				2	5.0	77	-
			overall	12		79	16
11-2554	Winter rape	seed	a.s.	7	0.01	97	3
				3	1.0	98	5
			overall	10		98	4
			DFEAF	6	0.01	102	4
				4	1.0	98	3
			overall	10		101	4
			DFA	7	0.02	70	15
				3	0.20	90	3
			overall	10		76	17

Table 7.9-4: Summary of field rotational crop trials in Northern and Southern Europe at 125 g as/ha

Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) expressed as BYI 02960					DALT (days)	Remarks
			kg a.s./ha	Water l/ha	kg a.s./hl				BYI 02960	DFA as BYI 02960	DFE AF	DFA as DFA	Total BYI 02960 + DFA, as BYI 02960		
Trial 11-2550-01 F-80700 Fresnoy les Roye N-France	Preceeding: - Succeeding: early potato Var. Resy	Preceeding: - Succeeding: 1. 18-04-2011 2. 10-06.2011 3. 15-07-2011	Preceeding: 125 125	Preceeding: 300 300	Preceeding: 41.7 41.7	Preceeding: 08-03-2011 18-03-2011	Preceeding: -	Succeeding: tuber	<0.01	0.19	<0.01	0.063	0.20	122	PBI = 31 days
Trial 11-2550-02 NL-1681 ND, Zwaagdjik The Netherlands	Preceeding: - Succeeding: early potato Var. Riviera	Preceeding: - Succeeding: 1. 04-05-2011 2. not reported 3. not reported	Preceeding: 125 125	Preceeding: 400 400	Preceeding: 31.3 31.3	Preceeding: 22-03-2011 01-04-2011	Preceeding: -	Succeeding: tuber	<0.01	0.028	<0.01	0.009	0.04	98	PBI = 33 days
Trial 11-2550-03 E-08520 Les Franqueses del Valles Spain	Preceeding: - Succeeding: early potato Var. Red Pontiac	Preceeding: - Succeeding: 1. 05-04-2011 2. 25-05.2011 3. 24-06-2011	Preceeding: 125 125	Preceeding: 300 300	Preceeding: 41.7 41.7	Preceeding: 02-03-2011 11-03-2011	Preceeding: -	Succeeding: tuber	<0.01	0.25	<0.01	0.083	0.26	126	PBI = 25 days
Trial 11-2550-04 I-70032 Bitonto (Bari) Italy	Preceeding: - Succeeding: early potato Var. Arinda	Preceeding: - Succeeding: 1. 20-07-2011 2. 20-09-2011 3. 20-10-2011	Preceeding: 125 125	Preceeding: 300 300	Preceeding: 41.7 41.7	Preceeding: 10-06-2011 20-06-2011	Preceeding: -	Succeeding: tuber	<0.01	0.036	<0.01	0.012	0.05	137	PBI = 30 days

Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) expressed as BYI 02960					DALT (days)	Remarks
			kg a.s./ha	Water l/ha	kg a.s./hl				BYI 02960	DFA as BYI 02960	DFE AF	DFA as DFA	Total BYI 02960 + DFA, as BYI 02960		
Trial 11-2551-01 F-78410 Bouafle N-France	Preceeding: - Succeeding: Leek Var. Bleu de Solaize leek	Preceeding: - Succeeding: 1. 13-07-2011 2. - 3. 23-09-2011	Preceeding: 125 125	Preceeding: 300 300	Preceeding: 41.7 41.7	Preceeding: 07-06-2011 17-06-2011	Preceeding: -	Succeeding: plant without root	<0.01	0.23	<0.01	0.076	0.24	103	PBI = 26 days
Trial 11-2551-02 D-51399 Burscheid Germany	Preceeding: - Succeeding: Leek Var. Prelina	Preceeding: - Succeeding: 1. 13-04-2011 2. - 3. 15-07-2011	Preceeding: 125 125	Preceeding: 300 300	Preceeding: 41.7 41.7	Preceeding: 01-03-2011 11-03-2011	Preceeding: -	Succeeding: plant without root	<0.01	0.095	<0.01	0.032	0.11	130	PBI = 33 days
Trial 11-2551-03 E-46230 Alginet Spain	Preceeding: - Succeeding: early Leek Var. Atal Temprano	Preceeding: - Succeeding: 1. 28-04-2011 2. - 3. 01-07-2011	Preceeding: 125 125	Preceeding: 300 300	Preceeding: 41.7 41.7	Preceeding: 21-03-2011 31-03-2011	Preceeding: -	Succeeding: plant without root	<0.01	0.020	<0.01	0.007	0.03	97	PBI = 28 days
Trial 11-2551-04 I-40128 Bologna Italy	Preceeding: - Succeeding: Leek Var. Libertas	Preceeding: - Succeeding: 1. 21-04-2011 2. - 3. 10-07-2011	Preceeding: 125 125	Preceeding: 400 400	Preceeding: 31.3 31.3	Preceeding: 08-03-2010 21-03-2010	Preceeding: -	Succeeding: plant without root	<0.01	0.039	<0.01	0.013	0.05	112	PBI = 31 days
Trial 11-2552-01 F-2734 Criquebeuf sur Seine N-France	Preceeding: - Succeeding: cucumber Var. Raider	Preceeding: - Succeeding: 1. 31-05-2011 2. 28-06-2011 3. 14-07-2011	Preceeding: 125 125	Preceeding: 300 300	Preceeding: 41.7 41.7	Preceeding: 22-04-2011 02-05-2011	Preceeding: -	Succeeding: fruit	<0.01	0.28	<0.01	0.093	0.29	79	PBI = 29 days

Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) expressed as BYI 02960					DALT (days)	Remarks
			kg a.s./ha	Water l/ha	kg a.s./hl				BYI 02960	DFA as BYI 02960	DFE AF	DFA as DFA	Total BYI 02960 + DFA, as BYI 02960		
Trial 11-2552-02 D-51399 Burscheid Germany	Preceeding: - Succeeding: cucumber Var. Melody cucumber	Preceeding: - Succeeding: 1. 13-05-2011 2. 10-06-2011 3. 01-07-2011	Preceeding: 125 125	Preceeding: 300 300	Preceeding: 41.7 41.7	Preceeding: 08-04-2011 18-04-2011	Preceeding: -	Succeeding: fruit	<0.01	0.32	<0.01	0.106	0.33	73	PBI = 25 days
Trial 11-2552-03 E-46230 Alginet Spain	Preceeding: - Succeeding: cucumber Var. Dahser Híbrido ciclo corto	Preceeding: - Succeeding: 1. 28-04-2011 2. 15-05-2011 3. 15-06-2011	Preceeding: 125 125	Preceeding: 300 300	Preceeding: 41.7 41.7	Preceeding: 21-03-2011 31-03-2011	Preceeding: -	Succeeding: fruit	<0.01	0.047	<0.01	0.016	0.06	83	PBI = 28 days
Trial 11-2552-04 I -00055 Ladispoli Italy	Preceeding: - Succeeding: cucumber Var. Marketmore	Preceeding: - Succeeding: 1. 18-05-2011 2. 13-06-2011 3. 26-06-2011	Preceeding: 125 125	Preceeding: 400 400	Preceeding: 31.3 31.3	Preceeding: 08-04-2010 18-04-2010	Preceeding: -	Succeeding: fruit	<0.01	0.41	<0.01	0.136	0.42	69	PBI = 30 days
Trial 11-2553-01 F-78410 Bouafle N-France	Preceeding: - Succeeding: onion Var. Paille des vertus	Preceeding: - Succeeding: 1. 06-04-2011 2. not reported 3. 25-08-2011	Preceeding: 125 125	Preceeding: 300 300	Preceeding: 41.7 41.7	Preceeding: 25-02-2011 07-03-2011	Preceeding: -	Succeeding: bulb	<0.01	0.16	<0.01	0.053	0.17	175	Plants sown at location PBI = 30 days
Trial 11-2553-02 D-51399 Burscheid Germany	Preceeding: - Succeeding: onion Var. Sherpa F1	Preceeding: - Succeeding: 1. 13-04-2011 2. not reported 3. 22-07-2011	Preceeding: 125 125	Preceeding: 300 300	Preceeding: 41.7 41.7	Preceeding: 01-03-2011 11-03-2011	Preceeding: -	Succeeding: bulb	<0.01	0.071	<0.01	0.024	0.08	133	Plants sown at location PBI = 33 days

Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) expressed as BYI 02960					DALT (days)	Remarks
			kg a.s./ha	Water l/ha	kg a.s./hl				BYI 02960	DFA as BYI 02960	DFE AF	DFA as DFA	Total BYI 02960 + DFA, as BYI 02960		
Trial 11-2553-03 I-37044 Cologna Veneta Italy	Preceeding: - Succeeding: onion Var. Dorata di Parma	Preceeding: - Succeeding: 1. 15-04-2011 2. not reported 3. 01-07-2011	Preceeding: 125 125	Preceeding: 300 300	Preceeding: 41.7 41.7	Preceeding: 10-03-2011 21-03-2011	Preceeding: -	Succeeding: bulb	<0.01	0.058	<0.01	0.019	0.07	116	Plants sown elsewhere and planted at location PBI = 25 days
Trial 11-2553-04 E-08840 Gavà Spain	Preceeding: - Succeeding: onion Var. Figueras	Preceeding: - Succeeding: 1. 18-05-2011 2. not reported 3. 15-07-2011	Preceeding: 125 132	Preceeding: 317 300	Preceeding: 41.8 41.7	Preceeding: 08-04-2011 18-04-2011	Preceeding: -	Succeeding: bulb	<0.01	<0.02	<0.01	0.007	0.03	118	Plants sown elsewhere and planted at location PBI = 28 days
Trial 11-2555-01 F-27340 Criquebeuf sur Seine N-France	Preceeding: - Succeeding: French bean Var. Banga	Preceeding: - Succeeding: 1. 31-05-2011 2. 12-07-2011 3. 01-08-2011	Preceeding: 125 125	Preceeding: 300 300	Preceeding: 41.7 41.7	Preceeding: 22-04-2011 02-05-2011	Preceeding: -	Succeeding: bean with pod	<0.01	1.1	<0.01	0.365	1.11	93	PBI = 29 days
Trial 11-2555-02 D-51399 Burscheid Germany	Preceeding: - Succeeding: French bean Var. Saxa	Preceeding: - Succeeding: 1. 13-05-2011 2. 03-07-2011 3. 27-07-2011	Preceeding: 125 125	Preceeding: 300 300	Preceeding: 41.7 41.7	Preceeding: 08-04-2011 18-04-2011	Preceeding: -	Succeeding: bean with pod	<0.01	0.57	<0.01	0.189	0.58	105	PBI = 25 days
Trial 11-2555-03 I-00055 Ladispoli Italy	Preceeding: - Succeeding: French bean Var. OR Amo	Preceeding: - Succeeding: 1. 18-05-2011 2. 28-06-0211 3. 12-07-2011	Preceeding: 125 125	Preceeding: 300 300	Preceeding: 31.0 31.0	Preceeding: 08-04-2011 18-04-2011	Preceeding: -	Succeeding: bean with pod	<0.01	0.38	<0.01	0.126	0.39	85	PBI = 30 days

Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) expressed as BYI 02960					DALT (days)	Remarks
			kg a.s./ha	Water l/ha	kg a.s./hl				BYI 02960	DFA as BYI 02960	DFE AF	DFA as DFA	Total BYI 02960 + DFA, as BYI 02960		
Trial 11-2555-04 E-46230 Alginet Spain	Preceeding: - Succeeding: French bean Var. Cleo Temprana	Preceeding: - Succeeding: 1. 28-04-2011 2. 01-06.2011 3. 16-07-2011	Preceeding: 125 125	Preceeding: 317 300	Preceeding: 41.8 41.7	Preceeding: 21-03-2011 31-03-2011	Preceeding: -	Succeeding: bean with pod	<0.01	0.26	<0.01	0.086	0.27	90	PBI = 28 days
Trial 11-2556-01 F-78410 Bouafle N-France	Preceeding: - Succeeding: pea Var. Montana	Preceeding: - Succeeding: 1. 11-03-2011 2. 07-05-2011 3. 29-06-2011	Preceeding: 125 125	Preceeding: 300 300	Preceeding: 41.7 41.7	Preceeding: 03-02-2011 14-02-2011	Preceeding: -	Succeeding: dry pea seed	<0.01	2.3	<0.01	0.764	2.31	136	1.5 mm of rain starting 20h after 2md Application PBI = 25 days
Trial 11-2556-02 D-51399 Burscheid Germany	Preceeding: - Succeeding: pea Var. Mascara	Preceeding: - Succeeding: 1. 28-03-2011 2. 01-06-2011 3. 15-07-2011	Preceeding: 125 125	Preceeding: 300 300	Preceeding: 41.7 41.7	Preceeding: 18-02-2011 28-02-2011	Preceeding: -	Succeeding: dry pea seed	<0.01	0.99	<0.01	0.329	1.0	144	PBI = 28 days DFA residue in control sample 0.11 mg/kg
Trial 11-2556-03 I- 40134 Bologna Italy	Preceeding: - Succeeding: pea Var. Lambado	Preceeding: - Succeeding: 1. 20-04-2011 2. 18-05-0211 3. 01-07-2011	Preceeding: 125 125	Preceeding: 400 400	Preceeding: 31.3 31.3	Preceeding: 09-03-2011 21-03-2011	Preceeding: -	Succeeding: dry pea seed	<0.01	0.65	<0.01	0.216	0.66	100	PBI = 30 days
Trial 11-2556-04 E- 41310 Brenes Spain	Preceeding: - Succeeding: pea Var. Lincoln	Preceeding: - Succeeding: 1. 17-03-2011 2. 10-05.2011 3. 20-05-2011	Preceeding: 125 125	Preceeding: 317 300	Preceeding: 41.8 41.7	Preceeding: 31-01-2011 10-02-2011	Preceeding: -	Succeeding: dry pea seed	<0.01	2.1	<0.01	0.698	2.11	132	PBI = 35 days DFA residue in control sample 0.031 mg/kg

Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) expressed as BYI 02960					DALT (days)	Remarks
			kg a.s./ha	Water l/ha	kg a.s./hl				BYI 02960	DFA as BYI 02960	DFE AF	DFA as DFA	Total BYI 02960 + DFA, as BYI 02960		
Trial 11-2554-01 F-95510 Vilers en Arthies N-France	Preceeding: - Succeeding: winter rape Var Dynastie hybrid 00	Preceeding: - Succeeding: 1. 31-08-2011 2. 26-03-2012 3. 23-07-2012	Preceeding: 125 125	Preceeding: 300 300	Preceeding: 41.7 41.7	Preceeding: 11-07-2011 21-07-2011	Preceeding: -	Succeeding: seed	<0.01	0.09	<0.01	0.030	0.10	369	1 mm of rain starting 22h after 2md Application PBI = 41 days
Trial 11-2554-02 D-59514 Welver-Flerke Germany	Preceeding: - Succeeding: winter rape Var ES-Astrid Rape	Preceeding: - Succeeding: 1. 01-09-2011 2. 02-04-2012 3. 18-07-2012	Preceeding: 125 125	Preceeding: 300 300	Preceeding: 41.7 41.7	Preceeding: 19-07-2011 29-07-2011	Preceeding: -	Succeeding: seed	<0.01	0.15	<0.01	0.050	0.16	361	1.5 mm of rain starting 13h after 2md Application PBI = 34 days
Trial 11-2554-03 I- 40128 Bologna Italy	Preceeding: - Succeeding: winter rape Var Primus winter variety	Preceeding: - Succeeding: 1. 14-09-2011 2. 20-03-2012 3. 01-06-2012	Preceeding: 125 125	Preceeding: 400 400	Preceeding: 31.3 31.3	Preceeding: 08-08-2011 18-08-2011	Preceeding: -	Succeeding: seed	<0.01	0.05	<0.01	0.017	0.06	307	PBI = 27 days
Trial 11-2554-04 E- 08520 Les Franqueses des Valles (Llerona) Spain	Preceeding: - Succeeding: winter rape Var Pacific winter rape	Preceeding: - Succeeding: 1. 11-10-2011 2. 27-03.2012 3. 20-05-2012	Preceeding: 125 125	Preceeding: 300 300	Preceeding: 41.7 41.7	Preceeding: 24-08-2011 02-09-2011	Preceeding: -	Succeeding: seed	<0.01	0.07	<0.01	0.023	0.08	290	PBI = 39days

Guidelines & Limitations

None. The precipitation measured in the French trials is not considered to impact the trial results.

B.7.9.1 Summary of studies in plants (succeeding crops)

In the large-scale field rotational crop trial, using multiple rotations (simulating crop failure, re-use of the field in the same season, and re-use in the following season) and multiple crop groups (a root crop, a leafy crop, and a cereal), it was evident that total residues in all relevant food and feed matrices were generally highest in the first rotation. Residues in *root*, *leafy*, and *cereal* crops due to field re-use ranged from 0.05-0.14 mg/kg, <0.04-0.16 mg/kg, and 0.11-0.65 mg/kg, respectively, in the primary food commodities. In straw and fodder, they were <0.07-0.39 mg/kg and 0.05-0.41 mg/kg, respectively for total of DFA, DFEAF and flupyradifurone,expressed as flupyradifurone.

Since it was evident that residues of flupyradifurone are to be expected in rotational crops, additional rotational crop studies were performed representative for each type of crop at short plant back interval (PBI 30 days) and in accordance with the supported maximum dose rate of 2 × 125 g as/ha. Potatoes, leek, cucumber, onion, bean, peas and winter rape were grown at 2 locations in Northern and at 2 locations in Southern Europe. BYI 02960 and DFEAF levels were always <0.01 mg/kg while soil metabolite DFA was present in all trials except one onion trial. Residues at harvest showed considerable variation which could not be attributed to geographical zone, soil type, pH, organic matter content, and temperature during treatment, rainfall after treatment or growth period (results not shown). Therefore, results for Northern and Southern Europe are considered as one data set with considerable variation.

HRs of DFA were 0.27, 0.25, 0.43, 0.18, 1.1, 2.3 and 0.17 mg/kg in potatoes, leek, cucumber, onion, bean, peas and winter rape, respectively.

In the Expert meeting of November 2014 it was concluded that rotational crop trials should be submitted, conducted at realistic-worst-case plant back intervals with regard to the following crop selected, and providing in addition soil analyses. Proposed MRLs for DFA for rotational crops are provisional, pending the availability of the new data.

B.7.10 Proposed pre-harvest intervals for envisaged use, or withholding periods, in the case of post-harvest uses (IIA 6.8; IIIA 8.8)

The envisaged pre-harvest intervals are as described above under points B.7.4 and B.7.5. For:

- protected lettuce, the critical PHI is 3 days.
- unprotected lettuce, the critical PHI is 3 days.
- hops, the PHI is 21 days.

B.7.11 Community MRLs

Flupyradifurone is a new active substance not on the market in Europe or outside Europe

B.7.12 Proposed EU MRLs and justification for the acceptability of those MRLs (IIA 6.7.2; IIIA 8.7.2)

B.7.12.1 Proposed MRLs

The supported use for flupyradifurone for approval under Reg (EC) 1107/2009 is on lettuce and hops. The notifier proposes the following MRLs; The proposed MRLs are 9 mg/kg for lettuce and 4 mg/kg for hops (kiln-dried). The applicant used a residue definition for monitoring as the sum of flupyradifuone and DFA, expressed as flupyradifurone.

Use of flupyradifurone is expected to result in residues in succeeding crops. MRLs are proposed based on the field rotational crops for succeeding crops:

Brassica vegetables:	0.3 mg/kg
Leafy vegetables:	0.3 mg/kg
Potatoes:	0.7 mg/kg
Root vegetables:	0.3 mg/kg
Bulb vegetables:	0.4 mg/kg
Fruiting vegetables:	1.0 mg/kg
Legume vegetables:	2.0 mg/kg
Stem vegetables:	0.6 mg/kg
Pulses:	6.0 mg/kg
Oilseeds:	0.8 mg/kg
Cereals:	1.5 mg/kg

Furthermore, the applicant applies for a number of EU-MRLs ~~and import tolerances~~ in parallel to the peer review (art 47 of Reg (EC) 1107/2009).

Table 7.12.1-1: Proposed EU-MRLs from uses of BYI 02960 supported in EU ~~and Import tolerances proposed by the Global Joint Review of BYI 02960~~

Commodity	Proposed EC MRL (mg/kg)	~~Proposed import tolerance (mg/kg)~~
~~Citrus fruits~~		~~3~~
~~Almonds~~		~~0.15~~
~~Pecan~~		~~0.15~~
~~Peanut~~		~~0.15~~
Apple	0.5	~~1.5~~
Pear	0.5	~~1.5~~
Grape	1	~~3~~
~~Blueberry~~		~~4~~
~~Prickly pear~~		~~0.7~~
Tomato	0.9	~~3~~
Egg plant	0.9	~~3~~

Commodity	Proposed EC MRL (mg/kg)	~~Proposed import tolerance (mg/kg)~~
Pepper	1	
~~Chilli pepper~~		~~3.0~~
Cucumber/gherkin	1.5	
Zucchini (courgette)	1.5	
Watermelon	0.9	
Sweet corn	0.03* (1.5)†	~~0.4~~
~~Celery~~		~~40~~
~~Dry bean seed~~		~~0.8~~
~~Dry pea seed~~		~~8~~
~~Soy bean seed~~		~~4~~
~~Barley, sorghum and wheat grain~~		~~4~~
~~Field corn grain~~		~~0.5~~
~~Cotton~~		~~0.9~~
~~Coffee~~		~~2~~
Hops	4	~~20~~
Bulb vegetables	0.4	~~0.8*~~
Legume vegetables	2	~~10*~~

† proposal depends on whether sweet corn is covered by EU rotational crop value for "cereals" or not

* extrapolation from EU rotational crop data to AUS use pattern

For MRL proposals for animal commodities, results from the transfer factors derived from the livestock feeding studies and the estimated dietary burden for livestock (on the basis of the field residue trials).

The feeding studies performed at or about the 1x rate resulted in the residue levels presented in table 7.12.1-2.

Table 7.12.1-2: Combined residues of BYI 02960 and DFA in poultry and bovine matrices as determined in the feeding studies (1X dose group)

Commodity	Residue levels (mg/kg)		
	combined residue	individual components	
		BYI 02960*	DFA**
chicken			
eggs	0.061†	<0.01†	0.051†
muscle	0.093	<0.01	0.083
fat	0.039	<0.01	0.029
liver	0.114	<0.01	0.104
cow			

Commodity	Residue levels (mg/kg)		
	combined residue	individual components	
		BYI 02960*	DFA**
milk	0.043††	0.023††	<0.02††
muscle	0.063	0.043	<0.02
fat	0.041	0.021	<0.02
liver	0.165	0.145	<0.02
kidney	0.179	0.159	<0.02

* LOQ for BYI 02960 = 0.01 mg/kg
† egg value based on the day-24 egg sample
** LOQ for DFA = 0.02 mg/kg, expressed in BYI 02960 equivalents
†† milk value based on the day-28 milk sample

Based on the feeding study results, transfer factors were calculated both for the *combined residue* of BYI 02960 and DFA derived from the feeding of BYI 02960 as well as for *DFA alone* derived from the exposure levels of the hens and cattle to DFA in the feeding studies. Using these transfer factors, estimations of the residue levels reasonably expected to be determined after feeding of a *mixture* of BYI 02960 and DFA, as would be anticipated in the feed crops, can be calculated.

The notifier separated the residues fed into individual components to perform these calculations. A dietary burden of 1.401 mg/kg feed was calculated for chickens and 4.290 mg/kg feed for dairy cows, for the *combined residue* of BYI 02960 and DFA. The separated components are calculated to be 0.421 mg/kg feed of BYI 02960 and 0.980 mg/kg feed of DFA for chicken and 2.811 mg/kg feed of BYI 02960 and 1.479 mg/kg feed of DFA for cows. Using these dietary burden levels and and the transfer factors derived in the feeding studies (for the low-dose groups re. the total residue, and for the most relevant exposure scenarios), the notifier calculates that the following levels of total residues of BYI 02960 and DFA (expressed in parent compound equivalents) can be expected after feeding of realistic worst-case residues in feed crops to poultry, presented in table 7.12.1-3.

Table 7.12.1-3: Theoretical residues resulting from feeding BYI 02960 and DFA to poultry and to dairy cows in a ratio relevant to the actual residues expected in feed commodities

Dietary burden (mg/ kg feed)	Matrix	Transfer factor*	Resulting residue (mg/kg)
chicken			
BYI 02960			
0.421	egg	0.038	0.016
	muscle	0.062	0.026
	fat	0.026	0.11
	liver	0.076	0.032
DFA			
0.980	egg	0.097	0.095
	muscle	0.172	0.169
	fat	0.060	0.059

Dietary burden (mg/ kg feed)	Matrix	Transfer factor*	Resulting residue (mg/kg)
	liver	0.216	0.212
cattle			
BYI 02960			
2.811	milk	0.009	0.025
	muscle	0.013	0.037
	fat	0.009	0.025
	liver	0.036	0.101
	kidney	0.037	0.104
DFA			
1.479‡	milk	0.028	0.041
	muscle	0.094	0.139
	fat	0.068	0.101
	liver	0.091	0.135
	kidney	0.140	0.207

* for derivation of transfer factors, see 7.8.1
† when deriving factors, residue values of <LOQ were calculated as being at the LOQ, thus representing a worst case for exposure
‡ based on this dietary burden, the transfer factor chosen for DFA reflects the lowest dosed exposure rate, 1.5 mg/kg feed for poultry and 4.8 mg/kg feed for lactating ruminants

Table 7.12.1-4 summarizes the results of the theoretical residues calculated above by the notifier, matrix for matrix. The following levels of total residues of BYI 02960 and DFA (expressed in parent compound equivalents) can be expected after feeding of realistic worst-case residues in feed crops to poultry and dairy cows.

Table 7.12.1-4: Combined residues of BYI 02960 and DFA in poultry and bovine matrices as determined in the feeding study (1X)

Matrix	Residue levels* (mg/kg) based on feeding of			Proposed MRL
	BYI 02960	DFA	*Sum*	
chicken				
egg	0.016	0.095	*0.111*	**0.15**
muscle	0.026	0.169	*0.195*	**0.20**
fat	0.011	0.059	*0.070*	**0.07**
liver	0.032	0.212	*0.244*	**0.30**
cattle				
milk	0.025	0.041	*0.067*	**0.07**
muscle	0.037	0.139	*0.176*	**0.20**
fat	0.025	0.101	*0.126*	**0.15**
liver	0.101	0.135	*0.236*	**0.30**
kidney	0.104	0.207	*0.311*	**0.40**

* all residue levels expressed in parent compound equivalents

B.7.12.2 Justification of proposed MRLs

Protected lettuce was presented to be the worst case with regard to residue levels (see table 7.6.2-1). For the MRLs proposed by the RMS, see tables 7.12.2-1 and 7.12.2-2. For plant products from ptrimary crops, the OECD MRL calculator was used. For MRLs based on succeeding crops, the HR was rounded up and this values is proposed a the MRL, in absence of guidance how to calculate

MRLs for rotational crops. ~~The proposed import tolerances are presented in the Evaluation Report on flupyradifurone, as it is considered that the imported products will in generally not be used for livestock feed.~~

Table 7.12.2-1 Proposed MRL, STMR and HR by the RMS

Crop	Zone	Total BYI 02960 + DFA, as BYI 02960		Flupyradifuone	DFA	Unrounded MRL (mg/kg)	Remarks
		STMR (mg/kg)	HR (mg/kg)	MRL proposal (mg/kg)	MRL proposal (mg/kg)		
Lettuce	EU	2.12	6.04	**9**	0.06	BYI 02960: 8.597 DFA: 0.060	
	SEU	0.22	3.18	6	0.08	BYI 02960: 5.526 DFA: 0.081	
	NEU	0.22	0.82	1.5	0.04	BYI 02960: 1.265 DFA: 0.036	
	SEU H&G	1.12	3.18	6	0.08	Unrounded MRL BYI 02960: 5.526 DFA: 0.081	
	NEU H&G	0.60	3.00	5	**0.1**	Unrounded MRL BYI 02960: 4.430 DFA: 0.091	
hops	NEU	1.05	2.3	**4**	**0.4**	Unrounded MRL BYI 02960: 3.488 DFA: 0.357	
Rotational root vegetables	EU	0.12	0.26	**0.01**	**0.09**	HR BYI 02960: 0.01 DFA:0.083	Based on four rotational crop trials in potato
Rotational stem vegetables	EU	0.08	0.24	**0.01**	**0.08**	HR BYI 02960: 0.01 DFA:0.076	Based on four rotational crop trials in leek
Rotational bulb vegetables	EU	0.07	0.17	**0.01**	**0.06**	HR BYI 02960: 0.01 DFA:0.053	Based on four rotational crop trials in onion
Rotational fruiting vegetables	EU	0.31	0.42	**0.01**	**0.15**	HR BYI 02960: 0.01 DFA:0.136	Based on four rotational crop trials in cucumber
Rotational legume vegetables	EU	0.49	1.11	**0.01**	**0.4**	HR BYI 02960: 0.01 DFA:0.365	Based on four rotational crop trials in French bean

Crop	Zone	Total BYI 02960 + DFA, as BYI 02960		Flupyradifuone	DFA	Unrounded MRL (mg/kg)	Remarks
		STMR (mg/kg)	HR (mg/kg)	MRL proposal (mg/kg)	MRL proposal (mg/kg)		
Rotational leafy vegetables	EU	0.06	0.20	**0.08**	**0.04**	HR BYI 02960: 0.08 DFA:0.04	Based on four rotational crop trials in lettuce
Rotational cereals and sweet corn	EU	0.34	0.64	**0.01**	**0.3**	HR BYI 02960: 0.01 DFA:0.209	Based on three rotational crop trials in barley.
Rotational pulses	EU	1.56	2.31	**0.01**	**0.8**	HR BYI 02960: 0.01 DFA:0.764	Based on four rotational crop trials in field peas
Rotational oilseeds	EU	0.09	0.16	**0.01**	**0.05**	HR BYI 02960: 0.01 DFA:0.05	Based on four rotational crop trials in rape seed

~~Crop~~	~~Zone~~	~~STMR (mg/kg)~~	~~HR (mg/kg)~~	~~Mean + 4 SD~~	~~CF x 3 MEan~~	~~Unrounded MRL (mg/kg)~~	~~MRL proposal (mg/kg)~~	~~Remarks~~
~~Lettuce~~	~~EU~~	~~2.2~~	~~6.0~~	~~8.565~~	~~7.567~~	~~8.565~~	~~9~~	~~STMRs and HRs are based on residue definition for RA, MRL on residue definition for monitoring~~
	~~SEU~~	~~0.32~~	~~0.83~~	~~1.365~~	~~0.930~~	~~1.365~~		
	~~NEU~~	~~0.23~~	~~0.83~~	~~1.299~~	~~0.967~~	~~1.299~~		
~~hops~~	~~NEU~~	~~1.15~~	~~2.4~~	~~4.031~~	~~3.720~~	~~4.031~~	~~5~~	
~~Rotational root vegetables~~	~~EU~~	~~0.12~~ ~~0.13~~	~~0.26~~ ~~0.27~~	~~0.591~~	~~0.438~~	~~0.581~~ ~~0.591~~	~~0.6~~	~~Based on four rotational crop trials in potato~~
~~Rotational stem vegetables~~	~~EU~~	~~0.08~~ ~~0.09~~	~~0.24~~ ~~0.25~~	~~0.494~~	~~0.344~~	~~0.485~~ ~~0.494~~	~~0.5~~	~~Based on four rotational crop trials in leek~~
~~Rotational bulb vegetables~~	~~EU~~	~~0.08~~ ~~0.09~~	~~0.17~~ ~~0.18~~	~~0.334~~	~~0.243~~	~~0.324~~ ~~0.344~~	~~0.4~~	~~Based on four rotational crop trials in onion~~
~~Rotational fruiting vegetables~~	~~EU~~	~~0.05~~ ~~0.32~~	~~0.42~~ ~~0.43~~	~~0.903~~	~~0.853~~	~~0.897~~ ~~0.903~~	~~0.9~~	~~Based on four rotational crop trials in cucumber~~
~~Rotational legume vegetables~~	~~EU~~	~~0.49~~ ~~0.50~~	~~1.1~~	~~2.039~~	~~1.778~~	~~2.050~~ ~~2.039~~	~~2.0~~	~~Based on four rotational crop trials in French bean~~
~~Rotational leafy vegetables~~	~~EU~~	~~0.07~~ ~~0.08~~	~~0.20~~ ~~0.21~~	~~0.385~~	~~0.323~~	~~0.375~~ ~~0.385~~	~~0.4~~	~~Based on four rotational crop trials in lettuce~~
~~Rotational cereals and sweet corn~~	~~EU~~	~~0.34~~ ~~0.35~~	~~0.65~~	~~1.452~~	~~1.110~~	~~1.442~~ ~~1.452~~	~~1.5~~	~~Based on three rotational crop trials in barley.~~
~~Rotational pulses~~	~~EU~~	~~1.6~~	~~2.3~~	~~4.732~~	~~4.553~~	~~4.771~~ ~~4.732~~	~~5~~	~~Based on four rotational crop trials in field peas~~
~~Rotational oilseeds~~	~~EU~~	~~0.09~~ ~~0.10~~	~~0.16~~ ~~0.17~~	~~0.283~~	~~0.330~~	~~0.330~~ ~~0.300~~	~~0.4~~	~~Based on four rotational crop~~

Crop	Zone	STMR (mg/kg)	HR (mg/kg)	Mean + 4 SD	CF x 3 MEan	Unrounded MRL (mg/kg)	MRL proposal (mg/kg)	Remarks
Lettuce	EU	2.2	6.0	8.565	7.567	8.565	9	STMRs and HRs are based on residue definition for RA, MRL on residue definition for monitoring
	SEU	0.32	0.83	1.365	0.930	1.365		
	NEU	0.23	0.83	1.299	0.967	1.299		
hops	NEU	1.15	2.4	4.031	3.720	4.031	5	
Rotational root vegetables	EU	0.12 0.13	0.26 0.27	0.591	0.438	0.581 0.591	0.6	Based on four rotational crop trials in potato
								trials in rape seed

MRLs for products of animal origin are based on the study reflecting the 1N dose rate, calculated with the dietary burden calculator according to SANCO guideline 7031/VI/95 and taking into account the results and the transfer factors derived from the livestock feeding studies. The results from the feeding study from the all 2N dose rates (dairy cow) were first normalised to 1N, the hen study was performed at 1N dose rate and was therefore not normalised. The theoretical exposure to DFA was included in the N-tae calcaulation, i.e 32.1% for poultry and 2.9% for cattle. This does however not take into account that livestock is exposed to mostly DFA in feed,and not to flupyradifurone.

DFA makes up 89-96% of the diet for chicken,dairy and beef cattle and pigs, whilst in the feeding studies, animals were exposed only to flupyradifurone. It is therefore expected that residues of DFA will be higher in practice.Therefore, the expert meeting concluded that feeding studie in poultry in cattle with DFA are required. The approach below is accepted in the meantime.

Table 7.12.2-2: Conversion of combined residues of BYI 02960 and DFA in poultry and bovine matrices as determined in the feeding studies to 1N dose level

Matrix	Residue levels (mg/kg) based on feeding study nominal dose			Residue levels (mg/kg) based on 1N calculated dietary burden			
	BYI 02960	DFA	*Sum*	BYI 02960	DFA as DFA	*Sum** *STMR*	*Sum** *HR*
chicken							
egg	<0.01	0.051	*0.061*	<0.01	0.026	*0.07*	*0.09*
fat	<0.01	0.029	*0.039*	<0.01	0.018	*0.05*	*0.07*
liver	<0.01	0.104	*0.114*	<0.01	0.061	*0.18*	*0.23*
muscle	<0.01	0.083	*0.093*	<0.01	0.042	*0.12*	*0.16*
cattle							
milk	0.023	<0.02	*0.043*	0.001	0.025	*0.03*	*0.08*
fat	0.021	<0.02	*0.041*	0.002	0.094	*0.14*	*0.29*
kidney	0.159	<0.02	*0.176*	0.008	0.134	*0.20*	*0.41*
liver	0.145	<0.02	*0.165*	0.006	0.094	*0.14*	*0.29*
muscle	0.043	<0.02	*0.063*	0.003	0.093	*0.14*	*0.28*

* Sum as cumulative total of 1N BYI 02960 and DFA, not a conversion of sum from feeding studies

The HR values are rounded up for MRL proposal

Secondly, the livestock dietary burden intake calculations were performed for parent flupyradifurone and DFA separately, with the following input values (see also table 7.2-1)

Table 7.12-2-3: Input values for livestock dietary burden calculations for BYI 02960 and DFA separately

Commodity	BYI 02960		DFA		Comment
	STMR	HR	STMR	HR	
grass	0.01	0.04	0.08	0.37	Rotational crop, barley field study green material
Rape forage	0.01	0.04	0.08	0.37	
Silage (alfalfa, clover, grass and maize)	0.01	0.04	0.08	0.37	
Cabbage, kale	0.03	0.08	0.05	0.12	Rotational crop, lettuce
Beet leaves (sugar and fodder)	0.03	0.08	0.05	0.12	
Hay (clover, alfalfa, grass)	0.02	0.04	0.09	0.34	Rotational crop, barley field study straw
Cereals straws	0.02	0.04	0.09	0.34	
Cereal grain	0.01	0.01	0.33	0.63	Rotational crop, barley field study grain Whiteflour bran: PF 6.50
Pulses	0.01	0.01	1.55	2.3	Rotational crop, field peas
Roots and tubers	0.01	0.01	0.11	0.25	Rotational crop, potatoes
Oilseeds	0.01	0.01	0.08	0.15	Rotational crop, winter rape Oilseeds meal: PF 1.0
Apple pomace, wet	0.07 STMR-P 0.10	0.32	0.02 STMR-P 0.02	0.07	Residue trial P-factor of 1.49 for BYI 02960 and 0.75 for DFA

	BYI 02960		DFA		Source
	STMR [mg/kg]	HR [mg/kg]	STMR [mg/kg]	HR [mg/kg]	
grass	0.01	0.02	0.08	0.37	Rotation; Cereal green plant
Rape forage	0.01	0.02	0.08	0.37	Rotation; Cereal green plant
Cabbage, kale	0.03	0.08	0.05	0.12	Rotation; leafy crops
Beet leaves (sugar and fodder)	0.03	0.08	0.05	0.12	Rotation; leafy crops
Silage (alfalfa, clover, grass and maize)	0.01	0.02	0.08	0.37	Rotation; Cereal green plant
Hay (clover, alfalfa, grass)	0.02	0.04	0.09	0.34	Rotation; cereal straw
Cereals straws	0.02	0.04	0.09	0.34	Rotation; cereal straw
Cereal grain	0.01	0.01	0.33	0.63	Rotation: cereal grain
Pulses	0.01	0.01	1.6	2.3	Rotation; pulses
Roots and tubers	0.01	0.01	0.11	0.25	Rotation; roots and tubers
Oilseeds	0.01	0.01	0.08	0.15	Rotation; oilseeds

	BYI 02960		DFA		Source
	STMR [mg/kg]	HR [mg/kg]	STMR [mg/kg]	HR [mg/kg]	
Apple pomace, wet	0.10	-	0.015		Residue trial

The maximum estimated dietary burden of separate parent BYI 02960 and metabolite DFA residues in livestock commodities, based on EU crop residue data and the European dietary burden calculator, are presented for poultry and cattle below in table 7.12.2-4. To maintain a uniform approach, the commodities in their ratio from the total BYI 02960 residues in table 7.2-2 were used, even though the EFSA dietary burden calculator would selected other commodities in different ratios based on the input values of DFA and BYI 02960 separately.

Table 7.12.2-4: Theoretical residues resulting from feeding BYI 02960 and DFA to poultry and cattle in a ratio relevant to the actual residues expected in feed commodities

Crop	Highest Residue levels (mg/kg)			Levels in dry matter		DM intake (%)	Dietary burden	
	total res.*	BYI 02960	DFA	% in crop	residue (mg/kg)		mg/kg bw/d	(mg/kg feed)
Cattle								
Turnips	0.26	0.01	-	10	0.10	30	0.001	0.030
Wheat bran*	2.21	0.07	-	89	0.07	20	0.001	0.015
Grass (fresh)	0.39	0.01	-	20	0.05	50	0.001	0.025
Subtotal BYI 02960:		0.11					**0.003**	**0.054**
Turnips	0.26	-	0.25	10	2.50	30	0.027	0.750
Wheat bran*	2.21	-	2.15	89	2.41	20	0.018	0.482
Grass (fresh)	0.39	-	0.37	20	1.85	50	0.034	0.925
Subtotal DFA:		-	2.77	-	-	100	**0.078**	**2.157**
Totals:		0.085	2.77	-	-	100	**0.081**	**2.227**
Poultry								
Turnips	0.26	0.01	-	10	0.10	20	0.001	0.020
Wheat bran	2.21	0.07	-	89	0.07	15	0.001	0.011
Peas (dry)	2.31	0.01	-	86	0.01	30	0.000	0.003
Cabbage	0.2	0.08	-	14	0.57	5	0.002	0.029
Rape seed	0.16	0.01	-	86	0.01	10	0.000	0.001
Subtotal BYI 02960:		0.175				80	**0.004**	**0.064**
Turnips	0.26		0.25	10	2.50	20	0.032	0.500
Wheat bran	2.21		2.15	89	2.41	15	0.023	0.362
Peas (dry)	2.31		2.3	86	2.67	30	0.051	0.802
Cabbage	0.2		0.12	14	0.86	5	0.003	0.043
Rape seed	0.16		0.15	86	0.17	10	0.001	0.017
Subtotal DFA:			4.97			80	**0.109**	**1.724**
Totals:		0.175	4.97			80	**0.140**	**2.224**
Pigs								

Crop	Highest Residue levels (mg/kg)			Levels in dry matter		DM intake (%)	Dietary burden	
	total res.*	BYI 02960	DFA	% in crop	residue (mg/kg)		mg/kg bw/d	(mg/kg feed)
Rape forage	0.39	0.01		14	0.07	15	0.001	0.011
Turnips	0.26	0.01		10	0.10	60	0.004	0.060
Wheat bran	2.21	0.07		89	0.07	20	0.001	0.015
Peas (dry)	2.31	0.01		86	0.01	5	0.000	0.001
Subtotal BYI 02960:		0.095				100	**0.005**	**0.086**
Rape forage	0.39		0.37	14	2.64	15	0.025	0.396
Turnips	0.26		0.25	10	2.50	60	0.095	1.500
Wheat bran	2.21		2.15	89	2.41	20	0.030	0.482
Peas (dry)	2.31		2.30	86	2.67	5	0.008	0.134
Subtotal DFA:			5.07		-	100	**0.164**	**2.598**
Totals:		0.095	5.07		-	100	**0.170**	**2.684**

* obtained from rotational crop studies: total of BYI 02960, DFA and DFEAF. Residue levels below LOQ are reported as 0.01 mg/kg

Crop	Highest Residue levels (mg/kg)			Levels in dry matter		DM intake (%)	Dietary burden	
	total res.*	BYI 02960	DFA	% in crop	residue (mg/kg)		mg/kg bw/d	(mg/kg feed)
Poultry								
Turnips	0.27	0.01		10	0.10	20	0.001	0.020
Wheat grain	0.65	0.01		86	0.01	45	<0.001	0.002
Peas (dry)	2.3	0.01		86	0.01	30	<0.001	0.003
Cabbage	0.2	0.08		10	0.57	5	0.002	0.029
Subtotal BYI 02960:		0.11					**0.003**	**0.054**
Turnips	0.27		0.25	10	2.50	20	0.032	0.500
Wheat grain	0.65		0.63	89	2.45	45	0.011	0.173
Peas (dry)	2.3		2.3 (1.5)	86	1.74	30	0.033	0.052
Cabbage	0.2		0.12	10	0.86	5	0.003	0.043
Subtotal DFA:			3.30				**0.078**	**1.24**
Totals:		0.11	3.30			100	**0.082**	**1.29**
Cattle								
Turnip	0.27	0.01		10	0.10	60	0.003	0.060
Rape forage	0.41	0.02		20	0.10	35	0.002	0.050
Pea (dry))	2.3	0.01		86	0.01	5	<0.001	0.005
Subtotal BYI 02960:		0.03					**0.005**	**0.115**
Turnip	0.27		0.25	10	2.50	60	0.064	1.5
Rape forage	0.41		0.37	20	1.85	35	0.040	0.925
Pea (dry))	2.3		2.3 (1.5)	86	1.74	5	0.004	0.087
Subtotal DFA:			2.80				**0.108**	**2.512**

Crop	Highest Residue levels (mg/kg)			Levels in dry matter		DM intake (%)	Dietary burden	
	total res.*	BYI 02960	DFA	% in crop	residue (mg/kg)		mg/kg bw/d	(mg/kg feed)
Totals:		0.03	2.80			100	**0.113**	**2.627**
Pigs								
Rape forage	0.41	0.02		14	0.02	15	0.001	0.021
Turnips	0.27	0.01		10	0.10	60	0.002	0.060
Peas (dry)	2.3	0.01		86	0.01	25	<0.001	0.003
Subtotal BYI 02960:		0.04					**0.003**	**0.084**
Rape forage	0.41		0.37	14	2.64	15	0.016	0.396
Turnips	0.27		0.25	10	2.5	60	0.060	1.500
Peas (dry)	(2.3) 1.6		(2.3) 1.6	86	1.86	25	0.019	0.465
Subtotal DFA:			5.10				**0.095**	**2.361**
Totals:		0.04	5.10			100	**0.098**	**2.445**

Using the dietary burdens calculated in table 7.12.2-4 and the transfer factors derived from the livestock feeding studies and comparing the resulting levels to the residue levels recalculated to a 1N dose, the following MRLs are proposed by RMS.

Table 7.12.2-5: Theoretical residues resulting from feeding BYI 02960 and DFA to poultry and to dairy cows in a ratio relevant to the actual residues expected in feed commodities

Dietary burden (mg/ kg bw/d)	Matrix	Transfer factor*	Resulting residue (mg/kg)	Dietary burden (mg/ kg bw/d)	Transfer factor*	Resulting residue (mg/kg)	Sum of BYI 02960 and DFA (mg/kg)
chicken							
BYI 02960				*DFA*		-	
0.004	egg	0.57	<0.01	0.109‡	1.12	0.12	**0.12**
	muscle	0.93	<0.01		2.00	0.22	**0.22**
	fat	0.39	<0.01		0.81	0.09	**0.09**
	liver	1.14	<0.01		2.85	0.31	**0.32**
cattle							
BYI 02960			-	*DFA*		-	
0.003	milk	0.24	<0.01	0.078	0.77	0.06	**0.06**
	muscle	0.35	<0.01		2.49	0.19	**0.20**
	fat	0.23	<0.01		2.11	0.16	**0.17**
	liver	0.92	<0.01		2.30	0.18	**0.18**
	kidney	0.98	<0.01		3.26	0.25	**0.26**
pigs							
BYI 02960			-	*DFA*		-	
0.005	muscle	0.35	<0.01	0.164	2.49	0.41	**0.41**
	fat	0.23	<0.01		2.11	0.35	**0.35**
	liver	0.92	<0.01		2.30	0.38	**0.38**
	kidney	0.98	<0.01		3.26	0.53	**0.54**

~~* for derivation of transfer factors, see 7.8.1, Transfer factors calculated on basis of dietary burden in mg/kg bw/d were used. when deriving factors, residue values of <LOQ were calculated as being at the LOQ, thus representing a worst case for exposure~~
~~‡ based on this dietary burden, the transfer factor chosen for DFA reflects the closest exposure rate, 1.724 1.45 mg/kg feed~~

~~Dietary burden (mg/ kg bw/d)~~	~~Matrix~~	~~Transfer factor*~~	~~Resulting residue (mg/kg)~~	~~Dietary burden (mg/ kg bw/d)~~	~~Transfer factor*~~	~~Resulting residue (mg/kg)~~	~~Sum of BYI 02960 and DFA (mg/kg)~~
~~chicken~~							
~~*BYI 02960*~~				~~*DFA*~~		~~-~~	
~~0.003‡~~	~~egg~~	~~0.57~~	~~<0.01~~	~~0.078~~	~~1.47~~	~~0.11~~	~~**0.12**~~
	~~muscle~~	~~0.93~~	~~<0.01~~		~~2.59~~	~~0.20~~	~~**0.21**~~
	~~fat~~	~~0.39~~	~~<0.01~~		~~0.91~~	~~0.07~~	~~**0.07**~~
	~~liver~~	~~1.14~~	~~<0.01~~		~~3.25~~	~~0.25~~	~~**0.26**~~
~~cattle~~							
~~*BYI 02960*~~			~~-~~	~~*DFA*~~		~~-~~	
~~0.005‡~~	~~milk~~	~~0.24~~	~~<0.01~~	~~0.108~~	~~0.77~~	~~0.08~~	~~**0.08**~~
	~~muscle~~	~~0.35~~	~~<0.01~~		~~2.49~~	~~0.27~~	~~**0.27**~~
	~~fat~~	~~0.23~~	~~<0.01~~		~~2.11~~	~~0.23~~	~~**0.23**~~
	~~liver~~	~~0.92~~	~~<0.01~~		~~2.30~~	~~0.25~~	~~**0.25**~~
	~~kidney~~	~~0.98~~	~~<0.01~~		~~3.26~~	~~0.35~~	~~**0.36**~~
~~pigs~~							
~~*BYI 02960*~~			~~-~~	~~*DFA*~~		~~-~~	
~~0.003‡~~	~~muscle~~	~~0.35~~	~~<0.01~~	~~0.095~~	~~2.49~~	~~0.24~~	~~**0.24**~~
	~~fat~~	~~0.23~~	~~<0.01~~		~~2.11~~	~~0.20~~	~~**0.20**~~
	~~liver~~	~~0.92~~	~~<0.01~~		~~2.30~~	~~0.22~~	~~**0.22**~~
	~~kidney~~	~~0.98~~	~~<0.01~~		~~3.26~~	~~0.31~~	~~**0.31**~~

Table 7.12.2-6 Proposed MRLs in animal commodities

Commodity	MRL proposal (mg/kg)		~~MRL proposal (mg/kg)~~
	Flupyradifurone	DFA, expressed as DFA	
eggs	0.01*	0.03	~~0.15~~
poultry meat (muscle)	0.01*	0.05	~~0.20~~
poultry fat	0.01*	0.03	~~0.09 0.07~~
poultry liver/offal	0.01*	0.1	~~0.30~~
milk	0.01*	0.03	~~0.06 0.08~~
Ruminant, pig and horse meat (muscle)	0.01*	0.1	~~0.20 0.30~~
Ruminant, pig and horse fat	0.01*	0.1	~~0.20 0.30~~
Ruminant, pig and horse liver	0.01*	0.1	~~0.20 0.30~~
Ruminant, pig and horse kidney	0.01*	0.15	~~0.30 0.40~~
other ruminant, horse and pig offal	0.01*	0.15	~~0.30 0.40~~

~~Pig meat (muscle)~~			~~0.40 0.30~~
~~Pig fat~~			~~0.40 0.20~~
~~Pig liver~~			~~0.40 0.30~~
~~Pig kidney~~			~~0.6 0.30~~
~~Other pig offal~~			~~0.6 0.30~~

Note there is a slight difference is MRLs proposed by the notifier and the RMS

B.7.12.3 Summary of proposed MRLs

Table 7.12.3-1 Summary of proposed MRLs

Crop	MRL proposal (mg/kg)		Commodity	MRL proposal (mg/kg)	
	Flupyradifurone	DFA, expressed as DFA		Flupyradifurone	DFA, expressed as DFA
Lettuce	9	0.1	eggs	0.01*	0.03
Hops	4	0.4	poultry meat (muscle)	0.01*	0.05
Rotational root and tuber vegetables	0.01	0.09	poultry fat	0.01*	0.03
Rotational stem vegetables	0.01	0.08	poultry liver/offal	0.01*	0.1
Rotational bulb vegetables	0.01	0.06	milk	0.01*	0.03
Rotational fruiting vegetables	0.01	0.15	Ruminant, pig and horse meat (muscle)	0.01*	0.1
Rotational legume vegetables	0.01	0.4	Ruminant, pig and horse fat	0.01*	0.1
Rotational leafy vegetables	0.08	0.04	Ruminant, pig and horse liver	0.01*	0.1
Rotational cereals and sweet corn	0.01	0.3	Ruminant, pig and horse kidney	0.01*	0.15
Rotational pulses	0.01	0.8	other ruminant, horse and pig offal	0.01*	0.15
Rotational oilseeds	0.01	0.05			

~~Crop~~	~~MRL proposal (mg/kg)~~	~~Commodity~~	~~MRL proposal (mg/kg)~~
~~Lettuce~~	~~9~~	~~eggs~~	~~0.15~~
~~Hops~~	~~5~~	~~poultry meat (muscle)~~	~~0.20~~
~~Rotational root and tuber vegetables~~	~~0.6~~	~~poultry fat~~	~~0.09 0.07~~
~~Rotational stem vegetables~~	~~0.5~~	~~poultry liver/offal~~	~~0.30~~
~~Rotational bulb vegetables~~	~~0.4~~	~~milk~~	~~0.06 0.08~~

~~Rotational fruiting vegetables~~	~~0.9~~	~~Ruminant and horse meat (muscle)~~	~~0.20 0.30~~
~~Rotational legume vegetables~~	~~2.0~~	~~Ruminant and horse fat~~	~~0.20 0.30~~
~~Rotational leafy vegetables~~	~~0.4~~	~~Ruminant and horse liver~~	~~0.20 0.30~~
~~Rotational cereals and sweet corn~~	~~1.5~~	~~Ruminant and horse kidney~~	~~0.30 0.40~~
~~Rotational pulses~~	~~5~~	~~other ruminant, horse and pig offal~~	~~0.30 0.40~~
~~Rotational oilseeds~~	~~0.4~~	~~Pig meat (muscle)~~	~~0.40 0.30~~
		~~Pig fat~~	~~0.40 0.20~~
		~~Pig liver~~	~~0.40 0.30~~
		~~Pig kidney~~	~~0.6 0.30~~
		~~Other pig offal~~	~~0.6 0.30~~

B.7.13 Proposed EU Import tolerances and justification for the acceptability of those residues

Flupyradifurone is to be registered worldwide and undergoing a Global Joint Review in the USA, Canada, Mexico, Brazil, and Australia.

~~Therefore, a~~ A large number of import tolerances have been applied for in the MRL application, parallel to the peer review of flupyradifurone. It was however decided during the peer review, not to consider these Its until they have een established as MRLs in the country of origin.

~~Crop~~	~~MRL proposal (mg/kg)~~	~~Commodity~~	~~MRL proposal (mg/kg)~~
~~Citrus fruits~~	~~3~~	~~Egg~~	~~0.5~~
~~Almonds~~	~~0.15~~	~~Poultry muscle~~	~~0.8~~
~~Pecan~~	~~0.15~~	~~Poultry fat~~	~~0.3~~
~~Peanut~~	~~0.15~~	~~Poultry liver/offal~~	~~1.0~~
~~Apple~~	~~1.5~~	~~Milk~~	~~0.3~~
~~Pear~~	~~1.5~~	~~Cattle muscle~~	~~1.0~~
~~Grape~~	~~3~~	~~Cattle fat~~	~~0.5~~
~~Blueberry~~	~~4~~	~~Cattle liver~~	~~2.0~~
~~Prickly pear~~	~~0.7~~	~~Cattle kidney~~	~~2.0~~
~~Tomato~~	~~3~~	~~Catlle offal~~	~~2.0~~
~~Chilli pepper~~	~~3~~		
~~Sweet corn~~	~~0.4~~		
~~Celery~~	~~40~~		
~~Dry bean seed~~	~~10~~		
~~Dry pea seed~~	~~10~~		
~~Soy bean seed~~	~~4~~		
~~Barley, sorghum and wheat grain~~	~~4~~		

~~Field corn grain~~	~~0.5~~		
~~Coffee~~	~~2~~		
~~hops~~	~~20~~		
~~Rotational bulb vegetable~~	~~0.8~~		
~~Rotational oilseeds~~	~~0.8~~		
~~Rotational tuber vegetables~~	~~1.5~~		

B.7.14 Basis for differences, if any, in conclusions reached having regard to established or proposed CAC MRLs

Not applicable, because there are no established or proposed CAC MRLs.

B.7.15 Estimates of potential and actual dietary exposure through diet and other means (IIA 6.9; IIIA 8.8)

B.7.15.1 Intakes by domestic animals

The supported use on hops and lettuce are not relevant for intake by domestic animals. However, as flupyradifurone and its metabolites DFEAF and DFA occur in succeeding crops, exposure to livestock from the use of flupyradifurone on lettuce can occur.

Based on the metabolism studies in laying hen and lactating ruminants, a residue definition as the sum of flupyradifurone and metabolite DFA, expressed as flupyradifurone is proposed for risk assessment and for monitoring flupyradifurone and DFA, expressed as DFA, separately.

A livestock feeding study was performed in lactating cows and laying hens. As the animals were dosed only with the parent, whilst exposure to DFA can occur via feed, transfer factors were calculated to estimate the amount of DFA in products of animal origin in practice when livestock is also exposed to DFA. These transfer factors were not used for MRL proposal.

Based on the livestock feeding studies and estimated exposure, MRLs in animal products are proposed. See B.7.8.1 and B.7.12.2.

B.7.15.2 Intakes by humans (long-term)

Chronic exposure for the consumer was estimated using the input values in table 7.15.2-01~~, including the import tolerances~~ (see also Evaluation report on flupyradifurone), the ADI of 0.064 ~~0.08~~ ~~0.12~~ mg/kg bw/d and EFSAs PRIMo model rev.2. The IEDI ~~TMDI~~ uses maximally 15.1% ~~47.3~~ ~~75.6~~% of the ADI for the WHO Cluster diet B. All other European (national) diets use less of the ADI. The 5 highest outcomes are found in Table 7.15.2-02.

Table 7.15.2-01 Input values for the consumer risk assessment

Commodity	Chronic risk assessment		Acute risk assessment	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
Flupyradifurone + difluoroacetic acid, expressed as flupyradifurone equivalents				

Commodity	Chronic risk assessment		Acute risk assessment	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
Apple	0.09	STMR	0.36	HR
Pear	0.09	STMR	0.36	HR
Grape	0.26	STMR	0.51	HR
0210000 Root & tuber vegetables	0.08	STMR, rotation	0.26	HR, rotation
0220000 Bulb vegetables	0.07	STMR, rotation	0.17	HR, rotation
Tomato	0.31	STMR, rotation	0.47	HR primary crop
Egg plant	0.31	STMR, rotation	0.47	HR primary crop
pepper	0.31	STMR, rotation	0.60	HR primary crop
Other solanacea	0.31	STMR, rotation	0.42	HR, rotation
Cucumber, gherkin	0.31	STMR, rotation	0.73	HR primary crop
zucchini (courgette)	0.31	STMR, rotation	0.73	HR primary crop
Other cucurbits, edible peel	0.31	STMR, rotation	0.42	HR, rotation
Cucurbits, inedible peel	0.31	STMR, rotation	0.42	HR, rotation
Watermelon	0.31	STMR, rotation	0.42	HR, rotation
Sweet corn	0.34	STMR, rotation	0.64	HR, rotation
Other fruiting vegetables	0.31	STMR, rotation	0.42	HR, rotation
024000 Brassicacaea	0.06	STMR, rotation	0.20	HR, rotation
Lettuce	2.12	STMR, primary	4.59	HR, primary: 6.04 PF: 0.76 inner leaves
All others of group 0250000 Leaf vegetables & fresh herbs	0.06	STMR, rotation	0.20	HR, rotation
0260000 Legumes	0.49	STMR, rotation	1.11	HR, rotation
0270000 Stem vegetables~~, except celery~~	0.08	STMR, rotation	0.24	HR, rotation
Pulses, dry	1.56	STMR, rotation	2.31	HR, rotation
Fungi	0.03*	Proposed MRL	0.03*	Proposed MRL
Oilseeds	0.09	STMR, rotation	0.16	HR, rotation
Cereals	0.34	STMR, rotation	0.64	HR, rotation
hops	1.05	STMR, primary	0.04	HR-P (beer)
Sugar beet	0.12	STMR, rotation	0.26	HR, rotation
Chicory root	0.12	STMR, rotation	0.26	HR, rotation
Egg	0.07	STMR	0.09	HR
Poultry muscle	0.12	STMR	0.16	HR
Poultry fat	0.05	STMR	0.07	HR

Commodity	Chronic risk assessment		Acute risk assessment	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
Poultry liver	0.18	STMR	0.23	HR
Milk	0.03	STMR	0.08	HR
Ruminant, pig and horse meat (muscle)	0.14	STMR	0.28	HR
Ruminant, pig and horse fat	0.14	STMR	0.29	HR
Ruminant, pig and horse liver	0.14	STMR	0.29	HR
Ruminant, pig and horse kidney	0.20	STMR	0.41	HR
other ruminant, horse and pig offal	0.20	STMR	0.41	HR
All other commodities	0.03*	Perennial crops	0.03*	Perennial crops

~~Commodity~~	~~Chronic risk assessment~~		~~Acute risk assessment~~	
	~~Input value (mg/kg)~~	~~Comment~~	~~Input value (mg/kg)~~	~~Comment~~
~~Flupyradifurone + difluoroacetic acid, expressed as flupyradifurone equivalents~~				
~~Apple~~	~~0.4~~	~~Proposed MRL~~	~~0.36~~	~~HR~~
~~Pear~~	~~0.4~~	~~Proposed MRL~~	~~0.36~~	~~HR~~
~~Grape~~	~~1~~	~~Proposed MRL~~	~~0.51~~	~~HR~~
~~0210000 Root & tuber vegetables~~	~~0.6~~	~~Proposed MRL, rotation~~	~~0.6~~	~~Proposed MRL, rotation~~
~~0220000 Bulb vegetables~~	~~0.4~~	~~Proposed MRL, rotation~~	~~0.4~~	~~Proposed MRL, rotation~~
~~Tomato~~	~~0.9~~	~~Proposed MRL, rotation~~	~~0. 9~~	~~Proposed MRL, rotation HR: 0.49~~
~~Egg plant~~	~~0.9~~	~~Proposed MRL, rotation~~	~~0. 9~~	~~Proposed MRL, rotation HR: 0.49~~
~~pepper~~	~~0.9~~	~~Proposed MRL~~	~~0.9~~	~~Proposed MRL, rotation HR: 0.60~~
~~Other solanacea~~	~~0.9~~	~~Proposed MRL, rotation~~	~~0.9~~	~~Proposed MRL, rotation~~
~~Cucumber, gherkin~~	~~1.5~~	~~Proposed MRL~~	~~0.9~~	~~Proposed MRL, rotation HR: 0.73~~
~~zucchini (courgette)~~	~~1.5~~	~~Proposed MRL~~	~~0.9~~	~~Proposed MRL, rotation HR: 0.73~~
~~Other cucurbits, edible peel~~	~~0.9~~	~~Proposed MRL, rotation~~	~~0.9~~	~~Proposed MRL, rotation~~

Commodity	Chronic risk assessment		Acute risk assessment	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
Cucurbits, inedible peel	0.9	Proposed MRL, rotation	0.9	Proposed MRL, rotation, PF: 0.7 HR: 0.73
Watermelon	0.9	Proposed MRL, rotation	0.9	Proposed MRL, rotation, PF 0.7 HR: 0.30
Sweet corn	1.5	Proposed MRL, rotation	1.5	Proposed MRL, rotation
Other fruiting vegetables	0.9	Proposed MRL, rotation	0.9	Proposed MRL, rotation
024000 Brassicacaea	0.4	Proposed MRL, rotation	0.4	Proposed MRL, rotation
Lettuce	9	Proposed MRL	4.56	HR-P 6.0 x 0.76
All others of group 0250000 Leaf vegetables & fresh herbs	0.4	Proposed MRL, rotation	0.4	Proposed MRL, rotation
0260000 Legumes	2	Proposed MRL, rotation	2	Proposed MRL, rotation
0270000 Stem vegetables, except celery	0.5	Proposed MRL, rotation	0.5	Proposed MRL, rotation
Pulses, dry	5	Proposed MRL, rotation	5	Proposed MRL, rotation
Fungi	0.03*	Proposed MRL	0.03*	Proposed MRL
Oilseeds	0.3	Proposed MRL, rotation	0.3	Proposed MRL, rotation
Cereals	1.5	Proposed MRL, rotation	1.5	Proposed MRL, rotation
hops	5	Proposed MRL (DAR, 2014)	0.04	HR-P (beer)
Sugar beet	0.6	Proposed MRL, rotation	0.6	Proposed MRL, rotation
Chicory root	0.6	Proposed MRL, rotation	0.6	Proposed MRL, rotation
Egg	0.15	Proposed MRL	0.15	Proposed MRL
Poultry muscle	0.20	Proposed MRL	0.20	Proposed MRL
Poultry fat	0.09	Proposed MRL	0.09	Proposed MRL
Poultry liver	0.30	Proposed MRL	0.30	Proposed MRL
Milk	0.06	Proposed MRL	0.06	Proposed MRL
Cattle muscle	0.20	Proposed MRL	0.20	Proposed MRL
Cattle fat	0.20	Proposed MRL	0.20	Proposed MRL
Cattle liver	0.20	Proposed MRL	0.20	Proposed MRL
Cattle kidney	0.30	Proposed MRL	0.30	Proposed MRL
Cattle offal	0.30	Proposed MRL	0.30	Proposed MRL

Commodity	Chronic risk assessment		Acute risk assessment	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
Goat and sheep milk	0.06	Proposed MRL	0.06	Proposed MRL
Other ruminant and horse muscle	0.20	Proposed MRL	0.20	Proposed MRL
Other ruminant and horse fat	0.20	Proposed MRL	0.20	Proposed MRL
Other ruminant and horse liver	0.20	Proposed MRL	0.20	Proposed MRL
Other ruminant and horse kidney	0.30	Proposed MRL	0.30	Proposed MRL
Other ruminant and horse offal	0.30	Proposed MRL	0.30	Proposed MRL
Pig muscle	0.4	Proposed MRL	0.4	Proposed MRL
Pig fat	0.4	Proposed MRL	0.4	Proposed MRL
Pig liver	0.4	Proposed MRL	0.4	Proposed MRL
Pig kidney	0.6	Proposed MRL	0.6	Proposed MRL
Pig offal	0.6	Proposed MRL	0.6	Proposed MRL
All other commodities	0.03*	Perennial crops	0.03*	Perennial crops

Commodity	Chronic risk assessment		Acute risk assessment	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
Flupyradifurone + difluoroacetic acid, expressed as flupyradifurone equivalents				
Citrus	3	MRL - IT	2.2	HR - IT
Almonds	0.15	MRL - IT	0.15	MRL - IT
Pecan	0.15	MRL - IT	0.15	MRL - IT
Peanut	0.15	MRL - IT	0.15	MRL - IT
Apple	1.5	MRL - IT	1.5	MRL - IT
Pear	1.5	MRL - IT	1.5	MRL - IT
Grape	3	MRL - IT	3	MRL - IT
Blueberry	4	MRL - IT	4	MRL - IT
Prickly pear	0.7	MRL - IT	0.7	MRL - IT
0210000 Root & tuber vegetables	1.5	MRL-IT, rotation	0.6	MRL, rotation
0220000 Bulb vegetables	0.8	MRL-IT, rotation	0.4	MRL, rotation
Tomato	3	MRL -IT	3	MRL -IT
Egg plant	3	MRL -IT	3	MRL -IT
pepper	3	MRL – IT Chili pepper	3	MRL – IT Chili pepper
Other solanacea	0.9	MRL, rotation	0.9	MRL, rotation
Cucumber, gherkin	1.5	MRL	1.5	MRL

Commodity	Chronic risk assessment		Acute risk assessment	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
zucchini (courgette)	1.5	MRL	1.5	MRL
Other cucurbits, edible peel	0.9	MRL, rotation	0.9	MRL, rotation
Cucurbits, inedible peel	0.9	MRL, rotation	0.9	MRL, rotation
Watermelon	0.9	MRL, rotation	0.9	MRL, rotation
Sweet corn	1.5	MRL, rotation	1.5	MRL, rotation
Other fruiting vegetables	0.9	MRL, rotation	0.9	MRL, rotation
024000 Brassicacaea	0.4	MRL, rotation	0.4	MRL, rotation
Lettuce	9	MRL (DAR, 2013)	9	MRL (DAR, 2013)
All others of group 0250000 Leaf vegetables & fresh herbs	0.4	MRL, rotation	0.4	MRL, rotation
0260000 Legumes	2	MRL, rotation	2	MRL, rotation
0270000 Stem vegetables, except celery	0.5	MRL, rotation	0.5	MRL, rotation
Celery	40	MRL- IT	6.1	HR- IT
Other pulses, dry	10	MRL - IT	10	MRL- IT
Peas (pulses, dry)	10	MRL - IT	10	MRL- IT
Fungi	0.03*	MRL	0.03*	MRL
Oilseeds	0.90	MRL - IT	0.90	MRL - IT
Soy bean seed	4	MRL- IT	4	MRL- IT
Barley, sorghum and wheat grain	4	MRL- IT	4	MRL- IT
Other cereals	1.5	MRL, rotation	1.5	MRL, rotation
hops	20	MRL- IT	20	MRL- IT
Coffee	2	MRL- IT	2	MRL- IT
Sugar beet	0.6	MRL, rotation	0.6	MRL, rotation
Chicory root	0.6	MRL, rotation	0.6	MRL, rotation
Egg	0.5	IT-MRL	0.15	MRL
Poultry muscle	0.8	IT-MRL	0.20	MRL
Poultry fat	0.3	IT-MRL	0.07	MRL
Poultry liver	0.3	IT-MRL	0.30	MRL
Milk	1.0	IT-MRL	0.15	MRL
Cattle muscle	1.0	IT-MRL	0.50	MRL Cattle: cow, goat, sheep, horses

~~Commodity~~	~~Chronic risk assessment~~		~~Acute risk assessment~~	
	~~Input value (mg/kg)~~	~~Comment~~	~~Input value (mg/kg)~~	~~Comment~~
~~Cattle fat~~	~~0.5~~	~~IT-MRL~~	~~0.40~~	~~MRL Cattle: cow, goat, sheep, horses~~
~~Cattle liver~~	~~2.0~~	~~IT-MRL~~	~~0.40~~	~~MRL Cattle: cow, goat, sheep, horses~~
~~Cattle kidney~~	~~2.0~~	~~IT-MRL~~	~~0.60~~	~~MRL Cattle: cow, goat, sheep, horses~~
~~Cattle offal~~	~~2.0~~	~~IT-MRL~~	~~2.0~~	~~IT-MRL~~
~~Goat and sheep milk~~	~~0.15~~	~~MRL~~	~~0.15~~	~~MRL~~
~~Other ruminant and horse muscle~~	~~0.30~~	~~MRL goat, sheep, horses~~	~~0.30~~	~~MRL goat, sheep, horses~~
~~Other ruminant and horse fat~~	~~0.30~~	~~MRL goat, sheep, horses~~	~~0.30~~	~~MRL goat, sheep, horses~~
~~Other ruminant and horse liver~~	~~0.30~~	~~MRL goat, sheep, horses~~	~~0.30~~	~~MRL goat, sheep, horses~~
~~Other ruminant and horse kidney~~	~~0.40~~	~~MRL goat, sheep, horses~~	~~0.40~~	~~MRL goat, sheep, horses~~
~~Other ruminant and horse offal~~	~~0.40~~	~~MRL goat, sheep, horses~~	~~0.40~~	~~MRL goat, sheep, horses~~
~~Pig muscle~~	~~0.30~~	~~MRL~~	~~0.30~~	~~MRL~~
~~Pig fat~~	~~0.20~~	~~MRL~~	~~0.20~~	~~MRL~~
~~Pig liver~~	~~0.30~~	~~MRL~~	~~0.30~~	~~MRL~~
~~Pig kidney~~	~~0.30~~	~~MRL~~	~~0.30~~	~~MRL~~
~~All other commodities~~	~~0.04*~~	~~Perennial crops~~	~~0.03*~~	~~Perennial crops~~

(*): Indicates that the MRL is set at the limit of analytical quantification.

Table 7.15.2-02 Worst-case results of the TMDI calculation in PRIMo model rev.2

Highest calculated TMDI values in % of ADI	MS Diet
15.1	WHO Cluster diet B
12.1	UK Toddler
9.8	NL child
9.5	IE adult
9.5	UK Infant

~~Highest calculated TMDI values in % of ADI~~	~~MS Diet~~
~~47,3~~	~~WHO Cluster diet B~~
~~31,6~~	~~DK child~~

~~30,9~~	~~NL child~~
~~29,4~~	~~IE adult~~
~~28,4~~	~~DE child~~

~~**Highest calculated TMDI values in % of ADI**~~	~~**MS Diet**~~
~~75.6~~	~~WHO Cluster diet B~~
~~60.1~~	~~DE child~~
~~59.2~~	~~NL child~~
~~50.4~~	~~UK Toddler~~
~~48.6~~	~~IE adult~~

Two ground water metabolites were identified, DFA and 6-~~CAN~~ CNA. The intake of these metabolites that can reach the ground water (and thus possibly occur in drinking water) was assumed to be insignificant as was discussed under B.6.9.6 (assessment of toxicological relevance of metabolites) and B.8.6.3.

Figure 7.15.2-1 Outcomes of chronic consumer intake calculations using EFSA Primo rev 2

flupyradifurone

Status of the active substance:	pending	Code no.	
LOQ (mg/kg bw):	0,03	proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	**0,064**	ARfD (mg/kg bw):	**0,15**
Source of ADI:	**peer review**	Source of ARfD:	**peer review**
Year of evaluation:	**2014**	Year of evaluation:	**2014**

Prepare workbook for refined calculations

Undo refined calculations

The risk assessment has been performed on the basis of the MRLs collected from Member States in April 2006. For each pesticide/commodity the highest national MRL was identified (proposed temporary MRL = pTMRL).
The pTMRLs have been submitted to EFSA in September 2006.

Chronic risk assessment

TMDI (range) in % of ADI minimum	maximum
2	15

No of diets exceeding ADI: ---

Highest calculated TMDI values in % of ADI	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	pTMRLs at LOQ (in % of ADI)
15,1	WHO Cluster diet B	6,3	CEREALS	1,9	Solanacea	1,2	Lettuce	0,5
12,1	UK Toddler	4,3	Sugar beet (root)	2,4	CEREALS	2,0	PULSES, DRY	1,2
9,8	NL child	3,0	CEREALS	1,4	Milk and cream,	0,9	Apples	1,7
9,5	IE adult	3,5	CEREALS	1,3	PULSES, DRY	0,5	Legume vegetables (fresh)	0,5
9,5	UK Infant	2,4	CEREALS	1,9	Sugar beet (root)	1,8	Milk and cream,	2,0
9,2	DK child	5,5	CEREALS	0,8	Cucurbits - edible peel	0,6	Milk and cream,	0,7
8,9	DE child	3,0	CEREALS	1,7	Apples	0,7	Milk and cream,	1,1
8,7	FR toddler	1,9	Milk and cream,	1,6	CEREALS	1,1	Legume vegetables (fresh)	2,1
8,7	ES child	2,8	CEREALS	1,4	Lettuce	1,2	PULSES, DRY	0,8
8,2	WHO cluster diet D	4,5	CEREALS	0,6	Root and tuber vegetables	0,6	Solanacea	0,3
8,0	WHO cluster diet E	3,2	CEREALS	0,7	Root and tuber vegetables	0,7	Wine grapes	0,3
7,4	WHO regional European diet	2,0	CEREALS	1,2	Lettuce	0,6	Solanacea	0,4
7,4	IT kids/toddler	4,5	CEREALS	1,0	Lettuce	0,8	Solanacea	0,1
7,1	WHO Cluster diet F	2,9	CEREALS	1,0	Lettuce	0,5	Root and tuber vegetables	0,3
6,4	ES adult	1,8	Lettuce	1,7	CEREALS	0,5	PULSES, DRY	0,4
6,2	PT General population	2,9	CEREALS	1,0	Wine grapes	0,6	PULSES, DRY	0,1
5,8	SE general population 90th percentile	2,7	CEREALS	0,7	Root and tuber vegetables	0,6	Milk and cream,	0,8
5,7	IT adult	2,7	CEREALS	1,3	Lettuce	0,7	Solanacea	0,1
5,6	FR infant	1,2	Milk and cream,	0,9	Root and tuber vegetables	0,8	Legume vegetables (fresh)	1,3
5,4	FR all population	1,8	CEREALS	1,6	Wine grapes	0,3	Lettuce	0,2
5,3	UK vegetarian	1,3	CEREALS	1,0	PULSES, DRY	0,7	Sugar beet (root)	0,2
4,8	NL general	1,5	CEREALS	0,4	Lettuce	0,4	Root and tuber vegetables	0,4
4,5	UK Adult	1,1	CEREALS	0,7	Sugar beet (root)	0,6	PULSES, DRY	0,2
3,6	LT adult	1,4	CEREALS	0,4	Root and tuber vegetables	0,3	Solanacea	0,2
3,6	DK adult	1,5	CEREALS	0,6	Wine grapes	0,3	Solanacea	0,3
2,6	FI adult	1,0	CEREALS	0,3	Milk and cream,	0,3	Lettuce	0,4
1,9	PL general population	0,5	Root and tuber vegetables	0,5	Solanacea	0,3	Apples	0,0

Conclusion:
The estimated Theoretical Maximum Daily Intakes (TMDI), based on pTMRLs were below the ADI.
A long-term intake of residues of flupyradifurone is unlikely to present a public health concern.

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B.7.15.3 National estimate for short term intake by humans (acute)

Acute exposure for the consumer was estimated using the proposed MRLs in table 7.15.2-1~~, including the import tolerances~~ and the ARfD of 0.15 ~~0.35~~ mg/kg bw and EFSAs PRIMo model rev.2. The NESTI uses maximally 82.3 ~~69.2~~% of the ARfD for the German children for lettuce. All other commodities use less than 100% of the ARfD. Since the EU uses do not result in an exceeded ARfD, a refinement of the acute intake calculations was performed, using the HR values and processing factors where available. ~~but the import tolerances for celery and oranges do.~~

~~The refined IESTI calculations show that the ARfD is taken up for maximally 81.8% for lettuce.~~

Refining the acute intake calculations by using the HRs, results in a safe use.

Figure 7.15.3-1 Outcomes of acute consumer intake calculations using EFSA Primo rev 2

Acute risk assessment /children | **Acute risk assessment / adults / general population**

The acute risk assessment is based on the ARfD.

For each commodity the calculation is based on the highest reported MS consumption per kg bw and the corresponding unit weight from the MS with the critical consumption. If no data on the unit weight was available from that MS an average European unit weight was used for the IESTI calculation.

In the **IESTI 1** calculation, the variability factors were 10, 7 or 5 (according to JMPR manual 2002), for lettuce a variability factor of 5 was used.
In the **IESTI 2** calculations, the variability factors of 10 and 7 were replaced by 5. For lettuce the calculation was performed with a variabilty factor of 3.

Threshold MRL is the calculated residue level which would leads to an exposure equivalent to 100 % of the ARfD.

Unprocessed commo

No of commodities for which ARfD/ADI is exceeded (IESTI 1):		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 2):**		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 1):**		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 2):**		---
IESTI 1	*)	**)	**IESTI 2**	*)	**)	**IESTI 1**	*)	**)	**IESTI 2**	*)	**)
Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)
82,3	Lettuce	4,59 / -	49,4	Lettuce	4,59 / -	33,6	Lettuce	4,59 / -	20,2	Lettuce	4,59 / -
42,5	Melons	0,42 / -	42,5	Melons	0,42 / -	14,8	Pumpkins	0,42 / -	14,8	Pumpkins	0,42 / -
34,2	Watermelons	0,42 / -	34,2	Watermelons	0,42 / -	13,1	Courgettes	0,73 / -	11,4	Watermelons	0,42 / -
31,3	Sweet corn	0,64 / -	28,5	Cucumbers	0,73 / -	11,4	Watermelons	0,42 / -	11,1	Melons	0,42 / -
28,5	Cucumbers	0,73 / -	28,1	Beans	2,31 / -	11,1	Melons	0,42 / -	10,8	Table grapes	0,51 / -

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No of critical MRLs (IESTI 1)	---	**No of critical MRLs (IESTI 2)**	---

Processed commo

No of commodities for which ARfD/ADI is exceeded:		---	**No of commodities for which ARfD/ADI is exceeded:**		---
		**)			**)
Highest % of ARfD/ADI	Processed commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Processed commodities	pTMRL/ threshold MRL (mg/kg)
12,2	Apple juice	0,36 / -	1,9	Bread/pizza	0,64 / -
11,2	Grape juice	0,51 / -	1,6	Apple juice	0,36 / -
7,4	Carrot, juice	0,26 / -	1,3	Wine	0,51 / -
5,5	Tomato juice	0,47 / -	0,6	Tomato (preserved-	0,47 / -
5,0	Wheat flour	0,64 / -	0,2	Orange juice	0,03 / -

*) The results of the IESTI calculations are reported for at least 5 commodities. If the ARfD is exceeded for more than 5 commodities, all IESTI values > 90% of ARfD are reported.
**) pTMRL: provisional temporary MRL
***) pTMRL: provisional temporary MRL for unprocessed commodity

Conclusion:

For flupyradifurone IESTI 1 and IESTI 2 were calculated for food commodities for which pTMRLs were submitted and for which consumption data are available.
No exceedance of the ARfD/ADI was identified for any unprocessed commodity.

For processed commodities, no exceedance of the ARfD/ADI was identified.

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B.7.16 Summary and evaluation of residue behaviour (IIA 6.10; IIIA 8.9)

Storage stability

Flupyradifurone, DFEAF, and DFA are stable in orange fruit (high acid content), spinach leaves and tomato fruit (high water content), wheat grain (high starch content), bean seed (high protein content), coffee bean (specific matrix), soybean seed (high oil content), and sugar cane (high water content) for at least 18 months when stored frozen.

DFA is stable in animal fat, kidney, liver, and muscle for at least 43 days, when stored frozen.

In the confined rotational crop studies, stability of flupyradifurone residues was additionally demonstrated for 18-24 months at ≤ -18 °C for Swiss chard and wheat straw.

Primary metabolism in crops

The metabolism of flupyradifurone (BYI 02960) was investigated in five target crops (tomato, potato, apple, cotton and rice) following application of [furanone-4-^{14}C] flupyradifurone and [pyridinylmethyl-^{14}C] flupyradifurone. Additionally, [ethyl-1-^{14}C] flupyradifurone was applied in one tomato metabolism study. In all other plant metabolism studies, the fate of the difluoroethane moiety of the active substance was tracked by analysing extracts of all RACs for non-radiolabelled difluoroacetic acid, the most plausible metabolite which can be formed from the moiety after cleavage of the parent compound. The analysis of difluoroacetic acid was initiated after detecting difluoroacetic acid as a major soil metabolite in the aerobic soil degradation studies.

The active substance was applied either as an SL formulation for drench and foliar application, an FS formulation for seed/tuber treatment or as a granule to cover the special application technique in rice in Japan.

The metabolism studies conducted in these five crops represent four different crop groups (fruiting crops, root and tuber vegetables, pulses and oilseeds and cereals) showed similar profiles, with the unchanged parent compound and metabolite difluoroacetic acid representing the major part of the residue.

The metabolism in all plants was very similar. The main reactions involved were:

- Oxidative cleavage of the difluoroethylamine bond and formation of difluoroacetic acid,
- complete degradation of the furanone moiety and incorporation of carbon atoms into the natural compound pool, e.g. into glucose/carbohydrates,
- cleavage of the pyridinylmethylamine bond and formation of BYI 02960-difluoroethylamino-furanone and BYI 02960-CHMP / BYI 02960-6-CNA,

- formation of BYI 02960-CHMP was followed by conjugation with carbohydrates and sulphate or by oxidation of the methylene group to a carboxylic group and subsequent conjugation with glycerol and glucuronic acid,
- hydroxylation of the furanone or the difluoroethyl moiety followed by conjugation with carbohydrates and sulphate, and
- oxidative degradation of the furanone moiety to an acetic acid group followed either by conjugation with a carbohydrate or by further oxidation or degradation of the moiety.
- Halogenation (bromination/chlorination) of the furanone moiety of the parent compound was most probably a process which took place in the soil.

Thus, oxidative cleavage of the molecule, oxidation and hydroxylation were the primary transformation steps followed by conjugation reactions with glycosides, sulphate and glucuronic acid.

Generally, these main metabolic reactions were also observed in the rat studies on absorption, distribution, metabolism and elimination (ADME). Parent compound and all major plant metabolites, or at least subsequent metabolites which imply the presence of the plant metabolites as transient metabolites were detected, although flupyradifurone shows a more moderate degradation behaviour in rats compared to plants.

Metabolism in rotational crops

The metabolism of flupyradifurone (BYI 02960) residues in the rotational crops wheat (small grain), Swiss chard (leafy crop) and turnip (root crop) was investigated at a nominal application rate of 400 g a.s./ha. The plant back intervals were 29, 135 and 296 days for all crops. The studies were conducted with [furanone-4-^{14}C]BYI 02960 and [pyridinylmethyl-^{14}C]BYI 02960. A study with [ethyl-1-^{14}C]BYI 02960 was not conducted and therefore non-radiolabelled difluoroacetic acid was determined in all extracts obtained in the CRC study conducted with [furanone-4-^{14}C]BYI 02960 to gain information on the fate of the difluoroethane moiety of the active substance.

Corresponding TRR values showed similar residue levels when comparing the studies performed with [furanone-4-^{14}C]BYI 02960 and [pyridinylmethyl-^{14}C]BYI 02960. Generally, the TRR values were slightly higher in the crops of 1st and 2nd rotation of the study performed with [pyridinylmethyl-^{14}C]BYI 02960, except for wheat grains, where it was the opposite way round due to the selective transport of glucose as a label specific metabolite into the RAC.

The levels of parent compound decreased with increasing plant back intervals but nevertheless parent formed a main portion of the residues in all of the RACs in all rotations, except for wheat grains. Other major metabolites (accounting for >10% of the TRR in at least one RAC in one rotation) were

- DFA, which was the main metabolite in wheat grains (all rotations) and in turnip roots (1st rotation) and a major metabolite in all other RACs

- 6-CNA-glucerol-gluA (3 isomers), which were major in and specific for all wheat commodities and accounted in sum for 11% to 33% of the TRR considering all rotations
- BYI 02960-difluoroethyl-amino-furanone, which was a major metabolite in all Swiss chard samples in all rotations and accounted for up to 17% of the TRR
- BYI 02960-OH-glyc, which was major in Swiss chard and turnip leaves in all rotations
- BYI 02960-glyoxylic acid, which was only a major metabolite in wheat forage, hay and straw and in turnip roots of the first rotation, in subsequent rotations it was detected in minor amounts, only
- BYI 02960-bromo-amino-furanone, which was only major in wheat hay of the 3rd rotation
- BYI 02960-OH, which was major in wheat grains of the 1st rotation, only
- Glucose/carbohydrates, which were identified as the main portion in wheat grains after exhaustive extraction and as a major metabolite in turnip roots after conventional extraction.

The proposed metabolic pathway is very similar to the one of primary crops. As observed in target plants the metabolic reactions involved were hydroxylation and oxidative cleavage of the active substance followed by additional oxidation and conjugation processes. No additional metabolic routes were detected, only additional metabolites were identified, e.g. additional conjugates or existing metabolites were subjected to further oxidation reactions.

Metabolism in livestock

The metabolism of flupyradifurone (BYI 02960) was investigated in laying hens and lactating goats following oral administration of [pyridinylmethyl-^{14}C] flupyradifurone and [furanone-4-^{14}C] flupyradifurone for both species.

The concentrations of the identified metabolites in the different matrices are basically in the same order of magnitude independent of the label employed. However, after administration of the furanone-labelled test compound, the concentrations of the total radioactivity in eggs and edible tissues of laying hens as well as in milk and fat of lactating goats are higher. This is attributable to the fact that the radioactivity originating from this label is incorporated into natural compounds like fatty acids in all poultry tissues and lactose in the milk of goats.

Notwithstanding these observations, the unchanged parent compound is a significant, if not the dominating constituent of the residue in milk, eggs and edible tissues of both species. Other metabolites determined in comparable concentrations are the natural compound lactose in the milk of goats after administration of [furanone-4-^{14}C]BYI 02960, and BYI 02960-acetyl-AMPC in eggs and tissues of poultry after administration of [pyridinylmethyl-^{14}C] BYI 02960.

The main metabolic reactions involved were:

- Hydroxylation in position 5 of the furanone ring forming BYI 02960-hydroxy followed by conjugation with sulfuric acid to BYI 02960-OH-SA
- Hydroxylation followed by conjugation with glucuronic acid forming two diastereomeric conjugates of BYI 02960-OH (BYI 02960-OH-gluA, isomer 2 and 3), the hydroxylation and conjugation being in the 5-position of the furanone ring. One isomer (BYI 02960-OH-gluA, isomer 4) with hydroxylation and conjugation in the difluoroethyl side chain and one isomer (BYI 02960-OH-gluA, isomer 1) with hydroxylation and conjugation in an unknown position
- Oxidative cleavage of the pyridinylmethyl moiety forming BYI 02960-6-CNA as well as subsequent total degradation of the furanone ring forming smaller carbon units (C-1- or C-2-fragments), entering the carbon pool of endogenous compounds and then being used either for the biosynthesis of fatty acids of lactose
- Substitution of the chloro group of BYI 02960-6-CNA with glutathione followed by degradation resulting in the conjugates BYI 02960-acetyl-cysteinyl-nicotinic acid and BYI 02960-lactato-mercaptyl-nicotinic acid
- Cleavage of the difluoroethyl group forming BYI 02960-des-difluoroethyl followed by hydroxylation and conjugation with sulfuric acid to BYI 02960-des-difluoroethyl-OH-SA
- Cleavage of the furanone ring and conjugation with sulfonic acid forming BYI 02960-AMCP-difluoroethanamine-SA
- Cleavage of the furanone ring and the difluoroethyl group forming an amine followed by acetylation to BYI 02960-acetyl-AMCP and BYI 02960-AMCP-difluoroethanamine
- Cleavage of the difluoroethyl group forming BYI 02960-des-difluoroethyl and difluoroacetic acid

These main metabolic reactions were also observed in the rat studies on absorption, distribution, metabolism and elimination (ADME) of BYI 02960 (see B.6 ~~refer to KIIA 5.1~~) and therefore the toxicological coverage of the livestock metabolites is given. Parent compound and all major plant metabolites, or at least subsequent metabolites which imply the presence of the livestock metabolites as transient metabolites were detected.

Difluoroacetic acid was determined in selected livestock samples by high resolution LC-MS subsequently to the metabolism studies since rat studies conducted with [ethyl-1-14C]BYI 02960 showed major amounts of this metabolite in organs and tissues. Extrapolation of rat data suggested high difluoroacetic acid levels in livestock tissues as well which was confirmed by the non-radioactive LC-MS analyses. Based on these findings, it was concluded that difluoroacetic acid is a major livestock metabolite and should be - besides parent compound - part of the residue definition for data collection, risk assessment and enforcement.

Definition of the Residue

Plants

Residue definition for monitoring:

Results from supervised residue trials show that flupyradifurone and difluoroacitic acid (DFA) are, by far, the most prominent components of the residues in target plants and rotational crops. The residues of metabolite BYI 02960-difluoroethyl-amino-furanone (DFEAF) were below the LOQ of 0.01 mg/kg in virtually all samples. Considering these results as well as those from the metabolism studies, the proposed residue definition for monitoring for target plants and rotational crops is:

~~**The sum of parent compound and DFA, expressed in flupyradifurone equivalents.**~~

1) flupyradifurone

2) DFA, expressed as DFA

Separatly

Residue definition risk assessment:

For flupyradifurone residues in target plants (primary crops) and rotational crops (succeeding crops), the proposed residue definition for data collection and risk assessment is:

The sum of parent compound flupyradifurone and its metabolites DFA ~~and BYI 02960-difluoroethyl-amino-furanone (DFEAF~~), expressed in flupyradifurone equivalents. ~~A conversion factor is not required to cover the difference between the residue definition for monitoring and risk assessment as both DFA and flupyradifurone are part of the residue definition for monitoring and as DFEAF is found in levels below <0.01 mg/kg in supervised residue trials and in rotational crops.~~

Animals

Residue definition for monitoring:

~~**The sum of parent compound and DFA, expressed in flupyradifurone equivalents.**~~

1) flupyradifurone

2) DFA, expressed as DFA

Separatly

Residue definition risk assessment:

The sum of Flupyradifurone and metabolite DFA, expressed in flupyradifurone equivalents.

Supervised residue trials

Protected Lettuce

Nine acceptable residue trials performed in accordance with the cGAP for greenhouse use have been provided.

In the greenhouse trials a highest residue value (HR) of 6.04 mg/kg was determined and an the STMR of 2.12 mg/kg for the sum of flupyradifurone and DFA, expressed as flupyradifurone.

Unprotected Lettuce

For the envisaged outdoor use in Northern and Southern Europe with a 10d PHI the required number of trials are available (nine acceptable trials for each region).
For the envisaged agricultural outdoor use in Northern and Southern Europe with a 3d PHI, the residue trials performed with lettuce in Northern and Southern Europe are not acceptable, except for two trials with sampling 2 days after the 1st treatment. No samples were taken 3 days after the first application. However, the results from the trials performed with outdoor lettuce in Northern and Southern Europe at 3 days after the 2nd treatment were valid to determine that the intended use on protected lettuce can be identified as the worst case residue situation, based on a comparison of the residue values from field and greenhouse testing using the most critical use pattern (2x125 g as/ha, int. 10d, PHI 3d).
Moreover, the tested GAP with two applications and a pre-harvest interval of 3 days is the "home and garden" use, which was submitted in parallel and is envisaged for registration shortly after the agricultural use (see the Evaluation Report for Flupyradifurone as an appendix to Vol. 3, B7). Since this field use is more critical than the agricultural field use, it was agreed with the RMS to conduct all field residue trials according to this GAP to identify the most critical consumer exposure and to avoid re-evaluation of MRLs due to this use. Hence, the trials in the DAR are not in accordance with the cGAP in the DAR, but with the cGAP supported in EU for MRL setting.

Thus, the open field trials in lettuce in Northern and Southern Europe are in compliance with the envisaged "home and garden"use. In addition, sufficient trials were performed providing evidence that the envisaged greenhouse can be identified as the worst case residue situation. Therefore no further trials are required.

Hops

In hops, eight valid trials were conducted in the northern European residue region in the years 2010-2011. Flupyradifurone was applied once as an SL 200 formulation at an active substance rate of 120 - 150 g/ha. All applications were at acceptable rates, and all trials were conducted according to GLP. in dried cones, residue levels also tended to decline with time. When evaluating the highest residues at relevant sampling intervals (either day 21 or, in three trials, day 28), residues ranged from 0.34-2.3 ~~0.61-2.4~~ mg/kg, with a median of 1.05 ~~1.15~~ mg/kg for the sum of flupyradifurone and DFA, expressed as flupyradifurone.

Processing studies

Nature of residues

Flupyradifurone stable under all conditions of high temperature hydrolysis for simulation of food processing. The following conditions were tested:

- pH 4 / 90°C (20 min) - pasteurisation
- pH 5 / 100°C (60 min) - baking, brewing, and boiling

- pH 6 / 120°C (20 min) - sterilisation

Metabolite DFEAF is a substructure of the parent compound. All functional groups, chemical moieties and their constitution of DFEAF are also present in the chemical structure of the parent compound. Therefore the chemical stability DFEAF is covered by studies with the parent compound. As parent compound was proved to be stable under the processing conditions, it is concluded that this is also true for DFEAF. In addition, in almost all uses worldwide – and certainly in all EU-relevant uses – DFEAF does not play any significant role in the field residues, generally comprising <5% of the total residue.

Due to the similarity of the chemical structures, the properties of DFA (= difluoroacetic acid) can be interpolated from the properties of TFA (= trifluoroacetic acid) and HOAc (acetic acid). The latter compounds are very common in organic chemistry, have often been used in chemical synthesis and many properties have been investigated. It can be deduced from literature data that these two compounds are stable under the conditions to be tested in a processing study. By interpolation of these properties, it is concluded that DFA is also stable under the processing conditions.

Magnitude of Residues

Household preparation of lettuce heads, i.e. removing outer leaves and washing the remaning head (separated in inner and outer head) resulted in processing factors of 1.8 for outer leaves, 0.72 ~~0.73~~ for inner head parts and 0.87 ~~0.76~~ for inner leaves.

Using hops in the production of beer, the transfer factors was determined to be 0.01. In the intermediates in the production of beer, the average processing factor was <0.1.

Field rotational crop studies

Four multi-plantback multi-crop rotational crop trials were conducted in Europe (2 each in the northern and southern residue regions) in the years 2010-2011. Flupyradifurone was applied once as an SL 200 formulation either to bare soil or to a target crop (lettuce) at an active substance rate of 200 g/ha, the target crop was then harvested, and crops representing 3 different botanical groups (roots, leafy veg., cereals) were planted on the plots at 3 intervals thereafter. All applications were at the required rates, and all trials were conducted according to GLP.

Quantifiable residues were measured in all rotations. Total residues of BYI 02960 in all rotational crops tended to be highest in the first, earliest rotation with a plant back interval of 25-33d.

Residues ranged from 0.05-0.14 mg/kg (median 0.06 mg/kg) in early harvest root crops, from 0.06-0.21 mg/kg (median 0.08 mg/kg) in early harvest leaf crops, 0.05-0.41 mg/kg (median 0.10 mg/kg) in cereal green forage, <0.07-0.39 mg/kg (median 0.12 mg/kg) in barley straw and from 0.11-0.65 mg/kg (median 0.35 mg/kg) in cereal grain grown in the first rotation, residues as the sum of flupyradifurone and DFA, expressed as flupyradifurone.

Up to the third rotation, residues above the LOQ were detected.

Additionally, field rotational crop trials were performed representative for each type of crop at short plant back intervals (PBI 30 days) and in accordance with the supported maximum dose rate of 2 × 125 g a.s./ha. Potatoes, leek, cucumber, onion, bean (fresh), peas (dry) and winter rape were grown at 2 locations in Northern and at 2 locations in Southern Europe. BYI 02960 and DFEAF residues were always <0.01 mg/kg while soil metabolite DFA was present in all trials except for one onion trial. Residues at harvest showed considerable variation which could not be attributed to geographical zone, soil type, pH, organic matter content, and temperature during treatment, rainfall after treatment or growth period (results not shown). Therefore, results for Northern and Southern Europe are considered as one data set with considerable variation.

HRs of DFA, expressed as DFA were 0.083, 0.076, 0.136, 0.053, 0.365, 0.764 and 0.050 ~~0.27, 0.25, 0.43, 0.18, 1.1, 2.3 and 0.17~~ mg/kg in potatoes, leek, cucumber, onion, bean, peas and winter rape, respectively.

Livestock Feeding studies

Flupyradifurone was administered orally to laying hens for 29 consecutive days at average dose rates of 1.5 mg/kg feed (1.2N) 6.5 mg/kg feed (5.4N ~~4.3N~~ ~~4.9N~~), 19.4 mg/kg feed (16N ~~13N~~ ~~14.7N~~), and 65.1 mg/kg feed (54N ~~43N~~ ~~49N~~). After the final dose, the animals were sacrificed and the key edible tissues were analyzed for the relevant residues of flupyradifurone. The combined residues of flupyradifurone + DFA in poultry tissues at sacrifice in the 1.2N dosing group were 0.093 mg/kg in muscle, 0.039 mg/kg in fat, and 0.11 mg/kg in liver, expressed in parent compound equivalents. Prior to sacrifice, residues in eggs were measured at various intervals in the high-dose group, and on days 24 and 28 in the three lower dose groups. In the 1N dose group, residues (flupyradifurone + DFA) in eggs amounted to 0.061 and 0.057 mg/kg, respectively.

Residue analysis of the high-dose eggs showed that levels reached a plateau in eggs. Though residues were highest at day 28, residue data from the 54N ~~43N~~ ~~49N~~ egg samples, when evaluated against the dose rate increases calculated as mg a.s./kg bw, suggest that flupyradifurone residues actually reached a plateau between day 4 and day 7; increases in residue levels were only due to increased dose rates.

Depuration occurred quickly. Total flupyradifurone residues in eggs, fat, liver, and muscle from the 54N ~~43N~~ ~~49N~~ dose level hens declined from 1.722, 1.230, 3.480, and 2.410 mg/kg, respectively, to <LOQ at 14 days after cessation of dose administration (=day 42 of the study).

DFA residues represented 65% to 74% of the total flupyradifurone residues in the excreta samples, suggesting significant exposure of the laying hens to DFA residues following the administration of BYI 02960 for 29 days.

Flupyradifurone was administered orally to dairy cows for 29 consecutive days at average *actual* dose rates of 4.8 mg/kg feed (1.6N ~~1.8N~~ ~~2N~~ dose), 23 mg/kg feed (8N ~~9N~~ ~~8N~~), 50 mg/kg feed (17N ~~19N~~ ~~18N~~), and 135 mg/kg feed (44N ~~52N~~ ~~49N~~).

After the final dose, the animals were sacrificed and the key edible tissues were analyzed for the relevant residues of BYI 02960.

The combined residues of BYI 02960 + DFA in bovine tissues at sacrifice in the 1.6N ~~1.8N~~ ~~2N~~ dosing group were 0.063 mg/kg in muscle, 0.041 mg/kg in fat, 0.18 mg/kg in kidney, and 0.17 mg/kg in liver, expressed in parent compound equivalents. Prior to sacrifice, residues in milk were measured at various intervals in the high-dose group, and on day 28 in the three lower dose groups. In the 1.6N ~~1.8N~~ ~~2N~~ dose group, the residues (BYI 02960 + DFA) amounted to 0.043 mg/kg.

Residue levels reached a plateau in milk. Highest residue were determined at day 17 in the 44N ~~52N~~ ~~49N~~ milk samples, remaining at similar levels for the remainder of the study.

Depuration occurred quickly. Total BYI 02960 residues in milk, fat, liver, kidney, and muscle from the 44N ~~52N~~ ~~49N~~ dose level cows declined from 0.81, 1.40, 1.59, 5.39, and 1.91 mg/kg, respectively, to <LOQ at 6-7 days after cessation of dose administration (=day 35-36 of the study).

Livestock will be exposed to DFA and flupyradifurone through feed as these are the major residues in plants. Based on the data from the feeding studies in which only parent flupyradifurone was dosed, transfer factors were proposed to estimate consumer exposure and to calculate MRLs for the sum of DFA and flupyradifurone. The feeding studies were designed to allow "material balancing" in order to evaluate levels and calculate transfer factors for both the total residue (parent BYI 02960 and DFA) and DFA, despite feeding parent compound only. Since this not an accepted approach, it was concluded in the expert meeting thatfeeding studies with DFA are required. In the meantime, the available study will be used for provisional MRL setting.

Consumer risk assessment

Based on the supervised residue trials, the field rotational crop trials, the livestock feeding studies and the calculated livestock transfer factors, MRLs are proposed:

Crop	MRL proposal (mg/kg)	
	Flupyradifurone	**DFA, expressed as DFA**
Lettuce	9	0.1
Hops	4	0.4
Rotational root and tuber vegetables	0.01	0.09
Rotational stem vegetables	0.01	0.08
Rotational bulb vegetables	0.01	0.06
Rotational fruiting vegetables	0.01	0.15
Rotational legume vegetables	0.01	0.4
Rotational leafy vegetables	0.08	0.04
Rotational cereals and sweet corn	0.01	0.3

Rotational pulses	0.01	0.8
Rotational oilseeds	0.01	0.05
eggs	0.01*	0.03
poultry meat (muscle)	0.01*	0.05
poultry fat	0.01*	0.03
poultry liver/offal	0.01*	0.1
milk	0.01*	0.03
Ruminant, pig and horse meat (muscle)	0.01*	0.1
Ruminant, pig and horse fat	0.01*	0.1
Ruminant, pig and horse liver	0.01*	0.1
Ruminant, pig and horse kidney	0.01*	0.15
other ruminant, horse and pig offal	0.01*	0.15

~~Lettuce 9 mg/kg~~

~~Hops 5 mg/kg~~

~~Rotational root vegetables 0.6 mg/kg~~

~~Rotational tuber vegetables 0.6 mg/kg~~

~~Rotational stem vegetables 0.5 mg/kg~~

~~Rotational bulb vegetables 0.4 mg/kg~~

~~Rotational fruiting vegetables 0.9 mg/kg~~

~~Rotational legume vegetables 2 mg/kg~~

~~Rotational leafy vegetables 0.4 mg/kg~~

~~Rotational cereals and sweet corn 1.5 mg/kg~~

~~Rotational pulses 5 mg/kg~~

~~Rotational oilseeds 0.3~~ ~~0.4~~ mg/kg

~~eggs 0.15 mg/kg~~

~~poultry meat (muscle) 0.20 mg/kg~~

~~poultry fat 0.09~~ ~~0.07~~ mg/kg

~~poultry liver/offal 0.30 mg/kg~~

~~milk 0.06~~ ~~0.08~~ mg/kg

~~Ruminant and horse meat (muscle) 0.20~~ ~~0.30~~ mg/kg

~~Ruminant and horse fat 0.20~~ ~~0.30~~ mg/kg

~~Ruminant and horse liver 0.20~~ ~~0.30~~ mg/kg

~~Ruminant and horse kidney 0.30~~ ~~0.40~~ mg/kg

~~other ruminant, horse and pig offal 0.30~~ ~~0.40~~ mg/kg

~~Pig meat (muscle) 0.40~~ ~~0.30~~ mg/kg

~~Pig fat 0.40~~ ~~0.20~~ mg/kg

~~Pig liver 0.40~~ ~~0.30~~ mg/kg

~~Pig kidney 0.60~~ ~~0.30~~ mg/kg

~~Other pig offal 0.60 0.30~~ mg/kg

~~Using these MRLs, and import tolerances applied for, dietary exposure assessments were performed.~~
Chronic and acute exposure for the consumer was estimated using the proposed MRLs, the ADI of 0.064 ~~0.08 0.12~~ mg/kg bw/d, the ARfD of 0.15 ~~0.35~~ mg/kg bw and EFSAs PRIMo model rev.2. The IEDI ~~TMDI~~ uses maximally 15.1% ~~47.3 75.6~~% of the ADI for the WHO Cluster diet B. All other European (national) diets use less of the ADI.
The NESTI uses maximally 82.3 ~~69.2~~% ~~of the ARfD UK infants for oranges (import tolerance) and 69.2%~~ of the ARfD for German children for lettuce after refinement with the HR-P. All other commodities use less of the ARfD in other consumer diets.

B.7.17 References relied on

A literature search and review was provided by the applicant. The search has been conducted in line with the EFSA guidance. Of all identified papers (342) the relevant ones (136) have been selected by the applicant in a transparent way using a text mining filter. The applicant provided a general justification for evaluating the relevant papers. As no study-by-study justifications were given, the RMS cannot properly verify whether the 136 relevant papers were correctly evaluated in terms of reliability/validity/acceptability for regulatory use. The one study that was considered relevant by the applicant is evaluated in the DAR. RMS notes that flupyradifurone is a new active substance worldwide. Therefore, public literature is not expected to be an important new information source for the active substance itself.

References for the active substance

Annex point / reference number	**Author(s)**	**Year**	**Title** **Source** (where different from company) **Company name, Report No., Date, GLP/GEP status** (where relevant)**, published or not**	**Data protect. claimed**	**Owner**
KIIA 6.1.1 /01	Netzband, D.; Timberlake, B. C.; Harbin, A. M.	2012	Storage stability of BYI 02960, difluoroacetic acid, and difluoroethyl-amino-furanone in plant matrices (18-month data) Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: RARVP046-1, Edition Number: M-428412-02-1 Date: 2012-04-03 ...Amended: 2012-11-01 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 6.1.1 /02	Moore, S. M.; Harbin, A. M.	2012a	BYI 02960 - Magnitude of the residue in dairy cows Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: RARVP050, Edition Number: M-428416-01-1 Date: 2012-04-03 GLP/GEP: yes, unpublished **...also filed: KIIA 4.3 /08** **...also filed: KIIA 6.4.2 /01**	Yes	BCS
KIIA 6.2.1 /01	Justus, K.	2011a	Metabolism of [furanone-4-^{14}C]BYI 02960 in tomatoes Bayer CropScience, Report No.: MEF-11/016, Edition Number: M-411352-01-2 Date: 2011-07-25 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 6.2.1 /02	Justus, K.	2011b	Metabolism of [pyridinylmethyl-^{14}C]BYI 02960 in tomatoes Bayer CropScience, Report No.: MEF-11/182, Edition Number: M-411500-01-2 Date: 2011-07-26 GLP/GEP: yes, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title **Source** (where different from company) **Company name, Report No., Date, GLP/GEP status** (where relevant), **published or not**	Data protect. claimed	Owner
KIIA 6.2.1 /03	Unold, M.; Justus, K	2011	Metabolism of [ethyl-1-^{14}C]BYI 02960 in tomatoes Bayer CropScience, Report No.: MEF-11/498, Edition Number: M-413996-01-2 Date: 2011-09-05 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 6.2.1 /04	Justus, K.	2011c	Metabolism of [furanone-4-^{14}C]BYI 02960 in potatoes Bayer CropScience, Report No.: MEF-10/769, Edition Number: M-415234-01-2 Date: 2011-09-14 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 6.2.1 /05	Justus, K.	2011d	Metabolism of [pyridinylmethyl-^{14}C]BYI 02960 in potatoes Bayer CropScience, Report No.: MEF-10/710, Edition Number: M-415078-01-2 Date: 2011-09-14 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 6.2.1 /06	Justus, K.	2011e	Metabolism of [furanone-4-^{14}C]BYI 02960 in apples Bayer CropScience, Report No.: MEF-11/499, Edition Number: M-422562-01-1 Date: 2011-12-29 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 6.2.1 /07	Justus, K.	2011f	Metabolism of [pyridinylmethyl-^{14}C]BYI 02960 in apples Bayer CropScience, Report No.: MEF-11/198, Edition Number: M-414678-01-2 Date: 2011-09-09 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 6.2.1 /08	Schmeling, S.; Weber, E.	2011a	Metabolism of [furanone-4-14C]BYI 02960 in cotton after spray application Bayer CropScience, Report No.: MEF-11/392, Edition Number: M-421625-01-2 Date: 2011-12-12 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 6.2.1 /09	Schmeling, S.; Weber, E.	2011b	Metabolism of [pyridinylmethyl-^{14}C]BYI 02960 in cotton after spray application Bayer CropScience, Report No.: MEF-11/393, Edition Number: M-421691-01-2 Date: 2011-12-12 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 6.2.1 /10	Schmeling, S.; Weber, E.	2011c	Metabolism of [furanone-4-^{14}C]BYI 02960 in paddy rice Bayer CropScience, Report No.: MEF-11/058, Edition Number: M-414219-01-2 Date: 2011-09-09 GLP/GEP: yes, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title **Source** (where different from company) **Company name, Report No., Date, GLP/GEP status** (where relevant), **published or not**	Data protect. claimed	Owner
KIIA 6.2.1 /11	Schmeling, S.; Weber, E.	2011d	Metabolism of [pyridinylmethyl-^{14}C]BYI 02960 in paddy rice Bayer CropScience, Report No.: MEF-11/059, Edition Number: M-414328-01-2 Date: 2011-09-09 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 6.2.1 /12	Schoening, R.; Ruhl, S.	2012	Determination of residues of difluoroacetic acid in extracts of samples from plant metabolism and confined rotational crops studies after application of BYI 02960 Bayer CropScience, Report No.: MR-11/050, Edition Number: M-422550-01-1 Date: 2012-01-17 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 6.2.2 /01	[redacted]	2012a	[Pyridinylmethyl-^{14}C]BYI 02960: Metabolism in the laying hen [redacted] Report No.: MEF-11/199, Edition Number: M-422162-01-2 Date: 2012-01-10 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 6.2.2 /02	[redacted]	2012b	[Furanone-4-^{14}C]BYI 02960: Metabolism in the laying hen [redacted] Report No.: MEF-11/200, Edition Number: M-422263-01-2 Date: 2012-01-10 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 6.2.3 /01	[redacted]	2011a	[Pyridinylmethyl-14C]BYI 02960: Metabolism in the lactating goat [redacted] Report No.: MEF-11/269, Edition Number: M-419701-01-2 Date: 2011-12-07 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 6.2.3 /02	B[redacted]	2011b	[Furanone-4-^{14}C]BYI 02960: Metabolism in the lactating goat [redacted] Report No.: MEF11/268, Edition Number: M-421995-01-2 Date: 2011-12-16 GLP/GEP: yes, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title **Source** (where different from company) **Company name, Report No., Date, GLP/GEP status** (where relevant)**, published or not**	Data protect. claimed	Owner
KIIA 6.3.1.1 /01	Noss, G.; Bauer, J.	2012	Determination of the residues of BYI 02960 in/on lettuce after spraying of BYI 02960 SL 200 in the field in the Netherlands, Belgium, France (North) and Germany Bayer CropScience, Report No.: 10-2223, Report includes Trial Nos.: 10-2223-01 10-2223-02 10-2223-03 10-2223-04 10-2223-05 Edition Number: M-424742-01-1 Date: 2012-02-08 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 6.3.1.1 /02	Uceda, L.	2012a	Amendment No. 1 - Determination of the residues of BYI 02960 in/on lettuce after spray application of BYI 02960 SL 200 in the field in Germany, northern France and Belgium Bayer S.A.S., Bayer CropScience, Lyon, France Bayer CropScience, Report No.: 11-2082, Report includes Trial Nos.: 11-2082-01 11-2082-02 11-2082-03 11-2082-04 Edition Number: M-425941-02-1 Date: 2012-02-23 ...Amended: 2012-03-05 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 6.3.1.1 /03	Schoening, R.; Bauer, J.	2011	Determination of the residues of BYI 02960 in/on lettuce, head after spray application of BYI 02960 SL 200 in the field in France (South), Spain and Italy - Amendment no. 0001 to report no. 10-2213 Bayer CropScience, Report No.: 10-2213, Report includes Trial Nos.: 10-2213-01 10-2213-02 10-2213-03 10-2213-04 10-2213-05 Edition Number: M-425913-02-1 Date: 2011-09-14 ...Amended: 2012-02-27 GLP/GEP: yes, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title **Source** (where different from company) **Company name, Report No., Date, GLP/GEP status** (where relevant)**, published or not**	Data protect. claimed	Owner
KIIA 6.3.1.1 /04	Uceda, L.	2012b	Amendment No.1 - Determination of the residues of BYI 02960 in/on lettuce after spray application of BYI 02960 SL 200 in the field in Spain, Italy, Southern France and Portugal Bayer CropScience, Lyon, France Bayer CropScience, Report No.: 11-2071, Report includes Trial Nos.: 11-2071-01 11-2071-02 11-2071-03 11-2071-04 Edition Number: M-425784-02-1 Date: 2012-02-23 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 6.3.1.1 /05	Schulte, G.	2012a	Determination of the residues of BYI 02960 in/on lettuce after spraying of BYI 02960 SL 200 in the greenhouse in France (North), Germany, the Netherlands and Italy Bayer CropScience, Report No.: 10-2212, Report includes Trial Nos.: 10-2212-01 10-2212-02 10-2212-03 10-2212-04 10-2212-05 Edition Number: M-425829-01-1 Date: 2012-02-22 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 6.3.1.1 /06	Uceda, L.	2012c	Determination of the residues of BYI 02960 in/on lettuce after spray application of BYI 02960 SL 200 in the greenhouse in northern France, Italy, Spain and Germany Bayer CropScience, Lyon, France Bayer CropScience, Report No.: 11-2070, Report includes Trial Nos.: 11-2070-01 11-2070-02 11-2070-03 11-2070-04 Edition Number: M-425786-01-1 Date: 2012-02-23 GLP/GEP: yes, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title **Source** (where different from company) **Company name, Report No., Date, GLP/GEP status** (where relevant)**, published or not**	Data protect. claimed	Owner
KIIA 6.3.1.2 /01	Noss, G.; Ballmann, C.	2012a	Determination of the residues of BYI 02960 in/on hop after spraying of BYI 02960 SL 200 in the field in Germany Bayer CropScience, Report No.: 10-2225, Report includes Trial Nos.: 10-2225-01 10-2225-02 10-2225-03 10-2225-04 Edition Number: M-425351-01-1 Date: 2012-02-13 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 6.3.1.2 /02	Noss, G.; Ballmann, C.	2012b	Determination of the residues of BYI 02960 in/on hop after spray application of BYI 02960 SL 200 in Germany Bayer CropScience, Report No.: 11-2076, Report includes Trial Nos.: 11-2076-01 11-2076-02 11-2076-03 11-2076-04 Edition Number: M-425339-01-1 Date: 2012-02-13 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 6.4.1 /01	[redacted]	2012	BYI 02960 - Magnitude of the residue in laying hens [redacted] Bayer CropScience, Report No.: RARVP041, Edition Number: M-428933-01-1 Date: 2012-04-05 GLP/GEP: yes, unpublished **...also filed: KIIA 4.3 /09**	Yes	BCS
KIIA 6.4.2 /01	[redacted]	2012b	BYI 02960 - Magnitude of the residue in dairy cows [redacted] Bayer CropScience, Report No.: RARVP050, Edition Number: M-428416-01-1 Date: 2012-04-03 GLP/GEP: yes, unpublished **...also filed: KIIA 4.3 /08** **...also filed: KIIA 6.1.1 /02**	Yes	BCS
KIIA 6.5.1 /01	Weber, E.	2011	Nature of the residues of [pyridinylmethyl-14C]BYI02960 in processed commodities - High temperature hydrolysis Bayer CropScience, Report No.: MEF-10/856, Edition Number: M-402311-01-2 EPA MRID No.: 48843966 Date: 2011-02-09 GLP/GEP: yes, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title **Source** (where different from company) **Company name, Report No., Date, GLP/GEP status** (where relevant), **published or not**	Data protect. claimed	Owner
KIIA 6.5.4 /01	Schulte, G.; Bauer, J.	2012a	Determination of the residues of BYI 02960 in/on lettuce and head lettuce and the processed fractions (head, inner parts; leaf, outer; leaf, inner; leaf, inner, washed and washings) after spraying of BYI 02960 SL 200 in the field in the Netherlands, Belgium and Germany Bayer CropScience, Report No.: 10-3223, Report includes Trial Nos.: 10-3223-01 10-3223-02 10-3223-04 10-3223-05 Edition Number: M-426982-01-1 Date: 2012-03-07 GLP/GEP: no, unpublished	Yes	BCS
KIIA 6.5.4 /02	Schulte, G.; Bauer, J.	2012b	Determination of the residues of BYI 02960 in/on hop (cone, green and cone, kiln-dried) and the processed fractions (hops draff, brewer's yeast and beer) after spraying of BYI 02960 SL 200 in the field in Germany Bayer CropScience, Report No.: 10-3407, Report includes Trial Nos.: 10-3407-01 10-3407-02 Edition Number: M-425311-01-1 Date: 2012-02-13 GLP/GEP: no, unpublished	Yes	BCS
KIIA 6.6.2 /01	A. Klempner	2011	Metabolism of [furanone-4-^{14}C]BYI 02960 in confined rotational crops Bayer CropScience, Report No.: MEF-11/365, Edition Number: M-421861-01-2 Date: 2011-12-16 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 6.6.2 /02	Breuer-Rehm, M.	2011	Metabolism of [pyridinylmethyl-^{14}C]BYI 02960 in confined rotational crops Bayer CropScience, Report No.: MEF-10/892, Edition Number: M-419853-02-1 Date: 2011-12-02 ...Amended: 2012-02-22 GLP/GEP: yes, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title **Source** (where different from company) **Company name, Report No., Date, GLP/GEP status** (where relevant), **published or not**	Data protect. claimed	Owner
KIIA 6.6.3 /01	Schoening, R.; Bauer, J.	2012	Determination of the residues of BYI 02960 in/on the field rotational crops barley, carrot, lettuce and turnip after spray application of BYI 02960 SL 200 on lettuce and soil in the field in Germany, the Netherlands, France (South) and Spain Bayer CropScience, Report No.: 10-2503, Report includes Trial Nos.: 10-2503-01 10-2503-02 10-2503-03 10-2503-04 Edition Number: M-429091-01-1 Date: 2012-04-11 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 6.6.3.1.2 /01	Schulte, G.	2012b	Determination of the residues of BYI 02960 in/on the field rotational crop potato after spray application of BYI 02960 SL 200 to bare soil in the field in northern France, the Netherlands, Spain and Italy - Limited rotational crop study Bayer CropScience, Report No.: 11-2550, Report includes Trial Nos.: 11-2550-01 11-2550-02 11-2550-03 11-2550-04 Edition Number: M-438341-01-1 Date: 2012-09-10 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 6.6.3.1.3 /01	Schulte, G.	2012c	Determination of the residues of BYI 02960 in/on the field rotational crop leek after spray application of BYI 02960 SL 200 to bare soil in the field in northern France, Germany, Spain and Italy - Limited rotational crop study Bayer CropScience, Report No.: 11-2551, Report includes Trial Nos.: 11-2551-01 11-2551-02 11-2551-03 11-2551-04 Edition Number: M-438384-01-1 Date: 2012-09-13 GLP/GEP: yes, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title **Source** (where different from company) **Company name, Report No., Date, GLP/GEP status** (where relevant), **published or not**	Data protect. claimed	Owner
KIIA 6.6.3.1.4 /01	Schulte, G.	2012d	Determination of the residues of BYI 02960 in/on the field rotational crop cucumber after spray application of BYI 02960 SL 200 to bare soil in the field in northern France, Germany, Spain and Italy - Limited rotational crop study Report No.: 11-2552, Report includes Trial Nos.: 11-2552-01 11-2552-02 11-2552-03 11-2552-04 Edition Number: M-438343-01-1 Date: 2012-09-13 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 6.6.3.1.5 /01	Schulte, G.	2012fe	Determination of the residues of BYI 02960 in/on the field rotational crop onion after spray application of BYI 02960 SL 200 on bare soil in the field in France (North), Germany, Italy and Spain - Limited rotational crop study Bayer CropScience, Report No.: 11-2553, Report includes Trial Nos.: 11-2553-01 11-2553-02 11-2553-03 11-2553-04 Edition Number: M-438397-01-1 Date: 2012-09-14 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 6.6.3.1.6 /01	Schulte, G.	2012f	Amendment no. 1 to report no: 11.2555 - Determination of the residues of BYI 02960 in/on the field rotational crop french bean after spray application of BYI 02960 SL 200 to bare soil in the field in northern France, Germany, Italy and Spain - Limited rotational crop study Bayer CropScience, Report No.: 11-2555, Report includes Trial Nos.: 11-2555-01 11-2555-02 11-2555-03 11-2555-04 Edition Number: M-438394-02-1 Date: 2012-09-14 ...Amended: 2012-09-27 GLP/GEP: yes, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title **Source** (where different from company) **Company name, Report No., Date, GLP/GEP status** (where relevant), **published or not**	Data protect. claimed	Owner
KIIA 6.6.3.1.7 /01	Schulte, G.	2012g	Determination of the residues of BYI 02960 in/on the field rotational crop pea after spray application of BYI 02960 SL 200 on bare soil in the field in France (North), Germany, Italy and Spain - Limited rotational crop study Bayer CropScience, Report No.: 11-2556, Report includes Trial Nos.: 11-2556-01 11-2556-02 11-2556-03 11-2556-04 Edition Number: M-438582-01-1 Date: 2012-09-17 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 6.6.3.1.8 /01	Schulte, G.	2012h	Determination of the residues of BYI 02960 in/on the field rotational crop winter rape after spray application of BYI 02960 SL 200 on bare soil in the field in northern France, Germany, Italy and Spain - Limited rotational crop study Bayer CropScience, Report No.: 11-2554, Report includes Trial Nos.: 11-2554-01 11-2554-02 11-2554-03 11-2554-04 Edition Number: M-443092-01-l Date: 2012-12-07 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 6.10 /01	Thielert, W.; Schoening, R.	2012	Systemicity of flupyradifurone and specific metabolites in tomato (foliar/soil application), melon (soil application) and cucumber (foliar application) under greenhouse conditions - Flupyradifurone SL 200 (200 g/L) Bayer CropScience Bayer CropScience, Report No.: MR-12/052, Edition Number: M-434311-01-1 Date: 2012-07-11 GLP/GEP: n.a., unpublished	Yes	BCS

Annex I

List of Metabolites

In the original study reports on BYI 02960 the metabolites are sometimes named by different synonyms. In order to present a common basis for the evaluation of the active substance and its degradation products a complete list of metabolites is presented.

The following abbreviations were used:

gluc = glucoside (conjugation with glucose)
glyc = glycoside (conjugation with a hexose)
gluA = glucuronide (conjugation with glucuronic acid)
OH = hydroxy
SA = sulfate

	Report name, Structure **IUPAC name** **CAS name, [CAS number]**	**Molecular formula** **molar mass** **Other names / codes**	**Occurrence**
a.s.	**BYF 02960 (parent compound)** 4-[[(6-chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino]furan-2(5H)-one (IUPAC) 2(5H)-furanone, 4-[[(6-chloro-3-pyridinyl)methyl](2,2-difluoroethyl)amino]- (CAS) CAS-No.: 951659-40-8	C_{12} H_{11} Cl F_2 N_2 O_2 288.68 g/mol flupyradifurone	all matrices
M01	**BYI 02960-chloro** 3-chloro-4-{[(6-chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino}furan-2(5H)-one	C_{12} H_{10} Cl_2 F_2 N_2 O_2 323.13 g/mol BCS-CD27046	Plant: rice cotton Swiss chard (CRC) turnips (CRC) wheat (CRC) (co-elution with BYI 02960-bromo) Environment: aerobic soil (minor)

	Report name, Structure IUPAC name CAS name, [CAS number]	Molecular formula molar mass Other names / codes	Occurrence
M02	**BYI 02960-bromo** 3-bromo-4-{[(6-chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino}furan-2(5H)-one	$C_{12}H_{10}BrClF_2N_2O_2$ 367.58 g/mol BCS-CD27042	Plant: rice cotton Swiss chard (CRC) turnips (CRC) wheat (CRC) (co-elution with BYI 02960-chloro)
M03	**BYI 02960-OH** 4-{[(6-chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino}-5-hydroxyfuran-2(5H)-one	$C_{12}H_{11}ClF_2N_2O3$ 304.68 g/mol BYI 02960-hydroxy BCS-CQ74364	<u>Animal:</u> rat lactating goat laying hen <u>Plant:</u> tomato potato apple cotton rice Swiss chard (CRC) turnips (CRC) wheat (CRC)
M04	**BYI 02960-OH-gluA (isomer 1)** + O glucuronide	$C_{18}H_{19}ClF_2N_2O_9$ 480.81	Animal: rat lactating goat

	Report name, Structure IUPAC name CAS name, [CAS number]	Molecular formula molar mass Other names / codes	Occurrence
M05	**BYI 02960-OH-gluA (isomer 2)** 3-{[(6-chloropyridin-3-yl) methyl](2,2-difluoroethyl) amino}-5-oxo-2,5-dihydrofuran-2-yl beta-D-glucopyranosiduronic acid	C_{18} H_{19} Cl F_2 N_2 O_9 480.81	Animal: lactating goat
M06	**BYI 02960-OH-gluA (isomer 3)** 3-{[(6-chloropyridin-3-yl) methyl](2,2-difluoroethyl) amino}-5-oxo-2,5-dihydrofuran-2-yl beta-D-glucopyranosiduronic acid	C_{18} H_{19} Cl F_2 N_2 O_9 480.81	Animal: rat lactating goat
M07	**BYI 02960-OH-gluA (isomer 4)** + O glucuronide	C_{18} H_{19} Cl F_2 N_2 O_9 480.81	Animal: lactating goat
M08	**BYI 02960-OH-glyc** 3-{[(6-chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino}-5-oxo-2,5-dihydrofuran-2-yl beta-D-glucopyranoside	C_{18} H_{21} Cl F_2 N_2 O_8 466.83 g/mol BYI 02960-OH-gluc BCS-CR46036	Plant: tomato potato apple cotton (co-elution with BYI 02960-acetic acid) Swiss chard (CRC) turnips (CRC) wheat (CRC)

	Report name, Structure IUPAC name CAS name, [CAS number]	Molecular formula molar mass Other names / codes	Occurrence
M09	**BYI 02960-OH-SA** 3-{[(6-chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino}-5-oxo-2,5-dihydrofuran-2-yl hydrogen sulfate	C_{12} H_{11} Cl F_2 N_2 O_6 S 384.75 g/mol	Animal: rat laying hen
M10	**BYI 02960-iso-OH** 	C_{12} H_{11} Cl F_2 N_2 O3 304.68 g/mol	Animal: rat lactating goat
M11	**BYI 02960-difluoroethyl-OH-glyc** 	C_{18} H_{21} Cl F_2 N_2 O_8 466.83 g/mol	Plant: apple
M12	**BYI 02960-OH-glyc-SA (isomer 1)** 	C_{18} H_{21} Cl F_2 N_2 O_{11} S 546.89 g/mol	Plant: Swiss chard (CRC) turnips (CRC)

	Report name, Structure IUPAC name CAS name, [CAS number]	Molecular formula molar mass Other names / codes	Occurrence
M13	**BYI 02960-OH-glyc-SA (isomer 2)** 	C_{18} H_{21} Cl F_2 N_2 O_{11} S 546.89 g/mol	Plant: Swiss chard (CRC) turnips (CRC) wheat (CRC)
M14	**BYI 02960-cysteine** S-(5-{[(2,2-difluoroethyl)(5-oxo-2,5-dihydrofuran-3-yl)amino]methyl}pyridin-2-yl)cysteine	C_{15} H_{17} F_2 N_3 O_4 S 373.38 g/mol	Animal: lacating goat
M15	**BYI 02960-acetic acid** N-[(6-chloropyridin-3-yl)methyl]-N-(2,2-difluoroethyl)glycine	C_{10} H_{11} Cl F_2 N_2 O_2 264.66 g/mol BCS-CQ74373	Plant: apple rice cotton (co-elution with BYI 02960-OH-glyc) Swiss chard (CRC) turnips (CRC) wheat (CRC) Animal: laying hen
M16	**BYI 02960-acetic acid-glyc** 	C_{16} H_{21} Cl F_2 N_2 O_7 426.8 g/mol	Plant: apple rice cotton Swiss chard (CRC) turnips (CRC) wheat (CRC)

	Report name, Structure IUPAC name CAS name, [CAS number]	Molecular formula molar mass Other names / codes	Occurrence
M17	**BYI 02960-glyoxylic acid** N-(6-chloropyridin-3-ylmethyl)-N-(2,2-difluoroethyl)oxamic acid	C_{10} H_9 Cl F_2 N_2 O_3 278.64 g/mol BCS-CQ74372	Plant: rice cotton Swiss chard (CRC) turnips (CRC) wheat (CRC)
M18	**BYI 02960-methylthio-glyoxylic acid** ({[6-(methylsulfanyl)pyridin-3-yl]methyl}amino)(oxo)acetic acid	C_9 H_{10} N_2 O_3 S 278.64 g/mol	Animal: lactating goat
M19	**BYI 02960-des-difluoroethyl** 4-[(6-chloropyridin-3-ylmethyl)amino]furan-2(5H)-one	C_{10} H_9 Cl N_2 O_2 224.65 g/mol BCS-AB49019	Animal: rat lactating goat laying hen Environment: Aerobic soil (minor)
M20	**BYI 02960-des-difluoroethyl-OH-SA** + O + SO_3	C_{10} H_9 Cl N_2 O_6 S 320.71 g/mol	Animal: laying hen

	Report name, Structure IUPAC name CAS name, [CAS number]	Molecular formula molar mass Other names / codes	Occurrence
M21	**BYI 02960-CHMP** 6-chloropyridin-3-ylmethanol (IUPAC) 3-pyridinemethanol, 6-chloro- (CAS) CAS-No.: 21543-49-7	$C_6 H_6 Cl N O$ 143.57 g/mol BCS-AA52175 6-CPA CPA (6-chloro-3-pyridyl)methanol BCS-AA52175 IM-0	Plant: tomato potato apple turnips (CRC) wheat (CRC)
M22	**BYI 02960-CHMP-glyc** glycoside	$C_{12} H_{16} Cl N O_6$ 305.72 g/mol	Plant: tomato potato apple
M23	**BYI 02960-CHMP-di-glyc** diglycoside	$C_{18}H_{26}ClNO_{11}$ 467.86 g/mol	Plant: tomato potato apple Swiss chard (CRC) turnips (CRC) wheat (CRC)
M24	**BYI 02960-CHMP-glyc-di-SA** glycosyl-disulfate	$C_{12} H_{16} Cl N O_{12} S_2$ 465.84 g/mol	Plant: Swiss chard (CRC)
M25	**BYI 02960-CHMP-glyc-tri-SA** glycosyl-trisulfate	$C_{12} H_{16} Cl N O_{15} S_3$ 545.9 g/mol	Plant: Swiss chard (CRC)

	Report name, Structure IUPAC name CAS name, [CAS number]	Molecular formula molar mass Other names / codes	Occurrence
M26	**BYI 02960-CHMP-serinate** 	$C_9 H_{11} Cl N_2 O_3$ 230.65 g/mol	Animal: laying hen
M27	**6-CNA** 6-chloronicotinic acid (IUPAC) CAS-No.: 5326-23-8	$C_6 H_4 Cl N O_2$ 157.56 g/mol 6-chloronicotinic acid IC-0 BYI 02960-6-CNA BCS-AA35572	Animal: rat laying hen Plant: tomato potato apple cotton rice Swiss chard (CRC) turnips (CRC) wheat (CRC) Environment Aerobic soil (major)
M28	**BYI 02960-6-CNA-glycerol-gluA (isomer 1)** 	$C_{15} H_{18} Cl N O_{10}$ 407.76 g/mol	Plant: potato (isomer was not assigned) wheat (CRC)
M29	**BYI 02960-6-CNA-glycerol-gluA (isomer 2 & 3)** 	$C_{15} H_{18} Cl N O_{10}$ 407.76 g/mol	Plant: wheat (CRC)

	Report name, Structure IUPAC name CAS name, [CAS number]	Molecular formula molar mass Other names / codes	Occurrence
M30	**BYI 02960-hippuric acid** N-[(6-chloropyridin-3-yl)carbonyl]glycine	C8 H7 Cl N2 O3 214.61 g/mol	Animal: rat lactating goat
M31	**BYI 02960-cysteinyl-nicotinic acid** 6-[(2-amino-2-carboxyethyl)sulfanyl]nicotinic acid	C9 H10 N2 O4 S 242.26 g/mol	Animal: lactating goat
M32	**BYI 02960-acetyl-cysteinyl-nicotinic acid** 	C_{11} H1$_2$ N_2 O_5 S 284.29 g/mol	Animal: laying hen
M33	**BYI 02960-lactato-mercaptyl-nicotinic acid** 	C_9 H_9 N O_5 S 243.24 g/mol	Animal: laying hen

	Report name, Structure IUPAC name CAS name, [CAS number]	Molecular formula molar mass Other names / codes	Occurrence
M34	**BYI 02960-difluoroethyl-amino-furanone** 4-[(2,2-difluoroethyl)amino]furan-2(5H)-one	$C_6 H_7 F_2 N O_2$ 163.12 g/mol DFEAF BCS-CC98193	Plant: tomato potato apple Swiss chard (CRC) turnips (CRC) wheat (CRC) Animal: rat
M35	**BYI 02960-difluoroethyl-amino-furanone-OH-glyc** + O glycoside	$C_{12} H_{17} F_2 N O_8$ 341.27 g/mol DFEAF-OH-glyc	Plant: Swiss chard (CRC) wheat (CRC)
M36	**BYI 02960-AMCP-difluoroethanamine** N-[(6-chloropyridin-3-yl)methyl]-2,2-difluoroethanamine	$C_8 H_9 Cl F_2 N_2$ 206.62 g/mol BCS-AA10710	Plant: apple Animal: lactating goat
M37	**BYI 02960-AMCP-difluoroethanamine-SA** 	$C_8 H_9 Cl F_2 N_2 O_3 S$ 286.69 g/mol	Animal: laying hen

	Report name, Structure IUPAC name CAS name, [CAS number]	Molecular formula molar mass Other names / codes	Occurrence
M38	**BYI 02960-N-formyl-AMCP-difluoroethanamine** N-[(6-chloropyridin-3-yl)methyl]-N-(2,2-difluoroethyl)formamide	$C_9 H_9 Cl F_2 N_2 O$ 234.63 g/mol	Plant: Swiss chard (CRC) turnips (CRC)
M39	**BYI 02960-N-acetyl-AMCP-difluoroethanamine** N-[(6-chloropyridin-3-yl)methyl]-N-(2,2-difluoroethyl)acetamide	$C_{10} H_{11} Cl F_2 N_2 O$ 248.66 g/mol	Plant: Swiss chard (CRC) turnips (CRC)
M40	**BYI 02960-acetyl-AMCP** 	$C_8 H_9 Cl N_2 O$ 184.63 g/mol AMCP BYI 02960-AMCP	Animal: laying hen
M41	**BYI 02960-mercapto-lactic acid** 3-({4-[(2,2-difluoroethyl)amino]-2-oxo-2,5-dihydrofuran-3-yl}sulfanyl)-2-hydroxypropanoic acid	$C_9 H_{11} F_2 N O_5 S$ 283.25 g/mol DFEAF-mercapto lactic acid	Plant: wheat (CRC)

	Report name, Structure IUPAC name CAS name, [CAS number]	Molecular formula molar mass Other names / codes	Occurrence
M42	**BYI 02960-amino-furanone** 4-aminofuran-2(5H)-one	$C_4 H_5 N O_2$ 99.09 g/mol	Plant: Swiss chard (CRC) turnips (CRC) wheat (CRC)
M43	**BYI 02960-bromo-amino-furanone** 4-amino-3-bromofuran-2(5H)-one	$C_4 H_4 Br N O_2$ 177.99 g/mol	Plant: wheat (CRC)
M44	**DFA** difluoroacetic acid (IUPAC) Acetic acid, 2,2-difluoro- (CAS) CAS-No.: 381-73-7	$C_2 H_2 F_2 O_2$ 96.03 g/mol difluroacetic acid BYI 02960-DFA BYI 02960-difluoroacetic acid BYI 0260-Difluoro-acetic-acid BCS-AA56716	Animal: rat lactating goat laying hen Plant: tomato potato apple cotton rice Swiss chard (CRC) turnips (CRC) wheat (CRC) Environment aerobic soil (major) aerobic water/sediment (major)
M45	**Glucose (hexose)** assumed structure D-glucose	$C_6 H_{12} O_6$ 180.16 g/mol glucose/carbohydrates D-glucose	Plant: tomato apple rice turnips (CRC) wheat (CRC)

	Report name, Structure IUPAC name CAS name, [CAS number]	Molecular formula molar mass Other names / codes	Occurrence
M46	**Lactose** 	$C_{10}H_{18}O_{11}$ 314.25 g/mol	Animal: lactating goat
M47	**BYI 02960-azabicyclosuccinamide** 4-{(2,2-difluoroethyl)[(3-oxo-2-azabicyclo[2.2.0]hex-5-en-6-yl)methyl]amino}-4-oxobutanoic acid (IUPAC)	$C_{12}H_{14}F_2N_2O_4$ 288.25 g/mol BCS-CS64875	Environment: water – aquatic photolysis (major)
M48	**BYI 02960-succinamide** 4-{[(6-chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino}-4-oxobutanoic acid (IUPAC)	$C_{12}H_{13}ClF_2N_2O_3$ 306.69 g/mol BCS-CR74729	Environment: water – aquatic photolysis (major)
M49	**BYI 02960-deschlorohydroxysuccinamide** 4-{(2,2-difluoroethyl)[(6-hydroxypyridin-3-yl)methyl]amino}-4-oxobutanoic acid (IUPAC)	$C_{12}H_{14}F_2N_2O_4$ 288.25 g/mol DCHS	Environment: water – aquatic photolysis (minor)

European Commission

FLUPYRADIFURONE

VOLUME 3

ANNEX B

B.8 Fate and behaviour

Rapporteur Member State: The Netherlands

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Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of flupyradifurone in Annex I of Council Directive 1107/2009/EEC

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B.8 Environmental Fate and Behaviour

Information is provided in this chapter with respect to the fate and the behavior in soil, water and air of flupyradifurone (BYI 02960). This active substance is an insecticide which is active against various pests such as aphids, white flies and hoppers in many target crops such as fruits, vegetables, plantations, cereals and soybean.

Internal code:	BYI 02960
CAS-No.:	951659-40-8
Chemical name:	4-[(6-chloro-3-pyridylmethyl)(2,2-difluoroethyl)amino]furan-2(5H)-one
Molecular mass:	288.68 g/mol (non-labelled)
Molecular formula:	$C_{12}H_{10}ClF_2N_2O_2$

The studies concerning the fate and behavior of BYI 02960 in the environment were conducted using different radiolabelled forms, [pyrindinyl-methyl-^{14}C], [pyridine-2,6-^{14}C], [furanone-4-^{14}C] and [ethyl-1-^{14}C] BYI 02960 as well as the non-labeled parent compound. These label positions are sufficient to define the degradation pathway. In the Tier II summaries that follow, the different radiolabels are referred to as PYM (= pyrindinyl-methyl-^{14}C-label), PYR (= pyridine-2,6-^{14}C-label), FUR (= furanone-4-^{14}C-label) and ETH (= ethyl-1-^{14}C-label). The structure of BYI 02960 and the positions of the different radiolabels are as follows:

[Pyridinyl-methyl-^{14}C]
BYI 02960
(PYM-^{14}C)

[Pyridine-2,6-^{14}C]
BYI 02960
(PYR-^{14}C)

[Furanone-4-^{14}C]
BYI 02960
(FUR-^{14}C)

[Ethyl-1-^{14}C]
BYI 02960
(ETH-^{14}C)

* indicates position of radiolabel

In addition, studies were performed with the following metabolites (radiolabelled or non-radiolabelled):

Difluoroacetic acid
(DFA)

6-Chloronicotinic acid
(CNA)

In original reports study authors may have used different names or codes for degradation products of BYI 02960. In this summary, a single name and a single code number is used for each metabolite, details are given at the end of the section.

List of concordances for names and designations of compounds

compound designation	Reference Code	Molecular weight / formula	Structure
Flupyradifurone (active substance)	BYI 02960	288.68 $C_{12}H_{10}ClF_2N_2O_2$	
M27 6-chloroniotinic acid	BYI 02960-CNA BCS-AA35572	157.56 $C_6H_4ClNO_2$	
M44 difluoroacetic acid	BYI 02960-DFA BCS-AA56716	96.03 $C_2H_2F_2O_2$	
M47	BYI 02960-azabicyclosuccinamide BCS-CS64875	288.25 $C_{12}H_{14}F_2N_2O_4$	
M48	BYI 02960-succinamide BCS-CR74729	306.69 $C_{12}H_{13}ClF_2N_2O_3$	
The following are minor metabolites in environmental matrices and are not considered in PEC calculations			
M01	BYI 02960-chloro BCS-CD27046	323.13 $C_{12}H_{10}Cl_2F_2N_2O_2$	
M49	BYI 02960-deschlorohydroxysuccinamide DHS	288.25 $C_{12}H_{14}F_2N_2O_4$	

B.8.1 Route and rate of degradation in soil (Annex IIA 7.1.1 and Annex IIIA 9.1.1)

B.8.1.1 Route of degradation in soil (Annex IIA 7.1)

B.8.1.1.1 Aerobic degradation (Annex IIA 7.1.1)

STUDY IIA, 7.1.1/01

Characteristics

Reference	:	Menke, U., 2011	study type	:	aerobic soil degradation
year of execution	:	2007-2009	incubation time	:	120 days
GLP statement	:	yes	nominal concentration	:	0.53 mg / kg soil
Guideline	:	OECD 307 (2002) US EPA, OPPTS 835.4100 (2008) OECD106 (2001)	temperature	:	20°C
test substance	:	[pyridinyl-methyl-^{14}C] BYI 02960	DT50	:	see results
Purity	:	> 99%	metabolites	:	see results
Soils	:	four soils; sandy loam, silt loam, loam, clay loam	acceptability	:	**acceptable**

Summary

The biotransformation and time dependent sorption of [pyridinylmethyl-^{14}C] BYI 02960 was studied in four European soils: Laacher Hof AXXa (AX), Höfchen am Hohenseh (HF), Hanscheiderhof Plot 611 (HN), and Dollendorf II (DD) for a maximum period of 120 days under aerobic conditions in the dark at approx. 20°C and 55% WHC_{max} (max. water holding capacity). BYI 02960 was applied at the nominal rate of 0.53 mg/kg soil, which is equivalent to 200 g/ha field application rate.

At each sampling date the soil samples were shaken for 24 hours with 400 mL $CaCl_2$-solution in order to measure the time-dependent desorption of the test item. Subsequently they were extracted by shaking at ambient temperature and in a microwave at 70 °C with acetonitrile/water mixtures, and the BYI 02960 residues were analyzed and quantified by TLC with HPLC as the confirmatory method.

Material balances were complete throughout the study, and the test item declined from 97.1, 96.1, 96.5 and 93.1% AR at DAT-0 to 37.1, 24.5, 50.2 and 28.7% in soils AX, HF, HN and DD, respectively, at the end of the study. Applying double first-order kinetics a half-life (geometric mean) of 68.8 days was calculated for BYI 02960 in the tested soils under aerobic conditions.

The mineralization of [PYM-^{14}C] BYI 02960 in this study was high. At the end of the study (DAT-120) up to 45.3 (AX), 58.6 (HF), 29.4 (HN) and 57.3% AR (DD) of $^{14}CO_2$ were generated. Volatile organic compounds were negligible (≤ 0.1% AR). With the exception of carbon dioxide only very minor transformation products (all were below 3% AR) were detected. Non-extractable ^{14}C-residues (NER) increased from 1.0, 1.5, 1.9 and 4.1% AR at DAT-0 to 12.6, 13.2, 16.8 and 12.5% AR at the end of the study period.

The part of the study related to time dependent sorption of [PYM-^{14}C] BYI 02960 is summarized in the mobility chapter (see B.8.2).

Materials and methods

Materials

1. Test Item: Flupyradifurone: Code = BYI 02960; Label PYM = [Pyridinyl-methyl-^{14}C] BYI 02960 (sample ID: BECH 2123); Specific activity 4.37 MBq/mg; Radiochemical purity: >99% (acc. radio-HPLC and -TLC); Chemical purity: >99% (HPLC, UV detection at 210 nm); Identity and purity of test item in the application solution were confirmed.

2. Soil: The biotransformation of [PYM-^{14}C] BYI 2960 was studied in four different soils (Table B.8.1.1.1-01). These soils are representative for agricultural use areas as required by the guidelines and cover a representative range of physico-chemical properties. All soils were taken fresh from the fields. Two days later, i.e. four days before starting the test the air dried soils were sieved through a 2 mm sieve. Three days before application aliquots equivalent to 100 g dry matter were weighed into individual 300 mL Erlenmeyer flasks and fitted with trap attachments. The soils were pre-equilibrated at 20 °C in the dark.

Table B.8.1.1.1-01 Properties of study soil

Parameter	Results/Units			
Soil Batch ID	Laacher Hof AXXa (AX) 20070306	Höfchen am Hohenseh (HF) 20070306	Hanscheiderhof (HN) 20070306	Dollendorf II (DD) 20070307
Location	Monheim, Germany	Burscheid, Germany	Burscheid, Germany	Blankenheim, Germany
Soil Taxonomic Classification (USDA)	Sandy floodplain deposits of the lower terrace of the Rhine river, material from the Pleistocene Ice Age	Loess or loess colluvium (Pleistocene, Holocene)	Not available	Not available
Soil Series	Sandy, mixed, mesic Typic Cambudolls	Loamy, mixed, mesic Typic Argudalfs	Not available	Not available
Texture Class (USDA)	Sandy Loam	Silt Loam	Loam	Clay loam
Sand Silt Clay	71 % 16 % 13 %	19 % 62 % 19 %	43 % 39 % 18 %	37 % 34 % 29 %
pH in Water pH in $CaCl_2$ pH in KCl	6.8 6.4 6.2	7.0 6.5 6.2	5.9 5.4 5.0	7.7 7.4 7.1
Organic Matter	2.1 %	3.1 %	4.0 %	7.9 %
Organic Carbon	1.2 %	1.8 %	2.3 %	4.6 %
Soil Microbial Biomass (mg microbial carbon per kg of soil)				
0 days	437	664	495	1908
59 days	283	468	371	1683
120 days	220	418	308	1375
Cation Exchange Capacity (CEC)	7.5 meq/100 g	11.9 meq/100 g	9.7 meq/100 g	20.5 meq/100 g
WHCmax	42.2 %	53.9 %	57.1 %	83.5 %

Methods

1. Experimental conditions: The study was performed in static incubation test systems under aerobic conditions in the dark at 19.8 ± 0.26 °C. The test system consisted of Erlenmeyer flasks (300 mL)

attached with a trap attachment (permeable to oxygen) containing soda lime for absorption of $^{14}CO_2$ and a polyurethane foam plug for adsorption of volatile organic compounds. Aliquots of 100 g of dry soil were weighed into the test flasks (each 21 flasks/soil). For all soils replicates were set up for each sampling (9 sampling dates including time 0). Three flasks for each soil were used for determination of the microbial biomass. The final soil moisture was adjusted to 55% of WHCmax by adding pure water.

2. Test Item Stock Solution: The entire amount of [PYM-^{14}C] BYI 02960 delivered was dissolved in 5 mL acetonitrile / water 1:1 (v/v).

3. Test Item Application Solution: An application solution (total volume 120 mL) was made by diluting 2082 µL of the stock solution with 117.92 mL of distilled water.

4. Mode of Application: aliquots of 991 µL of the application solution were applied in droplets onto each of the 100 g pre-incubated sub-samples from each soil. BYI 02960 was applied to the test soils at a treatment rate of 50.22 µg (219.442 kBq) per vessel. This value is equivalent to 94% of the nominal value of the application rate of 53.33 µg per vessel (calculated for a single application of 200 g BYI 02960 per hectare).

5. Sampling: Microbial biomass was determined prior to commencement of the test (soils sampled at the day of application), after 59 days, and at the end of the study 120 Days After Treatment (DAT-120). Entire test flasks were taken for processing and analysis at DAT-0, DAT-1, DAT-3, DAT-7, DAT-14, DAT-21, DAT-30, DAT-59, and DAT-120.

Prior to opening the incubation flasks (for moistening or sampling of soil), volatile (radioactive) compounds, possibly still present in the flasks, were transferred into the trap attachment by subjecting the flasks to vacuum in an excicator. At each sampling date the entire amount of soil in each test vessel was transferred into a centrifuge beaker and extracted using a mechanical shaker.

6. Description of analytical procedures: The soil processing procedure was optimized to obtain >90% extraction efficiency and >90% recovery of the test item at time zero. First, the test soils were shaken for 24 hours with 400 mL $CaCl_2$-solution to measure the time-dependent desorption of the test item. Subsequently they were extracted 4 times by shaking at ambient temperature and once in a microwave at 70 °C with acetonitrile/water mixtures, the [PYM-^{14}C] BYI 02960 residues were analyzed and quantified by LSC and normal phase Si-60 TLC with HPLC as the confirmatory method. Solid samples (i.e. soil and paper filters) were combusted and ^{14}C levels were measured using LSC. The identity of individual residues was established initially by spectroscopic methods. Within the course of the study compound identities were confirmed by co-chromatography using non-labeled reference substances.

Results

Results indicated that anticipated standardized conditions were maintained, and the soils were microbial active over the duration of the laboratory study (see Table B.8.1.1.1-01).

Data

The respective data for the four soils are shown in Table B.8.1.1.1-02 to Table B.8.1.1.1-05. The DAT-0 extraction efficiency of total radioactivity was 94.4 to 97.8% applied radioactivity (AR; mean 96.6% AR; sum of extracts only). The stability of the test item was verified by DAT 0 values of 93.1 to 97.1% AR for the soil extracts (mean 95.2% AR). These results indicated that the extraction method was appropriate for extraction of the applied [^{14}C]-labeled test item from the soil matrix.

Table B.8.1.1.1-02 Biotransformation of [PYM-^{14}C] BYI 02960 in sandy loam soil AX under aerobic conditions; mean values and standard deviations expressed as % of AR

Compound	Days After Treatment (DAT)								
	0	1	3	7	14	21	30	59	120
BYI 02960	97.1 ±0.4	95.9 ±0.1	90.1 ±0.2	86.7 ±0.4	76.5 ±0.3	71.4 ±0.2	63.1 ±0.5	49.9 ±0.0	37.1 ±0.7
ROI 1	n.d.	0.2 ±0.0	1.5 ±0.1	1.4 ±0.0	1.5 ±0.1	0.6 ±0.1	0.5 ±0.0	0.1 ±0.0	0.1 ±0.0
ROI 2	n.d.	0.2 ±0.0	0.2 ±0.0	0.5 ±0.0	1.1 ±0.3	1.5 ±0.1	2.0 ±0.1	2.6 ±0.0	1.9 ±0.6
ROI 3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.3 ±0.0
ROI 4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ROI 5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ROI 6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Non-characterized radioactivity	0.7 ±0.0	0.2 ±0.1	0.5 ±0.0	0.4 ±0.1	1.0 ±0.1	0.4 ±0.0	0.6 ±0.1	0.4 ±0.0	2.0 ±1.4
Total extractable residues	97.8 ±0.4	96.5 ±0.0	92.3 ±0.4	88.9 ±0.3	80.0 ±0.1	73.8 ±0.0	66.2 ±0.3	53.0 ±0.0	41.3 ±0.2
$^{14}CO_2$	n.a.	0.3 ±0.0	1.4 ±0.0	4.7 ±0.0	10.8 ±0.2	15.4 ±0.3	20.2 ±0.2	32.3 ±0.9	45.3 ±2.8
Volatile organics	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.0 ±0.0	<0.1
Non-extractable residues (NER)	1.0 ±0.0	1.9 ±0.0	4.1 ±0.0	4.9 ±0.1	6.7 ±0.0	8.2 ±0.1	9.7 ±0.0	11.7 ±0.3	12.6 ±0.1
Total recovery	98.8 ±0.4	98.7 ±0.0	97.8 ±0.4	98.5 ±0.2	97.6 ±0.2	97.5 ±0.2	96.1 ±0.1	97.1 ±0.6	99.2 ±3.1

n.d. = not detected; n.a. = not analyzed; ROI = region of intrest

Table B.8.1.1.1-03 Biotransformation of [PYM-^{14}C] BYI 02960 in sandy loam soil HF under aerobic conditions; mean values and standard deviations expressed as % of AR

Compound	Days After Treatment (DAT)								
	0	1	3	7	14	21	30	59	120
BYI 02960	96.1 ±0.0	95.9 ±1.0	92.1 ±0.3	87.1 ±0.5	77.1 ±0.9	71.4 ±0.6	63.2 ±0.3	45.2 ±1.0	24.5 ±0.2
ROI 1	n.d.	0.3 ±0.0	1.6 ±0.1	1.2 ±0.0	1.1 ±0.1	0.4 ±0.0	0.5 ±0.0	0.2 ±0.0	0.5 ±0.0
ROI 2	n.d.	0.1 ±0.0	n.d.	0.2 ±0.0	0.5 ±0.0	0.3 ±0.0	0.6 ±0.1	0.9 ±0.1	0.6 ±0.0
ROI 3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.2	0.1

								±0.0	±0.0
ROI 4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ROI 5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ROI 6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Non-characterized radioactivity	0.8 ±0.2	0.3 ±0.0	0.6 ±0.1	0.4 ±0.1	0.6 ±0.0	0.5 ±0.1	0.6 ±0.2	0.5 ±0.0	3.6 ±0.2
Total extractable residues	97.0 ±0.2	96.6 ±1.0	94.4 ±0.2	89.0 ±0.6	79.3 ±0.9	72.5 ±0.7	64.9 ±0.4	47.0 ±1.0	29.3 ±0.4
$^{14}CO_2$	n.a.	0.3 ±0.0	1.7 ±0.1	5.3 ±0.1	11.8 ±0.2	17.0 ±0.5	23.0 ±0.4	37.1 ±1.0	58.6 ±0.2
Volatile organics	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extractable residues (NER)	1.5 ±0.1	3.1 ±0.9	3.1 ±0.1	4.8 ±0.1	6.6 ±0.1	8.0 ±0.0	9.3 ±0.1	12.0 ±0.0	13.2 ±0.1
Total recovery	98.4 ±0.1	100.0 ±0.1	99.2 ±0.2	99.1 ±0.7	97.7 ±0.6	97.4 ±0.3	97.2 ±0.7	96.1 ±0.1	101.1 ±0.6

n.d. = not detected; n.a. = not analyzed; ; ROI = region of intrest

Table B.8.1.1.1-04 Biotransformation of [PYM-^{14}C] BYI 02960 in sandy loam soil HN under aerobic conditions; mean values and standard deviations expressed as % of AR

Compound	Days After Treatment (DAT)								
	0	1	3	7	14	21	30	59	120
BYI 02960	96.5 ±0.1	98.8 ±0.1	92.0 ±0.2	88.6 ±0.2	82.3 ±0.0	79.5 ±0.3	74.2 ±0.1	63.2 ±0.2	50.2 ±0.3
ROI 1	n.d.	0.2 ±0.0	0.8 ±0.0	0.5 ±0.1	<LOQ	n.d.	n.d.	0.2 ±0.0	n.d.
ROI 2	n.d.	n.d.	<LOQ	0.5 ±0.0	0.7 ±0.0	0.6 ±0.0	1.1 ±0.1	1.6 ±0.1	1.0 ±0.2
ROI 3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.1 ±0.0
ROI 4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ROI 5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ROI 6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Non-characterized radioactivity	0.8 ±0.0	0.1 ±0.1	0.5 ±0.1	0.5 ±0.0	0.4 ±0.1	0.4 ±0.0	0.4 ±0.1	0.4 ±0.0	2.0 ±1.0
Total extractable residues	97.2 ±0.0	99.1 ±0.0	93.3 ±0.2	90.0 ±0.3	83.5 ±0.1	80.5 ±0.3	75.7 ±0.2	65.3 ±0.3	53.4 ±0.5
$^{14}CO_2$	n.a.	0.3 ±0.0	1.2 ±0.0	3.3 ±0.0	3.9 ±2.5	9.1 ±0.1	11.8 ±0.0	19.7 ±0.2	29.4 ±0.1
Volatile organics	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extractable residues (NER)	1.9 ±0.0	3.0 ±0.0	4.7 ±0.1	6.3 ±0.1	8.2 ±0.1	9.6 ±0.1	11.2 ±0.1	14.0 ±0.3	16.8 ±0.3
Total recovery	99.2 ±0.0	102.4 ±0.0	99.2 ±0.1	99.6 ±0.2	95.6 ±2.5	99.2 ±0.3	98.6 ±0.1	99.1 ±0.2	99.5 ±0.1

n.d. = not detected; n.a. = not analyzed; ; ROI = region of intrest

Table B.8.1.1.1-05 Biotransformation of [PYM-^{14}C] BYI 02960 in sandy loam soil DD under aerobic conditions; mean values and standard deviations expressed as % of AR

Compound	Days After Treatment (DAT)								
	0	1	3	7	14	21	30	59	120

BYI 02960	93.1 ±0.4	91.9 ±1.7	89.8 ±0.5	86.0 ±0.0	75.9 ±0.3	70.8 ±0.0	63.1 ±0.0	46.1 ±0.5	28.7 ±0.2
ROI 1	<LOQ	0.2 ±0.0	1.2 ±0.1	0.6 ±0.1	0.3 ±0.0	n.d.	0.2 ±0.0	n.d.	0.3 ±0.3
ROI 2	n.d.	n.d.	<LOQ	<LOQ	<LOQ	n.d.	0.2 ±0.0	0.4 ±0.0	0.2 ±0.0
ROI 3	n.d.	n.d.	0.1 ±0.0	n.d.	n.d.	n.d.	n.d.	0.4 ±0.0	0.1 ±0.0
ROI 4	0.6 ±0.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ROI 5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.
ROI 6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.2 ±0.1
Non-characterized radioactivity	0.7 ±0.1	0.2 ±0.0	0.5 ±0.0	0.4 ±0.0	0.6 ±0.1	0.4 ±0.1	0.6 ±0.0	0.4 ±0.0	2.0 ±0.3
Total extractable residues	94.4 ±0.4	92.2 ±1.7	91.6 ±0.4	87.1 ±0.1	76.9 ±0.4	71.3 ±0.0	64.3 ±0.1	47.4 ±0.5	31.5 ±0.3
$^{14}CO_2$	n.a.	0.3 ±0.0	1.8 ±0.0	5.7 ±0.1	12.1 ±0.0	17.1 ±0.2	23.5 ±0.2	39.5 ±0.3	57.3 ±0.3
Volatile organics	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	0.0 ±0.0	<0.1	<0.1
Non-extractable residues (NER)	4.1 ±0.7	5.5 ±1.6	6.2 ±0.7	6.3 ±0.3	8.9 ±0.1	8.9 ±0.1	9.5 ±0.1	11.8 ±0.2	12.5 ±0.1
Total recovery	98.5 ±0.2	98.1 ±0.1	99.6 ±0.3	99.2 ±0.1	97.8 ±0.5	97.2 ±0.1	97.3 ±0.2	98.7 ±0.1	101.3 ±0.0

n.d. = not detected; n.a. = not analyzed; ROI = region of intrest

Mass balance

The material balances were 96.1 to 99.2% (soil AX), 96.1 to 101.1% (soil HF), 95.6 to 102.4% (soil HN), and 97.1 to 101.3% (soil DD) of the applied radioactivity (% AR; mean values). There was no decrease over the incubation time.

Extractable and Bound Residues (NER)

Extractable ^{14}C-residues decreased from 97.8, 97.0, 97.2, and 94.4% AR at DAT-0 to 41.3, 29.3, 53.4 and 31.5% AR at study end (DAT-120) in soils AX, HF, HN, and DD, respectively. Non-extractable ^{14}C-residues (NER) increased from 1.0, 1.5, 1.9 and 4.1% at DAT-0 to 12.6, 13.2, 16.8 and 12.5% of AR at the end of the study period. These portions of NER are comparatively low.

Table B.8.1.1.1-06 Summary of extractable and non-extractable residues

Soil		AX	HF	HN	DD
Extracted RA (%)	Day 0	97.8±0.4	97.0±0.2	97.2±0.0	97.4±0.4
	Day 120	41.3±0.2	29.3±0.4	53.4±0.5	31.5±0.3
Non-Extracted RA (%)	Day 0	1.0±0.0	1.5±0.1	1.9±0.0	4.1±0.7
	Day 120	12.6±0.1	13.2±0.1	16.8±0.3	12.5±0.1

Volatilization

The mineralization of [PYM-^{14}C] BYI 02960 was high. At the end of the study (DAT-120) up to 45.3 (AX), 58.6 (HF), 29.4 (HN) and 57.3% AR (DD) of $^{14}CO_2$ were generated. Volatile organic compounds were negligible (≤ 0.1% AR).

Transformation of Test Item

The test item declined from 97.1, 96.1, 96.5 and 93.1% AR at DAT-0 to 37.1, 24.5, 50.2 and 28.7% in soils AX, HF, HN and DD, respectively, at the end of the study. Only very minor transformation products (all were below 3% AR) were detected. In all soils three very minor metabolites, designated ROI 1, ROI 2 and ROI 3 were quantified and characterized by their chromatographic behavior. ROI 2 reached maximum levels eat DAT-59 of 2.6 (in soil AX), 0.9 (soil HF), 1.6 (soil HN) and 0.4% AR (soil DD).In the different soil ROI 1 did not exceed 1.5 (AX), 1.6 (HF), 0.8 (HN) and 1.2% AR (DD). ROI 3 was a maximum of 0.4% AR in the four soils. In soil DD, three additional very minor peaks were detected with maximum levels of 0.6% AR. The total of non-characterized extracted radioactivity did not exceed 3.1% AR. The mentioned results were included in the proposed overall pathway of degradation of BYI 02960 in soil shown in Figure B.8.1.3-01.

Kinetics of Test Item Degradation

A summary of the DT_{50} and DT_{90} calculations for the test item is given under paragraph B.8.1.2. Overall, the amount of BYI 02960 declined during the test period of 120 days. The geometric mean of the DT_{50} and the DT_{90} values for degradation of BYI 02960 in the tested soils under aerobic conditions at 20 °C were 68.8 and 331.3 days, respectively. A summary of the DT_{50} and DT_{90} calculations for the test item is given under paragraph B.8.1.2.

Conclusions

Major Outcomes of Study

The data gathered in the current laboratory investigation demonstrate that [PYM-^{14}C] BYI 02960 is degraded in the four soils. Three very minor metabolites were detected and quantified together with the test item. All further formed metabolites are regarded as transient, which is confirmed by the high mineralization rate of [PYM-^{14}C] BYI 02960 to $^{14}CO_2$ observed in this study, i.e. between 29.4 % (soil HN) and 58.6% of AR (soil HF) at the end of the study. Volatile organic compounds were very low (≤ 0.1% AR) at all sampling dates.

Table B.8.1.1.1-07 Synopsis of overall results

Soil	AX	HF	HN	DD
Total Recovery (%)	96.1 – 99.2	96.1 – 101.1	95.6 – 102.4	97.1 – 101.3
Extracted RA (%)	41.3 – 97.8	29.3 – 97.0	53.4 – 99.1	31.5 – 94.4
RA desorbed (%)	13.5 – 64.5	9.2 – 56.8	12.2 - 45.4	6.3 – 35.4
Max. CO_2 (%)	45.3	58.6	29.4	57.3
Bound Residues (%)	1.0 – 12.6	1.5 – 13.2	1.9 – 16.8	4.1 – 12.5
Extraction Efficiency of Test Substance DAT-0 (%)	97.1	96.1	96.5	93.1
Major metabolites	-	-	-	-

Significance of Results to Environmental Behaviour of BYI 02960

The current laboratory study demonstrated that [PYM-^{14}C] BYI 02960 is microbially degradable in soils under aerobic conditions. With respect to the radiolabel used mineralization to $^{14}CO_2$ is significant,

minor metabolites formed did not accumulate in soil and can therefore be regarded as transient. Non-extractable residues were low, maximum 16.8%.

Table B.8.1.1.1- 08 Synopsis of results of biotransformation of [PYM-^{14}C] BYI 02960 in soils incubated at 20 °C and 55 % of WHCmax under aerobic conditions

Soil	Laacher Hof AXXa	Hoefchen am Hohenseh	Hanscheiderhof	Dollendorf II
Soil type	Sandy loam	Silt loam	Loam	Clay loam
Major transformation products *	CO_2 (max. 58.6%) NER (comparatively low, max 16.8%)			
Minor transformation products	N/A			

*): Criteria for term "major": >10% of AR at any DAT or >5% of AR at two successive DATs or steadily increasing until the end of the study.

Remarks

The aerobic soil degradation, apart of the time dependent soption study, is acceptable and the results are reliable.

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Characteristics

reference	:	Menke, U., Unold, M. (2011)	study type	:	aerobic degradation
year of execution	:	2008	incubation time	:	120 days
GLP statement	:	yes	nominal concentration	:	1.07 mg/kg
guideline	:	OECD 307 (2002) US EPA, OPPTS 835.4100 (2008)	temperature	:	10°C or 20°C
test substance	:	[Furanone-4-^{14}C] BYI 02960	DT_{50}	:	see results
purity	:	> 98 %	metabolites	:	see results
soils	:	Four soils; sandy loam, silt loam, silt loam, silty clay	acceptability	:	**acceptable**

Summary

The biotransformation of [furanone-4-^{14}C] BYI 02960 was studied in four European soils: Laacher Hof AXXa (AX), Höfchen am Hohenseh (HF), Hanscheiderhof Plot 611 (HN), and Dollendorf II (DD) for a maximum period of 120 days under aerobic conditions in the dark at approx. 20 °C and 55% WHC_{max} (max. water holding capacity). BYI 02960 was applied at the nominal rate of 1.07 mg/kg dry weight of soil, which is equivalent to 400 g/ha field application rate.

At each sampling date, the soil samples were extracted with 2 x 80 mL acetonitrile/water (50/50, v/v), 1 x acetonitrile/water (80/20, v/v) and 1 x 80 mL acetonitrile by shaking at ambient temperature. Another extraction step was performed with acetonitrile/water (80/20, v/v) at 70°C using a microwave. The BYI 02960 residues and transformation products were analyzed and quantified by HPLC. TLC was used as confirmation method.

Material balances were complete throughout the study, and the test item declined from 96.9, 95.9, 96.4 and 94.3% of AR at DAT-0 to 37.3, 20.2, 45.2 and 26.9% at the end of the study for soils AX, HF, HN and DD, respectively. Applying double first-order kinetics a half-life (geometric mean) of 56.2 days was calculated for [furanone-4-^{14}C] BYI 02960 in the tested soils under aerobic conditions.

The mineralization of [furanone-4-^{14}C] BYI 02960 in this study was high. At the end of the study (DAT-120) up to 27.6 (soil AX), 38.9 (soil HF), 18.0 (soil HN) and 32.0% AR (soil DD) of $^{14}CO_2$ were generated. Volatile organic compounds were negligible (≤ 0.1% AR). Non-extractable ^{14}C-residues (NER) increased from 2.4, 3.8, 3.3 and 4.6% of AR at DAT-0 to 27.8, 33.6, 31.0 and 34.1% AR at the end of the study period for soils AX, HF, HN and DD, respectively. The major portions of NER radioactivity were found in the insoluble humin fraction.

Materials and methods

Materials

1. Test Item: Flupyradifurone: Code = BYI 02960; Label FUR = [Furanone-4-^{14}C] BYI 02960 (sample ID: KATH 6101); Specific activity 3.94 MBq/mg; Radiochemical purity: >98% (acc. radio-HPLC), >99% (acc. radio-TLC); Chemical purity: >98% (HPLC, UV detection at 210 nm). Identity and purity of test item in the application solution were confirmed.

2. Soil: The biotransformation of [FUR-^{14}C] BYI 2960 was studied in four different soils. These soils are representative for agricultural use areas as required by the guidelines and cover a representative range of physico-chemical properties. All soils were taken on fresh from the fields. Three days later, i.e. three days before starting the test the air dried soils were sieved through a 2 mm sieve and aliquots equivalent to 100 g dry matter were weighed into individual 300 mL Erlenmeyer flasks and fitted with trap attachments. The soils were pre-equilibrated at 20 °C in the dark.

Methods

1. Experimental conditions: The study was performed in static incubation test systems under aerobic conditions in the dark at 20.6 ± 0.3 °C. The test system consisted of Erlenmeyer flasks (300 mL) attached with a trap attachment (permeable to oxygen) containing soda lime for absorption of $^{14}CO_2$ and a polyurethane foam plug for adsorption of volatile organic compounds. Aliquots of 100 g of dry soil were weighed into the test flasks (each 28 flasks/soil). For all soils replicates were set up for each sampling (10 sampling dates including time 0, 4 spare flasks, and 1 flask for metabolite identification purposes treated with a 10x rate). Three flasks from each soil were used for determination of the microbial biomass. The final soil moisture was adjusted to 55% of WHCmax by adding deionized water.

2. Test Item Stock Solution: The entire delivered amount of [FUR-14C] BYI 02960 was dissolved in 4 mL methanol.

Table B.8.1.1.1-09 Soil physicochemical properties

Parameter	Results/Units			
Soil Batch ID	Laacherhof AXXa 20080325	Hoefchen am Hohenseh 4a 20080325	Hanscheiderhof 20080325	Dollendorf II 20080325

Location	Monheim, Germany	Burscheid, Germany	Burscheid, Germany	Blankenheim, Germany
Soil Taxonomic Classification (USDA)	Sandy floodplain deposits of the lower terrace of the Rhine river, material from the Pleistocene Ice Age	Loess or loess colluvium (Pleistocene, Holocene)	Not available	Not available
Soil Series	Sandy, mixed, mesic Typic Cambudolls	Loamy, mixed, mesic Typic Argudalfs	Not available	Not available
Texture Class (USDA)	Sandy Loam	Silt Loam	Silt Loam	Silty Clay
Sand Silt Clay	67% 19% 14%	21% 59% 20%	27% 53% 20%	17% 41% 42%
pH in Water pH in CaCl2 pH in KCl	6.3 6.1 5.9	6.6 6.5 6.2	5.1 4.8 4.4	7.2 7.1 6.8
Organic Matter	3.4%	4.3%	5.7%	7.1%
Organic Carbon	2.0%	2.5%	3.3%	4.1%
Soil Microbial Biomass	mg microbial carbon per kg of soil			
0 days	561	1140	751	2541
59 days	433	719	477	1999
120 days	367	635	336	1797
Cation Exchange Capacity (CEC)	9.6 meq/100 g	13.4 meq/100 g	10.5 meq/100 g	19.2 meq/100 g
WHCmax	51.6 g H_2O/ 100 g DM	66.3 g H_2O/ 100 g DM	75.2 g H_2O/ 100 g DM	77.6 g H_2O/ 100 g DM

3. Test Item Application Solution: An application solution (total volume 60 mL) was made by diluting 2853 µL of the stock solution with 57.1 mL of purified water.

4. Mode of Application: Aliquots of 391 µL of the application solution were applied in droplets onto the 100 g pre-incubated subsamples of each soil. By the addition of the application solution, the water content was finally adjusted to 55% of WHCmax. The test vessels for DAT-0 were immediately processed for analysis. All other test vessels, including the biomass flasks which were not spiked with application solution, were fitted with trap attachments and incubated in the dark at nominal 20 ± 1°C.

[FUR-14C] BYI 02960 was applied at a rate of 416200 Bq per vessel. This corresponds to 105.63 µg per vessel which is equivalent to 99% of the application rate of 106.7 µg per vessel (calculated. a single application rate of 400 g BYI 02960 per hectare).

5. Sampling: Microbial biomass was determined prior to commencement of the test (soils sampled at the day of application), after 59 days, and at the end of the study 120 Days After Treatment (DAT-120). Entire test flasks were taken for processing and analysis at DAT-0, DAT-1, DAT-3, DAT-7, DAT-14, DAT-30, DAT-45, DAT-59, DAT-85, and DAT-120.

Prior to opening the incubation flasks (for moistening or sampling of soil), volatile (radioactive) compounds, possibly still present in the flasks, were transferred into the trap attachment by subjecting the flasks to vacuum in an excicator. At each sampling date the entire amount of soil in each test vessel was transferred into a centrifuge beaker and extracted using a mechanical shaker.

6. Description of analytical procedures: The soil processing procedure was optimized to obtain >90% extraction efficiency and >90% recovery of the test item at time zero. At each sampling date, the soil samples were extracted with 2 x 80 mL acetonitrile/water (50/50, v/v), 1 x acetonitrile/water (80/20, v/v) and 1 x 80 mL acetonitrile by shaking at ambient temperature. Another extraction step was performed with acetonitrile/water (80/20, v/v) at 70°C using a microwave.

The BYI 02960 residues and transformation products were analyzed and quantified by LSC and reversed phase radio-HPLC. Normal-phase Si-60 radio-TLC was used as a confirmatory method. The limit of quantification (LOQ) was derived from the LOD by the operation: LOQ = 3 x LOD resulting in a LOQ of about 0.9% of AR. However, values between LOD and LOQ were also used for quantification. The limit of the detection in ambient and aggressive organic extracts was in the range of 0.3% of AR. The limit of detection for a single TLC peak was estimated from the data sheets used for the comparison of the results obtained by HPLC and TLC. The lowest peaks quantified were assigned to DFA in the aggressive and account for 0.1% of AR.

The identity of the test item in stock solution and in extracts was confirmed by spectroscopic methods. In addition, spectroscopic methods were used to identify one metabolite.

Results

Results indicated that anticipated standardized conditions were maintained, and the soils were microbially active over the duration of the laboratory study (see Table B.8.1.1.1-09).

Data

The respective data for the four soils are shown in Table B.8.1.1.1-10 to Table B.8.1.1.1-13. The DAT-0 extraction efficiency was in the range of 94.6 to 97.3% of AR (mean 96.2% AR; sum of extracts only). The stability of the test item was verified by DAT 0 values of 94.3 to 96.9% AR for the soil extracts (mean 95.9% AR). These results indicate that the extraction method was appropriate to extract the applied [^{14}C]-labeled test item from the soil matrix.

Table B.8.1.1.1-10 Biotransformation of [FUR-^{14}C] BYI 02960 in sandy loam soil AX under aerobic conditions; mean values and standard deviations expressed as % of AR

Compound	Days After Treatment (DAT)									
	0	1	3	7	14	30	45	59	85	120
BYI 02960	96.9 ±0.2	94.4 ±0.1	89.3 ±0.0	83.0 ±0.0	74.2 ±0.1	62.6 ±0.3	54.3 ±0.1	48.4 ±0.1	42.3 ±0.3	37.3 ±0.5
ROI 1	n.d.	n.d.	n.d.	0.4 ±0.0	0.8 ±0.2	n.d.	1.2 ±0.0	1.0 ±0.2	1.1 ±0.1	1.0 ±0.0
BYI 02960-chloro	n.d.	n.d.	n.d.	0.4 ±0.0	0.8 ±0.2	0.8 ±0.1	1.3 ±0.2	1.5 ±0.1	1.7 ±0.1	1.8 ±0.0
ROI 3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.4 ±0.1	0.4 ±0.2	0.3 ±0.0	0.1 ±0.1
ROI 4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ROI 5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.1	n.d.

									±0.1	
Non-charact. radioactivity	0.4 ±0.0	0.4 ±0.1	0.3 ±0.1	0.4 ±0.0	0.3 ±0.1	0.3 ±0.0	0.3 ±0.1	0.2 ±0.0	0.3 ±0.0	0.2 ±0.0
Total extractable residues	97.3 ±0.2	94.8 ±0.0	89.7 ±0.1	84.1 ±0.0	76.0 ±0.3	63.7 ±0.1	57.5 ±0.5	51.6 ±0.0	45.7 ±0.1	40.4 ±0.6
$^{14}CO_2$	n.a.	0.5 ±0.0	1.6 ±0.0	3.7 ±0.0	6.8 ±0.1	13.2 ±0.1	16.9 ±0.8	20.4 ±0.2	24.5 ±0.1	27.6 ±0.2
Volatile organics	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extractable residues (NER)	2.4 ±0.1	4.5 ±0.1	6.9 ±0.0	11.0 ±0.1	16.1 ±0.0	21.2 ±0.1	23.7 ±0.1	25.2 ±0.2	27.1 ±0.3	27.8 ±0.1
Total recovery	99.7 ±0.1	99.7 ±0.1	98.1 ±0.1	98.8 ±0.2	99.0 ±0.4	98.0 ±0.3	98.1 ±0.2	97.2 ±0.1	97.3 ±0.3	95.8 ±0.3

n.d. = not detected; n.a. = not analyzed; ROI = region of interest
ROI 2 was identified as compound BYI 02960-chloro

Table B.8.1.1.1-11 Biotransformation of [FUR-^{14}C] BYI 02960 in silt loam soil HF under aerobic conditions; mean values and standard deviations expressed as % of AR

Compound	Days After Treatment (DAT)									
	0	1	3	7	14	30	45	59	85	120
BYI 02960	95.9 ±0.3	93.7 ±0.1	90.4 ±0.7	80.6 ±0.5	71.1 ±0.0	52.7 ±0.4	40.6 ±0.5	33.3 ±0.4	26.2 ±0.1	20.2 ±0.2
ROI 1	n.d.	n.d.	0.2 ±0.2	0.4 ±0.1	0.7 ±0.0	0.8 ±0.2	1.6 ±0.0	1.4 ±0.0	1.4 ±0.1	1.2 ±0.1
BYI 02960-chloro	n.d.	n.d.	n.d.	0.4 ±0.1	0.5 ±0.1	1.0 ±0.2	1.3 ±0.1	1.3 ±0.3	1.3 ±0.0	1.5 ±0.3
ROI 3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.3 ±0.3	0.5 ±0.1	n.d.	n.d.
ROI 4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ROI 5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Non-charact. radioactivity	0.3 ±0.0	0.4 ±0.1	0.4 ±0.1	0.2 ±0.0	0.2 ±0.0	0.4 ±0.0	0.3 ±0.0	0.2 ±0.0	0.3 ±0.0	0.1 ±0.0
Total extract. residues	96.3 ±0.3	94.1 ±0.1	91.0 ±0.7	81.7 ±0.5	72.5 ±0.1	54.9 ±0.1	44.2 ±0.2	36.8 ±0.0	29.2 ±0.0	23.0 ±0.5
$^{14}CO_2$	n.a.	0.5 ±0.0	1.6 ±0.0	4.3 ±0.0	8.3 ±0.0	17.3 ±0.0	23.6 ±0.6	27.7 ±0.1	32.7 ±0.1	38.9 ±0.2
Volatile organics	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extract. residues (NER)	3.8 ±0.1	5.3 ±0.0	7.9 ±0.2	12.5 ±0.1	17.7 ±0.1	25.5 ±0.1	29.7 ±0.2	31.7 ±0.1	33.6 ±0.2	33.0 ±0.4
Total recovery	100.1 ±0.4	99.9 ±0.2	100.5 ±0.9	98.4 ±0.6	98.6 ±0.1	97.7 ±0.0	97.5 ±0.2	96.1 ±0.0	95.5 ±0.3	94.9 ±0.2

n.d. = not detected; n.a. = not analyzed; ROI = region of interest
ROI 2 was identified as compound BYI 02960-chloro

Table B8.1.1.1-12 Biotransformation of [FUR-^{14}C] BYI 02960 in silt loam soil HN under aerobic conditions; mean values and standard deviations expressed as % of AR

Compound	Days After Treatment (DAT)									
	0	1	3	7	14	30	45	59	85	120
BYI 02960	96.4 ±0.2	92.0 ±0.2	88.4 ±0.0	82.3 ±0.2	77.7 ±0.0	66.9 ±0.6	60.9 ±0.4	56.6 ±0.2	50.7 ±0.2	45.2 ±0.3
ROI 1	n.d.	n.d.	n.d.	0.4 ±0.0	0.8 ±0.0	1.3 ±0.1	1.7 ±0.0	1.6 ±0.0	1.2 ±0.0	1.1 ±0.1
BYI 02960-chloro	n.d.	n.d.	n.d.	0.4 ±0.0	0.6 ±0.1	1.2 ±0.1	1.2 ±0.2	1.2 ±0.0	1.3 ±0.1	1.3 ±0.1
ROI 3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.2 ±0.2	0.1 ±0.1	n.d.	n.d.

ROI 4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ROI 5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Non-charact. radioactivity	0.4 ±0.0	0.5 ±0.0	0.3 ±0.1	0.4 ±0.0	0.4 ±0.1	0.4 ±0.1	0.3 ±0.0	0.2 ±0.0	0.2 ±0.0	0.3 ±0.1
Total extract. residues	96.7 ±0.2	92.5 ±0.2	88.7 ±0.1	83.6 ±0.2	79.5 ±0.2	69.9 ±0.3	64.4 ±0.4	59.7 ±0.1	53.4 ±0.1	48.0 ±0.4
$^{14}CO_2$	n.a.	0.4 ±0.0	1.1 ±0.0	2.3 ±0.0	3.9 ±0.0	7.0 ±0.0	9.2 ±0.1	11.0 ±0.2	14.0 ±0.1	18.0 ±0.1
Volatile organics	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extract. residues (NER)	3.3 ±0.1	6.9 ±0.3	9.4 ±0.1	12.8 ±0.1	16.5 ±0.2	21.7 ±0.1	24.9 ±0.1	26.7 ±0.4	29.8 ±0.1	31.0 ±0.1
Total recovery	100.0 ±0.3	99.8 ±0.4	99.2 ±0.0	98.7 ±0.3	99.9 ±0.4	98.6 ±0.3	98.4 ±0.3	97.5 ±0.1	97.2 ±0.2	96.9 ±0.1

n.d. = not detected; n.a. = not analyzed; ROI = region of interest
ROI 2 was identified as compound BYI 02960-chloro

Table B.8.1.1.1-13 Biotransformation of [FUR-^{14}C] BYI 02960 in silty clay soil DD under aerobic conditions; mean values and standard deviations expressed as % of AR

Compound	Days After Treatment (DAT)									
	0	1	3	7	14	30	45	59	85	120
BYI 02960	94.3 ±0.7	93.3 ±0.2	89.5 ±0.0	84.5 ±0.3	75.5 ±0.1	63.5 ±0.9	50.9 ±1.4	41.4 ±0.0	34.0 ±0.5	26.9 ±0.3
ROI 1	n.d.	n.d.	n.d.	n.d.	0.8 ±0.2	0.9 ±0.1	1.6 ±0.2	1.7 ±0.1	1.6 ±0.1	1.6 ±0.0
BYI 02960-chloro	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.2 ±0.2	0.4 ±0.1	0.4 ±0.0	n.d.
Reg 3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.5 ±0.0	n.d.	n.d.	n.d.
ROI 4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.4 ±0.1	n.d.	n.d.
ROI 5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Non-charact. radioactivity	0.4 ±0.1	0.5 ±0.0	0.5 ±0.0	0.3 ±0.1	0.3 ±0.1	0.4 ±0.0	0.3 ±0.0	0.2 ±0.1	0.3 ±0.1	0.3 ±0.0
Total extract. residues	94.6 ±0.8	93.8 ±0.1	90.0 ±0.0	84.8 ±0.4	76.7 ±0.0	64.8 ±0.9	53.4 ±0.9	44.2 ±0.1	36.2 ±0.4	28.8 ±0.3
$^{14}CO_2$	n.a.	0.3 ±0.0	1.0 ±0.0	2.8 ±0.0	5.5 ±0.0	11.7 ±0.4	17.4 ±0.4	21.6 ±0.1	26.8 ±0.0	32.0 ±0.5
Volatile organics	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extract. residues (NER)	4.6 ±0.6	5.5 ±0.0	8.2 ±0.4	11.0 ±0.0	15.8 ±0.3	21.6 ±0.7	24.9 ±0.9	30.2 ±0.1	32.4 ±0.1	34.1 ±0.1
Total recovery	99.3 ±0.1	99.6 ±0.1	99.2 ±0.3	98.6 ±0.5	98.0 ±0.3	98.1 ±0.2	95.8 ±0.4	96.0 ±0.2	95.4 ±0.3	94.9 ±0.2

n.d. = not detected; n.a. = not analyzed; ROI = region of interest
Reg 2 was identified as compound BYI 02960-chloro
Reg 4 was tentatively identified as compound BYI 02960-des-difluoroethyl

Mass Balance

Material balances ranged from 95.8 to 99.7% (soil AX), 94.9 to 100.5% (soil HF), 96.9 to 100.0% (soil HN) and 94.9 to 99.6% (soil DD) of the applied radioactivity [AR]. The high material balances shown for all sampling intervals demonstrate that no significant RA dissipated from the flasks or was lost during processing.

Extractable and Bound Residues (NER)

Extractable ^{14}C-residues decreased from 97.3, 96.3, 96.7, and 94.6% AR at DAT-0 to 40.4, 23.0, 48.0 and 28.8% AR at study end (DAT-120) in soils AX, HF, HN, and DD, respectively.

Non-extractable ^{14}C-residues increased from 2.4, 3.8, 3.3 and 4.6% of AR at DAT-0 to 27.8, 33.6, 31.0 and 34.1% AR at the end of the study period for soils AX, HF, HN and DD, respectively. The major portions of non-extractable radioactivity were found in the insoluble humin fraction.

Table B.8.1.1.1-14 Summary of extractable and non-extractable residues

Soil	AX	HF	HN	DD
Extracted RA (%) Day 0	97.3±0.2	96.3±0.3	96.7±0.2	94.6±0.8
Day 120	40.4±0.6	23.0±0.5	48.0±0.4	28.8±0.3
Non-Extracted RA (%) Day 0	2.4±0.1	3.8±0.1	3.3±0.1	4.6±0.6
Day 120	27.8±0.1	33.0±0.4	31.0±0.1	34.1±0.1

Volatilization

The mineralization of [FUR-^{14}C] BYI 02960 was high. At the end of the study (DAT-120), $^{14}CO_2$ accounted for 27.6 (soil AX), 38.9 (soil HF), 18.0 (soil HN) and 32.0% (soil DD) of AR. Volatile organic compounds were not detected in significant amounts (≤ 0.1% AR).

Transformation of Test Item

The test item declined from 96.9, 95.9, 96.4 and 94.3% of AR at DAT-0 to 37.3, 20.2, 45.2 and 26.9% at the end of the study for soils AX, HF, HN and DD, respectively. Only very minor transformation products (all were below 2% AR) were detected in the extracts. Three metabolites, designated ROI 1, ROI 2 and ROI 3, were detected in all four soils and were characterized by their retention times. ROI 1 reached maximum levels of 1.2 (AX), 1.6 (HF), 1.7 (HN) and 1.7% (DD) of AR at DAT-45 or DAT-59. ROI 2 amounted to maximally 1.8 (AX), 1.5 (HF), 1.3 (HN) and 0.4% (DD) of AR with increasing amounts towards the end of the study. This metabolite was identified as BYI 02960-chloro by spectroscopic methods. ROI 3 accounted for up to 0.4 (AX), 0.5 (HF), 0.2 (HN) and 0.5% (DD) of AR, respectively. The transformation products assigned to ROI 4 and ROI 5 appeared only once with mean values of 0.4% (DD) and 0.1% (AX) of applied radioactivity, respectively. ROI 4 was tentatively identified as BYI 02960-des-difluoroethyl via co-chromatography. The total of non-characterized extracted radioactivity did not exceed 0.5% of AR.

The results were included in the proposed overall pathway of degradation of BYI 02960 in soil shown in Figure B.8.1.3-01.

Kinetics of Test Item Degradation

A summary of the DT_{50} and DT_{90} calculations for the test item is given under paragraph B.8.1.2.

Conclusions

Major Outcomes of Study

The data gathered in the current laboratory investigation demonstrate that [furanone-4-^{14}C] BYI 02960 is degraded in the four soils; a mean DT_{50} value of 56.2 days (geometric mean, n = 4 soils) was calculated. A synopsis of results is shown in Table B.8.1.1.1-15.

A few very minor metabolites were detected and quantified together with the test item. All further formed metabolites are regarded as transient, which is confirmed by the high mineralization rate of [FUR-^{14}C] BYI 02960 to $^{14}CO_2$ observed in this study, i.e. between 18.0 % (soil HN) and 38.9% of AR (soil HF) until the end of the study. Volatile organic compounds were very low (≤ 0.1% AR) at all sampling dates.

Table B.8.1.1.1-15 Synopsis of overall results

Soil	AX	HF	HN	DD
Total Recovery (%)	95.8 – 99.7	94.9 – 100.5	96.9 – 100.0	94.9 – 99.6
Extracted RA (%)	40.4 – 97.3	23.0 – 96.3	48.0 – 96.7	28.8 – 94.6
Max. CO_2 (%)	27.6	38.9	18.0	32.0
Bound Residues (%)	2.4 – 27.8	3.8 – 33.6	3.3 – 31.0	4.6 – 34.1
Extraction Efficiency on DAT-0 (%)	97.3	96.3	96.7	94.6

Significance of Results to Environmental Behavior of BYI 02960

The current laboratory study demonstrated that [FUR-^{14}C] BYI 02960 is degradable in soils under aerobic conditions. With respect to the radiolabel used mineralization to $^{14}CO_2$ is significant, however, other metabolites are not to be expected in soil, since – after their formation – they are degraded further (see Table B.8.1.1.1-16).

Table B.8.1.1.1-16 Synopsis of results of biotransformation of [FUR-^{14}C] BYI 02960 in soils incubated at 20 °C and 55 % of WHCmax under aerobic conditions

Soil	Laacher Hof AXXa	Hoefchen am Hohenseh	Hanscheiderhof	Dollendorf II
Soil type	Sandy loam	Silt loam	Silt loam	Silty clay
Major transformation products *	$^{14}CO_2$ (max. 38.9% NER (max. 34.1%)			
Minor transformation products	BYI 02960-chloro (max. 1.8%) BYI 02960-des-difluoroethyl (max. 0.4%)			

*): Criteria for term "major": >10% of AR at any DAT or >5% of AR at two successive DATs or steadily increasing until the end of the study.

Remarks

The aerobic soil degradation study is acceptable and the results are reliable.

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Characteristics

reference	:	Ripperger, R.J. (2011)	study type	:	aerobic degradation
year of execution	:	2010	incubation time	:	120 days
GLP statement	:	yes	nominal concentration	:	1.07 mg/kg
guideline	:	OECD 307 (2002) US EPA, OPPTS 835.4100 (2008)	temperature	:	20°C

test substance	:	[Furanone-4-^{14}C] BYI 02960	DT_{50}	:	see results
purity	:	> 98 %	metabolites	:	see results
soils	:	Two soils; silt loam, sandy loam	acceptability	:	**acceptable**

Summary

The biotransformation of [FUR-^{14}C] BYI 02960 was studied in two US soils: silt loam Springfield, NE, and sandy loam Sanger, CA, for a maximum period of 120 days under aerobic conditions in the dark at approx. 20 °C and soil moistures maintained between pF 2.0 to 2.5. Gamma irradiated samples were investigated along with microbial active test systems. BYI 02960 was applied at the rate of 1.1 µg a.i./g soil, equivalent to 410 g a.i./ha assuming a 2.5 cm soil depth.

Duplicate test systems were analyzed at 0, 3, 7, 14, 28, 60, 92, and 120 days of incubation. The 50-g soil samples were extracted by shaking with acetonitrile:water (70:30) and acetonitrile (100%), followed by microwave extraction at 70 °C(aggressive extract) using acetonitrile:water (70:30). Extract aliquots were concentrated and analyzed by HPLC. Identification of the transformation products was performed by LC/MS, co-chromatography, and/or retention time comparison with reference standards.

Material balances were complete throughout the study, and the test item declined from 98.0 and 99.4% and of the applied amount at day 0 to 67.3 % and 30.8% of the applied at the end of the study. The first-order half-life of BYI 02960 in silt loam was 228 days. The first-order half-life in the sandy loam was 65.7 days.

BYI 02960 mineralizes relatively rapidly under aerobic condition to $^{14}CO_2$ (12.3% Springfield, 36.1% Sanger) and becomes increasingly bound to soil (16.4% Springfield, 30.6% Sanger, CA) by study end. The amount of $^{14}CO_2$ and bound residue formed in gamma-irradiated soils was significantly less than in non-sterile soils indicating a biological component to degradation and the formation of non-extractable residues from BYI 02960. Additionally, soil fractionation shows that even with extraction using strong base, BYI 02960 related residues remain bound to the solid (humin) fraction indicating very strong and irreversible binding to soil.

Materials and Methods

Materials

1. Test Item: Flupyradifurone: Code = BYI 02960; Label FUR = [Furanone-4-14C] BYI 02960 (sample ID: KATH 6252); Specific activity: 30.74 mCi/mMole; Supplied substance was repurified (vial C-1116A, 429.4 µCi/mL); the final radiochemical purity was 100%. Identity and purity of test item in the application solution were confirmed.

2. Soil: The biotransformation of [FUR-^{14}C] BYI 2960 was studied in two different soils (see Table B.8.1.1.1-17). These soils are representative for agricultural use areas as required by the guidelines and cover a representative range of physico-chemical properties. NE and CA soils were taken fresh from the fields. Each soil was collected from the top 0 to 8 inches of the field and shipped to the testing

facility subsequently. No pesticides were applied in the past 5 years to these sites where soils were collected. The NE soil was planted with alfalfa and maintained in a greenhouse prior to use (40 days). The CA soil was maintained in a walk-in refrigerator prior to use (7 days).

The soils were air dried and sieved through a 2-mm sieve. Subsequently, aliquots equivalent to 50 g dry matter were weighed into individual 250 mL glass flasks until starting the test.

Test systems were pre-equilibrated at 20 ± 1°C with soil moisture between pF 2.0 to 2.5 for six days prior to the treatment.

Table B.8.1.1.1-17 Soil physicochemical properties

Parameter	Results/Units	Results/Units
Geographic Location	Springfield, NE, USA	Sanger, CA, USA
	Dozier AG Research Services LLC, PO Box 308 Hwy 50 & Cornish Rd Springfield, NE 68059	SynTech Research, Inc. 17915 E. Annadale Ave Sanger, CA. 93657
Soil Mapping Unit	41.03725 96.15085	N 36° 42' 22.16" W 119° 28' 00.12"
Taxonomic Name:	Marshal fine-silty mixed superactive mesic typic Hapludolls	Hanford Fine Sandy Loam Gravely substrate
Texture Class (USDA)	Silt Loam	Sandy Loam
Sand Silt Clay	13.4% 63.8% 22.8%	67.8% 25.0% 7.2%
pH Saturated paste 1:1 soil:water 0.01 M CaCl2	 6.7 6.7 6.5	 7.4 7.3 7.0
Organic Matter	4.0%	0.97%
Organic Carbon[B]	2.3%	0.57%
Cation Exchange Capacity (CEC)	17.8 meq/100 g	6.3 meq/100 g
Maximum Water Holding Capacity	49.6 g/100 g	27.7 g/100 g
Soil Moisture at 0.1 bar (pF 2.0)	37.9	15.8
Soil Moisture at 0.33 bar (pF 2.5)	26.4	9.1
Bulk Density (disturbed)	1.04 g/m3	1.29 g/m3
Microbial Biomass:[C] Initial (day 6) Middle (day 71) Final (day 120)	(mg Microbial C/kg soil) 833 686 660	Microbial Biomass:[C] Initial (day 6) Middle (day 71) Final (day 120)

Methods

1. Experimental conditions: All test systems were incubated at 20 ± 1°C in the dark in a temperature-controlled environmental chamber. The test systems containing 50 g soil (dry-weight basis) consisted of a 250-mL glass flask (21 cm long and 7.2 cm internal diameter) connected to a flow-through system, containing an ethylene glycol trap for volatile organics followed by two 2 M potassium hydroxide traps for collecting CO_2 and a 1 M sulfuric acid trap for volatile acids. The headspace above the soil was continuously purged with humidified air throughout the study.

For both soils twenty test systems were treated at 1X for the kinetic study rate with each soil (1.1 µg/g; see next section). Three test systems were prepared for metabolite identification (MID) purposes, and these systems were treated at 10x the kinetic rate. MID test systems were not used for kinetic evaluation. Four control test systems were prepared as biomass test systems to demonstrate biological activity of the soil.

For the sterile portion of this study, soil was sent to Food Technology Service, Inc. (FTSI; Mulberry, FL) for gamma irradiation (actual delivered absorbed dose of 24.67 kGy). Gamma-irradiated soils were transferred to sterilized 250-mL glass flasks within a BioGuard Laminar Flow Hood using aseptic techniques. Soils were treated at the kinetic rate in the laminar flow hood using aseptic techniques, and then connected to the flow-through system. Sterility was tested at each sampling interval on each test system by plating soil dilutions on 3M Petrifilm Agar plates which were incubated at ~34°C for a minimum of 48 hours.

In total, 64 test systems were prepared. All the soils were adjusted to the appropriate moisture level for each soil, and the moisture was maintained throughout the course of the study.

2, Test Item Application Solution: The application (treatment) solution was prepared by diluting 955 µL of [FUR-^{14}C] BYI 02960 (C-1116A) with 36 mL of H_2O:MeOH (4:1) for a theoretical concentration of 0.11 µg/µL. The extensive radio-assays to determine the concentration of kinetic treatment solution resulted in 25,996,470 dpm/mL or 110 µg/mL. Radiochemical purity and identity were confirmed by HPLC and mass spectral analysis.

3. Mode and Rate of Application: Aliquots of 500-µL of the kinetic treatment solution were applied in droplets onto the 50 g pre-incubated subsamples of soils, using a 500-µL gas-tight syringe. This rate is equal to the field-use rate of 410 g a.i./ha at 2.5 cm depth and 1.5 g/cm^3 soil density. The final concentration of a.i. in each test system was 1.1 µg BYI 02960 per g soil (dry weight). By the addition the water contained the soil moisture was finally adjusted to approx. pF 2.0. Material balance for the study was based on the day 0 recovery of radioactivity (12,632,000 dpm Springfield, NE and 12,557,000 dpm Sanger, CA).

4.Test System Maintenance and Sampling: Test systems were checked for air flow through the traps and water levels in the moisture flasks. The test systems were incubated at 20 ± 1°C in the dark in an environmental chamber. The moisture loss in each system was monitored periodically by comparing the weight of the test system with the weight on Day 0. The moisture of the test systems was measured at each interval. The moisture was maintained at pF 2.5 to pF 2.0 for each soil by the addition of HPLC grade water, as needed, at periodic intervals.

Duplicate test systems were extracted and analyzed on days 0, 3, 7, 14, 28, 60, 92 and 120 days after treatment. Gamma-irradiated test systems were processed at 0, 60, 61 and 122 days after treatment.

For the sterile portion of this study, the goal was to compare the formation of bound residues with non-sterile test systems and therefore, soil extracts were radio-assayed but not analyzed by HPLC. Extracted soils were analyzed by combustion.

On the day of sampling, the test system and associated volatile traps were removed from the incubator for analysis. Prior to removing samples, the air flow was increased for approx. 15 minutes to ensure all headspace volatiles had been purged and trapped. The two KOH trap solutions were combined, volumes of the traps were recorded and triplicate aliquots were radio-assayed.

The soil was extracted and analyzed by LSC on the day of sampling. The soil extracts were analyzed by HPLC within 16 days after sample extraction. Concentrated extracts were stored in a laboratory freezer.

5. Description of analytical procedures: The soil processing procedure was optimized to obtain >90% extraction efficiency and >90% recovery of the test item at time zero. At each sampling date the entire amount of soil in each test vessel was transferred into a centrifuge bottle and extracted for 30 minutes with approximately 40 mL of acetonitrile:water (70:30, v/v) and centrifuged (5 minutes at 1850 g).

The supernatant was decanted, and the remaining soil was extracted two additional times with acetonitrile:water (70:30,v/v) followed by an extraction with approximately 40 mL of acetonitrile. The supernatants were pooled, and the volume was recorded. Extracts were radioassayed by LSC in triplicate (0.25 to 1.0 mL). An aliquot (approximately 4 mL) of the combined extracts was concentrated by a stream of nitrogen and analyzed by HPLC.

Soil remaining after the ambient acetonitrile/water and acetonitrile extractions was extracted by microwave extraction using 50 mL of acetonitrile:water (70:30, v/v) at 70°C for 10 minutes. The volume was recorded, and the extract was radioassayed by LSC in triplicate (0.25 to1.0 mL). An aliquot (approximately 4 mL) was concentrated by a stream of nitrogen and then analyzed by HPLC.

Extracted soils were air-dried under a fume hood and thoroughly homogenized. Subsamples (0.3 to 1.0 g) of the non-extracted residue (NER) were quantified by combustion.

To characterize the NER, the extracted soils from the day 92 sandy loam samples were fractionated to quantify the amounts of radioactivity associated with humic acid, fulvic acid, and humin. The BYI 02960 residues and transformation products were analyzed and quantified by LSC and radio-HPLC. Recovery of radioactivity from the HPLC column was determined by comparing radioactivity of the collected HPLC effluent (triplicate aliquots, radio-assayed) to the calculated radioactivity of the injected representative samples.

The LOD for HPLC was determined empirically by a series of injections of increasing radioactivity. The lowest amount of radioactivity detected resulted in minimum peak height of 3 times the background level in the chromatogram (520 dpm) and was considered to be the instrument LOD. The minimum amount of radioactivity injected for a sample was 3,274 dpm from the Sanger, CA day 14, replicate 2 microwave extract. This extract contained 2.8% of the applied activity which results in a LOQ of 0.4% of the applied radioactivity.

Liquid chromatography/electrospray ionization-mass spectrometry (LC/ESI-MS) and liquid chromatography/electrospray ionization-mass spectrometry/mass spectrometry analyses of standards and isolated compounds was performed.

Results and discussion

Results indicated that anticipated standardized conditions were maintained, and the soils were microbial active over the duration of the laboratory study (see Table B.8.1.1.1-17).

Data

The respective data for the two soils are shown in Table B.8.1.1.1-18 and Table B.8.1.1.1-19. The ambient and aggressive extracts were effective in extracting essentially all of the ^{14}C-residues in both soils at DAT-0, the beginning of the study (silt loam = 99.2%, sandy loam = 99.8%).

The stability of the test item was verified by DAT 0 values of 98.0 and 99.4% AR (mean of duplicates) for the soil extracts. These results indicate that the extraction method was appropriate for extraction of the applied [^{14}C]-labeled test item from the soil matrix.

Table B.8.1.1.1-18 Biotransformation of [FUR-^{14}C] BYI 02960 in silt loam Springfield (NE) under aerobic conditions; mean values and standard deviations expressed as % of AR

Compound	Days After Treatment (DAT)							
	0 b)	3 b)	7	14	28 c)	60	92	120
BYI 02960	98.0 ±0.4	93.1 ±0.4	94.0 ±0.8	89.8 ± 0.7	87.1 ± n.a.	78.2 ±0.0	72.1 ±0.6	67.3 ±2.3
Non-characterized radioactivity a)	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ± n.a.	0.0 ±0.0	2.4 ±0.4	3.6 ±1.4
Total extractable residues	99.2 ±0.4	95.8 ±0.3	94.0 ±0.8	89.8 ±0.7	87.1 ± n.a.	78.2 ±0.0	74.5 ±0.2	70.9 ±0.9
$^{14}CO_2$	0.0 ±0.0	0.5 ±0.0	1.4 ±0.1	2.2 ±0.1	4.2 ± n.a.	7.8 ±0.1	9.8 ±0.7	12.3 ±1.1
Volatile organics	0.0 ±0.0	0.0 ± 0.0	± 0.0 ± 0.0	± 0.0 ± 0.0	± 0.0 ± n.a.	± 0.0 ± 0.0	± 0.0 ± 0.0	± 0.0 ± 0.0
Non-extractable residues (NER)	0.8 ±0.0	2.6 ±0.2	4.5 ±0.2	6.7 ±0.0	9.0 ± n.a.	13.4 ±0.3	14.4 ±0.2	16.4 ±0.3
Total recovery	100.0 ±0.4	99.0 ±0.5	99.9 ±1.2	98.9 ±0.6	100.4 ± n.a.	99.3 ±0.2	98.6 ±0.7	99.6 ±1.8

a) No individual peak accounted to more than 1.5%.
b) Aggressive extracts were not analyzed since the RA content was less than 3% of total applied.
c) SD not to be calculated because just a single replicate was considered: moisture level of one replicate was less than pF 2.5.
n.a. = not analyzed
Note: ppm analyte = analyte % of AR x (ppm parent applied/100%) x MW analyte/288.7)

Table B.8.1.1.1-19 Biotransformation of [FUR-^{14}C] BYI 02960 in sandy loam Sanger (CA) under aerobic conditions; mean values and standard deviations expressed as % of AR

Compound	Days After Treatment (DAT)							
	0 b)	3 b)	7 b)	14	28	60	92	120
BYI 02960	99.4 ±0.3	95.2 ±0.8	86.5 ±0.4	79.8 ± 0.2	67.7 ± 0.6	48.3 ±0.9	37.7 ±1.4	30.8 ±0.0
Non-characterized radioactivity a)	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.6 ±0.6	2.8 ±0.2	2.8 ±0.3
Total extractable residues	99.8 ±0.3	96.2 ±0.6	89.0 ±0.4	79.8 ±0.2	67.7 ± 0.6	49.0 ±0.0	40.5 ±1.1	33.7 ±0.2
$^{14}CO_2$	0.0 ±0.0	0.7 ±0.0	2.7 ±0.1	6.2 ±0.0	11.7 ± 0.1	23.9 ±0.2	30.1 ±0.6	36.1 ±0.1
Volatile organics	0.0 ±0.0	0.0 ± 0.0	± 0.0 ± 0.0	± 0.0 ± 0.0	± 0.0 ± n.a.	± 0.0 ± 0.0	± 0.0 ± 0.0	± 0.0 ± 0.0
Non-extractable residues (NER)	0.2 ±0.0	2.8 ±0.1	8.4 ±0.5	13.6 ±0.4	19.6 ± 0.5	27.9 ±0.2	28.7 ±0.7	30.6 ±0.0
Total recovery	100.0 ±0.2	99.6 ±0.8	100.1 ±0.8	99.7 ±0.2	99.1 ± 1.1	100.8 ±0.3	99.3 ±0.2	100.4 ±0.0

a) No individual peak accounted to more than 1.8%.
b) Aggressive extracts were not analyzed since the RA content was less than 3% of total applied.
Note: ppm analyte = analyte % of AR x (ppm parent applied/100%) x MW analyte/288.7)

Mass Balance

Material balances ranged from 98.6 to 100% (NE soil) and from 99.1 to 100.8% (CA soil) of the applied radioactivity [AR]. The high material balances shown for all sampling intervals demonstrate that no significant RA dissipated from the flasks or was lost during processing.In gamma-irradiated silt loam and sandy loam test systems, the material balances were complete as well.

Table B.8.1.1.1-20 Summary of material balances of radioactivity on four soils

Soil	Springfield	Sanger
Total Recovery (%)	100-99.6	100-100.4
Mean (%)	99.5	99.4
Relative SD (%)	0.6	0.6

Extractable and Bound Residues (NER)

Extractable ^{14}C-residues decreased from 99.2 and 99.8% of AR at DAT-0 to 70.9 and 33.7% of AR at study end (DAT-120) in NE and CA soil, respectively. In gamma-irradiated test systems extractable ^{14}C-residues only decreased from 100.8 and 99.4% of AR at DAT-0 to 90.7 and 82.4 % of AR at study end (DAT-120) in NE and CA soil, respectively.

Non-extractable ^{14}C-residues increased from 0.8 and 0.2% of AR at DAT-0 to 16.4 and 30.6% of AR at study end (DAT-120) in NE and CA soil, respectively. The majority of the NER was associated with the humin. In the sandy loam after extraction with the strong acid and base, an average of 74% still remained with the solid fraction (humin). Thus, even with exposure to strong base a significant portion of non-extractable BYI 02960 residues still remained bound to the solid phase of the soil, supporting the observations of biologically-mediated bound residues.

In gamma-irradiated test systems NER only increased from 0.3 and 0.2% of AR at DAT-0 to 6.2 and 8.7% of AR at study end (DAT-120) in NE and CA soil, respectively. Thus, NER were significantly lower in gamma-irradiated compared to non-sterile soils, indicating a biological component to the formation of bound residue.

Table B.8.1.1.1-21 Summary of extractable and non-extractable residues

Soil	Springfield	Springfield gamma-irradiated	Sanger	Sanger gamma-irradiated
Extracted RA (%) Day 0	99.2	100.8	99.8	99.4
Day 120 (122[#])	71.9	90.7	33.8	82.4
Non-Extracted RA (%) Day 0	0.8	0.3	0.2	0.2
Day 120	16.4	6.2	30.6	8.7

[#] gamma-irradiated soils

Volatilization

The mineralization of [FUR-^{14}C] BYI 02960 was high. At the end of the study (DAT-120), $^{14}CO_2$ accounted for 12.3% (NE soil) and 36.1% (CA soil) of AR. Volatile organic compounds were not detected in significant amounts (≤ 0.1% AR) throughout the study.

In gamma-irradiated test systems the mineralization of [FUR-^{14}C] BYI 02960 was low. At the end of the study (DAT-120), $^{14}CO_2$ accounted for 2.9% (NE soil) and 4.1% (CA soil) of AR, only. This indicates a biological component to the formation of $^{14}CO_2$.

Transformation of Test Item

The test item declined from 98.0 and 99.4% of AR at DAT-0 to 67.3 and 30.8% at the end of the study for soils NE and CA, respectively. Other than parent, only unidentified metabolites were measured and accounted for a maximum of 3.6 and 2.8% for soils NE and CA, respectively. The results are included in the proposed overall pathway of degradation of BYI 02960 in soil shown in Figure B.8.1.3-01.

Kinetics of Test Item Degradation

The degradation of the parent compound during the study is given under paragraph B.8.1.2.

Conclusions

Major Outcomes of Study

The data gathered in the current laboratory investigation demonstrate that BYI 02960 is degraded microbially in the soils. One part becomes increasingly bound to soil (16.4% Springfield, 30.6% Sanger) by study end. The other part is well mineralized to $^{14}CO_2$ (12.3% Springfield, 36.1% Sanger). A synopsis of results is shown in Table B.8.1.1.1-22.

All formed metabolites are regarded as transient, The mineralization rate of [FUR-^{14}C] BYI 02960 to $^{14}CO_2$ was high in this study. Volatile organic compounds were very low (≤ 0.1% AR) at all sampling dates.

The comparison of the behavior of the sterile and non-sterile soils, significantly lower amounts of bound residues and $^{14}CO_2$ in the sterile soils, indicate a biological component to the degradation / mineralization and formation of non-extractable residues from BYI 02960.

Additionally, soil fractionation shows that even with extraction using strong base, BYI 02960 related residues remain bound to the solid (humin) fraction indicating very strong and irreversible binding to soil.

Table B.8.1.1.1-22 Synopsis of overall results

Soil	Springfield Viable	Springfield Sterile	Sanger Viable	Sanger Sterile
Total Recovery (%)	98.9-100.4	98.1-101.1	99.6-100.8	95.2-99.6
Extracted RA (%)	70.9-99.2	90.7-100.8	33.7-99.8	82.4-99.4
Max. CO_2 (%)	12.3	2.9	36.1	4.1
Bound Residues (%)	0.8-16.4	0.3-6.2	0.2-30.6	0.2-8.7
Major metabolites	-	Not determined	-	Not determined

Significance of Results to Environmental Behavior of BYI 02960

The current laboratory study demonstrated that a viable aerobic soil environment will contribute significantly to the degradation of [FUR-^{14}C] BYI 02960. With respect to the radiolabel used mineralization to $^{14}CO_2$ is significant, however no metabolites are expected to be present in soil (see Table B.8.1.1.1-23).

Table B.8.1.1.1-23 Synopsis of results of biotransformation of [FUR-^{14}C] BYI 02960 in two soils incubated at 20 °C and pF 2.0 - 2.5 % under aerobic conditions

Soil	Springfield (NE)		Sanger (CA)	
Soil type	Silt loam		Sandy loam	
Soil status	Viable	Sterile	Viable	Sterile
DT_{50} of BYI 02960 [days]	228	Not calculated	66	Not calculated
Major transformation products *	CO_2 NER	(NER)	CO_2 (max. 36.1%) NER (max. 30.6%)	(NER)
Minor transformation products	-	CO_2	-	CO_2

*): Criteria for term "major":>10% of AR at any DAT or >5% of AR at two successive DATs or steadily increasing until the end of the study.
(NER): Major acc. to *, but did not exceed 10% of AR during the entire study period.

Remarks

The aerobic soil degradation study is acceptable and the results are reliable.

STUDY IIA, 7.1.1/04

Characteristics

reference	:	Menke, U., Unold, M., (2011)	study type	:	aerobic degradation
year of execution	:	2009-2011	incubation time	:	118 days
GLP statement	:	Yes	nominal concentration	:	1.07 mg/kg
guideline	:	OECD 307 (2002)	temperature	:	20°C

		US EPA, OPPTS 835.4100 (2008)			
test substance	:	[Ethyl-1-^{14}C] BYI 02960	DT_{50}	:	see results
purity	:	> 98 %	metabolites	:	see results
soils	:	three soils, silt loam, loamy sand and clay loam	acceptability	:	**acceptable**

Summary

The biotransformation of [ethyl-1-^{14}C] BYI 02960 was studied in three European soils: Laacher Hof AXXa (AX), Höfchen am Hohenseh (HF), and Dollendorf II (DD) for a maximum period of approx. 120 days under aerobic conditions in the dark at approx. 20°C and 55% WHC_{max} (max. water holding capacity). BYI 02960 was applied at the nominal rate of 1.07 mg/kg dry weight of soil, which is equivalent to 400 g/ha field application rate.

At each sampling date, the soil samples were extracted with 2 x 80 mL acetonitrile/water (50/50, v/v), 1 x acetonitrile/water (80/20, v/v) and 1 x 80 mL acetonitrile by shaking at ambient temperature. Another extraction step was performed with acetonitrile/water (80/20, v/v) at 70°C using a microwave. The BYI 02960 residues and transformation products were analyzed and quantified by LCS and radio-HPLC. Radio-TLC was used as confirmation method.

Material balances were complete throughout the study, and the test substance declined from 96.0, 96.6 and 97.1% of AR at DAT-0 to 17.7, 39.6 and 23.8% of AR at the end of the study for soils DD, AX and HF, respectively. Applying double first-order kinetics a half-life (geometric mean) of 41.6 days was calculated for BYI 02960 in the tested soils under aerobic conditions.

The mineralization of [ETH-^{14}C] BYI 02960 was high. At the end of the study (DAT-118), up to 42.3 (DD), 25.9 (AX) and 33.9% AR (HF) of $^{14}CO_2$ were generated. Volatile organic compounds were not detected in significant amounts (≤ 0.1% AR).

In addition to carbon dioxide, one major transformation product was detected in the extracts of all three soils. It was identified as difluoroacetic acid (DFA) via HPLC-MS and accurate mass determination. DFA reached maximum values of 30.2, 22.0 and 33.8% of AR on DAT-45 or DAT-48 in soils DD, AX and HF, respectively. Towards the end of the study, the levels of DFA declined to 17.0, 16.3 and 23.8% of AR in soils DD, AX and HF, respectively.

Non-extractable ^{14}C-residues increased from 2.7, 2.8 and 3.2% of AR at DAT-0 to 17.9, 14.3 and 15.4% AR at the end of the study period for soils DD, AX and HF, respectively.

Materials and methods

Materials

1. Test Item: Flupyradifurone: Code = BYI 02960; Label ETH = [Ethyl-1-^{14}C] BYI 02960; Specific activity 3.93 MBq/mg; Batch ID KATH 6350 used for soil Dollendorf II on 2009-08-25; Batch KATH 6431 used soils Laacherhof AXXa and Hoefchen am Hohenseh 4a; Radiochemical purity: >98% (for

both batches); Chemical purity: >98% (for both batches); Identity and purity of test items in the application solution were confirmed.

2. Soil: The biotransformation of [ETH-^{14}C] BYI 2960 was studied in three different soils (see Table B.8.1.1.1-24). Soils Dollendorf II was taken on 2009-08-18, soils , Laacherhof AXXa and Hoefchen am Hohenseh 4a were taken on 2010-03-08 fresh from the field, and shipped subsequently. Few days later, i.e. five (DD) and four days before starting the test the gently air dried soils were sieved through a 2 mm sieve. One (soil DD) or three days (soils AX and HF) before application, aliquots equivalent to 100 g dry matter were weighed into the individual test flasks and fitted with trap attachments. Water was added in order to reach 55% of the maximum water holding capacity by the addition of application solution. The soils were pre-equilibrated at study conditions.

Table B.8.1.1.1-24 Soil physicochemical properties

Parameter	Results/Units		
Soil Batch ID	Dollendorf II, 20090818	Laacher Hof AXXa, 20100308	Hoefchen am Hohenseh 4a, 20100308
Location	Blankenheim, Germany	Monheim, Germany	Burscheid, Germany
Soil Taxonomic Classification (USDA)	N/A	Sandy floodplain deposits of the lower terrace of the Rhine river, material from the Pleistocene Ice Age	Loess or loess colluvium (Pleistocene, Holocene)
Soil Series	N/A	Sandy, mixed, mesic Typic Cambudolls	Loamy, mixed, mesic Typic Argudalfs
Texture Class (USDA)	Clay loam	Loamy sand	Silt Loam
Sand Silt Clay	43% 26% 31%	81% 10% 9%	23% 60% 17%
pH in Water pH in $CaCl_2$ pH in KCl pH in Saturated Paste	7.3 7.1 6.9 7.3	6.5 6.2 6.1 6.6	6.8 6.5 6.3 6.8
Organic Matter	8.8%	3.3%	4.1%
Organic Carbon	5.1%	1.9%	2.4%
Soil Microbial Biomass mg microbial carbon per kg of soil			
-1/0 days	2831	627	979
69/61 days	2208	398	731
114/117 days	1753	224	455
Cation Exchange Capacity (CEC)	25.6 meq/100 g	9.3 meq/100 g	13.4 meq/100 g
WHCmax	83.9 g H_2O /100 g DM	50.7 g H_2O /100 g DM	64.7 g H_2O /100 g DM

Methods

1. Experimental conditions: The study was performed in static incubation test systems under aerobic conditions in the dark at 19.2 ± 0.2°C (soil DD) or 20.2 ± 0.3 C (soils AX and HF) for a maximum period of 118 days. The test system consisted of Erlenmeyer flasks (300 mL) attached with a trap attachment (permeable for oxygen) containing soda lime for absorption of $^{14}CO_2$ and a polyurethane foam plug for adsorption of volatile organic compounds. Aliquots of 100 g of dry soil were weighed into the test flasks (each 23 flasks/soil). For all soils replicates were set up for each sampling (10 sampling dates including time 0, and each three flasks were used for determination of the microbial biomass. In

addition four (soil DD) and 16 (soils AX and HF) spare flasks were set up. The final soil moisture was adjusted to 55% of WHC_{max} by adding pure water.

2. Test Item Stock Solution: The entire delivered amount of [ETH-^{14}C] BYI 02960 was dissolved in 4 mL methanol. Identity and purity of test items were confirmed by HPLC-MS, HPLC-MS/MS and NMR.

3. Test Item Application Solution: The application solution for soil DD was made by diluting 1806 µL of respective stock solution with 10.2 mL of purified water, resulting in a mean concentration of 21076 Bq/20 µL. The application solution used for soils AX and HF was made by diluting 3080 µL of respective stock solution with 28.92 mL of purified water, resulting in a mean concentration of 526.4 Bq/500 µL.

4. Mode of Application: Aliquots of 398 µL of resp. application solution were applied in droplets onto the 100 g subsamples of soil Dollendorf II. In the same way aliquots of 398 µL of resp. application solution were applied onto the 100-g subsamples of soils Laacherhof AXXa and Hoefchen am Hohenseh 4a. By addition of the application solution the water content was finally adjusted to 55% of WHCmax. The test vessels for DAT-0 were immediately worked up. All other test vessels, including four reserve vessels and the biomass flasks which were not spiked with application solution, were fitted with trap attachments and incubated in the dark at nominal 20 ± 1°C. BYI 02960 was applied at a rate of 436,341.7 Bq per vessel containing soil DD and at a rate of 412,663.0 Bq per vessel containing soils AX and HF. This corresponds to 111.03 µg per DD and to 105.0 µg per AX or HF soil containing vessel, which is equivalent to 104.1% and 98.4% of the intended application rate of 106.7 µg per vessel (calculated for a single application rate of 400 g BYI 02960 per hectare).

5. Sampling: Microbial biomass was determined prior to commencement of the test (soils sampled one day before or at the day of application), after 69 or 61 days, and at the end of the study (114 or 117 days after treatment (DAT). Entire DD test flasks were taken for processing and analysis at DAT-0, DAT-1, DAT-3, DAT-7, DAT-14, DAT-30, DAT-45, DAT-62, DAT-90, and DAT-118, entire AX and HF test flasks were taken for processing and analysis at DAT-0, DAT-1, DAT-4, DAT-7, DAT-14, DAT-29, DAT-48, DAT-61, DAT-90, and DAT-117.

Prior to opening the incubation flasks (for moistening or sampling of soil), volatile (radioactive) compounds, possibly still present in the flasks, were transferred into the trap attachment by subjecting the flasks to vacuum in an exicator. At each sampling date the entire amount of soil in each test vessel was transferred into a centrifuge beaker and extracted using a mechanical shaker.

6. Description of analytical procedures: The soil processing procedure was optimized to obtain >90% extraction efficiency and >90% recovery of the test item at time zero. At each sampling date, the soil samples were extracted with 2 x 80 mL acetonitrile/water (50/50, v/v), 1 x acetonitrile/water (80/20, v/v) and 1 x 80 mL acetonitrile by 30-min shaking at ambient temperature. Another extraction step was

performed with acetonitrile/water (80/20, v/v) at 70°C using a microwave for 10 minutes. The supernatants were decanted and filtered using a folded filter. The folded filters were compressed to pills and combusted. The evolved $^{14}CO_2$ was trapped in a scintillation cocktail and analyzed for radioactivity by LSC.

The BYI 02960 residues and transformation products were analyzed and quantified by LSC and reversed phase radio-HPLC.

For HPLC analysis, 4 mL of the combined ambient organic extracts were concentrated to about 2 mL. It was confirmed by a recovery test that that no radioactivity was lost during the concentration step. The microwave extracts ("aggressive" extracts") were analyzed without any further processing. The limit of detection (LOD) in HPLC was equal to or better than 0.49% AR. Normal-phase Si-60 radio-TLC was used as confirmation method. For TLC analysis, the extracts were not processed further.

The identity of the test item in stock solution and in extracts was confirmed by spectroscopic methods. In addition, spectroscopic methods were used to identify one of the minor metabolites.

Results and discussion

Results indicated that anticipated standardized conditions were maintained, and the soils were microbial active over the duration of the laboratory study (see Table B.8.1.1.1-24).

Data

The respective data for the three soils are shown in Table B.8.1.1.1-25 to Table B.8.1.1.1-27. The DAT-0 extraction efficiency was in the range 96.5 to 97.5% of AR (mean 97.0% AR; sum of extracts only). The stability of the test item was verified by DAT 0 values of 96.0 to 97.1% AR for the soil extracts (mean 96.6% AR). These results indicate that the extraction method was well suited to extract the applied [^{14}C]-labeled test item from the soil matrix. TLC confirmed the results of the HPLC measurements for the test item and DFA.

Table B.8.1.1.1-25 Biotransformation of [ETH-^{14}C] BYI 02960 in clay loam soil DD under aerobic conditions; mean values and SD expressed as % of AR

Compound	Days After Treatment (DAT)									
	0	1	3	7	14	30	45	62	90	118
BYI 02960	96.0 ±0.1	92.8 ±0.4	89.3 ±0.8	82.1 ±0.1	73.2 ±0.7	52.2 ±0.0	38.6 ±0.8	31.3 ±0.6	21.4 ±1.5	17.7 ±0.4
DFA	n.d.	0.8 ±0.1	3.2 ±0.2	7.7 ±0.4	15.5 ±0.0	26.4 ±0.5	30.2 ±0.2	28.0 ±0.7	23.4 ±0.9	17.0 ±0.2
ROI 2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.4 ±0.0
Non-charact. radioactivity	0.5 ±0.1	0.3 ±0.1	0.5 ±0.0	0.4 ±0.0	0.5 ±0.1	0.4 ±0.0	0.3 ±0.0	0.4 ±0.1	0.2 ±0.0	0.2 ±0.0
Total extract. residues	96.5 ±0.0	93.9 ±0.4	93.0 ±0.5	90.2 ±0.3	89.2 ±0.7	79.0 ±0.5	69.1 ±0.6	59.7 ±0.1	45.0 ±0.6	35.3 ±0.1
$^{14}CO_2$	n.a.	<0.1	0.1 ±0.0	0.7 ±0.0	2.2 ±0.0	7.8 ±0.3	14.3 ±0.1	22.3 ±0.0	33.4 ±0.0	42.3 ±0.2

Volatile organics	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extract. residues (NER)	2.7 ±0.0	3.8 ±0.3	4.6 ±0.1	6.9 ±0.0	8.6 ±0.2	11.1 ±0.2	13.9 ±0.1	15.7 ±0.0	17.5 ±0.0	17.9 ±0.0
Total recovery	99.2 ±0.0	97.7 ±0.2	97.7 ±0.6	97.8 ±0.3	100.0 ±0.9	97.8 ±0.0	97.4 ±0.6	97.7 ±0.0	96.0 ±0.6	95.5 ±0.3

n.d. = not detected; n.a. = not analyzed; ROI = region of interest

Table B.8.1.1.1-26 Biotransformation of [ETH-^{14}C] BYI 02960 in loamy sand soil AX under aerobic conditions; mean values and SD expressed as % of AR

Compound	Days After Treatment (DAT)									
	0	1	4	7	14	29	48	61	90	117
BYI 02960	96.6 ±1.0	94.3 ±1.0	90.5 ±1.3	83.7 ±0.3	76.7 ±1.4	59.6 ±2.7	53.8 ±1.6	49.4 ±0.6	44.5 ±0.1	39.6 ±0.3
DFA	n.d.	0.7 ±0.1	3.0 ±0.1	6.5 ±0.2	14.1 ±1.5	17.3 ±0.0	22.0 ±1.1	19.6 ±0.5	16.2 ±0.0	16.3 ±0.5
ROI 2	n.d.	n.d.	n.d.	n.d.	n.d.	1.2 ±0.1	1.5 ±0.1	1.6 ±0.1	1.8 ±0.1	1.3 ±0.3
Non-charact. radioactivity	0.4 ±0.1	0.4 ±0.0	0.5 ±0.1	0.4 ±0.1	0.3 ±0.0	0.4 ±0.2	0.3 ±0.1	0.2 ±0.1	0.3 ±0.1	0.4 ±0.0
Total extract. residues	97.0 ±1.1	95.4 ±0.9	93.9 ±1.2	90.6 ±0.3	91.1 ±0.0	78.6 ±3.0	77.5 ±0.3	70.8 ±0.1	62.8 ±0.0	57.6 ±0.1
$^{14}CO_2$	n.a.	<0.1	0.2 ±0.0	0.4 ±0.0	1.6 ±0.0	5.4 ±0.1	10.8 ±0.0	14.4 ±0.2	21.2 ±0.2	25.9 ±0.2
Volatile organics	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extractable residues (NER)	2.8 ±0.1	3.1 ±0.1	4.6 ±0.2	5.3 ±0.1	7.7 ±0.3	15.0 ±4.3	11.9 ±0.1	13.2 ±0.0	14.8 ±0.4	14.3 ±0.1
Total recovery	99.8 ±1.2	98.4 ±0.8	98.6 ±1.0	96.3 ±0.5	100.3 ±0.3	98.9 ±1.3	100.2 ±0.2	98.4 ±0.4	98.8 ±0.3	97.8 ±0.4

n.d. = not detected; n.a. = not analyzed; ROI = region of interest

Table B.8.1.1.1- 27 Biotransformation of [ETH-^{14}C] BYI 02960 in silt loam soil HF under aerobic conditions; mean values and SD expressed as % of AR

Compound	Days After Treatment (DAT)									
	0	1	4	7	14	29	48	61	90	117
BYI 02960	97.1 ±0.5	94.5 ±1.5	88.5 ±0.1	80.1 ±1.9	71.9 ±0.9	55.6 ±0.2	39.8 ±1.4	32.7 ±0.1	27.0 ±1.2	23.0 ±0.2
DFA	n.d.	0.9 ±0.1	5.0 ±0.2	8.7 ±0.3	17.1 ±0.1	25.8 ±0.0	33.9 ±1.2	33.1 ±1.0	28.0 ±1.4	23.8 ±0.1
ROI 2	n.d.	n.d.	n.d.	n.d.	n.d.	1.3 ±0.1	1.4 ±0.0	1.1 ±0.1	1.6 ±0.1	1.5 ±0.1
Non-characterized radioactivity	0.4 ±0.0	0.4 ±0.1	0.5 ±0.1	0.4 ±0.0	0.4 ±0.1	0.5 ±0.0	0.3 ±0.0	0.3 ±0.0	0.3 ±0.0	0.3 ±0.0
Total extractable residues	97.5 ±0.5	95.8 ±1.3	93.9 ±0.0	89.1 ±1.5	89.4 ±1.1	83.3 ±0.1	75.4 ±0.2	67.1 ±0.7	57.0 ±0.3	48.6 ±0.0
$^{14}CO_2$	n.a.	<0.1	0.2 ±0.0	0.5 ±0.0	1.9 ±0.0	6.3 ±0.0	13.0 ±0.1	17.5 ±0.1	27.1 ±0.2	33.9 ±0.1
Volatile organics	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extractable residues (NER)	3.2 ±0.1	4.2 ±0.4	5.2 ±0.2	7.3 ±0.4	8.2 ±0.0	11.0 ±0.1	12.6 ±0.1	14.5 ±0.4	14.4 ±0.4	15.4 ±0.2
Total recovery	100.7 ±0.6	100.0 ±0.9	99.3 ±0.2	97.0 ±1.9	99.4 ±1.1	100.6 ±0.0	101.0 ±0.1	99.1 ±0.3	98.5 ±0.5	97.9 ±0.1

n.d. = not detected; n.a. = not analyzed; ROI = region of interest

Mass Balance

Material balances ranged from 95.5 – 100.0% (soil DD), 96.3 – 100.3% (soil AX) and 96.3 – 100.3% (soil HF) of the applied radioactivity [AR]. The high material balances shown for all sampling intervals demonstrate that no significant RA dissipated from the flasks or was lost during processing.

Extractable and Bound Residues (NER)

Extractable ^{14}C-residues decreased from 96.5, 97.0 and 97.5% AR at DAT-0 to 35.3, 57.6 and 48.6% of AR at study end in soils DD, AX and HF, respectively.

Non-extractable ^{14}C-residues increased from 2.7, 2.8 and 3.2% of AR at DAT-0 to 17.9, 15.0 and 15.4% AR at the end of the study period for soils DD, AX and HF, respectively. The major portions of radioactivity were found in the insoluble humin fraction.

Volatilization

The mineralization of [ETH-^{14}C] BYI 02960 was high. At the end of the study, $^{14}CO_2$ accounted for up to 42.3 (soil DD), 25.9 (soil AX) and 33.9% (soil HF) of AR. Volatile organic compounds were not detected in significant amounts (≤ 0.1% AR).

Transformation of Test Item

The test item declined from 96.0, 96.6 and 97.1% of AR at DAT-0 to 17.7, 39.6 and 23.0% at the end of the study for soils DD, AX and HF, respectively.

One major transformation product was detected in all three soils. It was identified as difluoroacetic acid (DFA) via HPLC-MS and accurate mass determination in ambient organic extracts originating from soil Dollendorf II. The amount of DFA reached maximum values of 30.2, 22.0 and 33.9% of AR on DAT-45 or DAT-48 in soils DD, AX and HF, respectively. At the end of the study, the amounts of DFA declined to 17.0, 16.3 and 23.8% of AR in soils DD, AX and HF, respectively.

In addition, one minor transformation product (ROI 2) was detected in all three soils with maximum amounts of 0.4 (DAT-118), 1.8 (DAT-90) and 1.6% (DAT-90) of applied radioactivity for soils DD, AX and HF, respectively. The total of unknown extracted radioactivity did not exceed 0.5% AR.

The results were included in the proposed overall pathway of degradation of BYI 02960 in soil shown in Figure B.8.1.3-01.

Kinetics of Test Item Degradation

A summary of the DT_{50} and DT_{90} calculations for the test item is given in under paragraph B.8.1.2.

Conclusions

Major Outcomes of Study

The data gathered in the current laboratory investigation demonstrate that BYI 02960 is well degraded in the four soils to form one major and one very minor metabolite. The major metabolite was identified as difluoroacetic acid (max. 33.9% of AR). The amounts of the minor metabolite detected did not exceed 1.8% of AR; no identification or characterization was made. Significant amounts of $^{14}CO_2$ (up to 42.3% AR) were measured at the end of the study indicating that mineralization of the test item and/or degradates occurred. The maximum amount of non-extractable radioactivity was 17.9% of AR.

Table B.8.1.1.1-28 Synopsis of results of biotransformation of [ETH-14C] BYI 02960 in soils incubated at 20 °C and 55 % of WHCmax under aerobic conditions)

Soil	DD	AX	HF
Total Recovery (%)	95.5-100.0	97.8-100.3	97.9-100.7
Extracted RA (%)	35.3-95.5	57.6-97.0	48.6-97.5
Max. CO_2 (%)	42.3	25.9	33.9
Bound Residues (%)	2.7-17.9	2.8-15.0	3.2-15.4
Major metabolites	DFA (30.2%)	DFA (22.0%)	DFA (33.9%)

Significance of Results to Environmental Behavior of BYI 02960

The current laboratory study demonstrated that [ETH-^{14}C] BYI 02960 is degradable in soils under aerobic conditions. With respect to the radiolabel used mineralization to $^{14}CO_2$ is highly significant. Difluoroacetic acid was detected as a further major transformation product. Other metabolites are not be expected to accumulate in soil see Table B.8.1.1.1-29.

Table B.8.1.1.1-29 Synopsis of results of biotransformation of [ETH-14C] BYI 02960 in soils incubated at 20 °C and 55 % of WHCmax under aerobic conditions

Soil	Dollendorf II	Laacher Hof AXXa	Hoefchen am Hohenseh
Soil type	Clay loam	Loamy sand	Silt loam
Major transformation products *	CO2 (max. 42.3% AR); NER (max 17.9%) DFA (max. 33.9% AR)		
Minor transformation products	-		

*): Criteria for term “major”: >10% of AR at any DAT or >5% of AR at two successive DATs or steadily increasing until the end of the study.

Remarks

The aerobic soil degradation study is acceptable and the results are reliable.

STUDY IIA, 7.1.1/05

Characteristics

reference	:	Menke, U., Unold, M., (2011)	study type	:	aerobic degradation
year of execution	:	2010	incubation time	:	117 days
GLP statement	:	yes	nominal concentration	:	1.07 mg/kg
guideline	:	OECD 307 (2002) US EPA, OPPTS 835.4100 (2008)	Temperature	:	20°C
test substance	:	[Pyridine-2,6-^{14}C] BYI 02960	DT_{50}	:	see results
purity	:	> 98 %	metabolites	:	see results
soils	:	One soils; silt loam	acceptability	:	**acceptable**

Summary

The biotransformation of [pyridine-2,6-^{14}C] BYI 02960 was studied in one soil, Höfchen am Hohenseh 4a (HF, Burscheid, Germany), a silt loam soil of pH 6.5 ($CaCl_2$) and 2.4% organic carbon content. The

incubation was conducted for max. 117 days under aerobic conditions in the dark at ca. 20°C and 55% WHC_{max} (max. water holding capacity). BYI 02960 was applied at the nominal rate of 106.7 µg/ 100 g soil, which is equivalent to a field application rate of 400 g/ha.

At each sampling date, i.e. 0, 1, 4, 7, 14, 29, 48, 61, 90 and 117 days after treatment (DAT), the duplicate soil samples were extracted with 2 x 80 mL acetonitrile/water (50/50, v/v), 1 x acetonitrile/water (80/20, v/v) and 1 x 80 mL acetonitrile by shaking at ambient temperature. Another extraction step was performed with acetonitrile/water (80/20, v/v) at 70°C using a microwave. The BYI 02960 residues and transformation products were analyzed and quantified by LCS and radio-HPLC. Radio-TLC was used as a confirmatory method.

Material balances were complete throughout the study (ranged on mean from 94.1 to 100.3% of AR), and the test substance declined from 96.7% at DAT-0 to 24.6% of AR at the end of the study.

The mineralization of [PYR-^{14}C] BYI 02960 was high. At the end of study (DAT-117), $^{14}CO_2$ accounted for 57.4% of AR. Volatile organic compounds were not detected in significant amounts (≤ 0.1% AR).

Only minor transformation products (all mean values were ≤ 2.5% of AR) were detected in the extracts and were characterized by their retention time. The transformation product assigned to ROI 2 reached a mean maximum level of 2.0% of AR at DAT-48. The maximum amount of the second transformation product (ROI 3) was detected on DAT-7 (2.5% of AR). Towards the end of the study, the amounts decreased below the detection limit. ROI 3 was identified as 6-chloronicotinic acid (6-CNA) by co-chromatography. The third minor transformation product was detected only once (DAT-48, 0.3% of AR). Non-extractable ^{14}C-residues increased from 3.3% of AR at DAT-0 to 16.7% of AR at the end of the study period.

Materials and methods

Materials

1. Test Item: Flupyradifurone: Code = BYI 02960; Label PYR = [Pyridine-2,6-^{14}C] BYI 02960 (sample ID: KATH 6432); Specific activity: 4.49 MBq/mg ;Radiochemical purity: >98% (acc. radio-HPLC and radio-TLC; Chemical purity: >98% (HPLC, UV detection at 210 nm); Identity and purity of test item in the application solution were confirmed.

2. Soil: The biotransformation of BYI 02960 was studied in one soil.. The soil was taken fresh from the field. On the day of sampling, the soil was broken up stepwise and gently air dried. Then, the soil was successively sieved to ≤ 2 cm, filled into plastic bags and stored in a climatic cabinet at 20 ± 2°C.

100 g aliquots (dry matter) of the sieved soil were weighed into individual 300 mL Erlenmeyer flasks. Water was added in order to finally reach 55% of the maximum water holding capacity by the addition of application solution (353 µL) on the day of application.

Three additional test systems were prepared which were used for microbial biomass determinations. Since no application solution was added to these vessels, they were directly adjusted to 55% of maximum water holding capacity. All vessels were fitted with trap attachments and pre-equilibrated at 20 ± 2°C in the dark for three days.

Table B.8.1.1.1-30 Soil physicochemical properties

Parameter	Results/Units
Batch ID	Hoefchen am Hohenseh 4a, 20100308
Location	Burscheid, Germany
Soil Taxonomic Classification (USDA)	Loess or loess colluvium (Pleistocene, Holocene)
Soil Series	Loamy, mixed, mesic Typic Argudalfs
Texture Class (USDA)	Silt Loam
Sand Silt Clay	23% 60% 17%
pH in Water pH in CaCl2 pH in KCl	6.8 6.5 6.3
Organic Matter	4.1%
Organic Carbon	2.4%
Initial & Final Soil Biomass or Microbial Activity	mg microbial C/g soil
0 days	970
61 days	756
117 days	554
Cation Exchange Capacity (CEC)	13.4 meq/100 g
WHCmax	64.7 g H2O/ 100 g DM

Methods

1. Experimental conditions: The study was performed in static incubation test systems under aerobic conditions in the dark at 20.2 ± 0.3 °C. The test system consisted of Erlenmeyer flasks (300 mL) attached with a trap attachment (permeable for oxygen) containing soda lime for absorption of $^{14}CO_2$ and a polyurethane foam plug for adsorption of volatile organic compounds. Aliquots of 100 g of dry soil were weighed into the test flasks. Replicates were set up and processed for each sampling (10 sampling dates including time 0, and 16 spare flasks were set up but not further processed in this study). Three flasks were used for determination of the microbial biomass. The final soil moisture was adjusted to 55% of WHC_{max} by adding pure water.

2. Test Item Stock Solution: The entire delivered amount of [pyridine-2,6-^{14}C] BYI 02960 was dissolved in 4 mL methanol.

3. Test Item Application Solution: An application solution was made by diluting 2048 µL of before-mentioned stock solution with 9.952 mL of purified water.

4. Mode of Application: Aliquots of 353 µL of the application solution were applied in droplets onto the 100 g pre-incubated subsamples of each soil. By the addition of the application solution, the water content was finally adjusted to 55% of WHC_{max}. The test vessels for DAT-0 were immediately processed for analysis. All other test vessels, including the biomass flasks which were not spiked with application solution, were fitted with trap attachments and incubated in the dark at nominal 20 ± 2°C.

[PYR-^{14}C] BYI 02960 was applied at a rate of 415954 Bq per vessel. This corresponds to 92.64 µg per vessel which is equivalent to 86.8% of the intended application rate of 106.7 µg per vessel (calculated for a single application rate of 400 g BYI 02960 per hectare).

5. Sampling: Microbial biomass was determined prior to commencement of the test (soils sampled at the day of application), after 61 days, and at the end of the study 117 days after treatment (DAT-117). Entire test flasks were taken for processing and analysis at DAT-0, DAT-1, DAT-4, DAT-7, DAT-14, DAT-29, DAT-48, DAT-61, DAT-90, and DAT-117.

Prior to opening the incubation flasks (for moistening or sampling of soil), volatile (radioactive) compounds, possibly still present in the flasks, were transferred into the trap attachment by subjecting the flasks to vacuum in a desiccator. At each sampling date the entire amount of soil in each test vessel was transferred into a centrifuge beaker and extracted using a mechanical shaker.

6. Description of analytical procedures: The soil processing procedure was optimized to get >90% extraction efficiency and >90% recovery of the test item at time zero. At each sampling date, the soil samples were extracted with 2 x 80 mL acetonitrile/water (50/50, v/v), 1 x acetonitrile/water (80/20, v/v) and 1 x 80 mL acetonitrile by shaking at ambient temperature. Another extraction step was performed with acetonitrile/water (80/20, v/v) at 70°C using a microwave.

The BYI 02960 residues and transformation products were analyzed and quantified by LSC and reversed phase radio-HPLC. Losses of radioactivity after concentration of extracts were minimal. Normal-phase Si-60 radio-TLC was used as confirmation method. The identity of the test item in stock solution and in extracts was confirmed by spectroscopic methods. In addition, spectroscopic methods were used to identify one of the minor metabolites.

Results and discussion

Results indicated that anticipated standardized conditions were maintained, and the soils were microbial active over the duration of the laboratory study (see Table B.8.1.1.1-30).

Data

The data for the soil are shown in Table B.8.1.1.1-31. The DAT-0 extraction efficiency was 96.7% of AR (ambient extracts 95.0% of AR). The stability of the test item was verified by a DAT-0 recovery of

96.3 ± 0.7% of AR. These results indicate that the extraction method was well suited to extract the applied [^{14}C]-labeled test item from the soil matrix.

Table B.8.1.1.1-31 Biotransformation of [PYR-^{14}C] BYI 02960 in sandy loam soil HF under aerobic conditions; mean values and SD expressed as % of AR

Compound	Days After Treatment (DAT)									
	0	1	4	7	14	29	48	61	90	117
BYI 02960	96.3 ±0.7	95.3 ±0.1	88.5 ±0.8	78.4 ±0.8	70.9 ±0.2	53.5 ±0.1	40.9 ±1.5	33.9 ±0.1	27.3 ±0.3	22.7 ±0.5
ROI 2	n.d.	n.d.	n.d.	n.d.	0.7 ±0.2	1.6 ±0.2	2.0 ±0.2	1.9 ±0.3	1.1 ±0.3	1.9 ±0.1
6-CNA	n.d.	n.d.	1.7 ±0.0	2.5 ±0.1	2.0 ±0.2	0.9 ±0.0	0.5 ±0.1	0.2 ±0.2	n.d.	n.d.
ROI 4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.3 ±0.3	n.d.	n.d.	n.d.
Non-characterized radioactivity	0.4 ±0.0	0.5 ±0.0	0.4 ±0.1	0.2 ±0.0	0.3 ±0.0	0.2 ±0.1	0.2 ±0.0	0.1 ±0.0	0.1 ±0.0	0.1 ±0.0
Total extractable residues	96.7 ±0.8	95.8 ±0.2	90.6 ±0.9	81.2 ±0.7	73.9 ±0.2	56.4 ±0.0	43.9 ±1.1	36.1 ±0.2	28.6 ±0.0	24.6 ±0.4
$^{14}CO_2$	n.a.	0.1 ±0.0	1.9 ±0.1	5.1 ±0.0	13.4 ±0.1	26.8 ±0.4	40.6 ±0.3	46.0 ±0.1	52.5 ±0.6	57.4 ±0.5
Volatile organics	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extractable residues (NER)	3.3 ±0.0	4.2 ±0.0	6.1 ±0.0	7.8 ±0.3	10.4 ±0.0	14.1 ±0.2	15.7 ±0.0	16.3 ±0.1	16.7 ±0.1	16.2 ±0.1
Total recovery	100.0 ±0.7	100.2 ±0.2	98.6 ±0.9	94.1 ±0.3	97.7 ±0.3	97.3 ±0.2	100.3 ±0.8	98.3 ±0.1	97.8 ±0.5	98.3 ±0.0

n.d. = not detected; n.a. = not analyzed; ROI = region of interest; ROI 3 was identified as compound 6-CNA

Mass Balance

Material balance ranged from 94.1 ±0.3 to 100.3 ±0.8% of the applied radioactivity [AR]. The high material balances shown for all sampling intervals demonstrate that no significant RA dissipated from the flasks or was lost during processing.

Extractable and Bound Residues (NER)

Extractable ^{14}C-residues decreased from 96.7 ±0.8% at DAT-0 to 24.6 ±0.4% of AR at study end (DAT-117). Non-extractable ^{14}C-residues increased from 3.3 ±0.0% at DAT-0 to 16.2 ±0.1% of AR at the end of the study period.

Volatilization

The mineralization of BYI 02960 was high. At the end of the study (DAT-117), $^{14}CO_2$ accounted for 57.4 ±0.5% of AR. Volatile organic compounds were not detected in significant amounts (< 0.1% AR).

Transformation of Test Item

The test item declined from 96.3 ±0.7% of AR at DAT-0 to 22.7 ±0.5% of AR at the end of the study. Three minor transformation products were detected in the extracts. The transformation product assigned to ROI 2 reached a maximum level of 2.0% of AR at DAT-48. The amount of the second transformation product (ROI 3) increased to a maximum amount of 2.5% of AR on DAT-7 and

decreased below the detection limit towards the end of the study. This transformation product was identified as 6-chloronicotinic acid (6-CNA) by co-chromatography. The third minor transformation product was detected only once (DAT-48), accounting for 0.3% of AR.

The total of unknown extracted radioactivity did not exceed 0.5% of AR and was assigned to the background signal in the HPLC-analysis. The mentioned results were included in the proposed overall pathway of degradation of BYI 02960 in soil shown in Figure B.8.1.3-01.

Kinetics of Test Item Degradation

A summary of the DT_{50} and DT_{90} calculation for the test item is given in paragraph B.8.1.2.

Conclusions

Major Outcomes of Study

The data gathered in the current laboratory investigation demonstrate that BYI 02960 is degraded in HF soil. A synopsis of results is shown by Table B.8.1.1.1-32. A few very minor metabolites were detected and quantified together with the test item. All further formed metabolites are regarded as transient, which is confirmed by the high mineralization rate of [PYR-^{14}C] BYI 02960 to $^{14}CO_2$ observed in this study, i.e. 57.4% of AR until the end of the study. Volatile organic compounds were very low (≤ 0.1% AR) at all sampling dates.

Significance of Results to Environmental Behavior of BYI 02960

The current laboratory study demonstrated that [PYR-^{14}C] BYI 02960 is degradable in soil under aerobic conditions. With respect to the radiolabel used mineralization to $^{14}CO_2$ is significant, however, significant metabolites are not expected to be present in soil, since – after their formation – they are well mineralized (see Table B.8.1.1.1-32).

Table B.8.1.1.1-32 Synopsis of results of biotransformation of [PYR-^{14}C] BYI 02960 in soil HF incubated at 20 °C and 55 % of WHCmax under aerobic conditions

Soil	**Hoefchen am Hohenseh 4a**
Soil type	Silt loam
Major transformation products *	CO_2 (max. 57.4%) NER (max. 16.7%)
Minor transformation products	6-CNA (max. 2.5%)

*): Criteria for term "major": >10% of AR at any DAT or >5% of AR at two successive DATs or steadily increasing until the end of the study.

Remarks

The aerobic soil degradation study is acceptable and the results are reliable.

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Characteristics

reference	:	Shepherd, J.J. (2011)	study type	:	aerobic degradation
year of execution	:	2010	incubation time	:	120 days
GLP statement	:	yes	nominal concentration	:	1.1 mg/kg

guideline	:	OECD 307 (2002) US EPA, OPPTS 835.4100 (2008)	Temperature	:	20°C
test substance	:	[Pyridine-2,6-^{14}C] BYI 02960	DT_{50}	:	see results
purity	:	> 98 %	metabolites	:	see results
soils	:	Two soils; silt loam, sandy loam	acceptability	:	**acceptable**

Summary

The biotransformation of [pyridine-2,6-^{14}C] BYI 02960 was studied in two US soils, silt loam Springfield, NE, and sandy loam Sanger, CA, for a maximum period of 120 days under aerobic conditions in the dark at approx. 20 °C and soil moistures maintained between pF 2.0 to 2.5. Gamma irradiated samples were investigated along with microbial active test systems. BYI 02960 was applied at the rate of 1.1 µg a.i./g soil, equivalent to 410 g a.i./ha assuming a 2.5 cm soil depth.

Duplicate test systems were analyzed at 0, 3, 7, 14, 28, 60, 92, and 120 days of incubation. The 50-g soil samples were extracted by shaking with acetonitrile:water (70:30) and acetonitrile (100%), followed by microwave extraction at 70 °C(aggressive extract) using acetonitrile:water (70:30). Extract aliquots were concentrated and analyzed by HPLC. Identification of the transformation products was performed by LC/MS, co-chromatography, and/or retention time comparison with reference standards.

Material balances were complete throughout the study, and the test item declined from 97.4 and 98.6% and of the applied amount at day 0 to 65.5 and 30.0% of the applied at the end of the study. In the silt loam, extractable ^{14}C-residues decreased from 99.3% of the applied amount at day 0 to 67.0% of the applied at the end of the study. Non-extractable 14C-residues increased from 0.7% of the applied amount at day 0 to a maximum of 11.3% at day 120. At study termination, evolved $^{14}CO_2$ reached 20.2%, and radioactive volatile organics were not detected. Total unidentified radioactivity ranged from 0.0% to 2.4% of the applied amount.

In the sandy loam, extractable ^{14}C-residues decreased from 99.9% of the applied amount at day 0 to 40.0% of the applied at the end of the study. One major degradate was identified as 6-chloronicotinic acid (6-CNA), which formed 6.9% on day 14, reached a maximum of 17.1% on day 60, and declined to 8.2% by the end of the study.

In the sterile silt loam, extractable 14C-residues decreased from 99.1% of the applied amount at day 0 to 89.4% of the applied at the end of the study. Non-extractable 14C-residues increased from 0.9% of the applied amount at day 0 to a maximum of 6.2% at day 122. At study termination, evolved $^{14}CO_2$ reached 0.4%, and radioactive volatile organics were not detected.

In the sterile sandy loam, extractable 14C-residues decreased from 99.8% of the applied amount at day 0 to 85.8% of the applied at the end of the study. Non-extractable ^{14}C-residues increased from 0.2% of the applied amount at day 0 to a maximum of 9.3% at day 122. At study termination, evolved $^{14}CO_2$ reached 0.9%, and radioactive volatile organics were not detected.

[PYR-^{14}C] BYI 02960 mineralizes relatively rapidly under aerobic condition to $^{14}CO_2$ (20.2% Springfield, 36.1% Sanger) and becomes increasingly bound to soil (11.3% Springfield, 25.5% Sanger, CA) by study end. Total unidentified radioactivity ranged from 0.0% to 2.4% (Springfield) and 0.0% to 3.0% (Sanger) of the applied amount.

The amount of $^{14}CO_2$ and bound residue formed in gamma-irradiated soils was significantly less than in non-sterile soils indicating a biological component to degradation and the formation of non-extractable residues from BYI 02960. Additionally, soil fractionation shows that even with extraction using strong base, BYI 02960 related residues remain bound to the solid (humin) fraction indicating very strong and irreversible binding to soil.

Materials and methods

Materials

1. Test Item: Flupyradifurone: Code = BYI 02960; Label PYR = [pyridine-2,6-^{14}C] BYI 02960 (Vial C-1135);Specific activity: 35.03 mCi/mMole; Radiochemical purity: >98%; Identity and purity of test item in the application solution were confirmed.

2. Soil: The biotransformation of [PYR-^{14}C] BYI 2960 was studied in two different soils NE and CA soils were taken fresh from the fields. Each soil was collected from the top 0 to 8 inches of the field and shipped to the testing facility subsequently. No pesticides were applied in the past 5 years to these sites where soils were collected.

The NE soil was stored under refrigeration for 8 days, transferred to a greenhouse and stored under crop cover (alfalfa) and after it was sieved with 2-mm sieve before use. The CA soil was maintained in a walk-in refrigerator prior to use at 3.9 ±0.8°C, sieved with 2-mm sievebefore use.

Aliquots equivalent to 50 g dry matter were weighed into individual 250 mL glass flasks until starting the test. Test systems were pre-equilibrated at 20 ± 1°C and soil moisture between pF 2.0 to 2.5 for six days prior to the treatment.

Table B.8.1.1.1-33 Soil physicochemical properties

Parameter	Results/Units	
Batch ID	Springfield,NE	Sanger, CA
Location	Dozier AG Research Services LLC, PO Box 308 Hwy 50 & Cornish Rd Springfield, NE 68059	SynTech Research, Inc. 17915 E. Annadale Ave Sanger, CA. 93657
Soil Taxonomic Classification (USDA)	Marshall fine-silty, mixed, superactive, mesic Typic Hapludolls	Hanford fine sandy loam, gravelly substrate
Soil Series		
Texture Class (USDA)	Silt Loam	Sandy Loam
Sand Silt Clay	13.4% 63.8% 22.8%	67.8% 25.0% 7.2%
Saturated paste	6.7	7.4

pH in $CaCl_2$ pH in H_2O	6.5 6.7	7.0 7.3
Organic Matter	4.0%	0.97%
Organic Carbon	2.3%	0.57%
Microbial Biomass Initial (day 6) Middle (day 71) Final (day 120)	(mg Microbial C/kg soil) 833 686 660	(mg Microbial C/kg soil) 234 169 202
Cation Exchange Capacity (CEC)	17.8 meq/100 g	6.3 meq/100 g
Maximum Water Holding Capacity	49.6 g/100 g	27.7 g/100 g
Soil Moisture at 0.1 bar (pF 2.0)	37.9%	15.8%
Soil Moisture at 0.33 bar (pF 2.5)	26.4%	9.1%
Bulk Density (disturbed)	1.04 g/cc	1.29 g/cc

Methods

1. Experimental conditions: All test systems were incubated at 20 ± 1°C in the dark in a temperature-controlled environmental chamber. The test systems containing 50 g soil (dry-weight basis) consisted of a 250-mL glass flask (21 cm long and 7.2 cm internal diameter) connected to a flow-through system, containing an ethylene glycol trap for volatile organics followed by two 2 M potassium hydroxide traps for collecting CO_2 and a 1 M sulfuric acid trap for volatile acids. The headspace above the soil was continuously purged with humidified air throughout the study.

For both soils twenty test systems were treated at 1X for the kinetic study rate with each soil (1.1 µg/g) Three test systems were prepared for metabolite identification (MID) purposes, and these systems were treated at 10x the kinetic rate. MID test systems were not used for kinetic evaluation. Four control test systems were prepared as biomass test systems to demonstrate biological activity of the soil.

For the sterile portion of this study, soil was sent out for gamma irradiation (actual delivered absorbed dose of 24.67 kGy). Gamma-irradiated soils were transferred to sterilized 250-mL glass flasks within a BioGuard Laminar Flow Hood using aseptic techniques. Soils were treated at the kinetic rate in the laminar flow hood using aseptic techniques, and then connected to the flow-through system. Sterility was tested at each sampling interval on each test system by plating soil dilutions on 3M Petrifilm Agar plates which were incubated at ~35°C for a minimum of 48 hours.

In total, 64 test systems were prepared. All the soils were adjusted to the appropriate moisture level for each soil, and the moisture was maintained throughout the course of the study.

2. Test Item Application Solution: The application (treatment) solution was prepared by diluting 975 µL of [PYR-^{14}C] BYI 02960 (C-1135) with 37.5 mL of H_2O:MeOH (4:1) for a theoretical concentration of 0.11 µg/µL. The extensive radio-assays to determine the concentration of kinetic treatment solution resulted in 31,227, 300 dpm/mL or 116 µg/mL; the radiochemical purity and identity were confirmed by HPLC and mass spectral analysis.

3. Mode and Rate of Application: On 2010-01-25, aliquots of 480-µL of the kinetic treatment solution were applied in droplets onto the 50 g pre-incubated subsamples of soils, using a 500-µL gas-tight syringe. This rate is equal to the field-use rate of 410 g a.i./ha at 2.5 cm depth and 1.5 g/cm^3 soil density. The final concentration of a.i. in each test system was 1.1 µg BYI 02960 per g soil (dry weight). By the addition the water contained the soil moisture was finally adjusted to approx. pF 2.0. Material balance for the study was based on the day 0 recovery of radioactivity (14,495,777 dpm for Springfield, NE and 14,438,374 dpm for Sanger, CA).

4. Test System Maintenance and Sampling: Test systems were checked for air flow through the traps and water levels in the moisture flasks. The test systems were incubated at 20 ± 1°C in the dark in an environmental chamber. The moisture loss in each system was monitored periodically by comparing the weight of the test system with the weight on Day 0. The moisture of the test systems was measured at each interval. The moisture was maintained at pF 2.5 to pF 2.0 for each soil by the addition of HPLC grade water, as needed, at periodic intervals.

Duplicate test systems were extracted and analyzed on days 0, 3, 7, 14, 28, 60, 92 and 120 days after treatment. Gamma-irradiated test systems were processed at 0, 60 and 122 days after treatment. For the sterile portion of this study, the goal was only to compare the formation of bound residues with non-sterile test systems and therefore, soil extracts were radio-assayed but not analyzed by HPLC. Extracted soils were analyzed by combustion.

On the day of sampling, the test system and associated volatile traps were removed from the incubator for analysis. Prior to removing samples, the air flow was increased for approx. 15 minutes to ensure all headspace volatiles had been purged and trapped. The two KOH trap solutions were combined, volumes of the traps were recorded and triplicate aliquots were radio-assayed.

The soil was extracted and analyzed by LSC on the day of sampling. The soil extracts were analyzed by HPLC within 27 days after sample extraction. Concentrated extracts were stored in a laboratory freezer.

5. Description of analytical procedures: The soil processing procedure was optimized to get >90% extraction efficiency and >90% recovery of the test item at time zero. At each sampling date the entire amount of soil in each test vessel was transferred into a centrifuge bottle and extracted for 30 minutes with approximately 40 mL of acetonitrile:water (70:30, v/v) using a horizontal shaker, and centrifuged (5 minutes at 1850 g).

The supernatant was decanted, and the remaining soil was extracted two additional times with acetonitrile:water (70:30,v/v) followed by an extraction with approximately 40 mL of acetonitrile. The supernatants were pooled, and the volume was recorded. Extracts were radioassayed by LSC in

triplicate (0.25 to 1.0 mL). An aliquot (approximately 4 mL) of the combined extracts was concentrated by a stream of nitrogen and analyzed by HPLC.

Soil remaining after the ambient acetonitrile/water and acetonitrile extractions was extracted by microwave extraction using 50 mL of acetonitrile:water (70:30, v/v) at 70°C for 10 minutes. The volume was recorded, and the extract was radioassayed by LSC in triplicate (0.25 to1.0 mL). An aliquot (approximately 4 mL) was concentrated by a stream of nitrogen and then analyzed by HPLC.

Extracted soils were air-dried under a fume hood and thoroughly homogenized. Subsamples (0.3 to 1.0 g) of the non-extracted residue (NER) were quantified by combustion.

To characterize the NER, the extracted soils from the day 92 sandy loam samples were fractionated to quantify the amounts of radioactivity associated with humic acid, fulvic acid, and humin.

The BYI 02960 residues and transformation products were analyzed and quantified by LSC and radio-HPLC. Recovery of radioactivity from the HPLC column was determined by comparing radioactivity of the collected HPLC effluent (triplicate aliquots, radio-assayed) to the calculated radioactivity of the injected representative samples.

The LOD for HPLC was determined empirically by a series of injections of increasing radioactivity. The lowest amount of radioactivity detected resulted in minimum peak height of 3 times the background level in the chromatogram (520 dpm) and was considered to be the instrument LOD. The minimum amount of radioactivity injected for a sample was 6,034 dpm from the sandy loam day 120, replicate 1 microwave extract. This extract contained 2.5% of the applied activity which results in a LOQ of 0.2% of the applied radioactivity.

Liquid chromatography/electrospray ionization-mass spectrometry (LC/ESI-MS) and LC/ESI-MS/MS analyses of standards and isolated compounds was performed using a triple quadrupole mass spectrometer interfaced to an quaternary HPLC system (Thermo Electron).

Results and discussion

Results indicated that anticipated standardized conditions were maintained, and the soils were microbial active over the duration of the laboratory study (see Table B.8.1.1.1-33). For the gamma irradiated soils, sterility was lost during the study indicated by plate counts. However, results indicate the biological activity was compromised, as evidenced by the significant reduction in $^{14}CO_2$ formation in the sterile soils at the end of the study.

Data

The respective data for the four soils are shown in Table B.8.1.1.1-34 and Table B.8.1.1.1-35. The ambient and aggressive extracts were effective in extracting essentially all of the ^{14}C-residues in both soils at DAT-0, the beginning of the study (silt loam = 99.3%, sandy loam = 99.9%). The stability of the test item was verified by DAT 0 values of 97.4 and 98.6% AR (mean of duplicates) for the soil extracts These results indicate that the extraction method was very well suited to extract the applied [^{14}C]-labeled test item from the soil matrix.

Table B.8.1.1.1-34 Biotransformation of [PYR-^{14}C] BYI 02960 in silt loam Springfield (NE) under aerobic conditions; mean values and SD expressed as % of AR

Compound	Days After Treatment (DAT)							
	0 [b)]	3	7	14	28	60	92	120
BYI 02960	97.4 ±1.2	94.1 ±1.3	93.5 ±0.4	89.3 ±1.6	83.2 ±0.2	76.8 ±0.7	70.1 ±0.4	65.5 ±0.8
Non-characterized radioactivity [a)]	0.8 ±0.1	2.4 ±2.3	0.6 ±0.1	0.6 ±0.8	0.7 ±0.1	0.0 ±0.0	1.0 ±0.1	1.4 ±0.3
Total extractable residues	99.3 ±1.1	96.5 ±1.0	94.1 ±0.3	89.8 ±0.8	84.0 ±0.2	76.8 ±0.7	71.2 ±0.3	67.0 ±0.5
$^{14}CO_2$	0.0 ±0.0	0.7 ±0.0	1.8 ±0.0	4.5 ±0.0	7.8 ±0.3	13.8 ±0.2	17.2 ±0.2	20.2 ±1.9
Volatile organics	0.0 ±0.0	0.0 ± 0.0	± 0.0 ± 0.0	± 0.0 ± 0.0	± 0.0 ± n.a.	± 0.0 ± 0.0	± 0.0 ± 0.0	± 0.0 ± 0.0
Non-extractable residues (NER)	0.7 ±0.0	2.6 ±0.1	3.0 ±0.0	5.0 ±0.2	7.0 ±0.7	9.4 ±0.1	10.4 ±0.0	11.3 ±0.1
Total recovery	100.0 ±1.1	99.8 ±1.2	98.9 ±0.3	99.3 ±0.6	98.8 ±1.1	99.9 ±0.8	98.8 ±0.2	98.4 ±1.3

a) No individual peak accounted to more than 1.6%.
b) Aggressive extracts were not analyzed since the RA content was less than 3% of total applied.

Table B.8.1.1.1-35 Biotransformation of [PYR-^{14}C] BYI 02960 in sandy loam Sanger (CA) under aerobic conditions; mean values and SD expressed as % of AR

Compound	Days After Treatment (DAT)							
	0 [b)]	3	7	14	28	60	92	120
BYI 02960	98.6 ±0.2	95.2 ±1.4	87.4 ±0.0	83.8 ±1.1	67.3 ±0.2	46.8 ±1.1	34.8 ±0.0	30.0 ±0.7
6-chloronicotinic acid	0.0 ±0.0	0.6 ±0.9	3.8 ±0.7	6.9 ±0.2	12.2 ±1.2	17.1 ±1.3	12.3 ±0.8	8.2 ±1.5
Non-characterized radioactivity [a)]	1.0 ±0.6	0.0 ±0.0	1.8 ±0.8	0.8 ±0.0	2.7 ±0.3	2.0 ±0.3	3.0 ±0.3	1.9 ±0.3
Total extractable residues	99.9 ±0.8	96.9 ±0.7	95.6 ±1.2	91.5 ±0.9	82.1 ±1.1	66.0 ±0.1	50.1 ±1.1	40.0 ±1.8
$^{14}CO_2$	0.0 ±0.0	0.2 ±0.0	0.5 ±0.1	1.8 ±0.0	5.2 ±0.3	15.6 ±0.1	26.1 ±1.3	36.1 ±6.3
Volatile organics	0.0 ±0.0	0.0 ± 0.0	± 0.0 ± 0.0	± 0.0 ± 0.0	± 0.0 ± n.a.	± 0.0 ± 0.0	± 0.0 ± 0.0	± 0.0 ± 0.0
Non-extractable residues (NER)	0.1 ±0.0	1.8 ±0.1	4.2 ±0.1	7.5 ±0.4	12.4 ±0.8	18.1 ±1.0	23.4 ±0.8	25.5 ±1.3
Total recovery	100.0 ±0.8	98.9 ±0.7	100.2 ±1.0	100.9 ±0.6	99.7 ±2.1	99.7 ±1.1	99.6 ±1.0	101.6 ±5.8

a) No individual peak accounted to more than 2.4%.
b) Aggressive extracts were not analyzed since the RA content was less than 3% of total applied.

Mass Balance

Material balances ranged from 98.4 to 100% (NE soil) and from 98.9 to 101.6% (CA soil) of the applied radioactivity [AR]. The high material balances shown for all sampling intervals demonstrate that no

significant RA dissipated from the flasks or was lost during processing.In gamma-irradiated silt loam and sandy loam test systems, the material balances were complete as well.

Extractable and Bound Residues (NER)

Extractable ^{14}C-residues decreased from 99.3 and 99.9% of AR at DAT-0 to 67.0 and 40.0% of AR at study end (DAT-120) in NE and CA soil, respectively.

In gamma-irradiated test systems extractable ^{14}C-residues only decreased from 99.1 and 99.8% of AR at DAT-0 to 89.4 and 85.8 % of AR at study end (DAT-120) in NE and CA soil, respectively.

Non-extractable ^{14}C-residues increased from 0.7 and 0.1% of AR at DAT-0 to 11.3 and 25.5% of AR at study end (DAT-120) in NE and CA soil, respectively. The majority of the NER was associated with the humin. In the sandy loam after extraction with the strong acid and base, an average of 60% still remained with the solid fraction (humin). Thus, even with exposure to strong base a significant portion of non-extractable BYI 02960 residues still remained bound to the solid phase of the soil, supporting the observations of biologically-mediated bound residues.

In gamma-irradiated test systems NER only increased from 0.2 and 0.0% of AR at DAT-0 to 6.2 and 9.3% of AR at study end (DAT-120) in NE and CA soil, respectively. Thus, NER were significantly lower in gamma-irradiated compared to non-sterile soils, indicating a biological component to the formation of bound residue.

Table B.8.1.1.1-36 Summary of extractable and non-extractable residues

Soil	Springfield	Springfield gamma-irradiated	Sanger	Sanger gamma-irradiated
Extracted RA (%) Day 0	99.3	99.1	99.9	99.8
89.4 Day 120 (122[#])	67.0	89.4	40.0	85.8
Non-Extracted RA (%) Day 0	0.7	0.9	0.1	0.2
Day 120	11.3	6.2	25.5	9.3

[#] gamma-irradiated soils

Volatilization

The mineralization of [PYR-^{14}C] BYI 02960 was high. At the end of the study (DAT-120), $^{14}CO_2$ accounted for 20.2% (NE soil) and 36.1% (CA soil) of AR. In gamma-irradiated test systems the mineralization of [FUR-^{14}C] BYI 02960 was low. At the end of the study (DAT-120), $^{14}CO_2$ only accounted for 0.4% (NE soil) and 0.9% (CA soil) of AR,. This indicates a biological component to the formation of $^{14}CO_2$. Volatile organic compounds were not detected in significant amounts (≤ 0.1% AR) throughout the study.

Transformation of Test Item

The test item declined from 97.4 and 98.6% of AR at DAT-0 to 65.5 and 30.0% at the end of the study for soils NE and CA, respectively.

In silt loam, other than parent, unidentified metabolites were measured and accounted for a maximum of 2.4%. In sandy loam, other than parent, one major degradate was identified as 6-chloronicotinic acid, which was formed 6.9% on day 14, reached a maximum of 17.1% on day 60, and declined to 8.2% by the end of the study. The total unidentified radioactivity accounted for a maximum of 3.0% of the applied amount.

The mentioned results were included in the proposed overall pathway of degradation of BYI 02960 in soil shown in Figure B.8.1.3-01.

Kinetics of Test Item Degradation
The degradation of the parent compound during the study is given in paragraph B.8.1.2.

Conclusion
Major Outcomes of Study
A synopsis of results is shown in Table B.8.1.1.1-37. The data gathered in the current laboratory investigation demonstrate that [PYR-^{14}C] BYI 02960 is degraded moderately rapid in the two soils;. One part of the residues becomes increasingly bound to soil (11.3% Springfield, NE, 25.5% Sanger, CA) by study end. The other part is well mineralized to $^{14}CO_2$ (20.2% Springfield, NE, 36.1% Sanger, CA). In the Sanger soil one interim metabolite, which was identified as 6-chloronicotinic acid, was to be observed, reaching its maximum of 17.1% on day 60 and declining to 8.2% of AR by the end of the study. All further formed metabolites are regarded as transient, which is confirmed by the high mineralization rate of [PYR-^{14}C] BYI 02960 to $^{14}CO_2$ observed in this study. Volatile organic compounds were very low (≤ 0.1% of AR) at all sampling dates.

Degradation, amount of bound residues as well as of $^{14}CO_2$ formed in gamma-irradiated soils was significantly less than in non-sterile soils, indicating a biological component to the degradation/ mineralization and formation of non-extractable residues from BYI 02960.

Additionally, soil fractionation shows that even with extraction using strong base, BYI 02960 related residues remain bound to the solid (humin) fraction indicating very strong and irreversible binding to soil.

Table B.8.1.1.1-37 Synopsis of overall results

Soil	Springfield Viable	Springfield Sterile	Sanger Viable	Sanger Sterile
Total Recovery (%)	100-99.6	96-100.0	100-100.4	96.0-100.0
Extracted RA (%)	70.9-99.2	89.4-99.1	33.7-99.8	85.8-99.8
Max. CO_2 (%)	12.3	0.4	36.1	0.9

Bound Residues (%)	0.8-16.4	0.9-6.2	0.2-30.6	0.2-9.3
Major metabolites	-	Not determined	6-CNA (17.1%)	Not determined

Significance of Results to Environmental Behavior of BYI 02960

The current laboratory study demonstrated that a viable aerobic soil environment will contribute significantly to the degradation of [PYR-^{14}C] BYI 02960. With respect to the radiolabel used mineralization to $^{14}CO_2$ is significant. One metabolite, which was identified as 6-chloronicotinic acid, was observed at a proportion of greater 10% of applied, and declined to the end of study. Other metabolites are not to be expected to be significant in soil, since – after their formation – they are well mineralized (see Table B.8.1.1.1-38).

Table B.8.1.1.1-38 Synopsis of results of biotransformation of [PYR-^{14}C] BYI 02960 in two soils incubated at 20 °C and pF 2.0 – 2.5 % under aerobic conditions

Soil	**Springfield (NE)**		**Sanger (CA)**	
Soil type	**Silt loam**		**Sandy loam**	
Soil status	**Viable**	**Sterile**	**Viable**	**Sterile**
Major transformation products *	CO_2 (max. 20.2%) NER (max. 11.3%)	(NER)	CO_2 (max. 36.1%) NER (max. 25.5%) 6-CNA (max. 17.1%)	(NER)
Minor transformation products	-	CO_2	-	CO_2

*): Criteria for term "major": >10% of AR at any DAT or >5% of AR at two successive DATs or steadily increasing until the end of the study.
(NER): Major acc. to *, but did not exceed 10% of AR during the entire study period.

Remarks

The aerobic soil degradation study is acceptable and the results are reliable.

B.8.1.1.2 Supplementary studies (Annex IIA 7.1.2 to 7.1.3)

B.8.1.1.2.1 Anaerobic studies

STUDY IIA, 7.1.2/01

Characteristics

reference	:	Menke, U., Unold, M. 2012	study type	:	anaerobic degradation
year of execution	:	2009-2011	incubation time	:	up to 123 d
GLP statement	:	yes	nominal concentration	:	1.1 mg/kg
guideline	:	OECD 307 (2002) US EPA, OPPTS 835.4100 (2008)	temperature	:	20°C
test substance	:	[Furanone-4-^{14}C] and [Ethyl-1-^{14}C] and [Pyridine-2,6-^{14}C] BYI 02960	DT_{50}	:	see results
purity	:	radiochemical purity 98-99 %.	metabolites	:	see results
soil	:	One soil; silt loam	acceptability	:	**acceptable**

Summary

The present laboratory study investigated the degradation of BYI 02960 in one soil (silt loam) under flooded anaerobic conditions. [Pyridine-2,6-^{14}C], [furanone-4-^{14}C] and [ethyl-1-^{14}C] BYI 02960 (equivalent to label PYR, FUR, and ETH, respectively) were applied at of 110.1, 104.6 and 105.9 µg/100 g soil (dry matter), i.e. equivalent to 103.2, 98.0 and 99.2% of the nominal application rate of 400 g/ha.

The soil in duplicate test flasks/interval was maintained under aerobic conditions for 30 days in the dark at 20 ± 2°C and approx. 55% of the maximum water holding capacity. Following the aerobic phase, the samples were flooded with oxygen-depleted de-ionized water (approx. 3 cm layer above soil level), set under nitrogen atmosphere, and maintained in the dark at 20 ± 2°C under anaerobic conditions for max. 123 days. During the aerobic study phase, air-permeable traps were attached for the collection of CO_2 and volatile organics (static test system design). At start of the anaerobic study phase, the trap systems were replaced by sealable two-valve glass stoppers connected with plastic gas sampling bags.

Soil samples and water layers were separated by decanting to allow for individual analysis. The soil was extracted four times with at ambient temperature (combined as "ambient organic extract"). Subsequently, the soil was extracted once at an elevated temperature ("aggressive extract"). BYI 02960 residues in water layers were directly analyzed by reversed phase HPLC; the soil extracts were subjected to solvent exchange prior to analysis (all labels). TLC was employed as second contrasting separation method for the confirmation of the results. A limit of quantification (LOQ) of equal or better than 0.9% of the applied radioactivity (% AR) was calculated for HPLC radioactivity detection within the sample matrices.

For all three radiolabels complete material balances found at all sampling intervals demonstrated that no significant portion of radioactivity dissipated from the flasks or was lost during processing. During the 30 days of aerobic incubation $^{14}CO_2$ accounted for up to 26.7 (PYR), 15.9 (FUR) and 6.9 % of AR (ETH). During the anaerobic incubation phase, mineralization to $^{14}CO_2$ was negligible (< 0.1 % of AR). Organic volatiles were not observed in the aerobic or in the anaerobic study phase (< 0.1 % AR at all sampling intervals).

The radioactivity extractable from soil decreased to 55.9, 55.2 and 82.0% of AR towards the end of the aerobic incubation phase (DAT-30), and further to 41.5, 40.2 and 53.4% of AR until the end of the anaerobic incubation period for labels PYR, FUR and ETH, respectively.

Within the aerobic phase of the study (30 days) the percentages of BYI 02960 in the entire systems decreased to 53.7, 52.6 and 54.7% of AR for labels PYR, FUR and ETH, respectively. During the anaerobic incubation period (i.e. flooded state) the portions of BYI 02960 slightly decreased further to 47.8, 47.2 and 47.7% of AR for labels PYR, FUR and ETH, respectively.

Only one transformation product exceeded 5% of AR over the entire study period. It was detected in the test systems of label ETH and it was identified as difluoroacetic acid (DFA). DFA levels increased to a level of 25.1% of AR during the initial 30 days of aerobic conditions. During the anaerobic phase, the amounts of DFA remained more or less stable (24.2 - 26.2% of AR).

In the aerobic incubation phase, non-extractable residues (NER) in soil increased from 2.7 to 12.9% (label PYR), 3.1 to 25.6% (Label FUR) and 2.8 to 10.8% of AR (Label ETH). During the anaerobic incubation phase the NER slightly increased further.

Applying single first order kinetics (SFO) to the BYI 02960 residues in the entire systems during the anaerobic phase of the study, the estimated DT_{50} values range from 581.8 to 693.2 days (geometric mean: 633.7 days).

Based on the results obtained within this study it can be expected that the amounts of BYI 02960 and its only significant metabolite DFA remain stable under flooded field conditions. Degradation would be expected to continue whenever the conditions become aerobic.

Materials and methods

Materials

1. Test Items: Flupyradifurone: Code = BYI 02960;
Label PYR = [Pyridine-2,6-^{14}C] BYI 02960 (sample ID: KATH 6403); Specific activity: 4.49 MBq/mg; Radiochemical purity: >98% (acc. radio-HPLC)
Label FUR = [Furanone-4-^{14}C] BYI 02960 (sample ID: KATH 6405); Specific activity: 3.94 MBq/mg Radiochemical purity: >98% (acc. radio-HPLC).
Label ETH = [Ethyl -1-^{14}C] BYI 02960 (sample ID: KATH 6404); Specific activity: 3.93 MBq/mgRadiochemical purity: >99% (acc. radio-HPLC)
Identity and purity of test items in the application solution were confirmed.

2. Soil: the biotransformation of BYI 02960, under anaerobic conditions, was studied in one soil. The soil was taken on 2009-12-07 fresh from the field. On the day of sampling, the soil was broken up stepwise and gently air dried. Then, the soil was successively sieved to ≤ 2 cm. Soil moisture after sieving was equivalent to 40.8% WHCmax. One day after preparation, soil portions were weighed into the incubation flasks, adjusted to 55% WHCmax and pre-incubated at about 20 ± 2°C until application, i.e. for approx. 7 days.

Table B.8.1.1.2-01 Soil physicochemical properties

Parameter	Results/Units
Batch ID	Hoefchen am Hohenseh 4a, 20091207
Location	Burscheid, Germany
Soil Taxonomic Classification (USDA)	N/A
Soil Series	N/A
Texture Class (USDA)	Silt Loam
Sand Silt Clay	25% 61% 14%
pH in $CaCl_2$ pH in KCl pH in Water	6.4 6.2 6.5
Organic Matter	4.7%

Organic Carbon	2.7%
Soil Microbial biomass mg microbial C/kg	
0 days	1263
30 days	1097
Anaerobic bacteria plate count	67000 CFU/g soil (non-treated) 33000 CFU/g soil (solvent control)
Cation Exchange Capacity (CEC)	14.7 meq/100 g
Water Holding Capacity	
at 0.33 bar [1/3 bar WHC]	22.7 g/100 g
Maximum Water Holding Capacity [WHCmax]	67.0 g/100 g
Bulk Density (disturbed)	1.07 g/cm3

Methods

1. Experimental conditions: The study was performed in static incubation test systems under aerobic followed by anaerobic conditions in the dark at 19.9 ± 0.1 °C. The test system consisted of Erlenmeyer flasks (300 mL) attached, first with a trap attachment (permeable for oxygen) containing soda lime for absorption of $^{14}CO_2$ and a polyurethane foam plug for adsorption of volatile organic compounds, second with a gastight gas sampling bag. Aliquots of 100 g of dry soil were weighed into the test flasks. Replicates were set up and processed for each sampling (3 sampling dates including time 0 for the aerobic phase, 9 sampling dates including time 0 for the anaerobic phase). Additional flasks were used for determination of the microbial biomass. The final soil moisture, for the aerobic phase was adjusted to 55% of WHC_{max} by adding pure water.

The switch to anaerobic (flooded) conditions was made 30 days after test item application: The trap system of all remaining test flasks was removed and stored for later analysis. The soil of each flask was flooded with 150 mL of oxygen-depleted de-ionized water leading to a water layer of approx. 3 cm above soil level. The flasks were then equipped with sealable double-valve glass stoppers. The flasks were connected to plastic gas sampling bags, which had been flushed with nitrogen gas. The valves were set to connect flask headspace and gas sampling bag, but closing the system from the outer atmosphere. Such setup allowed for pressure-less closed-flask incubation. To ensure maintenance of fully oxygen-free conditions, the test systems furthermore were placed in an argon, then nitrogen flooded box within the incubation chamber.

2. Test Item Stock Solution: The entire delivered amount of [^{14}C] BYI 02960 was dissolved in 4 mL methanol.

3. Test Item Application Solution: An application solution was made by diluting 2-3-mL aliquots of stock solutions in 20 mL of purified water.

4. Mode of Application: The test systems were removed from the incubation chamber and dosed with 447 µL, 549 µL or 464 µL of respective application solutions per flask. Treatment was made as small droplets applied directly onto the soil surface using a micropipette. Each flask was then gently shaken to incorporate the radiochemical into the test soil. Care was taken not to form clumps during the mixing process. Finally, the test systems were fitted with the volatiles traps and placed back into the

incubation chamber. Biomass and anaerobic bacteria determination test systems were either left untreated (native soil sample DAT-0), or dosed with 30 µL of methanol.

The test vessels for DAT-0 were immediately processed for analysis. All other test vessels, including the biomass flasks which were not spiked with application solution, were fitted with trap attachments and incubated in the dark at nominal 20 ± 2°C.

[PYR-^{14}C] BYI 02960 was applied at a rate of 494,261.3 Bq per vessel. This corresponds to 110.1 µg per vessel which is equivalent to 102.3 % of the intended field application rate of 106.7 µg per vessel (calculated an application rate of 400 g BYI 02960 per hectare). [FUR-^{14}C] BYI 02960 was applied at a rate of 412,122.5 Bq per vessel, this corresponds to 104.6 µg per vessel which is equivalent to 98.0 % of the intended application rate. [ETH-^{14}C] BYI 02960 was applied at a rate of 416,163.9 Bq per vessel. This corresponds to 105.9 µg per vessel which is equivalent to 99.2 % of the intended application rate.

5. Sampling: Characterization of the soil microbial viability was achieved by (a) determinations of soil microbial biomass during the aerobic incubation phase, and (b) by determinations of anaerobic bacteria during the anaerobic incubation phase. Biomass measurements were conducted at the beginning of the incubation period (DAT-0) for a pre-equilibrated but untreated test system, and at the end of aerobic incubation period (DAT-30) for a test system treated with a test item and a test system treated with the application solvent (30 µL of methanol). Determinations of anaerobic bacteria were performed at the end of the study (DASF-123) for an untreated test system and a test system treated with the application solvent, each. For procedure descriptions see Sections 3.6.2.7 and 3.6.2.8 of report.

Entire test flasks were taken for processing and analysis at 0, 23 and 30 days after treatment (DAT, (i.e. the aerobic phase) and 0, 1, 4, 7, 14, 29, 60, 90 and 123 days after soil flooding (DASF).

Aerobic systems: After collection of the respective test systems from the incubation chamber, samples taken on days 23 and 30 were exposed to vacuum for about 10 min to purge the volatiles possibly still present in the headspace into the traps. Then, the flask and volatile traps were separated. For the samples which were directly processed after application (DAT-0), no volatiles were collected.
At each sampling date the entire amount of soil in each test vessel was transferred into a centrifuge beaker and extracted using a mechanical shaker.

Anaerobic systems: After collection of the respective test systems from the incubation box, they were connected to a volatiles combustion oven unit. Using nitrogen, volatiles present in the headspace and gas sampling bag were slowly purged over a soda lime trap for absorption of $^{14}CO_2$, through the catalytic oven for oxidative combustion of organic volatiles (e.g. methane), and finally through three liquid scintillation flasks filled with LSC cocktail, in order to absorb $^{14}CO_2$ contained in the combustion exhaust. LSC cocktail traps were directly analyzed.

Next, the test flasks were opened, and the oxygen content of the water layers as well as the redox potential and the pH value of the water phases and soils layer were immediately determined by electrode measurements.

The water layers were separated from the soil layers by decanting. For removal of suspended particles, the decanted water layers were centrifuged for about 20 min at ca. 10000xg and filtered through a paper filter. The supernatants were analyzed without further extraction. The centrifugation pellets were added to the soil phases, by re-use of the centrifuge flasks for the extraction of the respective soil layers.

Soil extracts and decanted water layers were subjected to chromatography profiling within a maximum of 2 days after sampling. Samples were stored in a refrigerator prior to analysis. After analysis, they were stored deep frozen at about -20°C. Extracted soil was air-dried at room temperature. Combustion analysis was conducted within a maximum of 127 days. Absence of losses upon storage can be concluded from the complete radioactive material balances. After analysis, the soil samples were stored deep frozen at about -20°C. Volatiles traps were processed and analyzed within a maximum of 35 days. Sample stability can be concluded from the complete radioactive material balances.

6. Description of analytical procedures: The soil processing procedure was optimized to obtain >90% extraction efficiency and >90% recovery of the test item at time zero. Extraction was by cycles of heavily shaking for about 30 minutes on a mechanical shaker at room temperature followed by centrifugation (ca. 10 min, 10000xg) and decanting through a paper filter. At each sampling date, the soil samples were extracted with 3 x 80 mL acetonitrile/water (80/20, v/v) and 1 x 80 mL acetonitrile by shaking at ambient temperature. A further extraction step followed with acetonitrile/water (80/20, v/v) at 70°C using a microwave for 10 minutes. 'Ambient' and 'aggressive' extracts were kept separate for individual chromatography profiling.

The BYI 02960 residues and transformation products were analyzed and quantified by LSC and reversed phase radio-HPLC. Losses of radioactivity after concentration of extracts were minimal. Normal-phase Si-60 radio-TLC was used as a confirmatory method. The identity of the test item in stock solution and in extracts was confirmed by spectroscopic methods. In addition, spectroscopic methods were used to identify one of the minor metabolites.

The disappearance kinetics of BYI 02960 in the entire test systems during the anaerobic phase was individually calculated for each radiolabel. The calculation was done according to FOCUS kinetics guidelines.

Results and discussion

Results indicated that anticipated standardized conditions were maintained, and the soils were microbial active throughout the entire laboratory study, i.e. in the aerobic as well as the anaerobic phase where anaerobic bacteria plate count assays showed the presence of at least 33000 colony forming units per gram of soil dry weight for untreated soil or soil treated with application solvent. This confirmed the establishment of an anaerobic micro flora in the test systems.

Oxygen content in the water layer was below 1 mg/L 4 days after soil flooding (DASF) for all three labels. Throughout the rest of the anaerobic incubation phase oxygen concentration stayed below this value. Redox potential measurements indicated transition of the system to reducing conditions, in both soil and water layer from DASF-29 or DASF-60 onwards. The pH values scatter until DASF-29. Afterwards, they increased slightly (from about pH 6.8 to pH 7.3/7.4) and approached a plateau at around DASF-60. The pH values in the sediment were slightly lower than the pH-values in the water layers (see Table B.8.1.1.2-02 to Table B.8.1.1.2-04).

Table B.8.1.1.2-02 Redox Potential, Oxygen Content and pH of Test Systems (PYR label)

DASF	Repli-cate	Water Phase				Soil Layer		
		pH	Oxygen content [mg/L]	Redox potential SenTix ORP electrode[1] [mv]	Redox potential E_h 1) [mv]	pH	Redox potential SenTix ORP electrode[1] [mv]	Redox potential E_h 1) [mv]
0	P1	6.44	3.62	205	415	6.62	200	410
	P2	6.91	3.38	200	410	6.66	205	415
	Mean	**6.68**	**3.50**	**203**	**413**	**6.64**	**203**	**413**
1	P1	6.78	2.07	130	340	6.75	120	330
	P2	6.67	2.37	140	350	6.62	126	336
	Mean	**6.73**	**2.22**	**135**	**345**	**6.69**	**123**	**333**
4	P1	6.60	0.75	135	345	6.76	124	334
	P2	6.71	0.68	195	405	6.64	186	396
	Mean	**6.66**	**0.72**	**165**	**375**	**6.70**	**155**	**365**
7	P1	6.91	0.44	195	405	6.82	191	401
	P2	6.62	0.97	197	407	6.69	186	396
	Mean	**6.77**	**0.71**	**196**	**406**	**6.76**	**189**	**399**
14	P1	6.82	0.33	112	322	6.61	107	317
	P2	6.81	0.42	116	326	6.60	105	315
	Mean	**6.82**	**0.38**	**114**	**324**	**6.61**	**106**	**316**
29	P1	6.85	0.40	-25	185	6.64	-39	171
	P2	6.75	0.23	-41	169	6.59	-55	155
	Mean	**6.80**	**0.32**	**-33**	**177**	**6.62**	**-47**	**163**
60	P1	7.45	0.36	-147	63	7.31	-151	59
	P2	7.39	0.40	-165	45	7.16	-164	46
	Mean	**7.42**	**0.38**	**-156**	**54**	**7.24**	**-158**	**53**
90	P1	7.45	0.32	-145	65	7.25	-159	51
	P2	7.51	0.40	-146	64	7.28	-162	48
	Mean	**7.48**	**0.36**	**-146**	**65**	**7.27**	**-161**	**50**
123	P1	7.67	0.19	-254	-44	7.38	-373	-163
	P2	7.57	0.25	-253	-43	7.40	-278	-68
	Mean	**7.62**	**0.22**	**-254**	**-44**	**7.39**	**-326**	**-116**

DASF: Days after soil flooding
1): Eh = Emeasured + 210mV (reference potential of the SenTix ORP electrode vs. the standard electrode at 20 °C according to information of manufacturer WTW)

Table B.8.1.1.2-03 Redox Potential, Oxygen Content and pH of Test Systems (FUR label)

DASF	Repli-cate	Water Phase				Soil Layer		
		pH	Oxygen content [mg/L]	Redox potential SenTix ORP electrode[1] [mV]	Redox potential E_h[1)] [mV]	pH	Redox potential SenTix ORP electrode[1] [mV]	Redox potential E_h[1)] [mV]
0	F1	6.87	3.94	199	409	6.75	200	410
	F2	6.87	3.82	195	405	6.75	206	416
	Mean	**6.87**	**3.88**	**197**	**407**	**6.75**	**203**	**413**
1	F1	6.76	1.66	140	350	6.63	127	337
	F2	6.72	1.26	165	375	6.57	151	361
	Mean	**6.74**	**1.46**	**153**	**363**	**6.60**	**139**	**349**
4	F1	6.53	0.56	197	407	6.48	187	397
	F2	6.58	0.72	199	409	6.54	190	400
	Mean	**6.56**	**0.64**	**198**	**408**	**6.51**	**189**	**399**
7	F1	6.63	0.64	211	421	6.57	216	426
	F2	6.60	0.68	230	440	6.57	215	425
	Mean	**6.62**	**0.66**	**221**	**431**	**6.57**	**216**	**426**
14	F1	6.71	0.33	111	321	6.69	104	314
	F2	6.82	0.54	107	317	6.65	96	306
	Mean	**6.77**	**0.44**	**109**	**319**	**6.67**	**100**	**310**
29	F1	6.84	0.70	-61	149	6.63	-82	128
	F2	6.79	0.31	-44	166	6.62	-62	148
	Mean	**6.82**	**0.51**	**-53**	**158**	**6.63**	**-72**	**138**
60	F1	7.35	0.30	-151	59	7.13	-153	57
	F2	7.32	0.35	-155	55	7.14	-162	48
	Mean	**7.34**	**0.33**	**-153**	**57**	**7.14**	**-158**	**53**
90	F1	7.35	0.28	-159	51	7.19	-162	48
	F2	7.40	0.27	-163	47	7.21	-167	43
	Mean	**7.38**	**0.28**	**-161**	**49**	**7.20**	**-165**	**46**
123	F1	7.47	0.18	-287	-77	7.23	-283	-73
	F2	7.44	0.27	-252	-42	7.24	-282	-72
	Mean	**7.46**	**0.23**	**-270**	**-60**	**7.24**	**-283**	**-73**

DASF: Days after soil flooding
1): $E_h = E_{measured}$ + 210mV (reference potential of the SenTix ORP electrode vs. the standard electrode at 20 °C according to information of manufacturer WTW)

Table B.8.1.1.2-04 Redox Potential, Oxygen Content and pH of Test Systems (ETH label)

DASF	Repli-cate	Water Phase				Soil Layer		
		pH	Oxygen content [mg/L]	Redox potential SenTix ORP electrode[1] [mV]	Redox potential E_h[1)] [mV]	pH	Redox potential SenTix ORP electrode[1] [mV]	Redox potential E_h[1)] [mV]
0	E1	6.79	4.18	205	415	6.80	207	417
	E2	6.88	4.13	212	422	6.76	212	422
	Mean	**6.84**	**4.16**	**209**	**419**	**6.78**	**210**	**420**
1	E1	6.74	1.52	192	402	6.55	185	395
	E2	6.65	1.68	175	385	6.53	161	371
	Mean	**6.70**	**1.60**	**184**	**394**	**6.54**	**173**	**383**
4	E1	6.47	0.61	199	409	6.46	192	402
	E2	6.48	0.68	199	409	6.51	195	405
	Mean	**6.48**	**0.65**	**199**	**409**	**6.49**	**194**	**404**
7	E1	6.82	0.49	219	429	6.60	207	417
	E2	6.66	0.51	272	482	6.61	236	446
	Mean	**6.74**	**0.50**	**246**	**456**	**6.61**	**222**	**432**
14	E1	6.89	0.31	108	318	6.65	98	308
	E2	6.85	0.43	100	310	6.59	92	302
	Mean	**6.87**	**0.37**	**104**	**314**	**6.62**	**95**	**305**

29	E1	6.77	0.25	-46	164	6.59	-61	149
	E2	6.80	0.25	-67	143	6.59	-76	134
	Mean	**6.79**	**0.25**	**-57**	**154**	**6.59**	**-69**	**142**
60	E1	7.27	0.24	-153	57	7.06	-162	48
	E2	7.26	0.32	-162	48	7.00	-164	46
	Mean	**7.27**	**0.28**	**-158**	**53**	**7.03**	**-163**	**47**
90	E1	7.45	0.47	-167	43	7.13	-171	39
	E2	7.40	0.13	-168	42	7.16	-169	41
	Mean	**7.43**	**0.30**	**-168**	**43**	**7.15**	**-170**	**40**
123	E1	7.39	0.23	-291	-81	7.18	-306	-96
	E2	7.38	0.28	-284	-74	7.18	-295	-85
	Mean	**7.39**	**0.26**	**-288**	**-78**	**7.18**	**-301**	**-91**

DASF: Days after soil flooding
1):E_h = $E_{measured}$ + 210mV (reference potential of the SenTix ORP electrode vs. the standard electrode at 20°C according to information of manufacturer WTW)

Data

The respective data for the three different radiolabels are shown in Table B.8.1.1.2-05 to Table B.8.1.1.2-07. The DAT-0 extraction efficiency was 95.4, 96.7 and 95.2% of AR (sum of ambient and aggressive extract), and for labels PYR, FUR and ETH, respectively. The stability of BYI 02960 during processing was verified by mean purities >98.1, >98.2 and >98.2% in the ambient extracts and 100% in the aggressive extracts of labels PYR, FUR and ETH, respectively. These results indicate that the extraction method was well suited to extract the applied [^{14}C]-labeled test item from the soil matrix.

Table B.8.1.1.2-05 Biotransformation of [PYR-^{14}C] BYI 02960 in silt loam HF under aerobic then anaerobic conditions; mean values and SD expressed as % of AR

Com-pound	Compart-ment	Days After Treatment (DAT)			Days After Soil Flooding (DASF)								
		0	23	30	0	1	4	7	14	29	60	90	123
BYI 02960	Water layer	N/A	N/A	N/A	4.0	8.1	11.7	10.5	10.3	8.9	7.8	7.1	7.8
	Soil extracts	94.5	58.6	53.7	49.7	47.3	43.1	43.9	42.4	42.0	41.8	41.3	40.0
	Entire system	94.5 ±1.9	58.6 ±1.4	53.7 ±1.1	53.7 ±1.0	55.3 ±0.2	54.7 ±0.2	54.4 ±0.3	52.7 ±0.7	50.9 ±0.7	49.6 ±0.2	48.5 ±1.1	47.8 ±0.2
ROI 1	Water layer	N/A	N/A	N/A						0.9	0.9	0.9	0.9
	Soil extracts		1.0	0.9	0.5			0.7		0.8	0.5		
	Entire system		1.0 ±0.1	0.9 ±0.4	0.5			0.7		1.7 ±0.2	1.2 ±0.5	0.9	0.9 ±0.1
ROI 2	Water layer	N/A	N/A	N/A									
	Soil extracts		0.7	0.6	0.9	0.8	0.8	0.8	0.7	0.7	0.7	0.7	0.8
	Entire system		0.7	0.6 ±0.1	0.9 ±0.3	0.8 ±0.1	0.8 ±0.2	0.8 ±0.3	0.7	0.7	0.7 ±0.1	0.7 ±0.2	0.8 ±0.2
ROI 3	Water layer	N/A	N/A	N/A			0.5	0.5	0.4				
	Soil extracts												
	Entire system						0.5	0.5 ±0.2	0.4				
ROI 4	Water layer	N/A	N/A	N/A				0.3					
	Soil extracts												
	Entire system							0.3					
ROI 5	Water layer	N/A	N/A	N/A						0.4	0.4	0.3	0.3
	Soil extracts												
	Entire system									0.4	0.4	0.3	0.3
Diffuse RA	Water layer	N/A	N/A	N/A	0.1	0.2	0.5	0.3	0.3	<0.1	0.1	0.1	0.1
	Soil extracts	0.9	0.4	0.7	0.7	0.4	0.6	0.6	0.8	0.7	1.3	0.5	0.7
	Entire system	0.9 ±1.2	0.4 ±0.3	0.7 ±0.9	0.8 ±0.2	0.6 ±0.1	1.1 ±0.5	0.9 ±0.1	1.1 ±0.9	0.7 ±0.8	1.4 ±0.3	0.6 ±0.4	0.8 ±0.5

Total extractable residues	Water layer	N/A	N/A	N/A	4.0	8.3	12.5	11.4	10.8	10.1	9.1	8.4	9.0
	Soil extracts	95.4	60.3	55.9	51.6	48.4	44.4	45.7	43.9	44.1	44.0	42.5	41.5
	Entire system	95.4 ±0.7	60.3 ±1.2	55.9 ±0.5	55.6 ±0.8	56.7 ±0.3	56.9 ±0.4	57.1 ±0.1	54.7 ±0.2	54.2 ±0.2	53.1 ±0.5	50.9 ±0.5	50.5 ±0.4
$^{14}CO_2$	Sum entire period	N/A	20.7	26.2 ±0.3	26.3 ±0.2	25.8 ±0.6	26.1 ±1.1	25.2	26.3 ±0.7	25.6 ±1.3	26.7 ±0.2	24.9 ±0.1	26.6 ±0.4
Volatile organics	Sum entire period	N/A	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
NER	Soil extracts	2.7 ±0.1	12.4	12.9 ±0.1	13.2 ±0.4	13.2 ±0.2	12.2	12.9	13.7 ±0.4	14.6 ±0.2	16.4 ±0.1	17.8	17.2 ±0.3
Total % recovery	Entire system	98.1 ±0.6	93.4 ±1.2	95.0 ±0.1	95.1 ±0.2	95.7 ±0.6	95.3 ±0.6	95.2 ±0.1	94.7 ±1.0	94.4 ±1.4	96.3 ±0.4	93.6 ±0.5	94.2 ±1.1

N/A = not applicable; blank cell = not detected, no SD could be calculated;

Table B.8.1.1.2-06 Biotransformation of [FUR-14C] BYI 02960 in silt loam HF under aerobic then anaerobic conditions; mean values and SD expressed as % of AR

Com-pound	Compart-ment	Days After Treatment (DAT)			Days After Soil Flooding (DASF)								
		0	23	30	0	1	4	7	14	29	60	90	123
BYI 02960	Water layer	N/A	N/A	N/A	3.6	9.1	11.0	9.8	10.8	8.9	7.3	6.8	7.9
	Soil extracts	95.1	58.2	52.6	48.3	43.0	41.7	43.2	40.2	41.0	40.5	40.5	39.3
	Entire system	95.1 ±0.6	58.2 ±0.4	52.6 ±0.6	51.9 ±0.3	52.1 ±1.5	52.6 ±1.8	53.0 ±1.0	51.0 ±0.5	49.9 ±0.7	47.7	47.3 ±1.1	47.2 ±0.7
ROI A	Water layer	N/A	N/A	N/A						0.4	0.7	0.8	0.7
	Soil extracts									0.6			0.1
	Entire system									1.1 ±0.4	0.7 ±0.2	0.8 ±0.2	0.8
ROI B	Water layer	N/A	N/A	N/A									
	Soil extracts		0.7	1.0	1.2	0.7	0.6	0.9	0.5	0.7	1.0	0.7	0.6
	Entire system		0.7 ±0.2	1.0 ±0.3	1.2	0.7 ±0.1	0.6 ±0.1	0.9 ±0.1	0.5 ±0.1	0.7 ±0.3	1.0	0.7 ±0.2	0.6
ROI C	Water layer	N/A	N/A	N/A									
	Soil extracts		0.7	0.5	1.1	1.3	0.7						
	Entire system		0.7 ±<0.1	0.5 ±0.1	1.1	1.3	0.7						
ROI D	Water layer	N/A	N/A	N/A									
	Soil extracts											0.6	
	Entire system											0.6	
Diffuse RA	Water layer	N/A	N/A	N/A	<0.1	0.2	0.3	0.1	0.2	0.2	0.2	0.1	0.1
	Soil extracts	1.5	0.6	1.1	0.9	0.6	0.4	0.5	1.0	0.3	0.9	0.9	0.5
	Entire system	1.5 ±0.3	0.6 ±0.4	1.1 ±1.3	1.0 ±0.3	0.7 ±0.4	0.7 ±0.2	0.6 ±0.3	1.3 ±0.2	0.5 ±0.1	1.2 ±0.4	1.0 ±0.3	0.6 ±0.2
Total extract. residues	Water layer	N/A	N/A	N/A	3.6	9.2	11.3	9.9	11.0	9.5	8.2	7.7	8.7
	Soil extracts	96.7	60.3	55.2	50.4	44.9	43.1	44.6	41.8	42.6	42.4	42.3	40.2
	Entire system	96.7 ±0.3	60.3 ±1.0	55.2 ±0.4	54.0 ±0.6	54.1 ±1.1	54.3 ±1.0	54.5 ±0.6	52.8 ±0.4	52.2 ±0.1	50.6 ±0.2	50.0 ±1.1	48.9
$^{14}CO_2$	Sum entire period	N/A	12.3 ±0.3	15.1 ±0.2	15.0 ±0.1	15.6 ±0.1	15.5 ±0.1	15.8 ±0.1	15.9 ±0.3	15.7 ±0.1	15.5 ±0.1	15.5 ±0.3	15.9 ±0.2
Volatile organics	Sum entire period	N/A	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
NER	Soil extracts	3.1	23.9	25.6	25.2	26.7	25.1	25.5	26.7	27.4	29.0	30.1	29.3
		±0.1	±0.6	±0.3	±0.4	±0.3	±0.1	±0.2	±0.3	±0.9	±0.1	±1.0	±0.9
Total % recovery	Entire system	99.7	96.5	95.9	94.2	96.4	94.9	95.8	95.5	95.3	95.1	95.6	94.1
		±0.4	±0.7	±0.9	±0.2	±0.9	±0.8	±0.6	±0.3	±0.8	±0.3	±1.8	±0.7

N/A = not applicable; blank cell = not detected, no SD could be calculated;

Table B.8.1.1.2-07 Biotransformation of [ETH-^{14}C] BYI 02960 in silt loam HF under aerobic then anaerobic conditions; mean values and SD expressed as % of AR

Com-pound	Compart-ment	Days After Treatment (DAT)			Days After Soil Flooding (DASF)								
		0	23	30	0	1	4	7	14	29	60	90	123

BYI 02960	Water layer	N/A	N/A	N/A	5.5	9.2	11.0	8.9	10.7	9.2	7.7	7.0	7.0
	Soil extracts	93.5	60.9	54.7	48.7	44.6	41.7	44.8	42.1	42.1	42.2	40.7	40.7
	Entire system	93.5 ±0.5	60.9 ±0.5	54.7 ±0.2	54.1 ±0.3	53.8 ±0.6	52.7 ±1.5	53.7 ±<0.1	52.8 ±0.5	51.4 ±0.7	50.0 ±1.2	47.6 ±0.2	47.7 ±0.9
DFA	Water layer	N/A	N/A	N/A	4.8	9.1	12.5	14.2	15.7	16.6	15.7	14.6	15.1
	Soil extracts		21.8	25.1	20.4	16.0	13.5	11.3	10.5	9.0	8.5	10.2	11.1
	Entire system		21.8 ±0.5	25.1 ±0.2	25.2 ±0.4	25.1 ±0.6	26.0 ±0.6	25.5 ±1.6	26.2 ±0.8	25.6 ±0.5	24.2 ±0.6	24.7 ±0.4	26.2 ±0.6
ROI Z	Water layer	N/A	N/A	N/A									
	Soil extracts			1.3	1.1	0.7	0.9	0.9	0.9	1.0	0.7	1.0	1.0
	Entire system			1.3 ±0.2	1.1 ±0.2	0.7	0.9 ±0.3	0.9 ±0.5	0.9 ±0.1	1.0	0.7 ±0.3	1.0 ±0.4	1.0 ±0.3
Diffuse RA	Water layer	N/A	N/A	N/A	0.2	0.8	0.6	1.0	0.2	0.1	1.4	0.3	0.6
	Soil extracts	1.7	0.5	0.9	0.7	1.0	1.1	0.8	0.9	0.8	0.6	0.4	0.6
	Entire system	1.7 ±<0.1	0.5 ±0.3	0.9 ±0.3	0.9 ±0.3	1.8 ±<0.1	1.7 ±0.3	1.8 ±0.5	1.0 ±0.6	0.9 ±1.0	2.0 ±0.3	0.7 ±0.4	1.2 ±0.1
Total extract. residues	Water layer	N/A	N/A	N/A	10.5	19.2	24.1	24.1	26.6	26.0	24.9	21.9	22.7
	Soil extracts	95.2	83.3	82.0	70.9	61.9	57.1	57.8	54.3	52.9	52.0	52.2	53.4
	Entire system	95.2 ±0.4	83.3 ±0.3	82.0 ±0.1	81.3 ±0.8	81.1 ±0.6	81.2 ±0.9	81.9 ±1.6	80.9 ±0.5	78.8 ±0.8	76.9 ±1.2	74.1 ±0.2	76.1 ±0.1
$^{14}CO_2$	Sum entire period	N/A	4.3 ±0.1	6.5 ±0.2	6.1	6.4 ±0.2	6.8 ±0.1	5.9 ±0.7	6.9	6.4 ±0.2	6.5 ±0.4	6.4 ±0.3	6.6 ±0.1
Volatile organics	Sum entire period	N/A	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
NER	Soil extracts	2.8 ±0.1	11.0 ±0.1	10.8	10.7	10.6	9.7 ±0.1	9.8 ±0.4	10.6 ±0.5	12.4 ±0.2	13.8 ±0.1	15.2 ±0.3	14.7 ±0.4
Total % recovery	Entire system	98.0 ±0.5	98.6 ±0.3	99.4 ±0.1	98.2 ±0.8	98.1 ±0.3	97.7 ±1.0	97.5 ±0.5	98.4 ±1.0	97.7 ±0.4	97.2 ±1.7	95.7 ±0.2	97.5 ±0.2

N/A = not applicable; blank cell = not detected, no SD could be calculated;

Mass Balance

Material balance ranged from 93.4 to 98.1% (PYR), 94.1 – 99.7% (FUR) and 95.7 – 99.4% (ETH) of the applied radioactivity [AR]. The high material balances shown for all sampling intervals demonstrate that no significant RA dissipated from the flasks or was lost during processing.

Table B.8.1.1.2-08 Synopsis mass balance and recovery of radioactivity

Label	PYR	FUR	ETH
Total Recovery (%)	93.4 – 98.1	94.1 – 99.7	95.7 – 99.4
Extracted RA (%)	41.5 – 95.4	40.2 – 96.7	52.0 - 95.2
Max. Volatile RA (CO_2) (%)	26.7	15.9	6.9
Bound Residues (%)	2.7 – 17.8	3.1 - 30.1	2.8 - 15.2
Extraction Efficiency on DAT-0 (%)	95.4	96.7	95.2

Extractable and Bound Residues (NER)

Extractable ^{14}C-residues (plus the portions in the water layer after flooding) decreased from 95.4, 96.7 and 95.2% of AR at DAT-0 to 50.5, 48.9 and 76.1% of AR at study end (DASF-123) for labels PYR, FUR and ETH, respectively.

Non-extractable ^{14}C-residues increased from 2.7, 3.1 and 2.8% of AR at DAT-0 to max. 17.8% (PYR), 30.1% (FUR) and 15.2% (ETH) of AR at DASF-90, respectively, a slight decrease was observed at the final sampling point.

Volatilization

^{14}C-carbon dioxide accounted for up to 26.7, 15.9 and 6.9% of AR at maximum for labels PYR, FUR and ETH, respectively. Mineralization occurred during the 30 days of aerobic incubation, during the anaerobic phase after 30 days the percentages of BYI 02960 in the entire systems decreased from 94.5, 95.1 and 93.5% of AR to 53.7, 52.6 and 54.7% of AR for labels PYR, FUR and ETH, respectively. During the anaerobic incubation period (i.e. flooded state) the portions of BYI 02960 decreased further to 47.8, 47.2 and 47.7% of AR for labels PYR, FUR and ETH, respectively.

Transformation of Test Item

In the course of the study several transformation products were detected and quantified (see Table B.8.1.1.2-05 to Table B.8.1.1.2-07). Up to five minor transformation products were detected in the test systems of label PYR, and up to four minor transformation products were detected in the test systems of label FUR. In the test systems of label ETH, two transformation products were detected. One of these was identified as difluoroacetic acid (DFA) by HPLC-MS with accurate mass detection. During the aerobic incubation phase, the amounts of DFA increased up to 25.1% of AR at DAT-30. During the anaerobic phase, the amounts of DFA remained more or less constant (24.2 - 26.2% of AR). The second, minor, transformation product (ROI Z) appeared from DAT-30 onwards in amounts ranging from 0.7 - 1.3% of AR.

The results did not change the proposed overall pathway of degradation of BYI 02960 in soil shown in Figure B.8.1.3-01.

Kinetics of Test Item Degradation

A summary of the DT_{50} and DT_{90} calculation for the test item is given in Table B.8.1.1.2-09 (including the data for alternate kinetic model evaluations). The single first order model (SFO) was chosen as the best fit kinetic model for all labels (indicated bold typed in Table B.8.1.1.2-09).

Overall, the amount of BYI 02960 was declined very slowly during the test period of 123 days. The estimated DT_{50} values range from 581.8 to 693.2 days (geometric mean: 633.7 days).

Table B.8.1.1.2-09 Summary of the kinetic evaluation (according to FOCUS) of the degradation of [14C] BYI 02960 in Anaerobic Soil at 20 °C

		Parent BYI 02960		
Soil	**Kinetic model**	**DT_{50}**	**DT_{90}**	**Chi^2 value**
Label PYR	**SFO ***	**581.8**	**> 1000**	1.4
	FOMC	> 1000	> 1000	0.9
	DFOP	> 1000	> 1000	0.9
Label FUR	**SFO ***	**693.2**	**> 1000**	1.3
	FOMC	> 1000	> 1000	1.0
	DFOP	> 1000	> 1000	1.0
Label ETH	**SFO ***	**631.0**	**> 1000**	0.9
	FOMC	> 1000	> 1000	0.7
	DFOP	653.2	> 1000	0.9

*: best fit (values bold typed)
SFO: Single first-order

FOMC: First-order multi compartment
DFOP: Double first-order in parallel

Conclusions

Major Outcomes of Study

The data gathered in the laboratory investigation demonstrate that residues of BYI 02960 are degraded very slowly in silt loam HF under anaerobic conditions at 20°C without the formation of further metabolites. For parent compound a SFO geometric mean DT_{50} value of 633.7 days was calculated. Observed portions of $^{14}CO_2$, NER and one major metabolite (DFA) were formed during the 30 days of aerobic incubation phase. A synopsis of results is shown in Table B.8.1.1.2-10.

Significance of Results to Environmental Behavior of BYI 02960

Based on the results obtained within this study it can be expected that the amounts of BYI 02960 and its only major metabolite DFA remain stable under flooded field conditions.

Degradation would be expected to continue according to the proposed overall pathway of degradation of BYI 02960 (see Figure B.8.1.3-01) whenever the conditions in soil turn aerobic again.

Table B.8.1.1.2-10 Synopsis of results of biotransformation of [^{14}C] BYI 02960 in 30 days aerobic, then 123 days anaerobic silt loam HF at 20°C

	Radio-label position		
	PYR	**FUR**	**ETH**
Total ^{14}C-Recovery (%) * Extracted RA (%) * Max. Volatile RA (%) Bound Residues (%) * Extraction Efficiency DAT-0 (%)	93.4 – 98.1 41.5 – 95.4 26.7 2.7 – 17.8 95.4	94.1 – 99.7 40.2 – 96.7 15.9 3.1 - 30.1 96.7	95.7 – 99.4 52.0 - 95.2 6.9 2.8 - 15.2 95.2
Anaerobic SFO DT_{50} of BYI 02960 [days]	582	693	631
Major transformation products ** predominantly formed during aerobic phase	CO_2 (max. 26.7%) NER (max. 17.8%)	CO_2 (max. 15.9%) NER (max. 30.1%)	CO_2 (max. 6.9%) NER (max. 15.2%) DFA (max. 26.2%)
Minor transformation predominantly formed during aerobic phase	Up to 5 individual (each ≤ 1.7% of AR)	Up to 4 individual (each ≤ 1.3% of AR)	1 (≤ 1.3% of AR)

*: Minimum and maximum values (as % of AR, mean values)
**: Criteria for term “major”: >10% of AR at any DAT or >5% of AR at two successive DATs or steadily increasing until the end of the study.

Remarks

The anaerobic soil degradation study is acceptable and the results are reliable.

STUDY IIA, 7.1.2/02

Characteristics

reference	:	Mislankar, S. Woodard, D., 2012	study type	:	anaerobic degradation
year of execution	:	2011	incubation time	:	up to 121 d
GLP statement	:	yes	nominal concentration	:	1.17 mg/kg
guideline	:	OECD 307 (2002) US EPA, OPPTS 835.4100 (2008)	temperature	:	20°C
test substance	:	Pyridine-2,6-^{14}C] BYI 02960	DT50	:	see results
purity	:	radiochemical purity 99 %.	metabolites	:	see results
soil	:	Loamy sand, Sanger California, USA	acceptability	:	**acceptable**

Summary

The anaerobic biotransformation of [pyridine-2,6-^{14}C] BYI 02960 was studied in a loamy sand (pH 6.7 in 0.01M CaCl2, organic carbon 0.45%) from Sanger, California, USA. During the first phase of the study, the soil was maintained under aerobic conditions for 30 days in the dark at 20 ± 1 °C and at soil moisture of 55% maximum water holding capacity. Following the aerobic phase, the samples were flooded with water (water:soil ratio 3:1, w/w) and maintained in the dark under anaerobic conditions for 121 days at 20 ± 1 °C. [PYR-^{14}C] BYI 02960 was applied at a rate of 1.17 µg a.i./g, dry soil, equivalent to an application rate of 410 g a.i./ha.

Samples were analyzed at 0, 14 and 32 days of aerobic incubation, and at 0, 14, 30, 45, 59, 91 and 121 days of incubation following flooding (post treatment) of the samples (anaerobic phase). The water was decanted from each test system and the soil was extracted by a shaking method. In addition, aggressive extraction was conducted. The water layer, ambient extract and the microwave extracts were analyzed by HPLC. Identification of the parent compound and major metabolite was achieved by mass spectrometry (LC/ESI/MS) and co-chromatography using an authentic standard.

The average total material balance in the soil/water system for BYI 02960 was 96.5% ± 1.8% of AR. Non-extractable (bound) residues in soil increased from 0.6% at day 0 to 15.7% at day 32. At the end of the aerobic phase, 6.1% of the applied radioactivity was present as CO_2. No volatile organic compounds were present. The concentration of BYI 02960 in the aerobic phase decreased to 61.4% of AR at day 32.

In the anaerobic phase, radioactivity in the combined water and ambient extract decreased from 60.2% at day 0 to 35.8% by the end of the study. Aggressive extractions with both acetonitrile:water and methanol:water ranged from 3.1% to 2.9% of the applied radioactivity in the study, indicating that residue left after ambient and aggressive extraction was not easily extractable. Since non-extractable residues did not change during aerobic (15.7%) and anaerobic phases (16.1%), no further characterization was conducted. No CO_2 or volatile organic compounds were produced during the anaerobic phase of the study.

During the anaerobic phase, the concentration of BYI 02960 in soil decreased from 51.1% at day 0 to 26.2% of the applied amount at study termination. One major metabolite, 6-chloronicotinic acid (6-CNA), was detected during the aerobic phase of the study and it reached maximum of 12% (water/sediment) at day 0 of anaerobic phase and remained steady (12 to 14 %) throughout anaerobic phase. One minor metabolite, BYI 02960-chloro was detected during the aerobic phase and accounted for 2.8% at day 32. During the anaerobic phase, it remained steady (2%).

The observed DT_{50} values for BYI 02960 in the aerobic, then anaerobic soil/water system were determined using single first-order kinetics (SFO), first-order multi compartmental (DFOP) and double

first-order in parallel (FOMC) and half-lives were 152 days, 164 days and 584 days, respectively for the anaerobic phase. BYI 02960 degrades moderately under aerobic conditions and remains more or less stable during anaerobic phase in soil.

Materials and methods

Materials

1. Test Item: [Pyridine-2,6-^{14}C] BYI 02960 (Flupyradifurone) (sample IDC-1135A); Specific activity: 35.03 mCi/mMole; Radiochemical purity: 99; Identity and purity of test item in the application solution was confirmed.

2. Soil The biotransformation of BYI 2960 was studied in one soil. The soil was taken from Sanger, California, USA and transported by air to Stilwell, KS at ambient temperature. It was stored for 13 days at 4 °C prior to use. The soil was sieved through a 2 mm sieve. Soil moisture was adjusted to 50% WHCmax and pre-incubated at about 20 ± 2°C until application, i.e. for approx. 20 days.

Table B.8.1.1.2-11 Soil physicochemical properties

Parameter	Results/Units
Geographic Location (City / State / Country)	Sanger/California/USA
GPS coordinates of sampling site	N 36°42'22.16" W 119°28'00.12"
Taxonomic Name	Hanford fine sandy Loam, gravely substrate
USDA Texture Class	Loamy Sand
Sand / Silt / Clay (%)	80 / 15 / 5
pH (soil/water, 1/1) pH (Saturated Paste) pH (soil/0.01 M $CaCl_2$ 1:2)	7.3 7.2 6.7
Organic Matter	0.77%
Organic Carbon	0.45%
Initial soil biomass (Day 0 aerobic)	128 mg microbial C/kg soil
Soil biomass on flooding day (Day 0 anaerobic)	128 mg microbial C/kg soil (untreated control soil)
Biomass at the end of the study (anaerobic)	3.83 x 10^6 cells/mL (untreated control water)
	6.83 x 10^7 cells/mL (untreated control soil)
	4.00 x 10^6 cells/mL (untreated control water)
	7.51 x 10^7 cells/mL (untreated control water)
Cation Exchange Capacity (CEC)	5.7 meq/100 g
Maximum Water Holding Capacity	30.5
Bulk Density (disturbed)	1.27 g/cm^3

Methods

1. Experimental conditions: The study was performed in flow-through incubation test systems under aerobic, and later under static anaerobic conditions in the dark at 20 ± 0.1 °C. During the aerobic phase, the test system consisted of Erlenmeyer flasks (250 mL) with a trap attached for absorption of ^{14}C-volatiles and $^{14}CO_2$, consisting of ethylene-glycol, potassium hydroxide and sulfuric acid,

respectively. During the anaerobic phase, test systems were flooded with nitrogen to purge oxygen from the systems. Aliquots of 50 g of dry soil were weighed into the test flasks. Replicates were set up and processed for each sampling. The final soil moisture was adjusted to 55% of WHC_{max} by adding pure water. The switch to anaerobic (flooded) conditions was made 30 days after test item application: The trap system of all remaining test flasks was removed and stored for later analysis.

2. Test Item Application Solution: [Pyridine 2,6-^{14}C] BYI 02960 was mixed into an aliquot of 1.3 mL acetonitrile dissolved 8.5 mL methanol:water (1:1).

3. Mode of Application: The application solution was applied at 200 µL evenly across the soil surface with a 250 µL syringe. The flasks were gently rotated to mix the treated soil, connected to volatile traps and kept at 20 C in the environmental chamber. [PYR-^{14}C] BYI 02960 was applied at a rate of 15,922,150 dpm per vessel. This corresponds to 58.4 µg per vessel.

4. Sampling: Duplicate test systems were sampled at day 0, 14 and 32 under aerobic conditions and after the 30 day aerobic incubation period, duplicate anaerobic test systems were analyzed at 0, 14, 45, 59, 91 and 121 days. During the anaerobic phase, pH, redox potential and dissolved oxygen were measured.

During the aerobic phase on the day of sampling, test systems were removed from the incubator along with the attached traps for the volatile and CO_2 analysis. During the anaerobic phase, volatile traps were attached and nitrogen was purged through the head space to trap any volatiles.

Samples were processed and soil was extracted on the day of sampling. The water was decanted from each test system and the soil was extracted by a shaking method. Water and extracts were analyzed with 10 days after sampling.

5. Description of analytical procedures: Extraction was done by cycles of heavily shaking for about 30 minutes on a mechanical shaker at room temperature followed by centrifugation (ca. 5 min, 2100g) and filtering through a paper filter. The soil samples were extracted with 3 x 150 mL acetonitrile/water (70/30, v/v) and 1 x 150 mL acetonitrile by shaking at ambient temperature. A further extraction step followed with acetonitrile/water (70/30, v/v) at 70°C using a microwave for 10 minutes. An aliquot of the combined 'ambient' and 'aggressive' extracts was transferred to a flask and rotovapped at 30°C.

The extracted soil was air dried and subsamples were combusted to quantify the non-extractable residues (NER). The water layer, ambient extract and the microwave extracts were analyzed by LSC and HPLC using a flow-through ^{14}C detector. Identification of the parent compound and major metabolite was achieved by mass spectrometry (LC/ESI/MS) and co-chromatography using an authentic standard.

Results and discussion

Results indicated that anticipated standardized conditions were maintained, and the soils were microbial active over the duration of the laboratory study.

Table B.8.1.1.2-12 Dissolved oxygen, pH and redox potential of the test systems of the [PYR 2,6-^{14}C] BYI 02960 anaerobic soil metabolism study (mean of duplicates)

Measurement Interval [Days of post flooding]	Dissolved Oxygen (mg/L)	pH	Redox (E_o) in Water (mV)	Redox (E_h) in Water (mV)	Redox (E_o) in Soil (mV)	Redox (E_h) in Soil (mV)
0	3.2	7.9	NA	NA	155.6	352.6
14	0.5	7.8	81.6	278.6	87.0	284.0
30	0.4	6.5	-19.0	178.1	-28.9	168.1
45	0.9	7.4	-28.8	168.2	-31.6	165.4
59	0.4	7.1	-30.2	166.8	-60.3	136.8
91	0.1	7.1	-54.2	142.9	-118.8	78.3
121	0.4	7.1	-70.9	126.2	-128.1	69.0

NA = not analyzed, $E_h = E_{Obs} + E_{Ref}$
Where: E_h = Redox potential referred to the hydrogen scale
E_{Obs} = Observed redox potential of electrode (Ag/AgCl)
E_{Ref} = Redox potential of the electrode as related to the hydrogen electrode (Ag/AgCl = +197 mV)

Data

Data on biotransformation is shown in Table B.8.1.1.2-13. The DAT 0 extraction efficiency was 99.4% of AR. The aggressive extraction removed approximately 2% to 3% radioactive residues during the study. These results indicate that the extraction procedure was efficient in extracting the majority of radioactive residues from the soil. At the end, bound residue was 16.1% of applied.

Table B.8.1.1.2-13 Biotransformation of [PYR-^{14}C] BYI 02960 in silt loam under aerobic, then anaerobic conditions; mean values and SD expressed as % of AR)

Compound	Matrix	Aerobic Phase Interval (Days Post Treatment)			Anaerobic Phase Interval (Days Post-Flooding)						
		0	14	32	0	14	30	45	59	91	121
BYI 02960	Water	-	-	-	11.7 ±0.4	26.9 ±1.8	27.0 ±3.2	25.0 ±0.1	22.5 ±1.0	23.1 ±2.9	22.7 ±4.2
	Soil	98.5 ±1.5	75.3 ±0.5	61.4 ±0.4	51.1 ±0.8	32.5 ±3.0	30.9 ±1.1	32.0 ±0.7	32.0 ±0.4	29.6 ±1.3	26.2 ±5.3
	Subtotal	98.5 ±1.5	75.3 ±0.5	61.4 ±0.4	62.7 ±1.2	59.4 ±4.8	57.9 ±4.2	57.0 ±0.5	54.5 ±1.4	52.8 ±1.6	48.9 ±1.2
6-CNA	Water	-	-	-	4.8 ±0.1	9.8 ±1.8	8.7 ±3.9	10.5 ±0.0	9.6 ±1.0	9.5 ±1.1	9.4 ±0.7
	Soil	0.0 ±0.0	7.4 ±0.0	11.1 ±0.4	7.2 ±0.3	3.4 ±0.4	2.9 ±0.1	3.8 ±0.1	3.4 ±0.1	3.2 ±0.5	3.0 ±0.6
	Subtotal	0.0 ±0.0	7.4 ±0.0	11.1 ±0.4	12.0 ±0.4	13.3 ±2.2	11.6 ±3.9	14.2 ±0.1	13.0 ±1.1	12.7 ±1.5	12.4 ±0.1
BYI 02960-chloro	Water	-	-	-	0.2 ±0.0	0.8 ±0.2	0.7 ±0.3	0.9 ±0.1	0.9 ±0.0	0.7 ±0.1	0.7 ±0.3
	Soil	0.0 ±0.0	1.3 ±0.2	2.8 ±0.3	2.1 ±0.5	1.7 ±0.1	1.4 ±0.2	1.7 ±0.1	1.8 ±0.2	1.4 ±0.1	1.5 ±0.2
	Subtotal	0.0 ±0.0	1.3 ±0.2	2.8 ±0.3	2.4 ±0.5	2.5 ±0.3	2.1 ±0.5	2.6 ±0.0	2.7 ±0.1	2.1 ±0.2	2.2 ±0.1
A (Diffuse radioactivity)	Subtotal	0.0 ±0.0	0.5 ±0.7	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.9 ±1.3	2.5 ±0.7
B (Diffuse radioactivity)	Subtotal	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	1.7 ±0.1	3.1 ±0.1
C (Diffuse radioactivity)	Subtotal	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.7 ±0.9	3.1 ±0.4

Unknown radioactivity	Subtotal	0.5 ±0.7	0.6 ±0.9	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
Unidentified radioactivity	Subtotal	0.5 ±0.7	2.4 ±1.3	2.8 ±0.3	2.4 ±0.5	2.5 ±0.3	2.1 ±0.5	2.6 ±0.0	2.7 ±0.1	5.4 ±0.7	8.7 ±0.2
Total extractable Radioactivity	Water	-	-	-	16.7 ±0.4	37.5 ±0.3	39.1 ±2.9	36.4 ±0.0	33.0 ±0.0	35.2 ±0.9	38.0 ±6.2
	Soil	99.4 ±1.5	84.6 ±0.1	75.3 ±0.3	60.2 ±0.6	37.6 ±2.6	35.2 ±0.7	37.5 ±0.7	37.2 ±0.1	38.1 ±0.2	35.8 ±5.1
	Subtotal	99.4 ±1.5	84.6 ±0.1	75.3 ±0.3	76.9 ±1.0	75.2 ±2.3	74.3 ±3.6	73.9 ±0.7	70.2 ±0.1	73.3 ±1.0	73.8 ±1.1
CO_2		-	1.7 ±0.0	6.1 ±0.0	5.7 ±0.4	5.7 ±0.7	4.3 ±0.4	5.2 ±0.3	4.8 ±0.0	6.0 ±1.8	6.0 ±0.6
Volatile organics		-	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
Total volatile		-	1.7 ±0.0	6.1 ±0.0	5.7 ±0.4	5.7 ±0.7	4.3 ±0.4	5.2 ±0.3	4.9 ±0.0	6.0 ±1.7	6.0 ±0.6
Bound Residues		0.6 ±0.0	9.3 ±0.3	15.7 ±0.9	15.9 ±0.5	15.1 ±2.3	16.5 ±0.5	18.1 ±3.1	18.7 ±0.1	16.9 ±3.7	16.1 ±0.0
Total % Recovery		100.0 ±1.5	95.5 ±0.2	97.1 ±0.5	98.5 ±1.1	95.9 ±0.7	95.2 ±3.5	97.2 ±3.6	93.8 ±0.0	96.3 ±3.0	95.9 ±0.5

Mass Balance

The average material balance was 96.5% ± 1.8% (mean range = 93.8% to 100%)

Extractable and Bound Residues (NER)

In the aerobic phase, extractable ^{14}C-residues in soil decreased from 99.4% at day 0 to 75.3% at day 32. Bound residues increased from 0.6% at day 0 to 15.7% at day 32.

In the anaerobic phase extractable ^{14}C-residues in soil decreased from 60.2% at day 0 to 35.8% at day 121. In the water layer, ^{14}C-residues increased from 16.7% at day 0 to 38% at the end of the study. Bound residues reached a maximum of 18.7% at day 59 and declined slightly to 16.1% at day 121 post-flooding.

Volatilization

At the end of the aerobic and anaerobic phase 6.1 and 6.0% of AR were present as CO_2. No organic volatiles were detected.

Transformation of Test Item

Within the aerobic phase of the study (30 days) the percentages of BYI 02960 in soil decreased from 98.5% (day 0) to 61.4% of AR (day 32). The major metabolite 6-chloronicotinic acid (6-CNA) increased from 0.9 to 11.1% of AR. During the anaerobic phase, the concentration of BYI 02960 in water and soil decreased from 62.7% (day 0) to 48.9% of AR at the end of the study. The proportion of metabolite 6-CNA was more or less constant during the anaerobic phase (12.4% at day 121).

The minor metabolite BYI 02960-chloro was observed at day 14 of the aerobic phase, reaching a maximum of 2.8% on day 32. During the anaerobic phase, it remained between 2% and 3%.

Further, there was a diffuse area of radioactivity, consisting of multiple peaks, which reached a maximum of 8.7%. None of the peaks accounted for more than 3.1% of applied. The proposed degradation pathway is depicted in Figure B.8.1.3-01.

Kinetics of Test Item Degradation

A summary of the SFO DT_{50} and DT_{90} calculation for the test item is given in Table B.8.1.1.2-14. The degradation may be due to residual aerobic conditions following flooding and should not be considered anaerobic soil degradation, anaerobic conditions were achieved after 14 days post flooding. The calculated half-life values should be attributed to the initial aerobic conditions with the test systems and not anaerobic soil half-life.

Conclusions

Major Outcomes of Study

BYI 02960 degraded under aerobic/anaerobic conditions. Most of the degradation occurred during the aerobic phase and once anaerobic conditions were established, very little degradation occurred. During the aerobic phase, 6-CNA was the major metabolite formed and it remained more or less constant during the anaerobic phase (between 11 and 14% of AR), assuming that it needed further days post flooding until strictly anaerobic conditions were achieved. A minor degradate, BYI 02960-chloro, accounted for 2.8% at day 32 of the aerobic phase and remained more or less constant during the anaerobic phase, too. A synopsis of results is shown in Table B.8.1.1.2-14.

Significance of Results to Environmental Behavior of BYI 02960

BYI 02960 degrades under aerobic condition; however, once anaerobic conditions are reached its residues remain more or less stable in soil.

Table B.8.1.1.2-14 Summary of the kinetic evaluation (according to FOCUS) of the degradation of [^{14}C] BYI 02960 in aerobic, then anaerobic soil at 20°C

	Parent BYI 02960 (total system)					
Kinetic model (entire test system)	**Estimated initial % applied radioact. M(0)**	**Rate constant [d^{-1}]**	**DT_{50} [d]**	**DT_{90} [d]**	**Chi^2 value [%]**	**r^2**
SFO	41.6	0.0045	152	506	11.9	0.52

Table B.8.1.1.2-15 Synopsis of results of biotransformation of [^{14}C] BYI 02960 in 30 days aerobic, then 121 days anaerobic loamy sand Sanger at 20°C

Material Balance	**(% of AR)**
Mean ^{14}C-recovery during entire study Total extractable radioactivity during aerobic phase Total extractable radioactivity during anaerobic phase Max. volatile RA Range of bound residues (NER) Extraction Efficiency DAT-0	96.5 ± 1.8 75.3 – 99.4 35.8 – 60.2 6.1 15.7 – 18.7 99.4
Aerobic/anaerobic DT_{50} of BYI 02960 [days]	152 (SFO)
Transformation products *)	
Major transformation products observed in entire study but predominantly formed during aerobic phase	CO_2 (max. 6.1%) NER (max. 18.7%)

	6-CNA (max. 14.2%)
Minor transformation products observed in entire study but predominantly formed during aerobic phase	BYI 02960-chloro (2.8% of AR)

*) Criteria for term "major": >10% of AR at any DAT or >5% of AR at two successive DATs or steadily increasing until the end of the study

Remarks

The anaerobic soil degradation study is acceptable and the results are reliable.

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Characteristics

reference	:	Woodard, D., 2012	study type	:	anaerobic degradation
year of execution	:	2011	incubation time	:	up to 60 d
GLP statement	:	yes	nominal concentration	:	1.1 mg/kg
guideline	:	OECD 307 (2002) US EPA, OPPTS 835.4100 (2008)	temperature	:	20°C
test substance	:	Pyridine-2,6-^{14}C] BYI 02960	DT50	:	see results
purity	:	radiochemical purity 100 %.	metabolites	:	see results
soil	:	sand clay loam, Springfield, Nebraska, USA	acceptability	:	**acceptable**

Summary

The anaerobic biotransformation of [pyridine-2,6-^{14}C] BYI 02960 was studied in sandy clay loam (pH 6.5 in 0.01 M $CaCl_2$, organic carbon 1.9%) from Springfield, NE, USA. During the first phase of the study, the soil was maintained under aerobic conditions for 29 days in the dark at 20 ± 2°C and at soil moisture of 55% maximum water holding capacity. Following the aerobic phase, the samples were flooded with water (water:soil ratio 3:1, w/w) and maintained in the dark under anaerobic conditions for 60 days at 20 ± 2 °C. The study was terminated after the 60 day sampling due to a failure of: the temperature control which resulted in a temperature of 50°C and compromised the remaining samples. [PYR-^{14}C] BYI 02960 was applied at a rate of 1.1 µg a.i./g, dry soil, equivalent to an application rate of 410 g a.i./ha.

Samples were analyzed at 0, 14 and 29 days of aerobic incubation, and at 0, 19, 31, 45 and 60 days of incubation following flooding (post treatment) of the samples (anaerobic phase). The water was decanted from each test system and the soil was extracted using a shaking method. In addition, an aggressive extraction was conducted. The water layer, ambient extract and the microwave extracts were analyzed by HPLC using a flow-through 14C detector. Identification of the parent compound was achieved by mass spectrometry (LC/ESI/MS) and co-chromatography using an authentic standard.

The material balance for the study was complete (on average 105.8% ± 3.2%, mean range = 100.0 to 109.4%). Extractable [^{14}C] residues in soil decreased from 99.7% at day 0 to 79.0% by day 29. Non-extractable (bound) residues in soil increased from 0.3% at day 0 to 9.8% at day 29. At the end of the aerobic phase, 16.2% of the applied radioactivity was present as CO_2. No volatile organic compounds were present. In the aerobic phase the concentration of BYI 02960 decreased from 99.7% of the applied amount at day 0 to 79.0% at day 29. In the anaerobic phase, radioactivity in the combined ambient and aggressive extracts remained steady from 69.5% at day 0 to 71.7% by the end of the

study. Aggressive extractions ranged from 6.7% to 11.7% of the applied radioactivity in the study, indicating that residue left after ambient and aggressive extraction was not easily extractable. NER and CO_2 in soil remained more or less constant during the anaerobic phase of the study, and no volatile organic compounds were produced.

BYI 02960 degrades moderately under aerobic conditions and its residues remain more or less stable during anaerobic phase in soil.

The study was terminated after the 60 day sampling due to a failure of the temperature control which resulted in a temperature of 50°C and compromised the remaining samples, this does not affect the interpretation of results of the study.

Materials and methods

Materials

1. Test Item: [Pyridine-2,6-^{14}C] BYI 02960 (Flupyradifurone) (sample IDC-1135A);Specific activity: 35.03 mCi/mMole; Radiochemical purity: 100%; Identity and purity of test item in the application solution was confirmed.

2. Soil: The test matrix used in this study was sandy clay loam from Springfield, Nebraska, USA. This site had no prior history of pesticide application for 5 years. The soil was transported from Nebraska to the testing facility, KS, by air cargo at ambient temperature, and upon arrival at Bayer CropScience, Stilwell, KS, was stored in a refrigerator at 4 ºC. Prior to the use, the soil was maintained at an average temperature of 4°C at the testing facility for 6 days. Soil was sieved through a 2-mm sieve. Soil was acclimated for a period of 6 days before treatment. Moisture was not adjusted due to no significant moisture loss from the time the test systems were set up until the time of flooding.

Table B.8.1.1.2-16 Soil physicochemical properties

Parameter	Results/Units
Geographic Location (City / State / Country)	Springfield / Nebraska, USA
GPS coordinates of sampling site	96.15085 41.03725
Taxonomic Name	Fine, kaolinitic, thermic Typic Kanhapludults
USDA Texture Class	Sandy clay loam
Sand / Silt / Clay (%)	53.1 / 23.2 /23.7
pH (soil/water, 1/1) pH (Saturated Paste) pH (soil/0.01 M $CaCl_2$ 1:2)	6.7 6.6 6.5
Organic Matter	3.3%
Organic Carbon	1.9%
Initial soil biomass (Day 0 aerobic)	263 mg microbial C/kg soil
Soil biomass on flooding day (Day 0 anaerobic)	433 mg microbial C/kg soil (untreated control soil)

Biomass at the end of the study (anaerobic)	Since the study was terminated due to the increased temperature from a malfunctioning incubator (>50 °C), biomass determination was not assessed as the biological component may have been compromised from the higher temperature
Cation Exchange Capacity (CEC)	17.6 meq/100 g
Maximum Water Holding Capacity	61.4%
Bulk Density (disturbed)	1.03 g/cm^3

Methods

1. Experimental Conditions: The study was performed under static aerobic incubation conditions, and later under flow-through anaerobic incubation conditions in the dark at 20 ± 0.1 °C. The test system consisted of 250-mL Pyrex® Erlenmeyer flasks (containing 50 g soil (dry weight)) with side arms for attachment to traps for the collection of CO_2 and volatile organic compounds. During the aerobic phase, test systems were kept in an environmental chamber at 20 ± 2 °C. The final soil moisture was adjusted to 55% of WHC_{max}. During the anaerobic (flooded) phase, they were kept in a temperature-controlled incubator with a nitrogen-filled atmosphere at 20 ± 2 °C. During incubation, aluminum foil was wrapped around flasks to prevent exposure to light.

The switch to anaerobic (flooded) conditions was made 29 days after test item application. The trap system of all remaining test flasks was removed and stored for later analysis.

2. Test Item Application Solution: [Pyridine 2,6-^{14}C] BYI 02960 (vial no. C-1135A) was mixed into an aliquot of 1.3 mL acetonitrile dissolved 8.5 mL methanol:water (1:1). The solution was mixed using a vortex and sonicator to get a homogeneous solution.

3. Mode and Rate of Application: The application solution (200 µL) was applied evenly across the surface of the soil using a 250-µL syringe. After treatment, 9 mL of water was added to the flasks to bring soil moisture to ~ 55% of max water holding capacity (MWHC). The flasks were gently rotated to mix the treated soil. The flasks were labeled, wrapped in aluminum foil and weighed. Flasks were connected to volatile traps and kept at 20 ± 2 °C in the environmental chamber.
[PYR-^{14}C] BYI 02960 was applied at a rate of 15,122,786 dpm per vessel. This corresponds to 56.1 µg per vessel. Material balances for the kinetic treatment test systems of the study were based on the theoretical dpm applied to the soil.

4. Sampling: Duplicate test systems at day 0, 14 and day 29 were analyzed under aerobic conditions. After the 29-day aerobic incubation, duplicate anaerobic test systems were analyzed at 0, 19, 31, 45 and 60-days post-flooding intervals. During the anaerobic phase of the study, test systems were measured for pH, redox potential, and dissolved oxygen. Radioactive CO_2 and volatile organics were measured at each interval. The water was separated from the soil by decanting.

Samples were processed and soil was extracted on the day of sampling. Water and extracts were analyzed within 7 days of sampling. The extracts and water were stored in a laboratory refrigerator.

The concentrated extracts were stored in the laboratory freezer until analysis, and were moved to a central freezer for long-term storage.

During the aerobic phase on the day of sampling, test systems were removed from the incubator along with the attached traps for the volatile and CO_2 analysis. During the anaerobic phase test systems were removed from the incubator, volatile traps were attached and nitrogen was purged through the head space to trap any volatiles from the head space into the bubblers.
5. Description of Analytical Procedures: The volatile organics were collected from the headspace of the treated test systems using 2 M KOH, ethylene glycol, and 1 M H_2SO_4. The trapping solutions were radioassayed in triplicate 1-mL aliquots for KOH, ethylene glycol and H_2SO_4. The aqueous portion of the sample was filtered through a Whatman GF/F glass filter into a 250-mL graduated cylinder. The water was radioassayed in triplicate 1-mL aliquots by LSC.

The soil was transferred to a 250-mL bottle, and 40 mL of acetonitrile/water (70/30) was added to the bottle which was then extracted on a bench-top shaker for 30 minutes. The samples were centrifuged for 5 minutes at 1850 g. The extracts were filtered through a Whatman GF/F filter into a 250-mL graduated cylinder. The extraction procedure was repeated two additional times with acetonitrile/water (70/30) and another time with 100% acetonitrile. The combined aqueous portion (ambient extract) was radioassayed in triplicate 1-mL aliquots. A further extraction step followed once with 50 mL of acetonitrile/water (70/30) and once with 50 mL of methanol/water (50/50) at 70°C using a microwave for 10 minutes (650 Watts). After the acetonitrile/water (70/30) microwave extraction the sample was centrifuged for 5 minutes at 1850 g and decanted through a Whatman GF/F filter into a 250-mL graduated cylinder. Following the methanol/water (50/50) microwave extraction the entire sample were filtered through a Whatman GF/F filter into the same 250-mL graduated cylinder and aliquots (3 x 1-mL) were radioassayed. The remaining soil was allowed to air dry on the filter.

The extracted soil samples were air dried, homogenized thoroughly and weighed. Subsamples (approximately 0.5 g) of the soil were combusted to quantify the non-extractable residue (NER).

The water layer, ambient extract and the microwave extracts were analyzed by LSC and HPLC using a flow-through ^{14}C detector. Identification of the parent compound and major metabolite was achieved by mass spectrometry (LC/ESI/MS) and co-chromatography using an authentic standard.

Results and discussion

Results indicated that anticipated standardized conditions were maintained, and the soils were microbial active over the duration of the laboratory study.

Table B.8.1.1.2-17 Dissolved oxygen, pH and redox potential of the test systems of the [PYR 2,6-^{14}C] BYI 02960 anaerobic soil metabolism study (mean of duplicates)

Measurement Interval [Days of post flooding]	Dissolved Oxygen (mg/L)	pH	Redox (E_o) in Water (mV)	Redox (E_o) in Soil (mV)
0	4.52	6.13	250.7	264.8
19	0.45	6.64	150.2	182.1
33	0.30	6.69	-10.6	20.9
45	0.26	7.04	-64.6	-46.1
60	0.03	6.98	-152.1	-128.9

NA = not analyzed, $E_h = E_{Obs} + E_{Ref}$
Where: E_h = Redox potential referred to the hydrogen scale
E_{Obs} = Observed redox potential of electrode (Ag/AgCl)
E_{Ref} = Redox potential of the electrode as related to the hydrogen electrode (Ag/AgCl = +197 mV)

Data

Data on biotransformation is shown in Table 8.1.1.2-18. The DAT 0 extraction efficiency was 99.7% of applied radioactivity. The shake procedure extracted an average of 99.7% of the applied radioactivity at aerobic day 0. This indicated that the extraction procedure was efficient in extracting the majority of the radioactive residues from the soil, and did not degrade the parent. The bound residue at the end of the study was 12.9% of the applied radioactivity.

Table B.8.1.1.2-18 Biotransformation of [pyridine 2,6-^{14}C] BYI 02960 in sandy clay loam under aerobic, then anaerobic conditions; means values and SD expressed as % of AR

Compound	Matrix	Aerobic Phase Interval (Days Post Treatment)				Anaerobic Phase Interval (Days Post-Flooding)			
		0	14	29	0	19	31	45	60
BYI 02960	Water	-	-	-	7.3 ±2.1	10.7 ±0.1	9.5 ±0.3	7.2 ±0.4	5.7 ±0.2
	Soil	99.7 ±2.5	83.7 ±2.9	74.4 ±0.9	64.5 ±3.7	58.4 ±3.6	66.5 ±2.2	67.1 ±0.4	66.7 ±1.4
	Subtotal	99.7 ±2.5	83.7 ±2.9	74.4 ±0.9	71.9 ±1.6	69.1 ±3.7	76.0 ±2.5	74.3 ±0.1	72.5 ±1.6
Unidentified radioactivity	Water	-	-	-	0.4 ±0.5	0.6 ±01	0.4 ±0.0	0.9 ±0.4	0.6 ±0.1
	Soil	0.0 ±0.0	5.4 ±1.0	4.6 ±0.6	5.0 ±0.1	5.1 ±1.3	5.0 ±0.1	5.6 ±0.2	5.0 ±0.6
	Subtotal	0.0 ±0.0	5.4 ±1.0	4.6 ±0.6	5.3 ±0.4	5.7 ±1.4	5.4 ±0.1	6.4 ±0.5	5.6 ±0.6
Total extractable Radioactivity	Water	-	-	-	7.7 ±2.6	11.3 ±0.2	9.9 ±0.3	8.1 ±0.1	6.3 ±0.3
	Soil	99.7 ±2.5	89.1 ±1.9	79.0 ±0.2	69.5 ±3.8	63.5 ±4.9	71.6 ±2.2	72.7 ±0.5	71.7 ±2.0
	Subtotal	99.7 ±2.5	89.1 ±1.9	79.0 ±0.2	77.2 ±1.2	74.8 ±5.1	81.5 ±2.5	80.8 ±0.5	78.1 ±2.2
CO_2		-	7.0 ±1.0	16.2 ±0.4	16.0 ±1.3	18.4 ±0.2	15.2 ±3.9	16.8 ±0.8	17.9 ±0.0
Bound Residues		0.3 ±0.0	7.7 ±0.1	9.8 ±0.9	10.6 ±1.5	14.8 ±1.8	11.1 ±0.4	11.9 ±0.2	12.9 ±0.0
Total % Recovery		100.0 ±2.6	103.8 ±3.0	105.0 ±0.2	103.8 ±1.7	108.0 ±3.1	107.8 ±1.8	109.5 ±0.5	108.9 ±2.2

Mass Balance

The average material balance for the study was 105.8 ± 3.2% (mean range = 100.0 to 109.5%).

Extractable and Bound Residues (NER)

In the aerobic phase, extractable [^{14}C]residues in soil decreased from 99.7% at day 0 to 79.0% by day 29. Non-extractable (bound) residues in the soil increased from 0.3% at day 0 to 9.8% at day 29.

In the anaerobic phase, extractable [^{14}C]residues in soil remained constant with residues from 69.5% at day 0 to 71.7% at day 60. In the water layer [^{14}C]residues also remained constant from 7.7% at day 0 to 6.3% at the end of the study. Non-extractable residues in soil reached a maximum of 14.8% at day 19 and declined to 12.9% at day 60, post flooding.

Volatilization

At the end of the aerobic and anaerobic phase, 16.2 and 17.9% of the applied radioactivity was present as CO_2, and no organic volatile compounds were detected.

Transformation of Test Item

During the aerobic phase, the concentration of [PYR-^{14}C] BYI 02960 in the soil decreased from 99.7% at day 0 to 74.4% of the applied amount at day 29. Other unidentified radioactivity remained constant from aerobic day 14 until the end of the study, ranging from 4.6 to 6.4% of the applied amount of radioactivity. During the anaerobic phase, the concentration of BYI 02960 remained constant, starting at 71.9% on day 0 and ending at 72.5% at the termination of the study. Non-extractable residues were less than 20% of AR, therefore, no further fractionation was conducted.

Kinetics of Test Item Degradation

Following the flooding of the Springfield, NE, sandy clay loam test system, BYI 02960 levels remained stable through the end of the study. Since degradation of BYI 02960 did not occur during the anaerobic phase of the study, kinetic endpoints could not be derived.

Conclusions

Major Outcomes of Study

[PYR-^{14}C] BYI 02960 steadily degraded during the aerobic phase, but once the test systems were flooded with water, no further degradation of [PYR-^{14}C] BYI 02960 occurred during the anaerobic phase. Although the study was terminated after the 60 day sampling the results are consistent with those obtained with the other soils and labels.

Significance of Results to Environmental Behavior of BYI 02960

BYI 02960 degrades under aerobic conditions. Once anaerobic conditions are reached, BYI 02960 is stable. Thus, degradation will not be a major route of dissipation in a flooded anaerobic soil environment.

Remarks

The anaerobic soil degradation study is acceptable and the results are reliable, altough the study was terminated after 60 days due to a failure of the temperature control. The study did not complete the guidance subscribed 120 days, however the same trend was observed as in the other anaerobic degradation studies submitted. Limited degradation of the test substance was observered.

B.8.1.1.3 Soil Photolysis

STUDY IIA, 7.1.3/01

Characteristics

reference	:	Menke, U., Unold, M., 2011	study type	:	soil photolysis
year of execution	:	2008-2009	incubation time	:	up to 8 days
GLP statement	:	yes	nominal concentration	:	40.8 µg / 3 g soil
guideline	:	SETAC (1995), EPA 161-3 draft OECD (TG 2002)	temperature	:	22°C
test substance	:	[pyridinylmethyl-^{14}C] BYI 02960 and [furanone-4-^{14}C] BYI 02960	light intensity	:	1082 W m^{-2} for label PYM and 1116 W m^{-2} for label FUR (Cut off < 290 nm)
purity	:	radiochemical purity > 99%	Conclusion	:	study results reliable
soil	:	loam, Guadalupe, California, USA	acceptability	:	**acceptable**

Summary

Phototransformation of [pyridinylmethyl-^{14}C]- and [furanone-4-^{14}C] BYI 02960 was studied on a loam soil from Guadalupe, California, USA at an application rate of about 400 g/ha. The study was conducted for a period of eight days at 20°C ± 1°C and at soil moisture of about 75% of 1/3-bar water holding capacity (humid test samples). In addition, photolysis was studied on air dried samples.

[PYM-^{14}C] and [FUR-^{14}C] BYI 02960 were directly applied to the surface of the soil aliquots at an initial concentration of about 40.8 µg/ 3 g soil. The treated samples were continuously exposed to artificial irradiation (xenon lamp with <290 nm cut-off filter, 1082 W m^{-2} for label PYM and 1116 W m^{-2} for label FUR). In addition, dark controls were set up. Test vessels were connected to traps for the collection of CO_2 and organic volatiles. Samples from humid test systems were taken in duplicate 0, 0.2, 1, 4, 5, 7, and 8 days after application for the determination of the parent compound and the transformation product residues. Samples from additional test systems containing air dried soil were only taken at the end of the study period. The soil was extracted at ambient temperature with 2 x acetonitrile/water (1/1, v/v), 1 x acetonitrile/water (8/2, v/v) and pure acetonitrile at ambient temperature (ambient extraction). Afterwards, the soil was extracted once with acetonitrile/water (8/2, v/v) at an elevated temperature of 70°C (aggressive extraction). The BYI 02960 residues were analyzed by reversed phase HPLC with radioactivity detection. Selected samples were additionally analyzed by TLC as a confirmatory method. Identification and confirmation of the parent compound was by HPLC-MS, HPLC-MS/MS and NMR (stock solution) or co-chromatography (selected extracts).

The mass balances were complete indicating that no losses occurred during exposure or processing of samples. The mass balances were 99.3 ± 1.2% and 99.4 ± 0.9% of the AR in the irradiated and dark

soil samples of label PYM, respectively. For label FUR, the corresponding mass balances were 99.5 ± 0.6% and 99.5 ± 0.8% of the AR.

Extractable residues for radiolabel PYM decreased from 99.7% of the AR at day 0 to 98.0% and 98.9% of the AR at the end of the study for irradiated and dark systems respectively. Extractable residues for radiolabel FUR decreased from 99.3% of the AR at day 0 to 96.9% and 99.4% of the AR at the end of the study for irradiated and dark systems respectively. For both radiolabels tested the amounts of non-extractable residues (NER) in the irradiated and dark samples were low during the entire study.

In the irradiated test systems, BYI 02960 decreased to 93.8 and 93.5% of the AR by the end of the study for PYM and FUR radiolabel, respectively. No major transformation product was detected in the soil extracts, very minor transformation products were observed. The amounts of $^{14}CO_2$ at study termination amounted to 0.1% and 2.2% of the AR for PYM and FUR test systems, respectively. Organic volatile formation was negligible throughout the study (≤ 0.1% of the AR).

In the dark PYM and FUR test systems, BYI 02960 decreased just to 98.4 and to 97.3% of the AR by the end of the study. No transformation products were detected. At study termination, the amounts of $^{14}CO_2$ were 0.1% of the AR for both labels. Organic volatile formation was negligible throughout the study (≤ 0.1% of the AR).

In order to test the effects of the dryness of soil, a supplementary test was performed. As degradation was generally low, no significant differences between humid and dry soil samples were observed.

Overall the degradation of BYI 02960 was very slow. The degradation in irradiated test systems was slightly less slow compared to the degradation observed in dark test systems. The experimental DT50 values of BYI 02960 in the irradiated samples were 99.6 days (PYM) and 109.3 days (FUR), showing a good comparability for the results obtained with the two labels. The corresponding experimental DT_{50} values of BYI 02960 in the dark controls were > 1000 days (PYM) and 419.2 days (FUR).

The results of this study indicate that phototransformation on soil is of minor importance for the degradation of BYI 02960 under outdoor conditions and no major phototransformation products are expected.

Materials and methods

Materials

1. Test Items: Flupyradifurone: Code = BYI 02960; Label PYM = [Pyridinylmethyl-^{14}C] BYI 02960 (sample ID: KATH 6703); Specific activity: 4.37 MBq/mgRadiochemical purity: >99% (acc. radio-HPLC) Label FUR = [Furanone-4-^{14}C] BYI 02960 (sample ID: KATH 6702); Specific activity: 3.94 MBq/mg

;Radiochemical purity: >99% (acc. radio-HPLC); Identity and purity of test items in the application solution were confirmed.

2. Soil: The biotransformation of BYI 2960 was studied in one soil. The soil was taken fresh from the field. At the test facility, the soil was air dried broken up for sieving. Then, the soil was successively sieved to ≤ 10, 5, 3.35 and 2 mm. The sieved soil was filled in a plastic bag and stored at 4-8°C until further use.

Table B.8.1.1.3-01 Soil physicochemical properties

Parameter	**Test soil Guadalupe** **Results/Units**
SCS soil series	Camarillo
Texture class	Loam
Sand Silt Clay	40% 45% 15%
pH (water), ($CaCl_2$)	6.4 6.5
Organic matter	1.03%
Organic carbon	0.6%
Cation exchange capacity	20.7 meq/100 g soil dry wt
% Moisture at 0.33 bar	12.3%
Bulk density (disturbed)	1.23 g/cm^3
Microbial biomass Day 0	92 mg microbial C/kg soil dry wt
Soil mapping unit	Latitude N35° 01'05.6", Longitude W120° 36'10.1"

Methods

1. Preparation and application of the test item: The test systems for the kinetic photolysis test with humid soil consisted of 32 quartz glass vessels for each label (36-mm inner diameter, 35-mm height, base area 10.2 cm^2) each containing 3 g of soil (dry weight), providing about 3-mm soil depth. In addition, 4 vessels containing air dried soil were prepared for each label. A glass neck with ground joint (NS 10) was attached to the side of the wall. There, except for day 0) the flask is closed with a solid trap attachment, a small glass tube of 90 mm length and 12 mm inner diameter, in which volatile compounds were bound to soda lime and polyurethane foam. The trap is packed in the following sequence: PU-foam plug → quartz wool → 2 g soda lime → quartz wool → 0.5 g soda lime → quartz wool. Soil moisture was about 75% of 1/3-bar water holding capacity at the time of application. Moisture adjustment was performed on day 4 by replenishing the lost water with 0 to 50 µL of Milli-Q-water using a Hamilton injection device.

Duplicate treated test systems were analyzed at each sampling interval for both irradiated and dark test systems.

In addition to the main test with soil Guadalupe CA adjusted to a definitive moisture (75% of 1/3 bar moisture), a supplementary non kinetic experiment was conducted in order to assess the effect of dryness on phototransformation of BYI 02960. For this purpose, soil was air dried to a remaining soil

moisture of 0.04 g / 3 g dry soil (about 11% of 1/3 bar moisture). Application of the test items, maintenance procedures and sampling details of this test were in line with the main test, but samples were taken on the last sampling intervals only.

The test items [PYM -^{14}C] BYI 02960 and [FUR-^{14}C] BYI 02960 were dissolved in 5.0 mL Milli-Q-water/methanol (1/1, v/v). The purity of the ^{14}C-labeled test items was determined by HPLC, resulting in mean purities of 99.8% and 99.6% for PYM and FUR, respectively.

According to an intended application rate of 400 g/ha, 39.2 µL (PYM) and 37.9 µL (FUR) of application solution was pipetted into each incubation vessel. The solutions were applied evenly as drops across the surface of the soil. The soil was not mixed or agitated after application.

The material balance of the study for both dark and irradiated test systems was based on the average amount of radioactivity (RA) recovered with these measurements: 186,094 Bq or 42.58 µg BYI 02960 for PYM and 156,914 Bq or 39.83 µg BYI 02969 for FUR (per 3 g soil (dry weight)).

2. Irradiation and sampling: The photolysis vessels were placed in a Suntest CPS+ unit (Xenotest GmbH, Hanau, Germany) containing a xenon lamp simulating natural sunlight. The light emission was filtered with a 290 nm cut-off UV-filter, which eliminates all wavelengths <290 nm. The exposure time and intensity under experimental conditions can be related to natural solar radiation, e.g. of Houston (Texas; USA), Los Angeles (California; USA) or Tampa (Florida; USA), representing areas of high intensity of sunlight. For example the total radiant exposure representative of a summer day (July) at a horizontal plane is 26 MJ m^{-2} (at Los Angeles), 22 MJ m^{-2} (at Houston) or 23 MJ m^{-2} (at Tampa). For humid samples, two test systems for each label were processed for analysis immediately after the application on day 0 (0 h). Subsequently, duplicates of both irradiated and dark soil samples for each label were processed at 0.2, 1, 4, 5, 7, and 8 days post-application. Air dried samples were only processed and analyzed at day 8 after application.

Soil samples were extracted immediately on the day of sampling. Extracts were analyzed within three days of sample extraction and then stored in a freezer (-18°C or below). Samples for CO_2 and volatile organics were stored at ambient temperature (≤ 12 days) until processing for analysis.

Prior to opening a test vessel, volatile compounds possibly still present in the headspace were transferred into the trap attachment. For this purpose, the test vessels were placed in a desiccator for 15 min and the headspace was carefully purged through the trap.

3. Description of analytical procedures: Volatile organic compounds possibly contained in the PU foam plug were extracted with 5 mL of ethyl acetate. 500 µL aliquots of the extracts were analyzed by LSC in duplicate. The radioactivity (i.e. $^{14}CO_2$) absorbed by the soda lime was liberated with 18% aqueous HCl

and purged and absorbed in a series of three Zinsser vials each filled with 20-mL of ice-cooled scintillation cocktail intended for radio-assay by LSC.

The extraction of the soil samples was similar to the extraction procedure used in the aerobic soil degradation study. The total amount of soil of each vessel was transferred into a 40 mL Teflon® centrifuge beaker and extracted once with 8 mL acetonitrile/water (1/1, v/v) followed by 5 mL acetonitrile/water (1/1, v/v), 5 mL ACN/water (8/2, v/v) and 5 mL ACN. The extractions were performed at ambient temperature on a mechanical shaker for 30 min. Each extraction step was followed by centrifugation (15 min, 10 000 x g) and decanting of the supernatant. All ambient extracts were combined and the volume was determined. Aliquots thereof were analyzed by LSC and reversed phase HPLC with radioactivity detection. Extracts sampled on day 0, 4 and 8 were also analyzed by TLC. Afterwards, the soil was extracted once with acetonitrile/water (8/2, v/v) at an elevated temperature of 70°C (aggressive extraction). Identification and confirmation of the parent compound was done by HPLC-MS, HPLC-MS/MS and NMR (stock solution) or co-chromatography (selected extracts).

The extracted soil was air dried and the non-extractable Residues (NER) were quantified by combustion.

The limit of quantification (LOQ) was set to three times the maximum background radioactivity, i.e. about 1.5 Bq (0.34 ng/500 µL for label PYM and 0.38 ng/500 µL for label FUR). The lowest amount measured in ambient and aggressive organic extract samples was about 25 Bq/500 µL, i.e. 16.7 times higher than the LOQ. For CO_2 liberated from soda lime and organic volatiles extracted with ethylacetate the lowest radioactivity measured (after background subtraction) were 1.2 and 0.01 Bq, respectively. Although these values were lower than the LOQ they were evaluated, resulting in amounts of radioactivity < 0.1% of AR.

Results and discussion

Data

Data on phototransformation and dark controls is shown in Table B.8.1.1.3-02 and Table B.8.1.1.3-03.

Table B.8.1.1.3-02 Transformation of [PYM-^{14}C] BYI 02960 in loam soil Guadalupe; mean values and standard deviations expressed as % of AR

Compound	Test system	Sampling Time (Days Post Treatment)							
		Humid soil				Dry soil			
		0	0.2	1	4	5	7	8	8
BYI 02960	Irradiated	99.3 ±1.1	98.2 ±0.3	97.1 ±1.0	94.4 ±05	95.4 ±0.8	93.3 ±0.6	93.8 ±0.2	92.1 ±0.1
	Dark	99.3 ±1.1	98.4 ±0.4	98.2 ±0.6	97.7 ±0.3	98.0 ±0.0	98.9 ±0.6	98.4 ±1.0	99.3 ±0.9
ROI a	Irradiated	n.d	n.d	n.d	1.6 ±0.1	1.3 ±0.2	1.4 ±0.1	1.3 ±0.2	1.3 ±0.3
	Dark	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d

ROI b	Irradiated	n.d	n.d	n.d	0.5 ±0.1	0.5 ±0.1	0.9 ±0.1	0.6 ±0.1	1.6 ±0.2
	Dark	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
ROI c	Irradiated	n.d	n.d	n.d	0.9 ±0.1	0.8 ±0.0	1.2 ±0.0	0.8 ±0.2	0.7 ±0.2
	Dark	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
ROI d	Irradiated	n.d	n.d	n.d	n.d	n.d	n.d	n.d	1.0 ±0.4
	Dark	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
ROI e	Irradiated	n.d	n.d	n.d	1.0 ±0.2	1.0 ±0.1	1.8 ±0.1	1.0 ±0.1	0.8 ±0.0
	Dark	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Unidentified radioactivity	Irradiated	0.4 ±0.0	0.3 ±0.0	0.4 ±0.0	0.4 ±0.1	0.3 ±0.2	0.7 ±0.0	0.5 ±0.2	0.5 ±0.0
	Dark	0.4 ±0.0	0.3 ±0.1	0.3 ±0.1	0.3 ±0.1	0.4 ±0.0	0.4 ±0.1	0.5 ±0.0	0.5 ±0.0
Total extractable Radioactivity	Irradiated	99.7 ±1.1	98.5 ±0.3	97.5 ±1.0	98.7 ±0.9	99.3 ±0.7	99.2 ±0.4	98.0 ±0.7	98.0 ±0.6
	Dark	99.7 ±1.1	98.6 ±0.5	98.5 ±0.5	98.0 ±0.9	98.3 ±0.0	99.4 ±0.6	98.9 ±1.0	99.8 ±0.9
CO_2	Irradiated	n.a	<0.1	<0.1	0.0 ±0.0	0.1 ±0.0	0.2 ±0.0	0.1 ±0.0	0.1 ±0.0
	Dark	n.a	<0.1	<0.1	<0.1	0.0 ±0.0	0.1 ±0.0	0.1 ±0.0	<0.1
Volatile organics	Irrad.	n.a	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	Dark	n.a	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extractable residues (NER)	Irradiated	0.1±0.0	0.2 ±0.0	0.3 ±0.1	0.9 ±0.0	0.8 ±0.1	0.9 ±0.1	0.8 ±0.1	0.6 ±0.1
	Dark	0.1 ±0.0	0.2 ±0.0	0.4 ±0.0	0.6 ±0.0	0.9 ±0.3	0.8 ±0.1	1.0 ±0.1	0.1 ±0.0
Total % Recovery	Irradiated	99.7 ±1.1	98.7 ±0.2	97.9 ±0.9	99.7 ±0.9	100.1 ±0.7	100.3 ±0.6	98.9 ±0.8	98.7 ±0.7
	Dark	99.7 ±1.1	98.8 ±0.5	98.9 ±0.5	98.6 ±0.4	99.3 ±0.3	100.2 ±0.7	99.9 ±0.9	99.9 ±0.9

n.d. = not detected; n.a. = not analyzed; <0.1 = values below 0.05% of AR; ROI = region of interest

Table B.8.1.1.3-03 Transformation of [FUR-^{14}C] BYI 02960 in loam soil Guadalupe; mean values and standard deviations expressed as % of AR

Compound	Test system	Sampling Time (Days Post Treatment)							
		Humid soil							Dry soil
		0	0.2	1	4	5	7	8	8
BYI 02960	Irradiated	99.1 ±0.3	98.1 ±0.4	98.5 ±0.5	95.9 ±0.3	96.2 ±0.6	94.7 ±0.2	93.5 ±0.1	93.3 ±0.2
	Dark	99.1 ±0.3	99.0 ±0.5	99.0 ±0.5	98.0 ±0.3	98.8 ±0.4	98.3 ±0.3	97.3 ±1.4	99.1 ±1.0
ROI a	Irradiated	n.d.	n.d.	0.2 ±0.2	1.1 ±0.1	1.2 ±0.3	1.1 ±0.0	1.3 ±0.0	1.2 ±0.0
	Dark	n.d.	n.d.	n.d	n.d	n.d	n.d	n.d	n.d
ROI b	Irradiated	n.d.	n.d.	n.d.	0.3 ±0.3	n.d.	0.3 ±0.3	0.6 ±0.0	1.3 ±0.0
	Dark	n.d.	n.d.	n.d.	n.d	n.d.	n.d	n.d	n.d
ROI c	Irradiated	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.4 ±0.1
	Dark	n.d.	n.d.	n.d.	n.d	n.d.	n.d.	n.d.	n.d
ROI f	Irradiated	n.d.	n.d.	n.d.	0.4 ±0.4	n.d	0.5 ±0.0	0.5 ±0.0	0.4 ±0.0
	Dark	n.d.	n.d.	n.d.	n.d	n.d	n.d	n.d	n.d
Unidentified radioactivity	Irradiated	0.3 ±0.0	0.3 ±0.1	0.3 ±0.0	0.5 ±0.1	0.4 ±0.0	0.4 ±0.0	0.5 ±0.0	0.4 ±0.0
	Dark	0.3 ±0.0	0.3 ±0.0	0.3 ±0.0	0.3 ±0.0	0.3 ±0.1	0.3 ±0.0	0.1 ±0.0	0.3 ±0.2
Total extractable	Irradiated	99.3	98.4	99.0	98.3	97.8	96.9	96.4	96.9

Radioactivity		±0.2	±0.3	±0.3	±0.3	±0.3	±0.4	±0.2	±0.3
	Dark	99.3 ±0.2	99.4 ±0.6	99.3 ±0.6	98.3 ±0.3	99.1 ±0.3	98.7 ±0.3	97.4 ±1.4	99.4 ±0.9
CO_2	Irradiated	n.a	<0.1	0.1 ±0.0	0.4 ±0.1	1.4 ±0.6	1.6 ±0.0	2.2 ±0.2	2.3 ±0.0
	Dark	n.a	<0.1	<0.1	<0.1	0.1 ±0.0	0.1 ±0.0	0.1 ±0.0	<0.1
Volatile organics	Irrad.	n.a	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	Dark	n.a	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extractable residues (NER)	Irradiated	0.0 ±0.0	0.1 ±0.0	0.4 ±0.1	1.1 ±0.0	1.0 ±0.1	1.0 ±0.1	0.9 ±0.1	0.7 ±0.1
	Dark	0.0 ±0.0	0.2 ±0.0	0.4 ±0.1	1.1 ±0.0	1.0 ±0.1	1.0 ±0.1	0.9 ±0.1	0.7 ±0.0
Total % Recovery	Irradiated	99.4 ±0.2	98.5 ±0.3	99.4 ±0.5	99.8 ±0.4	100.1 ±0.3	99.6 ±0.3	99.5 ±0.1	99.9 ±0.3
	Dark	99.4 ±0.2	99.6 ±0.5	99.7 ±0.7	99.4 ±0.3	100.2 ±0.4	99.8 ±0.2	98.4 ±1.3	100.1 ±0.8

n.d. = not detected; n.a. = not analyzed; <0.1 = values below 0.05% of AR; ; ROI = region of interest

Mass Balance

The material balances were shown to be complete. For irradiated test systems the overall mean (±RSD) was of 99.3 (±1.2) and 99.5 (±0.6) % for label PYM and FUR, respectively. For dark test systems the overall mean (±RSD) was of 99.4 (± 0.9) and 99.5 (± 0.8) % for label PYM and FUR, respectively.

Distribution and Composition of Residues

For irradiated test systems PYM and FUR, the extractable amounts of radioactivity were 99.7% and 99.3% of the AR at day 0 and 98.0% and 96.4% at day 8, respectively. The amounts of NER were 0.1% and 0.0% of the AR at day 0 and remained at very low levels of 0.8% and 0.9% of the AR at the end of the test. $^{14}CO_2$ formation increased up to 0.1% and 2.2% by day 8 for label PYM and FUR, respectively. Organic volatile formation was negligible throughout the study (<1%).

For dark test systems PYM and FUR, the extractable amounts of radioactivity were 99.7% and 99.3% of the AR at day 0 and 98.9% and 97.4% at day 8, respectively. NER were 0.1% and 0.0% of the AR at day 0 and remained at the very low levels of 1.0% and 0.9% of the AR at the end of the test, respectively. $^{14}CO_2$ formation increased up to 0.1% at day 8 for both labels. Organic volatile formation was negligible throughout the study (<1%).

In the irradiated PYM test systems, BYI 02960 decreased from an average of 99.3% of the AR at day 0 to 93.8% of the AR by the end of the study. No major transformation product was detected in the soil extracts. From day 4 onwards, minor transformation products were detected. The maximum amount of a single peak accounted for 1.8% of AR (day 7).

In the irradiated FUR test systems, BYI 02960 decreased from an average of 99.1% of the AR at day 0 to 93.5% of the AR by the end of the study. From day 1 onwards, minor transformation products were detected. The maximum amount of a single peak was 1.3% of AR (day 8). The DT_{50} and DT_{90} values

of BYI 02960 in dark and irradiated samples were calculated using a single first order model (see Table B.1.1.3-04).

Table B.8.1.1.3-04 Degradation of BYI 02960 on surface of loam soil Guadalupe

Test System	Half-life		Experimental DT_{50} [days]	Experimental DT_{90} [days]
	Kinetic Model	Chi^2 Error		
Dark, Label PYM	SFO	0.41	> 1000	> 1000
Irradiated, Label PYM	SFO	0.61	99.6	331.0
Dark, Label FUR	SFO	0.29	419.2	> 1000
Irradiated, Label FUR	SFO	0.32	109.3	363.2

SFO = single first order

Conclusions

Major Outcomes of Study

Overall the soil photolytical degradation of BYI 02960 was very slow. The degradation in irradiated test systems was slightly less slow compared to the degradation observed in dark test systems. Based on the experimental DT_{50} values of 99.6 and 109.3 days for [pyridinymethyl-^{14}C] BYI 02960 and [furanone-4-^{14}C] BYI 02960, respectively, the DT_{50} values of BYI 02960 under environmental conditions were calculated to be 358 and 405 solar summer days at Los Angeles, California, USA, 466 and 527 days at Athens, Greece and 638 and 722 solar summer days at Tokyo, Japan.

Significance of Results to Environmental Behavior of BYI 02960

Direct phototransformation of BYI 02960 on soil surfaces is not regarded as a relevant degradation process in the environment. Based on the experimental DT_{50} values of 99.6 and 109.3 days for [PYM-^{14}C] BYI 02960 and [FUR-^{14}C] BYI 02960, respectively, the DT_{50} values of BYI 02960 under environmental conditions were calculated to be 358 and 405 solar summer days at Los Angeles, California, USA, 466 and 527 days at Athens, Greece and 638 and 722 solar summer days at Tokyo, Japan. The soil photolytic DT_{50} for North Europe (London) is 582 days for the [PYM-^{14}C] BYI 02960 and 659 days for the [FUR-^{14}C] BYI 02960.

Table B.8.1.1.3-05 Synopsis of degradation of [^{14}C] BYI 02960 on loam soil Guadalupe

	(% of AR)			
	PYM		FUR	
Material Balance	**Irradiated**	**Dark**	**Irradiated**	**Dark**
Mean ^{14}C-recovery during study	99.3 (±1.2)	99.4 (± 0.9)	99.5 (±0.6)	99.5 (± 0.8)
Total extractable ^{14}C during study	99.7-98.0	99.7-98.0	99.3-96.4	99.4-97.4
Max. of bound ^{14}C residues (NER)	0.9	0.9	1.1	1.1
Max. $^{14}CO_2$	0.2	0.1	2.2	0.1
Experimental SFO DT_{50} of BYI 02960 [days]	99.6	> 1000	109.3	419.2
Environmental DT_{50} of BYI 02960 [days]		-		-
Los Angeles, California, USA	358		405	
Athens, Greece	466		527	
Tokyo, Japan	638		722	
Major transformation *)	-			
Minor transformation products	NER, CO_2			

*) Criteria for term "major": >10% of AR at any DAT or >5% of AR at two successive DATs or steadily increasing until the end of the study

Remarks

The soil photolysis study is acceptable and the results are reliable.

B.8.1.2 Rate of degradation in soil (Annex IIA 7.2; Annex IIIA 9.1)

The laboratory studies presented in paragraph B.8.1.1 (route of degradation in soil) were also designed to derive information on the rate of degradation of flupyradifurone (BYI 02960) and its significant metabolites under standardized laboratory conditions in soil. In this chapter the methods and results of the respective kinetics calculations were described in more detail. In addition, a separate experimental degradation study was performed with 6-CNA, a major aerobic soil metabolite.

B.8.1.2.1 Laboratory studies (Annex IIA 7.2; Annex IIIA 9.1)

Rate of degradation; parent substance

The degradation rate for the studies performed for flupyradifurone is briefly discussed under chapter B.8.1.1 and these studies IIA 7.1.1/01-7.1.1/06 are on the route of degradation and are also used for rate IIA 7.2.1/01-7.2.1/10. Evaluation of the degradation kinetics of the aerobic soil degradation studies described under paragraph B.8.1.2.1 has been performed to derive EU trigger endpoints and model input parameters. The endpoints are derived accoriding to the FOCUS kinetics (2006) KINGUI was used to fit the measured parameters and obtain the endpoints.

STUDY IIA, 7.2.1/01

Characteristics

reference	:	Menke, U., 2011	study type	:	aerobic degradation, kinetic evaluation
year of execution	:	2007-2009	incubation time	:	120 days
GLP statement	:	yes	nominal concentration	:	0.53 mg / kg soil
guideline	:	OECD 307 (2002) US EPA, OPPTS 835.4100 (2008) OECD106 (2001)	temperature	:	20°C
test substance	:	[pyridinylmethyl-^{14}C] BYI 02960	DT50	:	see results
purity	:	> 99%	metabolites	:	see results
soils	:	four soils, sandy loam, silt loam, loam, clay loam	acceptability	:	**acceptable**

Summary

The route of degradation and experimental design for study 7.1.1/01 has been described under paragraph B.8.1.1. The best fit kinetic for “trigger evaluation” was obtained of 68.8 days was calculated for BYI 02960 in the tested soils under aerobic conditions.

Table B.8.1.2.1-01 Synopsis of ”trigger” best-fit degradation kinetics calculated for BYI 02960

		Parent BYI 02960		
Soil (Soil type)	**Kinetic model**	**DT_{50} [d]**	**DT_{90} [d]**	**Chi^2 error [%]**
AX (Sandy loam)	DFOP	63.4	443.3	1.1702
HF (Silt loam)	DFOP	52.4	209.3	0.5924
HN (Loam)	DFOP	120.0	489.7	1.2373
DD (Clay loam)	DFOP	56.4	265.1	1.6945
Geometric mean	**DFOP**	**68.8**	**331.3**	

DFOP: Double first-order in parallel

Materials and methods

Materials

Materials used in this study are comprehensively described in paragraph B.8.1.1.

Method

Determination of Degradation Kinetics

The parameters of all three kinetic models were estimated by non-linear optimization to the measured data with the software KinGUI v.1.1. The best fit model was chosen on the basis of the goodness of fit as judged by visual assessment and on the chi^2 scaled-error criterion.

Results

The input parameters used for the model of the four soils are given in Table B.8.1.2.1-02. The graphical results of the parameter estimation are given in Figure B.8.1.2.1-01 to Figure B.8.1.2.1-04.

Table B.8.1.2.1-02 Percentage of BYI 02960 used as input parameters for modelling

Soil (Soil type)	AX (Sandy loam)	HF (Silt loam)	HN (Loam)	DD (Clay loam)
Days after treatment				
0 *	98.4	98.6	99.2	98.3
0 *	99.2	98.3	99.2	98.7
1	95.9	94.9	98.7	90.2
1	95.8	96.9	98.9	93.6
3	89.9	91.8	91.8	90.4
3	90.3	92.5	92.2	89.3
7	86.2	86.6	88.4	86.0
7	87.1	87.6	88.8	86.0
14	76.2	76.3	82.4	75.6
14	76.9	78.0	82.3	76.3
21	71.1	70.8	79.9	70.8
21	71.6	72.0	79.2	70.9
30	62.6	62.8	74.1	63.1
30	63.5	63.5	74.3	63.2
59	49.9	44.3	63.0	45.6
59	49.8	46.2	63.3	46.6
120	37.8	24.3	50.6	28.5
120	36.4	24.6	49.9	28.9

* Total recovery of radioactivity used as input parameter for DAT 0 sample

Figure B.8.1.2.1-01 Results of the KinGUI parameter estimation for SFO (left), FOMC (right) and DFOP (under) for AX soil

Figure B.8.1.2.1-02 Results of the KinGUI parameter estimation for SFO (left), FOMC (right) and DFOP (under) for HF soil

Figure B.8.1.2.1-03 Results of the KinGUI parameter estimation for SFO (left), FOMC (right) and DFOP (under) for HN soil

Figure B.8.1.2.1-04 Results of the KinGUI parameter estimation for SFO (left), FOMC (right) and DFOP (under) for DD soil

Kinetics of TEST Item Degradation

A summary of the DT_{50} and DT_{90} calculations for the test item considering the alternative kinetic models is given inTable B.8.1.2.1-03**.**

Table B.8.1.2.1-03 Summary of the kinetic evaluation for trigger values according to FOCUS

Soil	Kinetic model	Parent BYI 02960		
		DT_{50} (d)	DT_{90} (d)	Chi^2 value
AXXa	SFO	70.3	233.6	5.0955
	FOMC	62.9	>1000	1.0484
	DFOP *	**63.4**	**443.3**	**1.1702**
HF	SFO	54.4	180.6	2.3139
	FOMC	50.8	270.2	0.8125
	DFOP *	**52.4**	**209.3**	**0.5924**
HN	SFO	112.6	373.9	3.7804
	FOMC	134.8	>1000	1.3412
	DFOP *	**120.0**	**489.7**	**1.2373**
DD	SFO	60.1	199.8	3.3408
	FOMC	55.2	398.9	1.5906
	DFOP *	**56.4**	**265.1**	**1.6945**
Geometric mean	**DFOP ***	**68.8**	**331.3**	

*: best fit (values bold typed)
SFO: Single first-order

FOMC: First-order multi compartment
DFOP: Double first-order in parallel

The fit of the single first-order (SFO) model was less good than the fits of the biphasic models first-order multi compartment (FOMC) and double first-order in parallel (DFOP). The latter two models however were equivalent, concerning the visual assessment as well as the marginal difference in chi^2 error (< 3 %).

Where there is significant extrapolation the FOMC model is not suitable for prediction of DT_{90} values, since unrealistic DT_{90} values are estimated that are far outside the study duration. Therefore as the FOMC and DFOP models gave equivalent fits the DFOP model was selected for evaluation of the trigger end-points for all soils.

Overall, the geometric mean of the DT_{50} and the DT_{90} values for degradation of BYI 02960 in the tested soils under aerobic conditions at 20 °C were 68.8 and 331.3 days, respectively.

Conclusions

The current laboratory study demonstrated that [pyridinylmethyl-^{14}C] BYI 02960 is degraded in soil under aerobic conditions with geometric mean DT_{50} and the DT_{90} values in the tested soils under aerobic conditions at 20°C of 68.8. An overall summary of the “best-fit” trigger values for all soils is given Table B.8.1.2.1-21.

Remarks

The RMS does not agree with the argument of the authors that DFOP is preferred because it gave lower values of the $DegT_{90}$ than FOMC. FOCUS (2006) developed procedures for selecting the most suitable kinetic model depending on the intended use of the endpoint (trigger value or modelling input).

1. The measurements show a small bi-phasic pattern, so the RMS agrees with the authors that the SFO fits are poor because the residuals show systematic deviations. Although, the authors did not perform the advised modified fitting procedure for the SFO fits (relevant for this case: constrain the initial concentration), the RMS thinks that constraining the initial concentration would improve the fit.
2. Considering deriving endpoints to be used as triggers (Figure 7-1, page 110 FOCUS, 2006), the next step in the flow chart is to determine which of the models (FOMC or DFOP) is best. For all four soils it is not possible to decide from the visual assessment of the fits which model (FOMC, DFOP) gives a better fit; fits are equally good. Comparing the statistical output showed that for soil AX and DD FOMC gives a better fit and for soils HF and HN DFOP gives a better fit based on chi^2 error.
3. Considering deriving degradation parameters for modelling (Figure 7-2, page 114 FOCUS, 2006), DFOP is preferred because 10% of the initially measured concentration was not

reached within the experimental period. Based on this the endpoints depending on its intended use and in line with FOCUS (2006) are shown below.

All endpoints for 'model input' were based on DFOP. The endpoints for 'trigger value' were based on DFOP for HF and HN and on FOMC for AX and DD.

Table B.8.1.2.1-03a Summary of the kinetic evaluation for and trigger and modelling values according to FOCUS

Soil	Kinetic model	Parent BYI 02960 Trigger		Parent BYI 02960 Modelling	
		DT_{50} (d)	DT_{90} (d)	DT_{50} (d)	DT_{90} (d)
AXXa	SFO	70.3	233.6	70.3	233.6
	FOMC	**62.9**	**>1000**	62.9	>1000
	DFOP	63.4	443.3	**63.4**	**443.3**
HF	SFO	54.4	180.6	54.4	180.6
	FOMC	50.8	270.2	50.8	270.2
	DFOP	**52.4**	**209.3**	**52.4**	**209.3**
HN	SFO	112.6	373.9	112.6	373.9
	FOMC	**134.8**	**>1000**	134.8	>1000
	DFOP	120.0	489.7	**120.0**	**489.7**
DD	SFO	60.1	199.8	60.1	199.8
	FOMC	**55.2**	**398.9**	55.2	398.9
	DFOP	56.4	265.1	**56.4**	**265.1**

SFO: Single first-order
FOMC: First-order multi compartment
DFOP: Double first-order in parallel

The extraction methods used are appropriate and the total recovery of the radioactivity was satisfactory (96-102%).

The RMS concludes that the study has been well conducted and that its results (i.e. decline curves of BYI 02960) are in line with the draft guidance on time dependent sorption and are suitable to be used in a kinetic analysis to calculate timedependent sorption parameters.

According to the autors of the study best fit rate of degradation values were all obtained with the DFOP model. As pointed out in the evaluation above RMS does not agree. However, the new geometric mean trigger endpoint is almost similar and therefor the RMS suggests to use the value obtained by the applicant. The geometric mean for persistence is 68.8 days (based on the DFOP values). The RMS agrees to use this value as endpoint for [PYR-^{14}C] BYI 02960.

STUDY IIA, 7.2.1/02

Characteristics

Reference	:	Menke, U., Unold, M. (2011)	study type	:	aerobic degradation, kinetic evaluation
year of execution	:	2008	incubation time	:	120 days
GLP statement	:	yes	nominal concentration	:	1.07 mg/kg
Guideline	:	OECD 307 (2002) US EPA, OPPTS 835.4100 (2008)	temperature	:	10°C or 20°C
test substance	:	[Furanone-4-^{14}C] BYI 02960	DT50	:	see results
Purity	:	> 98 %	metabolites	:	see results

Soils	:	Four soils, sandy loam, silt loam, silt loam, silty loam	acceptability	:	**acceptable**

Summary

The route of degradation and experimental design has been described under in paragraph B.8.1.1.

The best fit kinetic for "trigger evaluation" was obtained by double first-order kinetics. A geometric mean a half-life of 56.2 days was calculated for BYI 02960 in the tested soils.

Table B.81.2.1-04 Synopsis of "trigger" half-lives calculated for BYI 02960

Soil (soil type)	Kinetic model	Parent BYI 02960 DT_{50} [d]	DT_{90} [d]	Chi^2 error [%]
AX (Sandy loam)	DFOP	62.2	390.6	1.55
HF (Silt loam)	DFOP	33.2	229.5	1.71
HN (Silt loam)	DFOP	98.3	462.5	2.03
DD (Silty clay)	DFOP	49.3	303.1	2.26
Geometric mean		**56.2**	**334.8**	

DFOP: Double first-order in parallel

Materials and methods

Materials

Materials used in this study are comprehensively described in paragraph B.8.1.1.

Method

Determination of Degradation Kinetics

In order to determine the best-fit kinetic model for the degradation of BYI 02960 in soil over time SFO, DFOP and FOMC models were considered. The best fit model was chosen on the basis of the goodness of fit as judged by visual assessment and on the chi^2 scaled-error criterion.

Results

The input parameters used for the model of the four soils are given in Table B.8.1.2.1-05. The graphical results of the parameter estimation are given in Figure B.8.1.2.1-05 to Figure B.8.1.2.1-08.

Table B.8.1.2.1-05 Percentage of BYI 02960 used as input parameters for modelling

Soil (Soil type)	AX (Sandy loam)	HF (Silt loam)	HN (Silt loam)	DD (Silt clay)
Days after treatment				
0 *	99.6	99.7	100.3	99.2
0 *	99.8	100.4	99.7	99.4
1	94.2	93.6	91.8	93.5
1	94.5	93.8	92.2	93.1
3	89.3	89.6	88.4	89.5
3	89.3	91.1	88.3	89.5
7	83.0	80.1	82.6	84.1
7	83.0	81.1	82.1	84.8
14	74.4	71.1	77.7	75.4
14	74.1	71.1	77.6	75.6
30	62.4	53.1	66.3	64.4
30	62.9	52.3	67.5	62.5
45	54.2	40.1	61.3	52.3

45	54.4	41.1	60.7	49.6
59	48.5	32.9	56.8	41.4
59	48.4	33.7	56.4	41.4
85	42.1	26.3	50.9	34.5
85	42.6	26.1	50.6	33.5
120	37.8	20.0	45.5	27.1
120	36.9	20.4	44.9	26.6

* Total recovery of radioactivity used as input parameter for DAT 0 sample

Figure B.8.1.2.1-05 Results of the KinGUI parameter estimation for SFO (left), FOMC (right) and DFOP (under) for AX soil

Figure B.8.1.2.1-06 Results of the KinGUI parameter estimation for SFO (left), FOMC (right) and DFOP (under) for HF soil

Figure B.8.1.2.1-07 Results of the KinGUI parameter estimation for SFO (left), FOMC (right) and DFOP (under) for HN soil

Figure B.8.1.2.1-08 Results of the KinGUI parameter estimation for SFO (left), FOMC (right) and DFOP (under) for DD soil

Kinetics of test Item Degradation

The degradation of the parent compound during the study was evaluated by first-order non-linear regression analysis, a summary of the kinetic analyses for all models is shown in Table B.8.1.2.1-06. Where there is significant extrapolation the FOMC model is not suitable for prediction of DT_{90} values, since unrealistic DT_{90} values are estimated that are far outside the study duration. Therefore as the FOMC and DFOP models gave equivalent fits the DFOP model was selected for evaluation of the trigger end-points for all soils.

Table B.8.1.2.1-06 Summary of the Kinetic Evaluation (for Trigger Values According to FOCUS) of the Degradation of [FUR-^{14}C] BYI 02960 in Aerobic Soils at 20 °C and 55 % of WHCmax)

Soil	Kinetic model	Parent BYI 02960		
		DT50 (d)	DT90 (d)	Chi^2 value
AXXa	SFO	70.6	234.5	6.06
	FOMC	59.5	> 1000	1.20
	DFOP *	**62.2**	**390.6**	**1.55**
HF	SFO	40.5	134.6	5.30
	FOMC	33.2	244.2	1.58
	DFOP *	**33.2**	**229.5**	**1.71**
HN	SFO	96.3	320.1	5.47
	FOMC	104.7	> 1000	1.97
	DFOP *	**98.3**	**462.5**	**2.03**
DD	SFO	55.1	183.0	3.87
	FOMC	49.3	341.0	2.11
	DFOP *	**49.3**	**303.1**	**2.26**
Geometric mean	**DFOP ***	**56.2**	**334.8**	

*Bold : best fit (values bold typed)
SFO: Single first-order
FOMC: First-order multi compartment
DFOP: Double first-order in parallel

Conclusions

The current laboratory study demonstrated that [FUR-^{14}C] BYI 02960 is degradable in soils under aerobic conditions with a geomean DT_{50} (trigger endpoint) of 56.2 days for the current study. An overall summary of the "best-fit" trigger values for all soils is given Table B.8.1.2.1-21.

Remarks

The best fit rate of degdration obtained were all with the DFOP model. The geometric mean DT_{50} for persistence is 56.2 days. The RMS agrees to use this value as endpoint for [FUR-^{14}C] BYI 02960.

STUDY IIA, 7.2.1/03

Characteristics

reference	:	Ripperger, R.J. (2011)	study type	:	aerobic degradation, kinetic evaluation
year of execution	:	2010	incubation time	:	120 days
GLP statement	:	yes	nominal concentration	:	1.07 mg/kg
guideline	:	OECD 307 (2002) US EPA, OPPTS 835.4100 (2008)	temperature	:	20°C
test substance	:	[Furanone-4-^{14}C] BYI 02960	DT50	:	see results
purity	:	> 98 %	metabolites	:	see results
soils	:	two soils, silt loam, sandy loam	acceptability	:	**acceptable**

Summary

The route of degradation and experimental design has been described under paragraph B.8.1.1. The best fit kinetic for "trigger evaluation" was obtained by SFO in Springfield and DFOP in Sanger soil.

Table B.8.1.2.1- 07 Synopsis of half-lives (trigger values) calculated for BYI 02960

Soil (soil type)	Kinetic model	Parent BYI 02960 DT_{50} [d]	DT_{90} [d]	Chi^2 error [%]
Springfield (silt loam)	SFO	228	757	1.3
Sanger (sandy laom)	DFOP	58.3	273	1.1

SFO: Single first-order
DFOP: Double first-order in parallel

Materials and methods

Materials

Materials used in this study are comprehensively described within paragraph B.8.1.1.

Method

Determination of Degradation Kinetics

In order to determine the best-fit kinetic model for the degradation of BYI 02960 in soil over time SFO, DFOP and FOMC models were considered. The best fit model was chosen on the basis of the goodness of fit as judged by visual assessment and on the chi^2 scaled-error criterion.

Results

The input parameters used for the model of the two soils are given in Table B.8.1.2.1-08. The graphical results of the parameter estimation are given in Figure B.8.1.2.1-09 to Figure B.8.1.2.1-10.

Table B.8.1.2.1-08 Percentage of BYI 02960 used as input parameters for modelling

Soil (Soil type)	Springfield (Sandy loam)	Sanger (Sandy loam)
Days after treatment		
0 *	99.7	99.8
0 *	100.3	100.2
3	93.4	94.7
3	92.8	95.8
7	94.6	86.8
7	93.4	86.2
14	89.3	80.0
14	90.3	79.7
28	-	67.3
28	87.1	68.1
60	78.2	49.0
60	78.2	47.7
92	71.6	38.7
92	72.5	38.7
120	65.6	30.9
120	68.9	30.8

* Total recovery of radioactivity used as input parameter for DAT 0 sample

Figure B.8.1.2.1-09 Results of the KinGUI parameter estimation for SFO (left), FOMC (right) and DFOP (under) for Springfield soil

Kinetics of test Item Degradation

The degradation of the parent compound during the study was evaluated by first-order non-linear regression analysis, a summary of the kinetic analyses for all models is shown in Table B.8.1.2.1-09. In the Springfield soil all models resulted in equivalent fits, the DFOP and FOMC were regarded as less reliable due to the extrapolation required, therefore the SFO was selected. In the Sanger soil the DFOP and FOMC models resulted in better fits, therefore the DFOP was selected as FOMC is less reliable where extrapolation beyond the experimental period is required.

Figure B.8.1.2.1-10 Results of the KinGUI parameter estimation for SFO (left), FOMC (right) and DFOP (under) for Sanger soil

Table B.8.1.2.1-09 Summary of the Kinetic Evaluation (for Trigger Values According to FOCUS) of the Degradation of [FUR-^{14}C] BYI 02960 in Aerobic Soils at 20 °C and 55 % of WHCmax

Soil	Kinetic model	Parent BYI 02960		
		DT50 (d)	DT90 (d)	Chi^2 value
Springfield	**SFO***	**228**	**757**	**1.3**
	DFOP	374	> 1000	1.2
	FOMC	> 1000	> 1000	1.1
Sanger	SFO	65.7	218	3.4
	DFOP*	**58.3**	**273**	**1.1**
	FOMC	57.2	455	4.4

* Bold: best fit (values bold typed)
SFO: Single first-order
FOMC: First-order multi compartment
DFOP: Double first-order in parallel

Conclusions

The current laboratory study demonstrated that [FUR-^{14}C] BYI 02960 is degradable in soils under aerobic conditions with DT_{50} values of 58.3 and 228 days. An overall summary of the "best-fit" trigger values for all soils is given in Table B.8.1.2.1-21.

Remarks

The RMS agrees with the applicant to use the SFO fit as best fit for Springfield and DFOP fit for Sanger soil as best fit. The DT_{50} used for persistence is 228 and 58.3 days.

STUDY IIA, 7.2.1/04

Characteristics

Reference	:	Menke, U., Unold, M., (2011)	study type	:	aerobic degradation, kinetic evaluation
year of execution	:	2009-2011	incubation time	:	118 days
GLP statement	:	yes	nominal concentration	:	1.07 mg/kg
Guideline	:	OECD 307 (2002) US EPA, OPPTS 835.4100 (2008)	temperature	:	20°C
test substance	:	[Ethyl-1-^{14}C] BYI 02960	DT50	:	see results
Purity	:	> 98 %	metabolites	:	see results
Soils	:	three soils; silt loam, loamy sand, clay loam	acceptability	:	**acceptable**

Summary

The route of degradation and experimental design has been described under paragraph B.8.1.1. The best fit kinetic for "trigger evaluation" was obtained by double first-order kinetics a half-life (geometric mean) of 41.6 days was calculated for BYI 02960 in the tested soils under aerobic conditions.

Table B.8.1.2.1-10 Synopsis of half- lives calculated for BYI 02960

Soil (Soil type)	Kinetic model	Parent BYI 02960 DT_{50} [d]	DT_{90} [d]	Chi2 error [%]
DD (Clay loam)	DFOP	33.9	649.6	1.9
AX (Loamy sand)	DFOP	62.0	538.1	1.6
HF (Silt loam)	DFOP	34.1	329.8	2.3
Geometric mean		**41.6**	**486.7**	

DFOP: Double first-order in parallel

Materials and methods

Materials

Materials used in this study are comprehensively described within paragraph B.8.1.1.

Methods

Determination of Degradation Kinetics

In order to determine the best-fit kinetic model for the degradation of BYI 02960 in soil over time SFO, DFOP and FOMC models were considered. The best fit model was chosen on the basis of the goodness of fit as judged by visual assessment and on the chi^2 scaled-error criterion.

Results

The input parameters used for the model of the three soils are given in Table B.8.1.2.1-11. The graphical results of the parameter estimation are given in Figure B.8.1.2.1-11 to Figure B.8.1.2.1-13.

Table B.8.1.2.1-11 Percentage of BYI 02960 used as input parameters for modelling

Soil (Soil type)	DD (Clay loam)	AX (Loamy sand)	HF (Silt loam)
Days after treatment			
0 *	99.2	98.6	101.3
0 *	99.2	101.0	100.1
1	92.5	93.3	96.0
1	93.2	95.3	92.9
3 (4 AX HF)	88.5	89.1	88.4
3 (4 AX HF)	90.0	91.8	88.6
7	82.0	84.0	82.0
7	82.2	83.5	78.2
14	73.9	78.1	72.8
14	72.5	75.2	71.0
30 (29 AX HF)	52.2	56.9	55.8
30 (29 AX HF)	52.2	62.3	55.5
45 (48 AX HF)	37.9	52.2	38.4
45 (48 AX HF)	32.0	55.4	41.2
62 (61 AX HF)	30.7	48.8	32.9
62 (61 AX HF)	32.0	50.0	32.6
90	19.9	44.4	28.2
90	22.9	44.6	25.8
118 (117 AX HF)	17.3	39.8	22.8
118 (117 AX HF)	18.1	39.3	23.2

* Total recovery of radioactivity used as input parameter for DAT 0 sample

Figure B.8.1.2.1-11 Results of the KinGUI parameter estimation for SFO (left), FOMC (right) and DFOP (under) for DD soil

Figure B.8.1.2.1-12 Results of the KinGUI parameter estimation for SFO (left), FOMC (right) and DFOP (under) for AX soil

Figure B.8.1.2.1-13 Results of the KinGUI parameter estimation for SFO (left), FOMC (right) and DFOP (under) for HF soil

Kinetics of test Item Degradation

A summary of the DT_{50} and DT_{90} calculations for the test item is given in Table B.8.1.2.1-12. In all cases the fit of the single first-order (SFO) model was less good than the fits obtained with the two biphasic models first order multi compartment (FOMC) and double first order in parallel (DFOP). Although the difference between the latter two models was quite low concerning the chi^2 errors, the visual assessments indicated a better fit of the last sampling points using the DFOP model. Therefore, the DFOP kinetic model was chosen as best fit for all soils.

Table B.8.1.2.1-12 Summary of the kinetic evaluation (for trigger values according to FOCUS)

Soil	Kinetic model	Parent BYI 02960		
		DT50 (d)	DT90 (d)	Chi^2 value
DD	SFO	38.6	128.1	4.1
	FOMC	33.8	178.4	2.0
	DFOP*	**33.9**	**649.6**	1.9
AXXa	SFO	74.5	247.4	6.8
	FOMC	61.6	> 1000	1.7
	DFOP*	**62.0**	**538.1**	1.6
HF	SFO	43.0	142.9	5.9
	FOMC	34.1	287.5	2.2
	DFOP*	**34.1**	**329.8**	2.3
Geometric mean	**Best fit**	**41.6**	**486.7**	

* Bold: best fit according to chi2 error or visual assessment
SFO: Single first-order
FOMC:First-order multi compartment
DFOP: Double first-order in parallel

Conclusion

The current laboratory study demonstrated that BYI 02960 is degradable in soils under aerobic conditions The geometric mean of the DT_{50} and the DT_{90} values for degradation of BYI 02960 was 41.6 and 486.7 days, respectively. An overall summary of the “best-fit” trigger values for all soils is given in Table B.8.1.2.1-21.

Remarks

The best fit rate of degdration obtained were all with the DFOP model. The geometric mean for persistence is 41.6 days. The RMS agrees to use this value as endpoint for Ethyl-1-^{14}C BYI 02960.

STUDY IIA, 7.2.1/05

Characteristics

Reference	:	Menke, U., Unold, M., (2011)	study type	:	aerobic degradation, kinetic evaluation
year of execution	:	2010	incubation time	:	117 days
GLP statement	:	yes	nominal concentration	:	1.07 mg/kg
Guideline	:	OECD 307 (2002) US EPA, OPPTS 835.4100 (2008)	Temperature	:	20°C
test substance	:	[Pyridine-2,6-^{14}C] BYI 02960	DT50	:	see results
purity	:	> 98 %	metabolites	:	see results
Soils	:	One soils, silt loam	acceptability	:	**acceptable**

Summary

The route of degradation and experimental design has been described in paragraph 8.1.1. The best fit kinetic for “trigger evaluation” was obtained by double first-order kinetics a half-life (geometric mean) of 33.0 days was calculated for BYI 02960 in the tested soils under aerobic conditions.

Table B.81.2.1-15 Synopsis of half- lives calculated for BYI 02960

Soil	Kinetic model	Parent BYI 02960		
		DT_{50} (d)	DT_{90} (d)	Chi^2 value
HF	DFOP	33.0	221.3	2.0

DFOP: Double first-order in parallel

Materials and methods

Materials

Materials used in this study are comprehensively described within report paragraph 8.1.1.

Determination of Degradation Kinetics

In order to determine the best-fit kinetic model for the degradation of BYI 02960 in soil over time SFO, DFOP and FOMC models were considered. The best fit model was chosen on the basis of the goodness of fit as judged by visual assessment and on the chi^2 scaled-error criterion.

Results

The input parameters used for the model of the soil are given in Table B.8.1.2.1-16. The graphical results of the parameter estimation are given in Figure B.8.1.2.1-14.

Table B.8.1.2.1-16 Percentage of BYI 02960 used as input parameters for modelling

Soil (Soil type)	**HF (Silt loam)**
Days after treatment	
0 *	100.7
0 *	99.3
1	95.5
1	95.2
4	89.3
4	87.7
7	79.3
7	77.6
14	70.7
14	71.1
29	53.6
29	53.4
48	39.4
48	42.4
61	34.1
61	33.8
90	27.0
90	27.7
117	22.1
117	23.2

* Total recovery of radioactivity used as input parameter for DAT 0 sample

Figure B.8.1.2.1-14 Results of the KinGUI parameter estimation for SFO (left), FOMC (right) and DFOP (under) for HF soil

Kinetics of test Item Degradation

A summary of the DT_{50} and DT_{90} calculation for the test item is given in Table B.8.1.2.1-17. The fit of the single first-order (SFO) model was less good than the fits of the biphasic models first-order multi compartment (FOMC) and double first-order in parallel (DFOP). The latter two models however were equivalent, concerning the visual assessment as well as the marginal difference in chi^2 error. In all cases the chi^2 values of the fits of both, FOMC and DFOP model, were very low (< 3 %).

Where there is significant extrapolation the FOMC model is not suitable for prediction of DT_{90} values, since unrealistic DT_{90} values are estimated that are far outside the study duration. Therefore as the FOMC and DFOP models gave equivalent fits the DFOP model was selected for evaluation of the trigger end-point.

Table B.8.1.2.1-17 Summary of the kinetic evaluation (for trigger values according to FOCUS) of the degradation of [PYR-^{14}C] BYI 02960 in aerobic soil

Soil	Kinetic model	Parent BYI 02960		
		DT_{50} (d)	DT_{90} (d)	Chi^2 value
HF	SFO	43.4	144.2	6.2
	FOMC	33.1	327.0	1.7
	DFOP*	**33.0**	**221.3**	**2.0**

*: best fit (values bold typed)
SFO: Single first-order; FOMC: First-order multi compartment; DFOP: Double first-order in parallel

Conclusions

The current laboratory study demonstrated that [PYR-^{14}C] BYI 02960 is degradable in soils under aerobic conditions with a DT_{50} of 33 days. An overall summary of the "best-fit" trigger values for all soils is given in Table B.8.1.2.1-21.

Remarks

The RMS agrees with the notifier to use DFOP DT_{50} as persistence trigger.

STUDY IIA, 7.2.1/06

Characteristics

reference	:	Shepherd, J.J. (2011)	study type	:	aerobic degradation, kinetic evaluation
year of execution	:	2010	incubation time	:	120 days
GLP statement	:	yes	nominal concentration	:	1.1 mg/kg
guideline	:	OECD 307 (2002) US EPA, OPPTS 835.4100 (2008)	temperature	:	20°C
test substance	:	[Pyridine-2,6-^{14}C] BYI 02960	DT50	:	see results
purity	:	> 98 %	metabolites	:	see results
soils	:	two soils; silt loam, sandy laom	acceptability	:	**acceptable**

Summary

The route of degradation and experimental design has been described in paragraph B.8.1.1. The best fit kinetic for "trigger evaluation" was obtained by DFOP or FOMC.

Table B.8.1.2.1-18 Summary of the kinetic evaluation (for trigger values according to FOCUS)

Soil	Kinetic model	Parent BYI 02960		
		DT50 (d)	DT90 (d)	Chi2 value
Springfield	DFOP	242	898	0.7
Sanger	FOMC	56.3	324	1.8

FOMC: First-order multi compartment
DFOP: Double first-order in parallel

Materials and methods

Materials

Materials used in this study are comprehensively described within paragraph B.8.1.1.

Methods Determination of Degradation Kinetics

In order to determine the best-fit kinetic model for the degradation of BYI 02960 in soil over time SFO, FOMC and DFOP models were considered. The goodness of fit was assessed by visual inspection and an error criterion based on a chi-square (χ^2) significance test. In addition to these, the coefficient of determination (r^2) was calculated and reported by the kinetics modelling tool.

Results

The input parameters used for the model of the two soils are given in Table B.8.1.2.1-19. The graphical results of the parameter estimation are given in Figure B.8.1.2.1-15 to Figure B.8.1.2.1-16.

Table B.8.1.2.1-19 Percentage of BYI 02960 used as input parameters for modelling

Soil (Soil type)	Springfield (Silt loam)	Sanger (Sandy loam)
Days after treatment		
0 *	100.8	99.4
0 *	99.2	100.6
3	93.2	96.2
3	95.0	94.3
7	93.2	87.4
7	93.8	87.4
14	90.4	84.6
14	88.1	83.1
28	83.4	67.4
28	83.0	67.2
60	77.3	47.6
60	76.2	46.0
90	70.4	34.8
90	69.8	34.8
120	66.1	29.5
120	65.0	30.5

* Total recovery of radioactivity used as input parameter for DAT 0 sample

Kinetics of test Item Degradation

A summary of the DT_{50} and DT_{90} calculation for the test item is given in Table B.8.1.2.1-20. The fit of the single first-order (SFO) model was less good than the fits of the biphasic models first-order multi compartment (FOMC) and double first-order in parallel (DFOP). The letter two models however were equivalent, concerning the visual assessment as well as the marginal difference in chi^2 error. In all cases the chi^2 values of the fits of SFO, FOMC and DFOP model, were very low (< 3 %). Due to the extent of extrapolation required and the lower reliability of the derived parameters the SFO fit was regarded as more robust.

Figure B.8.1.2.1-15 Results of the KinGUI parameter estimation for SFO (left), FOMC (right) and DFOP (under) for Springfield soil

Figure B.8.1.2.1-16 Results of the KinGUI parameter estimation for SFO (left), FOMC (right) and DFOP (under) for Sanger soil

Table B.8.1.2.1-20 Summary of the kinetic evaluation (for trigger values according to FOCUS)

Soil	Kinetic model	Parent BYI 02960		
		DT50 (d)	DT90 (d)	Chi^2 value
Springfield	SFO	211	400	1.6
	FOMC	429	>1000	0.6
	DFOP*	**242**	**898**	**0.7**
Sanger	SFO	62.2	207	3.0
	FOMC*	**56.3**	**> 1000**	**1.7**
	DFOP	56.3	324	1.8

* Bold: best fit according to chi2 error or visual assessment
SFO: Single first-order
FOMC: First-order multi compartment
DFOP: Double first-order in parallel

Conclusions

The current laboratory study demonstrated that BYI 02960 is degradable in soils under aerobic conditions with "best fit" DT_{50} values of 242 and 56.3. An overall summary of the "best-fit" trigger values for all soils is given in Table B.8.1.2.1-21.

Remarks

The RMS agrees with the notifier to use DFOP for Springfield soil and FOMC for Sanger soils as the best fit DT_{50} of as persistence trigger.

Degradation rates

Summary of the persistence trigger DT_{50} and DT_{90} values in aerobic soil studies

A summary of the DT_{50} and DT_{90} "best-fit" trigger values calculated as described above is given in Table B.8.1.2.1-21.

Table B.8.1.2.1-21 Overall summary of derived DT_{50} and DT_{90} values for degradation of BYI 02960 in aerobic soil (trigger evaluation according to FOCUS kinetics)

Soil	Labled test substance	Study Reference	Kinetic model (best-fit)	Parent BYI 02960		
				DT50 (d)	DT90 (d)	$\chi 2$
Laacher Hof AXXa	[PYM] BYI 02960	IIA 7.2.1/01	DFOP	63.4	443.3	1.2
Laacher Hof AXXa	[FUR] BYI 02960	IIA 7.2.1/02	DFOP	62.2	390.6	1.6
Laacher Hof AXXa	[ETH] BYI 02960	IIA 7.2.1/04	DFOP	62.0	538.1	1.6
Laacher Hof AXXa (AX) geometric mean				**62.5**	**453.3**	
Höfchen am Hohenseh	[PYM] BYI 02960	IIA 7.2.1/01	DFOP	52.4	209.3	0.6
Höfchen am Hohenseh	[FUR] BYI 02960	IIA 7.2.1/02	DFOP	33.2	229.5	1.7
Höfchen am Hohenseh	[ETH] BYI 02960	IIA 7.2.1/04	DFOP	34.1	329.8	2.3
Höfchen am Hohenseh	[PYR] BYI 02960	IIA 7.2.1/05	DFOP	33.0	221.3	2.0
Höfchen am Hohenseh (HF) geometric mean				**37.4**	**243.3**	
Hanscheiderhof	[PYM] BYI 02960	IIA 7.2.1/01	DFOP	120.0	489.7	1.2
Hanscheiderhof	[FUR] BYI 02960	IIA 7.2.1/02	DFOP	98.3	462.5	2.0
Hanscheiderhof (HN) geometric mean				**108.6**	**475.9**	
Dollendorf II	[PYM] BYI 02960	IIA 7.2.1/01	DFOP	56.4	265.1	1.7
Dollendorf II	[FUR] BYI 02960	IIA 7.2.1/02	DFOP	49.3	303.1	2.3

Dollendorf II	[ETH] BYI 02960	IIA 7.2.1/04	DFOP	33.9	649.6	1.9
			DD geometric mean	**45.5**	**373.7**	
Springfield	[FUR] BYI 02960	IIA 7.2.1/03	SFO	228	757	1.3
Springfield	[PYR] BYI 02960	IIA 7.2.1/06	DFOP	242	898	0.7
			Springfield geometric mean	**234.9**	**824.5**	
Sanger	[FUR] BYI 02960	IIA 7.2.1/03	DFOP	58.3	273	1.1
Sanger	[PYR] BYI 02960	IIA 7.2.1/06	FOMC	55.3	> 1000	1.8
			Sanger geometric mean	**56.8**	**522.5**	
			Overall geometric mean *	**73.2**	**405.0**	
			Overall median *	**59.7**	**413.5**	

* Geometric mean and median of the geometric means of the different soils

Kinetic Evaluation of Laboratory Studies – Modelling endpoints

In addition to the evaluation of trigger values by considering "best-fit" as described above parameters suitable for use in environmental modelling have been separately evaluated in accordance with the procedures of FOCUS kinetics (2006).

STUDY IIA, 7.2.1/07

Characteristics

reference	:	Sur, R., Dorn, S. (2012)	study type	:	kinetic evaluation
year of execution	:	2012	incubation time	:	n.a
GLP statement	:	no	nominal concentration	:	n.a
guideline	:	FOCUS Kinectics (2006) US EPA, OPPTS 835	temperature	:	n.a.
test substance	:	n.a	DT_{50}	:	see results
purity	:	n.a	metabolites	:	see results
soils	:	n.a.	acceptability	:	**acceptable**

Summary

The present report is a supplement to Menke 2011 (study IIA, 7.1.1/01), summarized in paragraph B.8.1.1. It compiles additional raw data of the kinetic evaluation of the degradation of BYI 02960 in soil to determine modelling endpoints. The evaluation followed the recommendations of the FOCUS working group on degradation kinetics. The trigger endpoints of BYI 02960 were derived in the study IIA, 7.2.1/01 and are summarized in Table B.8.1.2.1-21.

Methods

The measured data were taken into account as reported (individual replicates). All experimental data sets and all data points were weighted equally (weighting factor 1), which corresponds to an absolute error model. The initial concentration was fitted.

The goodness of the fits was assessed visually and by the Chi^2 error as described in FOCUS (2006). A single-sided t-test was used to identify the probability that the degradation rate is not significant, that is equal or smaller than zero. A probability t-prob of 0.05 is considered as sufficiently small to obtain significant degradation parameters, which are suitable for modelling purposes (FOCUS 2006).

As outlined in the underlying main report, parameter optimization was carried out with the MATLAB evaluation tool 'KinGUI' (MATLAB 2005 and Bramley 2007) considering alternative kinetic models. The graphs of the model fits are shown in Figure B.8.1.2.1-01 to Figure B.8.1.2.1-04.

Conclusion

The degradation parameters for modelling input are summarized in Table B.8.1.2.1-22. For modelling endpoints the SFO model was considered acceptable for Dollendorf II (DD) and Hoefchen am Hohenseh 4a (HF) and the DFOP model was chosen for Laacher Hof AXXa (Ax) and Hanscheiderhof (HN).

Table B.8.1.2.1-22 Modelling endpoints of BYI 02960

Soil	Kinetic Model	BYI 02960			
		DT_{50} (d)	χ^2 err (%)	Visual Acceptability	t-prob
Ax	DFOP	169.1*	1.1702	Yes	k_1: <0.001 k_2: <0.001
HF	SFO	54.4	2.3139	Yes	<0.001
HN	DFOP	157.5*	1.2373	Yes	k_1: <0.001 k_2: <0.001
DD	SFO	60.1	3.3408	Yes	<0.001

* slow compartment of DFOP model

Remarks

The RMS agrees with the applicant to use the slow phase DT_{50} of the DFOP fits of Ax and HN soils and SFO DT_{50} of HF and DD soils as input parameters for environmental modelling.

STUDY IIA, 7.2.1/08

Characteristics

reference	:	Sur, R., Dorn, S. (2012)	study type	:	kinetic evaluation
year of execution	:	2012	incubation time	:	n.a
GLP statement	:	No	nominal concentration	:	n.a
guideline	:	FOCUS Kinectics (2006) US EPA, OPPTS 835	temperature	:	n.a.
test substance	:	n.a	DT_{50}	:	see results
purity	:	n.a	metabolites	:	see results
soils	:	n.a.	acceptability	:	**acceptable**

Summary

The present report is a supplement to Menke and Unold 2011 (study IIA, 7.1.1/02), summarized in paragraph B.8.1.1. It compiles additional raw data of the kinetic evaluation of the degradation of BYI 02960 in soil to determine modelling endpoints. The evaluation followed the recommendations of the FOCUS working group on degradation kinetics (FOCUS, 2006). The trigger endpoints of BYI 02960 were derived in study IIA, 7.2.1/02 and have been summarized in Table B.8.1.2.1-21.

Method

The measured data were taken into account as reported (individual replicates). All experimental data sets and all data points were weighted equally (weighting factor 1), which corresponds to an absolute error model. The initial amount of the parent was fitted.

The goodness of the fits was assessed visually and by the Chi^2 error as described in FOCUS (2006). A single-sided t-test was used to identify the probability that the degradation rate is not significant, that is equal or smaller than zero. A probability t-prob of 0.05 is considered as sufficiently small to obtain significant degradation parameters, which are suitable for modelling purposes (FOCUS 2006).

As outlined in the underlying main report, parameter optimization was carried out with the MATLAB evaluation tool 'KinGUI' (MATLAB 2005 and Bramley 2007) considering alternative kinetic models. The graphs of the model fits are shown in Figure B.8.1.2.1-05 to Figure B.8.1.2.1-08.

Conclusion

The degradation parameters for modelling input are summarized in Table B.8.1.2.1-23. For modelling endpoints the SFO model was considered acceptable for Dollendorf II (DD) and Hoefchen am Hohenseh 4a (HF) and the DFOP model was chosen for Laacher Hof AXXa (Ax) and Hanscheiderhof (HN).

Table B.8.1.2.1-23 Modelling endpoints of BYI 02960

Soil	Kinetic Model	BYI 02960			
		DT_{50} (d)	χ^2 err (%)	Visual Acceptability	t-prob
Ax	DFOP	141.5*	1.5528	Yes	k_1: <0.001 k_2: <0.001
HF	SFO	40.5	5.2961	Yes	<0.001
HN	DFOP	157.5*	2.0266	Yes	k_1: <0.001 k_2: <0.001
DD	SFO	55.1	3.8698	Yes	<0.001

*slow compartment of DFOP model

Remarks

The RMS agrees with the applicant to use the slow phase DT_{50} of the DFOP fits of Ax and HN soils and SFO DT_{50} of HF and DD soils as input parameters for environmental modelling.

STUDY IIA, 7.2.1/09

Characteristics

reference	:	Sur, R., Dorn, S. (2012)	study type	:	kinetic evaluation
year of execution	:	2012	incubation time	:	n.a
GLP statement	:	no	nominal concentration	:	n.a
guideline	:	FOCUS Kinectics (2006) US EPA, OPPTS 835	temperature	:	n.a.
test substance	:	n.a	DT_{50}	:	see results
purity	:	n.a	metabolites	:	see results
soils	:	n.a.	acceptability	:	**acceptable**

Summary

The present report is a supplement to Menke and Unold 2011 (study IIA, 7.1.1/04). It compiles additional raw data of the kinetic evaluation of the degradation of BYI 02960 and the major metabolite DFA in soil to determine modelling endpoints. The evaluation followed the recommendations of the FOCUS working group on degradation kinetics (FOCUS, 2006). The trigger endpoints of BYI 02960 were derived in the study IIA, 7.2.1/04 have been summarized Table B.8.1.2.1-21, the degradation kinetic for the metabolite is summarized in study IIA, 7.2.3/05.

The present kinetics evaluation followed the recommendations of the FOCUS working group on degradation kinetics for modelling endpoints.

Methods

The measured data were taken into account as reported (individual replicates). All experimental data sets and all data points were weighted equally (weighting factor 1).

For all residues used in the kinetic evaluation, the following procedure was applied. Any value below LOD directly before or after a value ≥ LOD was set to 0.5 LOD. The LOD was chosen as 0.5% of applied radioactivity (AR). The remaining values below LOD were excluded from the analysis. The values of the parent compound BYI 02960 at zero days after application (DAT 0) were set to the recovery rate. The reason for this modification is that exactly at the time of application only the parent compound can occur. If metabolites occur at DAT 0, those values are set to zero for the same reason. If recovery rates are >110% or <90%, the values from those sampling points are excluded from the analysis as well.

The kinetic evaluation was performed following the recommendations of FOCUS 2006 to derive environmental fate modelling. All data were equally weighted which corresponds to an absolute error model. Four kinetic models are considered in the testing procedure as recommended by FOCUS 2006: the single first-order (SFO), first-order multiple-compartment (FOMC, Gustafson-Holden), the double-first-order in parallel (DFOP) and the hockey-stick model (HS). The graphs of the model fits are shown in Figure B.8.1.2.1-11 to Figure B.8.1.2.1-13.

Conclusion

For modelling endpoints the SFO model was considered acceptable for Dollendorf II and Hoefchen am Hohenseh 4a and the DFOP model was chosen for Laacher Hof AXXa.

Table B.8.1.2.1-24 DT_{50} of BYI 02960 for modelling

Soil	Kinetic model	DT_{50} [d]	χ^2 err (%)	Visual acceptablity	t-prob
Dollendorf II (DD)	SFO	38.6	4.209	yes	<0.001
Laacher Hof AXXa (Ax)	DFOP	210.2*	1.556	yes	k_1 <0.001 k_2 <0.05
Hoefchen am Hohenseh 4a (HF)	SFO	43.0	6.166	yes	<0.001

*slow compartment of DFOP model

Remarks

The RMS agrees with the applicant to use the slow phase DT_{50} of the DFOP fit of Ax soil and the SFO DT_{50} for HF and DD soils as input parameters for environmental modelling.

STUDY IIA, 7.2.1/10

Characteristics

reference	:	Sur, R., Dorn, S. (2012)	study type	:	kinetic evaluation
year of execution	:	2012	incubation time	:	n.a
GLP statement	:	no	nominal concentration	:	n.a
guideline	:	FOCUS Kinectics (2006) US EPA, OPPTS 835	temperature	:	n.a.
test substance	:	n.a	DT_{50}	:	see results
purity	:	n.a	metabolites	:	see results
soils	:	n.a.	acceptability	:	**acceptable**

Summary

The present report is a supplement to Menke and Unold 2011 (IIA 7.1.1/05). It compiles additional raw data of the kinetic evaluation of the degradation of BYI 02960 and 6-CNA in soil to determine modelling endpoints. The evaluation followed the recommendations of the FOCUS working group on degradation kinetics (FOCUS, 2006). The trigger endpoints of BYI 02960 were derived in the study IIA, 7.2.1/05 have been summarized in Table B.8.1.2.1-21, the degradation kinetics for the metabolite is summarized in study IIA, 7.2.3/03.

Methods

For all residues used in the kinetic evaluation, any value below LOD directly before or after a value ≥ LOD was set to 0.5 LOD. The LOD was chosen as 0.5% of applied radioactivity (AR) which corresponds to 0.005 mg/kg. The values of the parent compound BYI 02960 at zero days after application (DAT = 0) were set to the recovery rate. The reason for this modification is that exactly at the time of application only the parent compound can occur. If metabolites occur at DAT 0, those values are set to zero for the same reason. If recovery rates are >110% or <90%, the values from those sampling points are excluded from the analysis.

For the kinetic modelling analysis the mathematical software tool MatLab with a user shell “KinGUI” were employed (MatLab 2005, Bramley, 2007). The kinetic evaluation was performed following the recommendations of FOCUS 2006 to derive degradation parameters for environmental fate modelling. Four kinetic models are considered in the testing procedure as recommended by FOCUS 2006: the single first-order (SFO), first-order multiple-compartment (FOMC, Gustafson-Holden), the double-first-order in parallel (DFOP) and the hockey-stick model (HS). The graphs of the model fits are shown in Figure B.8.1.2.1-14.

Conclusion

The degradation of BYI 02960 was best described with bi-phasic kinetics for modeling endpoints. The DFOP model was used to describe the kinetics for Hoefchen am Hohenseh 4a.

Table B.8.1.2.1-25 DT_{50} of BYI 02960 for modelling

Soil	Kinetic model	DT_{50} [d]	χ^2 err (%)	Visual acceptablity	t-prob
Hoefchen am Hohenseh 4a (HF)	DFOP	90.0*	1.7060	yes	$k_1 < 0.001$ $k_2 < 0.001$

*slow compartment of DFOP model

Remarks

The RMS agrees with the applicant to use the slow phase DT_{50} of the DFOP fit of HF soil as input parameters for environmental modelling.

STUDY IIA, 7.2.1/03

Characteristics

reference	:	Ripperger, R.J. (2011)	study type	:	kinetic evaluation
year of execution	:	2010	incubation time	:	n.a.
GLP statement	:	yes	nominal concentration	:	n.a.
guideline	:	FOCUS Kinectics (2006) US EPA, OPPTS 835 (2008)	temperature	:	n.a.
test substance	:	n.a.	DT_{50}	:	see results
purity	:	n.a.	metabolites	:	see results
soils	:	n.a.	acceptability	:	**acceptable**

The route of degradation and experimental design has been described under study IIA, 7.1.1/03. The degradation kinetics was described under study IIA, 7.2.1/03 for the trigger values. The selection of values for modelling input concluded that for both soils SFO was the appropriate model.

Table B.8.1.2.1-26 Synopsis of half-lives (modelling) calculated for BYI 02960

Soil	Kinetic model	DT_{50} [d]	χ^2 err (%)	Visual acceptablity	t-prob
Springfield	SFO	227.9	1.287	yes	< 0.001
Sanger	SFO	65.7	3.421	yes	< 0.001

Remarks

The RMS agrees with the applicant to use the SFO DT_{50} of Springfield and Sanger soils as input parameters for environmental modelling.

STUDY IIA, 7.2.1/06

Characteristics

reference	:	Shepherd, J.J. (2011)	study type	:	Kinetic evaluation
year of execution	:	2010	incubation time	:	n.a.
GLP statement	:	yes	nominal concentration	:	n.a.
guideline	:	FOCUS Kinectics (2006) US EPA, OPPTS 835 (2008)	temperature	:	n.a.
test substance	:	n.a.	DT_{50}	:	see results
purity	:	n.a.	metabolites	:	see results
soils	:	n.a.	acceptability	:	**acceptable**

The route of degradation and experimental design has been described under study IIA, 7.1.1/06. The degradation kinetics was described under study IIA, 7.2.1/06 for the trigger values. The selection of values for modelling input concluded that for both soils SFO was the appropriate model.

Table B.8.1.2.1-27 Synopsis of half-lives (modelling) calculated for BYI 02960

Soil	Kinetic model	DT_{50} [d]	χ^2 err (%)	Visual acceptablity	t-prob
Springfield	SFO	211	1.6488	yes	< 0.001
Sanger	SFO	62.2	3.016	yes	< 0.001

Remarks

The RMS agrees with the applicant to use the SFO DT_{50} of Springfield and Sanger soils as input parameters for environmental modelling.

Summary of Modelling Input DT_{50} values in aerobic soil studies

A summary of the DT_{50} values calculated for modelling input is given in Table B.8.1.2.1-28. Considering the moisture content of the soils during incubation the DT_{50} was corrected to pF2, for the studies performed on European soils no correction for soil moisture content was required, for the studies on US soils the correction factor as outlined below was employed.

Details of the soil moisture correction are given in the PEC reports included in the Annex III (Sur and Ellerich, 2012, M-427646-01-1, study IIA1, 9.7/01).

Table B.8.1.2.1-28 Overall summary of derived DT_{50} values for degradation of BYI 02960 in aerobic soil - modelling input parameters

Soil	Labled test substance	Study Reference	Kinetic model (best-fit)	Parent BYI 02960		
				DT50 (d) *	Moisture correction factor	DT_{50} at 20°C and pF2
Laacher Hof AXXa	[PYM] BYI 02960	IIA 7.2.1/07	DFOP	169.1	1	169.1
Laacher Hof AXXa	[FUR] BYI 02960	IIA 7.2.1/08	DFOP	141.5	1	141.5
Laacher Hof AXXa	[ETH] BYI 02960	IIA 7.2.1/09	DFOP	210.2	1	210.2
Laacher Hof AXXa (Ax) geometric mean						**171.3**
Höfchen Hohenseh	[PYM] BYI 02960	IIA 7.2.1/07	SFO	54.4	1	54.4
Höfchen Hohenseh	[FUR] BYI 02960	IIA 7.2.1/08	SFO	40.5	1	40.5
Höfchen Hohenseh	[ETH] BYI 02960	IIA 7.2.1/09	SFO	43.0	1	43.0
Höfchen Hohenseh	[PYR] BYI 02960	IIA 7.2.1/10	SFO	90.0	1	90.0
Hoefchen am Hohenseh (HF) 4a geometric mean						**54.0**
Hanscheiderhof	[PYM] BYI 02960	IIA 7.2.1/07	DFOP	157.5	1	157.5
Hanscheiderhof	[FUR] BYI 02960	IIA 7.2.1/08	DFOP	157.5	1	157.5
Hanscheiderhof (HN) geometric mean						**157.5**
Dollendorf II	[PYM] BYI 02960	IIA 7.2.1/07	SFO	60.1	1	60.1
Dollendorf II	[FUR] BYI 02960	IIA 7.2.1/08	SFO	55.1	1	55.1
Dollendorf II	[ETH] BYI 02960	IIA 7.2.1/09	SFO	38.6	1	38.6
Dollendorf II (DD) geometric mean						**50.4**
Springfield	[FUR] BYI 02960	IIA 7.2.1/03	SFO	227.9	0.79	179.7

Springfield	[PYR] BYI 02960	IIA 7.2.1/06	SFO	211.0	0.79	166.4
					Springfield geometric mean	**172.9**
Sanger	[FUR] BYI 02960	IIA 7.2.1/03	SFO	65.7	0.90	58.8
Sanger	[PYR] BYI 02960	IIA 7.2.1/06	SFO	62.2	0.90	55.5
					Sanger geometric mean	**57.1**
					Overall geometric mean **	**94.8**
					Overall median **	**107.3**

* For DFOP fits, DT_{50} from slow phase
** Geometric mean and median of the geometric means of the different soils

Studies performed with and for the aerobic degradation rate of metabolites are described here.

Rate of degradation; metabolite added as parent

Metabolite 6-CNA

The rate of degradation of the major metabolite 6-CNA has been investigated in 3 soils. The metabolite is also a common metabolite to the insecticide acetamiprid, this study has been evaluated in the context of the review report for the active substance acetamiprid, SANCO/1392/2001 – Final, 16 June 2004. Access to the study has been granted by the owners Nippon Soda (Nisso). Only a brief summary of the study is presented here. Due to changes in the requirements for kinetic evaluation a new kinetic evaluation of the study was performed and is described in study IIA, 7.2.3/02

STUDY IIA, 7.2.3/01

Characteristics

reference	:	Lowden, P., Oddy, A.M., Jones, M.K. (1997)	study type	:	aerobic degradation
year of execution	:	1997	incubation time	:	119 d
GLP statement	:	no	nominal concentration	:	130 g ha^{-1}
guideline	:	not specified	temperature	:	20°C
test substance	:	[14C]-IC-O (equivalent to 6-CNA)	DT50	:	see results
purity	:	radiochemical purity >988% 95.76% (batch no. CFQ 9603)	metabolites	:	not applicable
soils	:	three soils; sandy loam, clay, loam	acceptability	:	**acceptable**

Summary

The study was performed to examine the rate of degradation of [^{14}C]-IC-O (equivalent to 6-CNA) at 20± 2°C. Three soils were selected, which were a sandy loam (RPAL reference 97/03), a silty clay loam (RPAL reference 97/04) and a clay loam (RPAL reference 97/05). Portions of soil (approximately 100 or 500 g oven dried equivalent) were placed in uniquely labelled soil flasks and treated with [14C]-IC-O solution at a rate equivalent to 130 g ha^{-1}). The moisture content was adjusted to 45% of the maximum water holding capacity and maintained at that level throughout the study. The flasks were placed in a closed system maintained in the dark in which moist, carbon-dioxide-free air was continuously blown over the soils, then through ethylene glycol followed by potassium hydroxide. At intervals up to and including 119 days after treatment soil samples were extracted with solvent (methanol/ammonium acetate 2M (80:20 v/v)) or acetonitrile water (80:20 v/v) and the extracts containing sufficient radioactivity were examined by high performance liquid chromatography (HPLC).

The unextracted radioactivity was assayed using a combustion technique. Selected extracts were further examined by liquid chromatography followed by mass spectrometry (LC-MS). Parts for the original DAR from acetamiprid on metabolite [^{14}C]-IC-O are included.

Materials and Methods :

The rate of degradation of ^{14}C-IC-0 was studied in three soils (sandy loam, silty clay loam and clay loam, Table B.8.1.2.1-1) at 20 °C for a period of 119 days. Portions of soil were placed in labelled soil flasks and treated with ^{14}C-IC-0 solution at a rate equivalent to 130 g/ha. The soils were brought to 45% of their maximum moisture holding capacity by the addition of deionised water. Two flasks from each soil type were taken and extracted. At each sampling point two further flasks from each soil were taken along with their associated ethylene glycol and potassium hydroxide traps. The sampling points were 1, 3, 5, 7, 10, 14, 32, 60, and 119 days after treatment.

Table B.8.1.2.1-1 : Physico-Chemical Properties of Soils :

Soil Type	Sand (%)	Silt (%)	Clay (%)	Org. C (%)	pH	CEC [1)]	MWC (%)	Bio-mass [2)] (initial)	Bio-mass [2)] (final)
CL	25.2	50.7	24.2	1.7	7.2	23.2	63.1	230	338
SL	55.7	36.2	8.2	3.1	6.7	8.7	86.0	804	428
SiCL	19.9	49.5	30.6	3.8	7.8	78.9	106.7	1047	978

1) meq/100g

2) µg C per g of soil

The soil samples were extracted with solvent (methanol/ammonium acetate 2M, 80:20 v/v) or acetonitrilewater (80:20 v/v) and the extracts containing sufficient radioactivity were analysed by HPLC. The unextracted radioactivity was assayed using a combustion technique. Selected extracts were further examined by LC-MS.

Recoveries of applied radioactivity were generally good throughout the study with all but three of the individual flasks at each time point falling within the range 90 -110%. The major metabolite detected was carbon dioxide which was trapped in the potassium hydroxide traps and accounted for approximately 84% of the applied dose in the sandy loam, about 92% in the UK silty clay loam and 84% in the clay loam at the end of the study.

The percentage of material in the solvent extract declined with time from over 90% at day 0 to less than 2% at day 119. This fall was initially concomitant with an increase in unextractable residues and with the decrease in radioactivity associated with the soil as the compound was mineralised to carbon dioxide.

Results:

Recoveries of the applied radioactivity were within the range 90-110%. The non-extractable residues reached a maximum level which ranged in the three soils from 9% to 14% of the applied dose, by the end of the study (day 119). Respectively, volatile radioactivity reached maximum levels between 84 and 92%, at day 119, in the three soils used. Analysis showed that the only volatile product was carbon dioxide which was trapped in the potassium hydroxide traps. Chromatographic analysis of soil extracts showed the presence of IC-0 and two very minor metabolites. Spectroscopic examination of the extracts confirmed the presence of parent IC-0 (Table B.8.1.2.1-2). The two very minor compounds were transient metabolites and were both less than 3% maximum in all three soils, of applied dose.

Table B.8.1.2.1-2 : Summary table of IC-0 concentrations (% applied dose) based on HPLC results

	Days						
Soil Type	**0**	**1**	**3**	**5**	**7**	**10**	**14**
Sandy loam	100.06	90.38	60.42	35.6	12.76	8.06	2.5
Silty clay loam	94.58	75.54	47.84	19.11	8.57	1.78	1.47
Clay loam	93.35	89.36	76.68	62.39	44.32	20.21	9.7

The DT_{50} for IC-0 was calculated from the HPLC results, using the KIM computer program, and was found to be 3.5 days in the sandy loam, 2.9 days in the silty clay loam and 6.5 days in the clay loam. The DT_{90} was found to be 8.3 days in the sandy loam, 6.6 days in the silty clay loam and 13.3 days in the clay loam.

Chromatographic analysis of the soil extracts showed the presence of IC-O and two very minor metabolites. Spectroscopic examination of the extracts confirmed the presence of parent IC-O. The two very minor compounds were transient metabolites and were both less than 3% maximum in all three soils.

Remarks

The aerobic soil degradation study for 6-CNA is acceptable.

STUDY IIA, 7.2.3/02

Characteristics

reference	:	Sur, R., Dorn, S. (2012)	study type	:	kinetic evaluation
year of execution	:	2012	incubation time	:	n.a
GLP statement	:	no	nominal concentration	:	n.a
guideline	:	FOCUS Kinectics (2006) US EPA, OPPTS 835	temperature	:	n.a.
test substance	:	n.a	DT_{50}	:	see results
purity	:	n.a	metabolites	:	see results
soils	:	n.a.	acceptability	:	**acceptable**

Summary

The aerobic degradation of 6-chloronicotinic acid (6-CNA) was kinetically evaluated based on a laboratory study with three soils (Lowden et al. 1997, IIA 7.2.3/01). The evaluation followed the recommendations of the FOCUS working group on degradation kinetics and considered trigger and

modelling endpoints (Table B.8.1.2.1-29). Degradation of 6-CNA was best described by single first-order (SFO) kinetics in all cases.

Table B.8.1.2.1-29 Trigger and Modelling DT_{50} and DT_{90} of 6-CNA

Compound	Soil	Kinetic model	DT_{50} [d]	DT_{90} [d]
6-CNA	Aldham's Farm	SFO	2.9	9.7
	Flint Hall Farm	SFO	2.2	7.4
	Boarded Barns Farm	SFO	5.3	17.5

Materials and methods

The biotransformation of [^{14}C]-6-CNA, labelled in the 2,6 positions of the pyridine ring was studied in three soils from the UK.

Measured data of individual replicates were taken into account and all experimental data sets and all data points were weighted equally (weighting factor 1). For all residues used in the kinetic evaluation, any value below LOD directly before or after a value ≥ LOD was set to 0.5 LOD. The LOD was chosen as 3.88% of applied radioactivity (AR) which corresponds to 0.005 mg/kg. The remaining values below LOD were excluded from the analysis. The values of 6-CNA at zero days after application (DAT = 0) were set to the recovery rate because exactly at the time of application only the parent compound can occur. Values from sampling points with recovery rates >110% or <90% were excluded from the analysis.

Method

For the kinetic modelling analysis the mathematical software tool MatLab with a user shell "KinGUI" were employed (MatLab 2005, Bramley, 2007). The kinetic evaluation was performed following the recommendations of FOCUS 2006 to derive degradation parameters for comparison with triggers and for tier-1 PEC_{soil} calculation as well as for environmental fate modelling. Four kinetic models are considered in the testing procedure as recommended by FOCUS 2006: the single first-order (SFO), first-order multiple-compartment (FOMC, Gustafson-Holden), the double-first-order in parallel (DFOP) and the hockey-stick model (HS). Testing of DFOP and HS models was not required.

Results

The input parameters used for the model of the three soils are given in Table B.8.1.2.1-30. The graphical results of the parameter estimation are given in Figure B.8.1.2.1-17 to Figure B.8.1.2.1-19.

Table B.8.1.2.1-30 Percentage of 6-CNA used as input parameters for modelling

Soil (Soil type)	Aldham's Farm (Sandy loam)	Flint Hall Farm (Clay)	Boarded Barns Farm (Loam)
Days after treatment			
0	104.0	106.4	101.8
0	103.7	103.8	101.5
1	89.0	81.0	91.8
1	90.8	70.1	87.0
3	47.3	- [a]	78.0
3	73.5	50.0	75.3

5	- [a]	- [a]	64.7
5	33.0	23.0	60.1
7	14.1	9.4	42.0
7	33.0	7.8	46.6
10	8.4	1.9 [b]	19.6
10	7.1	1.9 [b]	20.1
14	1.9 [b]	- [c]	7.3
14	1.9 [b]	- [c]	12.1
32	- [c]	- [c]	1.9 [b]
32	- [c]	- [c]	1.9 [b]

[a] excluded because recovery blow 90%
[b] set to 0.5 LOD
[c] excluded because below LOD

Kinetics of test item degradation

The appropriate models for trigger and modelling endpoints were selected according to visual assessment and χ^2 error (see Table B.8.1.2.1-31). SFO provided the best fits for all soils.

Figure B.8.1.2.1-17 Results of the KinGUI parameter estimation for SFO (left) and FOMC (right) for Aldham's Frams soil

Figure B.8.1.2.1-18 Results of the KinGUI parameter estimation for SFO (left) and FOMC (right) for Flint Hall Farm soil

Figure B.8.1.2.1-19 Results of the KinGUI parameter estimation for SFO (left) and FOMC (right) for Boarded Barns Farm soil

Table B.8.1.2.1-31 χ^2 error, t-probability (significance of deg. rate) and visual acceptability of different kinetic models for 6-CNA; χ^2 error bold typed indicates the kinetic model chosen)

Soil	Criterion	SFO	FOMC
Aldham's Farm	X^2 err [%]	**8.5482**	9.3384
	Visual acceptability	Yes	Yes
	t-prob	<0.001	n.a.[a]
Flint Hall Farm	X^2 err [%]	**6.9131**	7.7869
	Visual acceptability	Yes	Yes
	t-prob	<0.001	n.a.[a]
Boarded Barns Farm	χ^2 err [%]	**8.5177**	9.1517
	Visual acceptability	Yes	Yes
	t-prob	<0.001	n.a.[a]

[a] not applicable

Conclusions

The current laboratory study demonstrated that 6-chloronicotinic acid (6-CNA) is well degradable under aerobic conditions in soils with a DT_{50} of 2.2 to 5.3 days. The values derived by SFO kinetics are acceptable for risk assessment of the persistence criteria for metabolite 6-CNA and as input for environmental exposure modelling.

Remarks

The kinetic evaluation of the soil degradation study of 6-CNA is acceptable and the values are used as trigger and model endpoints.

Rate of degradation; modelling of metabolite degradation from parent study

STUDY IIA, 7.2.3/03

Characteristics

reference	:	Sur, R., Dorn, S. (2012)	study type	:	kinetic evaluation
year of execution	:	2012	incubation time	:	n.a
GLP statement	:	no	nominal concentration	:	n.a
guideline	:	FOCUS Kinectics (2006) US EPA, OPPTS 835	temperature	:	n.a.
test substance	:	n.a	DT_{50}	:	see results
purity	:	n.a	metabolites	:	see results
soils	:	n.a.	acceptability	:	**acceptable**

Summary

The aerobic degradation of [pyridine-2,6-^{14}C] BYI 02960 was kinetically evaluated based on a laboratory study with one soil (Menke and Unold, 2011; IIA, 7.1.1/05). During the study the metabolite 6-chloronicotinic acid (6-CNA) reached a maximum percentage of 2.5% of AR at DAT 7. The present kinetics evaluation followed the recommendations of the FOCUS working group on degradation kinetics and considered trigger and modelling endpoints for the metabolite 6-CNA, the end-points for BYI 02960 were described under paragraph B.8.1.2.1 above.

Degradation of metabolite 6-CNA could be described with SFO kinetics. The pathway fit (FOMC parent / SFO metabolite) was considered acceptable for trigger endpoints while the pathway fit DFOP parent / SFO metabolite was used for modelling endpoints.

Table B8.1.2.1-32 Trigger DT_{50} and DT_{90} of 6-CNA

Compound	Soil	Kinetic model	DT_{50} [d]	DT_{90} [d]
6-CNA	Hoefchen am Hohenseh 4a	SFO	3.1	10.4

Table B.8.1.2.1-33 DT_{50} of 6-CNA for modelling

Compound	Soil	Kinetic model	DT_{50} [d]	Formation fraction pa→6-CNA
6-CNA	Hoefchen am Hohenseh 4a	SFO	3.0	0.2660

Materials and Methods

Materials

The biotransformation of [pyridine-2,6-^{14}C] BYI 02960 was studied in a one European soil (silt loam, Hoefchen am Hohenseh 4a) for 118 days under laboratory aerobic conditions at 20±1°C in the dark (Menke & Unold, 2011, IIA 7.1.1/05).

The test item in the extracts declined from 96.3% of AR at DAT 0 to 22.7% at the end of the study. Only minor transformation products (all mean values were ≤ 2.5% of AR) were detected in the extracts, the maximum amount was detected on DAT 7 with 2.5% of AR and was identified as 6-CNA by co-chromatography. Towards the end of the study, the amounts decreased below the detection limit. Measured data of individual replicates were taken into account and all experimental data sets and all data points were weighted equally (weighting factor 1).

For all residues used in the kinetic evaluation, any value below LOD directly before or after a value ≥ LOD was set to 0.5 LOD. The LOD was chosen as 0.5% of applied radioactivity (AR) which corresponds to 0.005 mg/kg. The values of the parent compound BYI 02960 at zero days after application (DAT = 0) were set to the recovery rate. The reason for this modification is that exactly at the time of application only the parent compound can occur. If metabolites occur at DAT 0, those values are set to zero for the same reason. If recovery rates are >110% or <90%, the values from those sampling points are excluded from the analysis.

Methods

Determination of Degradation Kinetics

For the kinetic modelling analysis the mathematical software tool MatLab with a user shell "KinGUI" were employed (MatLab 2005, Bramley, 2007). The kinetic evaluation was performed following the recommendations of FOCUS 2006 to derive degradation parameters for comparison with triggers and for tier-1 PEC_{soil} calculation as well as for environmental fate modelling. Four kinetic models are considered in the testing procedure as recommended by FOCUS 2006: the single first-order (SFO), first-order multiple-compartment (FOMC, Gustafson-Holden), the double-first-order in parallel (DFOP) and the hockey-stick model (HS).

Results

The input parameters used for the model of the three soils are given in Table B.8.1.2.1-33. The graphical results of the parameter estimation are given in Figure B.8.1.2.1-20 to Figure B.8.1.2.1-21.

Figure B.8.1.2.1-20 Results of the KinGUI parameter estimation for FOMC for BYI 02960 (left) and SFO for 6-CNA (right) used for trigger values for 6-CNA

Figure B.8.1.2.1-21 Results of the KinGUI parameter estimation DFOP for BYI 02960 (left) and SFO for 6-CNA (right) used for modelling values for 6-CNA

Table B.8.1.2.1-34 Percentage of BYI 02960 and 6-CNA used as input parameters for modelling

HF (silt loam)	BYI 0260 % AR	6-CNA % AR	BYI 0260 % AR	6-CNA % AR
Days after treatment	**Replicate 1**	**Replicate 1**	**Replicate 2**	**Replicate 2**
0 *	100.7	0.0 [a]	99.3	0.0 [a]
1	95.5	0.3 [b]	95.2	0.3 [b]
4	89.3	1.7	87.7	1.7
7	79.3	2.4	77.6	2.6
14	70.7	2.1	71.1	1.8
29	53.6	1.0	53.4	0.9
48	39.4	0.5	42.4	0.6
61	34.1	0.3 [b]	33.8	0.3 [b]
90	27.0	- [c]	27.7	- [c]
117	22.1	- [c]	23.2	- [c]

* total recovery of radioactivity used as input parameter for DAT 0 sample
[a] set to zero
[b] set to 0.5 LOD
[c] excluded because below detection limit

Kinetics of TEST Item Degradation

The appropriate models for trigger and modelling endpoints were selected according to visual assessment and χ^2 error and are presented in in Table B.8.1.2.1-35 for the metabolite 6-CNA.

Table B.8.1.2.1-35 χ^2 error, t-probability (significance of deg. rate) and visual acceptability of different kinetic models for 6-CNA in soil Hoefchen am Hohenseh

Compound	Criterion	FOMC parent/ SFO metabolite	DFOP parent/ SFO metabolite
6-CNA	χ^2 err [%]	16.9529	15.3680
	Visual acceptability	Yes	Yes
	t-prob	0.0937	<0.001
	Formation fraction 6-CNA	0.2534	0.2660
	Endpoint	Trigger	Modelling

Conclusions

The current laboratory study demonstrated that 6-chloronicotinic acid is very well degradable (DT_{50}, 3 days) under aerobic conditions in soils.

Remarks

The RMS considers the endpoint obtained acceptable and reliable. Altough, the χ^2 error was slightly above 15% this was still considered acceptable by the RMS in case of a metabolite and, thus, the results may be used for modelling endpoints.

STUDY IIA, 7.2.3/04

Characteristics

reference	:	Shepherd, J.J. (2011)	study type	:	soil degradation including kinetic evaluation
year of execution	:	2010	incubation time	:	n.a.
GLP statement	:	yes	nominal concentration	:	n.a.
guideline	:	FOCUS Kinectics (2006) US EPA, OPPTS 835 (2008)	temperature	:	n.a.
test substance	:	n.a.	DT50	:	see results
purity	:	n.a.	metabolites	:	see results
soils	:	n.a.	acceptability	:	**acceptable**

The route of degradation and experimental design has been described in study IIA, 7.1.1/06. A kinetic evaluation of the degradation and formation fraction of 6-CNA was performed.

Table B.8.1.2.1-36 Summary of the kinetic evaluation (trigger and modelling values according to FOCUS)

Soil		6-CNA		
		DT_{50} (d)	DT_{90} (d)	Formation fractions
Sanger	Trigger (DFOP-SFO)	36.6	121	0.5272
Sanger	Modelling (SFO-SFO)	24.8	82.4	0.6936

Materials and Methods

This study is comprehensively described within report IIA 7.1.1/06.

Determination of Degradation Kinetics

In order to determine the best-fit kinetic model for the degradation of 6-CNA the models SFO parent and SFO for 6-CNA plus DFOP for parent and SFO for 6-CNA were used. The goodness of fit was assessed by visual inspection and an error criterion based on a chi-square (χ^2) significance test. In addition to these, the coefficient of determination (r^2) was calculated and reported by the kinetics modelling tool.

Results

The input parameters used for the model of the soil is given in Table B.8.1.2.1-37. The graphical results of the parameter estimation are given in Figure B.8.1.2.1-22 to Figure B.8.1.2.1-23.

Table B.8.1.2.1-37 Percentage of BYI 02960 and 6-CNA used as input parameters for modelling

Sanger (Sandy loam)	BYI 0260 % AR	6-CNA % AR	BYI 0260 % AR	6-CNA % AR
Days after treatment	**Replicate 1**	**Replicate 1**	**Replicate 2**	**Replicate 2**

0	98.5	0.0	98.7	0.0
3	96.2	0.0	94.3	1.3
7	87.4	4.3	87.4	3.3
14	84.6	6.7	83.1	7.0
28	67.4	13.0	67.2	11.3
60	47.6	16.2	46.0	18.1
92	34.8	12.9	34.8	11.8
120	29.5	7.2	30.5	9.3

Figure B.8.1.2.1-22 Results of the KinGUI parameter estimation for DFOP for BYI 02960 and SFO for 6-CNA used for trigger values for 6-CNA

Figure B.8.1.2.1-23 Results of the KinGUI parameter estimation SFO for BYI 02960 and SFO for 6-CNA used for modelling values for 6-CNA

Kinetics of test Item Degradation

For the observed degradation of BYI 02960 and the formation and decline of 6-CNA in the Sanger sandy loam soil, SFO-SFO and DFOP-SFO kinetic models were used. Both approaches gave an acceptable fit to the measured data. However, the visual examination of the observed data and models, as well as the χ^2 scaled error statistic and the coefficient of determination (r^2), showed that DFOP-SFO is the best-fit model.

The parameter values from the best-fit DFOP-SFO model should be used with care for extrapolation purposes (e.g. for environmental modelling), since the DFOP parameters are highly inter-correlated (r^2 > 0.97 between k_1, k_2 and g) and the parameter estimates have large standard deviations. The parameter values from the SFO –SFO model are more robust for extrapolation purposes, despite the slightly worse fit of the model to the measured data.

Table B.8.1.2.1-38 Summary of the kinetic evaluation formation and decline of 6-CNA

Soil	Kinetic model		6-CNA					
	Parent	6-CNA	DT50 (d)	DT90 (d)	Chi^2 value	t-prob	Visual assessment	Formation fraction
Sanger	DFOP	SFO	36.6*	121	13.8	<0.001	+	0.5272
	SFO	SFO	24.8#	82.4	15.1	<0.001	+	0.6936

* best fit trigger value
# modelling value

Conclusions

The current laboratory study demonstrated that 6-CNA is degraded in soil with a best fit DT_{50} of 36.6 days and a modelling DT_{50} (non-normalised) of 24.8 days.

Remarks

The RMS considers the endpoints obtained acceptable and reliable. Altough, the χ^2 error was slightly above 15% this was still considered acceptable for metabolites and, thus, the results may be used for modelling endpoints.

STUDY IIA, 7.2.3/05

Characteristics

reference	:	Sur, R., Dorn, S. (2012)	study type	:	kinetic evaluation
year of execution	:	2012	incubation time	:	n.a
GLP statement	:	no	nominal concentration	:	n.a
guideline	:	FOCUS Kinectics (2006) US EPA, OPPTS 835	temperature	:	n.a.
test substance	:	n.a	DT_{50}	:	see results
purity	:	n.a	metabolites	:	see results
soils	:	n.a.	acceptability	:	**acceptable**

Summary

The present report is a supplement to Menke and Unold 2011, summarized in study IIA, 7.1.1/04. It compiles additional raw data of the kinetic evaluation of the degradation of BYI 02960 and the major metabolite DFA in soil to determine modelling endpoints. The evaluation followed the recommendations of the FOCUS working group on degradation kinetics (FOCUS, 2006). The degradation parameters for the metabolite are summarized below.

The present kinetics evaluation followed the recommendations of the FOCUS working group on degradation kinetics for modelling endpoints.

Table B.8.1.2.1-39 Trigger DT_{50} and DT_{90} of DFA

Compound	Soil	Kinetic model	DT_{50} [d]	DT_{90} [d]
DFA	Dollendorf II	SFO	44.9	149.0
	Laacher Hof AXXa	SFO	73.6	244.5
	Hoefchen am Hohenseh 4a	SFO	67.4	223.9

Table B.8.1.2.1-40 DT_{50} of DFA for modelling

Compound	Soil	Kinetic model	DT_{50} [d]	Formation fraction pa→DFA
DFA	Dollendorf II	SFO	32.0	0.9087
	Laacher Hof AXXa	SFO	73.6	0.5896
	Hoefchen am Hohenseh 4a	SFO	37.8	1.0000
		Geometric mean DT_{50}	**44.7**	
		Arithmetic mean formation fraction		**0.8328**

Materials and methods

Materials

This study is comprehensively described within report IIA 7.1.1/04

Measured data of individual replicates were taken into account and all experimental data sets and all data points were weighted equally (weighting factor 1).

For all residues used in the kinetic evaluation, the following procedure was applied. Any value below LOD directly before or after a value ≥ LOD was set to 0.5 LOD. The LOD was chosen as 0.5% of applied radioactivity (AR). The remaining values below LOD were excluded from the analysis. The values of the parent compound BYI 02960 at zero days after application (DAT 0) were set to the recovery rate. The reason for this modification is that exactly at the time of application only the parent compound can occur. If metabolites occur at DAT 0, those values are set to zero for the same reason. If recovery rates are >110% or <90%, the values from those sampling points were excluded from the analysis.

Methods

Determination of Degradation Kinetics

For the kinetic modelling analysis the mathematical software tool MatLab with a user shell “KinGUI” were employed (MatLab 2005, Bramley, 2007). The kinetic evaluation was performed following the recommendations of FOCUS 2006 to derive degradation parameters for comparison with triggers and for tier-1 PEC_{soil} calculation as well as for environmental fate modelling. Four kinetic models are considered in the testing procedure as recommended by FOCUS 2006: the single first-order (SFO), first-order multiple-compartment (FOMC, Gustafson-Holden), the double-first-order in parallel (DFOP) and the hockey-stick model (HS).

Results

The input parameters used for the model of the three soils are given in Table B.8.1.2.1-41 to Table B.8.1.2.1-43. The graphical results of the parameter estimation are given in Figure B.8.1.2.1-24 to Figure B.8.1.2.1-28.

Table B.8.1.2.1-41 Percentage of BYI 02960 and DFA used as input parameters for modelling in DD soil

DD (clay loam)	BYI 0260 % AR	DFA % AR	BYI 0260 % AR	DFA % AR
Days after treatment	**Replicate 1**	**Replicate 1**	**Replicate 2**	**Replicate 2**
0	99.2	0.0	99.2	0.0
1	92.5	0.6	93.2	0.9
3	88.5	3.4	90.0	3.1
7	82.0	8.2	82.2	7.3
14	73.9	15.5	72.5	15.5
30	52.2	25.8	52.2	26.9
45	37.9	30.4	39.4	30.0
62	30.7	28.6	32.0	27.3
90	19.9	24.4	22.9	22.5
118	17.3	17.2	18.1	16.7

Table B.8.1.2.1-42 Percentage of BYI 02960 and DFA used as input parameters for modelling in Ax soil

Ax (loamy sand)	BYI 0260 % AR	DFA % AR	BYI 0260 % AR	DFA % AR
Days after treatment	**Replicate 1**	**Replicate 1**	**Replicate 2**	**Replicate 2**
0	98.6	0.0	101.0	0.0
1	9.3	0.8	95.3	0.5
4	89.1	3.1	89.1	2.9
7	84.0	6.7	83.5	6.3
14	78.1	12.6	75.2	15.5
29	56.9	17.3	62.3	17.3
48	52.2	23.1	55.4	20.8
61	48.8	20.1	50.0	19.2
90	44.4	16.1	44.6	16.2
117	39.8	15.9	39.3	16.8

Table B.8.1.2.1-43 Percentage of BYI 02960 and DFA used as input parameters for modelling in HF soil

HF (silt loam)	BYI 0260 % AR	DFA % AR	BYI 0260 % AR	DFA % AR
Days after treatment	**Replicate 1**	**Replicate 1**	**Replicate 2**	**Replicate 2**
0	101.3	0.0	100.1	0.0
1	96.0	0.9	92.9	1.0
4	88.4	5.1	88.6	4.8
7	82.0	8.3	78.2	9.0
14	72.8	17.2	71.0	16.9
29	55.8	25.8	55.5	25.8
48	38.4	35.1	41.2	32.7
61	32.9	32.1	32.6	34.0
90	28.2	26.7	25.8	29.4
117	22.8	23.9	23.2	23.8

Figure B.8.1.2.1-24 Results of the KinGUI parameter estimation for DFOP for BYI 02960 (top) and SFO for DFA (bottom) used for trigger values for DFA in DD soil

Figure B.8.1.2.1-25 Results of the KinGUI parameter estimation for SFO for BYI 02960 (top) and SFO for DFA (bottom) used for modelling values for DFA in DD soil

Figure B.8.1.2.1-26 Results of the KinGUI parameter estimation for DFOP for BYI 02960 (top) and SFO for DFA (bottom) used for trigger and modelling values for DFA in Ax soil

Figure B.8.1.2.1-27 Results of the KinGUI parameter estimation for FOMC for BYI 02960 (top) and SFO for DFA (bottom) used for trigger values for DFA in HF soil

Figure B.8.1.2.1-28 Results of the KinGUI parameter estimation for SFO for BYI 02960 (top) and SFO (bottom) for DFA used for modelling values for DFA in HF soil

Kinetics of test Item Degradation

The appropriate models for trigger and modelling endpoints were selected according to visual assessment and χ^2 error and are presented in the following table.

Table B.8.1.2.1- 44 χ^2 error, t-probability (significance of degradation rate) and visual acceptability of different kinetic models for DFA in soil

Soil	Criterion	BYI 02960-DFA	
		DFOP or FOMC** parent/ SFO metabolite	SFO parent/ SFO metabolite
Dollendorf II	χ2 err [%]	5.412	5.169
	Visual acceptability	Yes	Yes
	t-prob	<0.001	<0.001
	Formation fraction DFA	0.7477	0.9087
	Endpoint	Trigger	Modelling
Laacher Hof AXXa	χ2 err [%]	8.359	n.r.
	Visual acceptability	Yes	n.r.
	t-prob	<0.001	n.r.
	Formation fraction DFA	0.5896	n.r.
	Endpoint	Trigger / Modelling	n.r.
Hoefchen am Hohenseh 4a	χ2 err [%]	7.394	4.033
	Visual acceptability	Yes	Yes
	t-prob	<0.001	<0.001
	Formation fraction DFA	0.7359	1.0000
	Endpoint	Trigger	Modelling

n.r. = relevant; ** for parent DFOP kinetic model used for DD and Ax soil and FOMC kinetic model used for HF soil

Conclusions

The current laboratory study demonstrated that difluoroacetic acid is degradable under aerobic conditions in soils. The tigger endpoints obtained for DFA, where parent was dosed, were obtained from DFOP (DD and Ax soil) or FOMC (HF soil) kinetic fit for the parent and SFO kinetic fit for DFA. The modelling endpoints obtained for DFA, where parent was, dosed were obtained from SFO (DD and HF soil) or DFOP (Ax) kinetic fit for the parent and SFO kinetic fit for DFA.

Remarks

The RMS considers the endpoints obtained for trigger and modelling acceptable and reliable.

B.8.1.2.2 Field studies (Annex IIA 7.1.1.2.2; Annex IIIA 9.1.1.2)

STUDY IIA, 7.3.1/01

Characteristics

reference	:	Heinemann, O., 2011	location	:	Germany three sites, UK, Italy, Spain
year of execution	:		dose	:	250 g as/ha
GLP statement	:	yes	residues	:	DFA
guideline	:	Incl. SETAC (1995)	DT_{50}	:	see results
test substance	:	BYI 02960 SL 200	DT_{90}	:	see results
purity	:		acceptability	:	**acceptable**
soils	:	sandy loam, clay loam, silt loam, clay loam, loam			

Summary

Soil dissipation of BYI 02960 under European field conditions was investigated after application of BYI 02960 SL 200 on bare soil plots at sites in Monheim, Burscheid and Hanscheider Hof (all Germany), Great Chishill (United Kingdom), Albaro (Italy), and Vilobi d'Onyar (Spain). An application was also made in Chilly (Northern France), however the trial was aborted due to heavy rain shortly after application. The sites are located in the ecoregions Northern and Southern Europe.

BYI 02960 SL 200 was sprayed once pre-emergence onto the soil at 1.25 L/ha, corresponding to 250 g BYI 02960/ha in 180 to 320 sqm plots. The measured initial zero-time concentrations corresponded from 91% to 107% of the intended dose rate (250 g/ha). The control plots were at least 5 m away from the treated plots.

Soil samples were taken (nominally) at 0 to 540 days post-application to a maximum depth of 100 cm and analyzed for BYI 02960 and its soil metabolite DFA (difluoroacetic acid).

Soil samples of 20 g were extracted in a microwave extractor with 50 mL of acetonitrile/water (4/1, v/v). Possible matrix effects were eliminated by using an internal standard solution of isotopically labeled reference items which was added to the extracts of samples. Then a subsample was filtrated to remove fine particles of the soil. Identification and quantitation of the test items was done by high performance liquid chromatography using MS/MS detection in the Multiple Reaction Monitoring mode. The method was validated using three different soils. The limit of quantitation (LOQ) was 5.0 µg/kg, and the limit of detection (LOD) was 1.5 µg/kg for both analytes.

At Monheim (Germany), the amount of BYI 02960 determined in 0-10 cm at day 0 was 237 g/ha, which is 95% of the nominal application rate. BYI 02960 declined from 237 g BYI 02960/ha in soil at day 0 to 35.6 g/ha at day 545, corresponding to 15% of the applied amount. BYI 02960 had a DT_{50} of 41.0 days, and a DT_{90} of 749 days.

At Great Chishill (United Kingdom), the amount of BYI 02960 determined in 0-10 cm at day 0 was 236 g/ha, which is 94% of the nominal application rate. BYI 02960 declined from 236 g BYI 02960/ha in soil

at day 0 to 70.2 g BYI 02960/ha in soil at day 552, corresponding to 29.8% of the applied amount. BYI 02960 had a DT_{50} of 251 days, and a DT_{90} of >1000 days.

At Burscheid (Germany), the amount of BYI 02960 determined in 0-10 cm at day 0 was 245 g/ha, which is 98% of the nominal application rate. BYI 02960 declined from 245 g BYI 02960/ha in soil at day 0 to 17.9 g/ha at day 540, corresponding to 7.3% of the applied amount. BYI 02960 had a DT_{50} of 42.8 days, and a DT_{90} value of 484 days.

At Albaro (Italy), the amount of BYI 02960 determined in 0-10 cm at day 0 was 268 g/ha, which is 107% of the nominal application rate. BYI 02960 declined from 268 g BYI 02960/ha in soil at day 0 to 7.8 g/ha at day 547, corresponding to 2.9% of the applied amount. BYI 02960 had a DT_{50} value of 8.3 days, and a DT_{90} value of 279 days.

At Vilobi d'Onyar (Spain), the amount of BYI 02960 determined in 0-10 cm at day 0 was 240 g/ha, which is 96% of the nominal application rate. BYI 02960 declined from 240 g BYI 02960/ha in soil at day 0 to 8.8 g/ha at day 533, corresponding to 3.7% of the applied amount. BYI 02960 had a DT_{50} of 22.6 days, and a DT_{90} value of 215 days.

At Hanscheider Hof (Germany), the amount of BYI 02960 determined in 0-10 cm at day 0 was 229 g/ha, which is 91% of the nominal application rate. BYI 02960 declined from 229 g BYI 02960/ha in soil at day 0 to 20.5 g/ha at day 540, corresponding to 9.0% of the applied amount. BYI 02960 had a DT_{50} of 39.0 days, and a DT_{90} value of 579 days.

In general, BYI 02960 residues remained in the upper 0-20 cm soil layer and only very small amounts (<LOQ) were translocated to a maximum depth of 30 cm. At one site (Vilobi d'Onyar, Spain), BYI 02960 residues just above the LOQ were found at day 13 up in the 20-30 cm soil layer. BYI 02960 dissipated from soil at all test sites with DT_{50} values ranging from 8.3 to 251 days.

The metabolite BYI 02960-DFA appeared in the upper soil layer 0-20 cm in amounts of up to 17.4 g/ha soil. However, the metabolite declined to values of max. 7.9 g/ha soil towards the end of the study (in Burscheid, Germany). An overview of the results for parent compound is given in Table B.8.1.2.2-01.

Table B.8.1.2.2-01 Results synopsis for BYI 02960

Location: Trial No.	Soil type (USDA)	Kinetic model	DT50 [d]	DT90 [d]
Monheim (Germany): 09-2702-01	Sandy loam (0-100 cm)	DFOP	41.0	749
Great Chishill (United Kingdom): 09-2702-02	Clay loam (0-100 cm)	DFOP	251	>1000
Burscheid (Germany): 09-2702-03	Silt loam (0-75 cm), Loam (75-100 cm)	DFOP	42.8	484
Albaro (Italy): 09-2702-05	Clay loam (0-30 cm), Loam (30-75 cm), Sandy loam (75-100 cm)	DFOP	8.3	279
Vilobi d'Onyar (Spain):	Loam (0-30 cm),	DFOP	22.6	215

09-2702-06	Sandy loam (30-75 cm), Sandy clay loam (75-100 cm)			
Hanscheider Hof (Germany): 09-2702-07	Loam(0-100 cm)	DFOP	39.0	579

Materials and Methods

Materials

1. Test item: BYI 02960 SL 200;Content of active substance: 200 g/L (soluble concentrate); Specification No: 102000021884, Batch No: 2009-001253

2. Trial locations: The study was conducted at six sites, typical for the ecoregions Southern and Northern Europe (Table B.8.1.2.2-02). At an additional site (Chilly, Northern France) was abandoned because of a heavy rain event of 25 mm occurring 7 hrs after application. The remaining six sites were neither subjected to erosion, flooding nor to run-off. A trial consisted of a treated and an untreated plot at each test site. The control plots were at least 5 m away from the treated plots.

Methods

1. Application: The representative formulation BYI 02960 SL 200, containing 200 g/L BYI 02960 was sprayed once with an application rate of 1.25 L/ha and 300 L water/ha, corresponding to 250 g BYI 02960 per hectare, using a knapsack sprayer.

2. Meteorological data: Air temperature, precipitation including irrigation, and sunshine data were recorded during the field tests. Temperatures were within the range of the long term average for all trial sites.

The overall precipitation was normal in all trials with some exceptions: In trial 09-2702-01 (Monheim, Germany) April and June 2010 were very dry, whereas May, July and August to September 2010 were very wet. In trial 09-2702-02 (Great Chishill, UK) July 2009 was wet but September and October 2009 was dry and April to July 2010 was dry but August 2010 was very wet compared to the long-term average. In trial 09-2702-03 (Burscheid, Germany) May to September 2009 was dry but October to December 2009 wetter compared to the long-term average. In addition, April 2010 was very dry compared to the long-term average. In trial 09-2702-05 (Albaro, Italy) the situation was very special during the trial period: May 2009 and April 2010 was very dry and December 2009 to March 2010, May/June 2010 and August to December 2010 was wet compared to the long-term average. In trial 09-2702-06 (Vilobi d'Onyar, Spain) the summer in 2009 and 2010 was dry compared to the long-term average, except for May 2010 which was wet compared to the long-term average. In trial 09-2702-07 (Hanscheider Hof, Germany) May to September 2009 was dry but October to December 2009 wetter compared to the long-term average, but April 2010 was very dry compared to the long-term average.

3. Sampling and sample processing: Before application samples were taken from control plots to a depth of 10 cm with a soil piercer (Ø 50 mm) and immediately after application from treated plots. All

samples at day 0 consisted of 20 soil cores. All subsequent samplings were performed using a ”Wacker Hammer” (Ø 48 to 50.0 mm). At each sampling interval 20 cores from the treated plots were taken, randomly distributed over the plots. From control plots 10 to 20 soil cores were taken.

In all treated plots the samples were taken to a maximum depth of 100 cm on the following days: 0 (post-application; each 0-10 cm depth), 6-7 (0-30 cm depth), 12-14, 28-30, 60-68 (each 0-50 cm depth), 90-96, 111-128, 177-204, 354-394, 432-478, 533-552 (each 0-100 cm depth) after treatment. From the control plot samples were taken on day 0 before application and 354-394 and 533-552 days after application. In addition, for soil characterisation samples were taken on day -3 (i.e. prior) to day 7 after application from the treated plots to a depth of 100 cm.

Table B.8.1.2.2-02 Location, site description and climatic data at the study sites

Location Trial Number		**Monheim, Germany 09-2702-01**	**Great Chishill, UK 09-2702-02**	**Burscheid, Germany 09-2702-03**	**Albaro, Italy 09-2702-05**	**Vilobi d'Onyar, Spain 09-2702-06**	**Hanscheider Hof, Germany, 09-2702-07**
Designation		Monheim, Germany, Plot 712/718, (Laacher Hof)	Great Chishill, United Kingdom, Chishill Orchard Farm Block 5.1	Burscheid, Germany, Plot 4011 (Hoefchen)	Albaro, Italy, Az. Marcomcini Sandro	Vilobi d'Onyar, Spain, Camps de Can Martorell Plot 8, Parcel 54	Burscheid, Germany Hanscheider Hof, Plot 606
Plot Size [sqm]		255	320	225	180	300	225
Geographic coordinates	Latitude	51° 06' N	52° 1'46.70" N	51° 04' N	45°21'12.92" N	41°53'15.09" N	51° 04' N
	Longitude	06° 54' E	0° 4'19.25"E	07° 08' E	11°11'30.32" E	2°44'23.65"E	07° 08' E
	Country	Germany	United Kingdom	Germany	Italy	Spain	Germany
	Ecoregion	Northern EU	Northern EU	Northern EU	Southern EU	Southern EU	Northern EU
Distance from weather station used for climatic measurements		At trial location	At trial location (sunshine hours 43 km)	At trial location	10 km (soil temp. from trial site)	At trial location (sunshine hours 13 km)	At trial location
Meteorological conditions compared to long-term average within normal levels (Yes/No)		Yes	Yes Winter 2009 colder, May and July drier, August wetter than compared to long-term average	Yes Summer 2009 drier and autumn 2009 wetter compared to the long-term average	No In 2009 dry May and August, wet winter, wet from May to December 2010 compared to the long-term average	No Dry 2009 compared to the long-term average.	Yes Dry summer 2009 and wet winter 2009 and dry April and June 2010 but wet August 2010 compared to the long-term average
Soil type (USDA)	Soil depth [cm]						
	0-30	Sandy loam	Clay loam	Silt loam	Clay loam	Loam	Loam
	30-50	Sandy loam	Clay loam	Silt loam	Loam	Sandy loam	Loam
	50-75	Loamy sand	Clay loam	Silt loam	Loam	Sandy loam	Loam
	75-100	Loamy sand	Clay loam	Loam	Sandy loam	Sandy clay loam	Loam

The soil samples were deep-frozen within 24 hours, stored and shipped to the test facility BCS, Monheim at maximum -18°C. The cores with the frozen field samples were cut into 10 cm segments. The control samples or the treated samples of each horizon separately (laboratory samples) were milled in a hammer mill and carefully homogenized. A portion of each of the homogenized laboratory samples was transferred into polystyrene boxes (analytical samples) and stored at or below -18 °C until preparation for analysis.

4. Analytical procedure: The modification M001 to the analytical method 01074 was developed for the determination of BYI 02960 and its metabolites in soil. Soil samples of 20 g are extracted in a microwave extractor with 50 mL of a mixture of acetonitrile/water (4/1, v/v). Possible matrix effects of BYI 02960 and its metabolite DFA are eliminated by using an internal standard solution of isotopically labeled reference items. This solution is added to the sample solutions after extraction. Then a subsample is filtrated to remove fine particles of the soil. Identification and quantitation of the test items is done by high performance liquid chromatography using MS/MS detection in the MRM mode (Multiple Reaction Monitoring mode). The method was validated using three different soils. The limit of quantitation (LOQ) for each single analyte is 5.0 µg/kg in soil. The limit of determination (LOD) for each single analyte is 1.5 µg/kg. Recoveries for each fortification level were in an acceptable range (70 - 110%).

Results

Residue Concentrations

The measured initial mean concentrations (n = 4) of BYI 02960 for the test sites were 237 g/ha (Monheim, Germany), 236 g/ha (Great Chishill, UK), 245 g/ha (Burscheid, Germany), 268 g/ha (Albaro, Italy), 240 g/ha (Vilobi d'Onyar, Spain), and 229 g/ha (Burscheid/Hanscheider Hof, Germany) representing 91 to 107 % of the intended dose rate.

Dissipation of BYI 02960 varied at the different test locations. At one site each a very fast and a rather slowly dissipation was observed, whereas the other four sites were similarly in a moderate range of dissipation of the residues. In general the dissipation of BYI 02960 showed a biphasic behavior.

After treatment, BYI 02960 dissipated in a first step very fast within one month followed by a second more slowly step until the end of the study to residue levels of 2.9 to 29.8 % of the total nominal applied amount. Mean residue levels of BYI 02960 reached 35.6 g/ha on day 545 in Monheim, Germany (15% of applied), 70.2 g/ha on day 552 in Great Chishill, UK (29.8% of applied), 17.9 g/ha on day 540 in Burscheid, Germany (7.3% of applied), 7.8 g/ha on day 547 in Albaro, Italy (2.9% of applied), 8.8 g/ha on day 533 in Vilobi d'Onyar, Spain (3.7% of applied), and 20.5 g/ha on day 540 in Hanscheider Hof, Germany (9.0% of applied). In general, BYI 02960 residues remained in the upper 0-20 cm soil layer and only very small amounts could be detected to a maximum depth of 30 cm.

The metabolite DFA appeared in the upper soil layer 0-20 cm in amounts of up to 17.4 g/ha soil. However, the metabolite declined to values of max. 7.9 g/ha soil towards the end of the study.

Kinetics Analysis

Based on the chi^2 error criterion and visual assessment the best fit kinetic model was chosen for the evaluation of the dissipation time. The calculated data are based on the quantifiable residues reported for the entire soil profile in [g/ha], and results are presented in Table B.8.1.2.2-03.

Table B.8.1.2.2-03 BYI 02960 dissipation values for field studies

Location and Trial No.	Kinetic	Parent BYI 02960			
		DT_{50}	DT_{90}	Visual	Chi^2 error
	model	[d]	[d]	Assessment	[%]
Monheim, Germany 09-2702-01	SFO	105	349	--	19.3
	FOMC	46.6	>1000	+	8.6
	DFOP *	**41.0**	**749**	**+**	**7.5**
Great Chishill, United Kingdom 09-2702-02	SFO	353	>1000	--	11.9
	FOMC	206	>1000	--	11.7
	DFOP *	**251**	**>1000**	**--**	**7.5**
Burscheid, Germany 09-2702-03	SFO	83.0	276	o	14.8
	FOMC	43.4	999	+	7.3
	DFOP *	**42.8**	**484**	**+**	**6.3**
Albaro, Italy 09-2702-05	SFO	28.8	95.8	--	29.4
	FOMC	9.9	465	o	10.3
	DFOP *	**8.3**	**279**	**+**	**7.1**
Vilobi d'Onyar, Spain 09-2702-06	SFO	45.7	152	o	19.5
	FOMC	19.3	445	+	11.4
	DFOP *	**22.6**	**215**	**+**	**6.6**
Hanscheider Hof, Germany 09-2702-07	SFO	55.6	185	--	18.3
	FOMC	40.9	477	+	11.8
	DFOP *	**39.0**	**579**	**+**	**11.3**

* Bold: best fit according to chi2 error or visual assessment
SFO: Single first-order
FOMC: First-order multi compartment
DFOP: Double first-order in parallel

Conclusions

Based on the results it can be concluded that BYI 02960 shows a biphasic degradation behavior under the investigated Northern and Southern European field conditions. BYI 02960 residues remained in the upper 0-20 cm soil layer. Only small amounts below the LOQ could be detected to a maximum depth of 30 cm. At study completion, i.e. 540 days post-application, the remaining BYI 02960 residues in soil corresponded to 2.9 to 29.8% of the applied amount. The calculated DT_{50} of BYI 02960 ranged between 8.3 and 251 days.

In general the field dissipation observed for BYI 02960 residues, i.e. for BYI 02960 and its main soil metabolite DFA, was comparable to that found within the standardized laboratory studies.

Remarks

The field dissipation study is acceptable and the endpoints are reliable. The field study results are not normalized because these are not used for further assessment since the laboratory DT_{50} is smaller

than 60 days and field study is not triggered. For completeness the field study is included in the DAR. The graphs of the fits are available in the study but also not included since the results are not used further. Only the worst case field DT_{50} of 251 days is used for PEC_{soil} calculations (kinetic parameters DFOP, k_1=34.66 and k_2=0.0015 and g =0.27).

B.8.1.2.3 Soil residue studies

No further studies have been performed.

B.8.1.2.4 Soil accumulation studies

No field accumulation studies have been performed as the accumulation of BYI 02960 can be calculated from the degradation data obtained. The results of modelling, considering specific application rates and crops are presented in the PEC_{soil} calculations for the representative uses.

B.8.1.3 Summary route and rate of degradation in soil

Aerobic soil degardation

The route of Flupyradifurone (BYI 02960) degradation in soil was studied using different radiolabel positions, [pyrindinyl-methyl-^{14}C = PYM], [pyridine-2,6-^{14}C = PYR], [furanone-4-^{14}C = FUR] and [ethyl-1-^{14}C = ETH] BYI 02960. The data gathered in the aerobic soil metabolism studies demonstrated that BYI 02960 is degraded in soil.

When using the PYM label in four different European aerobic soils no major metabolites were detected. All formed metabolites were regarded as transient, which was confirmed by the high mineralization rate to $^{14}CO_2$, i.e. up to 58.6% of AR from [PYM-^{14}C] BYI 02960. The portions of not extractable residues (NER) were comparatively low throughout the study (max 16.8% of AR) in case of [PYM-^{14}C] BYI 02960. As minor transformation products BYI 02960-chloro (max. 1.8%) and BYI 02960-des-difluoroethyl (max. 0.4%) were identified (for structures see Figure B.8.1.3-01).

In studies with the FUR label (four European and two US soils) similar results were obtained with regard to the formation of NER (maximum 34.1% of AR) and extensive mineralization to $^{14}CO_2$ (up to 38.9% of AR). With the exception of $^{14}CO_2$ no major metabolites were formed in any of the soils. Degradation, amount of NER as well as of $^{14}CO_2$ formed in sterilised soil was significantly less than in non-sterile soils, indicating a biological component to the degradation/mineralization and formation of non-extractable residues from BYI 02960. Additionally, soil fractionation showed that even with extraction using strong base, BYI 02960 related residues remain bound to the solid (humin) fraction indicating very strong and irreversible binding to soil.

When using [ETH-^{14}C] BYI 02960 in three aerobic soils one major metabolite, identified as difluoroacetic acid (max. 33.9% of AR), and one very minor metabolite was detected and quantified. Again, significant amounts of $^{14}CO_2$ (up to 42.3% AR) were measured, indicating that mineralization of

the test item and/or its metabolites occurred. The maximum amount of NER was relatively low at 17.9% of AR.

The biotransformation of [PYR-^{14}C] BYI 02960 was studied in one aerobic EU and two US soils. The mineralization to $^{14}CO_2$ was significant (max. 57.4, 20.2 and 36.1% of AR) with the formation of minor metabolites in two soils. However, in one US soil, one major metabolite, which was identified as 6-chloronicotinic acid, was formed at maximum of 17.1%. NER formation was in the range of max. 11.3 to 25.5% of AR in the three soils.

Considering the results from laboratory soil metabolism studies the major routes of degradation of BYI 02960, under aerobic conditions, are:

- cleavage of the difluoroethyl group producing difluoroacetic acid (DFA),
- cleavage of the molecule at the pyridinylmethylene bridge with subsequent oxidation to 6-CNA
- mineralization to CO_2 and formation of non-extractable residues.

Degradation under anaerobic conditions and due to photolysis on the soil are not relevant routes for BYI 02960.

Figure B.8.1.3-01 Proposed degradation pathway of BYI02960 in soil

Note: The degradates observed and the maximum values are those for all label positions and soils

The biotransformation of BYI 02960 was studied in several EU and US soils under standardized aerobic and anaerobic laboratory conditions, as well as in a terrestrial field dissipation study performed on six different sites in Europe. BYI 02960 was found to be moderately degradable in aerobic soil under laboratory as well as under field conditions. The clear bi-phasic degradation kinetics indicates that the compound is less available for biotransformation with time, probably due to a time-dependent sorption behavior in soil. The BYI 02960 residues remain stable under anaerobic conditions.

The following Table B.8.1.3-01 summarises the findings in the aerobic laboratory studies. The kinetics data for the metabolite 6-CNA indicate that it is very rapidly degraded in soil with a mean DT_{50} of < 1 week. The metabolite DFA indicated a slightly longer DT_{50}, i.e. it was calculated to be in the range of approx. 2 months.

Table B.8.1.3-01 Trigger and modelling endpoints for BYI 02960 and its major soil metabolites

Soil	Dossier position of kinetic evaluation	DT_{50} (d) Trigger values	DT_{50} (d) Modelling input	Formation Fraction
Parent BYI 02960				
Laacher Hof AXXa	IIA 7.2.1/01 IIA 7.2.1/07	63.4	169.1	-
Laacher Hof AXXa	IIA 7.2.1/02 IIA 7.2.1/08	62.2	141.5	-
Laacher Hof AXXa	IIA 7.2.1/04 IIA 7.2.1/09	62.0	210.2	-
Laacher Hof AXXa (Ax) geometric mean		**62.5**	**171.3**	
Höfchen am Hohenseh	IIA 7.2.1/01 IIA 7.2.1/07	52.4	54.4	-
Höfchen am Hohenseh	IIA 7.2.1/02 IIA 7.2.1/08	33.2	40.5	-
Höfchen am Hohenseh	IIA 7.2.1/04 IIA 7.2.1/09	34.1	43.0	-
Höfchen am Hohenseh	IIA 7.2.1/05 IIA 7.2.1/10	33.0	90.0	-
Hoefchen am Hohenseh (HF) geometric mean		**37..4**	**54.0**	
Hanscheiderhof	IIA 7.2.1/01 IIA 7.2.1/07	120.0	157.5	-
Hanscheiderhof	IIA 7.2.1/02 IIA 7.2.1/08	98.3	157.5	-
Hanscheiderhof (HN) geometric mean		**108.6**	**157.5**	
Dollendorf II	IIA 7.2.1/01 IIA 7.2.1/07	56.4	60.1	-
Dollendorf II	IIA 7.2.1/02 IIA 7.2.1/08	49.3	55.1	-
Dollendorf II	IIA 7.2.1/04 IIA 7.2.1/09	33.9	38.6	-
Dollendorf II (DD) geometric mean		**45.5**	**50.4**	
Springfield	IIA 7.2.1/03	228	164.4#	-
Springfield	IIA 7.2.1/06	242	179.7#	-
Springfield geometric mean		**234.9**	**172.9**	
Sanger	IIA 7.2.1/03	58.3	55.5#	-
Sanger	IIA 7.2.1/06	56.3	58.8#	-
Sanger geometric mean		**56.8**	**57.1**	
Overall geometric mean *		**73.2**	**94.8**	**-**
Overall median *		**59.7**	**107.3**	

6-CNA				
Aldham's Farm	IIA 7.2.3/02	2.9	2.9	-
Flint Halls Farm	IIA 7.2.3/02	2.2	2.2	-
B. Barns Farm	IIA 7.2.3/02	5.3	5.3	-
Höfchen am Hohenseh	IIA 7.2.3/03	3.1	3.0	0.266
Sanger	IIA 7.2.3/04	36.6	22.4#	0.694
Overall geometric mean		**5.2**	**4.7**	
Overall arithmetic mean				**0.480**
DFA				
Dollendorf II	IIA 7.2.3/05	44.9	32.0	0.9087
Laacher Hof AXXa	IIA 7.2.3/05	73.6	73.6	0.5896
Höfchen am Hohenseh	IIA 7.2.3/05	67.4	37.8	1.000
Overall geometric mean		**60.6**	**44.7**	
Overall arithmetic mean				**0.833**

normalised to pF2
* Geometric mean and median of the geometric means of the different soils

Anaerobic soil degradation

Three studies investigating the degradation of [PYR-^{14}C], [FUR-^{14}C] and [ETH-^{14}C] BYI 02960 in soils under aerobic, then flooded anaerobic conditions showed that it can be expected that the amounts of BYI 02960 and its major aerobic soil metabolites DFA and 6-CNA remain stable under flooded field conditions. Degradation would be expected to continue according to the proposed overall pathway of degradation of BYI 02960 (see Figure B.8.1.3-01) whenever the conditions in soil turn aerobic again.

Soil photolysis

Phototransformation of [PYM-^{14}C] and [FUR-^{14}C] BYI 02960 was studied on a loam soil. From the results it is concluded that direct phototransformation of BYI 02960 is not regarded as a relevant degradation process in the environment. The soil photolytic DT_{50} of BYI 02960 was 99.6 and 109.3 days.

Field studies

Soil dissipation of BYI 02960 under European field conditions was investigated after application of BYI 02960 SL 200 on bare soil plots at sites in Monheim, Burscheid and Hanscheider Hof (all Germany), Great Chishill (United Kingdom), Albaro (Italy), and Vilobi d'Onyar (Spain). An application was also made in Chilly (Northern France), however the trial was aborted due to heavy rain shortly after application. The sites are located in the ecoregions Northern and Southern Europe.

In general, BYI 02960 residues remained in the upper 0-20 cm soil layer and only very small amounts (<LOQ) were translocated to a maximum depth of 30 cm. At one site (Vilobi d'Onyar, Spain), BYI 02960 residues just above the LOQ were found at day 13 up in the 20-30 cm soil layer. BYI 02960 dissipated from soil at all test sites with DT_{50} values ranging from 8.3 to 251 days.

The metabolite BYI 02960-DFA appeared in the upper soil layer 0-20 cm in amounts of up to 17.4 g/ha soil. However, the metabolite declined to values of max. 7.9 g/ha soil towards the end of the study (in Burscheid, Germany). An overview of the results for parent compound is given in Table B.8.1.3-02.

The field data are not different from the laboratory data in that it does not result in different DT_{50}-values on average. The average DT_{50}-value for the non-normalized field studies is 38.4 days (geometric mean) and 40.0 days (median) compared to the laboratory DT_{50}-values of 72.2 (geometric mean) and 59.7 days (median). A normalization might be conducted, since these values are not required for modelling this is not conducted.

Table B.8.1.3-02 Results for BYI 02960 in field dissipation studies

Location: Trial No.	Soil type (USDA)	Kinetic model	DT50 [d]	DT90 [d]
Monheim (Germany): 09-2702-01	Sandy loam (0-100 cm)	DFOP	41.0	749
Great Chishill (United Kingdom): 09-2702-02	Clay loam (0-100 cm)	DFOP	251	>1000
Burscheid (Germany): 09-2702-03	Silt loam (0-75 cm), Loam (75-100 cm)	DFOP	42.8	484
Albaro (Italy): 09-2702-05	Clay loam (0-30 cm), Loam (30-75 cm), Sandy loam (75-100 cm)	DFOP	8.3	279
Vilobi d'Onyar (Spain): 09-2702-06	Loam (0-30 cm), Sandy loam (30-75 cm), Sandy clay loam (75-100 cm)	DFOP	22.6	215
Hanscheider Hof (Germany): 09-2702-07	Loam(0-100 cm)	DFOP	39.0	579
		Gemetric mean	**38.4**	**482.3**
		Median	**40.0**	**531.5**

B.8.2 Adsorption, desorption and mobility in soil (IIA 7.1.2, 7.1.3, IIIA 9.1.2)

B.8.2.1 Batch sorption

STUDY IIA, 7.4.1/01

Characteristics

reference	:	Menke, U., Telscher, M., 2008	soils	:	four soils; sandy laom, loam, loam, loam
year of execution	:	2007	concentrations	:	1.0, 0.3, 0.1, 0.03, and 0.01 mg/L
GLP statement	:	yes	temperature	:	20 ± 1 °C
guideline	:	Incl. OECD 106	$K_F$oc	:	74.9 – 107.0
test substance	:	[Pyridinylmethyl-^{14}C] BYI 02960	1/n	:	0.8445 – 0.8682
purity	:	radiochemical purity > 99%	acceptability	:	**acceptable**

Summary

Freundlich adsorption and desorption constants K_F and K_{OC} of BYI 02960 have been determined in batch equilibrium experiments with four different soils using radiolabeled test substance ([PYM-^{14}C] BYI 02960). The adsorption phase of the study was carried out using pre-equilibrated air-dried soils in 0.01 M aqueous $CaCl_2$ solution with soil/solution ratios of 1:4 for all soils. BYI 02960 was applied at concentrations of nominal 1.0, 0.3, 0.1, 0.03, and 0.01 mg/L. Desorption phase was performed by supplying pre-adsorbed soil samples with fresh 0.01 M aqueous $CaCl_2$ solution for one desorption cycle. Adsorption and desorption took place in the dark at 20 ± 1 °C for 24 hours, each. The test item was stable throughout the study, and the parental mass balance determined for all soils at the highest concentration was in the range of 92.9 - 94.7%.

For key data and results of study see Table B.8.2.1-01.The calculated adsorption constants $K_{f\ (ads)}$ of the Freundlich isotherms for the four test soils ranged from 2.08 to 3.82 mL/g, and the mean $K_{OC(ads)}$ was 93.3 mL/g. The Freundlich exponent 1/n was in the range of 0.8445 to 0.8682, and the mean 1/n was 0.86.

The desorption $K_{F(des)}$ and the normalized $K_{OC(des)}$ values were significantly higher (i.e. 2 times higher) than those obtained for the adsorption phase, indicating that the test item once adsorbed to soil is not readily desorbed.

Table B.8.2.1-01 Key data and results of study

Soil origin **Soil type (USDA)**	**Laacher Hof AXXa Sandy loam**	**Hoefchen am Hohenseh 4a Loam**	**Hanscheider Hof Loam**	**Dollendorf II Loam**
pH (aqueous $CaCl_2$ solution)	6.2	6.6	5.3	7.2
Organic carbon [%]	2.1	2.4	2.2	5.1
$K_{F(ads)}$ [mL/g]	2.077	2.213	2.354	3.822
1/n	0.8445	0.8682	0.8643	0.8648
$K_{oc(ads)}$ [mL/g]	98.9	92.2	107.0	74.9
Arithmetic mean $K_{OC(ads)}$ [mL/g]	**93.3**			

Materials and Methods

Materials

1. Test Item: Code BYI 02960, CAS no. 951659-40-8 (unlabeled substance); Label position = Pyridinylmethyl-^{14}C] BYI 02960, sample ID BECH 2135; Specific activity: 118.1 µCi/mg (4.37 MBq/mg); Radiochemical and chemical purity: >99% (at beginning of study); The test item was identified by LC-MS/MS).

The test item was dissolved in acetonitrile / Milli-Q-water 1:1 (v/v) (approx. 1 mg/mL) after arrival at the testing facility and stored in a freezer in the dark. Radiopurity of the labeled test item was checked by HPLC analysis before application.

2. Soils: Four soils originating from Germany were used in the batch equilibrium experiments. The pH values of the soil batches were measured in 0.01 M aqueous $CaCl_2$. The soils were air-dried and homogenized by sieving (≤ 2 mm). The detailed parameters of soils are shown in Table B.8.2.1-02.

Table B.8.2.1-02 Physico-chemical characteristics of test soils used

Parameter **Soil** **Batch ID**	**Results/Units** **Laacher Hof AXXa (AX) 20061129**	**Hoefchen am Hohenseh 4a (HF) 20061130**	**Hanscheiderhof (HN) 20061130**	**Dollendorf II (DD) 20061130**
Geographic Location (City/ State/ Country)	Monheim/ NRW/ Germany	Burscheid/ NRW/ Germany	Burscheid/ NRW/ Germany	Blankenheim/ NRW/ Germany
Texture Class[A]	Sandy loam	Loam	Loam	Loam
Sand[A] Silt[A] Clay[A]	73% 18% 9%	39% 46% 15%	35% 50% 15%	33% 42% 25%
pH ($CaCl_2$) pH (Water) pH (KCl)	6.2 6.6 6.1	6.6 7.0 6.4	5.3 5.7 4.9	7.2 7.4 7.0
Organic Matter[B]	3.6%	4.1%	3.8%	8.8%
Organic Carbon[C]	2.1%	2.4%	2.2%	5.1%
Cation Exchange Capacity (meq/100 g)	9.4	12.3	8.8	20.3
Moisture at 0.33 bar	12.5%	24.3%	23.9%	36.6%
Bulk Density	1.22 g/mL	1.04 g/mL	1.05 g/mL	0.98 g/mL
Soil Taxonomic Classification (USDA)	Sandy, mixed, mesic Typic Cambudolls	Loamy, mixed, mesic Typic Argudalfs	Not available	Not available
Soil Mapping Unit	N 51° 04.647' E 006° 53.5217	N 51° 04.011' E 007° 06.327	N 51° 04.482' E 007° 08.361'	N 50° 22.899' E 06° 43.001'

[A]) According to USDA classification
[B]) Calculated: organic matter = organic carbon * 1.724
[C]) Determination method: LECO

Methods

1. Experimental conditions: Adsorption and desorption constants of BYI 02960 were determined by batch equilibrium experiments. In pre-tests, the stability of the test substance, an adequate soil/solution ratio as well as appropriate adsorption and desorption equilibration times were determined. Following the preliminary tests the adsorption and desorption phases were carried out for 24 hours using a soil: solution ratio of 1:4.

The adsorption phase of the study was carried out using pre-equilibrated air-dried soils (each 5 g dry weight) in 0.01 M aqueous $CaCl_2$ solution with soil/solution ratios of 1:4 for all soils. [PYM-^{14}C] BYI 02960 was applied at concentrations of nominal 1.0, 0.3, 0.1, 0.03, and 0.01 mg/L. Desorption was performed by supplying pre-adsorbed soil samples with fresh 0.01 M aqueous $CaCl_2$ solution for one desorption cycle. The samples were incubated at constant temperature of 20 ± 1 °C for 24 hours in the dark and continuously agitated using an overhead shaker. Thereafter, the suspensions were centrifuged and the supernatants were analyzed by LSC. Additionally, the pH values of the supernatants were determined.
The experiments were performed in duplicate. The adsorption parameters were calculated using the Freundlich adsorption isotherm.

2. Analytical procedures: In the pre-test the supernatant was analyzed by HPLC. The recovery in HPLC was 97.6% of the injected RA. The limit of quantification (LOQ) of the HPLC analysis was calculated by considering the applied amount of radioactivity and the lowest amount of radioactivity which could be quantified (i.e. LOD = 3 Bq). As an example, for the supernatant phase in case of 0.1 mg/L application rate at soil Laacherhof AXXa a limit of quantification of 1.15% of AR was calculated, corresponding to a LOQ of approx. 1.2 µg/kg.

No degradation product was detected.
In the definitive test the BYI 02960 residue in the supernatant was analyzed by liquid scintillation counting (LSC). After the desorption step, for the calculation of the mass balance, the remaining soil was freeze-dried and combusted. The trapped $^{14}CO_2$ after combustion was measured by LSC. Due to the stability of the test item, the partition of the test item was determined based on the amount of radioactivity measured in the supernatant by LSC.

Results

Mass Balance

In pre-tests, the stability of the test substance, an adequate soil/solution ratio as well as appropriate adsorption and desorption equilibration times were determined, a summary of the recovery after adsorption is presented in Table B.8.2.1-03.

Table B.8.2.1-03 Preliminary study - Recovery of Test Item in Soil after Preliminary Equilibrium Test: Adsorption after incubation for 96 hours calculated as percentage of test item in solution and soil extract.

Soil ID	Laacherhof AXXa (Ax)	Hoefchen am Hohenseh 4a (HF)	Hanscheiderhof (HN)	Dollendorf II DD)
Supernatant [%AR]	51.4	49.4	46.1	34.9
Test item [% of injected]	97.3	97.5	97.7	96.7
Solid phase (ACN extract) [%AR]	45.0	47.5	50.0	60.6
Test item [% of injected]	97.9	98.0	97.7	97.7
Non-extractable residues	N/A	N/A	N/A	N/A
Total recovery of test item [% AR]	94.0	94.7	93.9	92.9

For the definitive study he overall material balance for all concentrations was in the range of 95.7 to 98.6% (overall mean: 97.4%) of the applied radioactivity.

Table B.8.2.1-04 Recovery of Total Radioactivity of BYI 02960 after Adsorption and Desorption Expressed as percentage of applied radioactivity

Soil ID Conc. ID	**AX Recovery (% of AR)**	**HF Recovery (% of AR)**	**HN Recovery (% of AR)**	**DD Recovery (% of AR)**
1.0 mg/L	98.4	98.2	97.6	97.2
0.30 mg/L	97.9	97.9	97.6	95.7
0.10 mg/L	97.8	97.1	97.4	96.3
0.030 mg/L	97.4	97.4	97.6	95.9
0.010 mg/L	98.1	98.6	98.3	95.7
Mean	**97.9**	**97.8**	**97.7**	**96.2**
Standard deviation	**± 0.32**	**± 0.53**	**± 0.30**	**± 0.56**

Transformation of Test Item

The stability of the BYI 02960 in the test system used was confirmed by performing HPLC analyses prior to the definitive test.

Findings

After 24 hours of equilibration, 34.9 - 52.9%, 36.6 - 52.3%, 38.1- 54.3%, and 50.2 - 66.4% of the applied test item were adsorbed in soils Laacher Hof AXXa, Hoefchen am Hohenseh 4a, Hanscheiderhof, and Dollendorf II, respectively. The adsorption behavior of BYI 02960 in the concentration range of two orders of magnitude (i.e. from 0.01 to 1.0 mg/L) was accurately described for all soils with the Freundlich equation (see Table B.8.2.1-07). The correlation coefficient of the individual isotherms was 0.9988 to 0.9999. The calculated adsorption constants Kf (ads) of the Freundlich isotherms ranged from 2.077 mL/g to 3.822 mL/g. The Freundlich exponents 1/n were in the range of 0.8445 to 0.8682, indicating that the concentration of the test item affected the adsorption behavior in the examined concentration range.

In general, the organic matter in soil, determined as organic carbon content, is the most important part to bind organic chemicals. Therefore, the adsorption coefficients Kf (ads) are correlated with the organic carbon content of the soil, in order to get a comparability of the adsorption behavior in different soils. For BYI 02960 the calculated KOC(ads) values varied between 74.9 and 107.0 mL/g (mean: 93.3 mL/g.

At the end of the desorption phase, 31.5 - 44.4%, 33.8 - 43.4%, 31.0 - 39.9%, and 22.0 - 31.9% of the initially adsorbed amount was desorbed in soils Laacher Hof AXXa, Hoefchen am Hohenseh 4a, Hanscheiderhof, and Dollendorf II, respectively. The calculated desorption constants K_f (des) of the Freundlich isotherms for the four test soils ranged from 4.115 - 7.056 mL/g. The $K_{OC(des)}$ values of the soils ranged from 138.4 - 236.7 mL/g (mean: 188.9 mL/g). Thus, the $K_{OC(des)}$ values were significantly (1.8 to 2.2 times) higher than the $K_{OC(ads)}$ values, indicating a strengthened binding of the test item once adsorbed to the soil.

Table B.8.2.1-05 Concentration of BYI 02960 in the Solid and Liquid Phases at the End of the Adsorption Period

Description	**Soil**			
Soil ID	**AX**			
Concentration	**Soil (mg/kg)**	**Solution (mg/L)**	**Percentage adsorbed**	
Control	N/A	N/A		
0.010 mg/L	0.022	0.005	52.9	± 0.03
0.030 mg/L	0.062	0.015	50.7	± 0.57
0.10 mg/L	0.187	0.053	47.0	± 1.10
0.30 mg/L	0.480	0.181	39.9	± 0.12
1.01 mg/L	1.417	0.660	34.9	± 0.52
Soil ID	**HF**			
Control	N/A	N/A		
0.010 mg/L	0.021	0.005	52.3	± 0.29
0.030 mg/L	0.060	0.015	49.1	± 0.35
0.10 mg/L	0.181	0.054	45.4	± 0.04
0.30 mg/L	0.494	0.177	41.1	± 0.58
1.01 mg/L	1.485	0.643	36.6	± 0.34
Soil ID	**HN**			
Control	N/A	N/A		
0.010 mg/L	0.022	0.005	54.3	± 0.00
0.030 mg/L	0.063	0.015	51.5	± 0.33
0.10 mg/L	0.187	0.053	47.1	± 0.48
0.30 mg/L	0.517	0.171	43.0	± 0.32
1.01 mg/L	1.544	0.628	38.1	± 0.05
Soil ID	DD			
Control	**N/A**	**N/A**		
0.010 mg/L	0.027	0.003	66.4	± 0.41
0.030 mg/L	0.078	0.011	64.5	± 0.30
0.10 mg/L	0.239	0.040	60.1	± 0.08
0.30 mg/L	0.679	0.131	56.5	± 0.46
1.01 mg/L	2.037	0.505	50.2	± 0.19

Table B.8.2.1-06 Concentration of BYI 02960 in the Solid and Liquid Phase at the End of Desorption Phases

Description	**Soil**			
Soil ID	**AX**			
Concentration of a.i.	**Soil (mg/kg)**	**Solution (mg/L)**	**Percentage Desorbed***	
Control	N/A	N/A		
0.010 mg/L	0.015	0.002	31.5	± 0.33
0.030 mg/L	0.041	0.005	34.0	± 0.40
0.10 mg/L	0.120	0.017	35.7	± 0.45
0.30 mg/L	0.286	0.048	40.4	± 0.74
1.01 mg/L	0.789	0.157	44.4	± 0.19
Soil ID	**HF**			
Control	N/A	N/A		
0.010 mg/L	0.014	0.002	33.8	± 0.88
0.030 mg/L	0.038	0.005	35.8	± 0.67
0.10 mg/L	0.112	0.017	38.1	± 0.05
0.30 mg/L	0.292	0.050	40.8	± 0.32
1.01 mg/L	0.840	0.161	43.4	± 0.31
Soil ID	**HN**			
Control	N/A	N/A		
0.010 mg/L	0.015	0.002	31.0	± 0.15
0.030 mg/L	0.043	0.005	31.3	± 0.18
0.10 mg/L	0.123	0.016	34.4	± 0.49
0.30 mg/L	0.328	0.047	36.6	± 0.00
1.01 mg/L	0.929	0.154	39.9	± 0.16

Soil ID	DD			
Control	N/A	N/A		
0.010 mg/L	0.021	0.001	22.0	± 0.81
0.030 mg/L	0.059	0.005	24.0	± 0.30
0.10 mg/L	0.175	0.016	27.0	± 0.21
0.30 mg/L	0.484	0.049	28.8	± 0.42
1.01 mg/L	1.387	0.162	31.9	± 0.46

* = expressed as a percentage of the initially adsorbed material
Desorption steps: One desorption step for all concentrations

Table B.8.2.1-07 Adsorption and Desorption Constants of BYI 02960 in the Soils

Soil Type	Adsorption				Desorption			
	K_F (mL/g)	1/n	R^2	Koc (mL/g)	K_F (mL/g)	1/n	R^2	Koc (mL/g)
LH AXXa	2.077	0.8445	0.9988	98.9	4.115	0.8786	0.9994	196.0
Hoefchen am Hohenseh 4a	2.213	0.8682	0.9999	92.2	4.431	0.9086	0.9999	184.6
Hanscheiderhof	2.354	0.8643	0.9998	107.0	5.208	0.9099	0.9996	236.7
Dollendorf II	3.822	0.8648	0.9995	74.9	7.056	0.8923	0.9998	138.4
Arithmetic mean	**2.616**	**0.8604**	**0.9995**	**93.3**	**5.202**	**0.8973**	**0.9997**	**188.9**

Conclusions

BYI 02960 can be classified as intermediate mobile for adsorption and low mobile for desorption. For a compilation of results see Table B.8.2.1-07.

Remarks

The results of the adsorption desorption study for BYI 02960 is acceptable and reliable. Flupyradifurone does no dissociate in aqueous solutions in the pH-range 1< pH <12 and hence there is no reason to expect a pH dependence of the sorption. The range of tested pH values is about two units and reflects typical agricultural soils. The Kom values seem to decrease with higher pH values, however, a statistical significant correlation could not be determined (e.g. by the Kendall test included in the German input decision tool). Moreover the coefficient of variation for the resulting Kom values accounts for 20 % and indicates a very good determination of this parameter. Looking at the Kf dependence on pH it becomes also clear that the magnitude of sorption is not dependent on pH. The endpoints will be used for risk assessment.

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Characteristics

reference	:	Stroech, K. , 2011	soils	:	two US soils; sandy loam, silt loam
year of execution	:	2009-2010	concentrations	:	1.0, 0.3, 0.1, 0.03, and 0.01 mg/L
GLP statement	:	yes	temperature	:	20 ± 1 °C
guideline	:	Incl. OECD 106	$K_F$oc	:	74.9 – 107.0
test substance	:	[Pyridinylmethyl-^{14}C] BYI 02960	1/n	:	0.8445 – 0.8682
purity	:	radiochemical purity > 99%	acceptability	:	**acceptable**

Summary

Freundlich adsorption and desorption constants K_F and K_{OC} of BYI 02960 have been determined in batch equilibrium experiments with two different soils using radiolabeled test substance ([PYM-^{14}C] BYI

02960). The adsorption phase of the study was carried out using pre-equilibrated air-dried soils in 0.01 M aqueous $CaCl_2$ solution with soil/solution ratios of 1:1 soil Sanger and 1/2 for soil Springfield. BYI 02960 was applied at concentrations of nominal 1.0, 0.3, 0.1, 0.03, and 0.01 mg/L. Desorption phase was performed by supplying pre-adsorbed soil samples with fresh 0.01 M aqueous $CaCl_2$ solution for one desorption cycle. Adsorption and desorption took place in the dark at 20 ± 1 °C for 24 hours, each. For the highest concentration, two additional desorption cycles were performed with 24 hours equilibration time each. The test item was stable throughout the study, and the parental mass balance determined for all soils at the highest concentration was in the range of 93.4 - 94.4%. For key data and results of study see Table B.8.2.1-08.

The calculated adsorption constants $K_{f\ (ads)}$ of the Freundlich isotherms for the two test soils were 0.597 and 2.512 mL/g, and the mean $K_{OC(ads)}$ was 1.554 mL/g. The Freundlich exponent 1/n was 0.8505 and 0.9021 (mean 0.8763).

The desorption $K_{f\ (des)}$ and the normalized $K_{OC(des)}$ values were significantly (i.e. 2.0 and 3.6 times) than those obtained for the adsorption phase, indicating that the test item once adsorbed to soil is not readily desorbed.

Table B.8.2.1-08 Key data and results of study

Soil origin	Sanger, CA, USA	Springfield, NE, USA
Soil type (USDA)	Sandy loam	Silt loam
pH (aqueous $CaCl_2$ solution)	6.8	6.5
Organic carbon [%]	0.7	1.9
$K_{f\ (ads)}$ [mL/g]	0.597	2.512
1/n	0.9021	0.8505
$K_{oc(ads)}$ [mL/g]	**85.2**	**132.2**

(Arithmentic mean $K_{OC(ads)}$: 108.7 mL/g and 1/n 0.8363)

Material and Methods

Materials

1. Test Item: Code BYI 02960, CAS no. 951659-40-8 (unlabelled substance); Label position = [Pyridinylmethyl-^{14}C] BYI 02960, sample ID MERVP017-SS; The test item was identified by HPLC-MS/MS; Specific activity: 118.1 µCi/mg (4.37 MBq/mg); Radiochemical and chemical purity: >99% (at beginning of study)

The test item was dissolved in acetonitrile after arrival at the testing facility and stored in a freezer in the dark. Radiopurity of the labeled test item was checked by HPLC analysis before application.

2. Soils: Two soils originating from the USA were used in the batch equilibrium experiments. The pH values of the soil batches were measured in 0.01 M aqueous $CaCl_2$. The soils were air-dried and homogenized by sieving (≤ 2 mm). The detailed parameters of soils are shown in Table B.8.2.1-09.

Methods

1. Experimental conditions: Adsorption and desorption constants K_{OC} of BYI 02960 were determined for two soils (see Table B.8.2.1-09) with batch equilibrium experiments using [PYM-^{14}C] BYI 02960. Preliminary tests were performed prior to the definitive test in order to optimize the test conditions.

The adsorption phase of the study was carried out using pre-equilibrated air-dried soils (each 5 g dry weight) in 0.01 M aqueous $CaCl_2$ solution with soil/solution ratios of 1:4, 1:2 and 1:1 for the soils. BYI 02960 was applied at concentrations of nominal 1.0, 0.3, 0.1, 0.03, and 0.01 mg/L. Desorption phase was performed by supplying pre-adsorbed soil samples with fresh 0.01 M aqueous $CaCl_2$ solution for one desorption cycle. The samples were incubated at constant temperature of 20 ± 1 °C for 24 hours in the dark. Thereafter, the suspensions were centrifuged and the supernatants were analyzed by LSC. Additionally, the pH values of the supernatants were determined. The experiments were performed in duplicate. The adsorption parameters were calculated using the Freunlich adsorption isotherm.

2. Analytical procedures: In the pre-test the supernatant was analyzed by HPLC. The recovery in HPLC was 97.4% of the injected radioactivity. The limit of quantification (LOQ) of the HPLC analysis was calculated by considering the applied amount of radioactivity and the lowest amount of radioactivity which could be quantified (LOD). The limit of detection (LOD) was set to 150 dpm, i.e. 0.3% of applied radioactivity, deduced from the chromatograms of the parental mass balance samples (supernatant, 96 hours equilibration time, highest concentration nominal 1.0 mg/L). The LOQ (limit of quantification) was set to three times the LOD, i.e. 450 dpm or 0.9% of applied radioactivity.

In the definitive test the BYI 02960 residue in the supernatant was analyzed by liquid scintillation counting (LSC). After the desorption step, for the calculation of the mass balance, the remaining soil was freeze-dried and combusted. The trapped $^{14}CO_2$ after combustion was measured by LSC.

Table B.2.1.1-09 Physico-chemical characteristics of test soils used

Parameter	Results/Units [C]	
Soil (Soil ID) Batch ID	Sanger (SA) 091609-S	Springfield (SP) 072209-S
Geographic Location (City / State / Country)	Sanger/ California/ USA	Springfield/ Nebraska/ USA
Soil Series	Hanford	Marshall
Texture Class [A]	Sandy Loam	Silt Loam
Sand [A] Silt [A] Clay [A]	64.3% 28.1% 7.6%	11.1% 62.2% 26.7%
pH (0.01 M $CaCl_2$, 1/1) pH (Water, 1/1) pH (Saturated Paste)	6.8 7.3 7.3	6.5 7.1 6.9
Organic Matter [B]	1.2%	3.3%
Organic Carbon	0.7%	1.9%
Cation Exchange Capacity (CEC)	6.7 meq/100 g	19.0 meq/100 g
Water Holding Capacity 0.1 bar Water Holding Capacity 0.33 bar	22.1% 11.5%	33.3% 25.0%
Maximum Water Holding Capacity	28.9 g/100 g	37.9 g/100 g
Bulk Density Particle Density	1.30 g/cm^3 N/A	0.96 g/cm^3 N/A

Biomass	N/A	N/A
Soil Taxonomic Classification (USDA)	Coarse-loamy, mixed, superactive, nonacid, thermic Typic Xerorthents	Fine-silty, mixed, superactive, mesic Typic Hapludolls
Soil Mapping Unit	N 36°70.4' W 119°46.3'	N 41 03.7' W 096 15.1'

[A]) According to USDA classification
[B]) Calculated: organic matter = organic carbon * 1.724
[C]) Analyses performed at Agvise Laboratories, 604 Highway 15 West, Northwood, ND 58267, USA

Results

Mass Balance

In pre-tests, the stability of the test substance, an adequate soil/solution ratio as well as appropriate adsorption and desorption equilibration times were determined, a summary of the recovery after adsorption is presented in Table B.8.2.1-10.

Table B.8.2.1-10 Preliminary study - Recovery of Test Item in Soil after Preliminary Equilibrium Test: Adsorption after incubation for 96 hours calculated as percentage of test item in solution and soil extract.

Soil ID	**SA**	**SP**
Supernatant [% AR]	36.8	26.7
a.i. in supernatant [area %]*	97.1	96.6
Recovery a.i. in supernatant [% AR]	**35.7**	**25.8**
Solid phase (organic extract) [% AR]	60.0	69.1
a.i. in solid phase (organic extract) [area %]*	97.8	97.8
Recovery a.i. in solid phase (organic extract) [% AR]	**58.7**	**67.6**
Non-extractable residues	N/A	N/A
Total recovery of AR [%]	96.8	95.9
Total recovery of a.i. [% AR]	**94.4**	**93.4**

* % of Regions of Interest from HPLC

The overall material balance for all concentrations was in the range of 96.0 - 98.7% (mean: 97.1%) of the applied radioactivity. The complete material balance found at all sampling intervals demonstrated that no significant RA dissipated from the test vessels or was lost during processing.

Table B.8.2.1-11 Recovery of Total Radioactivity of BYI 02960 after Adsorption and Desorption Expressed as percentage of applied radioactivity

Soil ID **Conc. ID**	**SA** **Recovery** **(% of AR)**	**SP** **Recovery** **(% of AR)**
1.0 mg/L	97.6	97.4
0.30 mg/L	97.1	96.0
0.10 mg/L	96.5	97.6
0.030 mg/L	98.7	96.9
0.010 mg/L	97.3	96.0
Mean	**97.5**	**96.8**
standard deviation	**± 0.7**	**± 0.7**

Transformation of Test Item

The stability of the BYI 02960 in the test system used was confirmed by performing HPLC analyses prior to the definitive test.

Findings

The adsorption behavior of BYI 02960 in the concentration range of two orders of magnitude (i.e. from 0.01 - 1.0 mg/L) was accurately described for all soils with the Freundlich equation (for summary of results see Table B.8.2.1-14). The correlation coefficient of the individual isotherms was 0.9993 and 0.9995. The calculated adsorption constants $K_{f\ (ads)}$ of the Freundlich isotherms were 0.597 and 2.512 mL/g (mean: 1.554 mL/g). The Freundlich exponents 1/n were 0.8505 and 0.9021 (mean: 0.8763), indicating that the concentration of the test item affected the adsorption behavior in the examined concentration range.

In general, the organic matter in soil, determined as organic carbon content, is the most important part to bind organic chemicals. Therefore, the adsorption coefficients $K_{f\ (ads)}$ were correlated with the organic carbon content of the soil, in order to get a comparability of the adsorption behavior in different soils. For BYI 02960 the calculated $K_{OC(ads)}$ values were 85.2 and 132.2 mL/g (mean: 108.7 mL/g).

At the end of desorption phase, 23.7-29.5 % and 13.2-23.1 % of the initially adsorbed amount was desorbed in soils Sanger and Springfield, respectively. The calculated desorption constants $K_{f\ (des)}$ of the FREUNDLICH isotherms for the two test soils were 2.143 and 5.096 mL/g (mean: 3.620 mL/g). The $K_{OC(des)}$ values of the soils were 268.2 and 306.2 mL/g (mean: 287.2 mL/g).

The $K_{OC(des)}$ values were significantly (2.0 and 3.6 times) higher than the $K_{OC(ads)}$ values, indicating a strengthened binding of the test item once adsorbed to the soil.

Table B.8.2.1-12 Concentration of Test Substance in the Solid and Liquid Phases at the End of Adsorption Equilibration (Mean ± SD)

Concentration of a.i.	Soil (mg/kg)	Solution (mg/L)	Percentage adsorbed	
Soil	**SA**			
Control	N/A	N/A		
0.010 mg/L	0.005	0.005	50.1	± 0.1
0.030 mg/L	0.014	0.016	47.2	± 1.5
0.10 mg/L	0.044	0.057	43.6	± 1.0
0.30 mg/L	0.128	0.175	42.3	± 0.2
1.01 mg/L	0.384	0.622	38.2	± 0.8
Soil	**SP**			
Control	N/A	N/A		
0.010 mg/L	0.015	0.003	74.9	± 0.5
0.030 mg/L	0.043	0.008	72.4	± 0.6
0.10 mg/L	0.136	0.032	67.9	± 0.4
0.30 mg/L	0.391	0.108	64.5	± 0.5
1.01 mg/L	1.166	0.422	58.0	± 0.6

Table B.8.2.1-13 Concentration of Test Substance in the Solid and Liquid Phases at the End of Desorption Equilibration (Mean ± SD)

Concentration of a.i.	Soil (mg/kg)	Solution (mg/L)	Percentage desorbed*	
Soil	**SA**			
Control	N/A	N/A		
0.010 mg/L	0.004	0.001	23.7	± 0.4
0.030 mg/L	0.011	0.004	25.4	± 1.2

0.10 mg/L	0.032	0.012	26.5	± 0.9
0.30 mg/L	0.094	0.035	26.9	± 0.6
1.01 mg/L	0.270	0.113	29.5	± 0.2
Soil	**SP**			
Control	N/A	N/A		
0.010 mg/L	0.013	0.001	13.2	± 0.3
0.030 mg/L	0.037	0.003	14.7	± 0.0
0.10 mg/L	0.113	0.012	17.3	± 0.0
0.30 mg/L	0.314	0.039	19.9	± 0.1
1.01 mg/L	0.897	0.135	23.1	± 0.0

***** Expressed as a percentage of the initially adsorbed material, one desorption step for all concentrations, two additional desorption steps for highest concentration only (not calculated in this evaluation).

Conclusions

BYI 02960 can be classified as intermediate mobile for adsorption and low mobile for desorption. For a compilation of results see Table B.8.2.1-14.

Table B.8.2.1-14 Adsorption and desorption of [^{14}C] BYI 02960 in tested soils

Soil	Adsorption				Desorption			
	K_f [mL/g]	1/n	R^2	K_{OC} [mL/g]	K_f [mL/g]	1/n	R^2	K_{OC} [mL/g]
SA	0.597	0.9021	0.9993	85.2	2.143	0.9407	0.9992	306.2
SP	2.512	0.8505	0.9995	132.2	5.096	0.8603	0.9999	268.2
Arithmetic mean	**1.554**	**0.8763**	**0.9994**	**108.7**	**3.620**	**0.9005**	**0.9995**	**287.2**

Remarks

The results of the adsorption desorption study for BYI 02960 is acceptable and reliable. The endpoints will be used for risk assessment.

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Characteristics

reference	:	Menke, U., 2011	study type	:	time dependent sorption
year of execution	:	2007-2009	incubation time	:	120 days
GLP statement	:	yes	nominal concentration	:	0.53 mg / kg soil
guideline	:	OECD 307 (2002) US EPA, OPPTS 835.4100 (2008) OECD106 (2001)	temperature	:	20°C
test substance	:	[pyridinylmethyl-^{14}C] BYI 02960	$K_F$oc	:	see results
purity	:	> 99%	1/n	:	see results
soils	:	four soils; sandy loam, silt loam, loam, clay loam	acceptability	:	**acceptable**

Summary

The biotransformation and time dependent sorption of [pyridinylmethyl-^{14}C] BYI 02960 was studied in four European soils: Laacher Hof AXXa (AX), Höfchen am Hohenseh (HF), Hanscheiderhof Plot 611 (HN), and Dollendorf II (DD) for a maximum period of 120 days under aerobic conditions in the dark at ca. 20 °C and 55% WHC_{max} (max. water holding capacity). BYI 02960 was applied at the nominal rate of 0.53 mg/kg soil, which is equivalent to 200 g/ha field application rate (see study IIA.7.1.1/01)

At each sampling date the soil samples were shaken for 24 hours with 400 mL $CaCl_2$-solution in order to measure the time-dependent desorption of the test item. Subsequently they were extracted by shaking at ambient temperature and in a microwave at 70 °C with acetonitrile/water mixtures, and the BYI 02960 residues were analyzed and quantified by TLC with HPLC as the confirmatory method.The degradation and metabolism part of the study was summarized earlier (see IIA 7.1.1/01), this summary only considers results relevant to the assessment of time dependent sorption.

At the beginning of the study (DAT-0; equivalent to approximately two hours) the distribution coefficient values R_{TDS} were 1.46, 1.91, 3.35 and 4.66 mL/g for soils AX, HF, HN and DD, respectively. Depending on the ageing time, these values increased with time until the end of the study to 6.50, 5.64, 10.78 and 12.16 mL/g for the four soils indicating a significant increase of sorption with time. Based on results from the ageing time of 120 days, the R_{TDS} values increased by a factor of 4.4, 2.9, 3.2 and 2.6 (mean of all four soils = 3.3).

Materials and Methods

Materials

1. Test Item: Flupyradifurone: Code = BYI 02960; Label PYM = [Pyridinyl-methyl-^{14}C] BYI 02960 (sample ID: BECH 2123); Specific activity 4.37 MBq/mg; Radiochemical purity: >99% (acc. radio-HPLC and -TLC); Chemical purity: >99% (HPLC, UV detection at 210 nm); Identity and purity of test item in the application solution were confirmed.

2. Soil: The time-dependent sorption of BYI 2960 was studied in four different soils (for physico-chemical properties (see study IIA, 7.1.1/01). These soils are representative for agricultural use areas as required by the guidelines and cover a representative range of physico-chemical properties. Other details were summarized earlier (see IIA, 7.1.1/01).

Methods

See for summary of study IIA, 7.1.1/01. In addition the changes of the sorption parameter R_{TDS} (equivalent to K_d) of BYI 02960 affected by a preceding ageing period in soil were investigated by time-dependent desorption experiments. This additional test was designed in analogy to a "batch equilibrium" study. The soils incubated with the test item were shaken for 24 hours with 400 mL 0.1 M $CaCl_2$ solution, i.e. a soil to solution ratio of 1:4 was applied, which was derived from the respective batch equilibrium adsorption/desorption study (see study IIA, 7.1.1/01).

After centrifugation, the distribution of the test item between supernatant and soil was determined by means of LSC. For TLC analysis, the extracts were not processed further. About 20 Bq of the extracts were spotted per cm lane onto silica plates. For HPLC, the desorption solution ($CaCl_2$ extract) was analyzed directly as well (designation: D).

The R_{TDS} values (ratio time-dependent sorption, equivalent to K_d values) were calculated by:

$$R_{TDS} = \frac{concentration\ of\ test\ item\ in\ soil\ extract\ [\frac{\mu g}{g}]}{concentration\ of\ test\ item\ in\ CaCl_2\ solution\ [\frac{\mu g}{mL}]} = \frac{c_{extr}}{c_{des}}$$

Results

Data

For basic data including recovery and degradation data see summary under study IIA, 7.1.1/01.

Findings

The results of desorption measurements affected by the ageing period are presented in Table B.8.2.1-15. The R_{TDS} values were 1.46, 1.91, 3.35 and 4.66 mL/g for soils AX, HF, HN and DD, respectively, at the beginning of the study (DAT-0; equivalent to approximately two hours). With time of ageing in soil, these values increased until the end of the study to 6.50, 5.64, 10.78 and 12.16 mL/g for the four soils. Based on these results during an ageing time of 120 days, the mean R_{TDS} value increased by a factor of 4.4, 2.9, 3.2 and 2.6 (mean of all four soils = 3.3).

Conclusions

Major Outcomes of Study

During the ageing period of 120 days a clear increase of the distribution coefficient R_{TDS} became apparent. Based on the results of an ageing time of 120 days, the R_{TDS} values increased by a factor of 2.6 to 4.4 (mean of all four soils = 3.3).

Table B.8.2.1-15 R_{TDS} values [mL/g] of time-dependent sorption of [^{14}C] BYI 02960 on four different soils; mean of duplicates

DAT	LH AXXa (sandy loam)	Hoefchen am Hohenseh (loam)	Hanscheiderhof (loam)	Dollendorf II (loam)
0	1.46	1.91	3.35	4.66
1	2.09	2.78	4.58	5.29
3	1.88	2.32	4.32	5.23
7	2.40	2.57	5.07	5.90
14	2.91	3.03	6.04	6.47
21	3.38	3.38	6.95	7.21
30	3.71	3.72	7.38	7.78
59	4.98	4.84	9.35	9.73
120	6.50	5.64	10.78	12.16
Factor DAT-120/DAT-0	4.4	2.9	3.2	2.6
Mean factor DAT-120/DAT-0	**3.3**			

Significance of Results to Environmental Behavior of BYI 02960

The current laboratory study demonstrated that whenever BYI 02960 has time to age in soil it is much less susceptible to leaching. Parameters for exposure modelling can be derived from the study as decribed in study IIA, 7.4.1/04.

Remarks

See study IIA, 7.4.1/04

STUDY IIA, 7.4.1/04

Characteristics

reference	:	Sur, R., Scherr, F. 2012	study type	:	evaluation of time dependent sorption
year of execution	:	2012	incubation time	:	n.a.
GLP statement	:	No	nominal concentration	:	n.a.
guideline	:	OECD 307 (2002) US EPA, OPPTS 835.4100 (2008) OECD106 (2001)	temperature	:	n.a.
test substance	:	n.a.	$K_F$oc	:	see results
purity	:	n.a.	1/n	:	see results
soils	:	four soils; sandy loam, silt loam, loam, clay loam	acceptability	:	**acceptable**

This report is a supplement to study IIA, 7.4.1/03 and evaluates the study to derive input parameters for implementation in environmental exposure modelling.

Summary

Experimental sorption data of BYI 02960 on four soils (see report IIA 7.1.1/01). BYI 02960 was incubated at initial concentrations of about 0.5 mg/kg for 120 d at constant soil moisture (55% MWHC) and 20°C in a number of four soils with varying properties. After the respective incubation period, BYI 02960 was first desorbed with aqueous $CaCl_2$ to derive kinetic sorption parameters via curve fitting. These parameters constitute the prerequisite to adequately address time-dependent sorption processes in regulatory exposure modelling. The kinetic sorption was evaluated according to the model of Boesten et al. (1989) implemented in the groundwater leaching models PEARL and FOCUS-PELMO (FOCUS, 2009).

Up to the end of the incubation period (120 days) the (desorption) $K_{d,oc}$ (defined as $R_{TDS,OC}$: ratio of concentration adsorbed to soil and in solution) increased by a factor of 4.4 at the maximum (see study IIA, 7.4.1/03 and Table B.8.2.1-16) indicating kinetically controlled sorption processes.

The experimental raw data were pre-processed to calculate the concentration in the $CaCl_2$ desorption solution, and the total mass. These data were used to fit the kinetic-sorption model using PEARLNEQ. The evaluation showed that the experimental data could be well described by the kinetic-sorption model, with excellent fits and reliable parameter estimates. The inferred parameters were the desorption rate constant k_{des} [1/d], the ratio between the Freundlich coefficients in the non-equilibrium and in the equilibrium compartment f_{ne} [-], the degradation half-life in the equilibrium compartment DT_{50_eq} [d], the organic matter normalized distribution coefficient in the equilibrium compartment K_{om_eq} [L/kg], and the initial total mass in the system M_{ini} [µg]. The corresponding values for all four soils are shown in Table B.8.2.1-16.

The kinetic desorption rate constant $k_{_des}$ indicates a time scale of about 22 d, calculated as "pseudo half-life" for the exchange between equilibrium and non-equilibrium domain.

Values for $f_{_ne}$ in the range of 0.387 – 0.779 indicate that the kinetically controlled "sorption capacity" is in the range of 39% - 78%, and accounts on average for about 58% of the instantaneous "sorption capacity". The DT_{50_eq} representing degradation in the equilibrium domain was on average (geomean) 58 d suggesting moderate degradation during the laboratory study.

Materials and Methods

Materials

1. Test Item: See study IIA, 7.1.1/01

2. Data: For basic data see study IIA, 7.1.1/01. In the present study the Freundlich exponent 1/n of the batch adsorption of the four soils was taken from study IIA, 7.4.1/01, see Table B.8.2.1-07.

Methods

Experimental sorption data of BYI 02960 (Flupyradifurone) on four soils were used to derive kinetic sorption parameters via curve fitting. These parameters constitute the prerequisite to adequately address time-dependent sorption processes in regulatory exposure modelling. The kinetic sorption was evaluated according to the model of Boesten et al. (1989) implemented in the groundwater leaching models PEARL and FOCUS-PELMO (FOCUS, 2009).

The sorption is described as rate limited between an instantaneous equilibrium and a non-equilibrium domain. Degradation of the compound occurs only in the equilibrium domain including a dissolved and an equilibrium sorbed phase. If kinetic sorption is relevant, a part of the substance is sorbed in the non-equilibrium domain where no degradation is assumed. Therefore, considering both domains the apparent degradation of the substance is lower than the observed degradation in the equilibrium domain only. Thus, the degradation curve of the total substance may show a bi-phasic behaviour as a slowdown of total degradation results from the increasing fraction of substance being sorbed in the non-equilibrium domain.

The experimental raw data were pre-processed to calculate the concentration in the $CaCl_2$ desorption solution, and the total mass. These data were used to fit the kinetic-sorption model using PEARLNEQ program (Version 5; Boesten et al. 2007).

Results

The evaluation showed that the experimental data could be well described by the kinetic-sorption model, with excellent fits and reliable parameter estimates. The inferred parameters were the desorption rate constant $k_{_des}$ [1/d], the ratio between the Freundlich coefficients in the non-equilibrium and in the equilibrium compartment $f_{_ne}$ [-], the degradation half-life in the equilibrium compartment

DT_{50_eq} [d], the organic matter normalized distribution coefficient in the equilibrium compartment K_{om_eq} [L/kg], and the initial total mass in the system $M_{_ini}$ [µg].

Results of the curve fitting procedure used to derive optimized kinetic sorption parameters for BYI 02960 are shown in Table B.8.2.1-16. The kinetic desorption rate constant $k_{_des}$ indicates a time scale of about 22 days, calculated as "pseudo half-life" for the exchange between equilibrium and non-equilibrium domain. Values for $f_{_ne}$ in the range of 0.387 - 0.779 indicate that the kinetically controlled "sorption capacity" is in the range of 39 - 78%, and accounts on average for about 58% of the instantaneous "sorption capacity". The DT_{50_eq} representing degradation in the equilibrium domain was on average (geomean) 58 days suggesting moderate degradation during the laboratory study.

Table B.8.2.1-16 Estimated parameters of the kinetic-sorption model for all soils

Soil	$M_{_ini}$ [µg]	K_{om_eq} [L/kg]	DT_{50_eq} [d]	$K_{_des}$ [1/d]	$F_{_ne}$ [-]
Laacher Hof AXXa (AX)	44.5	40.3	57.3	0.031	0.779
Dollendorf II (DD)	46.0	39.3	50.1	0.029	0.470
Hanscheider Hof (HN)	46.0	62.8	86.6	0.032	0.683
Hoefchen am Hohenseh (HF)	47.7	43.6	45.7	0.033	0.387
Arithmetic mean	**46.1**	**46.5**			**0.580**
Geometric mean			**58.0**	**0.031**	

The fits between experimental and modelled data were visually good. The statistical parameters also indicate a sufficient goodness of fit. The χ^2 statistics indicate a very good fit with values ranging from 3.1 for the AXXa soil to 1.6 for the Hoefchen soil (Table B.8.2.1-17). Relative standard error (RSE) values were generally small and never exceeded 0.25 implying parameter estimates with a high confidence.

Table B.8.2.1-17 χ^2 statistics and RSE values for the parameter estimates of the kinetic sorption model

Soil		RSE				
	χ^2	M_ini	Kom_eq	DT50_eq	k_des	f_ne
AX	3.12	0.014	0.121	0.041	0.133	0.105
DD	1.74	0.008	0.026	0.018	0.090	0.057
HN	1.93	0.009	0.038	0.031	0.090	0.061
HF	1.62	0.007	0.042	0.017	0.099	0.064

Conclusion

The time-dependent sorption data received for BYI 02960 constitute the prerequisite to adequately address the obvious TDS process in higher tier regulatory modelling, e.g. related to a potential groundwater contamination.

Remarks

It is not reported whether the optimisation was done using different starting values of the parameters. Beulke and van Beinum (2012)[1] advise to do so and to report the results of these different optimisations. However, this case seems a well posed optimisation problem, so the RMS does not expect that different starting values would lead to very different optimised parameter values.

Beulke and van Beinum (2012) also advise to calculate the apparent sorption coefficient for each measurement and plot it as a function of time and assess is visually. This was not done by the authors. Since, this case seems a well posed optimisation problem The RMS does not expect that adding this assessment would lead to rejection of the optimised parameter values.

Furthermore, Beulke and van Beinum (2012) advise to compare the optimised $K_{om,EQ}$ with the $K_{om,EQ}$ value that is calculated from the measured total mass and the concentration in the liquid phase at DAT0 ($K_{om,0}$). A difference of more than 20% between the fitted $K_{om,EQ}$ and the $K_{om,0}$ is considered not acceptable. This check was not done by the authors. Since, this is a very important criterion the RMS decided to do this calculation and found that the difference between fitted $K_{om,EQ}$ and the $K_{om,0}$ was below 20% for all four soils.

The RMS concludes that the fitting of the kinetic model to the data from the time dependent sorption study was performed adequately and that its resulting optimised parameter values are suitable for use in regulatory assessments.

STUDY IIA, 7.4.1/05

Characteristics

reference	:	Scherr, F. 2014	study type	:	evaluation of time dependent sorption
year of execution	:	2014	incubation time	:	n.a.
GLP statement	:	No	nominal concentration	:	n.a.
guideline	:	draft guidance TDS (Beulke en Van Beinmum, 2012)	temperature	:	n.a.
test substance	:	n.a.	$K_{F}oc$	:	see results
purity	:	n.a.	1/n	:	see results
soils	:	four soils; sandy loam, silt loam, loam, clay loam	acceptability	:	**acceptable**

This report is a supplement to study IIA, 7.4.1/02 and study IIA, 7.4.1/03 and evaluates the study to derive input parameters for implementation in environmental exposure modelling according to the draft guidance document on time dependent sorption (Beulke and van Beinmum, 2012). The results from study IIA, 7.4.1/03 are replaced by the results obtained in this study.

[1] Beulke, S. and van Beinum, W. (2012). Guidance on how aged sorption studies for pesticides should be conducted, analysed and used in regulatory assessments. The Food and Environment Research Agency. Sand Hutton, YO41 1LZ, UK.

The objective of this study is to describe the kinetic processes which control the sorption of Flupyradifurone by means of a kinetic-sorption model according to Boesten et al. (1989) [2] and to derive kinetic-sorption parameter values which can be used in higher-tier evaluation studies and exposure assessments. The dependence of the parameter estimates on starting values for desorption rate constant (k_des) and f_ne, the ratio between the Freundlich coefficients in the non-equilibrium and in the equilibrium compartment, was also investigated. The evaluation was conducted to comply with the currently available draft guidance on evaluating such studies (Beulke and van Beinmum, 2012).

Summary

The study reflects thorough evaluation of the magnitude of the kinetic sorption characteristics of Flupyradifurone resulted in 4 robust parameter sets, that serve requirements laid down by Beulke and van Beinum (2012). Kinetic sorption parameters for 4 soils were derived and showed virtually no dependence on the initial values of k_des and f_ne and the corresponding parameter estimates for Kom_eq did not deviate by more than 15 % from the matching value calculated at DAT0.

The summary of the results is given in Table B.8.2.1-18. Desorption rate constant *k_des* corresponds to 22.4 d ,the half-life for the exchange process between equilibrium and non-equilibrium domains. Values from 0.3875 – 0.7808 for *f_ne* indicate that kinetic sorption capacity is in the range of 39 - 78 % of the instantaneous sorption capacity. The *Kom_eq* is derived to be 47.5 L/kg on average and the degradation half-life in the equilibrium domain is 57.9 d, .

Table B.8.2.1-18 Kinetic sorption parameter optimization results, including their RSE in brackets for the 4 soils. The most appropriate parameter set out of 4 starting value combinations are given.

Soil	M_ini [µg]	k_des [1/d]	f_ne [-]	DT50_eq [d]	Kom_eq [L/kg]	Chi²-err [%]
AXXa	44.6 (0.0144)	0.0306 (0.1319)	0.7808 (0.1066)	57.1 (0.0397)	40.7 (0.1188)	9.68
Dollendorf	46.0 (0.0077)	0.0282 (0.0906)	0.4738 (0.0566)	49.9 (0.0181)	39.9 (0.0262)	4.90
Hanscheider Hof	46.0 (0.0090)	0.0316 (0.0906)	0.6865 (0.00610)	86.4 (0.0313)	63.4 (0.0380)	4.28
Hoefchen	47.7 (0.0071)	0.0333 (0.0995)	0.3875 (0.00654)	45.6 (0.0173)	46.1 (0.0419)	3.32
	average		**0.5821**		**47.5**	
	geometric mean	**0.0309**		**57.9**		

Method (hardware software)

Parameter estimates for the kinetic sorption processes were obtained by curve fitting employing the PEARLNEQ (Version 5, Boesten et al., 2007) program that integrates the kinetic sorption model

[2] Boesten, J.J.T.I., van der Pas, L.J.T. and Smelt, J.H. (1989): Field test of a mathematical model for non-equilibrium transport of pesticides in soil. Pesticide Science, 25, 187-203.

implemented in PEARL and the optimization package PEST (Doherty, 2005). In brief, the PEARLNEQ program is run consecutively by PEST until the criteria for the goodness of fit are achieved. The underlying optimization method follows least-squares and Gauss-Marquardt-Levenberg algorithm employing inverse weights to achieve a relative error model. The model is fitted against the total mass of pesticide in soil, M_tot [µg], and the concentration in the liquid phase of the equilibrium domain, C_liq [µg/L].

The PEARLNEQ summary output files including a listing of observed and predicted values, the initial and optimized parameters and their 95% confidence interval.

Fitting procedure

The above described model was fitted to the observed replicate data using the PEARLNEQ program as described in Beulke and van Beinum (2012).

- Following parameters were optimized: M_ini, k_des, f_ne, DT50_eq, Kom_eq. The corresponding 1/n value was taken from an accompanying batch sorption study including the same soils, albeit from different batches
- Optimization settings for PEST were set accordingly similar to the guidance provided in Beulke and van Beinum (2012)
- a log-normal distribution for k_des and f_ne was assumed
- A varying timestep was implemented
- A relative error model was assumed employing weights calculated as the inverse of the measurements
- Observed values at t < 48 h of incubation were omitted from the curve fitting.

A number of four starting value combinations for the parameters k_des and f_ne were used to derive a robust parameter set for each individual soil as recommended in Beulke and van Beinum (2012).

Table B.8.2.1-19 Starting values for f_ne and k_des

Version	f_ne	k_des
1	0.2	0.004
2	0.2	0.05
3	1.5	0.004
4	1.5	0.05

Goodness of fit

Three criteria for the assessment of the goodness of the fit are proposed by Beulke and van Beinum (2012).

1. A visual assessment of both the total mass and the liquid phase concentration as well as the apparent Kd over time

2. A residual assessment

3. χ2-test.

Additionally, the fitted Kom_eq is required to be within ±20% of the Kom_eq value calculated from the measurements of total mass and liquid phase concentration at DAT0.

Results

Soil AXXa

The equilibrium model assuming no kinetic sorption did not deliver a visually acceptable fit (Figure B.8.2.1-01), particularly the liquid phase concentration was describe very poorly. Therefore, it is appropriate to assume kinetic sorption process and to evaluate its magnitude.

Figure B.8.2.1-01 **Equilibrium model fit to AXXa soil data.**
Total mass depicted at top and liquid phase concentration at bottom.

Table B.8.2.1-20 **Parameter optimization for soil AXXa, using 4 different starting sets for *f_ne* and *k_des*, Chi2-error %and sum of RSE (single rel. standard error RSE in brackets); most appropriate parameter set given in bold**

Version	M_ini [µg]	k_des [1/d]	f_ne [-]	DT50_eq [d]	Kom_eq [L/kg]	Chi^2-err [%]	Sum of RSE
1	44.6 (0.0144)	0.0306 (0.1325)	0.7817 (0.1065)	57.1 (0.0340)	40.6 (0.1191)	9.69	0.4125
2	44.6 (0.0144)	0.0306 (0.1325)	0.7817 (0.1065)	57.1 (0.0340)	40.6 (0.1190)	9.69	0.4125
3	**44.6 (0.0144)**	**0.0306 (0.1319)**	**0.7808 (0.1066)**	**57.1 (0.0397)**	**40.7 (0.1188)**	**9.68**	**0.4115**
4	44.6 (0.0144)	0.0306 (0.1325)	0.7818 (0.1065)	57.1 (0.0340)	40.6 (0.1190)	9.69	0.4124

The optimization results for the 4 starting value combinations of *k_des* and *f_ne* are given in Table B.8.2.1-20. From the numbers it is clear that there are only very minor differences between the four estimations and thus a robust parameter set for the kinetic sorption magnitude in soil AXXa can be derived. By means of the Chi2-error percentage and the sum of RSE values the parameter set from version 3 appears as the most appropriate one and will be discussed in more details in the following.

The model fit described by parameter starting value combination 3 is depicted in detail in Figure B.8.2.1-02 - Figure B.8.2.1-04. Total mass dissipation and liquid phase concentration are represented very well by the model fit (Figure B.8.2.1-02), the residuals indicate a good representation of data (Figure B.8.2.1-03) and the apparent Kd value over time is reasonably well estimated by the model parameters (Figure B.8.2.1-04). Clearly, the kinetic sorption model is a better representation of the measured data than the equilibrium model (c.f. Figure B.8.2.1-01). Additionally, the parameter estimates are derived with low RSE values well below the proposed threshold of 0.4, f_ne is <10 and k_des < 0.5. The difference to the Kom_eq calculated from data at DAT0 accounts for only 15% and is thus also within the range of acceptable deviation (Beulke and van Beinum, 2012).

Figure B.8.2.1-02 Observed and fitted values for total mass (top) and liquid phase concentration (bottom) in soil AXXa and starting value combination version 3

Figure B.8.2.1-03 Residuals for total mass (top) and liquid phase concentration (bottom) in soil AXXa and starting value combination version 3

Figure B.8.2.1-04 Measured and fitted apparent Kd value (top) and distribution of mass between equilibrium and non-equilibrium sorption domain (bottom) in soil AXXa and for starting value combination version 3

Soil Dollendorf

The equilibrium model fit without kinetic sorption to the data for the soil Dollendorf is shown in Figure B.8.2.1-05. While the total mass is quite well predicted the liquid phase concentration is not very well represented. Therefore, a systematic evaluation of the kinetic sorption parameters for the soil Dollendorf was performed.

Figure B.8.2.1-05 Equilibrium model fit to Dollendorf soil data.
Total mass depicted at top and liquid phase concentration at bottom.

Table B.8.2.1-21 Parameter optimization for soil Dollendorf, using 4 different starting sets for f_ne and k_des, Chi2-error %and sum of RSE (single rel. standard error RSE in brackets); most appropriate parameter set given in bold

Version	M_ini [µg]	k_des [1/d]	f_ne [-]	DT50_eq [d]	Kom_eq [L/kg]	Chi²-err [%]	Sum of RSE
1	**46.0 (0.0077)**	**0.0282 (0.0906)**	**0.4738 (0.0566)**	**49.9 (0.0181)**	**39.9 (0.0262)**	**4.90**	**0.1992**
2	46.0 (0.0077)	0.0282 (0.0913)	0.4735 (0.0567)	50.0 (0.0183)	40.0 (0.0270)	4.92	0.2010
3	46.0 (0.0077)	0.0282 (0.0913)	0.4735 (0.0567)	50.0 (0.0183)	39.9 (0.0270)	4.91	0.2009
4	46.0 (0.0077)	0.0282 (0.0910)	0.4737 (0.0567)	50.0 (0.0184)	39.9 (0.0272)	4.92	0.2009

The optimization results for the four starting value combinations of k_des and f_ne are given in Table B.8.2.1-21. There are only very minor differences between the four estimations and thus a robust parameter set for the kinetic sorption magnitude in the Dollendorf soil can be derived. By means of the Chi2-error percentage and the sum of RSE values the parameter set from version 1 appears as the most appropriate one and will be discussed in more details in the following.

The model fit described by parameter starting value combination 1 is depicted in detail in Figure B.8.2.1-06 - Figure B.8.2.1-08. Total mass dissipation and liquid phase concentration are represented very well by the model fit (Figure B.8.2.1-06). Also the residuals indicate a good representation of data (Figure B.8.2.1-07) and the apparent Kd value over time is very well estimated by the model parameters and the corresponding fit (Figure B.8.2.1-08). Clearly, the kinetic sorption model is a better representation of the measured data than the equilibrium model (c.f. Figure B.8.2.1-05). Additionally, the parameter estimates are derived with very low RSE values well below the proposed threshold of 0.4, f_ne is <10 and k_des < 0.5 (c.f. Table B.8.2.1-21). The difference to the Kom_eq calculated from data at DAT0 accounts for only 1.5% and is thus also within the range of acceptable deviation (Beulke and van Beinum, 2012).

Figure B.8.2.1-06 Observed and fitted values for total mass (top) and liquid phase concentration (bottom) in soil Dollendorf and starting value combination version 1

Figure B.8.2.1-07 Residuals for total mass (top) and liquid phase concentration (bottom) in soil Dollendorf and starting value combination version 1

Figure B.8.2.1-08 Measured and fitted apparent Kd value (top) and distribution of mass between equilibrium and non-equilibrium sorption domain (bottom) in soil Dollendorf and for starting value combination version 1

Soil Hanscheider Hof

The equilibrium model fit assuming no kinetic sorption to data for the soil Hanscheider Hof is depicted in Figure B.8.2.1-09. The fit indicates that the equilibrium model does not represent the data very well. Both the total mass and the liquid phase concentration are not very well represented by the model fit. An evaluation of the kinetic sorption magnitude is therefore warranted.

Figure B.8.2.1-09 Equilibrium model fit to Hanscheider Hof soil data.
Total mass depicted at top and liquid phase concentration at bottom.

Table B.8.2.1-22 Parameter optimization for soil Hanscheider Hof, using 4 different starting sets for f_ne and k_des, Chi2-error %and sum of RSE (single rel. standard error RSE in brackets); most appropriate parameter set given in bold

Version	M_ini [µg]	k_des [1/d]	f_ne [-]	DT50_eq [d]	Kom_eq [L/kg]	Chi^2-err [%]	Sum of RSE
1	46.0 (0.0090)	0.0316 (0.0906)	0.6865 (0.0610)	86.4 (0.0313)	63.4 (0.0381)	4.28	0.2300
2	46.0 (0.0089)	0.0315 (0.0913)	0.6865 (0.00609)	86.5 (0.0317)	63.4 (0.0389)	4.29	0.2317
3	**46.0 (0.0090)**	**0.0316 (0.0906)**	**0.6865 (0.00610)**	**86.4 (0.0313)**	**63.4 (0.0380)**	**4.28**	**0.2299**
4	46.0 (0.0090)	0.0316 (0.00906)	0.6865 (0.0610)	86.4 (0.0313)	63.4 (0.0381)	4.28	0.2300

The optimization results for the four starting value combinations of *k_des* and *f_ne* are given in Table B.8.2.1-22. There are only very minor differences between the four estimations and thus a robust parameter set for the kinetic sorption magnitude in the Hanscheider Hof soil can be derived. By means

of the Chi2-error percentage and the sum of RSE values the parameter set from version 3 appears as the most appropriate one and will be discussed in more details in the following.

The model fit described by parameter starting value combination 3 is shown in detail in Figure B.8.2.1-10 -Figure B.8.2.1-12. Total mass dissipation and liquid phase concentration are represented very well by the model fit (Figure B.8.2.1-10). Also the residuals indicate a good representation of data (Figure B.8.2.1-11) and the apparent Kd value over time is very well estimated by the model parameters and the corresponding fit (Figure B.8.2.1-12). Clearly, the kinetic sorption model is a better representation of the measured data than the equilibrium model (c.f. Figure B.8.2.1-09). Additionally, the parameter estimates are derived with very low RSE values well below the proposed threshold of 0.4, *f_ne* is <10 and *k_des* < 0.5 (c.f.Table B.8.2.1-22). The difference to the *Kom_eq* calculated from data at DAT0 accounts for only 9.5% and is thus also within the range of acceptable deviation (Beulke and van Beinum, 2012).

Figure B.8.2.1-10 Observed and fitted values for total mass (top) and liquid phase concentration (bottom) in soil Hanscheider Hof and starting value combination version 3

Figure B.8.2.1-11 Residuals for total mass (top) and liquid phase concentration (bottom) in soil Hanscheider Hof and starting value combination version 3

Figure B.8.2.1-12 Measured and fitted apparent Kd value (top) and distribution of mass between equilibrium and non-equilibrium sorption domain (bottom) in soil Hanscheider Hof and for starting value combination version 3

Soil Hoefchen

The equilibrium model fit assuming no kinetic sorption to data for the soil Hoefchen soil is depicted in Figure B.8.2.1-13. Both the total mass and the liquid phase concentration are not very well represented by the model fit. An evaluation of the kinetic sorption magnitude is therefore warranted.

Figure B.8.2.1-13 Equilibrium model fit to Hoefchen soil data.
Total mass depicted at top and liquid phase concentration at bottom.

Table B.8.2.1-23 Parameter optimization for soil Hoefchen, using 4 different starting sets for f_ne and k_des, Chi2-error %and sum of RSE (single rel. standard error RSE in brackets); most appropriate parameter set given in bold

Version	M_ini [µg]	k_des [1/d]	f_ne [-]	DT50_eq [d]	Kom_eq [L/kg]	Chi^2-err [%]	Sum of RSE
1	47.7 (0.0071)	0.0332 (0.1008)	0.3866 (0.0659)	45.7 (0.0175)	46.2 (0.0428)	3.33	0.2340
2	47.7 (0.0071)	0.0332 (0.1012)	0.3867 (0.0661)	45.7 (0.0174)	46.2 (0.0427)	3.33	0.2344
3	**47.7 (0.0071)**	**0.0333 (0.0995)**	**0.3875 (0.00654)**	**45.6 (0.0173)**	**46.1 (0.0419)**	**3.32**	**0.2311**
4	47.7 (0.0070)	0.0332 (0.1008)	0.3868 (0.0662)	45.7 (0.0175)	46.2 (0.0430)	3.33	0.2344

The optimization results for the four starting value combinations of *k_des* and *f_ne* are given in Table B.8.2.1-23. There are only very minor differences between the four estimations and thus a robust parameter set for the kinetic sorption magnitude in the Hoefchen soil can be derived. By means of the Chi2-error percentage and the sum of RSE values the parameter set from version 3 appears as the most appropriate one and will be discussed in more details in the following.

The model fit described by parameter starting value combination 3 is shown in detail in Figure B.8.2.1-14 -Figure B.8.2.1-16. Total mass dissipation and liquid phase concentration are represented very well by the model fit (B.8.2.1-14). Also the residuals indicate a good representation of data (Figure B.8.2.1-15) and the apparent Kd value over time is very well estimated by the model parameters and the corresponding fit (Figure B.8.2.1-16). Clearly, the kinetic sorption model is a better representation of the measured data than the equilibrium model (c.f. Figure B.8.2.1-13). Additionally, the parameter estimates are derived with very low RSE values well below the proposed threshold of 0.4, *f_ne* is <10 and *k_des* < 0.5 (c.f. Table B.8.2.1-23). The difference to the *Kom_eq* calculated from data at DAT0 accounts for only 3.3% and is thus also within the range of acceptable deviation (Beulke and van Beinum, 2012).

Figure B.8.2.1-14 Observed and fitted values for total mass (top) and liquid phase concentration (bottom) in soil Hoefchen and starting value combination version 3

Figure B.8.2.1-15 Residuals for total mass (top) and liquid phase concentration (bottom) in soil Hoefchen and starting value combination version 3

Figure B.8.2.1-16 Measured and fitted apparent Kd value (top) and distribution of mass between equilibrium and non-equilibrium sorption domain (bottom) in soil Hoefchen and for starting value combination version 3

Remarks

The study showed that the four data sets obtained for time dependent sorption did meet the quality criteria set in the draft guidance for time dependent sorption (Beulke and van Beinum, 2012). Additionally fitting was done with four different sets of initial parameters. This only resulted in slightly different optimized model results than the initial fitting that was described in study IIA, 7.4.1/04. The values obtained in this study are used for higher tier PECgw calculations. The obtained parameters are reliable and acceptable and can be used for higher tier ground water calculations.

Metabolite 6-CNA

STUDY IIA, 7.4.2/01

Characteristics

reference	:	Liu, A.C., 1997	soils	:	five soils; loamy sand I, loamy sand II, silt loam, clay, and clay loam
year of execution	:	1997	concentrations	:	0.035, 0.070, 0.349, 1.170 and 2.319 ppm
GLP statement	:	yes	temperature	:	20±1°C
guideline	:	U. S. EPA-FIFRA, 40 CFR, Section 158.290, Subdivision N, Guideline 163-1 EU Commission Directive 95/3 6/EC, Annex I, Section 7.1.2 (14 July 1995) Canada PMRA DACO Number 8.2.4.2, 1987	$K_F$oc	:	70-258
test substance	:	[pyridyl -2,6-^{14}C] 6-chloronicotinic acid	1/n	:	0.894-1.01
purity	:	radiochemical purity 99%	acceptability	:	**acceptable**

Summary

The soil adsorption/desorption of 6-chloronicotinic acid [pyridyl-2,6-^{14}C], was studied in equilibrium experiments using five soils (loamy sand I, loamy sand II, silt loam, clay, and clay loam) as well as in an aquatic pond sediment. The study was conducted at 20±1°C in the dark at five different concentrations (0.035, 0.070, 0.349, 1.170 and 2.319 ppm) in a 0.01 M $CaCl_2$ solution (equivalent to 0.174, 0.349, 1.745, 5.847, and 11.586 ppm in the soil, respectively). The preliminary range-finding study conducted using loamy sand II, silt loam and pond sediment, showed that both adsorption equilibrium and desorption equilibrium were reached at 16 hours. Therefore, the adsorption cycle and each desorption cycle were carried out for 24 hours each in the definitive study. No adsorption of 6-chloronicotinic acid to glass was observed.

Total accountability for all the soils averaged 99% and ranged from 94% to 110% of the applied dose activity with the exception of 0.07 ppm clay (87%). The average % of applied 6-chloronicotinic acid remaining in the supernatants of each phase was as follows:

The Freundlich adsorption (K_f) and desorption (K_{des}) constants as well as adsorption K_{oc} are summarised in Table B.8.2.1-23.

The Freundlich adsorption (K_f) constants ranged from 0.569 for silt loam to 2.121 for pond sediment, averaging 0.981. Adsorption Koc values ranged from 70 for loamy sand II and clay to 258 for loamy sand I. Similar K_f and K_{desl} values for all of the soils and pond sediment shows the reversible equilibration between adsorption and first desorption phases.

Table B.8.2.1-23 ~~Average % of applied 6-CNA remaining in the supernatants~~ Adsorption and desorption constants of 6-CNA in six soils

Soil type	OC [%]	pH	Adsorption			Desorption		
			1/n	K_f	K_{oc}	K_{des1}	K_{des2}	K_{des3}
Loamy Sand I *	*0.43*	*4.4*	*0.967*	*0.643*	*258*	*0.762*	*2.394*	*6.789*
Loamy Sand II	2.54	6.2	1.007	1.027	70	1.391	1.243	2.551
Silt Loam	0.76	6.6	0.971	0.569	129	0.472	0.989	6.789
Clay	2.05	7.5	0.894	0.833	70	0.393	3.522	2.551
Clay Loam	1.41	8.3	0.926	0.690	84	0.505	2.394	6.789
Pond sediment (Sandy Loam) **	*4.32*	*5.6*	*0.867*	*2.121*	*85*	*3.144*	*1.243*	*2.551*
Average soils			**0.949**	**0.780**	**88**			

*: not considered for calculation of mean since the OC was regarded as too low
**: pond sediment not considered for calculation of mean in soils

Remarks

The results of the adsorption desorption study with 6-CNA is acceptable and reliable. The sorption did not show pH-dependency. The pH range of the used soils is within the typical range of agricultural soils and there is no indication of a pH dependence of the sorption. This is not unexpected as the pKa of 6-CNA (3.28) indicate full ionsiation above pH 5 and hence no influence of the pH of the soil would be expected. The endpoints will be used for risk assessment. In the calculation of the mean the values of loamy sand I and pond sediment are not taken into account.

Metabolite DFA

STUDY IIA, 7.4.2/02

Characteristics

reference	:	Menke, U., Unold, M., 2011	soils	:	five soils; silt loam, loam, clay loam, sandy loam, silty clay loam
year of execution	:	2010	concentrations	:	1.0, 0.3, 0.1, 0.03, and 0.01 mg/L
GLP statement	:	yes	temperature	:	20±1°C
guideline	:	Incl. OECD 106 (2000)	$K_F$oc	:	70-258
test substance	:	[1-^{14}C] BYI 02960-DFA	1/n	:	0.894-1.01
purity	:	radiochemical and chemical purity: >98% / 99.5%	acceptability	:	**acceptable**

Summary

The adsorption/desorption behavior of BYI 02960-DFA was studied in batch equilibrium experiments in five soils originating from Germany and the USA.

The adsorption phase of the study was carried out using pre-equilibrated air-dried soils in 0.01 M aqueous $CaCl_2$ solution with a soil to solution of 1:1.25 for soil Dollendorf II and soil to solution ratios of

1:1 for soils Hoefchen am Hohenseh 4a, Hanscheiderhof, Sanger and Springfield. BYI 02960-DFA was applied at concentrations of nominal 1.0, 0.3, 0.1, 0.03, and 0.01 mg/L supernatant.

Desorption experiments were performed by supplying pre-adsorbed soil samples with fresh 0.01 M aqueous $CaCl_2$ solution. One desorption cycle was performed for all concentrations and three desorption cycles were performed for the highest concentration only.

Adsorption and desorption took place in the dark at 20 ± 1 °C for 24 hours with triplicate samples, respectively. The aqueous supernatant after adsorption and desorption was separated by centrifugation, and the BYI 02960-DFA residue in the supernatant was analyzed by liquid scintillation counting (LSC). The adsorption and desorption parameters were calculated using the Freundlich equation.

The test item was stable throughout the study. The mass balance of the parent compound determined for all soils at the highest concentration was >90%. In the definitive adsorption test 16.9 - 25.3%, 16.6 - 34.9%, 22.3 - 26.6%, 2.6 - 10.7% and 2.4 - 5.7% of the applied test item were adsorbed to soils Hoefchen am Hohenseh 4a, Hanscheiderhof, Dollendorf II, Sanger and Springfield, respectively.

The calculated adsorption constant $K_{F(ads)}$ of the Freundlich isotherms for the five test soils ranged from 0.028 - 0.368 mL/g, the $K_{oc(ads)}$) from 1.7 – 9.5 mL/g. The Freundlich exponent 1/n was in the range of 0.6902 to 0.9579, indicating that the concentration of the test item affected the adsorption behavior in the examined concentration range.

The $K_{oc(des)}$ values were 12.7 - 57.1 times higher than the $K_{oc(ads)}$ values, indicating a strong binding of the test item to the soil. Soils Dollendorf II and Springfield did not show an acceptable linearity of the Freundlich desorption isotherms and therefore desorption constants could not be calculated. For the highest concentration of each soil, a second and a third desorption step were performed. In these steps, the desorbed amounts of BYI 02960-DFA range from 13.4 to 45.2% of the adsorbed amount, indicating that the binding is partly reversible. The following table summarizes the key data of this study. BYI 02960-DFA can be classified as very mobile for adsorption and low mobile for desorption.

Table B.8.2.1-24 Key data and results of study

Soil origin	**Hoefchen am Hohenseh 4a, Germany**	**Hanscheider Hof, Germany**	**Dollendorf II, Germany**	**Sanger, USA**	**Springfield, USA**
Soil type (USDA)	Silt Loam	Loam	Clay loam	Sandy Loam	Silty Clay loam
pH (aqueous $CaCl_2$ solution)	6.5	5.8	7.4	6.0	6.5
Organic carbon [%]	2.4	2.9	4.5	0.5	1.7
$K_{F(ads)}$ [mL/g]	0.228	0.226	0.368	0.033	0.028
1/n	0.9053	0.8013	0.9579	0.6902	0.8194
$K_{oc(ads)}$ [mL/g]	9.5	7.8	8.2	6.7	1.7
$K_{oc(des)}$ [mL/g]	121.3	155.5	*	380.7 **	*
Arithmetic mean $K_{oc(ads)}$: 6.8 mL/g; $K_{oc(des)}$: 219.1 mL/g					

* The correlation coefficient determined for the Freundlich desorption isotherms was not significant
** For soil Sanger (SA) the highest concentration (1 mg/L) was not included in the calculation of the Freundlich desorption isotherm due to a negative supernatant concentration calculated for one replicate

Material and Methods

Materials

1. Test Item: BYI 02960-DFA Code BCS-AB60481; CAS no. 2218-52-2 [sodium salt]; 381-73-7 [free acid]; [1-14C] BYI 02960-difluoro-acetic acid: sample ID KATH 6450; Specific activity: 76.68 µCi/mg (2.84 MBq/mg); Radiochemical and chemical purity: >98% / 99.5% (at beginning of study); The test item was identified by LC-MS, LC-MS/MS and NMR)

The ^{14}C-test item was dissolved in TKA-water (approx. 1.6 mg/mL) after arrival at the testing facility and stored in a freezer in the dark. Radiopurity of the labeled test item was checked by TLC analysis.

2. Soils: The adsorption/desorption behavior of DFA was studied in batch equilibrium experiments in five soils originating from Germany and the USA: Hoefchen am Hohenseh 4a, silt loam, pH 6.5, 2.4% organic carbon; Hanscheiderhof, loam, pH 5.8, 2.9% organic carbon; Dollendorf II, clay loam, pH 7.4, 4.5% organic carbon; Sanger, sandy loam, pH 6.0, 0.5% organic carbon; Springfield, silty clay loam, pH 6.5, 1.7% organic carbon (pH values derived from aqueous CaCl2 suspension). The pH values of the soil batches were measured in 0.01 M aqueous $CaCl_2$. The soils were air-dried and homogenized by sieving (≤ 2 mm) and stored in a climate chamber at 5 °C. The detailed parameters of soils are shown in Table B.8.2.1-25.

Methods

1. Experimental conditions: In the definitive test, 16 g of soil Dollendorf II and 20 g of soils Hoefchen am Hohenseh 4a, Hanscheiderhof, Sanger and Springfield were weighed into the centrifuge tubes and aqueous 0.01 M $CaCl_2$ solution was added to a solution volume of 18 mL (corrected for soil humidity). After pre-equilibration by shaking for about three days, 2 mL of the respective application solution were added. The adsorption/desorption measurements were performed over a range of five BYI 02960-DFA concentrations (0.01 mg/L to 1.0 mg/L), covering two orders of magnitude. The samples were incubated at constant temperature in the dark and continuously agitated using an overhead shaker. After a certain time the suspensions were centrifuged and the supernatants were analyzed by LSC. Additionally, the pH values of the supernatants were determined.

For the desorption experiments the supernatants were removed, measured, and replaced by an equivalent volume of aqueous 0.01 M $CaCl_2$ solution. After agitation (for e.g. 24 h in the definitive test) and centrifugation, the supernatant was decanted, measured, and analyzed by LSC. One desorption step was performed for the samples of all five concentration levels whereas three desorption steps were performed for the samples of the highest concentration level.

Mass balances were calculated for the one sample of each concentration level. For this purpose, the remaining soil was freeze-dried, grounded, combusted, and analyzed by LSC. Due to the stability of the test item, the partition of the test item was determined based on the amount of RA measured in the supernatant by LSC. The adsorption and desorption experiments were performed in triplicate.

2. Application procedures: The total amount of [1-^{14}C] BYI 02960-DFA delivered (6.5 mg) was dissolved in 4 mL TKA-water resulting in a solution containing approximately 1.6 mg test item/mL. In the definitive test, the soil to solution ratio for soils Hoefchen am Hohenseh 4a, Hanscheiderhof, Sanger and Springfield was 20 g soil to 20 mL of 0.01 M aqueous $CaCl_2$ solution. For soil Dollendorf II, the soil to solution ratio was 16 g soil to 20 mL 0.01 M aqueous $CaCl_2$ solution. The nominal concentrations of radiolabeled BYI 02960-DFA were 0.01, 0.03, 0.1, 0.3, and 1.0 mg/L. Using pipette aliquots of 2 mL, the radiolabeled BYI 02960-DFA application solutions were added to the pre-equilibrated suspensions consisting of 16 or 20 g soil and 18 mL 0.01 M aqueous $CaCl_2$ solution.

Table B.8.2.1-25 Physico-chemical characteristics of test soils

Soil ID / Batch #	**Hoefchen am Hohenseh 4a (HF) 20100308**	**Hanscheider Hof (HN) 20100308**	**Dollendorf II (DD) 20100308**	**Sanger (SA) 20090626**	**Springfield (SF) 20090629**
Geographic Location (City / State / Country)	Burscheid / Northrhine-Westfalia / Germany	Burscheid / Northrhine-Westfalia / Germany	Blankenheim / Northrhine-Westfalia / Germany	Sanger / California / USA	Springfield/ Nebraska / USA
Texture Class[A]	Silt Loam	Loam	Clay Loam	Sandy Loam	Silty Clay loam
Sand[A] Silt[A] Clay[A]	23% 60% 17%	37% 42% 21%	33% 36% 31%	63% 26% 11%	19% 50% 31%
pH ($CaCl_2$) pH (Water) pH (KCl)	6.5 6.8 6.3	5.8 6.1 5.4	7.4 7.5 7.1	6.0 6.4 5.7	6.5 6.6 6.0
Organic Matter[B]	4.1%	5.0%	7.8%	0.9%	2.9%
Organic Carbon[C]	2.4%	2.9%	4.5%	0.5%	1.7%
Cation Exchange Capacity (CEC)	13.4 meq/100 g	10.1 meq/100 g	20.6 meq/100 g	7.4 meq/100 g	19.4 meq/100 g
Moisture at 0.33 bar	22.3%	22.5%	32.7%	9.4%	23.0%
Bulk Density Particle Density	1.05 g/mL	1.08 g/mL	1.00 g/mL	1.39 g/mL	1.03 g/mL
Soil Mapping Unit	N 51° 04.011' E 007° 06.327	N 51° 04.482' E 007° 06.361	N 50°22.899' E 06° 43.001	N 36°42.157' W 119° 27.851	N 96° 09.051 W 41° 02.235

[A]) According to USDA classification
[B]) Calculated: Organic matter = organic carbon * 1.724
[C]) Determination method: Combustion

3. Analytical procedures: The stability of the test item was determined by TLC of the control samples and of supernatants and extracts of the soil samples (highest concentration) in the pre-tests. The test item was considered to be stable (parental mass balance >90). The limit of quantification (LOQ) was

set to three times the background radioactivity, i.e. about 0.96 Bq (0.33 ng). The lowest amount measured was about 5.5 Bq, i.e. about 5.7 times higher than the LOQ.

In the definitive test supernatants were analyzed by liquid scintillation counting (LSC). After the desorption step, for the calculation of the mass balance, the remaining soil was freeze-dried and combusted. The trapped $^{14}CO_2$ after combustion was measured by LSC. Due to the stability of the test item, the partition of the test item was determined based on the amount of RA measured in the supernatant by LSC.

Results and Discussion

Mass Balance

The overall material balance for all soils was in the range of 95.1-100.3% (mean: 97.2%) of the applied radioactivity. The complete material balances found at all sampling intervals demonstrated that no significant RA dissipated from the test vessels or was lost during processing.

Table B.8.2.1-26 Recovery of Total Radioactivity of BYI 02960 after Adsorption and Desorption Steps (Expressed as percentage of applied radioactivity)

Description Soil ID Conc. ID	Soil 1 HF (% of AR)	Soil 2 HN (% of AR)	Soil 3 DD (% of AR)	Soil 4 SA (% of AR)	Soil 5 SF (% of AR)
1.0 mg/L	98.2	97.6	97.7	100.3	98.7
0.30 mg/L	96.0	95.3	95.3	99.2	97.3
0.10 mg/L	96.2	94.8	95.1	98.9	97.0
0.030 mg/L	97.0	94.3	94.7	97.8	97.1
0.010 mg/L	96.2	93.6	95.1	105.2	101.2
Mean	**96.7**	**95.1**	**95.6**	**100.3**	**98.3**
Standard deviation	**± 0.8**	**± 1.4**	**± 1.1**	**± 2.6**	**± 1.6**

Stability and Recovery of Test Item

The stability of DFA in the test system used was confirmed by performing HPLC analyses prior to the definitive test. Recoveries of radioactivity in the 0.01 M $CaCl_2$ solutions of control samples without soil range from 99.8 to 100.7% of applied radioactivity (0 – 96 h). The test item was stable in control samples without soil.

Findings

In the definitive adsorption test 16.9 - 25.3%, 16.6 - 34.9%, 22.3 - 26.6%, 2.6 - 10.7% and 2.4 - 5.7% of the applied test item were adsorbed to soils Hoefchen am Hohenseh 4a, Hanscheiderhof, Dollendorf II, Sanger and Springfield, respectively. The adsorption behavior of BYI 02960-DFA in the concentration range of two orders of magnitude (i.e. from 0.01 - 1.0 mg/L) was accurately described for all soils with the Freundlich equation. The correlation coefficient of the individual isotherms was in the range of 0.9699 - 0.9984. The calculated adsorption constants $K_{F(ads)}$ of the Freundlich isotherms for the five test soils ranged from 0.028 - 0.368 mL/g. The Freundlich exponents 1/n were in the range of 0.6902 -

0.9579, indicating that the concentration of the test item affected the adsorption behavior in the examined concentration range.

Table B.8.2.1-27 Concentration of BYI 02960 in the Solid and Liquid Phases at the End of the Adsorption Period

Concentration	Soil (mg/kg)	Solution (mg/L)	Percentage adsorbed	
Soil ID	**HF**			
Control	N/A	N/A		
0.010 mg/L	0.002	0.008	24.4	± 1.3
0.030 mg/L	0.008	0.022	25.3	± 0.5
0.10 mg/L	0.025	0.075	24.8	± 0.3
0.30 mg/L	0.067	0.234	22.2	± 0.3
0.99 mg/L	0.167	0.825	16.9	± 0.2
Soil ID	**HN**			
Control	N/A	N/A		
0.010 mg/L	0.003	0.006	34.9	± 1.3
0.030 mg/L	0.010	0.020	34.4	± 0.7
0.10 mg/L	0.030	0.070	30.0	± 1.2
0.30 mg/L	0.078	0.222	26.0	± 0.3
0.99 mg/L	0.165	0.828	16.6	± 0.8
Soil ID	**DD**			
Control	N/A	N/A		
0.010 mg/L	0.003	0.007	25.4	± 1.5
0.030 mg/L	0.010	0.022	26.6	± 0.4
0.10 mg/L	0.032	0.075	25.5	± 0.3
0.30 mg/L	0.091	0.228	24.1	± 1.0
0.99 mg/L	0.276	0.772	22.3	± 0.4
Soil ID	**SA**			
Control	N/A	N/A		
0.010 mg/L	0.001	0.009	10.7	± 0.6
0.030 mg/L	0.003	0.027	10.0	± 0.1
0.10 mg/L	0.008	0.092	7.5	± 0.1
0.30 mg/L	0.017	0.284	5.5	± 0.3
0.99 mg/L	0.026	0.966	2.6	± 0.2
Soil ID	**SF**			
Control	N/A	N/A		
0.010 mg/L	0.001	0.009	5.5	± 1.4
0.030 mg/L	0.002	0.028	5.7	± 1.1
0.10 mg/L	0.005	0.095	4.9	± 0.8
0.30 mg/L	0.011	0.289	3.7	± 0.3
0.99 mg/L	0.024	0.968	2.4	± 0.4

Table B.8.2.1-28 Concentration of BYI 02960 in the Solid and Liquid Phase at the End of First Desorption Phase

Concentration	Soil (mg/kg)	Solution (mg/L)	Percentage desorbed*	
Soil ID	**HF**			
Control	N/A	N/A		
0.010 mg/L	0.002	0.000	19.8	± 3.6
0.030 mg/L	0.006	0.002	26.3	± 2.3
0.10 mg/L	0.019	0.006	25.3	± 2.5
0.30 mg/L	0.050	0.017	25.1	± 3.5
0.99 mg/L	0.133	0.034	20.5	± 1.7
Soil ID	**HN**			
Control	N/A	N/A		
0.010 mg/L	0.003	0.001	18.9	± 1.6
0.030 mg/L	0.008	0.002	19.4	± 1.7

0.10 mg/L	0.024	0.006	21.2	± 1.6
0.30 mg/L	0.062	0.016	21.0	± 1.5
0.99 mg/L	0.138	0.027	16.2	± 0.4
Soil ID	**DD**			
Control	N/A	N/A		
0.010 mg/L	0.003	**	**	**
0.030 mg/L	0.010	0.000	3.8	± 0.8
0.10 mg/L	0.030	0.001	5.2	± 1.1
0.30 mg/L	0.085	0.004	5.5	± 2.6
0.99 mg/L	0.281	**	**	**
Soil ID	**SA**			
Control	N/A	N/A		
0.010 mg/L	0.001	0.000	42.8	± 4.1
0.030 mg/L	0.002	0.001	44.2	± 2.7
0.10 mg/L	0.004	0.003	43.0	± 2.8
0.30 mg/L	0.010	0.006	38.5	± 2.6
0.99 mg/L	0.025	**	**	**
Soil ID	**SF**			
Control	N/A	N/A		
0.010 mg/L	0.000	0.0001	25.7	± 13.16
0.030 mg/L	0.001	0.0005	24.9	± 8.82
0.10 mg/L	0.004	**	**	**
0.30 mg/L	0.011	**	**	**
0.99 mg/L	0.033	**	**	**

* Expressed as a percentage of the initially adsorbed material, one desorption step for all concentrations
** The subtraction of the remaining radioactivity in solution from the measured concentrations resulted in negative liquid concentrations for some samples. Therefore the mean concentration was not presented and the percentage of desorbed test item was not calculated for this concentration level

The adsorption coefficients $K_{F(ads)}$ were correlated with the organic carbon content of the soil, in order to get a comparability of the adsorption behavior in different soils. For DFA the calculated $K_{oc(ads)}$ values varied between 1.7 and 9.5 mL/g (mean: 6.8 mL/g).

One desorption step was performed for all concentrations. For soils Hoefchen am Hohenseh 4a and Hanscheiderhof this first desorption step resulted in highly significant correlation coefficients for the Freundlich isotherm with values of 0.9788 and 0.9884. When omitting the highest concentration, the first desorption step of soil Sanger had a significant correlation coefficient as well (0.9801). At the end of the first desorption phase, 19.8 - 26.3%, 16.2 - 21.2% and 38.5 - 44.2% of the initially adsorbed amount was desorbed in soils Hoefchen am Hohenseh 4a, Hanscheiderhof and Sanger, respectively. The other soils (Dollendorf II and Springfield) did not show an acceptable linearity of the Freundlich desorption isotherms and therefore, desorption constants could not be calculated.

The calculated desorption constants $K_{F(des)}$ of the Freundlich isotherms for the three evaluated test soils ranged from 1.903 - 4.509 mL/g. The respective mean $K_{oc(des)}$ values was 219.1 mL/g). The $K_{oc(des)}$ values were 12.7 to 57.1 times higher than the $K_{oc(ads)}$ values, indicating a strong binding of the test item to the soil. For the summary of results see Table B.8.2.1-29.

Table B.8.2.1-29 Adsorption and desorption constants of BYI 02960-DFA in five soils

Soil	Adsorption				Desorption			
	K_F (mL/g)	1/n	R^2	Koc (mL/g)	K_F (mL/g)	1/n	R^2	Koc (mL/g]

HF	0.228	0.9053	0.9945	9.5	2.911	0.975	0.9788	121.3
HN	0.226	0.8013	0.9903	7.8	4.509	1.013	0.9884	155.5
DD	0.368	0.9579	0.9984	8.2	*	*	*	*
SA	0.033	0.6902	0.9769	6.7	1.903**	1.052**	0.9801**	380.7**
SF	0.028	0.8194	0.9699	1.7	*	*	*	*
Arithmetic mean	**0.177**	**0.8348**	**0.9860**	**6.8**	**3.1**	**1.0**	**1.0**	**219.1**

* The correlation coefficient determined for the Freundlich desorption isotherms was not significant
** For soil Sanger (SA) the highest concentration (1 mg/L) was not included in the calculation of the Freundlich desorption isotherm due to a negative supernatant concentration calculated for one replicate

Conclusions

DFA can be classified as very mobile for adsorption and low mobile for desorption.

Remarks

The results of the adsorption desorption study with DFA is acceptable and reliable. The sorption did not show pH-dependency. The pH range of the used soils is within the typical range of agricultural soils and there is no indication of a pH dependence of the sorption. This is not unexpected as the pKa of DFA (1.57) indicate full ionsiation above pH 5 and hence no influence of the pH of the soil would be expected. The endpoints will be used for risk assessment.

B.8.2.2 Column leaching studies, leaching of aged residues and field leaching studies (Annex IIA 7.1.3.1, 7.1.3.2, 7.1.3.3; Annex IIIA 9.1.2.1, 9.1.2.2)

Reliable adsorption values were determined by batch equilibrium experiments according to OECD guideline 106. Therefore, no column leaching studies have to be performed with BYI 02960 and soil metabolites. A colomn leaching study was submitted and included for completeness.

STUDY IIA, 7.4.3/01

Characteristics

Reference	:	De Souza, T.J.T., 2012	soils	:	four soils; clay I, clay II, loamy sand/sandy loam, loam
year of execution	:	2011	Application rate	:	1500 g a.i./ha
GLP statement	:	yes	temperature	:	ambient
Guideline	:	OECD 312 (2004)	$K_{F}oc$	:	not applicable
test substance	:	[pyridine-2,6-^{14}C]-BYI 02960	1/n	:	not applicable
Purity	:	radiochemical purity: 99.55%	acceptability	:	**not required**

Summary

In the EU and NAFTA this requirement is covered by the adsorption/desorption studies with the parent compound as presented in chapter B.8.2.1. However, a soil column leaching study for the active substance was performed in order to fulfill a requirement of Brazilian registration authorities. A short summary of that study is given as follows.

The leaching behaviour of [pyridine-2,6-^{14}C]-BYI 02960 was studied in four Brazilian soils under laboratory conditions according to OECD Guideline Method 312 (2004). The four soil types were

Argissolo (clay), Latossolo vermelho (clay), Neossolo (loamy sand/sandy loam) and Gleissolo humico (loam). Duplicate glass columns per soil type were filled with the respective soil to a height of 30 cm. After conditioning with 0.01 M $CaCl_2$ solution [PYR-^{14}C]-BYI 02960 was applied to the top of the columns at a dosage approximating a maximum application rate of 1500 g a.i./ha. Monuron was used as a reference substance and was applied at a rate of 5 kg a.i./ha. After the application of test and reference substance each column was irrigated with 200 mm of artificial rain over a 48-hrs period, and the respective leachate samples were collected. After percolation, the soil columns were divided into six layers of ca. 5 cm each, and the soil samples were extracted with acetonitrile:water (80:20 v/v). Layers containing more than 10% of applied radioactivity (AR) were sequentially extracted until the last extract represented less than 10% of AR. Extracted radioactivity was analyzed by high-performance liquid chromatography (HPLC) with radiometric detection. The results of the HPLC analysis did not show degradation of the parent compound during the timescale of the study.

The average mass balance ranged from 96.6 - 106.3% in the four tested soils. Extractable radioactivity ranged for all soils from 73.75 - 95.26%, and non-extractable radioactivity ranged from 3.64 - 25.22%.
No radioactivity (<LOQ) was detected in the leachate of Argissolo and Gleissolo soils. Thus the AR was completely retained in Argissolo and Gleissolo soils, however, percolated to the leachate in Latossolo and Neossolo soils after application of the artificial rain. In the leachates of Latossolo and Neossolo mean 14.93 and 23.50% of AR were detected, respectively. Analysis of the leachates showed that there was detectable Monuron in the leachate of Argissolo soil (one replicate), Latossolo and Neossolo soils.

The maximum (mean) depth penetration of the radioactivity in the soil profiles was < 10 cm for Argissolo soil, > 30 cm for Latossolo, > 30 cm for Neossolo, and ≤ 7.5 cm for Gleissolo soil, whereas the leaching depth of Monuron was ≤ 27.5, > 30, > 30 and < 7.5 cm in the respectively listed soils.

In order to compare leaching data from different experiments, a relative mobility factor (RMF) to a reference chemical such as Monuron was used. Monuron is known to be moderately mobile in the field. Knowing the RMF values of crop protecting compounds allows their classification into mobility classes. The relative mobility factor (RMF) for BYI 02960 was 0.45 for Argissolo soil, 1.0 for Latossolo, 1.0 for Neossolo soil and 1.0 for Gleissolo soil. Based on these values, BYI 02960 can be classified as little mobile in Argissolo and moderately mobile in Latossolo, Neossolo and Gleissolo soils.

B.8.2.3 Lysimeter studies (Annex IIA 7.4.7 to 7.4.8; Annex IIIA 9.3.2 to 9.3.3)

Reliable adsorption values for parent and metabolites were determined by batch equilibrium experiments according to OECD guideline 106 (2000). The potential of leaching to groundwater for the parent and metabolites can be predicted from the available data. Therefore, no lysimeter study is considered necessary.

B.8.2.4 Summary adsorption, desorption and mobility in soil

Batch adsorption studies BYI 02960

Batch adsorption studies were performed for BYI 02960 and two major soil metabolites. The equilibrium sorption BYI 02960 was studied in two batch equilibrium studies in the laboratory at 20°C with six different soils and one time dependent sorption experiment. The data of the batch experiments were evaluated to derive Freudlich isotherms. The resulting arithmetic mean values for 1/n, Kf (ads) and Koc were 0.866, 2.263 (L/kg) and 98.4 (L/kg (Table B.8.2.4-01). The desorption Kf (des) and the normalized K_{OC}(des) values were significantly higher (i.e. approx. 2 times) than those obtained for the adsorption phase, indicating that the test item once adsorbed to soil is not readily desorbed. BYI 02960 can be classified as intermediate mobile for adsorption and low mobile for desorption.

Table B.8.2.4-01 Adsorption measurements for BYI 02960

Soil	Soil Type (USDA)	pH ($CaCl_2$) [-]	K_f [mL g^{-1}]	1/n [-]	$K_{f,oc}$ [mL g^{-1}]
Laacherhof AXXa	Sandy loam	6.2	2.077	0.8445	98.9
Hoefchen am Hohenseh 4a	Loam	6.6	2.213	0.8682	92.2
Hanscheiderhof	Loam	5.3	2.354	0.8643	107.0
Dollendorf II	Loam	7.2	3.822	0.8648	74.9
Sanger	Sandy Loam	6.8	0.597	0.9021	85.2
Springfield	Silt loam	6.5	1.554	0.8505	132.2
Arithmetic mean		**-**	**-**	**0.8657**	**98.4**

* $K_{oc,EQ}$ restults of the TDS included in the average caluclation

Time dependent sorption study BYI 02960

Time dependent sorption (TDS) of BYI 02960 was studied in four soils a maximum period of 120 days under aerobic conditions in the dark at ca. 20°C and 55% WHCmax. At the beginning of the study the distribution coefficient values RTDS were 1.46, 1.91, 3.35 and 4.66 mL/g for soils AX, HF, HN and DD, respectively. Based on results from the ageing time of 120 days, the RTDS values increased by a factor of 4.4, 2.9, 3.2 and 2.6 (mean of all four soils = 3.3).

A more detailed evaluation showed that the before-mentioned experimental data can be well described by a kinetic-sorption model, with excellent fits and reliable parameter estimates. Such time-dependent sorption data received for BYI 02960 constitute the prerequisite to adequately address the obvious TDS process in higher tier regulatory modelling, e.g. related to a potential groundwater contamination. For BYI 02960 a fitted mean $K_{oc,eq}$ of 80.2 L/kg ($K_{om,eq}$ of 46.5 L/kg) and the Freundlich exponent 1/n of 0.8605 can be used in higher tier simulation runs (arithmetic mean of the 1/n of the four soils used for the batch sorption and identiacal as the soils used for TDS).

The RMS intendedat first to use both the batch adsorption and time dependent sorption (TDS) K_{oc} and $K_{oc,eq}$ to obtain a arithmetic mean K_{oc}. The RMS did this on basis of the EFSA opinion on the science behind the guidance for scenario selection and scenario parameterisation for predicting environmental concentrations of plant protection products in soil (ESFA Journal 2012;10(2)2562). It is advised to use in each tier, all available relevant scientific information. The RMS was aware that in the guidance on

how aged sorption studies for pesticides should be conducted, analysed and used in regulatory assessments (Beulke S. and Van Beimum W. 2012, DEFRA projects PS2235 and PS2244) a different opinion is presented, i.e. not to combine the TDS and batch adsorption/desorption data. This UK guidance on TDS is not yet adopted in Europe and also the opinion is not adopted. After a discussion with the applicant and external experts, the RMS decided to not include the TDS $K_{oc,EQ}$ data in the first tier data set for the K_{oc}. The first tier arithmetic mean K_{oc} value is 98.4 L/kg, this is the average of the six batch adsorption values.

Batch adsorption studies for metabolites

The equilibrium sorption of 6-chloronicotinic acid (6-CNA) was studied in experiments using five soils and an aquatic pond sediment. The arithmetic mean 1/n value for soil was 0.9495, adsorption Koc values in soils ranged from 70 to 258, averaging 88.0 (L/kg). Similar Kf and Kdes values for all soils indicated a reversible equilibration between adsorption and first desorption phases. 6-CNA can be classified as medium to high mobile for adsorption and desorption.

The equilibrium sorption of difluoroacetic acid (DFA) was studied in experiments using five soils. The arithmetic mean 1/n value for soil was 0.8348, adsorption Koc values ranged from 1.7 to 9.5, averaging 6.8 (L/kg). The Koc(des) values were 12.7 to 57.1 times higher than the Koc(ads) values, indicating a strong binding of the test item to the soil. BYI 02960-DFA can be classified as very mobile for adsorption and low mobile for desorption.

The results of the batch adsorption analyses performed for the metabolites are reported below (see Table B.8.2.4-02 and Table B.8.2.4-03).

Table B.8.2.4-02 Adsorption measurements for 6-chloronicotinic acid

Soil	Soil Type (USDA)	pH ($CaCl_2$) [-]	K_f [mL g^{-1}]	1/n [-]	$K_{f,oc}$ [mL g^{-1}]
Clayton	Loamy Sand	6.2	1.027	1.0069	70
Leland	Silt loam	6.6	0.569	0.9706	129
Leland	Clay	7.5	0.833	0.8941	70
San Juan Bautista	Clay loam	8.3	0.690	0.9262	84
Arithmetic mean		**-**	**-**	**0.9495**	**88.3**

Table B.8.2.4-03 Adsorption measurements for BYI 02960-DFA

Soil	Soil Type (USDA)	pH ($CaCl_2$) [-]	K_f [mL g^{-1}]	1/n [-]	$K_{f,oc}$ [mL g^{-1}]
Hoefchen am Hohenseh 4a	Silt loam	6.5	1.74	0.9053	9.5
Hanscheiderhof	Loam	5.8	1.07	0.8013	7.8
Dollendorf II	Clay loam	7.4	1.78	0.9579	8.2
Sanger	Sandy Loam	6.0	0.594	0.6902	6.7
Springfield	Silty clay loam	6.5	0.274	0.8194	1.7
Arithmetic mean		**-**	**-**	**0.8348**	**6.8**

B.8.3 Predicted environmental concentrations in soil (PECs) (Annex IIIA, 9.1.3)

Calculations were based on a simple first tier approach (Excel sheet) assuming even distribution of the compound in upper 0-5 cm soil layer. A standard soil density of 1.5 g/cm^3 was assumed. Crop interception data which correspond to the intended growth stages were taken from the FOCUS groundwater guidance paper (FOCUS 2010).

Crop interception will reduce the amount of a compound reaching the soil and therefore this has been taken into account depending on the growth stage at application. The interception rates follow the recommendations of the FOCUS groundwater guidance paper (FOCUS 2010) provided in Table B.8.3-01. As hop and lettuce have not been defined in the FOCUS groundwater guidance paper, vines and cabbage were chosen as surrogate crops. As there is currently no guidance on calculation of PEC_{soil} for greenhouse crops no specific calculation was performed and it is assumed that the glasshouse use is covered by the outdoor use on lettuce.

STUDY IIIA, 9.4/01 and STUDY IIIA, 9.4/02

Reference/notifier	:	Sur R., Ellerich C.; 2012 and 2013	GLP statement	:	no
type of study	:	PECs calculation	guideline	:	Soil Persistence Models and EU registration: Report of the FOCUS Soil Modelling Work Group, 1996. EC Document Reference 7617VI/96
year of execution	:	2012 and 2013	acceptability	:	**acceptable**
test substance	:	BYI 02960			

Study design

Methods and Materials: The predicted environmental concentrations in soil (PEC_{soil}) of BYI 02960 were estimated using a simple first tier approach. Detailed application data used for simulation of PEC_{soil} are compiled in Table B.8.3-01. PEC soil were not calculated for the glasshouse uses of the product.

Substance Specific Parameters: PEC_{soil} calculations were based on the DT_{50} of 0.02 days for the fast and 462 days for the slowly degrading compartments (DFOP, worst case of field dissipation studies, k_1=34.66 and k_2=0.0015 and g =0.27).

Table B.8.3-01 Application pattern used for PEC_{soil} calculations of BYI 02960

Individual Crop	FOCUS Crop Used for Interception	Application				Amount Reaching the Soil per Season application [g a.s. /ha]
		Rate per Season [g a.s. /ha]	Interval [days]	Plant Interception [%]	BBCH Stage	
Hops, GAP – identical with simulation	Vines	1 x 150	-	60	31-75	1 x 60

Lettuce 1 (F), GAP – identical with simulation [1)]	Cabbage	1 x 125	-	25	12-49	1 x 94
Lettuce 2 (F), GAP – identical with simulation [2)]	Cabbage	1 x 125	-	70	41-49	1 x 37.5

[1)] Max 1 application per 24 months (biennual)
[2)] Max 1 application per 12 months (annual)
F = field use

To account for potential accumulation of BYI 02960 in soil (worst-case non-normalised DFOP DT_{90} > 365 days), long-term soil concentrations were calculated.

Findings: The PEC_{soil} and the time weighted average values (TWA_{soil}) of BYI 02960 are summarised in Table B.8.3-02 for hops and in for lettuce Table B.8.3-03.

Table B.8.3-02 PEC_{soil} (actual) and TWA of BYI 02960 in hops in the upper 5 cm, DFOP decay

	Time [days]	BYI 02960 Hops, 1 x 150 g/ha	
		PEC_{soil} [mg/kg]	TWA_{soil} [mg/kg]
Initial	0	0.080	-
Short term	1	0.058	0.069
	2	0.058	0.066
	4	0.058	0.063
Long term	7	0.058	0.061
	14	0.057	0.059
	21	0.057	0.058
	28	0.056	0.058
	50	0.054	0.057
	100	0.050	0.054

Table B.8.3-03 PEC_{soil} (actual) and TWA of BYI 02960 in lettuce in the upper 5 cm, DFOP decay (1st or 2nd cropping per year)

	Time [days]	BYI 02960 Lettuce 1 [1)], 1 x 125 g/ha (field use, interception 25%)		BYI 02960 Lettuce 2 [2)], 1 x 125 g/ha (field use, interception 70%)	
		PEC_{soil} [mg/kg]	TWA_{soil} [mg/kg]	PEC_{soil} [mg/kg]	TWA_{soil} [mg/kg]
Initial	0	0.125	-	0.050	-
Short term	1	0.091	0.108	0.036	0.043
	2	0.091	0.102	0.036	0.041
	4	0.091	0.098	0.036	0.039
Long term	7	0.090	0.095	0.036	0.038
	14	0.089	0.093	0.036	0.037
	21	0.088	0.091	0.035	0.037
	28	0.087	0.091	0.035	0.036
	50	0.085	0.089	0.034	0.035
	100	0.079	0.085	0.031	0.034

[1)] Max 1 application per 24 months (biennial)
[2)] Max 1 application per 12 months (annual)

Accumulation in soil

The potential accumulation of BYI 02960 in soil was calculated considering the following approaches:

- Maximum soil residue in first year: maximum soil residue calculated for one season.
- Long-term plateau concentration C_{min}: maximum of the lower saw tooth curve, which can be considered as background concentration after multiple year use.
- Long-term maximum concentration C_{max}: maximum of the upper saw tooth curve after multiple year use.
- Background C_{min} + maximum of one year in 5 cm depth: to the long-term background concentration C_{min} in a certain depth (e.g. 5, 10 or 20 cm), the maximum residue of one year (distributed in 5 cm) will be added, to take into account a conservative shallow distribution just after an annual application.

Table B.8.3-04 Long-term soil concentrations of BYI 02960 following multi-year use

	Residues distributed over … [cm]	Seasonal $PEC_{s, max}$, max. soil residue in 1st year [mg/kg]	Long-term plateau / background conc. C_{min} [mg/kg]	Long-term maximum conc. C_{max} [mg/kg]	Background C_{min} + max. of 1 year in 5 cm [mg/kg]
Hops 1 x 150 g/ha	**5**	0.080	0.080	**0.160**	-
	10	0.040	0.040	0.080	0.120
	20	0.020	0.020	0.040	0.100
Lettuce 1 [1)] 1 x 125 g/ha	5	0.125	0.046	0.171	-
	10	0.063	0.023	0.085	0.148
	20	0.031	0.011	0.043	**0.136**
Lettuce 2 [2)] 1 x 125 g/ha	5	0.050	0.050	0.100	-
	10	0.025	0.025	0.050	0.075
	20	0.013	0.013	0.025	**0.063**

[1)] Max 1 application per 24 months (biennial)
[2)] Max 1 application per 12 months (annual)
In bold: Generally, for long-term assessments the substance distribution in soil for annual crops with tillage should be assumed over a depth of 20 cm (lettuce) and for perennial crops without tillage over a depth of 5 – 10 cm (e.g. hops).

Remarks by RMS

The RMS recalculated all the values with the DFOP worst case input of the UK field study. Using DFOP kinetics identical results were obtained by the RMS. Therefore the RMS accepts the PEC_{soil} calculations from the applicant. The long term soil concentrations for hop were assessed applying it every year. This differs from the applied biennial usage in hop in the GAP however the assessed value covers the biennial usage. The indoor use was not assessed since this soil is not considered a natural habitat for soil organisms. Soils are often sterilized before and during use in glasshouses. Therefore, PECsoil for the use in glasshouse is not included.

STUDY IIIA, 9.5/01 and STUDY IIIA, 9.5/02 and STUDY 9.5/03

Predicted environmental concentrations in soil were calculated for the major soil metabolites DFA and 6-CNA. These metabolites are not automatically "relevant" with regard to their environmental, biological, eco-toxicological or toxicological properties.

Reference/notifier	:	Sur R., Ellerich C.; 2012 and 2013 Scherr F., Ellertich C.; 2014	GLP statement	:	No
type of study	:	PECs calculation	guideline	:	Soil Persistence Models and EU registration: Report of the FOCUS Soil Modelling Work Group, 1996. EC Document Reference 7617VI/96
year of execution	:	2012 and 2013	acceptability	:	**acceptable**
test substance	:	BYI 02960-DFA and BYI 02960-CNA			

Methods and Materials: PEC_{soil} for the metabolites were calculated using the approach, scenarios and application rates described for the calculations for the parent compound in study IIIA, 9.4/01. Compound specific parameters are summarised in Table B.8.3-05.

Table B.8.3-05 Input parameters for PEC_{soil} for metabolites of BYI 02960

Compound	Max. DT_{50} [days]	Max. occurrence in soil [%]	Molar mass [g/mol]	Molar mass correction factor
Difluoroacetic acid	73.6	33.9	96.03	0.333
6-Chloronicotinic acid	36.6	17.1	157.56	0.546

Findings: The maximum PEC_{soil} values of metabolites of BYI 02960 are summarised in Table B.8.3-06.

Table B.8.3-06 Hops and Lettuce: PEC_{soil} (max) of BYI 02960 metabolites

Crop	DFA $PEC_{soil, max}$ [mg/kg]	6-CNA $PEC_{soil, max}$ [mg/kg]
Hops 1 x 150 g/ha of parent	0.009	0.007
Lettuce 1, 1 x 125 g/ha of parent [1)]	0.014	0.012
Lettuce 2, 1 x 125 g/ha of parent [2)]	0.006	0.005

[1)] Max 1 application per 24 months (biennial)
[2)] Max 1 application per 12 months (annual)

PEC_s accumulation of the metabolites

The long term maximal concentration of the active substance are reported in Table B.8.3-04. These concentrations are for hops 0.160 mg/kg over 5 cm, 0.043 mg/kg for lettuce (biennial application) and 0.025 mg/kg for lettuce (annual application) over 20 cm. Using these concentrations and taking the maximal percentage observed and molecular weight of the metabolites in to account (Table B.8.3-05) results in a seasonal $PEC_{s,max}$. When using these togehter with the maximal DT_{50} values of both metabolites the $PEC_{plateau}$ of the metaolbites and long term $PEC_{s,max}$ can be calculated. Table B.8.3-07 and -08 give the results for the metalobites DFA and 6-CNA.

Table B.8.3-07 Long-term soil concentrations of DFA following multi-year use

	Residues distributed over … [cm]	Seasonal $PEC_{s, max}$, max. soil residue in 1st year [mg/kg]	Long-term plateau / background conc. C_{min} [mg/kg]	Long-term maximum conc. C_{max} [mg/kg]
Hops 1 x 150 g/ha	**5**	0.018	0.001	0.019
Lettuce 1 [1)] 1 x 125 g/ha	**20**	0.005	<0.001	0.005
Lettuce 2 [2)] 1 x 125 g/ha	**20**	0.003	<0.001	0.003

[1)] Max 1 application per 24 months (biennial); [2)] Max 1 application per 12 months (annual)

Table B.8.3-08 Long-term soil concentrations of 6-CNA following multi-year use

	Residues distributed over … [cm]	Seasonal $PEC_{s, max}$, max. soil residue in 1st year [mg/kg]	Long-term plateau / background conc. C_{min} [mg/kg]	Long-term maximum conc. C_{max} [mg/kg]
Hops 1 x 150 g/ha	**5**	0.015	<0.001	0.015
Lettuce 1 [1)] 1 x 125 g/ha	**20**	0.004	<0.001	0.004
Lettuce 2 [2)] 1 x 125 g/ha	**20**	0.002	<0.001	0.002

[1)] Max 1 application per 24 months (bienniall);[2)] Max 1 application per 12 months (annual)

Remarks by RMS

The RMS agrees with the obtained PECsoil values for the parent and metabolites. The long term soil concentrations for hop were assessed applying it every year. This differs from the biennial usage in hop in the GAP however the assessed value covers the biennial usage.

B.8.4 Fate and behaviour in water (Annex IIA 7.2.1; Annex IIIA 9.2.1/9.2.3)

B.8.4.1 Hydrolysis (Annex IIA 7.2.1.1) OECD number 7.5

STUDY IIA 7.5/01

Characteristics

reference	:	Mislankar, S., Woodard, D., 2011		purity	:	radiochemical purity 99%.
study type	:	hydrolysis		nominal concentration	:	1.0 mg/L
year of execution	:	2009		pH	:	4, 7, 9
GLP statement	:	yes		temperature	:	50°C
guideline	:	JMAFF (2000), EPA 161-1, PMRA DACO Number 8.2.3.2, OECD 111 (2002)		conclusion	:	hydrolytically stable
test substance	:	[furanone-4-^{14}C] BYI 02960		acceptability	:	**acceptable**

Summary

Hydrolysis of radiolabeled [furanone-4-^{14}C] BYI 02960 at 1.0 mg a.i./L was studied in the dark at 50 °C in sterile aqueous buffers at pH 4 [acetate buffer], pH 7 [tris buffer], and pH 9 [borate buffer] for 5 days. Samples were analyzed at 0, 1, 2, 3, 4 and 5 days. The samples were analyzed directly by HPLC without extraction.

The results showed that BYI 02960 is hydrolytically stable at ambient temperature. Most of the applied radioactivity was associated with the parent compound at test termination in the pH 4, 7 and 9 buffer solutions. There were three minor components which accounted for a total of 4.9% of AR, none was more than 2.7% of AR in any of the pH.

A preliminary study was conducted at 50°C to determine if volatiles would be formed. It demonstrated that no volatiles were formed during hydrolysis. This result was further confirmed by the acceptable recoveries observed in the definitive experiments.

Materials amd Methods

Materials

1. Test Item: Code BYI 02960, CAS no. 951659-40-8 (unlabelled substance); Label position = [Furanone-4-^{14}C] BYI 02960 (vial No. C-1116); Specific activity: 30.74 mCi/mMole (3.94 MBq/mg) Radiochemical purity: 99% (at beginning of study); Identity and purity of test item in the application solutions were checked. The radiochemical purity of the dosing solution (test item in85 µL acetonitrile, each) was determined by HPLC and found to be 96%.

2. Test matrix: The test matrices for this study were a 0.01 M sodium acetate/acetic acid solution (pH 4), a 0.01 M tris(hydroxymethyl)aminomethane/HCl solution (pH 7), and a 0.01 M boric acid/NaOH solution (pH 9). The acetate, tris, and borate buffers were used since they are unlikely to affect the rate of hydrolysis at their respective pH.

Methods

1. Experimental conditions: The test system consisted of a 30 mL amber vial containing 10 mL of buffer and capped with a septum lined crimp cap. It was autoclaved for 60 minutes at >93 °C to sterilize the system. Forty-five test systems were prepared consisting of three groups of 15 vials, one group for each pH (4, 7, and 9). This included 6 time points, 2 replicates per interval and 3 extra vials. Each vial was filled with 10 mL of the appropriate buffer and sealed with a crimp cap equipped with a Teflon™-lined septum. The test systems were autoclaved for 60 minutes at >93°C to sterilize the system. Test systems were allowed to cool to room temperature before the addition of the test material.

No volatiles were observed in the preliminary experiment. Therefore, no trapping system was used.
The test systems were maintained in a covered water bath. The temperature was held at 50 ±0.5 °C. The pH and sterility were measured at each sampling interval.

2. Application Procedures: 100-µL Hamilton syringe was used to deliver 85 µL of application solution to each test containing 10 ml of buffer. The septum of the each vial was pierced by the syringe, and the application solution was added. To check the DPM applied to the system, using the same syringe, 85 µl of application solution was first put into two vials containing 10 ml of water. Then all the buffers were treated with 85 µl of application solution. After this another aliquot of 85 µl was put into two vials containing 10 ml of water. Three aliquots were taken from each vial for a total of 12 samples and analyzed by LSC which gave average of 240,371 dpm /mL equivalent to 1 ppm

3. Sampling: The sampling intervals for all three pH values were 0, 1, 2, 3, 4 and 5 days post treatment. Samples were typically analyzed the day of sampling with the maximum storage duration before analysis of less than 24 hrs. The samples were stored under refrigerated conditions before analysis if analysis was not conducted on the sampling date. No storage stability data was generated because samples were analyzed within one day of collection.

4. Analytical procedures: At each interval, two replicate test systems for each pH were removed from the water bath, three 1-mL aliquots were counted on the liquid scintillation counter (LSC) and 1 mL of each sample was directly analyzed by HPLC.

The retention time for [^{14}C] BYI 02960 using the HPLC system was 22.0 minutes. Recovery of radioactivity from the HPLC column ranged from 94.2 - 99.2% with a mean recovery of 97.1%.
The linearity of the detector's response to 14C was confirmed. The limit of quantitation (LOQ) was determined empirically by a series of injections at decreasing concentrations. The lowest concentration that resulted in a peak 2 to 3 times the background level was determined to be the LOQ. The limit of quantitation used in this experiment was 890 dpm. Defining 445 dpm as the limit of detection (LOD) of the HPLC radio detector a minimum of approximately 1.1% or 1.6 ppb of AR was detected by HPLC analysis.

No attempt was made to identify the minor transformation products observed in the study since they comprised <2.7% of the applied radioactivity, at any interval.

The rate of degradation was not determined for BYI 02960 since minimal degradation was observed in the pH 4, 7 and 9 buffer solutions.

Results

Data

All buffer solutions (pH 4 = acetate, pH 7 = tris, pH 9 = borate) had a concentration of < 0.01 M. The measured average pH of the buffers was 3.94, 6.89 and 8.99, respectively. Sterility was maintained throughout the study with the exception of a single time point in a single pH. The Day 4, pH 9.0, replicate 1 and 2 samples gave a positive result (sterility was lost) on the aerobic count plate. The samples were analyzed by HPLC to determine if a lack of sterility was an issue. The results showed comparable results to the other test system (stable), thus the lack of sterility did not impact results.

The test systems were maintained in the dark, in a covered water bath held at 50.1 ± 0.13°C (minimum = 49.7°C, maximum = 50.3°C). The resulting data based on LSC and analyses are shown in Table B.8.4.1-01 to Table B.8.4.1-03.

Mass Balance

For this study the AR (100% of applied radioactivity) was defined as the amount of radioactivity recovered in the day 0 sample (mean of label #1 and #2). Based on the results of LSC an RA balance was established for each buffer solution at each sampling interval. A summary of the total recoveries of the radioactivity is given in the following tables.

The mean material balance for pH 4 buffer ranged from 95.8 - 100.9% of AR, with a mean of 98.8% (Table B.8.4.1-01). The mean material balance for pH 7 ranged from 95.0 - 102.7% of AR, with a mean of 99.9% (Table B.8.4.1-02). The mean material balance for pH 9 ranged from 100.3 - 101.2% of AR, with a mean of 100.7% (Table B.8.4.1-03). Individual values are provided for pH 4, 7 and 9 in the report. Adsorption of the test compound to the test vessels was not indicated based on the material balances. The complete material balances found in all solutions demonstrated that no radioactivity dissipated from the solutions by means of volatilization or was lost during sampling/processing.

Table B.8.4.1-01 Transformation of BYI 02960 at 50°C, expressed as percentage of applied radioactivity (mean ± S.D.), in pH 4 buffer solution

Sampling times [days]	0	1	2	3	4	5
BYI 02960	96.2 ± 1.2	96.1 ± 1.9	91.2 ± 0.2	93.7 + 1.6	96.3 ± 0.5	92.1 ± 3.6
Unknown A	2.7 ± 0.1	2.2 ± 0.0	1.9 ± 0.1	1.7 ± 0.0	1.4 ± 0.0	1.4 ± 0.0
Unknown B	0.0 ± 0.0	1.0 ± 0.3	1.4 ± 0.0	1.5 ± 0.0	1.8 ± 0.2	1.7 ± 0.1
Unknown C	1.6 ± 0.2	1.5 ± 0.0	1.3 ± 0.1	1.5 ± 0.1	1.3 ± 0.1	1.3 ± 0.2
Total% recovery	100.6 ± 0.9	100.9 + 1.6	95.8 + 0.1	98.4 + 1.6	100.7 ± 0.7	96.5 + 3.8

Table B.8.4.1-02 Transformation of BYI 02960 at 50°C, expressed as percentage of applied radioactivity (mean ± S.D.), in pH 7 buffer solution

Sampling times [days]	0	1	2	3	4	5
BYI 02960	94.5 ± 1.0	90.7 ± 6.0	94.3 ± n/a	95.1 ± 2.1	97.8 ± 0.1	96.6 ± 0.4
Unknown A	2.6 ± 0.1	1.8 ± 0.2	1.8 ± n/a	1.6 ± 0.0	1.6 ± 0.0	1.5 ± 0.2
Unknown B	0.4 + 0.6	1.1 ± 0.1	1.6 ± n/a	1.8 ± 0.0	1.8 + 0.3	1.8 + 0.1
Unknown C	1.4 ± 0.1	1.4 ± 0.1	1.4 ± n/a	1.2 ± 0.0	1.5 ± 0.1	1.3 ± 0.1
Total% recovery	98.8 ± 1.6	95.0 ± 5.9	99.0 ± n/a	99.7 ± 2.2	102.7 ± 0.2	101.3 ± 0.3

Table B.8.4.1-03 Transformation of BYI 02960 at 50°C, expressed as percentage of applied radioactivity (mean ± S.D.), in pH 9 buffer solution

Sampling times [days]	0	1	2	3	4	5
BYI 02960	95.8 ± 0.7	97.5 ± 1.1	96.2 ± 0.2	95.8 ± 0.1	96.9 ± 0.2	95.8 ± 1.1
Unknown A	1.8 + 0.0	1.4 + 0.3	1.5 ± 0.3	1.5 + 0.1	1.2 ± 0.0	1.4 + 0.1
Unknown B	1.5 ± 0.2	0.9 ± 1.3	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.0	1.9 ± 0.1
Unknown C	1.4 ± 0.0	1.4 ± 0.2	1.2 ± 0.1	1.1 ± 0.0	1.2 ± 0.1	1.1 ± 0.1
Total% recovery	100.6 ± 0.9	101.2 ± 0.2	100.5 ± 0.4	100.3 ± 0.1	101.2 ± 0.1	100.3 ± 1.2

Bound and Extractable Residues

Not applicable

Volatilization

Since the test system was sealed and a loss in material balance was not observed, no attempt was made to trap volatiles.

Transformation of test item

The amounts of BYI 02960 and degradates were quantified by HPLC. The results for all tests, expressed as percent AR, are given in tables above. Throughout, the concentration of parent compound remained almost constant from day 0 to day 5, with 96.2 - 92.1% of AR at pH 4, 94.5 - 96.6% of AR at pH 7, and 95.8 - 95.8% of AR at pH 9, respectively.

Minimal degradation of the parent compound occurred at all three pH values. No major transformation products were formed at any pH. Three minor degradates “Unknown A, B and C” at maximum amounts of 2.7% of AR.

Kinetics of Test Item Degradation

The rate of degradation was not determined for BYI 02960 since minimal degradation was observed in the pH 4, 7 and 9 buffer solutions. Thus, BYI 02960 can be considered to be hydrolytically stable under environmental conditions.

Conclusions

From the current laboratory study it is concluded that hydrolysis is not relevant for the degradation of BYI 02960 in the environment, and that a further study with another radiolabel is not needed.

Remarks

The study clearly indicates that BYI 02960 is hydrolytically stable under environmental conditions. The RMS agrees with the applicant that no further studies with other radiolabels are required. The results are acceptable and were also evaluated under study IIA, 2.9.1/01.

B.8.4.2 Photolytic degradation (Annex IIA 7.2.1.2) OECD number 7.6

In accordance with points IIA, 2.9.2 and IIA, 2.9.3, tests on photolysis (direct photo-transformation) of BYI 02960 in water are also presented here to provide a complete and comprehensive overview on the fate and behaviour of this substance in this corresponding section B.8.4 of the dossier. For the respective route of phototransformation study (compare study IIA 2.9.2) radiolabeled FUR BYI 02960 was investigated in pure buffers. A summary of these studies is repeated here, focusing on formation and degradation of transformation products. In addition, the determination of the quantum yield of direct phototransformation in water and the derived environmental half-lives in surface water are also given for the active substance. Further a supplementary study on the phototransformation of BYI 02960 in natural water is submitted and presented.

STUDY IIA, 7.6/01

Characteristics

reference	:	Hall, L.R., 2011	incubation time	:	up to 35 hours
study type	:	aqueous photolysis	nominal concentration	:	~1.0 mg/L
year of execution	:	2010	pH/temperature	:	pH 7/temp 25°C
GLP statement	:	Yes	light intensity	:	680 W/cm2
guideline	:	OPPTS 835.2240, 2008; Japanese JMAFF New Test Guidelines, 2000 Canada PMRA DACO Number 8.2.3.3.2	conclusion	:	photolytic DT_{50} under study conditions 13.8 hours
test substance	:	[furanone-4-^{14}C] BYI 02960	acceptability	:	**Acceptable**
purity	:	radiochemical purity 99.3%			

Summary

The aqueous phototransformation of [FUR-^{14}C] BYI 02960 was studied at 25°C in sterile buffer (pH 7) at an initial concentration of approx. 1.0 mg a.i./L under artificial irradiation (xenon lamp, >290 nm, quartz and Suprax® filter) for 35 hours. At this timepoint the DT_{75} for BYI 02960 was exceeded.

Duplicate irradiated test systems and single dark control test systems were analyzed at 0, 4, 8, 12, 16, 22, 28, and 35 hours by direct injection of samples into HPLC. Identification of parent and major transformation products was accomplished by co-elution with authentic reference standards, LC/MS and/or NMR analysis.

The anticipated test conditions (temperature, sterility and pH 7) were maintained, and material balances for the irradiated and dark test systems were complete throughout the study. In the dark test systems (i.e. the controls), BYI 02960 was found to be stable.

In the irradiated test systems, BYI 02960 decreased from 98.1% of AR at time 0 (mean of replicates) to 8.4% of AR at 35 hours of irradiation. The major degradates included BYI 02960-succinamide (max. 39.6% of AR at 28 hours) and BYI 02960-azabicyclosuccinamide (maximum 25.9% of AR at 35 hours). A minor degradate was identified as BYI 02960-deschlorohydroxysuccinamide (DCHS, maximum of 2.5% at 35 hours) which is considered as intermediate between the two major photodegradates. No other single detected component exceeded 3% of AR at 35 hours.

The first-order half-life for photolytic degradation of BYI 02960 in sterile buffer of pH 7 was 13.8 experimental hours. Based on this experimental half-life of BYI 02960, the direct phototransformation half-life in top layer of surface water is calculated to be 1.75 days under environmental sunlight conditions in Phoenix, AZ, USA (latitude 33.3°N) and 2.7 days in Athens, Greece (latitude 38.03N).. Based on this finding, BYI 02960 should rapidly degrade in the aqueous environment if exposed to sunlight.

Table B.8.4.2-01 Half-life, DT_{75} and DT_{90}

Test System	Kinetic Model	Equation	χ^2 test error (%)	Experimental hours		
				DT_{50}	DT_{75}	DT_{90}
Irradiated	SFO	$M_{(t)} = 103.3^{-0.0503\,t}$	10.4	13.8	27.6	45.8
Dark	SFO	$M_{(t)} = 99.7e^{-(1.2e-10)\,t}$	0.50	>>1000	nc	Nc

nc = not calculated since compound is stable

Materials and Methods

Materials

1. Test Item: Code BYI 02960, CAS no. 951659-40-8 (unlabelled substance); Label position = [Furanone-4-^{14}C] BYI 02960 (vial No. C-1116); Specific activity: 30.74 mCi/mMole (3.94 MBq/mg) Radiochemical purity: 99.3% (at beginning of study);Identity and purity of test item in the application solutions were checked.

2. Test system: The irradiated test system consisted of 20 mL of filter-sterilized sterile buffer (pH 7) treated with BYI 02960 at ~1.0 µg/mL and sealed in a quartz glass vessel. The dark control test systems consisted of 20 mL of filter-sterilized buffer (pH 7) treated with BYI 02960 at ~1.0 µg/mL and crimp-sealed in a 20-mL amber bottle.

3. Test matrix: The test matrix for this study was comprised of sterile 10 mM potassium phosphate buffer (pH 7). Buffer was prepared by dissolving 2.72 g of potassium dihydrogen phosphate in ~1.9 L of water. The pH was adjusted to 7 by the addition of 1 M potassium hydroxide. After adjustment to pH 7, the buffer was diluted to 2.0 L and thoroughly mixed. The buffer was stored at room temperature overnight and used immediately the following morning.

Methods

1. Experimental conditions: Twenty four test systems were prepared for irradiation. Duplicate test systems were prepared for each of the nine planned sampling intervals as well as six spare test systems for additional sampling intervals or for unforeseen losses. Each quartz glass vessel was filled with 20 mL of filter-sterilized sterile buffer (pH 7) containing approximately 1.0 µg/mL of test substance. Ten control test systems were prepared and were maintained in the dark at 25 ± 1°C in an incubator.

2. Application procedures: Treated buffer (pH 7) was sterilized by passing it through a 0.22-µm sterile filter into each autoclaved test vessel using aseptic techniques in a laminar flow hood. The amount of water added to each vessel was determined by the weight (approximately 20 g) added to a tared vessel. The irradiated test vessels were closed with ground glass stoppers. The quartz photolysis vessels were placed in a Suntest CPS unit containing a Heraeus xenon-arc lamp. Light emission was filtered using a Suprax® filter that eliminated wavelengths <290 nm. For the dark control test systems, the same amount of treated sterile buffer (pH 7) was filter-sterilized and transferred into sterile amber bottles which were sealed with crimp caps.

The test substance concentration was 1.11 µg/mL which is much less than half of the water solubility of BYI 02960 at pH 7 (water solubility is 3.2 mg/mL).

3. Sampling: Duplicate irradiated test systems and individual dark control test systems were analyzed at 0, 4, 8, 12, 16, 22, 28, and 35 hours post treatment. All test systems were checked for pH and sterility. Samples were immediately analyzed after removal from either the incubator or the Suntest.

4. Analytical procedures: Triplicate aliquots (100 µL) from each test system were radioassayed. For all samples, a 1-mL aliquot was removed and used for sterility testing. An aliquot (approximately 4 mL) was transferred to an HPLC autosampler vial, and the remaining test solution was transferred to a glass vial. All samples were directly analyzed by HPLC (1 mL injection) without any concentration or extraction.

Major degradates, including the parent compound, were either isolated by HPLC from an MID sample or a kinetic sample was analyzed directly by LC/MS. Collected fractions were dissolved in water and analyzed by LC/MS. Some degradates were isolated and analyzed by high resolution MS and NMR.

Results

Data

The target temperature of 25 ± 1°C was maintained during the study, and the mean pH of the water was 6.99 in the irradiated systems and 7.01 in the dark test systems. Sterile conditions were maintained in all test systems. The irradiated systems were continuously exposed to artificial sunlight with an intensity of 680 W/m^2 for a period of 35 hours. The analytical results of the study are presented in Table B.8.4.2-02.

Mass Balance

The mean material balance of the irradiated [^{14}C] BYI 02960 test systems was 100.6% and ranged from 99.3 to 101.6% in individual systems. The mean material balance for the dark control test systems was 101.1% and ranged from 99.6 to 101.4% in individual test systems.

Bound and Extractable Residues

Not applicable.

Volatilization

No attempt was made to trap volatiles. This was justified since the mass balances were complete throughout the study.

Transformation of Test Item

Four radioactive components exceeded 10% of AR at any interval, one of these was [^{14}C] BYI 02960. Two major degradates were identified as BYI 02960-succinamide and BYI 02960-azabicyclosuccinamide. The fourth region that exceeded 10% of AR was a group of components ("polar mixture") which eluted early in the chromatogram in reverse-phase HPLC. By TLC it was shown to consist of multiple components, and no single component exceeded 3% of the applied radioactivity at 35 hours. One minor degradate was identified as BYI 02960-dechlorohydroxy-succinamide (DCHS) by comparison to a synthetic standard.

Table B.8.4.2-02 Phototransformation of [^{14}C] BYI 02960 in irradiated test systems (mean ± SD) as a percentage of applied radioactivity (1)

Compound	Sampling times [hours]			
	0	4	8	12
BYI 02960	98.1 ± 0.2	86.8 ± 0.1	71.8 ± 2.2	55.3 ± 2.5
BYI 02960-succinamide	0.0 ± 0.0	9.40 ± 0.1	17.30 ± 0.8	26.0 ± 4.2
BYI 02960-azabicyclosuccinamide	0.0 ± 0.0	0.0 ± 0.0	0.9 ± 0.1	2.4 ± 0.8
DCHS (2)	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.1	0.9 ± 0.3
Polar Region 2 (3)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.4 ± 0.7
Polar Region 1 (3)	0.0 ± 0.0	0.0 ± 0.0	2.4 ± 0.6	3.8 ± 1.4
Minor others (4)	1.4 ± 0.3	4.6 ± 0.6	7.3 ± 0.3	11.4 ± 0.3
Total volatile organic	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Total% recovery	99.5 ± 0.0	100.8 ± 0.6	100.3 ± 1.0	101.2 ± 0.4
	16	**22**	**28**	**35**
BYI 02960	55.7 ± 1.8	41.3 ± 4.7	14.1 ± 2.2	8.4 ± 1.4
BYI 02960 – succinamide	26.6 ± 0.7	32.7 ± 1.6	39.6 ± 0.1	37.6± 1.2
BYI 02960-azabicyclosuccinamide	2.8 + 0.3	4.9 ± 1.1	19.4 ± 3.2	25.9 ± 1.9
DCHS (2)	0.8 ± 0.2	1.2 ± 0.3	2.1 ± 0.3	2.5 ± 0.4
Polar Region 2 (3)	1.5 ± 0.4	1.9 ± 0.1	3.0 ± 0.9	3.5 ± 0.3
Polar Region 1 (3)	2.5 ± 0.4	4.8 ± 0.2	6.9 ± 0.0	8.2 ± 1.5
Minor others (4)	10.3 ± 0.3	13.1 ± 1.2	16.4 ± 0.0	15.1 ± 0.9
Total volatile organic	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Total% recovery	100.3 ± 1.4	100.1 ± 0.7	101.5 ± 0.2	101.2 ± 0.2

(1) Ppb analyte can be calculated as follows: Ppb analyte = Analyte as % of applied x (ppb parent applied ÷ 100%) x (MW analyte/MW parent); MW of BYI 02960 = 288.7
(2) DCHS – BYI 02960-deschlorohydroxysuccinamide - intermediate between BYI 02960-succinamide and BYI 02960-azabicyclosuccinamide
(3) Polar regions combined and analyzed by TLC. No single component comprised >3% of the applied radioactivity
(4) Minor others is comprised of several compounds, none of which comprised >7% of the applied radioactivity at any interval

Kinetics of the test item degradation

Half-life, DT_{75} and DT_{90} of [^{14}C] BYI 02960 are presented in Table B.8.4.2-03. The first-order half-life for photolytic degradation of BYI 02960 in sterile buffer of pH 7 was 13.8 experimental hours. Based on this experimental half-life of BYI 02960, the direct phototransformation half-life in top layer of surface water is calculated to be 1.75 days under environmental sunlight conditions, e.g. in Phoenix, AZ, USA (latitude 33.3°N), 2.7 days in Athens, Greece (latitude 38.03N) and 3.4 days in London, UK (51.32 N). Based on this finding, BYI 02960 should rapidly degrade in the aqueous environment if exposed to sunlight.

Table B.8.4.2-03 Half-life, DT_{75} and DT_{90}

Test System	Kinetic Model	Equation	χ^2 test error (%)	Experimental hours			Equivalent days in Phoenix, AZ; USA		
				DT_{50}	DT_{75}	DT_{90}	DT_{50}	DT_{75}	DT_{90}
Irradiated	SFO	$M_{(t)} = 103.3^{-0.0503\,t}$	10.4	13.8	27.6	45.8	1.8	3.5	5.8
Dark	SFO	$M_{(t)} = 99.7e^{-(1.2e-10)\,t}$	0.50	>>1000	nc	nc	nc	nc	nc

nc = not calculated since compound is stable

Conclusions

[^{14}C] BYI 02960 photolytically degraded in sterile buffer (pH 7) with a half-life of 13.8 experimental hours. Based on this finding, BYI 02960 should degrade within a few days in the aqueous environment if exposed to sunlight. The major degradates (>10% of applied radioactivity) were identified as [^{14}C] BYI 02960-succinamide and [^{14}C] BYI 02960-azabicyclosuccinamide. The findings were included in the proposal for the pathway of degradation of BYI 02960 in an aqueous environment (see Figure B.8.4.4-01).

Remarks

The study is acceptable and results are reliable. Only the FUR BYI 02960 was tested and not an other radiolabel. The other ring structure is also photolytically active. A test with this radiolabel might result in different metabolites. Therefore, in principle PYR BYI 02960 should also be tested. However, for BYI 02960 (flupyradifurone) in the aqueous photolysis study, two major degradates BYI 02960-succinamide and BYI 02960-azabicyclosuccinamide were identified and contained the same number of carbons as the parent compound as demonstrated in the pathway. Thus the bond between the two rings (furanone and chloropyridine) was not broken during the course of this study. In addition, the bond between the difluoroethyl side chain and the amino moiety remained intact. Therefore conducting this study with only one radiolabel position is sufficient for tracking all the major degradates formed in the photolyis of BYI 02960.

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Characteristics

reference	:	Heinemann, O., 2011	incubation time	:	500 min
study type	:	aqueous photolysis, quantum yield	nominal concentration	:	5.03 mg/L in water
year of execution	:	2011	temperature	:	temp 25°C
GLP statement	:	Yes	pH	:	4, 7, 9
guideline	:	Phototransformation of Chemicals in	quantum yield	:	Φ = 0.000138

		Water, Part A: Direct Phototransformation, UBA, Berlin, Germany (Dec 1992); Test Method: ECETOC (Polychromatic Light Source)			
test substance	:	BYI 02960	acceptability	:	**acceptable**
purity	:	99.4%			

Summary

The quantum yield of direct phototransformation of BYI 02960 was determined in aqueous solutions using polychromatic light according to the ECETOC method. Degradation of BYI 02960 in aqueous solution of ≤10% was measured by HPLC-UV after a maximum irradiation period of 500 minutes. This indicated moderate degradability of BYI 02960 via direct phototransformation in aqueous and buffered solutions. A mean quantum yield of Φ = 0.000138 was calculated on the basis of UV absorption data and the degradation kinetics determined from both experiments.

The estimates based on the two modelling concepts (Zepp & Cline or Frank & Kloepffer) are comparable. Both estimates consider the quantum yield Φ and the absorption in the UV-VIS spectrum being in the range of wavelengths relevant for the environment (see tables below). Environmental direct phototransformation half-lives of BYI 02960 in sunlight exposed surface water layers are estimated to in a range of 10 to 14 days during periods of main use, i.e. in spring to summer.

Thus, direct phototransformation in aqueous solution may contribute to the dissipation of BYI 02960 from the environment. This assessment does not consider other potential mechanisms which may enhance the degradation in natural water, e.g. by indirect photolytic processes.

Table B.8.4.2-04 Zepp and Cline modelling using GC Solar program

Season	Environmental DT_{50} of Direct Phototransformation of BYI 02960 [days]			
	30th degree lat.	40th degree lat.	50th degree lat.	60th degree lat.
Spring	11.4	12.2	13.6	16.2
Summer	10.3	10.3	10.7	11.3
Fall	15.9	20.2	29.5	54.2
Winter	21.2	32.2	63.2	195

Marginal conditions: pure surface water at 0-5 cm depth, 10th degree longitude, clear sky, typical ozone concentrations in the atmosphere, half-lives integrated over the entire day. The columns of the 40-50th degree of latitude is more or less relevant to the conditions of Europe.

Table B.8.4.2-05 Frank and Kloepffer modelling (MEF-11/554)

Month	Photolysis Constant [1/sec]	Environmental DT_{50} of Direct Phototransformation of BYI 02960 [days]		
		Minimum	Mean	Maximum
January	0.501×10^{-7}	76	160	730
February	0.104×10^{-6}	37	77	340
March	0.203×10^{-6}	21	40	160
April	0.340×10^{-6}	13	24	94
May	0.434×10^{-6}	12	18	74
June	0.486×10^{-6}	11	17	66
July	0.433×10^{-6}	12	19	62

August	0.423×10^{-6}	13	19	63
September	0.246×10^{-6}	19	33	120
October	0.135×10^{-6}	31	59	270
November	0.601×10^{-7}	58	130	670
December	0.316×10^{-7}	120	250	1300

Marginal conditions: pure stagnant surface water at 0-5 cm depth, geographic and climatic conditions of Germany (50th degree latitude); no contribution of another mono- or bimolecular elimination process.

Materials and Methods

Materials

1. Test Item:Common name: Flupyradifurone (CAS no. 951659-40-8); Chem. Code: BYI 02960; Sample ID: AZ16895, Batch NLL7780-47-4; Certified assay: 99.4% w/w.

2. Test Solutions: The study was conducted using highly pure water (taken from a TKA-Genpure unit): conductivity = 0.055 µS; TOC = 2 ppb. UV-VIS spectra were measured in 0.01 M aqueous buffer solutions (acetate pH 4, phosphate pH 7 and borate pH 9). A solution containing 5.03 mg BYI 02960 (1.74×10^{-5} mol)/L pure water was prepared for irradiation experiments.

Methods

The quantum yield for direct phototransformation of BYI 02960 in aqueous solution was determined in a merry-go-round apparatus (Type 13/150 Mangels Co.) that was equipped with a mercury arc lamp (Type TQ 150 Original Hanau Co.) in a Duran® 50 filter and cooling finger. The filter absorbed light with a wavelength below 290 nm and let pass the polychromatic light above this cut-off wavelength. The merry-go-round irradiation apparatus is warmed up for at least 15 minutes prior to the exposure of the samples in order to guarantee a constant radiation of the light source as well as the projected sample temperature of 25°C ± 1°C at the beginning of the experiment. Subsequently, only the merry-go-round but not the lamp or the cycle cooling is switched off for adding or removing samples.

After equilibration, two measuring cells with 3.0 mL of actinometer solution (A) are first exposed to the light in the system for 10 minutes. The measuring cells containing 3.0 mL of test solution are swiftly placed onto the 10 positions of the merry-go-round apparatus. At the respective sampling interval a single sample is removed. Finally, *i.e.* after both degradation experiments were finished, two samples with solution (A) were irradiated and used for actinometry.

The results of BYI 02960 analysis (usually means of duplicates) were evaluated on the basis of linear regression in order to receive the time (in minutes) after which 10% of the molecules of the test item have been degraded. That value is necessary to calculate the quantum yield of phototransformation.

Results

Data

Intensity of irradiation was calculated to 6.023 x 10^{16} (experiment #1) and 6.1599 x 10^{16} (experiment #2) photons absorbed per second for the 3 mL actinometry solution in the range of wavelength from 295 to 490 nm. Phototransformation results are presented in Table B.8.4.2-06.

Table B.8.4.2-06 Phototransformation of BYI 02960 in water

Duration of Irradiation [min]	Experiment #1 BYI 02960 [mg/L]	Experiment #2 BYI 02960 [mg/L]
0	5.03 (= 100%)	5.03 (= 100%)
50	4.90	4.97
100	4.89	4.91
150	4.93	4.87
200	4.89	4.83
250	4.81	4.83
300	4.71	4.77
350	4.72	4.79
400	4.64	4.70
450	4.58	4.61
500	4.56 (= 90.7%)	4.69 (= 93.2%)

A degradation of BYI 02960 of 7 and 10% was determined by HPLC-UV after a maximum irradiation period of 500 minutes. This indicated moderate photostability of BYI 02960 in the aqueous buffered solution.

Based on both degradation experiments quantum yields Φ of 1.5538 x 10^{-4} (experiment #1) and 1.1983 x 10^{-4} (experiment #2) were calculated. Environmental half-lives were calculated according to Zepp & Cline and Frank & Kloepffer (see Table B.8.4.2-04 and Table B.8.4.2-05).

Conclusions

A mean quantum yield of direct phototransformation of Φ = 0.000138 was calculated for BYI 02960 on the basis of UV absorption data and the degradation kinetics determined from both experiments. Thus, direct phototransformation in aqueous solution may contribute to the dissipation of BYI 02960 from the environment. A comparison of the estimates derived from models of Zepp & Cline and Frank & Kloepffer shows that both approaches are well comparable. Environmental direct phototrans-formation half-lives of BYI 02960 in sunlight exposed surface water layers are estimated to in a range of 10 to 14 days during periods of main use, i.e. in spring to summer.

However, this assessment does not consider any other potential mechanisms which may enhance the degradation in unpurified water, i.e. caused by indirect photolytic processes. Maybe such was the difference to the kinetics result of the earlier study not performed in pure water but in buffer (study IIA, 7.6/01). Thereby, BYI 02960 degraded faster, i.e. with an estimated DT_{50} of just a few days in an aqueous environment if exposed to sunlight.

Remarks

The study is acceptable.

STUDY IIA, 7.6/03

Characteristics

reference	:	Hall, L.R., 2011	incubation time	:	28 hours
study type	:	aqueous photolysis in sterile natural water	nominal concentration	:	1 mg/L
year of execution	:		pH/temperature	:	8.2 / 25°C
GLP statement	:	Yes	light intensity	:	680 W/cm^2
guideline	:	JMAFF (2000)	conclusion	:	DT_{50} in sterile buffer pH 7 14 hours days, equivalent to <3.8 calendar days at Tokyo (35°N)
test substance	:	[furanone-4-^{14}C] BYI 02960	acceptability	:	**acceptable**
purity	:	radiochemical purity 99.3%			

Summary

The aqueous phototransformation of [FUR-^{14}C] BYI 02960 was studied at 25°C in sterile natural water at an initial concentration of 1 mg a.i./L under artificial irradiation (xenon lamp, >290 nm, quartz and Suprax® filter) for max. 28 hours, equivalent to max 7.5 environmental days at Tokyo, Japan. This duration of irradiation exceeded the DT_{75} for BYI 02960 in the experiment. Duplicate irradiated and single dark test systems were analyzed at 0, 4, 8, 12, 16, 22 and 28 hours by direct injection of samples into HPLC. Identification of parent and major transformation products was accomplished by co-elution with authentic reference standards, LC/MS and/or NMR analysis.

The anticipated test conditions (temperature and sterility) were maintained, and material balances for the irradiated and dark test systems were complete throughout the study. In the dark test systems (i.e. the controls), BYI 02960 was found to be stable, showed very little degradation at pH 8 and 25°C.

In the irradiated test systems, [^{14}C] BYI 02960 degraded from 95.1% of AR at time 0 (mean of replicates) to 17.2% of AR after 28 hours of irradiation. The major degradates included BYI 02960-succinamide (max. 38.2% at 28 hours) and BYI 02960-azabicyclosuccinamide (max. 14.3% at 28 hours). A minor degradate was identified as BYI 02960-deschlorohydroxysuccinamide (maximum of 2.2% at 22 hours) and is considered an intermediate leading to the formation of BYI 02960-azabicyclosuccinamide. A group of components ("polar mixture") that eluted near the HPLC column's void volume was shown by TLC to consist of multiple components, and none of these components exceeded 7.3% of the applied radioactivity.

The first-order half-life for photolytic degradation of BYI 02960 in the natural water was 14.0 experimental hours. Based on this value the half-life of BYI 02960 under environmental conditions is calculated to be, e.g., 3.8 days at Tokyo, Japan (latitude 35°N). Thus, phototransformation in aqueous solution contributes very well to the dissipation of BYI 02960 from the environment. Therefore, the findings were included in the proposal for the pathway of degradation of BYI 02960 in an aqueous environment (see Figure B.8.4.4-01).

Materials and Methods

Materials

1. Test Item: Flupyradifurone: Code = BYI 02960; CAS no. 951659-40-8 (unlabeled); Designation of label: [furanone-4-^{14}C] BYI 02960 or [FUR-^{14}C] BYI 02960; Specific Activity: 30.74 mCi/mmol (~236,400 dpm/µg; 3.94 MBq/mg); Radiochemical Purity: 99.3%

2. Test System: The test matrix for this study was comprised of natural water collected on March 29, 2010 from the lake at Heritage Park, Olathe (Johnson County), Kansas. Water was collected at the surface, 0-6 inches deep. The natural water was stored at room temperature overnight and used immediately the following morning.

Table B.8.4.2-07 Physicochemical characteristics of unfiltered natural water

Parameter	Results/Units
pH	8.2
Dissolved oxygen	8.43 mg/L
Calcium	67 mg/L (ppm)
Magnesium	12 mg/L (ppm)
Hardness ($CaCO_3$ equivalent)	217 mg/L
Electrical conductivity	0.88 mmho/cm
Total Dissolved Solids	486 mg/L (ppm)
Turbidity	3.53 NTU
Alkalinity	158 mg $CaCO_3$/L
Total Organic Carbon	4.3 mg/L (ppm)
Dissolved Organic Carbon	3.7 mg/L (ppm)
Carbonates	0.58 meq/L
Bicarbonates	2.16 meq/L
Total Nitrogen	1.1 mg/L (ppm)
Total Phosphorus	0.9 mg/L (ppm)

Methods

1. Experimental conditions: The tests were performed using individual static test systems held at aerobic conditions at 25 ± 1 °C for a period of 6 experimental days. They consisted of 24 quartz glass vessels [50 mm x 25 mm x 15 mm (height)] each containing 20 mL of the test solution (buffer solution + 1.0 µg/mL test item), and were closed (except in case of time 0) with a trap attachment (permeable for oxygen) containing soda lime for absorption of $^{14}CO_2$ and a polyurethane foam plug for adsorption of volatile organic compounds. The test systems were either incubated in the dark as controls or continuously exposed to artificial irradiation (Suntest® unit equipped with a xenon lamp and <290 nm cut-off filter). The Suntest unit was operated at approximately 680 W/m^2 (290-800 nm) to simulate highly photoreactive conditions. At this intensity it would take 3.71 hours in the Suntest unit to equal one solar day in Tokyo, Japan. Since 3.71 hours represents 1 environmental day, 111.4 hours (4.64 days) of continuous irradiation at 680 W/m^2 is equivalent to 30 environmental days.

2. Sampling: Duplicate irradiated test systems and individual dark control test systems were analyzed at 0, 4, 8, 12, 16, 22, and 28 hours post treatment. All samples were immediately analyzed after removal from either the incubator or the Suntest, with analyzes completed within 3 hrs. Volatile traps were not used.

3. Description of analytical procedures: The radioactivity of the test solutions was radio-assayed by triplicate 100-µL aliquots. All samples were directly analyzed by HPLC without any concentration or extraction. Identification and confirmation of the parent compound and transformation products was done by co-chromatography (LC-MS/MS and NMR techniques).

Determination of Degradation Kinetics

The photolytic degradation of BYI 02960 in the natural water was characterized using kinetics modelling. A simple first order (SFO) model was used for determination of rate constants. The mean percentage (two replicates) of applied radioactivity as BYI 02960 of two replicates at all sampling times was used to determine the degradation rate and kinetic endpoints (modelling tool MATLAB (Ver. 7.0.4)).

Results and discussion

Data

The pH of the test solutions was about pH 8 at the beginning irradiated and dark test systems. The test water was maintained sterile throughout the test period. The 22-hr dark control (sample 5C) showed the presence of seven colony forming units. However, this contamination did not have a significant impact on the degradation of BYI 02960 since the percentage of parent in the dark control sample at 22 hours was slightly higher than the percentage of parent in either the 16 or 28-hour samples.

Mass Balance

The mean material balance of the irradiated test systems was 98.1% and ranged from 95.7 – 99.7% in individual test systems (see Table). The mean material balance for the dark control test systems was 98.5% and ranged from 97.1 – 99.8% in individual test systems.

Transformation of Test Item

Irradiated samples: During the study, the concentration of [^{14}C] BYI 02960 in irradiated test systems decreased from 95.1 to 17.2% of the theoretical applied amount of radioactivity (mean of replicates) after 28 hours (see Table B.8.4.2-08). Only two degradates exceeded 10% of AR in 28 hours in the irradiated test systems. The major degradate was identified as [^{14}C] BYI 02960-succinamide which reached a max. of 38.2% of AR after 28 hours. [^{14}C] BYI 02960-azabicyclosuccinamide reached a max. of 14.3% of AR after 28 hours. A group of radioactive components eluted near the solvent front by HPLC in the irradiated samples (polar regions 1 and 2). These two regions were collected together and reanalyzed by TLC which showed that the polar region was comprised of many components present at low levels**.** No single component in the polar region comprised more than 7.3% of AR at 22 hours or 4.5% of AR at 28 hours. A minor degradate, [^{14}C] BYI 02960-deschlorohydroxysuccinamide, was identified by LC/MS comparison to a synthetic standard. The max. concentration of BYI 02960-deschloro-hydroxysuccinamide was 2.2% of AR at 22 hours.

Dark control samples: In the dark test systems, [^{14}C] BYI 02960 was more or less constant (98.0 and 96.0% (single values) after 28 hours. No single degradate exceeded 0.8% of the applied radioactivity at any sampling interval.

Table B.4.2-08 Phototransformation of [^{14}C] BYI 02960 in natural water test systems (mean ± SD) as a percentage of applied radioactivity (1)

Compound	Sampling times [days]						
	0	4	8	12	16	22	28
BYI 02960	95.1 ± 0.3	85.0 ± 1.0	72.7 ± 2.3	64.0 ± 1.0	55.7 ± 1.2	21.8 ± 3.1	17.2 ± 1.5
A	0.2 ± 0.2	0.2 ± 0.3	0.1 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.4 ± 0.5	0.0 ± 0.0
B	0.2 ± 0.3	1.2 ± 0.0	2.2 ± 0.2	2.8 ± 0.1	3.2 ± 0.1	4.6± 0.8	5.3 ± 0.1
C	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.3	0.0 ± 0.0	0.6 ± 0.0
D	0.2 ± 0.2	1.3 ± 0.0	2.6 ± 0.1	3.4 ± 0.2	4.0 ± 0.1	3.6 ± 0.0	4.0 ± 0.2
E	0.0 ± 0.0	0.6 ± 0.2	1.4 ± 0.0	1.6 ± 0.2	2.2 ± 0.0	2.8 ± 0.2	2.8 ± 00
F	0.4 ± 0.1	1.0 ± 0.1	1.2 ± 0.0	1.0 ± 0.3	1.0 ± 0.4	1.3 ± 1.1	0.0 ± 0.0
-Succinamide	0.0 ± 0.0	8.4 ± 0.6	15.6 ± 1.7	20.9 ± 0.3	22.7 ± 0.5	35.2 ± 1.2	38.2 ± 0.1
H	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.4	0.6 ± 0.2	1.5 ± 0.2	1.6 ± 0.1
I	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.2	0.6 ± 0.0
-Azabicyclo succinamide	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.0	1.4 ± 0.3	1.9 ± 0.2	10.4 ± 0.6	14.3 ± 0.8
DCHS (2)	0.0 ± 0.0	0.5 ± 0.1	0.5 ± 0.2	0.8 ± 0.3	0.9 ± 0.2	2.2 ± 0.1	2.0 ± 0.0
Polar Region2 (3)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.7	1.4 ± 0.2	2.2 ± 0.6	2.8 ± 0.6
Polar Region1 (3)	0.0 ± 0.0	0.0 ± 0.0	2.1 ± 0.2	2.9 ± 0.4	3.7 ± 0.2	10.5 ± 2.7	8.8 ± 0.8
Total extractable residues	96.1 ± 0.6	98.3 ± 0.6	99.0 ± 0.1	99.7 ± 0.0	97.6 ± 0.2	96.9 ± 1.2	98.9 ± 0.7
CO_2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Bound to walls	n/a ± 0.0	n/a ± 0.0	n/a ± 0.0	n/a ± 0.0	n/a ± 0.0	n/a ± 0.0	n/a ± 0.0
Total% recovery	96.1 ± 0.6	98.3 ± 0.6	99.0 ± 0.1	99.7 ± 0.0	97.6 ± 0.0	96.9 ± 1.2	98.9 ± 0.7

(1) Ppb analyte can be calculated as follows: Ppb analyte = Analyte as % of applied x (ppb parent applied ÷ 100%) x (MW analyte/MW parent); MW of BYI 02960 = 288.7
(2) DCHS – BYI 02960-deschlorohydroxysuccinamide – intermediate between BYI 02960-succinamide and BYI 02960-azabicyclosuccinamide
(3) Polar regions combined and analyzed by TLC. No single component comprised >3% of the applied radioactivity

Kinetics of Test Item Degradation

Degradation kinetics of [^{14}C] BYI 02960 (DT_{50}, DT_{75}, and DT_{90}) is shown in Table B.8.4.2-09.

Table B.8.4.2-09 BYI 02960 degradation kinetics

Test System	Kinetic Model	Equation	χ^2test error (%)	Experimental hours			Equivalent days, at Tokyo, JAP		
				DT_{50}	DT_{75}	DT_{90}	DT_{50}	DT_{75}	DT_{90}
Irradiated	SFO	$M_{(t)} = 101.7e^{-0.0495\,t}$	10.7	14.0	28.0	46.5	3.8	7.5	12.5
Dark	SFO	$M_{(t)} = 98.2e^{-0.00062\,t}$	0.50	1118	>2200	>3700	46.6	>92	n/a

Conclusions

BYI 02960 photolytically degraded in sterile natural water with a half-life of 14.0 experimental hours. The major degradates (>10% of AR) were identified as BYI 02960-succinamide and BYI 02960-azabicyclosuccinamide. A minor degradate was identified as BYI 02960-deschlorohydroxy-succinamide. In the dark controls BYI 02960 was stable. Based on the experimental half-life of 14.0

hours, the half-life of BYI 02960 under environmental conditions in Tokyo, Japan was calculated to be 3.8 days. The phototransformation study in natural water is regarded more relevant for the aqueous environment as the pure buffer study (compare study IIA, 7.6/01). The following data represent the synopsis for phototransformation of BYI 02960, and the findings were included in the proposal for the pathway of degradation of BYI 02960 in an aqueous environment (see Figure B.8.4.4-01).

Table B.8.4.2-10 Result Synopsis

Test substance	**[Furanone-4-^{14}C] BYI 02960**
Test medium	Sterile natural water from lake in Heritage Park, Olathe, Kansas.
Source of irradiation	Xenon lamp with cut-off filter to remove light of waves <290 nm
Experimental DT_{50} in Suntest	14.0 hours
Environmental DT_{50}, e.g. in Tokyo, Japan	3.8 days
Dark control DT_{50} in Tokyo, Japan	>46 days. Very little degradation observed
Major transformation products after irradiation: BYI 02960-succinamide BYI 02960-azabicyclosuccinamide 	
Minor transformation products after irradiation BYI 02960-deschlorohydroxysuccinamide 	
Total number of photodegradates formed by irradiation	At least 18 compounds formed, but only two products exceeded 10% of applied radioactivity.
Total number of degradates observed in dark control samples	Maximum of 3 degradates observed, and all were <1%.

Remarks

The RMS evaluated the photolysis study and agrees that photolysis is an important process in the aqueous environment. No major difference caused by indirect photolysis. Results ar not used for EU risk assessment.

B.8.4.3 Biological degradation

Biological degradation of BYI 02960 in aquatic systems was investigated in studies on anaerobic and aerobic biodegradation under dark laboratory conditions using natural water-sediment systems. Results also relevant for the behavior of BYI 02960 in natural water, i.e. those of the abiotic hydrolysis and aqueous photolysis study in water, were shown in studies IIA 7.5/01 and IIA, 7.6/01 to IIA, 7.6/02, already. All those findings are considered for the overall pathway of BYI 02960 in water shown by Figure B.8.4.4-01.

B.8.4.3.1 Ready biodegradability (Annex IIA 7.7)

A respective study was not performed. The parent compound BYI 02960 is considered to be "Not Readily Biodegradable.

B.8.4.3.2 Degradation in water-sediment systems (Annex IIA 7.8)

STUDY IIA, 7.8.2/01

Characteristics

reference	:	Xu, T., 2012	incubation time	:	102 days
year of execution	:	2010	nominal concentration	:	0.233 and 0.203 mg/L
GLP statement	:	Yes	temperature	:	24 ± 2 °C
guideline	:	incl. OECD 308	DT_{50}	:	415 and > 1000 days
test substance	:	[Pyridine-2,6-^{14}C] BYI 02960	metabolites	:	see results
purity	:	radiochemical purity 99%	acceptability	:	**acceptable supplementary**
test system	:	see materials			

Summary

The anaerobic biotransformation of [pyridine-2,6-^{14}C] BYI 02960 was studied in two pond water/sediment systems. One system was collected from a pond near Lawrence, KS, USA and the other was taken from a pond in Pikeville, NC, USA. The study was conducted for 102 days in the dark at 24 ± 2 °C. BYI 02960 was applied at a rate of 0.233 and 0.203 mg a.i./L for Lawrence, KS and Pikeville, NC. The treatment rate was based an application rate of 410 g a.i./ha. The test systems consisted of an Erlenmeyer flask containing 50 g (dry weight) sediment and 150 mL pond water, i.e. a sediment/water ratio of 1:3. Eight sampling intervals were conducted and included 0, 7, 14, 21, 29, 43, 70 (71 for Pikeville, NC), and 102 days post treatment. The water samples were filtered and analyzed by direct sample injection into HPLC. The sediment was extracted sequentially at ambient temperature, followed by two aggressive extractions using a microwave extractor at 70 ºC. Appropriate volumes of both the ambient and aggressive extracts were concentrated and analyzed by HPLC coupled to a ^{14}C detector to characterize ^{14}C-BYI 02960 residues. Identification of the BYI 02960 was achieved by co-chromatography and liquid chromatography-electrospray ionization mass spectrometry (LC-ESI/MS).

The anticipated test conditions (incubation temperature, anaerobicity and microbial viability) were maintained, and material balances for both test systems were complete throughout the study.

<u>Lawrence test system</u>: The radioactive residues in the water phase decreased from an average of 99.1% at Day 0 to 48.6% at Day 7 and declined to 25.1% at the end of the study (Day 102). The radioactive residues in the sediment increased from an average of 8.8% on Day 0 to 46.8% at Day 7 and increased to 70.4% at Day 43, and 68.9% at the end of the study. Unextractable residues were increasing from 0.2% at Day 0 to 4.9% at the end of the study. Volatile compounds remained low with $^{14}CO_2$ ≤0.1% and organic volatiles below the LOQ. No major degradates were formed in the test systems with Lawrence, KS sediment. Total minor unidentified ranged from 1.3 – 5.3% throughout the study.

Pikeville test system: The radioactive residues in the water phase decreased from an average of 87.3% at Day 0 to 48.4% at Day 7 and further declined to 18.5% by the end of the study (Day 102). The radioactive residues in the sediment increased from an average of 11.4% on Day 0 to 49.2% at Day 7 and continued to increase to 78.7% at Day 71 and 64.8% at the end of the study. Unextractable residues were increase from 0% at Day 0 to 12% at the end of the study. Volatile compounds remained low with $^{14}CO_2$ ≤0.1% and organic volatiles below the LOQ. No major degradates were formed in the test systems. Total minor unidentified ranged from 0.0 -. 0.7% throughout the study.

Kinetics: BYI 02960 dissipated from the water phase to the sediment with a half-life of 7.2 and 9.6 days for Lawrence and Pikeville test system, respectively. The half-lives of BYI 02960 in the sediment under anaerobic conditions were 415 days for Pikeville sediment and greater than 1000 days for Lawrence sediment. Thus the compound is regarded as stable under anaerobic conditions.

Materials and Methods

Materials

1. Test Item: [Pyridine-2,6-^{14}C] BYI 02960; Sample ID: C-1135; Specific activity = 269,387 dpm/µg (35.03 mCi/mMole); Radiochemical purity: 99%

2. Test system: The test matrices used in this study were a water/sediment collected from pond near Lawrence, KS, USA (water: pH 8.3, sediment: texture silty clay, pH 7.7 (saturated paste), OC% 1.1) and the other was taken from a pond in Pikeville, NC, USA (water: pH 7.4; sediment: texture loamy sand, pH 5.1 (saturated paste), OC% 1.5). The top 6 inches of sediment were collected using a shovel. Description of the test matrix collection and storage data is given in Table B.8.4.3.2-01. Selected physico-chemical characteristics of water are presented in Table B.8.4.3.2-02, and of sediment in Table B.8.4.3.2-03.

Methods

1. Experimental conditions: Each twenty-two kinetics test systems were treated. In addition, 6 test systems were dosed at 4 times the kinetic rate for the NC sediment and 5 for KS sediment to be used for identification of metabolites. Seven additional test systems for each sediment/water system were used as controls for determining biomass at the beginning and end of the study. The experimental design is depicted in Table B.8.4.3.2-04.

Table B.8.4.3.2-01 Description of water and sediment collection and storage

Parameter	Lawrence, KS	Pikeville, NC
Geographic location	350 Wild Horse Road, Lawrence, KS	703 Nor AM Road, Pikeville, NC
Site description	Research farm pond	Pond in mix land used area
Latitude and longitude	35.050283 N; 95.1935 W	35.484401 N ; 78.043249 W
Pesticide use history at the collection site	No pesticide used	Roundup® spot treatments on slope and water edge Reglone® (diquat) spot treatment at water's edge, more than two years

		ago.
Collection procedures for Water	Collected water and placed over sediment in 5-gallon bucket	
Sediment	Collected ca. top 15 cm of sediment with shovel device; placed in 5-gallon buckets	
Collection date	05/21/2010	07/07/2010
Shipping conditions and date	Vehicle transport on the same day of sampling	FedEx at ambient temperature, received on 07/08/2010
Sampling depth (cm)	Water 0-20 cm Sediment 0-15 cm	
Storage conditions	Ambient conditions during transport; stored at 4 ºC upon arrival at BRP until used for study. Acclimated to ambient temperature for 4 days prior to start of pre-incubation period	Ambient conditions during shipment; stored at 4 ºC upon arrival at BRP until used for study. Acclimated to ambient temperature for 4 days prior to start of pre-incubation period
Storage length	7 days to pre-incubation	7 days to pre-incubation
Preparation of water and sediment samples (e.g., water –filtered/not filtered; sediment sieved/not sieved)	Water and sediment were filtered or wet sieved under water with a 2-mm sieve	

Table B.8.4.3.2-02 Physico-chemical characteristics of water

Property	Lawrence, KS		Pikeville, NC	
pH	8.3		7.4	
Hardness (mg $CaCO_3$/L)	65		24	
Conductivity (mmhos/cm)	0.18		0.09	
Total Organic Carbon (ppm)	7.8		11.9	
Dissolved organic carbon (ppm)	6.3		9	
Total nitrogen (ppm)	<LOQ of 0.2		0.6	
Total phosphorus (ppm)	0.7		1.0	
Temperature (°C)	24 ± 2			
Redox potential E_h (mV)	Initial	Final	Initial	Final
	112	82	115	158
Dissolved Oxygen (mg/L)	Initial	Final	Initial	Final
	0.04	0.19	0.03	0.08
Biomass (cells / mL water)	Initial	Final	Initial	Final
	5.75E+06	8.66E+05	5.71E+06	7.95E+06

Table B.8.4.3.2-03 Physico-chemical characteristics of sediment

Property		Lawrence, KS		Pikeville, NC	
Textural classification		Silty Clay		Loamy Sand	
	% Sand	7.0		80.7	
	% Silt	47.9		16.3	
	% Clay	45.1		3.0	
pH	1:1 soil:water	7.9		4.9	
	saturated paste	7.7		5.1	
	0.01 M $CaCl_2$	7.6		4.7	
Bulk Density (gram/cc)		1.09		1.15	
Organic carbon (%)		1.1		1.5	
Organic matter (%)		1.8		2.6	
CEC (meq/100 g)		26.8		4.3	
Total nitrogen (%)		0.08		0.08	
Total phosphorus (ppm)		163		95	
Soluble Salts (mmhos/cm)		0.46		0.18	
Redox potential E_h (mV)		Initial	Final	Initial	Final
		85	95	109	155
Biomass (cells / g soil)		Initial	Final	Initial	Final
		1.22E+08	1.07E+08	5.76E+07	8.21E+07

Table B.8.4.3.2-04 Experimental design

Parameter	Description

Duration of test		102 days
Water		2-mm sieved
Sediment		2-mm sieved
Sample Size	Water: Sediment:	150 mL 50 g (dry weight)
Water:Sediment ratio		3:1
Test concentrations	mg a.i./L water mg a.i./kg sediment mg a.i./kg total system	0.233 (KS), 0.203 (NC) 0.700 (KS), 0.608 (NC) 0.175 (KS), 0.152 (NC)
Control conditions (if used)		24 ± 2 °C, dark
Number of replications per interval	Treatments	2
	Control (untreated)	0
Test apparatus		250-mL Pyrex Erlenmeyer flask with side-arm and double-valve sealable top (or mineral oil bubblers during pre-incubation).
Traps for CO_2 & organic volatiles		2 N KOH, ethylene glycol, 1 M H_2SO_4
Test material application	Identity of solvent	Methanol (< 0.1%)
	Volume of test solution used per test system	0.230 mL (KS) 0.190 mL (NC)
	Application method	250-µL Hamilton syringe to water surface
	Evaporation of application of solvent	No
Indication of test material adsorbing to walls of test apparatus		None
Experimental conditions	Temperature (ºC)	24 ± 2 °C
	Continuous darkness (Yes/No)	Yes
Other details		None

2. Sampling:

Sampling intervals	Kinetics interval, pH, redox, and dissolved oxygen	0, 7, 14, 21, 29, 43, 70 (71 for NC), and 102 days post-application
	Sterility Checks	None
Sampling method	Water Sediment	Aqueous Phase: Water decanted through filter paper. Ambient Shake: Sediment shaken at ambient temperature 3X with ACN:water (70:30 v:v), 1X ACN, centrifuged, supernatant filtered and combined. Microwave extraction: Sediment extracted 1X with ACN:water (70:30 v: v) and 1X methanol:water (70:30 v:v)
Method of collection of CO2 and volatile organics		Test systems flushed with nitrogen through volatile traps containing 2 N KOH, ethylene glycol, and 1 M H2SO4
Sample storage before analysis		Stored in freezer below -15 ºC. (LIMS ID: BLDG2WF)
Other observations, if any		None

3. Description of analytical procedures:

At each sampling interval the water and sediment were separated by decantation. The sediment was extracted sequentially three times with 30-minute ambient shakes using 50 mL 70:30 (v/v) ACN:water and one 30-minute shake with 50 mL ACN. The sediment/solvent mixture was centrifuged after each

shake extraction, and the supernatants of all ambient extracts were filtered into the same graduated cylinder. The sediment then underwent two aggressive extractions using a microwave extractor at 70 ºC for 10 minutes with first 70:30 ACN: water and one more microwave extract with 70:30 methanol/water was added after Day 14. The two aggressive extracts were combined.

Appropriate volumes of both the ambient and aggressive extracts were concentrated and analyzed by HPLC coupled to a ^{14}C detector to characterize ^{14}C-BYI 02960 residues. Water was analysed by radio-HPLC directly without concentration. Identification of the BYI 02960 was achieved by co-chromatography and liquid chromatography-electrospray ionization mass spectrometry (LC-ESI/MS). Identification of metabolites was confirmed by mass spectrometry at day 102.

The LOD was determined empirically to be the lowest concentration resulting in a peak height of approximately 3 times the background level in the chromatogram was approximately 500 dpm. T Assuming 500 dpm as the detection limit, the calculated limit of detection for the radioactivity detector would be 500 dpm/specific activity (dpm/µg) = 0.002 µg.

Triplicate 1-mL aliquots of the aqueous and the sediment extracts of the sample were radioassayed by LSC. The extracted sediment samples (soil cakes) were air dried, weighed and homogenized thoroughly with coffee grinder. Triplicate aliquots of the sediment were analyzed by combustion.

Determination of Degradation Kinetics

Dissipation rates from the water phase and rates of degradation for the total system were calculated by use of the software KinGui, version 1.1, which was built within the frame-work of MATLAB (Ver.7.0.4). The kinetic evaluation included the fitting of data with kinetic models. The bi-phasic model DFOP was chosen.

Results

Findings

The redox potential and dissolved oxygen content of the test systems indicated an anaerobic and reducing environment throughout the study (Table B.8.4.3.2-05).

Table B.8.4.3.2-05 Dissolved oxygen, pH, Eh and temperature measurements taken throughout the study period (average of replicates)

Sediment	Interval (days)	Temperature (ºC)[a]	Dissolved Oxygen (mg/L)	pH	E_h[c] Water (mV)	E_h[c] Sediment (mV)
Lawrence, KS	0	19.9	0.04	7.1	112	85
	7	20.2	0.04	7.1	56	67
	14	20.0	0.04	6.7	85	104
	21	-[b]	0.71	6.7	66	55
	29	20.5	0.06	7.3	29	41
	43	20.4	0.05	6.8	62	62
	70	21.3	0.04	6.8	39	44
	102	19.7	0.19	6.8	82	95
	Mean	20.3	0.1	6.9	66	69

	Min	19.7	0.04	6.7	29	41
	Max	21.3	0.71	7.3	112	104
Pikeville, NC	0	20.2	0.0	6.4	115	109
	7	21.2	0.1	6.5	137	146
	14	20.0	0.0	6.0	147	151
	21	21.3	0.0	5.9	128	123
	29	21.4	0.6	5.7	184	146
	43	20.7	0.1	5.7	162	140
	71	21.8	0.3	5.4	173	167
	102	19.5	0.1	6.0	158	155
	Mean	20.7	0.17	5.9	151	142
	Min	19.5	0.03	5.4	115	109
	Max	21.8	0.63	6.5	184	167

[a]: Temperature was taken after the flasks had been taken out of incubator for measurement during the intervals.
[b]: No temperature reading was taken for this interval
[c]: Eh = E_{obs} +197 mv (Ag/AgCl reference electrode)

Data

The distribution of radioactive residues for the test system of Lawrence during the course of the study is summarized in Table B.8.4.3.2-06. The distribution of radioactive residues for the test system Pikeville during the course of the study is summarized in Table B.8.4.3.2-07.

Table B.8.4.3.2-06 Distribution of [pyridine-2,6-^{14}C] BYI02960 residues, expressed as percentage of AR (mean ± s.d.) in Lawrence sediment/water system under anaerobic aquatic conditions

Compound	Sample		DAT 0	7	14	21	29	43	70	102
BYI 02960	Water Layer	Mean	94.0	47.4	34.9	24.2	31.1	24.2	28.9	25.1
		SD ±	5.3	0.2	1.4	0.3	1.1	0.4	0.9	2.7
	Sediment	Mean	8.8	45.4	54.7	64.7	61	65.2	65.9	66.5
		SD ±	5.2	0.7	0.8	0.4	0.8	0.5	0	0.7
	Entire System	Mean	103	92.8	89.5	88.9	92.0	89.4	94.8	91.6
		SD ±	0.1	0.5	0.6	0.1	1.9	0.1	1.0	2.0
Sum of Unidentified Minor Radio-activity[a]	Water Layer	Mean	5.1	1.2	1.2	1.2	0	0	0	0
		SD ±	1.4	1.7	0.1	0.1	0	0	0	0
	Sediment	Mean	0	1.4	2.9	3.4	2.8	5.3	1.3	2.4
		SD ±	0	0.7	0.1	0.4	0.1	0.7	1.9	3.4
	Entire System	Mean	5.1	2.6	4.1	4.5	2.8	5.3	1.3	2.4
		SD ±	1.4	2.4	0.3	0.3	0.1	0.7	1.9	3.4
Total Extractable Radio-activity	Water Layer	Mean	99.1	48.6	36	25.4	31.1	24.2	28.9	25.1
		SD ±	6.8	1.4	1.3	0.4	1.1	0.4	0.9	2.7
	Sediment	Mean	8.8	46.8	57.6	68.1	63.7	70.4	67.3	68.9
		SD ±	5.1	1.4	0.9	0.8	0.8	1.0	1.8	4.1
	Entire System	Mean	108	95.5	93.6	93.4	94.8	94.6	96.1	94.0
		SD ±	1.7	2.8	0.3	0.4	2.0	0.6	0.9	1.4
CO_2		Mean	0	0	0.1	0	0.1	0.1	0.1	0.1
		SD ±	0	0	0	0	0	0	0	0
Volatile organics		Mean	0	0	0	0	0	0	0	0
		SD ±	0	0	0	0	0	0	0	0
Total volatile		Mean	0	0.1	0.1	0.1	0.1	0.1	0.1	0.1
		SD ±	0	0	0	0	0	0	0	0
Bound residues		Mean	0.2	1.4	3.0	2.0	3.3	3.5	3.5	4.9
		SD ±	0.1	1.7	0.2	0.1	0.2	0.1	0	0.6
Total % recovery		Mean	108	96.9	96.6	95.5	98.1	98.2	99.8	99.0
		SD ±	1.6	1.1	0.2	0.3	1.7	0.5	1.0	2.0

[a]: individual minor degradates did not exceed a mean of 3.1 %

Table B.8.4.3.2-07 Distribution of [Pyridine-2,6-14C] BYI02960 residues, expressed as percentage of AR (mean ± s.d.) in Pikeville sediment/water system under anaerobic aquatic conditions

Compound	Sample		DAT							
			0	7	14	21	29	43	70	102
BYI 02960	Water Layer	Mean	86.6	48.4	33	25.6	20.7	13.6	7.7	18.5
		SD ±	3.4	0.2	1.4	0.8	2.2	0.7	0.4	3.1
	Sediment	Mean	11.3	49.0	62.5	70.6	72.4	78.2	78.7	64.8
		SD ±	1.9	0.2	0.6	0.1	0.3	0.7	1.1	1.2
	Entire System	Mean	97.9	97.4	95.5	96.2	93.1	91.8	86.4	83.3
		SD ±	1.4	0.1	0.8	0.8	1.8	0	1.6	1.9
Sum of Unidentified Minor Radio-activity[a]	Water Layer	Mean	0.7	0	0	0	0.3	0	0.2	0
		SD ±	0.9	0	0	0	0.4	0	0.3	0
	Sediment	Mean	0	0.2	0.1	0	0.4	0	0	0
		SD ±	0	0	0.2	0	0.5	0	0	0
	Entire System	Mean	0.7	0.2	0.1	0	0.7	0	0.2	0
		SD ±	0.9	0	0.2	0	1.0	0	0.3	0
Total Extractable Radio-activity	Water Layer	Mean	87.3	48.4	33.0	25.6	21.0	13.6	7.9	18.5
		SD ±	2.4	0.2	1.4	0.8	1.7	0.7	0.1	3.1
	Sediment	Mean	11.4	49.2	62.6	70.6	72.8	78.2	78.7	64.8
		SD ±	2.0	0.2	0.8	0.1	0.9	0.7	1.1	1.2
	Entire System	Mean	98.6	97.6	95.6	96.2	93.8	91.8	86.6	83.3
		SD ±	0.5	0.1	0.6	0.8	0.8	0	1.2	1.9
CO_2		Mean	0	0.1	0	0.1	0	0	0.1	0.1
		SD ±	0	0	0	0	0	0	0	0
Volatile organics		Mean	0	0	0	0	0	0	0	0
		SD ±	0	0	0	0	0	0	0	0
Total volatile		Mean	0	0.1	0	0.1	0	0	0.1	0.1
		SD ±	0	0	0	0	0	0	0	0
Bound residues		Mean	0	1.5	3.0	3.6	4.8	7.4	11.6	12.0
		SD ±	0	0	0.2	0.1	0.1	0.2	0.6	0.1
Total % recovery		Mean	98.7	99.2	98.7	99.9	98.7	99.2	98.4	95.4
		SD ±	0.5	0.1	0.5	0.9	0.9	0.2	1.8	1.8

[a]: individual minor degradates did not exceed a mean of 0.7 %

Mass balance

The material balances per sampling interval for the test systems of Lawrence, KS ranged from 95.5 to 108% of the applied radioactivity, with an overall mean material balance of 99.1 ± 3.9%. The material balances per sampling interval for the test systems of Pikeville, NC ranged from 95.4 to 99.9% of the applied radioactivity, with an overall mean material balance of 98.5 ± 1.3%.

Residues in water, bound and extractable residues in sediment

In the Lawrence system the radioactive residues in the water phase decreased from an average of 99.1% at Day 0 to 48.6% at Day 7 and declined to 25.1% at the end of the study (Day 102). The radioactive residues in the sediment increased from an average of 8.8% on Day 0 to 46.8% at Day 7 and increased to 70.4 at Day 43 and 68.9% at the end of the study. Unextractable residues increased from 0.2% at Day 0 to 4.9% at the end of the study. Volatile compounds remained low with $^{14}CO_2$ ≤0.1% and organic volatiles below the LOQ. BYI 02960 decreased from 103% at Day 0 to 91.6% at the end of the study in the total Lawrence water/sediment system. No major degradates were formed in the test systems with Lawrence sediment; total minor unidentified ranged from 1.3 to 5.3% throughout the study.

In the Kansas system radioactive residues in the water phase decreased from an average of 87.3% at Day 0 to 48.4% at Day 7 and declined to 18.5% at the end of the study (Day 102). The radioactive residues in the sediment increased from an average of 11.4% on Day 0 to 49.2% at Day 7 and increased to 78.7% at Day 71 and 64.8% at the end of the study. Unextractable residues increased from 0% at Day 0 to 12% at the end of the study. Volatile compounds remained low with $^{14}CO_2$ ≤0.1% and organic volatiles below the LOQ. BYI 02960 decreased from 97.9% at Day 0 to 83.3% at the end of the study in the total water/sediment system. No major degradates were formed; the total minor unidentified ranged from 0 to 0.7% throughout the study.

Volatilization

No volatile compounds were detected in the study.

Transformation of parent compound

BYI 02960 dissipates to the sediment from aqueous phase with a half-life of 7.2 and 9.6 days for Lawrence and Pikeville, respectively. BYI 02960 is stable in the sediment under anaerobic condition with a half-life 415 days for Pikeville and greater than 1000 days for Lawrence sediment.

Table B.8.4.3.2-08 Kinetic analysis

Sediment Origination	Test System	Double First Order Parallel				DT_{90} (days)
		DT_{50} (days)	k_1 day^{-1}	k_2 day^{-1}	χ2 %	
Lawrence, KS	Water	7.2	0.1672	2.0E-4	6.0403	>1000
	Total	>1000	2.3E-14	3.5E-4	4.1304	>1000
Pikeville, NC	Water	9.6	0.0947	2.6E-14	9.0418	>1000
	Total	415	0.0026	0.0017	0.6588	>1000

Conclusions

BYI 02960 dissipated from aqueous phase to sediment with a half-life of 7.2 and 9.6 days for Lawrence and Pikeville test system, respectively. With respect to the entire test system, BYI 02960 is regarded stable under anaerobic condition, no major metabolite were formed.

Remarks

The study is acceptable. It is noted that the two systems bothe are in the low range of organic carbon content as prescribed in the OECD guideline. The water/sediment system was tested under anaerobic conditions, however the redox potential and dissolved oxygen meseaurements show that the system is not completely anaerobic. The RMS agrees with the applicant that BYI 02960 stable under anaerobic conditions. Results of this anaerobic study are not used for further assessment in the aquatic environment. The study is accepted as supplementary information.

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Characteristics

Reference	:	Hellpointner, E., Unold, M., 2012	incubation time	:	119 days
year of execution	:	2011	nominal concentration	:	0.040 mg/L
GLP statement	:	yes	temperature	:	20 ± 1 °C

guideline	:	incl. OECD 308	DT_{50}	:	193.1 and 246.9 days
test substance	:	[Pyridine-2,6-^{14}C] BYI 02960	metabolites	:	see results
purity	:	radiochemical purity >99%	acceptability	:	**acceptable**
test system	:	see materials			

Summary

The aerobic transformation of [pyridine-2,6-^{14}C] BYI 02960 was studied in two different water/sediment systems (Hönniger and Angler Weiher) for a maximum of 119 days in the dark at 20 ± 1 °C. In a supplementary test, the degradation of [PYR-^{14}C] BYI 02960 was studied in samples which were sterilized by gamma radiation and (separate vessels) by steam pressure. The test systems consisted of laboratory microcosm flasks attached to traps for collection of CO_2 and volatile organic compounds. Individual flasks filled a volume ratio of water to sediment of 3:1 were treated with [PYR-^{14}C] BYI 02960 with an application rate of 20.9 µg/batch corresponding to approx. 40 µg/L water assuming a maximum field application rate of 400 g BYI 02960/ha. During incubation the supernatant water was agitated gently.

Duplicate test systems were processed and analyzed after 0, 3, 7, 14, 28, 63, 91, and 119 days of incubation. The water samples were analyzed for radioactivity after centrifugation. Prior to radio-HPLC analysis, a concentration step was performed. The sediment samples were extracted at ambient temperature three times with acetonitrile/water (70/30, v/v), followed by one extraction step with pure acetonitrile. Afterwards, the sediment was extracted once more using a microwave-accelerated solvent extraction (aggressive organic extract) with 80 mL acetonitrile/water (70/30, v/v). The combined extracts from the ambient extractions and the aggressive extraction were analyzed by LSC and, after a concentration step, via radio-HPLC. Identification of BYI 02960 residues was by HPLC-MS, HPLC-MS/MS, NMR and HPLC co-chromatography.

The non-extractable residues (NER) in sediment samples were determined, and those from Hönniger Weiher in case of the last sampling date were separated into humin, humic acid and fulvic acid fractions.

The test conditions outlined in the study protocol were maintained throughout the study, and the material balance in the two test series was complete (on average 98.9% for HW and 98.0% for AW test system). The complete material balance demonstrates that no significant portion of radioactivity dissipated from the vessels or was lost during processing.

The radioactivity in the water phase of HW test systems decreased to 12.9% of AR, and in the water phase of AW test systems to 24.6% of AR at study termination. Extractable ^{14}C sediment residues in test systems from HW increased from 1.9% of the applied radioactivity at DAT-0 to 59.8% at DAT-63 and declined to 53.0% towards the end of the study. Extractable ^{14}C residues in the sediments from AW increased from 1.1% of the applied radioactivity at day 0 to 49.7% at study termination. The maximum amount of non-extractable ^{14}C residues (NER) in the sediment was 25.0% of AR for test

systems from HW and 13.6% of AR for AW (DAT-119). The fractionation of NER of HW resulted in portions of 4.5 and 5.4% of AR within the humic acids, whereas similar amounts of radioactivity were associated with the fulvic acids (9.1 and 9.3% of AR) or strongly integrated into the insoluble humin of the soil matrix (10.7 and 9.8% of AR). At the end of the study, 6.8% and 8.5% of AR were present as $^{14}CO_2$ in the test systems from HW and AW, respectively. Organic volatile compounds were not detected in significant amounts (< 0.1% of AR in all test systems).

In the water phase of HW and AW, the amount of [PYR-^{14}C] BYI 02960 decreased from 96.7 and 96.5% of AR at day 0 to 11.4 and 22.3% at study termination, respectively. In the sediment phase of HW, the amounts of BYI 02960 increased from 1.8% of AR at day 0 to a maximum of 59.4% at DAT-63, followed by a decrease to 52.6% towards the end of the study. In the AW sediment the amounts of BYI 02960 increased from 1.0% of AR at day 0 to 49.5% at study termination. Not any major transformation products were to be detected in the water phase and the sediment of both test systems. The dissipation of BYI 02960 from the water phases was mainly characterized by rapid partitioning into the sediment. The best-fit kinetic models for the determination of trigger values were the FOMC kinetic model for HW and the DFOP kinetic model for AW with DT_{50} values of 8.5 and 34.5 days, respectively. The corresponding modelling endpoints were calculated using the DFOP kinetic model. The DT_{50} values for the slow degradation phase were 63.0 and 63.6 days for the water phase of HW and AW, respectively. In the entire water/sediment systems, BYI 02960 was degraded slowly which was best described using single first-order kinetics (SFO). The estimated DT_{50} values were 193.1 and 246.9 days for test systems from HW and AW, respectively.

In the supplemental test, i.e. the test systems sterilized by gamma radiation or steam pressure and then incubated for 0, 60 or 120 days, no CO_2 was formed (≤ 0.1% AR). The amount of radioactivity in the water phase, predominantly represented by parent compound, was about two times higher than in that from the microbial active samples. Further, there was a clear trend that NER formation in sediment was lower than in the microbial active test flasks, especially seen for both test systems HW and AW sterilized by steam pressure. This shows that the NER were at least partly formed by microbial processes.

Materials and Methods

Materials

1. Test Item: BYI 02960, CAS No: 951659-40-8

[Pyridine-2,6-^{14}C] BYI 02960, sample ID: KML 9090; Specific activity = 4.49 MBq/mg (121.35 µCi/mg); Radiochemical purity: >99% (acc. Radio-HPLC and –TLC;Chemical purity: >99% (HPLC, UV detection at 210 nm)

2. Test System: The study was carried out using the natural water/sediment systems Hoenniger Weiher (HW), near Wipperfürth, Germany and Anglerweiher (AW), Leverkusen, Germany.

Hoenniger Weiher is an artificially dammed pond in the course of the Hoenniger Creek. Due to its inlet and outlet the pond (about 1000 m² in surface area) has strong water current. Anglerweiher is a reclaimed gravel pit, which is used for fishing only. The chosen systems are well characterized, physical-chemical characteristics of the water/sediment systems are summarized in Table B.8.4.3.2-08.

Water and sediment samples were taken separately and poured into plastic containers. The collected sediments were sieved down to 2 mm mesh-size to remove parts of e.g. plants and stones. The collected water phases were filtered through a 0.06 mm sieve.

Table B.8.4.3.2-08 Physico-chemical characteristics of water and sediment

Parameter	Hönniger Weiher (HW)	Angler Weiher (AW)
Geographic Location	Close to Wipperfürth North Rhine-Westphalia Germany	Leverkusen North Rhine-Westphalia Germany
	51°08.217 latitude North, 7°27.129' longitude East	51°01.088' latitude North, 7°00.693' longitude East
Properties of Water		
Temperature [°C][1]	5.2	6.8
pH[1]	7.4	7.7
Hardness [°dH] [,2]	3.1 / 2.6 / 4.8	9.8 / 12.5 / 14.2
Oxygen Conc. (saturation) [mg/L][1]	13.33	13.48
Total Org. Carbon (TOC) [mg/L][,2]	<2 / 3 / 9	<2 / 4 / 16
Dissolved Org. Carbon (DOC) [mg/L][*,2]	<2 / 3 / 5	< 2 / 4/ 11
Total Nitrogen [mg/L] [,2]	4.7 / < 1.0 / 3.4	4.7 / 2.8 / 1.3
Total Phosphorus [mg/L] [,2]	< 0.03 / < 0.03 / < 0.03	< 0.03 / 0.14 / 0.20
Redox Potential E_h [mV][1,4]	436.7	505.1
Properties of Sediment		
Soil Taxonomic Classification (USDA)[#]	Sandy Loam	Loamy Sand
Sand (2000 – 50 µm) [%][#]	65	85
Silt (< 50 – 2 µm) [%][#]	30	12
Clay (< µm) [%][#]	5	3
pH[1]	7.1	7.0
pH[#]	5.2 ($CaCl_2$); 5.4 (H_2O)	6.7 ($CaCl_2$); 7.1 (H_2O)
Temperature [°C][1]	3.9	6.2
Organic Matter [%][2,3]	6.19 / 6.34 / 6.14	2.07 / 2.34 / 2.53
Organic Carbon [%][*,2]	3.59 / 3.68 / 3.56	1.20 / 1.36 / 1.47
% $CaCO_3$ [#]	n.a.	n.a.
Sediment Microbial Activity [mg CO_2 /hr/kg sediment (dry wt)][2]	16.25 / 12.25 / 7.50	15.42 / 14.17 / 10.08
Cation Exchange Capacity [meq/100 g] [#]	7.6	6.6
Total Nitrogen [%][*,2]	0.26 / 0.25 / 0.31	0.09 / 0.12 / 0.12
Total Phosphorus (Olsen) [mg/kg] [*,2]	600 / 590 / 730	330 / 330 / 340
Redox Potential E_h [mV][1,4]	227.7	500.1
1 Measurement at day of sampling	2 start of acclimation / DAT-0 / DAT-120	
3 %organic matter =%organic carbon x 1.724	n.a. = not analyzed	
4 Eh = Eobs + Uref [Uref = reference potential of SenTix ORP electrode (WTW) vs. standard hydrogen electrode at given Temperature; 210 mV at 20 °C, extrapolated from manufacturer information]		

Materials

1. Experimental conditions: The test systems consisted of laboratory microcosm flasks attached to traps for collection of CO_2 and volatile organic compounds. The individual static test systems were kept

at aerobic conditions at 20 ± 2 °C for a maximum period of 120 experimental days. Each vessel was filled with 175 mL of wet sediment (i.e. 118.3 and 247.9 g dry weight sediment for HW and AW) and about 525 mL of water, equivalent to approx. 6 cm in height, resulting in a volume ratio of water to sediment of 3:1. After pre-equilibration, aliquots of the application solution were directly applied onto the water surface of each system. Thereafter, the systems were closed with the trap attachment for absorbing volatile compounds from DAT 1 onwards. During incubation the supernatant water was agitated gently.

The amount of radiolabelled BYI 02960 for the treatment of the individual test systems was based on a single field use rate of 400 g/ha, calculated to a water depth of 100 cm, which resulted in a nominal application rate was 20.8 µg/batch. The actual application rate set as 100 % of applied radioactivity (100 % AR) corresponding to 1887.68 Bq/500 µL, 1885.15 Bq/500 µL and 1871.07 Bq/500 µL in the regular water/sediment systems. The material balance for the regular test systems was based on the average amount of radioactivity (RA) recovered from these measurements: 94065 Bq or 20.9 µg of test item per vessel.

2. Sampling: Duplicate samples were taken on DAT 0, 3, 7, 14, 28, 63, 91, and 119 days after application for both test systems. The water was decanted and centrifuged. The sediment was extracted with 3 x 80 mL acetonitrile/water (70/30, v/v), followed by one extraction with 80 mL acetonitrile (combination: ambient extracts). Finally, the sediment was extracted once with 80 mL acetonitrile/water (70/30, v/v) by microwave-accelerated solvent extraction (aggressive extract). Volatile organics and $^{14}CO_2$ were trapped with solid trapping attachments containing soda lime for absorption of $^{14}CO_2$ and polyurethane foam for volatile organic compounds.

3. Description of analytical procedures: The radioactivity of the supernatant and sediment samples was radio-assayed by LSC and HPLC. Volatiles were analysed by LSC. For HPLC analysis of the water phase, 10 mL of the water phase were concentrated to about 1 mL. For representative sampling dates (DAT 0, 14, 28, 63, 91 and 119), aliquots of 200 µL of the water phases were investigated by TLC without a concentration step (confirmation method).

Sediment samples were exhaustively extracted once with 80 mL acetonitrile/water (70/30, v/v) by microwave-accelerated solvent extraction for 10 minutes (temperature approx. 70°C) with magnetic stirring. After extraction and centrifugation (approx. 15 min, 2500 x *g*) the supernatant was decanted (aggressive organic extracts) and analysed with LSC. Prior to HPLC-analysis, aliquots of 10.0 mL from the ambient organic extracts and aliquots of 4 mL from the aggressive organic extracts were concentrated to volumes of about 1 mL. 200 µL aliquots of representative ambient and aggressive organic extracts (taken on DAT-0, 14, 28, 63, 91 and 119) were investigated by TLC as a confirmatory method.

Determination of Degradation Kinetics

Dissipation rates from the water phase and rates of degradation for the total system were calculated by use of the software KinGui, version 1.1. The kinetic evaluation included the fitting of data with kinetic models SFO, FOMC and DFOP to the experimental data and their assessment according to FOCUS guidance to result in values for comparison with trigger endpoints.

Results

Findings

The test conditions outlined in the study protocol were maintained throughout the study. Oxygen Saturation, pH and Redox Potential Measurements of the Aerobic Test Systems throughout the Study Period.

Table B.8.4.3.2-09 Oxygen Saturation, pH and Redox Potential Measurements of the Aerobic Test Systems throughout the Study Period (HW)

		Water Phase				Sediment Layer		
DAT	Replicate	pH	Oxygen Conc.	Redox Potential		pH	Redox Potential	
				SenTix ORP Electrode	E_h*		SenTix ORP Electrode	E_h*
	JR53-		[mg O_2/L]	[mV]	[mV]		[mV]	[mV]
Pre-incubation								
-19		7.1	8.3	250	460	6.7	-22	188
-15		6.7	8.3	252	462	6.8	37	247
-11		6.6	8.4	178	388	6.6	53	263
-8		7.1	8.6	181	391	6.9	91	301
-5		7.1	8.4	201	411	6.6	55	265
-1		7.1	8.8	183	393	7.1	42	252
0	HW1-D0	7.1	8.7	174	384	6.6	58	268
	HW2-D0	7.0	8.9	168	378	6.8	36	246
	Mean	**7.1**	**8.8**	**171**	**381**	**6.7**	**47**	**257**
3	HW1-D3	6.9	8.6	198	408	6.9	69	279
	HW2-D3	7.1	8.6	210	420	6.8	59	269
	Mean	**7.0**	**8.6**	**204**	**414**	**6.9**	**64**	**274**
7	HW1-D7	6.9	8.2	226	436	6.8	54	264
	HW2-D7	6.9	8.4	236	446	6.8	64	274
	Mean	**6.9**	**8.3**	**231**	**441**	**6.8**	**59**	**269**
14	HW1-D14	7.1	8.8	198	408	6.5	51	261
	HW2-D14	6.8	8.6	208	418	6.4	66	276
	Mean	**7.0**	**8.7**	**203**	**413**	**6.5**	**59**	**269**
28	HW1-D28	6.7	8.3	228	438	6.9	72	282
	HW2-D28	7.1	8.5	236	446	6.8	61	271
	Mean	**6.9**	**8.4**	**232**	**442**	**6.9**	**67**	**277**
63	HW1-D63	7.3	8.8	234	444	6.9	97	307
	HW2-D63	7.3	8.8	238	448	6.9	86	296
	Mean	**7.3**	**8.8**	**236**	**446**	**6.9**	**92**	**302**
91	HW1-D91	6.7	8.6	246	456	6.6	67	277
	HW2-D91	6.8	8.5	238	448	6.5	78	288
	Mean	**6.8**	**8.5**	**242**	**452**	**6.5**	**73**	**283**
119	HW1-D119	7.0	8.7	213	423	6.5	60	270
	HW2-D119	6.9	8.7	211	421	6.5	64	274
	Mean	**7.0**	**8.7**	**212**	**422**	**6.5**	**62**	**272**
overall Mean#		7.0	8.6	216	426	6.7	65	275
overall Minimum#		6.7	8.2	168	378	6.4	36	246
overall Maximum#		7.3	8.9	246	456	6.9	97	307

* $E_h = E_{obs}$ + 210 mV [reference potential of SenTix ORP electrode (WTW) vs. standard hydrogen electrode at 20°C; manufacturer information]

without pre-incubation

Table B.8.4.3.2-10 Oxygen Saturation, pH and Redox Potential Measurements of the Aerobic Test Systems throughout the Study Period (AW)

		Water Phase				Sediment Layer		
DAT	Replicate	pH	Oxygen Conc.	Redox Potential		pH	Redox Potential	
				SenTix ORP Electrode	E_h*		SenTix ORP Electrode	E_h*
	JR53-		[mg O_2/L]	[mV]	[mV]		[mV]	[mV]
Pre-incubation								
-19		8.1	9.3	180	390	7.3	135	345
-15		8.2	8.9	233	443	7.3	230	440
-11		8.2	8.6	187	397	7.4	237	447
-8		8.2	8.8	186	396	7.4	156	366
-5		8.3	8.8	221	431	7.4	195	405
-1		8.4	9.0	170	380	7.5	181	391
0	AW1-D0	8.4	9.0	201	411	7.6	186	396
	AW2-D0	8.4	9.0	196	406	7.6	178	388
	Mean	**8.4**	**9.0**	**199**	**409**	**7.6**	**182**	**392**
3	AW1-D3	8.3	9.0	196	406	7.5	180	390
	AW2-D3	8.3	9.0	203	413	7.4	184	394
	Mean	**8.3**	**9.0**	**200**	**410**	**7.4**	**182**	**392**
7	AW1-D7	8.5	8.8	238	448	7.5	207	417
	AW2-D7	8.5	8.8	225	435	7.5	196	406
	Mean	**8.5**	**8.8**	**232**	**442**	**7.5**	**202**	**412**
14	AW1-D14	8.3	9.0	192	402	7.5	184	394
	AW2-D14	8.4	9.0	189	399	7.3	181	391
	Mean	**8.4**	**9.0**	**191**	**401**	**7.4**	**183**	**393**
28	AW1-D28	8.5	8.8	205	415	7.7	171	381
	AW2-D28	8.4	8.8	220	430	7.7	196	406
	Mean	**8.4**	**8.8**	**213**	**423**	**7.7**	**184**	**394**
63	AW1-D63	8.5	8.9	224	434	7.9	181	391
	AW2-D63	8.5	8.9	212	422	7.8	174	384
	Mean	**8.5**	**8.9**	**218**	**428**	**7.8**	**178**	**388**
91	AW1-D91	8.5	8.8	204	414	7.5	171	381
	AW2-D91	8.5	8.8	215	425	7.5	166	376
	Mean	**8.5**	**8.8**	**210**	**420**	**7.5**	**169**	**379**
119	AW1-D119	8.5	8.8	203	413	7.5	183	393
	AW2-D119	8.5	8.8	210	420	7.6	176	386
	Mean	**8.50**	**8.81**	**207**	**417**	**7.5**	**180**	**390**
overall Mean#		8.4	8.9	208	418	7.5	182	392
overall Minimum#		8.3	8.8	189	399	7.3	166	376
overall Maximum#		8.5	9.0	238	448	7.9	207	417

* $E_h = E_{obs}$ + 210 mV [reference potential of SenTix ORP electrode (WTW) vs. standard hydrogen electrode at 20°C; manufacturer information]

without pre-incubation

Data

A summary of key data on total recovery and the distribution of radioactivity into the various components formed in water and sediment is given for system Hönniger Weiher in Table B.8.4.3.2-11 and in Table B.8.4.3.2-12 for system Angler Weiher.

Mass Balance

During the study, the total recovery of radioactivity in individual test vessels ranged from 96.5 to 100.6% (mean 98.9%, RSD 1.2 %) for Hönniger Weiher and from 95.7 to 100.8% (mean 98.0%,

RSD 1.6%) for Angler Weiher. Complete material balances found for all sampling dates demonstrating that no significant portion of radioactivity dissipated from the vessels or was lost during processing.

Table B.8.4.3.2- 11 Biotransformation of [PYR-^{14}C] BYI 02960 in Hönniger Weiher under aerobic conditions, expressed as percent of AR, mean ± SD

Compound	Source	Days After Treatment (DAT)							
		0	3	7	14	28	63	91	119
Parent BYI 02960	Water Layer	96.7	68.2	52.1	40.2	26.3	17.7	14.7	11.4
		± 0.3	± 0.1	± 1.7	± 5.3	± 4.7	± 3.4	± 1.4	± 0.4
	Sediment	1.8	27.0	42.5	53.3	59.2	59.4	55.8	52.6
		± 0.1	± 0.2	± 1.5	± 4.3	± 3.5	± 1.4	± 2.3	± 0.3
	Entire System	98.5	95.2	94.6	93.6	85.4	77.2	70.5	64.0
		± 0.4	± 0.3	± 0.2	± 1.1	± 1.1	± 4.8	± 3.7	± 0.1
Reg 1	Water Layer	n.d.	n.d.	n.d.	n.d.	0.8	0.6	n.d.	n.d.
						± 0.0	± 0.0		
	Sediment	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Entire System	n.d.	n.d.	n.d.	n.d.	0.8	0.6	n.d.	n.d.
						± 0.0	± 0.0		
Reg 2	Water Layer	n.d.	n.d.	n.d.	n.d.	0.4	n.d.	0.4	1.4
						± 0.0		± 0.0	± 0.2
	Sediment	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Entire System	n.d.	n.d.	n.d.	n.d.	0.4	n.d.	0.4	1.4
						± 0.0		± 0.0	± 0.2
Non-characterized Radioactivity	Water Layer	0.4	< LOD	0.3	<LOD	< LOD	< LOD	< LOD	< LOD
		± 0.1		± 0.0					
	Sediment	< LOD	< LOD	< LOD	< LOD	< LOD	0.3	< LOD	0.4
							± 0.2		± 0.0
	Entire System	0.4	0.5	0.6	0.4	0.4	0.4	0.3	0.5
		± 0.1	± 0.1	± 0.1	± 0.0	± 0.1	± 0.2	± 0.0	± 0.0
Total Extractable Residues	Water Layer	97.0	68.5	52.4	40.4	27.6	18.5	15.1	12.9
		± 0.2	± 0.0	± 1.7	± 5.4	± 5.1	± 2.8	± 1.8	± 0.2
	Sediment	1.9	27.2	42.8	53.6	59.4	59.8	56.1	53.0
		± 0.1	± 0.2	± 1.5	± 4.3	± 3.5	± 1.5	± 2.3	± 0.3
	Entire System	98.9	95.7	95.1	94.0	87.0	78.3	71.2	65.9
		± 0.3	± 0.2	± 0.2	± 1.1	± 1.6	± 4.3	± 4.0	± 0.2
$^{14}CO_2$	Entire System	n.a.	< 0.1	0.1	0.3	1.2	2.8	5.3	6.8
			± 0.0	± 0.0	± 0.1	± 0.3	± 0.0	± 0.2	± 0.1
Organic Volatiles	Entire System	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
			± 0.0	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0
Non-Extractable Residues (NER)	Sediment	0.4	2.6	4.5	6.3	11.6	18.3	20.1	25.0
		± 0.0	± 0.1	± 0.1	± 0.7	± 1.4	± 3.0	± 3.6	± 0.1
Total Recovery	Water Layer	97.0	68.5	52.4	40.4	27.6	18.5	15.1	12.9
		± 0.2	± 0.0	± 1.7	± 5.4	± 5.1	± 2.8	± 1.8	± 0.2
	Sediment	2.2	29.8	47.2	59.9	71.0	78.0	76.1	78.0
		± 0.1	± 0.1	± 1.5	± 5.0	± 4.9	± 1.4	± 1.3	± 0.3
	Entire System	99.3	98.3	99.7	100.6	99.8	99.3	96.5	97.7
		± 0.4	± 0.1	± 0.2	± 0.4	± 0.1	± 1.4	± 0.6	± 0.0

± = SD, standard deviation, < LOD = < 0.3% of AR (Minimum LOD)

Table B.8.4.3.2-12 Biotransformation of [PYR-^{14}C] BYI 02960 in Angler Weiher under aerobic conditions, expressed as percent of AR, mean ± SD (MEF-11/907)

Compound	Source	Days After Treatment (DAT)							
		0	3	7	14	28	63	91	119
Parent BYI 02960	Water Layer	96.5 ± 0.0	75.7 ± 0.3	69.5 ± 0.8	63.3 ± 0.1	47.6 ± 0.0	32.1 ± 1.4	26.8 ± 0.0	22.3 ± 0.0
	Sediment	1.0 ± 0.2	20.4 ± 1.0	26.5 ± 0.5	33.4 ± 0.1	42.4 ± 0.1	47.5 ± 0.8	48.7 ± 0.3	49.5 ± 0.3
	Entire System	97.5 ± 0.2	96.1 ± 0.6	96.0 ± 0.4	96.7 ± 0.0	90.0 ± 0.1	79.6 ± 2.3	75.5 ± 0.3	71.7 ± 0.2
Reg 2	Water Layer	n.d.	n.d.	n.d.	n.d.	n.d.	0.4 ± 0.0	0.6 ± 0.1	0.8 ± 0.0
	Sediment	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Entire System	n.d.	n.d.	n.d.	n.d.	n.d.	0.4 ± 0.0	0.6 ± 0.1	0.8 ± 0.0
Reg 3	Water Layer	n.d.	n.d.	n.d.	n.d.	n.d.	1.3 ± 0.0	1.1 ± 0.1	1.3 ± 0.2
	Sediment	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Entire System	n.d.	n.d.	n.d.	n.d.	n.d.	1.3 ± 0.0	1.1 ± 0.1	1.3 ± 0.2
Non-characterized Radioactivity	Water Layer	0.5 ± 0.0	0.3 ± 0.0	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	Sediment	< LOD	< LOD	< LOD	< LOD	< LOD	0.3 ± 0.0	< LOD	< LOD
	Entire System	0.6 ± 0.1	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.0
Total Extractable Residues	Water Layer	97.1 ± 0.0	76.0 ± 0.3	69.8 ± 0.8	63.5 ± 0.1	47.8 ± 0.0	34.0 ± 2.0	28.8 ± 0.0	24.6 ± 0.2
	Sediment	1.1 ± 0.2	20.4 ± 0.9	26.7 ± 0.4	33.6 ± 0.1	42.5 ± 0.1	47.8 ± 0.8	48.9 ± 0.4	49.7 ± 0.2
	Entire System	98.1 ± 0.3	96.5 ± 0.6	96.4 ± 0.3	97.1 ± 0.0	90.3 ± 0.1	81.8 ± 2.8	77.7 ± 0.3	74.3 ± 0.0
$^{14}CO_2$	Entire System	n.a.	0.1 ± 0.0	0.2 ± 0.0	0.7 ± 0.0	3.0 ± 0.0	6.0 ± 1.5	7.4 ± 0.4	8.5 ± 0.5
Organic Volatiles	Entire System	n.a.	< 0.1 ± 0.0	< 0.1 ± 0.0	< 0.1 ± 0.0	< 0.1 ± 0.0	< 0.1 ± 0.0	< 0.1 ± 0.0	< 0.1 ± 0.0
Non-Extractable Residues (NER)	Sediment	< 0.1 ± 0.0	1.1 ± 0.0	1.8 ± 0.0	3.0 ± 0.0	6.5 ± 0.0	9.3 ± 0.5	10.7 ± 0.3	13.6 ± 0.3
Total Recovery	Water Layer	97.1 ± 0.0	76.0 ± 0.3	69.8 ± 0.8	63.5 ± 0.1	47.8 ± 0.0	34.0 ± 2.0	28.8 ± 0.0	24.6 ± 0.2
	Sediment	1.1 ± 0.2	21.5 ± 1.0	28.4 ± 0.5	36.6 ± 0.1	49.1 ± 0.1	57.1 ± 0.4	59.6 ± 0.7	63.3 ± 0.5
	Entire System	98.2 ± 0.3	97.6 ± 0.6	98.4 ± 0.3	100.8 ± 0.0	99.9 ± 0.1	97.2 ± 0.8	95.7 ± 0.2	96.4 ± 0.2

± = SD, standard deviation, < LOD = < 0.3% of AR (Minimum LOD)

Volatilization

Formation of $^{14}CO_2$, mineralization of [PYR-^{14}C] BYI 02960, was observed in both water/sediment test systems. At termination of the study, the $^{14}CO_2$ recovery (mean values of duplicates) in test system from HW was 6.8% of AR. In AW test system, $^{14}CO_2$ accounted for 8.5% of AR at study termination. Most of the $^{14}CO_2$ was collected in the soda lime fraction of the trap attachments, ≤ 0.3% of AR in Hönniger Weiher and ≤ 1.5% of AR in Angler Weiher was dissolved CO_2 in water. No significant amounts of organic volatiles were found (< 0.1% of AR).

Residues in water, bound and extractable residues in sediment

The radioactivity in the water phase of HW test system decreased steadily from 97.0% at day 0 to 12.9% of AR at study termination. The extractable ^{14}C residues in sediment increased from 1.9% at DAT 0 to 59.8% of AR at DAT 63 and declined to 53.0% of AR towards the end of the study. NER was 0.4% of AR at day 0 which increased to 25.0% of AR at study termination.

The radioactivity in the water phase of AW test system decreased from 97.1% at day 0 to 24.6% of AR towards the end of the study. Extractable ^{14}C residues in the sediment increased from 1.1% at day 0 to 49.7% of AR at study termination. The amount of bound residues was <0.1% of the applied radioactivity at day 0 and increased to 13.6% towards the end of the incubation period.

The NER of extracted sediments of DAT 119 from HW (both replicates) was found to be of heterogeneous nature. Portions of 4.5 and 5.4% of AR were fractionated together with the humic acids, whereas similar amounts of radioactivity were associated with the fulvic acids (9.1 and 9.3% of AR) or strongly integrated into the insoluble humin of the sediment matrix (10.7 and 9.8% of AR).

Transformation of Parent Compound

The percentages of BYI 02960 and its residues determined in water and sediment extracts are presented in Table B.8.4.3.2-11 for the system HW, and in Table B.8.4.3.2-12 for the system AW. The elimination of BYI 02960 from the water body occurred mainly via partition into the sediment phase and partly via degradation. In the sediment phase of HW, the amounts of BYI 02960 increased from 1.8% at day 0 to a maximum of 59.4% of AR at DAT-63, followed by a decrease to 52.6% of AR towards the end of the study. In the AW sediment the amounts of BYI 02960 increased from 1.0% at day 0 to 49.5% of AR at study termination.

Three very minor transformation products were observed in the water phase. One of those was detected in the water phase of both test systems, and accounted for up to 1.4 and 0.8% of AR, respectively. The others were either detected in the water phase from HW (≤ 0.8% of AR) or Angler Weiher (≤ 1.3% of AR). The maximum amount of the non-characterized radioactivity in the water phase was 0.5% of AR. No transformation products were detected in the sediment extracts from HW and AW. The maximum amount of the non-characterized radioactivity was 0.4% of AR.

Dissipation and Degradation Kinetics

After fitting of data to the three kinetic models SFO, FOMC (Gustafson-Holden) and DFOP the quality of fits was assessed according to FOCUS kinetic guidance. For the dissipation from water the best-fit kinetic models for the determination of trigger values were the FOMC kinetic model for HW and the DFOP kinetic model for AW with DT_{50} values of 8.5 and 34.5 days, respectively (Table B.8.4.3.2-13). The corresponding modelling endpoints were calculated using the DFOP kinetic model. The DT_{50} values for the slow degradation phase were 63.0 and 63.6 days for the water phases of Hönniger Weiher and Angler Weiher, respectively.

In the entire water/sediment systems, [PYR] BYI 02960 was degraded more slowly and the best fit kinetics was single first-order kinetics (SFO). The DT_{50} values were 193.1 and 246.9 days for systems from Hönniger Weiher and Angler Weiher, respectively. These values were used as trigger values and modelling endpoints (Table B.8.4.3.2-14).

Table B.8.4.3.2-13 Summary of dissipation kinetics of BYI 02960 from the supernatant water

Water Phase of Test System	Kinetic Model	DT_{50} [days]	DT_{90} [days]	Chi2 Error [%]
Hönninger Weiher	SFO	14.9	49.6	19
	FOMC	**8.5**	**174.6**	**1.6**
	DFOP	8.2	125.4	3.8
Angler Weiher	SFO	48.5	161	10.2
	FOMC	27.3	705.5	4.6
	DFOP	**34.5**	**181.8**	**4.4**

Bold: best fit, SFO = Single First Order Model, FOMC = First Order Multi Compartment Model, DFOP = Double First Order in Parallel Model

Table B.8.4.3.2-14 Summary of degradation kinetics of BYI 02960 from the entire water/sediment test system

Entire System	Kinetic Model	DT_{50} [days]	DT_{90} [days]	Chi^2 Error [%]
Hönninger Weiher	**SFO**	**193.1**	**641.3**	**1.2**
	FOMC	251.9	> 1000	1.1
	DFOP	204.2	716.3	1.1
Angler Weiher	**SFO**	**246.9**	**820.1**	**1.3**
	FOMC	360.3	> 1000	1.2
	DFOP	> 1000	> 1000	1.3

Bold: best fit, SFO = Single First Order Model, FOMC = First Order Multi Compartment Model, DFOP = Double First Order in Parallel Model

In the supplemental test, i.e. the test systems sterilized by gamma radiation or steam pressure and then incubated for 0, 60 or 120 days, no $^{14}CO_2$ was formed (≤ 0.1% AR). The amount of radioactivity in the water phase, predominantly represented by parent compound, was about two times higher than in that from the microbial active samples. Further, there was a clear trend that NER formation in sediment was lower than in the microbial active test flasks. This shows that the NER were at least partly formed by microbial processes.

Conclusions

[PYR] BYI 02960 is microbially degraded in aerobic water/sediment systems. The DT_{50} values for BYI 02960 in the entire water/sediment systems were 193 and 247 days for Hönniger Weiher and Angler Weiher, respectively. The only major transformation products were carbon dioxide and formation of NER.

Table B.8.4.3.2-15 Results Synopsis

Parameter	Hönniger Weiher	Angler Weiher
Material Balance [% AR]	96.5 – 100.6	95.7 – 100.8
Water Phase [% AR]	12.9 – 97.0	24.6 – 97.1
Sediment Extract [% AR]	1.9 – 59.8	1.1 – 49.7
Major transformation products *	CO_2 (max. 6.8%) NER (max. 25.0%)	CO_2 (max. 8.5%) NER (max. 13.6%)

* Criteria for "major": ->10% of AR at any DAT; >5% of AR at two successive DATs, increasing towards study end

Kinetics Evaluation, Trigger Values		DT_{50} [days]	Chi² Err [%]	Best Fit Kinetic Model	t-probability
Supernatant water	Hönniger Weiher	8.5	1.6	FOMC	-
	Angler Weiher	34.5	4.4	DFOP	-
Entire system	Hönniger Weiher	193.1	1.2	SFO	-
	Angler Weiher	246.9	1.3	SFO	-
Kinetics Evaluation, Modelling Endpoints					
Supernatant water	Hönniger Weiher	63.0*	3.8	DFOP	< 0.001
	Angler Weiher	63.6*	4.4	DFOP	< 0.001
Entire system	Hönniger Weiher	193.1	1.2	SFO	< 0.001
	Angler Weiher	246.9	1.3	SFO	< 0.001

SFO = Single First-Order Model, FOMC = First Order Multi Compartment Model, DFOP = Double First Order in Parallel Model
* DT_{50} value of the slow degradation phase

Remarks

The RMS finds the water/sediment study acceptable. The modelling endpoints for water phase are not used for exposure modelling since they are not level PII true degradation values. Level PI whole system values are considered acceptable for use in environmental exposure modelling.

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Characteristics

reference	:	Menke, U., Unold, M., 2012	incubation time	:	120 days
year of execution	:	2009-2010	nominal concentration	:	0.04 mg/L
GLP statement	:	yes	temperature	:	20 ± 2 °C
guideline	:	incl. OECD 308	DT_{50}	:	208.2 and 202.4 days 246.1 and 285.0 days
test substance	:	[Furanone-4-^{14}C] and [Ethyl-1-^{14}C] BYI 02960	metabolites	:	see results
purity	:	radiochemical purity >98% [FUR] >99% [ETH]	acceptability	:	**acceptable**
test system	:	see materials			

Summary

The aerobic transformation of [FUR-^{14}C] and [ETH-^{14}C] BYI 02960 was studied in two water/sediment systems, Hönniger Weiher (HW) and Angler Weiher (AW) for a maximum of 120 days in the dark at 20 ± 2°C. The test systems consisted of laboratory microcosm flasks attached to traps for collection of CO_2 and volatile organic compounds. Individual flasks filled a volume ratio of water to sediment of 3:1 were treated with [^{14}C] BYI 02960. The actual application rates were 20.86 and 20.80 µg/batch for label FUR and label ETH test systems from Hönniger Weiher and 20.63 and 20.53 µg/batch for label FUR and label ETH test systems from Angler Weiher, corresponding to approx. 40 µg/L water assuming a field application rate of 400 g BYI 02960/ha. During incubation the supernatant water was agitated gently.

Duplicate test systems were processed and analyzed after 0, 1, 3, 7, 14, 30, 45/44, 60/58, 87/86 and 120 days of incubation for test systems from HW and AW (both labels), respectively. The water samples were decanted and analyzed for radioactivity after centrifugation and filtration. Prior to HPLC analysis, a concentration step was performed. The sediment samples were extracted three times with

acetonitrile/water (70/30, v/v), followed by one extraction step with pure acetonitrile, all at ambient temperature (ambient organic extracts). Afterwards, the sediment was extracted once more using a microwave-accelerated solvent extraction (aggressive organic extract) with 80 mL acetonitrile/water (70/30, v/v). The combined extracts from the ambient extractions and the aggressive extract were analyzed by LSC and via HPLC after a concentration step. For selected samples, the HPLC analysis was confirmed by TLC. Identification of the two test items was achieved by HPLC-MS, HPLC-MS/MS, NMR and/or Co-chromatography. The major metabolite detected in label ETH test systems was characterized by HPLC-MS. The non-extractable residues (NER) in sediment samples were determined, and those from the last sampling date were separated into humin, humic acid and fulvic acid fractions.

The test conditions outlined in the study protocol were maintained throughout the study, and the mean material balances in all four test series ranged from 98.2 – 100.4% of AR. The complete material balance demonstrates that no significant portion of radioactivity dissipated from the vessels or was lost during processing.

The radioactivity in FUR and ETH test systems from HW water decreased steadily from 96.0 and 97.2% of AR at day 0 to 14.3 and 14.2% at study termination. The radioactivity in FUR and ETH test systems from AW water decreased from 97.9 and 98.3% of AR at day 0 to 37.3 and 44.1% towards the end of the study. Extractable ^{14}C sediment residues in FUR and ETH test systems from HW increased from 4.2 and 3.8% of AR at day 0 to 54.7 and 54.1% at study termination, respectively. Extractable ^{14}C residues in FUR and ETH sediments from AW increased from 2.2 and 2.4% of AR at day 0 to maxima of 37.7 and 40.1% of AR at study termination. The maxima of non-extractable ^{14}C residues (mean values of duplicates) in the sediments were 22.6 and 26.6% of AR for FUR and ETH test systems from HW, and 17.9 and 15.2% of AR for FUR and ETH test systems from AW, respectively.

At the end of the study period, 3.9 and 1.5% of AR were present as $^{14}CO_2$ in systems from HW, and 5.5 and 0.9% of AR were present as $^{14}CO_2$ in systems from AW, each for FUR and ETH label, respectively. The total amount mainly accounted for of $^{14}CO_2$ trapped in soda lime, but as well for the amount of $^{14}CO_2$ present in the water phase (all sampling intervals) and sediments (only DAT-120). Organic volatile compounds amounted to ≤ 0.2% of the applied radioactivity in both systems and for both labels.

In the water phase from HW, BYI 02960 decreased from 95.5 and 96.8% of AR at day 0 to 14.3 and 14.1% at study termination for FUR and ETH test systems, respectively. In AW water of FUR and ETH test systems, BYI 02960 decreased from 97.4 and 97.9% of AR at day 0 to 35.6 and 36.8% at study termination. In the sediment phase of FUR and ETH test systems from HW, BYI 02960 increased from 4.1 and 3.7% of AR at day 0 to maximum amounts of 58.7 and 58.3% at DAT-60 or DAT-45, and then slightly declined to 54.3 and 52.6% towards study termination, respectively. In AW sediment (FUR and

ETH, respectively) BYI 02960 increased from 2.2 and 2.3% of AR at day 0 to 37.6 and 38.9% at study termination.

DFA (difluoroacetic acid) was observed as a degradation product of [ETH] BYI 02960 in the water phases and in the sediment extracts of both water/sediment systems. In the water phases it accounted for up to 1.1% (HW) and 6.0% (AW) of AR, in the sediment extracts, DFA accounted for only 0.8% and 0.9% of AR, respectively. Two very minor metabolites were detected in the water phases of ETH test systems (≤ 1.1% of AR), and three very minor metabolites were detected in the water phases of FUR test systems (≤ 1.0% of AR). In the sediments, one very minor metabolite was detected in the ETH test systems (≤ 0.5% of AR) and the FUR test systems (≤ 0.1% of AR). The maximum amount of the non-characterized radioactivity was 0.5% of AR for all test systems and compartments.

The dissipation of BYI 02960 from the water phase was mainly characterized by a fast translocation into the sediment. The best-fit kinetic model for the determination of trigger values was the DFOP kinetic model with DT_{50} values of 9.8 and 9.4 days for FUR and ETH test systems from HW, and DT_{50} values of 59.2 and 66.2 days for FUR and ETH test systems from AW. The corresponding modelling endpoints were also calculated using the DFOP kinetic model. The DT50 values for the slow degradation phase were 48.5 and 50.2 days for the water phase of HW, but 123.8 and 117.5 days for the water phase of AW.

In the entire water/sediment systems, BYI 02960 was degraded slowly, the degradation was best described using single first-order kinetics (SFO). The estimated DT_{50} values were 208.2 and 202.4 days for FUR and ETH systems from HW, and 246.1 and 285.0 for FUR and ETH test systems from AW. These values are to be used as trigger values and modelling endpoints.

Materials and Methods

Materials

1. Test Items:

[Furanone-4-^{14}C] BYI 02960, sample ID: KATH 6352; Specific activity = 3.94 MBq/mg (106.46 µCi/mg); Radiochemical purity: >99% (acc. Radio-HPLC and –TLC); Chemical purity: >99% (HPLC, UV detection at 210 nm)

[Ethyl-1-^{14}C] BYI 02960, sample ID: KATH 6358; Specific activity = 3.93 MBq/mg (106.28 µCi/mg); Radiochemical purity: >98% (acc. Radio-HPLC and –TLC); Chemical purity: >98% (HPLC, UV detection at 210 nm)

2. Test System: The study was carried out using the natural water/sediment systems Hoenniger Weiher (HW, near Wipperfürth, Germany;) and Anglerweiher (AW, Leverkusen).

Hoenniger Weiher is an artificially dammed pond in the course of the Hoenniger Creek forming Hoenniger Weiher. Due to its inlet and outlet the pond (about 1000 m² in surface area) has strong water current. Anglerweiher is a reclaimed gravel pit, which is used for fishing only.. The chosen systems are well characterized.

Physical-chemical characteristics of the water/sediment systems are summarized in Table B.8.4.3.2-16.

Water and sediment samples were taken separately and poured into plastic containers. The collected sediments were sieved down to 2 mm mesh-size to remove parts of e.g. plants and stones. The collected water phases were filtered through a 0.06 mm sieve.

Table B.8.4.3.2-16 Physico-chemical characteristics of water and sediment

Parameter	Hönniger Weiher (HW)	Angler Weiher (AW)
Geographic Location	close to Wipperfürth North Rhine-Westphalia Germany	Leverkusen North Rhine-Westphalia Germany
Properties of Water		
Temperature [°C][1]	13.1	16.5
pH[1]	6.5	6.9
Hardness [°dH]*	2.3	9.7
Oxygen Concentration (saturation) [mg/L][1]	8.59	9.02
Total Organic Carbon (TOC) [mg/L][2]	5 / 3 /11	<2 / <2 / 2
Dissolved Organic Carbon (DOC) [mg/L]	4	< 2
Total Nitrogen [mg/L]	< 10	4.6
Total Phosphorus [mg/L]	< 0.3	< 0.03
Redox Potential E_h [mV][1,4]	+ 445	+ 418
Properties of Sediments		
Soil Taxonomic Classification (USDA)	Loam	Sand
Sand (2000 – 50 µm) [%]	44	88
Silt (< 50 – 2 µm) [%]	45	8
Clay (< µm) [%]	11	4
pH[#]	4.8 ($CaCl_2$); 5.0 (H_2O)	6.8 ($CaCl_2$); 7.2 (H_2O)
Organic Matter [%][2,3]	8.8	0.7
Organic Carbon [%][2]	5.1 / n.a. / 4.8	0.4 / n.a. / 0.6
% $CaCO_3$ [#]	0.2	0.4
Sediment Microbial Activity [mg CO_2 /hr/kg sediment (dry wt)][2]	22.50 / 20.83 / 12.42	7.92 / 6.67 / 4.5
Cation Exchange Capacity [meq/100 g]	8.5	3.5
Total Nitrogen [%]	0.39	0.02
Total Phosphorus (Olsen) [ppm]	784	188
Redox Potential E_h [mV][1,4]	+ 142	+ 331

[1] Measurement at day of sampling
[2] start of acclimation / DAT-0 / DAT-120
[3] %organic matter =%organic carbon x 1.724
n.a. = not analyzed
[4] $E_h = E_{obs} + U_{ref}$ [U_{ref} = reference potential of SenTix ORP electrode (WTW) vs. standard hydrogen electrode at given Temperature; 210 mV at 20 °C, extrapolated from manufacturer information]

Materials

1. Experimental conditions: The test systems consisted of laboratory microcosm flasks attached to traps for collection of CO_2 and volatile organic compounds. The individual static test systems were kept at aerobic conditions at 20 ± 1 °C for a maximum period of 120 days. Entire vessels filled with either 82.2 g (HW) or 279.7 g (AW) dry weight sediment and 525 mL of supernatant water (volume ratio of water to sediment: 3:1) were applied with [FUR-^{14}C] or [ETH-^{14}C] BYI 02960, resulting in four parallel

test series. After pre-equilibration, aliquots of the application solution were directly applied onto the water surface of each microcosm system. Thereafter, the systems were closed with the trap attachment for absorbing volatile compounds from DAT 1 onwards. During incubation the supernatant water was agitated gently.

The nominal application rate of 20.8 µg/batch corresponds to about 40 µg/L water and was selected based on a field application rate of 400 g/ha. The actual application rates were 20.86 and 20.80 µg/flask for FUR and ETH test systems from Hönniger Weiher and 20.63 and 20.53 µg/flask for FUR and ETH test systems from Angler Weiher.

2. Sampling: Duplicate flasks were processed and analyzed after 0, 1, 3, 7, 14, 30, 45/44, 60/58, 87/86 and 120 days of incubation for test systems from HW and AW, respectively. The water phase was decanted and analyzed for radioactivity after centrifugation and filtration. Prior to HPLC analysis, a concentration step was performed. The sediment was extracted three times with acetonitrile/water (70/30, v/v), followed by one extraction step with pure acetonitrile, all at ambient temperature (ambient organic extracts). Afterwards, the sediment was extracted once more using a microwave-accelerated solvent extraction (aggressive organic extract) with 80 mL acetonitrile/water (70/30, v/v). The extracted sediment phase was freeze-dried, homogenized and combusted in an oxidizer. The evolved CO_2 was trapped in a scintillation cocktail and measured by LSC to determine the non-extractable residues.

3. Description of analytical procedures: The radioactivity of the supernatant and sediment samples was radio-assayed by LSC and HPLC. The combined extracts from the ambient extractions and the aggressive extract were analyzed by LSC and via HPLC after a concentration step. For selected samples, the HPLC analysis was confirmed by TLC. Identification of the two test items was achieved by HPLC-MS, HPLC-MS/MS, NMR and/or Co-chromatography. The major metabolite detected in the ETH test systems was characterized by HPLC-MS. Volatile organics and $^{14}CO_2$ were trapped with solid trapping attachments containing soda lime for absorption of $^{14}CO_2$ and polyurethane foam for volatile organic compounds. The non-extractable residues were separated into humin, humic acid and fulvic acid fractions for the last sampling interval.

Determination of Degradation Kinetics

Dissipation rates from the water phase and rates of degradation for the total system were calculated by use of the software KinGui, version 1.1. The kinetics evaluation included the fitting of data with kinetic models SFO, FOMC and DFOP to the experimental data and their assessment according to FOCUS guidance to result in values for comparison with trigger endpoints.

Results

The test conditions outlined in the study protocol were maintained throughout the study

Table B.8.4.3.2-17 Oxygen Saturation, pH and Redox Potential Measurements of the Aerobic Test Systems throughout the Study Period (HW)

		Water Phase								Sediment Layer					
DAT	Replicate	pH		Oxygen Conc.		Redox				pH		Redox			
						Elec-trode	E_h*	Elec-trode	E_h*			Elec-trode	E_h*	Elec-trode	E_h*
	JR48-H-			[mg O_2/L]		[mV]	[mV]	[mV]	[mV]			[mV]	[mV]	[mV]	[mV]
Pre-incubation/Label		F	E	F	E	F	F	E	E	F	E	F	F	E	E
-11		6.1	6.2	8.1	8.1	130	340	130	340	6.2	6.2	-41	169	-41	169
-7		6.1	6.1	7.8	7.8	141	351	141	351	6.1	6.1	42	252	42	252
-5		6.1	6.0	8.5	8.5	218	428	218	428	6.0	6.0	70	280	70	280
-3		5.9	6.0	8.3	8.3	212	422	212	422	6.0	6.0	100	310	100	310
0	D0-F/E1	5.8	5.8	7.7	7.6	220	430	205	415	5.9	5.9	70	280	98	308
	D0-F/E2	5.8	5.8	7.6	7.7	210	420	202	412	5.9	6.0	65	275	110	320
	Mean	**5.8**	**5.8**	**7.6**	**7.6**	**215**	**425**	**204**	**414**	**5.9**	**6.0**	**68**	**278**	**104**	**314**
1	D1-F/E1	6.3	6.2	7.7	7.7	248	458	238	448	6.2	6.1	98	308	100	310
	D1-F/E2	6.2	6.2	7.7	7.7	246	456	236	446	6.2	6.1	95	305	108	318
	Mean	**6.2**	**6.2**	**7.7**	**7.7**	**247**	**457**	**237**	**447**	**6.2**	**6.1**	**97**	**307**	**104**	**314**
3	D3-F/E1	6.5	6.5	7.6	7.6	237	447	234	444	6.3	6.2	98	308	104	314
	D3-F/E2	6.5	6.5	7.6	7.5	239	449	231	441	6.2	6.3	113	323	109	319
	Mean	**6.5**	**6.5**	**7.6**	**7.5**	**238**	**448**	**233**	**443**	**6.2**	**6.3**	**106**	**316**	**107**	**317**
7	D7-F/E1	6.2	6.2	7.7	7.9	245	455	242	452	6.0	6.1	106	316	110	320
	D7-F/E2	6.2	6.2	7.5	7.8	240	450	236	446	6.0	6.0	111	321	104	314
	Mean	**6.2**	**6.2**	**7.6**	**7.8**	**243**	**453**	**239**	**449**	**6.0**	**6.0**	**109**	**319**	**107**	**317**
14	D14-F/E1	6.4	6.5	7.4	7.7	251	461	243	453	6.2	6.1	114	324	101	311
	D14-F/E2	6.5	6.5	7.4	7.8	249	459	238	448	6.1	6.2	108	318	108	318
	Mean	**6.4**	**6.5**	**7.4**	**7.7**	**250**	**460**	**241**	**451**	**6.2**	**6.1**	**111**	**321**	**105**	**315**
30	D30-F/E1	6.7	6.7	7.6	7.6	241	451	228	438	6.2	6.2	128	338	116	326
	D30-F/E2	6.7	6.7	7.5	7.7	237	447	233	443	6.2	6.2	116	326	110	320
	Mean	**6.7**	**6.7**	**7.5**	**7.6**	**239**	**449**	**231**	**441**	**6.2**	**6.2**	**122**	**332**	**113**	**323**
45	D45-F/E1	6.8	7.0	7.3	7.5	238	448	228	438	6.2	6.2	120	330	113	323
	D45-F/E2	6.8	7.0	7.3	7.4	234	444	238	448	6.2	6.2	111	321	106	316
	Mean	**6.8**	**7.0**	**7.3**	**7.4**	**236**	**446**	**233**	**443**	**6.2**	**6.2**	**116**	**326**	**110**	**320**
60	D60-F/E1	6.9	6.8	7.4	7.6	252	462	242	452	6.2	6.2	131	341	128	338
	D60-F/E2	6.7	6.9	7.5	7.6	246	456	246	456	6.3	5.9	122	332	115	325
	Mean	**6.8**	**6.9**	**7.4**	**7.6**	**249**	**459**	**244**	**454**	**6.2**	**6.0**	**127**	**337**	**122**	**332**
87	D87-F/E1	6.8	6.8	7.3	7.3	238	448	244	454	6.2	6.1	138	348	148	358
	D87-F/E2	6.8	6.8	7.3	7.3	240	450	248	458	6.2	6.1	145	355	148	358
	Mean	**6.8**	**6.8**	**7.3**	**7.3**	**239**	**449**	**246**	**456**	**6.2**	**6.1**	**142**	**352**	**148**	**358**
120	D120-F/E1	6.6	6.6	7.2	7.3	232	442	238	448	6.0	5.9	141	351	136	346
	D120-F/E2	6.6	6.6	7.2	7.2	238	448	243	453	5.9	5.9	133	343	134	344
	Mean	**6.6**	**6.6**	**7.2**	**7.3**	**235**	**445**	**241**	**451**	**6.0**	**5.9**	**137**	**347**	**135**	**345**
overall Mean#		6.5	6.5	7.5	7.6	239	449	235	445	6.1	6.1	113	323	115	325
overall Minimum#		5.8	5.8	7.2	7.2	210	420	202	412	5.9	5.9	65	275	98	308
overall Maximum#		6.9	7.0	7.7	7.9	252	462	248	458	6.3	6.3	145	355	148	358

* $E_h = E_{obs}$ + 210 mV [reference potential of SenTix ORP electrode (WTW) vs. standard hydrogen electrode at 20°C; manufacturer information]

without pre-incubation

Table B.8.4.3.2-18 Oxygen Saturation, pH and Redox Potential Measurements of the Aerobic Test Systems throughout the Study Period (AW)

		Water Phase								Sediment Layer					
DAT	Replicate	pH		Oxygen Conc.		Redox Potential				pH		Redox Potential			
						Elec-trode	E_h*	Elec-trode	E_h*			Elec-trode	E_h*	Elec-trode	E_h*
	JR48-A-			[mg O_2/L]		[mV]	[mV]	[mV]	[mV]			[mV]	[mV]	[mV]	[mV]
Pre-incubation/Label		F	E	F	E	F	F	E	E	F	E	F	F	E	E
-7		7.5	7.5	7.7	7.7	234	444	234	444	6.9	6.9	199	409	199	409
-5		7.7	7.7	8.3	8.3	236	446	236	446	7.0	7.0	203	413	203	413
-3		7.8	7.8	8.3	8.3	234	444	234	444	7.1	7.1	211	421	211	421
0	D0-F/E1	7.9	7.8	8.3	8.4	236	446	233	443	7.1	7.1	204	414	211	421
	D0-F/E2	7.9	7.4	8.4	8.4	233	443	230	440	7.1	7.0	207	417	208	418
	Mean	**7.9**	**7.6**	**8.3**	**8.4**	**235**	**445**	**232**	**442**	**7.1**	**7.1**	**206**	**416**	**210**	**420**
1	D1-F/E1	8.1	8.1	8.0	8.1	205	415	210	420	7.1	7.1	215	425	208	418
	D1-F/E2	8.1	8.1	8.1	7.9	203	413	208	418	7.1	7.1	208	418	204	414
	Mean	**8.1**	**8.1**	**8.0**	**8.0**	**204**	**414**	**209**	**419**	**7.1**	**7.1**	**212**	**422**	**206**	**416**
3	D3-F/E1	7.9	8.0	8.1	8.0	215	425	210	420	7.1	7.1	222	432	222	432
	D3-F/E2	8.0	8.0	8.0	8.1	208	418	211	421	7.1	7.2	225	435	218	428
	Mean	**8.0**	**8.0**	**8.0**	**8.0**	**212**	**422**	**211**	**421**	**7.1**	**7.1**	**224**	**434**	**220**	**430**
7	D7-F/E1	8.0	8.0	8.4	8.4	203	413	219	429	7.2	7.1	228	438	221	431
	D7-F/E2	8.1	8.0	8.3	8.5	199	409	217	427	7.2	7.0	230	440	216	426
	Mean	**8.0**	**8.0**	**8.3**	**8.4**	**201**	**411**	**218**	**428**	**7.2**	**7.0**	**229**	**439**	**219**	**429**
14	D14-F/E1	8.3	8.2	8.1	8.2	228	438	213	423	7.0	7.1	185	395	193	403
	D14-F/E2	8.3	8.2	8.2	8.2	218	428	214	424	7.0	7.1	190	400	195	405
	Mean	**8.3**	**8.2**	**8.2**	**8.2**	**223**	**433**	**214**	**424**	**7.0**	**7.1**	**188**	**398**	**194**	**404**
30	D30-F/E1	8.4	8.3	8.3	8.3	230	440	229	439	7.2	7.2	183	393	198	408
	D30-F/E2	8.4	8.3	8.4	8.3	226	436	230	440	7.2	7.2	196	406	201	411
	Mean	**8.4**	**8.3**	**8.3**	**8.3**	**228**	**438**	**230**	**440**	**7.2**	**7.2**	**190**	**400**	**200**	**410**
44	D44-F/E1	8.3	8.2	8.3	8.2	236	446	238	448	7.1	7.1	193	403	209	419
	D44-F/E2	8.3	8.3	8.2	8.2	237	447	234	444	7.1	7.0	205	415	196	406
	Mean	**8.3**	**8.2**	**8.2**	**8.2**	**237**	**447**	**236**	**446**	**7.1**	**7.0**	**199**	**409**	**203**	**413**
58	D58-F/E1	8.4	8.4	8.1	8.2	198	408	201	411	7.0	7.0	186	396	190	400
	D58-F/E2	8.4	8.4	8.2	8.2	205	415	206	416	7.0	7.0	194	404	188	398
	Mean	**8.4**	**8.4**	**8.2**	**8.2**	**202**	**412**	**204**	**414**	**7.0**	**7.0**	**190**	**400**	**189**	**399**
86	D86-F/E1	8.5	8.5	8.5	8.5	221	431	217	427	7.0	6.9	186	396	186	396
	D86-F/E2	8.5	8.5	8.5	8.5	223	433	218	428	7.0	7.0	180	390	188	398
	Mean	**8.5**	**8.5**	**8.5**	**8.5**	**222**	**432**	**218**	**428**	**7.0**	**7.0**	**183**	**393**	**187**	**397**
120	D120-F/E1	8.6	8.6	8.2	8.2	228	438	225	435	7.2	7.2	194	404	198	408
	D120-F/E2	8.6	8.6	8.2	8.2	225	435	226	436	7.1	7.1	198	408	196	406
	Mean	**8.6**	**8.6**	**8.2**	**8.2**	**227**	**437**	**226**	**436**	**7.2**	**7.1**	**196**	**406**	**197**	**407**
overall Mean#		8.3	8.2	8.2	8.2	219	429	219	429	7.1	7.1	201	411	202	412
overall Minimum#		7.9	7.4	8.0	7.9	198	408	201	411	7.0	6.9	180	390	186	396
overall Maximum#		8.6	8.6	8.5	8.5	237	447	238	448	7.2	7.2	230	440	222	432

* $E_h = E_{obs}$ + 210 mV [reference potential of SenTix ORP electrode (WTW) vs. standard hydrogen electrode at 20°C; manufacturer information]

without pre-incubation

Data

A summary of key data on total recovery and the distribution of radioactivity into the various components formed in water and sediment is given in Table B.8.4.3.2-19 and Table B.8.4.3.2-20 for

test system Hönniger Weiher and in Table B.8.4.3.2-21 and Table B.8.4.3.2-22 for test system Angler Weiher.

Mass Balance

During the study, the total recovery of radioactivity in individual test vessels of the HW test systems ranged from 94.9 to 100.7% (mean 98.2%, RSD 2.0%) for FUR and from 96.5 to 101.4% (mean 98.7%, RSD 1.4%) for ETH. The radioactivity in individual test vessels of the AW test systems ranged from 98.4 to 101.8% (mean 100.4%, RSD 1.1%) for FUR and from 99.4 to 101.2% (mean 100.3%, RSD 0.5%) for ETH. The complete material balance found for all sampling intervals demonstrates that no significant portion of radioactivity dissipated from the vessels or was lost during processing.

Table B.8.4.3.2-19 Biotransformation of [FUR-^{14}C] BYI 02960 in Hönniger Weiher under aerobic conditions, expressed as percent of AR, mean ± SD

Compound	Source	Days After Treatment (DAT)									
		0	1	3	7	14	30	45	60	87	120
Parent BYI 02960	Water Layer	95.5	86.4	69.3	57.5	47.0	32.7	27.0	21.1	15.6	14.3
		±0.6	±1.1	±1.1	±0.7	±1.5	±1.0	±0.8	±1.2	±1.8	±1.2
	Sediment	4.1	10.9	27.1	37.2	47.4	55.1	57.2	58.7	54.9	54.3
		±0.7	±0.3	±0.6	±0.3	±0.9	±0.4	±0.5	±1.5	±0.7	±2.9
	Entire System	99.6	97.3	96.4	94.7	94.4	87.9	84.2	79.8	70.5	68.6
Reg 2	Water Layer	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0
		±0.0	±0.0	±0.0	±0.0	±0.0	±0.0	±0.0	±0.0	±0.1	±0.0
	Sediment	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
		±0.0	±0.0	±0.0	±0.0	±0.0	±0.0	±0.0	±0.0	±0.0	±0.1
	Entire System	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.1
Non-characterized radioactivity	Water Layer	0.5	0.3	0.4	0.3	0.2	0.2	0.1	0.1	0.1	0.0
		±0.0	±0.1	±0.0	±0.0	±0.1	±0.1	±0.0	±0.0	±0.0	±0.0
	Sediment	0.1	0.4	0.1	0.1	0.2	0.2	0.3	0.2	0.3	0.2
		±0.0	±0.0	±0.0	±0.0	±0.1	±0.1	±0.0	±0.0	±0.1	±0.1
Total extractable residues	Water Layer	96.0	86.7	69.7	57.7	47.3	32.9	27.1	21.3	16.3	14.3
		±0.6	±1.0	±1.1	±0.7	±1.5	±1.1	±0.8	±1.2	±1.9	±1.2
	Sediment	4.2	11.3	27.3	37.3	47.6	55.3	57.5	58.9	55.3	54.7
		±0.8	±0.3	±0.6	±0.3	±0.0	±0.5	±0.5	±1.5	±0.6	±3.1
Total $^{14}CO_2$	Entire System	0.0	0.0	0.1	0.2	0.4	1.0	1.2	1.8	3.3	3.9
		±0.0	±0.0	±0.0	±0.0	±0.0	±0.0	±0.1	±0.0	±0.7	±0.3
Total volatile organics	Entire System	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.0
		±0.0	±0.0	±0.0	±0.1	±0.1	±0.0	±0.0	±0.0	±0.0	±0.0
NER	Sediment	0.4	1.0	2.5	4.1	5.2	8.9	11.9	14.9	19.9	22.6
		±0.0	±0.0	±0.3	±0.1	±0.3	±0.6	±0.5	±0.6	±1.0	±3.7
Total recovery	Water Layer	96.0	86.7	69.7	57.7	47.3	32.9	27.1	21.3	16.3	14.3
		±0.6	±1.0	±1.1	±0.7	±0.2	±1.1	±0.8	±1.2	±1.9	±1.2
	Sediment	4.6	12.3	29.7	41.4	52.8	64.3	69.4	73.8	75.2	77.3
		±0.8	±0.3	±0.6	±0.3	±0.9	±0.5	±0.5	±1.5	±0.6	±3.1
	Entire System	100.7	99.0	99.6	99.4	100.5	98.2	97.8	97.0	94.9	95.5
		±0.1	±0.7	±0.1	±0.5	±0.2	±0.1	±0.3	±0.9	±0.8	±0.3

n.d.: not detected; n.a. : not analysed; DAT : day after treatment, ± = standard deviation.

Table B.8.4.3.2-20 Biotransformation of [ETH-^{14}C] BYI 02960 in Hönniger Weiher under aerobic conditions, expressed as percent of AR, mean ± SD

Compound	Source	Days After Treatment (DAT)									
		0	1	3	7	14	30	45	60	87	120
Parent BYI 02960	Water Layer	96.8 ±0.3	82.1 ±0.1	70.1 ±0.4	57.4 ±0.6	44.6 ±0.8	31.9 ±1.0	28.2 ±0.4	21.5 ±0.1	14.8 ±0.2	14.1 ±0.7
	Sediment	3.7 ±0.1	14.2 ±0.2	26.8 ±0.0	37.2 ±0.1	48.6 ±0.6	56.0 ±0.6	58.3 ±0.2	57.2 ±0.6	55.9 ±0.6	52.6 ±1.8
	Entire System	100.6	96.2	96.8	94.6	93.2	87.9	86.5	78.8	70.7	66.7
DFA	Water Layer	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.6 ±0.0	0.0 ±0.0	1.1 ±0.0	0.7 ±0.1	0.0 ±0.0
	Sediment	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.8 ±0.1	0.7 ±0.5
	Entire System	0.0	0.0	0.0	0.0	0.0	0.6	0.0	1.1	1.4	0.7
Reg 2	Water Layer	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	1.0 ±0.0	0.8 ±0.0	0.0 ±0.0
	Sediment	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.5 ±0.5
	Entire System	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.8	0.5
Non-characterized radioactivity	Water Layer	0.4 ±0.0	0.3 ±0.0	0.4 ±0.0	0.3 ±0.0	0.2 ±0.0	0.1 ±0.0	0.1 ±0.0	0.1 ±0.0	0.1 ±0.0	0.1 ±0.0
	Sediment	0.1 ±0.0	0.1 ±0.0	0.1 ±0.0	0.2 ±0.0	0.2 ±0.0	0.2 ±0.0	0.3 ±0.0	0.2 ±0.1	0.3 ±0.0	0.2 ±0.1
Total extractable residues	Water Layer	97.2 ±0.3	82.4 ±0.1	70.4 ±0.4	57.6 ±0.5	44.8 ±0.8	32.6 ±1.0	28.3 ±0.4	23.8 ±0.1	16.4 ±0.2	14.2 ±0.7
	Sediment	3.8 ±0.1	14.2 ±0.2	26.9 ±0.0	37.5 ±0.1	48.8 ±0.0	56.2 ±0.6	58.6 ±0.2	57.5 ±0.6	57.0 ±0.8	54.1 ±0.7
Total $^{14}CO_2$	Entire System	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.1 ±0.0	0.2 ±0.0	0.6 ±0.0	1.1 ±0.0	1.5 ±0.3
Total volatile organics	Entire System	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.1 ±0.0	0.1 ±0.1	0.0 ±0.0	0.1 ±0.0	0.1 ±0.0
NER	Sediment	0.3 ±0.0	1.5 ±0.3	2.1 ±0.1	4.2 ±0.0	6.0 ±0.6	9.4 ±0.1	12.0 ±0.3	16.3 ±0.5	22.5 ±1.0	26.6 ±0.8
Total recovery	Water Layer	97.2 ±0.3	82.4 ±0.1	70.4 ±0.4	57.6 ±0.5	44.8 ±0.8	32.6 ±1.0	28.3 ±0.4	23.8 ±0.1	16.4 ±0.2	14.2 ±0.7
	Sediment	4.1 ±0.1	15.8 ±0.2	29.0 ±0.0	41.6 ±0.1	54.7 ±0.6	65.6 ±0.6	70.6 ±0.2	73.8 ±0.6	79.5 ±0.8	54.1 ±0.7
	Entire System	101.4 ±0.4	98.2 ±0.5	99.4 ±0.3	99.3 ±0.4	99.6 ±0.8	98.4 ±0.3	99.2 ±0.3	98.2 ±0.0	97.0 ±0.2	96.5 ±0.3

n.d.: not detected; n.a. : not analysed; DAT : day after treatment, ± = standard deviation.

Table B.8.4.3.2-21 Biotransformation of [FUR-^{14}C] BYI 02960 in Angler Weiher under aerobic conditions, expressed as percent of AR, mean ± SD

Compound	Source	Days After Treatment (DAT)									
		0	1	3	7	14	30	45	60	87	120
Parent BYI 02960	Water Layer	97.4 ±0.8	89.4 ±1.6	85.1 ±0.3	78.8 ±0.2	69.6 ±0.1	59.3 ±2.8	52.3 ±0.8	46.0 ±0.9	41.5 ±0.8	35.6 ±1.6
	Sediment	2.2 ±0.1	10.4 ±1.9	14.2 ±0.3	19.4 ±0.0	26.4 ±0.2	31.9 ±1.8	34.8 ±0.3	34.9 ±0.8	37.6 ±0.4	37.6 ±0.3
	Entire System	99.6	99.8	99.4	98.2	96.0	91.2	87.1	80.9	79.1	73.1
Reg 4	Water Layer	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	1.0 ±0.1	0.8 ±0.0	0.6 ±0.2
	Sediment	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
	Entire System	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.8	0.6
Reg 2	Water Layer	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.8 ±0.0	0.7 ±0.1	0.9 ±0.1
	Sediment	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
	Entire System	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.7	0.9

Reg 3	Water Layer	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.5 ±0.0	0.0 ±0.0	0.0 ±0.0
	Sediment	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
	Entire System	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0
Non-characterized radioactivity	Water Layer	0.5 ±0.1	0.2 ±0.0	0.3 ±0.1	0.4 ±0.0	0.4 ±0.0	0.2 ±0.1	0.3 ±0.1	0.2 ±0.0	0.2 ±0.0	0.2 ±0.0
	Sediment	0.1 ±0.0	0.0 ±0.0	0.0 ±0.0	0.1 ±0.0	0.1 ±0.1	0.2 ±0.0	0.2 ±0.0	0.2 ±0.0	0.2 ±0.0	0.1 ±0.1
Total extractable residues	Water Layer	97.9 ±0.7	89.6 ±1.6	85.5 ±0.2	79.1 ±0.2	70.0 ±0.1	59.5 ±2.7	52.6 ±0.9	48.6 ±1.0	43.2 ±0.8	37.3 ±1.4
	Sediment	2.2 ±0.1	10.4 ±1.9	14.2 ±0.3	19.5 ±0.0	26.5 ±0.0	32.1 ±1.8	35.0 ±0.3	35.1 ±0.8	37.8 ±0.5	37.7 ±0.2
Total $^{14}CO_2$	Entire System	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.3 ±0.0	0.5 ±0.0	0.7 ±0.1	1.6 ±0.1	2.4 ±0.1	3.3 ±0.3	5.5 ±0.7
Total volatile organics	Entire System	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.1 ±0.1	0.0 ±0.0	0.0 ±0.0	0.1 ±0.1	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
NER	Sediment	0.5 ±0.0	0.9 ±0.0	1.6 ±0.0	2.6 ±0.1	4.7 ±0.0	8.4 ±0.1	10.3 ±0.4	13.0 ±0.0	15.1 ±0.1	17.9 ±0.2
Total recovery	Water Layer	97.9 ±0.7	89.6 ±1.6	85.5 ±0.2	79.1 ±0.2	70.0 ±0.0	59.5 ±2.7	52.6 ±0.9	48.6 ±1.0	43.2 ±0.8	37.3 ±1.4
	Sediment	2.7 ±0.1	11.3 ±1.9	15.9 ±0.3	22.1 ±0.0	31.3 ±0.2	40.5 ±1.8	45.3 ±0.3	48.1 ±0.8	52.9 ±0.5	37.7 ±0.2
	Entire System	100.6 ±0.6	101.0 ±0.4	101.4 ±0.5	101.6 ±0.3	101.8 ±0.0	100.7 ±1.1	99.5 ±0.3	99.1 ±0.0	99.4 ±0.0	98.4 ±0.2

n.d.: not detected; n.a. : not analysed; DAT : day after treatment, ± = standard deviation.

Table B.8.4.3.2-22 Biotransformation of [ETH-^{14}C] BYI 02960 in Angler Weiher under aerobic conditions, expressed as percent of AR, mean ± SD

Compound	Source	Days After Treatment (DAT)									
		0	1	3	7	14	30	45	60	87	120
Parent BYI 02960	Water Layer	97.9 ±1.1	91.6 ±0.1	87.4 ±1.5	80.6 ±0.7	73.9 ±0.1	62.1 ±0.7	54.7 ±0.0	49.7 ±0.0	43.2 ±0.9	36.8 ±0.3
	Sediment	2.3 ±0.2	7.5 ±0.1	11.5 ±0.6	17.3 ±0.5	22.3 ±0.5	29.4 ±0.2	33.6 ±0.6	34.4 ±0.0	38.0 ±0.2	38.9 ±0.0
	Entire System	100.2	99.1	98.8	97.8	96.2	91.5	88.3	84.1	81.2	75.7
DFA	Water Layer	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	1.5 ±0.0	2.2 ±0.1	2.6 ±0.1	4.2 ±0.7	6.0 ±0.2
	Sediment	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.5 ±0.0	0.6 ±0.0	0.9 ±0.1
	Entire System	0.0	0.0	0.0	0.0	0.0	1.5	2.2	3.1	4.8	6.9
Reg 2	Water Layer	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.8 ±0.2	0.9 ±0.1	1.0 ±0.0	1.1 ±0.2
	Sediment	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
	Entire System	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.9	1.0	1.1
Reg 3	Water Layer	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.6 ±0.1	0.4 ±0.0	0.0 ±0.0	0.0 ±0.0
	Sediment	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
	Entire System	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.4	0.0	0.0
Non-characterized radioactivity	Water Layer	0.4 ±0.0	0.3 ±0.1	0.4 ±0.1	0.4 ±0.0	0.5 ±0.2	0.1 ±0.0	0.3 ±0.0	0.3 ±0.1	0.3 ±0.1	0.2 ±0.0
	Sediment	0.1 ±0.0	0.0 ±0.0	0.0 ±0.0	0.1 ±0.0	0.1 ±0.0	0.1 ±0.0	0.2 ±0.0	0.2 ±0.0	0.1 ±0.0	0.2 ±0.0
Total extractable	Water Layer	98.3 ±1.1	91.9 ±0.0	87.7 ±1.4	81.0 ±0.7	74.3 ±0.1	63.8 ±0.6	58.6 ±0.2	53.9 ±0.1	48.6 ±0.3	44.1 ±0.1

residues	Sediment	2.4 ±0.3	7.5 ±0.1	11.5 ±0.6	17.3 ±0.5	22.3 ±0.0	29.5 ±0.2	33.8 ±0.6	35.1 ±0.0	38.7 ±0.1	40.1 ±0.0
Total $^{14}CO_2$	Entire System	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.1 ±0.0	0.2 ±0.0	0.3 ±0.0	0.5 ±0.0	0.9 ±0.0
Total volatile organics	Entire System	0.0 ±0.0	0.0 ±0.0	0.1 ±0.1	0.1 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.1 ±0.0
NER	Sediment	0.5 ±0.0	0.6 ±0.0	1.1 ±0.0	2.0 ±0.1	3.4 ±0.0	6.0 ±0.1	7.4 ±0.1	10.5 ±0.1	13.1 ±0.3	15.2 ±0.2
Total recovery	Water Layer	98.3 ±1.1	91.9 ±0.0	87.7 ±1.4	81.0 ±0.7	74.3 ±0.6	63.8 ±0.6	58.6 ±0.2	53.9 ±0.1	48.6 ±0.3	44.1 ±0.1
	Sediment	2.9 ±0.3	8.2 ±0.1	12.6 ±0.6	19.3 ±0.5	25.7 ±0.5	35.5 ±0.2	41.3 ±0.6	45.6 ±0.0	51.8 ±0.1	40.1 ±0.0
	Entire System	101.2 ±0.8	100.1 ±0.1	100.4 ±0.9	100.3 ±0.2	100.1 ±0.6	99.4 ±0.7	100.1 ±0.7	99.9 ±0.1	100.9 ±0.6	100.3 ±0.0

n.d.: not detected; n.a. : not analysed; DAT : day after treatment, ± = standard deviation.

Volatilization

Formation of $^{14}CO_2$, i.e. mineralization of BYI 02960, was observed in all water/sediment systems. At termination of the study, the $^{14}CO_2$ recovery (mean values of duplicates) in systems from HW was 3.9 and 1.5% of AR for FUR and ETH, respectively. In AW water/sediment systems, $^{14}CO_2$ accounted for 5.5 and 0.9% of AR at study termination for FUR and ETH, respectively. Organic volatiles were < 0.2% of the applied radioactivity for both systems and labels.

Residues in water, bound and extractable residues in sediment

The radioactivity in FUR and ETH test systems from Hönniger Weiher water decreased steadily from 96.0 and 97.2% of AR at day 0 to 14.3 and 14.2% of AR at study termination. The radioactivity in FUR and ETH test systems from Angler Weiher water decreased from 97.9 and 98.3% of AR at day 0 to 37.3 and 44.1% of AR towards the end of the study. The elimination of BYI 02960 from the water body occurred mainly via translocation into the sediment phase and partly via degradation.

The extractable radioactivity in FUR and ETH test systems from HW increased from 4.2 and 3.8% of AR at day 0 to 54.7 and 54.1% of AR at study termination, respectively. Extractable ^{14}C residues in FUR and ETH sediment from Angler Weiher increased from 2.2 and 2.4% of AR at day 0 to maxima of 37.7 and 40.1% of AR at study termination. This indicates a rapid partitioning of BYI 02960 residues from the water phases into the sediments.

The NER for FUR and ETH test systems from HW were 0.4 and 0.3% of AR at day 0 and increased to 22.6 and 26.6% of AR at study termination, respectively. For AW water/sediment systems (FUR and ETH, respectively), the amount of NER was 0.5% of AR at day 0 and increased to 17.9 and 15.2% of AR towards the end of the incubation period. By further characterization it was found that the NER was of a heterogeneous nature in case of both types of sediments and for both radiolabels. In the sediments from HW, portions of only 1.4 and 1.8% of the NER were fractionated together with humic acids, whereas the major portion of radioactivity (53.9 and 59.0% of NER for FUR and ETH, respectively) was associated with fulvic acids. Amounts of 45.2% (FUR) and 42.9% (ETH) of the NER were found strongly integrated into the insoluble humin of the sediment matrix. In the sediment from

AW, only 4.8 and 2.4% of the NER were fractionated together with humic acids for FUR and ETH, respectively, whereas similar amounts of radioactivity were associated with fulvic acids or strongly integrated into the insoluble humin of the sediment matrix (45.4 and 47.2% of the NER for FUR; 47.9 and 49.1 for ETH).

Transformation of Parent Compound

In the water phase from HW, the amount of BYI 02960 decreased from 95.5 and 96.8% of AR at day 0 to 14.3 and 14.1% of AR at study termination for FUR and ETH test systems, respectively. In the AW water of FUR and ETH test systems, the amount of BYI 02960 decreased from 97.4 and 97.9% of AR at day 0 to 35.6 and 36.8% of AR at study termination.

One major peak was observed in the water phase of ETH test systems, accounting for up to 1.1% of AR in the water phase from HW, and 6.0% of AR in water phases from AW. The metabolite was identified as DFA using HPLC-MS. Further, two very minor metabolites were detected in the water phases of ETH test systems (≤ 1.1% of AR) and three very minor metabolites were detected in the water phases of FUR test systems (≤ 1.0% of AR). The maximum amount of the non-characterized radioactivity was 0.5% of AR for all water phases.

In the sediment phase of FUR and ETH test systems from HW, the amount of BYI 02960 increased from 4.1 and 3.7% of AR at day 0 to maximum amounts of 58.7 and 58.3% of AR at DAT-60 or DAT-45, and then slightly declined to 54.3 and 52.6% of AR towards study termination. In the AW sediment (FUR and ETH, respectively) the amount of BYI 02960 increased from 2.2 and 2.3% of AR at day 0 to 37.6 and 38.9% of AR at study termination. –DFA was also observed in the ETH sediments with maximum amounts of 0.8% of AR (DAT-87) for HW and 0.9% of AR (DAT 120) for sediment from AW. Additionally one very minor metabolite was detected in the ETH sediments (≤ 0.5% of AR) and FUR sediments (≤ 0.1% of AR), respectively. The maximum amount of the non-characterized radioactivity was 0.4% of AR for all sediments.

The BYI 02960 residues in the entire test systems from HW declined to 68.6 and 66.7% of AR at study termination, FUR and ETH, respectively. In the entire FUR and ETH test systems from AW, 73.1 and 75.7% of AR were found as unchanged test item at the end of the incubation period.

The metabolite DFA was found at maximum amounts of 1.4% (Hönniger Weiher, DAT-87) and 6.9% of AR (Angler Weiher, DAT-120). The maximum amount of a single minor radioactivity zone in both systems and with both labels was 1.1% of the AR.

Dissipation Kinetics

The dissipation of BYI 02960 from the water phase is mainly characterized by a fast translocation into the sediment which is best described using bi-phasic kinetic models. The best-fit kinetic model for the determination of trigger values was the DFOP kinetic model with DT_{50} values of 9.8 and 9.4 days for

FUR and ETH test systems from Hönniger Weiher and DT_{50} values of 59.2 and 66.2 days for FUR and ETH test systems from Angler Weiher. The corresponding modelling endpoints were also calculated using the DFOP kinetic model. The DT_{50} values for the slow degradation phase were 48.5 and 50.2 days for the water phase of Hönniger Weiher, but 123.8 and 117.5 days for the water phase of Angler Weiher which is much more of sandy type.

In the entire water/sediment systems, BYI 02960 the degradation was best described using single first-order kinetics (SFO). The estimated DT_{50} values were 208.2 and 202.4 days for FUR and ETH systems from Hönniger Weiher and 246.1 and 285.0 for FUR and ETH test systems from Angler Weiher. These values were used as trigger values and modelling endpoints.

Table B.8.4.3.2-23 Summary of dissipation kinetics of BYI 02960 from the supernatant water; evaluation for trigger values according to FOCUS

Water Phase of Test System	Kinetic Model	DT_{50} [days]	DT_{90} [days]	Chi^2 Error [%]
Hönninger Weiher (FUR)	SFO	24.6	81.6	15.6
	FOMC	10.2	292.3	3.7
	DFOP	9.8	118.4	4.1
Hönninger Weiher (ETH)	SFO	24.7	82.0	16.2
	FOMC	9.8	308.8	4.4
	DFOP	9.4	120.9	4.9
Angler Weiher (FUR)	SFO	69.3	230.1	7.6
	FOMC	53.2	> 1000	2.7
	DFOP	59.2	344.9	2.9
Angler Weiher (ETH)	SFO	73.1	242.8	6.5
	FOMC	61.4	> 1000	2.4
	DFOP	66.2	341.2	2.4

Bold: best fit; SFO = Single First Order Model; FOMC = First Order Multi Compartment Model; DFOP = Double First Order in Parallel Model

Table B.8.4.3.2-24 Summary of degradation kinetics of BYI 02960 from the entire water/sediment test system; evaluation for trigger values according to FOCUS

Entire Test System	Kinetic Model	DT_{50} [days]	DT_{90} [days]	Chi^2 Error [%]
Hönninger Weiher (FUR)	**SFO**	208.2	691.6	1.5
	FOMC	283.6	> 1000	1.3
	DFOP	*205.4*	706.8	1.4
Hönninger Weiher (ETH)	**SFO**	202.4	672.2	1.6
	FOMC	249.3	> 1000	1.6
	DFOP	197.6	685.2	1.3
Angler Weiher (FUR)	**SFO**	246.1	817.4	1.3
	FOMC	475.1	> 1000	0.9
	DFOP	626.4	> 1000	0.9
Angler Weiher (ETH)	**SFO**	285.0	946.9	1.0
	FOMC	633.6	> 1000	0.6
	DFOP	420.4	> 1000	0.6

Bold: best fit; SFO = Single First Order Model; FOMC = First Order Multi Compartment Model; DFOP = Double First Order in Parallel Model

Table B.8.4.3.2-25 Modelling endpoints according to FOCUS obtained for dissipation or degradation of BYI 02960 from the supernatant water

Water Phase of Test System	Kinetic Model	k_2	DT_{50} for k2 [days]	Visual Acceptability	Chi^2 Err. [%]	t-prob-ability
HW (FUR)	DFOP	0.0143	48.5	Yes	4.1	< 0.001
HW (ETH)	DFOP	0.0138	50.2	Yes	4.9	< 0.001
AW (FUR)	DFOP	0.0056	123.8	Yes	2.9	< 0.001
AW (ETH)	DFOP	0.0059	117.5	Yes	2.4	< 0.001

DFOP = Double First Order in Parallel Model, k_2 = slow degradation rate constant of the DFOP kinetic model

Table B.8.4.3.2-26 Modelling endpoints according to FOCUS obtained for dissipation or degradation of BYI 02960 from the entire water/sediment system

Entire System	Kinetic Model	DT_{50} [days]	Visual Acceptability	Chi^2 Err [%]	t-probability
HW(FUR)	SFO	208.2	Yes	1.5	< 0.001
HW(ETH)	SFO	202.4	Yes	1.6	< 0.001
AW (FUR)	SFO	246.1	Yes	1.3	< 0.001
AW (ETH)	SFO	285.0	Yes	1.0	< 0.001

SFO = Single First Order Model

Conclusions

BYI 02960 is translocated from the water phase into the sediment where it is slowly degraded and mineralized. Major transformation products were mineralization to carbon dioxide, formation of NER and one major metabolite, DFA, (max. 6.9% of AR). An overall result synopsis is shown by Table B.8.4.3.2-27.

Table B.8.4.3.2-27 Results Synopsis

	Hönniger Weiher		Angler Weiher	
Parameter	**Label FUR**	**Label ETH**	**Label FUR**	**Label ETH**
Material Balance [% AR]	94.9 – 100.7	96.5 – 101.4	98.4 – 101.8	99.4 – 101.2
Water Phase [% AR]	14.3 – 96.0	14.2 – 97.2	37.3 – 97.9	44.1 – 98.3
Sediment Extract [% AR]	4.2 – 58.9	3.8 – 58.6	2.2 – 37.8	2.4 – 40.1
$^{14}CO_2$ [max. %]	3.9	1.5	5.5	0.9
Major transformation products *	NER, max. 22.6%	NER, max. 26.6%	NER, max. 17.9	NER, max. 15.2% DFA, max. 6.9%
DT_{50} (trigger values)				
Water	9.8	9.4	59.2	66.2
Total system	208.2	202.4	246.1	285.0
DT_{50} (modelling values)				
Water	48.5*	50.2*	123.8*	117.5*
Total system	208.2	202.4	246.1	285.0

* DT_{50} value of the slow degradation phase of DFOP model

Remarks

The RMS finds the water/sediment study acceptable. The modelling endpoints for water phase are not used for exposure modelling since they are not level PII true degradation values. Level PI whole system values SFO kinetics are considered acceptable for use in modelling.

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Characteristics

reference	:	Hellpointner, E., Unold, M., T., 2012	incubation time	:	99 days
year of execution	:	2011	nominal concentration	:	4.7 µg DFA per test

					system
GLP statement	:	yes	temperature	:	19.2 ± 0.1 °C
guideline	:	incl. OECD 308	DT50	:	see results
test substance	:	[1-^{14}C] BYI 02960-DFA	metabolites	:	see results
purity	:	radiochemical purity > 99.5%	acceptability	:	**acceptable**
test system	:	see materials			

Summary

The aerobic transformation of the metabolite [1-^{14}C] DFA was studied in two water/sediment systems Hönniger Weiher (HW) and Angler Weiher (AW) for a maximum of 99 days in the dark at 19.2 ± 0.1 °C. The application rate of 4.7 µg DFA per test system was the ten-fold overdose of the application rate calculated based on the use rate of the parent compound BYI 02960 (400 g/ha) and the maximum amount of BYI 02960-DFA formed in the parent aerobic aquatic metabolism study (6.9%). The test systems consisted of laboratory microcosm flasks attached to traps for the collection of CO_2 and volatile organic compounds. Entire vessels filled with either 88.4 g (HW) or 210.3 g (AW) dry weight sediment and 520 mL of supernatant water (volume ratio of water to sediment: 3:1) were treated with [1-^{14}C] BYI 02960-DFA. During incubation the supernatant water was in smooth motion.

Samples were taken after 0, 7, 19, 33, 61, 79 and 99 days of incubation. The water layers were decanted and centrifuged. The sediment samples were extracted stepwise with acetonitrile/water mixtures at ambient temperature (ambient organic extracts). Afterwards, the sediment was extracted once more using a microwave-accelerated solvent extraction (aggressive organic extract). The extracted sediment phase was air-dried or freeze-dried, homogenized and combusted in an oxidizer in order to determine the non-extractable residues (NER). The water phases and the combined extracts of the ambient extraction steps were analyzed by LSC and TLC in order to determine the amounts of the test item and its transformation products. The aggressive extracts were only analyzed by LSC since they contained only low amounts of radioactivity. Identification of the test item in application solution was achieved by HPLC-MS, HPLC-MS/MS and NMR.

The test conditions outlined in the study protocol were maintained throughout the study. The mean material balances in the two test series ranged from 97.7 to 105.8% for test systems from Hönniger Weiher and from 97.7 to 107.6% for test systems from Angler Weiher.

The radioactivity in the water phase of HW test systems decreased steadily from 97.6% at day 0 to 34.6% of AR at study termination. The radioactivity in the water phase of AW test systems decreased from 100.0% of AR at day 0 to 80.1% at DAT-7 and varied between 75.0% (DAT-79) and 83.8% (DAT-33) afterwards. Extractable sediment ^{14}C residues in test systems from HW increased from 2.8% of at day 0 to 27.7% of AR at DAT-61 and declined to 24.1% towards the end of the study. Extractable ^{14}C residues in the sediment from AW accounted for 1.5% of at day 0 and varied between 16.2% (DAT-7) and 17.2% of AR (DAT-79) afterwards. The maximum of non-extractable ^{14}C residues (NER) in the sediment were 15.8% and 6.5% of AR for test systems HW and AW at DAT-61, respectively. The maximum of $^{14}CO_2$ in the test systems from HW and AW were 25.1% and 7.5% of AR, respectively.

The majority of total $^{14}CO_2$ accounted for volatile $^{14}CO_2$ trapped by the soda lime. A very minor portion included in those values was $^{14}CO_2$ present dissolved in the water phases (all sampling dates) and sediments (only DAT-99). Organic volatile compounds were not detected in significant amounts (< 0.1% of the applied radioactivity in all test systems).

In the water phase of HW, BYI 02960-DFA decreased from 93.8% of AR at day 0 to 32.3% at study termination. In the water of AW, BYI 02960-DFA decreased from 95.4% of AR at day 0 to 78.3% at DAT-7 and varied between 72.3% (DAT-79) and 81.1% (DAT-33) afterwards. In the sediment phase of HW, BYI 02960-DFA increased from 2.8% of AR at day 0 to a maximum of 25.2% at DAT-79 and then declined 22.7% towards the end of the study. In the sediment phase of AW, BYI 02960-DFA accounted for 1.5% of AR at day 0 and varied between 13.3% (DAT-33) and 16.5% (DAT-79) afterwards. Just minor transformation products were observed in the water and sediment phase of both test systems.
The dissipation of DFA from the supernatant water phase was characterized by translocation into the sediment and by degradation. This was best described using the DFOP kinetic model with DT_{50} values of 54.2 and 583.9 days for Hönniger Weiher and Angler Weiher, respectively. The corresponding modelling endpoints were either determined using the DFOP kinetic model (Hönniger Weiher), resulting in a DT_{50} value of 75.3 days for the slow degradation compartment, or the SFO kinetic model (Angler Weiher) with a DT_{50} value of 371.5 days. In the entire water/sediment systems, -DFA was degraded slowly which was best described using the DFOP kinetic model. The estimated DT_{50} values were 106.5 and 967.1 days for test systems from Hönniger Weiher and Angler Weiher. The corresponding modelling endpoints were determined using the SFO kinetic model with estimated DT_{50} values of 109.0 and 567.2 days for test systems from Hönniger Weiher and Angler Weiher,.

Materials and Methods

Materials

1. Test Item: DFA: CAS No: 2218-52-2 (sodium salt), 381-73-7 (free acid), [1-^{14}C] BYI 02960-DFA, sample ID: KATH 6450; Specific activity = 2.84 MBq/mg (76.68 µCi/mg); Radiochemical purity: >98% (acc. Radio-HPLC –RA); Chemical purity: >99.5% (HPLC); [1-^{14}C-] BYI 02960-DFA was identified via NMR, HPLC-MS and HPLC-MS/MS in stock solution, and via NMR and HPLC-MS with accurate mass detection in application solution

2. Test System: The water/ sediment systems were taken from Hönniger Weiher (HW), near Wipperfürth, Germany and Angler Weiher (AW), Leverkusen, Germany. Hoenniger Weiher is an artificially dammed pond in the course of the Hoenniger Creek forming Hoenniger Weiher. Due to its inlet and outlet the pond (about 1000 m² in surface area) has strong water current. Anglerweiher is a reclaimed gravel pit, which is used for fishing only. The chosen systems are well characterized. Physical-chemical characteristics of the water/sediment systems are summarized in Table B.8.4.3.2-28. Water and sediment samples were taken separately and poured into plastic containers. The

collected sediments were sieved down to 2 mm mesh-size to remove parts of e.g. plants and stones. The collected water phases were filtered through a 0.06 mm sieve.

Table B.8.4.3.2-28 Physico-chemical Characteristics of Water and Sediment

Parameter	Hönniger Weiher (HW)	Angler Weiher (AW)
Geographic Location	close to Wipperfürth North Rhine-Westphalia Germany	Leverkusen North Rhine-Westphalia Germany
	51°08.217 latitude North, 7°27.129' longitude East Greenwich	51°01.088' latitude North, 7°00.693' longitude East Greenwich
Properties of Water		
Temperature [°C]1	13.1	17.0
pH1	6.9	7.5
Oxygen Concentration (saturation) [mg/L][1]	9.9	10.3
Total Organic Carbon (TOC) [mg/L] [2]	2 / 20 / 4	2 / 5 / 15
Redox Potential Eh [mV][1,4]	462.2	458.8
Properties of Sediment		
Soil Taxonomic Classification (USDA)	Loam	Loamy Sand
Sand (2000 – 50 µm) [%]	49	85
Silt (< 50 – 2 µm) [%]	39	9
Clay (< µm) [%]	12	6
Organic Carbon [%]	3.9	1.4
Organic Matter [%][3]	6.7	2.4
pH[1]	6.6	7.0
pH	5.2 (CaCl2); 5.4 (H2O)	7.0 (CaCl2); 7.2 (H2O)
Temperature [°C][1]	11.3	15.7
Organic Carbon [%][2]	4.4 / 4.5 / 4.3	1.4 / 1.4 / 1.7
Organic Matter [%][3]	7.6 / 7.8 / 7.4	2.4 / 2.4 / 2.9
Sediment Microbial Activity [mg CO_2 /hr/kg sediment (dry wt)][2]	14.17 / 9.58 / 6.67	20.00 / 22.08 / 9.00
Cation Exchange Capacity [meq/100 g]	7.9	7.1
Total Nitrogen [%]	0.3	0.11
Total Phosphorus [ppm]	692	266
Redox Potential Eh [mV][1,4]	244.3	387.2
1 Measurement at day of sampling	2 start of acclimatization / DAT-0 / DAT-99	
3 %organic matter =%organic carbon x 1.724	n.a. = not analyzed	
4 Potential difference between used electrode and H2-electrode at 20°C: 210 [mV] Theoretical potential of used buffer solution for Pt-Ag/AgCl electrode at 25°C: 220 [mV]		

Methods

1. Experimental conditions: The tests were performed using individual static test systems held at aerobic conditions at 19.2 ± 0.1 °C for a maximum period of 99 experimental days. Each vessel was filled with 175 mL of wet sediment and about 520 mL of water, equivalent to approx. 6 cm in height, resulting in a volume ratio of water to sediment of 3:1. After pre-equilibration, aliquots of the application solution were directly applied onto the water surface of each microcosm system. Thereafter, the systems were closed with a solid trap attachment for absorbing volatile compounds from DAT 1 onwards.

The amount of radiolabelled DFA for the treatment of the test systems was based an application rate of the parent compound (400 g/ha, calculated to a water depth of 100 cm). The actual application rate set as 100 % of applied radioactivity (100 % AR) corresponding to 266.78 Bq/500 µL (prior to application), 265.82 Bq/500 µL (during application) and 270.17 Bq/500 µL (after application) in the water/sediment

systems. The material balance was based on the average amount of radioactivity (RA) recovered from these measurements: 13380 Bq or 4.7 µg BYI 02960-DFA per test system.

2. Sampling: Duplicate samples were taken on DAT 0, 7, 19, 33, 61, 79, and 99 days after application for test systems from Hönniger Weiher and Angler Weiher. The water was decanted and centrifuged. The sediment samples were exhaustively extracted with 3 x 80 mL acetonitrile/water (50/50, v/v), followed by one extraction 80 mL acetonitrile/water (70/30, v/v) all at ambient temperature (combination: ambient extract). Afterwards, the sediment was extracted once more using a microwave-accelerated solvent extraction (aggressive organic extract) with 80 mL acetonitrile/water (70/30, v/v) for 10 minutes (temperature approx. 70°C) with magnetic stirring.

Volatile organics and $^{14}CO_2$ were trapped with solid trapping attachments containing soda lime for absorption of $^{14}CO_2$ and polyurethane foam for volatile organic compounds. The extracted sediment phase was air-dried or freeze-dried, homogenized and combusted in an oxidizer in order to determine the non-extractable residues (NER).

3. Description of analytical procedures: The water phase and combined extract of the ambient extraction steps were analyzed by LSC and TLC in order to determine the amounts of the test item and its transformation products. The aggressive extract was only analyzed by LSC since they contained only low amounts of radioactivity. The very minor transformation products detected in the water layers and sediment extracts were characterized according to their R_f-values in TLC-analysis. No identification procedures were needed or performed. Quantification of the microbial activity in the sediments was based on the method of substrate-induced initial respiratory response (SIR).

Determination of Degradation Kinetics

Dissipation rates from the water phase and rates of degradation for the total system were calculated by use of the software KinGui, version 1.1. The kinetic evaluation included the fitting of data with kinetic models SFO, FOMC and DFOP to the experimental data and their assessment according to FOCUS guidance to result in values for comparison with trigger endpoints.

Results

The test conditions outlined in the study protocol were maintained throughout the study.

Table B.8.4.3.2-29 Oxygen Saturation, pH and Redox Potential Measurements of the Aerobic Test Systems throughout the Study Period (HW)

DAT	Sample	Water Layer				Sediment			Buffer solution
		O_2	Redox E_{obs}	Redox E_H	pH	Redox E_{obs}	Redox E_H	pH	Redox E_{obs}
	WU53 H-	[mg/L]	[mV]	[mV]		[mV]	[mV]		[mV]
0*	D0-A	9.0	173	372	7.3	-13	186	6.3	231
	D0-B	9.0	180	379	7.3	49	248	6.9	

7	D7-A	8.9	211.8	414	8.5	31	233	6.7	228*
	D7-B	8.8	212.2	414	8.0	68	270	6.5	
19	D19-A	8.5	214	416	6.8	47	249	6.7	228
	D19-B	8.5	211	413	6.8	43	245	6.7	
33	D33-A	8.5	276	482	5.5	-96	111	6.0	224
	D33-B	8.6	239	445	5.5	-89	117	6.1	
61	D61-A	8.4	209	407	7.2	219	417	6.2	232
	D61-B	8.4	205	403	7.2	227	425	6.2	
79	D79-A	8.5	245	453	4.9	119	327	5.2	222
	D79-B	8.6	239	447	4.9	121	329	5.2	
99	D99-A	8.4	211	412	7.2	196	397	6.5	229
	D99-B	8.4	209	410	7.3	212	413	6.5	
	min	**8.4**	**173**	**372**	**4.9**	**-96**	**111**	**5.2**	**222**
	max	**9.0**	**276**	**482**	**8.5**	**227**	**425**	**6.9**	**232**
	mean	**8.6**	**217**	**419**	**6.7**	**81**	**283**	**6.3**	**228**

* The redox potential of the buffer solution was not determined at DAT-7. Therefore, the mean value of the other measurements as used to calculate the Redox E_h Potentials at this sampling date.

Table B.8.4.3.2-30 Oxygen Saturation, pH and Redox Potential Measurements of the Aerobic Test Systems throughout the Study Period (AW)

DAT	Sample ID	Water Layer				Sediment			Buffer solution
		O_2	Redox E_{obs}	Redox E_H	pH	Redox E_{obs}	Redox E_H	pH	Redox E_{obs}
	WU53 A-	**[mg/L]**	**[mV]**	**[mV]**		**[mV]**	**[mV]**		**[mV]**
0*	D0-A	8.9	248	447	8.2	82	281	7.7	231
	D0-B	8.8	244	443	8.2	89	288	7.7	
7	D7-A	8.7	230.6	433	8.5	153.2	355.2	7.7	228*
	D7-B	9.2	218.6	421	8.5	19.1	221.1	7.6	
19	D19-A	8.3	196	398	8.7	203	405	7.6	228
	D19-B	8.3	197	399	8.7	202	404	7.6	
33	D33-A	8.9	182	388	8.3	-43	163	7.3	224
	D33-B	8.8	191	397	8.4	-42	164	7.4	
61	D61-A	8.7	207	405	8.2	153	351	7.7	232
	D61-B	8.7	202	400	8.3	155	353	7.7	
79	D79-A	8.5	154	362	8.1	226	434	7.9	222
	D79-B	8.5	160	368	8.1	230	438	7.9	
99	D99-A	8.6	202	403	8.4	151	352	7.7	229
	D99-B	8.7	199	400	8.5	159	360	7.7	
	min	**8.3**	**153.6**	**362**	**8.1**	**-43**	**163**	**7.3**	**222**
	max	**9.2**	**248.0**	**447**	**8.7**	**230**	**438**	**7.9**	**232**
	mean	**8.7**	**202.2**	**404**	**8.4**	**124**	**326**	**7.7**	**228**

* The redox potential of the buffer solution was not determined at DAT-7. Therefore, the mean value of the other measurements as used to calculate the Redox E_h Potentials at this sampling date.

Data

A summary of key data on total recovery and the distribution of radioactivity into the various components formed in water and sediment is given for system Hönniger Weiher in Table B.8.4.3.2-31 and in Table B.8.4.3.2-32 for system Angler Weiher.

Mass Balance

During the study, the total recovery of radioactivity in individual test vessels ranged from 97.7 to 105.8% of AR (mean 100.7%, RSD 2.7 %) for HW and from 97.7 to 107.6% of AR (mean 103.0%, RSD 2.9%) for AW. The complete material balance found for all sampling dates demonstrated that no significant portion of radioactivity dissipated from the vessels or was lost during processing (for detailed results see resp. tables).

Volatilization

Formation of $^{14}CO_2$ was observed in all water/sediment systems. At termination of the study, the $^{14}CO_2$ recovery (mean values of duplicates) in systems from HW was 25.1% of the applied radioactivity. In AW test system, $^{14}CO_2$ accounted for max. 7.5% of the applied radioactivity at DAT-79. From these data it can be concluded that DFA is mineralized in water/sediment systems. No significant amounts of organic volatiles were found (< 0.1% of AR).

Residues in Water, Bound and Extractable Residues in Sediment

The radioactivity in the water phase of HW test system decreased steadily from 97.6% of AR at day 0 to 34.6% at study termination. The extractable radioactivity increased from 2.8% of AR at DAT 0 to 27.7% at DAT 61 and declined to 24.1% towards the end of the study. The bound residues were 5.4% of the applied radioactivity at day 0 and slightly increased during the course of the study. The maximum amount of bound residues (15.8% of AR) was detected on DAT 61.

The radioactivity in the water phase of AW test systems decreased to 80.1% of AR at day 7 and varied between 75.0% (DAT 79) and 83.8% (DAT 33) afterwards. Extractable ^{14}C residues in the sediments accounted for 1.5% of AR at day 0 and varied between 16.2% (DAT-7) and 17.2% (DAT-79) afterwards. The amount of bound residues was 4.6% of AR at day 0. During the entire study period the amounts of bound residues varied between 1.0% (DAT 19) and 6.5% (DAT 61). Due to their low amounts (< 20%), the non-extractable residues were not further characterized.

Along with the overall metabolism of BYI 02960-DFA bound residues were formed. Their maximum amounts were comparatively low and accounted for 15.8 and 6.5% of AR for water/sediment systems from Hönniger Weiher and Angler Weiher, respectively.

Transformation of Test Item

The BYI 02960-DFA residues in the entire test systems from HW declined from 96.6% of AR at DAT 0 to 55.0% of AR at study termination. In the entire test systems from AW, the decrease of the test item was less pronounced. There, unchanged test item accounted for 89.7% of AR at the end of the incubation period. The maximum amount of a single minor radioactivity zone in the water phase of both test systems was 2.7% of AR; in the sediment it was max. 3.3% of AR.

Table B.8.4.3.2-31 Biotransformation of DFA in Hönniger Weiher under aerobic conditions, expressed as percentage AR

Compound	Sample	DAT						
		0	7	19	33	61	79	99
DFA	Water Layer	93.8	72.1	64.3	58.3	43.9	35.7	32.3
		± 0.1	± 1.5	± 1.6	± 0.6	± 1.0	± 1.5	± 0.1
	Sediment	2.8	21.4	20.8	21.8	24.8	25.2	22.7
		± 0.2	± 0.6	± 1.0	± 1.4	± 0.1	± 0.1	± 0.6
	Entire System	96.6	93.5	85.0	80.0	68.6	60.9	55.0
		± 0.1	± 0.1	± 0.6	± 0.8	± 1.2	± 1.4	± 0.5

Origin on the TLC plate	Water Layer	0.4	0.2	n.d.	< LOD	< LOD	n.d.	n.d.
		± 0.0	± 0.0					
	Sediment	n.d.	n.d.	< LOD	0.3	0.4	n.d.	0.4
					± 0.0	± 0.1		± 0.1
	Entire System	0.4	0.2	< LOD	0.5	0.5	n.d.	0.4
		± 0.0	± 0.0		± 0.0	± 0.2		± 0.1
Minor metabolite 3	Water Layer	0.5	0.3	0.4	0.2	0.7	1.1	1.5
		± 0.1	± 0.1	± 0.0	± 0.0	± 0.2	± 0.1	± 0.1
	Sediment	n.d.	n.d.	0.3	0.9	0.8	n.d.	n.d.
				± 0.0	± 0.0	± 0.2		
	Entire System	0.5	0.3	0.8	1.1	1.6	1.1	1.5
		± 0.1	± 0.0	± 0.0	± 0.2	± 0.0	± 0.1	± 0.1
Minor metabolite 4	Water Layer	n.d.	n.d.	0.4	n.d.	< LOD	n.d.	n.d.
				± 0.0				
	Sediment	n.d.	n.d.	0.2	0.6	0.4	n.d.	n.d.
				± 0.0	± 0.3	± 0.2		
	Entire System	n.d.	n.d.	0.7	0.6	0.6	n.d.	n.d.
				± 0.1	± 0.3	± 0.2		
Non-characterized Radioactivity	Water Layer	2.9	1.8	1.7	1.8	1.3	0.9	0.7
		± 0.2	± 0.2	± 0.0	± 0.3	± 0.2	± 0.2	± 0.2
	Sediment	n.d.	1.7	2.1	3.3	1.3	1.8	1.0
			± 0.1	± 0.0	± 0.1	± 0.1	± 0.2	± 0.0
	Entire System	2.9	3.6	3.7	5.1	2.6	2.7	1.8
		± 0.2	± 0.1	± 0.0	± 0.2	± 0.1	± 0.0	± 0.1
Total Extractable Residues	Water Layer	97.6	74.4	66.8	60.5	46.1	37.7	34.6
		± 0.1	± 1.1	± 1.7	± 0.7	± 1.3	± 1.2	± 0.2
	Sediment	2.8	23.1	23.5	26.9	27.7	27.1	24.1
		± 0.2	± 0.5	± 1.0	± 1.8	± 0.1	± 0.1	± 0.5
	Entire System	100.4	97.6	90.3	87.4	73.9	64.7	58.7
		± 0.3	± 0.6	± 0.6	± 1.1	± 1.3	± 1.4	± 0.7
$^{14}CO_2$	Entire System	n.a.	1.2	3.7	6.3	14.5	21.1	25.1
			± 0.0	± 0.3	± 0.2	± 0.2	± 1.0	± 0.1
Organic Volatiles	Entire System	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
			± 0.0	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0
Non-Extractable Residues	Sediment	5.4	1.8	3.7	6.8	15.8	13.2	14.0
		± 0.1	± 0.1	± 0.2	± 0.8	± 11.7	± 0.5	± 0.1
Total Recovery *	Water Layer	97.6	74.4	66.8	60.5	46.1	37.7	34.6
		± 0.1	± 1.1	± 1.7	± 0.7	± 1.3	± 1.2	± 0.2
	Sediment	8.2	24.9	27.3	33.7	55.3	40.3	38.2
		± 0.3	± 0.5	± 1.2	± 2.6	± 11.6	± 0.3	± 0.4
	Entire System	105.8	100.5	97.7	100.5	103.1	99.0	97.9
		± 0.5	± 0.5	± 0.2	± 2.1	± 12.8	± 0.1	± 0.5

DAT: day after treatment, SD: standard deviation, < LOD = Peak contained less than 0.22 Bq
* Values taken from Material Balance, ± = SD = standard deviation

Table B.8.4.3.2-32 Biotransformation of DFA in Angler Weiher under aerobic conditions, expressed as percentage AR

Compound	Source	DAT						
		0	7	19	33	61	79	99
DFA	Water Layer	95.4	78.3	79.0	81.1	75.9	72.3	74.8
		± 0.3	± 1.2	± 0.7	± 2.1	± 1.2	± 0.6	± 1.7
	Sediment	1.5	15.1	14.3	13.3	15.3	16.5	14.9
		± 0.0	± 0.3	± 1.7	± 1.1	± 0.4	± 0.2	± 1.9
	Entire System	97.0	93.3	93.3	94.5	91.2	88.8	89.7
		± 0.3	± 1.5	± 1.1	± 1.1	± 0.8	± 0.8	± 0.2
Origin on the TLC plate	Water Layer	0.5	n.d.	n.d.	0.3	< LOD	n.d.	1.2
		± 0.0			± 0.1			± 0.0
	Sediment	n.d.	< LOD	n.d.	< LOD	< LOD	n.d.	0.2
								± 0.0
	Entire System	0.5	< LOD	n.d.	0.5	< LOD	n.d.	1.4
		± 0.0			± 0.1			± 1.2
Minor metabolite 3	Water Layer	1.3	0.2	1.0	0.8	0.9	1.2	1.4
		± 0.4	± 0.0	± 0.1	± 0.1	± 0.2	± 0.3	± 0.1

	Sediment	n.d.	< LOD	0.5	0.6	0.7	< LOD	< LOD
				± 0.2	± 0.1	± 0.2		
	Entire System	1.3	0.4	1.4	1.3	1.7	1.4	1.5
		± 0.4	± 0.2	± 0.2	± 0.0	± 0.1	± 0.3	± 0.1
Minor metabolite 4	Water Layer	n.d.	n.d.	0.4	n.d.	0.7	n.d.	n.d.
				± 0.0		± 0.0		
	Sediment	n.d.	< LOD	0.3	0.2	0.2	n.d.	< LOD
				± 0.1	± 0.0	± 0.1		
	Entire System	n.d.	< LOD	0.7	0.2	0.9	n.d.	< LOD
				± 0.3	± 0.0	± 0.1		
Non-characterized Radioactivity	Water Layer	2.7	1.6	2.1	1.6	1.2	1.5	1.3
		± 0.4	± 0.3	± 0.2	± 0.3	± 0.1	± 0.7	± 0.1
	Sediment	n.d.	0.8	1.8	1.9	0.8	0.6	0.5
			± 0.0	± 0.5	± 0.2	± 0.3	± 0.1	± 0.0
	Entire System	2.7	2.4	3.9	3.5	2.0	2.1	1.8
		± 0.4	± 0.3	± 0.4	± 0.1	± 0.2	± 0.8	± 0.1
$^{14}CO_2$	Entire System	n.a.	0.3	1.4	1.3	4.1	7.5	5.6
			± 0.0	± 0.1	± 0.1	± 0.0	± 0.4	± 1.0
Organic Volatiles	Entire System	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
			± 0.0	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0
Non-Extractable Residues	Sediment	4.6	1.1	1.0	1.7	6.5	2.7	2.1
		± 0.0	± 0.1	± 0.1	± 0.0	± 24.5	± 0.2	± 0.2
Total Recovery	Water Layer	100.0	80.1	82.4	83.8	78.8	75.0	78.7
		± 0.3	± 1.3	± 0.2	± 2.6	± 1.4	± 0.4	± 2.8
	Sediment	6.1	17.3	17.8	17.9	48.1	19.9	18.0
		± 0.0	± 0.3	± 1.0	± 1.5	± 24.7	± 0.1	± 1.9
	Entire System	106.1	97.7	101.6	103.1	107.6	102.4	102.3
		± 0.3	± 1.5	± 0.7	± 1.0	± 23.4	± 0.0	± 0.2

DAT: day after treatment, SD: standard deviation, < LOD = Peak contained less than 0.22 Bq

Remark: Due to the low amounts of radioactivity present in the aggressive organic extracts, only the ambient organic extracts were subjected to TLC-analysis. The amounts of DFA and its metabolites in the sediment were calculated for the sum of ambient and aggressive organic extracts, assuming that the distribution of radioactivity in the aggressive extracts is equal to the distribution in the ambient extracts.

Dissipation Kinetics

The dissipation of BYI 02960-DFA from the supernatant water phase was characterized by translocation into the sediment and by degradation. This was best described using the DFOP kinetic model with DT_{50} values of 54.2 and 583.9 days for Hönniger Weiher and Angler Weiher, respectively (see Table B.8.4.3.2-33). The corresponding modelling endpoints were either determined using the DFOP kinetic model (Hönniger Weiher), resulting in a DT_{50} value of 75.3 days for the slow degradation compartment, or the SFO kinetic model (Angler Weiher) with a DT_{50} value of 371.5 days (see Table B.8.4.3.2-34).

In the entire water/sediment systems, BYI 02960-DFA was degraded slowly which was best described using the DFOP kinetic model. The estimated DT_{50} values were 106.5 and 967.1 days for test systems from Hönniger Weiher and Angler Weiher, respectively (see Table B.8.4.3.2-33). The corresponding modelling endpoints were determined using the SFO kinetic model with estimated DT_{50} values of 109.0

and 567.2 days for test systems from Hönniger Weiher and Angler Weiher, respectively (see Table B.8.4.3.2-34).

Table B.8.4.3.2-33 Kinetics evaluation (trigger and modelling values according to FOCUS) of the dissipation of DFA

Supernatant Water of Test System	Kinetic Model	DT_{50} [days]	DT_{90} [days]	Chi^2 Error [%]
Hönninger Weiher	SFO	63.2	210.1	6.6
	FOMC	50.7	> 1000	4.7
	DFOP	**54.2**	**229.9**	**1.6**
Angler Weiher	SFO	371.5	> 1000	4.8
	FOMC	> 1000	> 1000	2.2
	DFOP	**583.9**	**> 1000**	**2**
Entire Test System				
Hönninger Weiher	SFO	109.0	362.2	2.9
	FOMC	125.0	> 1000	2.1
	DFOP	**106.5**	**397.8**	**0.6**
Angler Weiher	SFO	567.2	> 1000	3.0
	FOMC	> 1000	> 1000	1.2
	DFOP	**967.1**	**> 1000**	**0.9**

Bold: best fit
Underlined: modelling endpoint

Conclusions

The dissipation of DFA from the supernatant water phase was characterized by translocation into the sediment and by slowly degradation. With the exception of mineralization to carbon dioxide and formation of NER no major metabolite was found. An overall result synopsis is shown by Table B.8.4.3.2-34.

Table B.8.4.3.2-34 Results Synopsis

Parameter	Hönniger Weiher	Angler Weiher
Material Balance [% AR]	97.7 – 105.8	97.7 – 107.6
Water Phase [% AR]	34.6 – 97.6	75.0 -100.0
Sediment Extract [% AR]	2.8 – 27.7	1.5 – 17.2
$^{14}CO_2$ [max. %]	25.1	7.5
Major transformation products * [max. %]	NER: 15.8	NER: 6.5
Kinetics Evaluation, Trigger Values		
Supernatant water	54.2	583.9
Entire system	106.5	967.1
Kinetics Evaluation, Modelling Endpoints		
Supernatant water	75.3#	371.5
Entire system (SFO)	109.0	567.2

* Criteria for "major": ->10% of AR; >5% of AR at two successive DAT, increasing towards study end
# DT50 value of the slow degradation phase as a worst case scenario for risk assessment

Remarks

The RMS considers the water/sediment study acceptable. Th modelling endpoints for water phase are not used for exposure modelling since they are not level PII true degradation values. Level PI whole system values are considered acceptable for use in modelling.

STUDY IIA, 7.8.3/04

Characteristics

reference	:	Burns, E., 2012	incubation time	:	148 days
year of execution	:	2010	nominal concentration	:	10 ang 100 µg/L
GLP statement	:	yes	temperature	:	ambient
guideline	:	OECD Guidance Document "Simulated Freshwater Lentic Field Tests (Outdoor Microcosms and Mesocosms)", April 2006 OECD 308, but only in part	DT_{50}	:	see results
test substance	:	BYI 02960 (tech.)	metabolites	:	see results
purity	:	96.2%	acceptability	:	**acceptable**
test system	:	see materials			

Summary

The fate of BYI 02960 (tech.) was determined in pond water and sediment in outdoor microcosms as an aquatic model ecosystem for lentic aquatic freshwater systems with different trophic levels. For this purpose, 4 microcosms were treated with two different concentrations of the test item. During the consecutive months samples of water and sediment were taken and the content of the test item in these compartments was analyzed. Additionally several parameters of water and sediment were monitored to characterize the system.

In November 2009 a pond with the shape of a stub pyramid (bottom 53 m^2, surface 80 m^2) was filled with a layer of natural sediment (0.15 m height) and water (0.7 m height). The walls and bottom of the pond is made of plastic foil. The sediment originated from a drinking water reservoir system, the water was composed of local ground water and water from a natural pond. Twenty-five enclosures (= microcosms) made of polycarbonate (diameter 0.97 m. height 0.9 m) were inserted into the pond on May 4, 2010 (= about 4 weeks before application). Overall, the microcosms are representative for small stagnant water bodies.

The test substance BYI 02960 (tech.) (Batch ID: 2009-000239) was applied once on June 02, 2010 onto the water surface of four microcosms. Two treatment levels, 10 and 100 µg a.i./L, were tested with two replicates each:. Two microcosms were kept untreated as controls. The microcosms were investigated for a period of 148 days after the treatment. Several times during the study period water and sediment samples were taken and analyzed to demonstrate the initial test concentrations and the fate of the test substance in these compartments. The physic-chemical water parameters were also evaluated. Furthermore the abundance of filamentous algae und the turbidity of the water was assessed.

The results of initial concentrations demonstrated that the nominal concentrations had been applied. A steady decline of BYI 02960 in the water phase was shown. At the end of the study (= 5 months after application) the a.i. concentration was 22% of applied amount at the test concentration of 10 µg a.s./L, and 40% of applied amount at 100 µg a.s./L concentration level. The analyzed concentrations of BYI 02960 in the sediment did not show a clear trend during the entire study period, although a slight

increase of BYI 02960 concentrations was observed towards the end of the study. For the 10 µg a.s./L-level, 2.3 to 7.5% of applied amount was found in the sediment; for the 100 µg a.s./L-level, 1.9 to 11.4 % of the applied amount were detected in the sediment.

The half-life calculations for BYI 02960 in the microcosm water using SFO kinetics model resulted on average (arithmetic mean) for both concentration level in 80.6 days, and in the entire system (water + sediment) in 95.1 days. A calculation for the fate in sediment was not given, since the fate of BYI 02960 in this matrix did not show a clear pattern of decline.

Materials and Methods

Materials

1. Test Item: BYI 02960 (tech.); Origin Batch No. 2009-000239; Batch code BYI 02960-01-03; Specification No. 102000022313); Analyzed a.i. content: 96.2% w/w;

Application solution: 6.34 g of test item was dissolved in 50 mL DMF, which was suspended in pure water to get nominal 10 and 100 µg a.s./L concentration level after treatment.

2. Test System: In Nov. 2009 a pond with the shape of a stub pyramid (bottom 56 m^2, surface 80 m^2) was filled with a layer of natural sediment (0.15 m height) and water (0.7 m height). The walls and bottom of the pond consist of a plastic foil. The sediment originated from “Hönniger Weiher”, part of the water reservoir system “Wuppertalsperre (Wupperverband)”, 50 km distant from the testing facility. The sediment was taken in November 2009 by means of an excavator, transported in containers to Monheim am Rhein and uniformly distributed among the pond bottom thereafter. The water was composed of local ground water and water from a natural pond.

Twenty-five cylindrical enclosures (= microcosms) made of polycarbonate (diameter 0.97 m. height 0.9 m, previous to light) were inserted into the pond on May 4, 2010 (= about 4 weeks before application). Six of these microcosms) were used for the present fate-o-cosm. To ensure conditions as close to nature as possible, the tanks are not roofed and are exposed to outdoor weather conditions. Overall, the microcosms are representative for small stagnant water bodies.

Methods

3. Experimental conditions: On day 0 an aqueous suspension of the test substance was applied using a pipette and a small glass plate. The glass plate was held onto the water surface. The application suspension was transferred onto the glass plate using a glass pipette. The application suspension did run via the glass plate into the microcosms simulating a run off scenario. The mean water height in the microcosms on day 0 was 0.66 m corresponding to 488 L water / microcosm.

Two treatment levels were tested, with two replications each: 10 and 100 µg a.i./L. Two microcosms were kept untreated as controls. The microcosms were investigated for a period of 148 days after the treatment. Several times during the study period water and sediment samples were taken and analyzed to demonstrate the initial test concentrations and the fate of the test substance in these compartments. The physic-chemical water parameters were also evaluated and reported. Furthermore the abundance of filamentous algae und the turbidity of the water was assessed.

At least once per week the oxygen content, temperature, pH and conductivity were measured in the centre of the water column. The measurements were made directly in the pond water with electronic measuring instruments. Several times during the study ammonium, nitrite, nitrate, total nitrogen, total phosphate, ortho-phosphate, carbonate hardness, sum of alkaline earth's (=total hardness) were determined. Carbonate hardness and sum of alkaline earth were measured by means of commercial tests. Ammonium, nitrite, nitrate, total nitrogen total phosphate and ortho-phosphate were determined by means of commercial photometric tests. Redox potential in water was determined monthly during the study. Total organic carbon (TOC) in water and dissolved organic carbon (DOC) in water were determined at study start and on days 84 and 148.

Parameters at day 0 were determined in non-treated sediment outside of the microcosms. At day 84 and 148 (=study end), the parameters were determined in the sediment of the control microcosms (mixed sample of both microcosms). The Redox potential was determined directly in the sediment of both control microcosms. At the start of the study, on day 84 and at the end of the study the following parameters of the sediment were determined: Total organic carbon and microbial biomass. The redox potential was measured monthly parallel to the water. This measurement was performed according to common guidelines. The characteristics of test system are summarized in Table B.8.4.3.2-35

Table B.8.4.3.2-35 Characteristics of test system

Redox potential TOC and DOC measured in the test water					
Date 2010	Study day	Redox potential		TOC (mg/L)	DOC(mg/L)
		Ct/1	Ct/2		
02.06.	0	233	175	9	9
30.06.	28	121	125		
27.07.	55	178	176		
25.08.	84	202	197	13	12
30.09	120	200	201		
28.10	148	209	212	11	10

Water levels in the microcosms (cm) around the time of sampling dates							
Date	Study	Control		10 µg a.i./L		100 µg a.i./L	
2010	Day	(1)	(2)	(1)	(2)	(1)	(2)
03.06	1	65	64	64	66	68.5	66
09.06	7	63	61.3	58.5	63.8	64	63
16.06.	14	61	58.5	53	61.5	59.5	60
01.07.	29*)	56.5	53	48	55	54	57
28.07.	56	61	58.5	53	61.5	58	60
25.08.	84	65	63	59	65.5	65.5	67.5
29.09.	119	69	68	64.5	68.5	69	68.5
28.10.	148	69	67	65	69	68	69
*) water level before refill with water							
Characterization of the sediment							

Particle size distribution	56 % sand	36 % Silt	8% Clay	= sandy loam[USDA]

Date 2010	Study day	Microbial biomass (mg CO_2/hr/kg soil DW	Redox potential		TOC (gew.-%)
			Ct/1	Ct/2	
02.06.	0	30.0	- 103	- 96	5.3
30.06.	28		- 148	- 142	
27.07.	55		- 124	- 131	
25.08.	84	29.6	- 100	- 109	5.6
30.09	120		- 118.3	- 85.7	
28.10	148	37.8	- 31.3	-24.6	4.8

Climatic data (recorded at the nearby weather station of the Bayer CropScience AG)

Month (2010)	Temperature (mean °C)	Precipitation (mm)	Sunshine duration (hrs)	Insolation (kWh/m2)
June	19.3	19.1	197	181
July	22.9	111	211	175
August	19.4	233	136	114
September	14.6	93.1	131	152
October	10.8	43.1	118	101

4. Sampling: Samples were taken on 0, 1, 7, 14, 28, 56, 84, 120 and 148 days after application.
Mixed water samples out of the entire water column were taken for analysis to investigate the fate of the substance in water during the study period. For this purpose the water samples were obtained with a flask attached to a metal rod. The flask (1.0 L glass bottle) was moved around in the microcosm during filling to obtain water from different sites. 6 x 20 mL of the water samples were filled into 50 mL amber glass bottles and deep frozen at < - 18 °C until analysis.

Sediment samples were taken by means of a corer (inner diameter of 5.0 cm) at two to three sampling points each sampling time. The sample points were noted to avoid multiple sampling at one sampling point. Water covering the sediment was decanted carefully and the sampled sediment-column was filled into a glass-beaker with the same dimensions as the core. As it can be assumed that the test substance primarily adsorbed to the upper layer of the sediment, the upper 2 to 3 cm of the sediment samples were mixed for analyses (= ca. 170 g wet weight). The resulting sediment samples were frozen and stored at < -18°C until analysis. Sediment samples of the control microcosms were also taken at each time.

5. Description of analytical procedures: The water samples were analysed according to Method 01182: Method 01182 for the determination of BYI 02960 in test water from aquatic toxicity tests by HPLC-MS/MS. In the method 01182 the linearity of the detector was checked for BYI 02960 in the range 0.05 to 11 µg/L with an injection volume of 10 µL. The correlation coefficient was 0.9993.

In the present study the method was validated concurrently with the sample analyses of the study by evaluation of the standard injections. To the samples from day 84 until 148 a volume of 1 mL acetonitrile was added. Before measurement 0.1 mL formic acid was added to the samples. The water samples were directly injected into the HPLC-MS/MS instrument. The injection volume was 10 µL. Each sample was injected in duplicate. The limit of quantification (LOQ) of BYI 02960 in the pond water was 1.141 µg a.i./L.

Sediment samples of 20 g were extracted with 40 mL of acetonitrile/water (1/4; v/v) for 3 minutes in a microwave at 250 W. An aliquot of 1.5 mL was taken and centrifuged for 5 minutes at > 12000 g to remove fine particles of the sediment. The supernatant was taken and injected into the HPLC-MS/MS system. Two replicates (A + B) of 20 g each were analysed from each sample. The mean value of both replicates is reported. The sediment samples were analysed according to the following method: Analytical Method 01074 for the Determination of BYI 02960 in Soil using LC/MS/MS. Additional recovery experiments for sediment at a LOQ level of 1 µg/kg were conducted. The limit of quantification (LOQ) was 5.0 µg BYI 02960/kg dry weight of sediment.

Determination of Degradation Kinetics

The dissipation kinetics of BYI 02960 in the microcosm ponds was evaluated based on a simple first order, single compartment model. An exponential decline curve was fitted to the experimental data, and the rate constant k, as well as the characteristic dissipation times DT50 (dissipation time of 50% of the test item) was derived from this calculation. The decline curves and regression analyses for BYI 02960 were calculated with the Single First Order mathematical model (SFO). The used program was KinGUI Version 2. Model input data sets were the residual concentrations of BYI 02960 in water found in each pond at each sampling interval (displayed as % of nominal content). The total residues in each pond were calculated based on the measured residues in water plus sediment (displayed as mg a.i./microcosm). All data points were weighted equally.

Results

The analyzed concentrations of the application solutions indicated that 118 and 112% of nominal test concentration were applied into the pond water in case of the two treatment levels of 10 and 100 µg a.i./L.

Water Layer

The analyzed concentrations of the mixed samples are shown in Table B.8.4.3.2-36 and in Table B.8.4.3.2-37 as percentage of nominal application. For the calculation of percentage values the actual water volume in the microcosms at the time of sampling was taken into consideration.

Table B.8.4.3.2-36 Results of analysis in water [as µg BYI 02960/L], average of two measurements

Date 2010	Study Day	Control		10 µg a.i./L		100 µg a.i./L	
		(1)	(2)	(1)	(2)	(1)	(2)
03.06	1	<LOQ	<LOQ	10.3	10.0	105	100
09.06	7	nd	nd	9.15	8.28	106	104
16.06.	14	nd	nd	9.61	8.38	98.9	87.9
30.06.	28	nd	nd	7.73	7.18	89.5	88.9
28.07.	56	nd	nd	5.85	5.09	69.7	68.2
25.08.	84	nd	nd	4.30	3.77	58.0	53.9
30.09.	119	nd	nd	2.63	2.55	44.1	42.3
28.10.	148	nd	nd	2.13	2.15	40.0	37.2

nd = not determined; (1)= replicate 1, (2) = replicate 2

Table B.8.4.3.2-37 Results of analysis in water [as % of nominal BYI 02960], average of two measurements

Date 2010	Study Day	Control (1)	Control (2)	10 µg a.i./L (1)	10 µg a.i./L (2)	100 µg a.i./L (1)	100 µg a.i./L (2)
03.06	1	<LOQ	<LOQ	99.8	99.9	108.9	99.9
09.06	7	nd	nd	81.1	79.9	102.7	99.2
16.06.	14	nd	nd	77.1	78.0	89.1	79.9
30.06.	28	nd	nd	56.2	59.8	73.2	76.7
28.07.	56	nd	nd	47.0	47.4	61.2	62.0
25.08.	84	nd	nd	38.4	37.4	57.5	55.1
30.09.	119	nd	nd	25.7	26.5	46.0	43.9
28.10.	148	nd	nd	21.0	22.5	41.2	38.9

nd = not determined; (1)= replicate 1, (2) = replicate 2

Sediment

The results of the analysis of BYI 02960 in sediment are summarized in Table B.8.4.3.2-38. The samples for analysis were taken from the uppermost 2-3 cm layer. The total dry weight of this sediment layer was around 7.13 kg per microcosm. The analyzed concentrations were multiplied by this value to calculate the % of the initial applied.

Table B.8.4.3.2-38 Results of analysis of BYI 02960 in sediment average of two measurements

Date 2010	Study Day	Control (1)	Control (2)	10 µg a.i./L (1) µg BYI 02960 /kg dry weight		10 µg a.i./L (2) % of applied BYI 02960		100 µg a.i./L (1) µg BYI 02960 /kg dry weight		100 µg a.i./L (2) % of applied BYI 02960	
03.06	1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
09.06	7	<LOQ	<LOQ	36.3	30.3	5.3	4.4	128	127	1.9	1.9
16.06	14	<LOQ	<LOQ	38.8	30.1	5.7	4.4	140	247	2.0	3.6
30.06	28	<LOQ	<LOQ	34.6	15.9	5.1	2.3	252	415	3.7	6.1
28.07	56	<LOQ	<LOQ	26.4	46.3	3.9	6.8	258	152	3.8	2.2
25.08	84	<LOQ	<LOQ	29.6	23.8	4.3	3.5	152	260	2.2	3.8
30.09	119	<LOQ	<LOQ	47.0	45.0	6.9	6.6	142	185	2.1	2.7
28.10	148	<LOQ	<LOQ	47.3	51.4	6.9	7.5	781	701	11.4	10.2

nd = not determined; (1)= replicate 1, (2) = replicate 2

Dissipation Kinetics

The dissipation of BYI 02960 from the supernatant water phase was characterized by translocation into the sediment and by degradation. The half-life calculations for BYI 02960 in the microcosm water and the total system (water + sediment) are displayed in Table B.8.4.3.2-39. A calculation for the decline in sediment was not possible, since the fate of BYI 02960 in this matrix did not show a continuous pattern.

Table B.8.4.3.2-39 SFO DT_{50}- values calculated for BYI 02960 in water and the entire test system

Nominal test concentration (µg a.i./L)	DT_{50} – Water (days)	DT_{50} – Total System (water+sediment)

		(days)
10	62.1	74.1
100	99.1	116
Arithmetic mean	**80.6**	**95.1**
Geometric mean	**78.4**	**92.7**

Conclusion

BYI 02960 dissipated from the supernatant water phase with a mean DT_{50} of 81 days due to translocation into the sediment and by degradation. The overall degradation (mean of 95 days) was faster under the prevailing outdoor conditions in comparison to the laboratory water sediment studies. Considering that BYI 02960 is rapidly degraded by photolysis there may be an enhanced degradation effect of sunlight under the outdoor test conditions.

Remarks

- The calculated half-lives for water and total system are not influenced by the use of the intended nominal concentrations instead of the presumably dosed nominal concentrations.
- The largest part of pesticide mass is expected to reside in the upper 1 or 2 cm sediment (see the results of Crum et al. (1997)) for linuron (K_{oc} of around 700 L/kg, so same order of magnitude as BYI 02960's value of around 50 L/kg) in 0.5 m deep ditches with high organic matter contents in the upper 2 cm; (see also Adriaanse et al. (2012)) for prosulfocarb (K_{oc} of around 1700 L/kg). Thus, dividing the pesticide mass by the sediment mass of a 2 cm depth results in another concentration than dividing by the sediment mass of a 3 cm depth. So, the variability is (at least partly) due to the calculation procedure and calculated concentrations (and thus also percentages) do not necessarily reflect the real penetration into sediment. It is therefore more logic to calculate the mass of BYI 02960 per unit area of sediment surface (i.e. using the cross sectional area of the core): in that way the concentration does not depend on the sampling depth. The errorneous procedure to calculate concentration and mass in the sediment has a negligible influence on the calculated half-lives, because few mass (max 11.4% of total applied) has penetrated into the sediment.
- It is unknown what the realised initial concentrations in the enclosures were because, these were not measured. Measuring the concentration in the application solution does not guarantee that the realised initial concentrations in the enclosures were equal to the intended or presumably dosed nominal concentrations. In this study the realised initial concentration might for instance be lower in case the glass plate used for applying BYI 02960 was not properly rinsed and so realised nominal concentrations might differ per enclosure. The calculation error in mass presumably dosed in the application solution results in higher initial concentrations than the ones reported. From day 1 concentrations have been measured (and so, they are assumed to reflect reality), so presumably dosed mass is not relevant anymore. By using the reported lower initial concentrations calculated dissipation half-lives are conservative.
- BYI 02960 dissipates considerably faster in the enclosure with the 10 µg a.s./L treatment level

than in the enclosure with the 100 µg a.s./L treatment level. The RMS is unable to explain this and recommends not to use an average value, but to maintain the two separate values. Next, the RMS recommends to select the half-life of the treatment level that is closest to the exposure concentration level in the exposure scenarios used in the registration procedure.

- For use in registration the DT_{50} has to be calculated back to 20°C with the Arrhenius equation. In this study the water temperature varied between 16 and 27°C during the first 3 to 4 months after application, i.e. temperatures seem somewhat higher than 20°C, so the presented half-lives may be slightly too short (i.e. not conservative).
- BYI 02960's dissipation and degradation half-life represents the half-life as a 'bulk' process, it does not yield explanatory, quantitative dissipation and degradation process descriptions. This implies that the half-lives found are system specific and cannot be generalised to other small outdoor waters across the EU. For use in registration the RMS recommends to study dissipation of BYI 02960 in outdoor surface waters under various conditions. Current practice for soil are degradation studies in 4 soils, of which the geometric mean degradation rate is determined. The geometric mean value is used for registration purposes. In analogy, we recommend to calculate a geometric mean DT_{50} of more than one system, representing small surface waters, e.g. two to four. The geometric mean DT_{50} should be calculated from DT_{50} values at 20°C.

Concluding: The study can be used for risk assessment but only in case i) the half-lives derived are calculated back to 20 °C (as well as possible, the weekly temperature measurements should preferably be supplemented, e.g. with temperature measurements nearby and correlating these to the experiment, because DT_{50} is a function of temperature and ii) as one of the 2-4 systems providing DT_{50} values to be used for calculating a geometric mean DT_{50}. The DT_{50} values of this study are system specific and cannot be generalised to other small outdoor waters across the EU.

B.8.4.4 Summary and assessment

Hydrolysis

BYI 02960 proved to be stable under sterile hydrolytically conditions at all pHs.

Photo degradation in water

Sterile buffer

[^{14}C] BYI 02960 photolytically degraded in sterile buffer (pH 7) with a half-life of 13.8 experimental hours. Based on this finding, BYI 02960 should degrade within a few days in the aqueous environment if exposed to sunlight. The major degradates (>10% of applied radioactivity) were identified as [^{14}C] BYI 02960-succinamide and [^{14}C] BYI 02960-azabicyclosuccinamide. The half-life was calculated to be equivalent to 2.7 days calculated for Athens, Greece and 3.4 days calculated for Lodon, U.K..

Natural waters

BYI 02960 photolytically degraded in sterile natural water with a half-life of 14.0 experimental hours. The major degradates (>10% of applied radioactivity) were identified as BYI 02960-succinamide and BYI 02960-azabicyclosuccinamide. A minor degradate was identified as BYI 02960-deschlorohydroxy-succinamide. In the dark controls BYI 02960 was stable. Based on the experimental half-life of 14.0 hours, the half-life of BYI 02960 under environmental conditions in Tokyo, Japan was calculated to be 3.8 days. The phototransformation study in natural water is regarded more relevant for the aqueous environment as the pure and sterile buffer study.

Ready biodegradability

A respective study was not performed. The parent compound BYI 02960 is considered to be “Not Readily Biodegradable.

Water/sediment studies

Five different water/sediment studies were submitted in the dossier. An anerobic water sediment study, two aerobic water sediment studies (one with one label and one with two labels). The fourth water sediment study was with the metabolite DFA. The final study was an outdoor microcosm study.

Anaerobic conditions

BYI 02960 dissipated from aqueous phase to sediment with a half-life of 7.2 and 9.6 days for Lawrence and Pikeville test system, respectively. With respect to the entire test system, BYI 02960 is regarded stable under anaerobic condition, no major metabolite were formed.

Aerobic conditions

[PYR] BYI 02960 is microbially degraded in aerobic water/sediment systems. The DT_{50} values for BYI 02960 in the entire water/sediment systems were 193.1 and 246.9 days for Hönniger Weiher and Angler Weiher, respectively. The only major transformation products were to carbon dioxide and formation of NER .

[FUR] and [ETH] BYI 02960 is translocated from the water phase into the sediment where it is slowly degraded and mineralized. Major transformation products were mineralization to carbon dioxide, formation of NER and one metabolite, DFA, (max. 6.9% of AR). The DT_{50} values for BYI 02960 in the entire water/sediment systems were 208.2 [FUR], 202.4 [ETH] and 246.1 [FUR], 285.0 [ETH] days for Hönniger Weiher and Angler Weiher, respectively.

The dissipation of DFA from the supernatant water phase was characterized by translocation into the sediment and by slowly degradation. With the exception of mineralization to carbon dioxide and formation of NER no major metabolite was found. The DT_{50} values for DFA in the entire water/sediment systems were 106.5 (persistence), 109.0 (modelling) and 967.1 (persistence) 567.2 (modelling) days for Hönniger Weiher and Angler Weiher, respectively.

Microcosm study

BYI 02960 dissipated from the supernatant water phase with a mean DT_{50} of 81 days due to translocation into the sediment and by degradation. The overall degradation (mean of 95.1 days) was faster under the prevailing outdoor conditions in comparison to the laboratory water sediment studies. Considering that BYI 02960 is rapidly degraded by photolysis there may be an enhanced degradation effect of sunlight under the outdoor test conditions.

Table B.8.4.4-01 Selected models and endpoints for BYI 02960 (anaerobic dark experiment)

System	kinetic model	chi^2 error	r^2	DT_{50} [days]	DT_{90} [days]
total system					
Lawrence, KS	DFOP	4.1	0.0576	> 1000	> 1000
Pikerville, NC	DFOP	0.66	0.9821	415.0	> 1000
			geometric mean	**664**	**>1000**
water					
Lawrence, KS	DFOP	6.0	0.9867	7.2	> 1000
Pikerville, NC	DFOP	9.0	0.9827	9.6	> 1000

Table B.8.4.4-02 Selected models and endpoints for BYI 02960 (aerobic dark experiment)

System	kinetic model	chi^2 error	r^2	DT_{50} [d]	DT_{90} [d]
total system					
Hönniger Weiher [PYR] label	SFO	1.2	0.9577	193.1	641.3
Angler Weiher [PYR] label	SFO	1.3	0.9721	246.9	820.1
Hönniger Weiher [FUR] label	SFO	1.5	-	208.2	691.6
Angler Weiher [FUR] label	SFO	1.3	-	246.1	817.4
Hönniger Weiher [ETH] label	SFO	1.6	-	202.4	672.2
Angler Weiher [ETH] label	SFO	1.0	-	285.0	946.9
			geometric mean	**228.1**	**757.7**
water					
Hönniger Weiher [PYR] label	FOMC	1.6	0.9888	8.5	174.6
Angler Weiher [PYR] label *	DFOP	4.4	0.9886	34.5	181.8
Hönniger Weiher [FUR] label *	DFOP	4.1	-	48.5	161.0
Angler Weiher [FUR] label *	DFOP	2.9	-	123.8	411.2
Hönniger Weiher [ETH] label *	DFOP	4.9	-	50.2	116.9
Angler Weiher [ETH] label *	DFOP	2.4	-	117.5	390.3

* DT_{50} and DT_{90} value of the slow degradation phase as a worst case scenario for risk assessment

Table B.8.4.4-03 Selected models and endpoints for DFA (aerobic dark experiment)

System	kinetic model	chi^2 error	r^2	DT_{50} [days]	DT_{90} [days]
total system					
Hönniger Weiher	SFO	2.9	0.9692	109.0	362.2
Angler Weiher	SFO	3.0	0.5323	567.2	> 1000
			geometric mean	**248.6**	**601.8**
water					
Hönniger Weiher *	DFOP	1.6	0.9949	75.3	250.3
Angler Weiher	SFO	4.8	0.5088	371.5	> 1000

* DT_{50} and DT_{90} value of the slow degradation phase as a worst case scenario for risk assessment

Table B.8.4.4-04 Selected models and endpoints for BYI 02960 in microcosm systems

System	kinetic model	chi^2 error	r^2	DT_{50} [d]	DT_{90} [d]

System	kinetic model	chi² error	r²	DT50 [d]	DT90 [d]
total system					
10 ug/L microcosm	SFO	-	-	74.1	
100 ug/L microcosm	SFO	-		116	
			geometric mean	**92.7**	
water					
10 ug/L microcosm	SFO	-	-	62.1	
100 ug/L microcosm	SFO	-	-	99.1	

The degradation route of BYI 02960 in aqueous environments is shown in Figure B.8.4.4-01.

Figure B.8.4.4-01 Proposed route of degradation of BYI 02960 in water/sediment systems (BYI 02960 rapidly partitioned into the sediment phase, DFA is mainly present in water phase)

Note: The degradates to be observed as well as the given maximum values are highly dependent on radiolabel and kind of study considered; Ph = photo-transformation

B.8.5 Impact on water treatment procedures (Annex IIIA 9.2.2)

No information is provided on the fate of BYI 02960 in wastewater treatment plants. The effects on activated sludge are reported under B.9.10.1.

STUDY IIIA 9.2.2/01

Characteristics

reference	:	Schäfer, D.; Ströch, K.; Garside C.M. 2014	statement	:	PEC at drink water abstraction points
year of execution	:	2014			
GLP statement	:	no			
guideline	:	not available			
test substance	:	BYI 02960 and metabolites	acceptability	:	**acceptable**

The applicant provided a statement including an assessment that, based on the obtained PEC_{gw} and PEC_{sw} concentrations of the parent substance and metabolites, the concentrations are below the 0.1 µg/L...

Concentrations in groundwater or surface water of the active substance flupyradifurone and its metabolites at raw water abstraction points will be clearly below 0.1 µg/L, or maximum about 0.1 µg/L for the non-relevant metabolite DFA in groundwater. It is therefore highly unlikely that water treatment processes such as ozonation or chlorination will result in the formation of significant levels of by-products that would require human or animal health risk assessment. Consequently, further information on the effect of water treatment processes on the nature of residues present in surface water and groundwater is not considered necessary.

REMARKS RMS

The RMS agrees with the applicant and statement that the concentration of the active substance and metabolites will not exceed 0.1 µg/L at drinking water abstraction points.

B.8.6 Predicted environmental concentrations in surface water, in sediment and in groundwater (PECsw, PECsed, PECgw) (Annex IIIA 9.2.1, 9.2.3)

B.8.6.1 Predicted concentrations in surface water (Annex IIIA 9.2.3)

Calculation of PEC values for the active substance and metabolites according to FOCUS

$FOCUS_{sw}$ is a four step tiered approach:

Step 1: In this, the most conservative step, all inputs are considered as a single loading to the water body and a worst-case PEC_{sw} and PEC_{sed} is calculated.

Step 2: A refinement is made whereby individual loadings into the water body from different entry routes are considered. Scenarios are also considered for Northern and Southern Europe separately but no specific crop scenarios are defined.

Step 3: An exposure assessment using realistic worst-case scenarios is made. The scenarios are representative of agricultural conditions in Europe and consider weather, soil, crop and different water-bodies. Simulations use the models PRZM, MACRO and TOXSWA.

Step 4: PEC values are refined by considering mitigation measures or specific scenario descriptions on a case-by-case basis.

The PEC values in water and sediment were calculated at Step 1, 2, 3 and 4 for the parent substance. Only global maximum values were calculated, which can be used as conservative estimates of the actual and time-weighted average values assuming no degradation. Study design, compound characteristics and application were the same as for the PEC_{sw} calculations as for the PEC_{sed}.

Note: There are currently no agreed European guidelines for the assessment of exposure of surface water from the use in glasshouses, therefore it was assumed that the use in glasshouses on lettuce is covered by the outdoor field use even considering the different use pattern.

STUDY IIIA, 9.7/01 and STUDY IIIA, 9.7/02

reference	:	Sur R., Ellerich C. (9.7/01) Scherr F., Ellerich C. (9.7/02)	GLP statement	:	no
type of study	:	PECsw calculations	guideline	:	FOCUS 2000, SANCO/321/2000-rev.2 FOCUS 2003, SANCO/4802/2001-rev.2 FOCUS 2006, SANCO/10058/2005 version 2.0 FOCUS 2007, SANCO 10422/2005 version 2.0
year of execution	:	2012 (9.7/01); 2013 (9.7/02)	acceptability	:	**acceptable**
test substance	:	BYI 02960			

Materials and Methods

Predicted environmental concentrations in surface water and sediment (PEC_{sw} and PEC_{sed}) of BYI 02960 have been calculated for the use in hop and lettuce in Europe employing the tiered FOCUS Surface Water approach. All relevant entry routes of a compound into surface water (principally a combination of spray drift and runoff/erosion or drain flow) were considered in these calculations.

Details of the parameters used in the calculations are summarised in Table B.8.6.1-01.

Table B.8.6.1-01 Comparison of actual and calculated use pattern (for FOCUS step 1 & 2)

Individual Crop	FOCUS Crop Used for Interception	Application				Amount Reaching the Soil per Season application [g a.i. /ha]
		Rate per Season [g a.i. /ha]	Interval [days]	Plant Interception [%]	Growth Stage	
Hops, GAP	hops	1× 150	-	-	31-75	-
Hops, FOCUS simulation		1× 150	-	50 (average crop cover)	31-75	1 x 75
Lettuce 1 (F), GAP	leafy vegetables	1 x 125	-	-	12-49	-
Lettuce 1 (F), FOCUS simulation [1)] *		1 x 125	-	25 (minimal crop cover)	12-49	1 x 93.75
Lettuce 2 (F), GAP	leafy vegetables	1 x 125	-	-	41-49	-
Lettuce 2 (F), FOCUS simulation [2)] *		1 x 125	-	50 (average crop cover)	41-49	1 x 63.5

[1)] Maximal 1 use per 24 months (minimal crop cover)
[2)] Maximal 1 use per 12 months (average crop cover)
* First or second cropping per year
F = field use

At FOCUS step 3 actual application dates were determined by the PAT (pesticide application timer) included within SWASH. Details on application timing are summarised in Table B.8.6.1-02.

Table B.8.6.1-02 Application dates of BYI 02960 (FOCUS Step 3)

Parameter	Hops		Lettuce 1		Lettuce 2	
PAT start date rel./absolute	Absolute		Absolute		Absolute	
Appl. Method (appl. Type)	ground spray (CAM 2)		ground spray (CAM 2)		ground spray (CAM 2)	
No of appl.	1		1		1	
PAT window range	98		var. Range		30	
Appl. Interval	1		1		1	
Application Details	PAT Start Date (Julian Day)	Appl. Date	PAT Start Date (Julian Day)	Appl. Date	PAT Start Date (Julian Day)	Appl. Date
D1 (1st)	-	-	-	-	-	-
D2 (1st)	-	-	-	-	-	-
D3 (1st)	-	-	30-Apr (120)	04-May	30-Jun (181)	08-Jul
D3 (2nd)	-	-	09-Aug (221)	17-Sep	02-Oct (275)	01-Oct
D4 (1st)	-	-	18-May (138)	18-May	24-Aug (236)	27-Aug
D5 (1st)	-	-	-	-	-	-
D6 (1st)	-	-	21-Aug (233)	25-Aug	05-Nov (309)	06-Nov
D6 (2nd)	-	-	-	-	-	-
R1 (1st)	01-May(121)	02-May	25-Apr (115)	26-Apr	25-Jun (176)	29-Jun
R1 (2nd)	-	-	04-Aug (216)	20-Aug	27-Sep (270)	06-Oct
R2 (1st)	-	-	07-Mar (66)	22-Mar	02-Jun (153)	04-Jun
R2 (2nd)	-	-	06-Aug (218)	06-Aug	21-Oct (294)	24-Oct
R3 (1st)	-	-	06-Mar (65)	10-Mar	10-May (130)	18-May
R3 (2nd)	-	-	20-Jun (171)	25-Jun	24-Aug (236)	28-Aug

R4 (1st)	-	-	06-Mar (65)	06-Mar	10-May (130)	27-May
R4 (2nd)	-	-	20-Jun (171)	23-Jun	24-Aug (236)	27-Aug

At Step 4, the mitigation according to the FOCUS Landscape and Mitigation Factors report (FOCUS 2007) is assessed. For this purpose, calculations using mitigation measures for drift and runoff were defined. The relevant parameters are summarised in Table B.8.6.1-03 and Table B.8.6.1-04.

Table B.8.6.1-03 Mitigation approaches used

Crop	Buffer length	Mitigation type	Drift reducing nozzles
Hops	5 m	Spraydrift	0%, 50%, 75% 90%
	10 m	Spraydrift & RunOff	0%, 50%, 75% 90%
	15 m	Spraydrift & RunOff	0%, 50%, 75% 90%
	20 m	Spraydrift & RunOff	0%, 50%, 75% 90%
Lettuce	5 m	Spraydrift	0%, 50%, 75% 90%
	10 m	Spraydrift & RunOff	0%, 50%, 75% 90%
	15 m	Spraydrift & RunOff	0%, 50%, 75% 90%
	20 m	Spraydrift & RunOff	0%, 50%, 75% 90%

Table B.8.6.1-04 Runoff mitigation parameters used for the assessment

Crop	Fraction in reduction		10 m	15 m	20m
Hops	Runoff:	Volume	0.60	0.80	0.80
		Flux	0.60	0.80	0.80
	Erosion:	Mass	0.85	0.95	0.95
		Flux	0.85	0.95	0.95
Lettuce	Runoff:	Volume	0.60	0.80	0.80
		Flux	0.60	0.80	0.80
	Erosion:	Mass	0.85	0.95	0.95
		Flux	0.85	0.95	0.95

Compound specific input data are summarised in Table B.8.6.1-05.

Table B.8.6.1-05 Substance specific and model related input parameter for PEC_{sw} calculation of BYI 02960

Parameter	Unit	BYI 02960
Molar Mass	g/mol	288.7
Water Solubility	mg/L	3200
Vapour pressure	Pa	9.1×10^{-7}
K_{oc}	mL/g	98.4
Freundlich Exponent (1/n)		0.8657
Degradation		
Soil	days	94.8
Total System	days	228
Water	days	228
Sediment	days	228 / 1000*
Max Occurrence		
Water / Sediment	%	100
Soil	%	100

* used for FOCUS STEP 3 and 4

Findings:

Step 1 and 2: The maximum PEC values for Steps 1 and 2 are given in Table B.8.6.1-06.

Table B.8.6.1-06 Maximum PEC_{sw} and PEC_{sed} values for BYI 02960 at Step 1 & 2

Crop Application rate	FOCUS Step	$PEC_{sw, max}$ [µg/L]	$PEC_{sed, max}$ [µg/kg]
Hops 1 x 150 g/ha	1	53.86	43.49
	2 (N-EU)	13.07	12.49
	2 (S-EU)	17.36	16.70
Lettuce 1 1 x 125 g/ha (F) [1)] *	1	37.89	36.24
	2 (N-EU)	6.410	6.249
	2 (S-EU)	11.78	11.51
Lettuce 2 1 x 125 g/ha (F) [2)] *	1	37.89	36.24
	2 (N-EU)	3.191	3.091
	2 (S-EU)	5.337	5.196

[1)] Maximal 1 use per 24 month
[2)] Maximal 1 use per 12 month
* First or second cropping per year

Step 3: The maximum PEC_{sw} and PEC_{sed} values for relevant FOCUS Step 3 scenarios are given in Table B.8.6.1-07 and B.8.6.1-08. Time dependent PEC values or time-weighted average concentrations are not included in this summary, because they were not used in the risk assessment. However, all values are given in the report.

Table B.8.6.1-07 Maximum PEC_{sw} and PEC_{sed} of BYI 02960 for relevant scenarios at Step 3 following application to hops

Step 3 Scenario	BYI 02960: Hops, 1 x 150 g/ha		
	Entry route	$PEC_{sw, max}$ [µg/L]	$PEC_{sed, max}$ [µg/kg]
R1, pond	Spray drift	0.394	0.795
R1, stream	Spray drift	5.531	0.362

Table B.8.6.1-08 Maximum PECsw and PEC_{sed} values of BYI 02960 for relevant scenarios at Step 3 following application to lettuce, field applications

Step 3 Scenario	BYI 02960: Lettuce 1 [1)*], 1 x 125 g/ha (F)			BYI 02960: Lettuce 2 [2)*], 1 x 125 g/ha (F)		
	Entry route	$PEC_{sw, max}$ [µg/L]	$PEC_{sed, max}$ [µg/kg]	Entry route	$PEC_{sw, max}$ [µg/L]	$PEC_{sed, max}$ [µg/kg]
D3 (ditch, 1st)	Spray drift	0.830	0.380	Spray drift	0.813	0.339
D3 (ditch, 2nd)	Spray drift	0.840	0.460	Spray drift	0.811	0.321
D4 (pond, 1st)	Drainage	1.035	4.545	Drainage	0.983	3.755
D4 (stream, 1st)	Spray drift	0.794	1.772	Drainage	0.969	1.395
D6 (ditch, 1st)	Drainage	1.268	1.766	Drainage	1.206	1.959
R1 (pond, 1st)	Run-off	0.060	0.162	Run-off	0.337	0.751
R1 (stream, 1st)	Run-off	0.858	0.211	Run-off	2.827	0.698
R1 (pond, 2nd)	Run-off	0.097	0.254	Run-off	0.139	0.411
R1 (stream, 2nd)	Run-off	1.186	0.334	Run-off	2.189	0.415
R2 (stream, 1st)	Run-off	1.586	0.521	Run-off	1.215	0.513
R2 (stream, 2nd)	Run-off	0.940	0.342	Run-off	1.044	0.246
R3 (stream, 1st)	Run-off	2.226	0.469	Run-off	3.698	0.796
R3 (stream, 2nd)	Run-off	3.570	1.011	Run-off	3.297	0.964
R4 (stream, 1st)	Spray drift	0.522	0.054	Run-off	0.830	0.241
R4 (stream, 2nd)	Run-off	4.808	1.255	Run-off	2.954	0.803

[1)] Maximal 1 use per 24 month
[2)] Maximal 1 use per 12 month
* First or second cropping per year
F = Field use

Step 4: The maximum PEC_{sw} and PEC_{sed} values for relevant FOCUS Step 4 scenarios with mitigation options are given in the following tables.

Table B.8.6.1-09 Maximum PEC_{sw} and PEC_{sed} values of BYI 02960 at Step 4 including drift reduction (without buffer) – Hops (1 x 150 g/ha)

Step 4		BYI 02960: Hops, 1 x 150 g/ha							
Buffer Width & Type	FOCUS Scenario	PECsw [µg/L] Drift Reduction				PECsed [µg/kg] Drift Reduction			
		25%	50%	75%	90%	25%	50%	75%	90%
0m (drift)	R1 (pond, 1st)	0.296	0.197	0.099	0.039	0.607	0.415	0.218	0.094
	R1 (stream, 1st)	4.149	2.766	1.383	0.553	0.273	0.184	0.093	0.072

Table B.8.6.1-10 Maximum PEC_{sw} and PEC_{sed} values of BYI 02960 at Step 4 including buffer zones and drift reduction – Hops (1 x 150 g/ha)

Step 4		BYI 02960: Hops, 1 x 150 g/ha							
Buffer Width & Type	FOCUS Scenario	PEC_{sw} [µg/L] Drift Reduction				PEC_{sed} [µg/kg] Drift Reduction			
		0%	50%	75%	90%	0%	50%	75%	90%
5m (drift)	R1 (pond, 1st)	0.445	0.223	0.111	0.045	0.891	0.465	0.244	0.105
	R1 (stream, 1st)	4.515	2.258	1.129	0.452	0.297	0.151	0.076	0.071
10m (drift & run-off)	R1 (pond, 1st)	0.253	0.126	0.063	0.025	0.522	0.273	0.142	0.061
	R1 (stream, 1st)	2.354	1.177	0.589	0.235*	0.157	0.080	0.04	0.031
15m (drift & run-off)	R1 (pond, 1st)	0.141	0.071	0.035	0.014*	0.302	0.158	0.083	0.036
	R1 (stream, 1st)	1.554	0.777	0.388	0.155*	0.104	0.053	0.032	0.03
20m (drift & run-off)	R1 (pond, 1st)	0.078	0.039	0.02*	0.008*	0.174	0.091	0.048	0.021
	R1 (stream, 1st)	0.708	0.354	0.177*	0.071*	0.048	0.025	0.017	0.016

* reduction of the overall drift percentage is >95%, compared to STEP3, in case drift leads to PECsw max.

The mitigation from 10 m onwards includes spray drift and concurrent run-off buffer. However, as can be seen from the linear decrease of PEC_{sw} values with increasing drift reduction, the PEC_{sw} is always drift dominated and the run-off buffer does not drive the PEC_{sw}.

The step 4 PEC_{sw} for the pond scenario with 5 m buffer and 0% drift reduction is higher compared to the step 3 value. This is due to the default buffer width of 6 m already included in step 3 calculations resulting in a lower drift percentage of 2.63% compared to 2.97% in step 4.

Table B.8.6.1-11 Maximum PEC_{sw} and PEC_{sed} values of BYI 02960 at Step 4 including drift reduction (without buffer) – Lettuce 1 (1 x 125 g/ha, field use)

Step 4		BYI 02960: Lettuce 1, 1 x 125 g/ha (field use)							
Buffer Width & Type	FOCUS Scenario	PECsw [µg/L] Drift Reduction				PECsed [µg/kg] Drift Reduction			
		25%	50%	75%	90%	25%	50%	75%	90%
0m (drift)	D3 (ditch, 1st)	0.632	0.434	0.235	0.117	0.335	0.334	0.333	0.332
	D3 (ditch, 2nd)	0.643	0.446	0.249	0.130	0.419	0.399	0.398	0.397
	D4 (pond, 1st)	1.034	1.034	1.033	1.033	4.538	4.531	4.524	4.520
	D4 (stream, 1st)	0.721	0.721	0.721	0.721	1.771	1.771	1.771	1.771
	D6 (ditch, 1st)	1.268	1.268	1.268	1.268	1.766	1.765	1.765	1.764
	R1 (pond, 1st)	0.055	0.050	0.045	0.043	0.148	0.134	0.119	0.111
	R1 (stream, 1st)	0.858	0.858	0.858	0.858	0.210	0.208	0.207	0.206
	R1 (pond, 2nd)	0.092	0.087	0.082	0.079	0.240	0.225	0.210	0.201
	R1 (stream, 2nd)	1.186	1.186	1.186	1.186	0.333	0.332	0.330	0.330
	R2 (stream, 1st)	1.586	1.586	1.586	1.586	0.520	0.520	0.519	0.518
	R2 (stream, 2nd)	0.940	0.940	0.940	0.940	0.342	0.341	0.341	0.340
	R3 (stream, 1st)	2.226	2.226	2.226	2.226	0.466	0.462	0.459	0.456
	R3 (stream, 2nd)	3.570	3.570	3.570	3.570	1.006	1.000	0.995	0.991
	R4 (stream, 1st)	0.392	0.261	0.131	0.074	0.041	0.027	0.025	0.024
	R4 (stream, 2nd)	4.808	4.808	4.808	4.808	1.253	1.251	1.249	1.248

Table B.8.6.1-12 Maximum PEC_{sw} and PEC_{sed} values of BYI 02960 at Step 4 including buffer zones and drift reduction – Lettuce 1 (1 x 125 g/ha, field use, 5 and 10m buffer zones)

Step 4		BYI 02960: Lettuce 1 , 1 x 125 g/ha (field use)							
Buffer Width & Type	FOCUS Scenario	PEC_{sw} [µg/L] Drift Reduction				PEC_{sed} [µg/kg] Drift Reduction			
		0%	50%	75%	90%	0%	50%	75%	90%
5m (drift)	D3 (ditch, 1st)	0.252	0.145	0.091	0.059*	0.333	0.332	0.332	0.332
	D3 (ditch, 2nd)	0.265	0.158	0.105	0.073*	0.398	0.397	0.397	0.396
	D4 (pond, 1st)	1.035	1.034	1.033	1.033	4.541	4.529	4.523	4.520
	D4 (stream, 1st)	0.721	0.721	0.721	0.721	1.771	1.771	1.771	1.771
	D6 (ditch, 1st)	1.268	1.268	1.268	1.268	1.765	1.764	1.764	1.764
	R1 (pond, 1st)	0.057	0.049	0.045	0.042	0.154	0.130	0.117	0.11
	R1 (stream, 1st)	0.858	0.858	0.858	0.858	0.208	0.207	0.206	0.206
	R1 (pond, 2nd)	0.094	0.086	0.082	0.079	0.247	0.221	0.208	0.201
	R1 (stream, 2nd)	1.186	1.186	1.186	1.186	0.331	0.330	0.33	0.329
	R2 (stream, 1st)	1.586	1.586	1.586	1.586	0.519	0.518	0.518	0.518
	R2 (stream, 2nd)	0.940	0.940	0.940	0.940	0.341	0.341	0.34	0.34
	R3 (stream, 1st)	2.226	2.226	2.226	2.226	0.460	0.458	0.456	0.455
	R3 (stream, 2nd)	3.570	3.570	3.570	3.570	0.997	0.993	0.991	0.99
	R4 (stream, 1st)	0.191	0.095	0.074	0.074	0.025	0.025	0.024	0.024
	R4 (stream, 2nd)	4.808	4.808	4.808	4.808	1.250	1.248	1.247	1.247
10m (drift and run-off)	D3 (ditch, 1st)	0.151	0.094	0.066*	0.049*	0.332	0.332	0.332	0.332
	D3 (ditch, 2nd)	0.165	0.108	0.080*	0.063*	0.397	0.397	0.396	0.396
	D4 (pond, 1st)	1.034	1.033	1.033	1.033	4.534	4.526	4.522	4.519
	D4 (stream, 1st)	0.721	0.721	0.721	0.721	1.771	1.771	1.771	1.771
	D6 (ditch, 1st)	1.268	1.268	1.268	1.268	1.764	1.764	1.764	1.764
	R1 (pond, 1st)	0.029	0.022	0.019	0.018	0.083	0.064	0.055	0.049
	R1 (stream, 1st)	0.389	0.389	0.389	0.389	0.097	0.097	0.097	0.096
	R1 (pond, 2nd)	0.043	0.037	0.034	0.032	0.122	0.103	0.094	0.088
	R1 (stream, 2nd)	0.540	0.540	0.540	0.540	0.157	0.157	0.157	0.156
	R2 (stream, 1st)	0.716	0.716	0.716	0.716	0.228	0.228	0.228	0.228
	R2 (stream, 2nd)	0.422	0.422	0.422	0.422	0.151	0.151	0.151	0.151
	R3 (stream, 1st)	1.009	1.009	1.009	1.009	0.219	0.217	0.216	0.216
	R3 (stream, 2nd)	1.630	1.630	1.630	1.630	0.452	0.450	0.449	0.448
	R4 (stream, 1st)	0.101	0.051	0.034	0.034	0.012	0.012	0.012	0.011
	R4 (stream, 2nd)	2.184	2.184	2.184	2.184	0.585	0.584	0.584	0.584

* reduction of the overall drift percentage is >95%, compared to STEP3, in case drift leads to PECsw max.

Table B.8.6.1-13 Maximum PEC_{sw} and PEC_{sed} values of BYI 02960 at Step 4 including buffer zones and drift reduction – Lettuce 1 (1 x 125 g/ha, field use, 15 and 20m buffer zone)

Step 4		BYI 02960: Lettuce 1, 1 x 125 g/ha (field use)							
Buffer Width & Type	FOCUS Scenario	PEC_{sw} [µg/L] Drift Reduction				PEC_{sed} [µg/kg] Drift Reduction			
		0%	50%	75%	90%	0%	50%	75%	90%

Step 4		BYI 02960: Lettuce 1, 1 x 125 g/ha (field use)							
Buffer Width & Type	FOCUS Scenario	PEC_{sw} [µg/L] Drift Reduction				PEC_{sed} [µg/kg] Drift Reduction			
		0%	50%	75%	90%	0%	50%	75%	90%
15m (drift and run-off)	D3 (ditch, 1st)	0.115	0.076*	0.057*	0.045*	0.332	0.332	0.332	0.332
	D3 (ditch, 2nd)	0.129	0.090*	0.071*	0.059*	0.397	0.396	0.396	0.396
	D4 (pond, 1st)	1.034	1.033	1.033	1.033	4.531	4.524	4.521	4.519
	D4 (stream, 1st)	0.721	0.721	0.721	0.721	1.771	1.771	1.771	1.771
	D6 (ditch, 1st)	1.268	1.268	1.268	1.268	1.764	1.764	1.764	1.764
	R1 (pond, 1st)	0.018	0.013	0.011	0.009	0.055	0.039	0.032	0.027
	R1 (stream, 1st)	0.204	0.204	0.204	0.204	0.053	0.052	0.052	0.052
	R1 (pond, 2nd)	0.025	0.021	0.018	0.017	0.076	0.060	0.052	0.047
	R1 (stream, 2nd)	0.283	0.283	0.283	0.283	0.086	0.085	0.085	0.085
	R2 (stream, 1st)	0.375	0.375	0.375	0.375	0.122	0.122	0.122	0.122
	R2 (stream, 2nd)	0.220	0.220	0.220	0.220	0.081	0.081	0.081	0.081
	R3 (stream, 1st)	0.528	0.528	0.528	0.528	0.119	0.118	0.117	0.117
	R3 (stream, 2nd)	0.856	0.856	0.856	0.856	0.245	0.243	0.242	0.242
	R4 (stream, 1st)	0.069	0.035	0.018	0.018	0.008	0.006	0.006	0.006
	R4 (stream, 2nd)	1.144	1.144	1.144	1.144	0.318	0.317	0.317	0.316
20m (drift and run-off)	D3 (ditch, 1st)	0.097	0.067*	0.052*	0.045*	0.332	0.332	0.332	0.332
	D3 (ditch, 2nd)	0.110	0.081*	0.066*	0.057*	0.397	0.396	0.396	0.396
	D4 (pond, 1st)	1.033	1.033	1.033	1.033	4.529	4.523	4.520	4.518
	D4 (stream, 1st)	0.721	0.721	0.721	0.721	1.771	1.771	1.771	1.771
	D6 (ditch, 1st)	1.268	1.268	1.268	1.268	1.764	1.764	1.764	1.764
	R1 (pond, 1st)	0.016	0.012	0.010	0.009	0.050	0.037	0.03	0.026
	R1 (stream, 1st)	0.204	0.204	0.204	0.204	0.052	0.052	0.052	0.052
	R1 (pond, 2nd)	0.024	0.020	0.018	0.016	0.071	0.057	0.05	0.046
	R1 (stream, 2nd)	0.283	0.283	0.283	0.283	0.086	0.085	0.085	0.085
	R2 (stream, 1st)	0.375	0.375	0.375	0.375	0.122	0.122	0.122	0.122
	R2 (stream, 2nd)	0.220	0.220	0.220	0.220	0.081	0.081	0.081	0.081
	R3 (stream, 1st)	0.528	0.528	0.528	0.528	0.118	0.117	0.117	0.117
	R3 (stream, 2nd)	0.856	0.856	0.856	0.856	0.244	0.243	0.242	0.242
	R4 (stream, 1st)	0.053	0.026	0.018	0.018	0.007	0.006	0.006	0.006
	R4 (stream, 2nd)	1.144	1.144	1.144	1.144	0.317	0.317	0.317	0.316

* reduction of the overall drift percentage is >95%, compared to STEP3, in case drift leads to PECsw max.

Table B.8.6.1-14 Maximum PEC_{sw} and PEC_{sed} values of BYI 02960 at Step 4 including buffer zones and drift reduction – Lettuce 2 (1 x 125 g/ha, field use, 5 and 10m buffer zones)

Step 4		BYI 02960: Lettuce 2 , 1 x 125 g/ha (field use)							
Buffer Width & Type	FOCUS Scenario	PEC_{sw} [µg/L] Drift Reduction				PEC_{sed} [µg/kg] Drift Reduction			
		0%	50%	75%	90%	0%	50%	75%	90%
5m (drift)	D3 (ditch, 1st)	0.234	0.127	0.073	0.040*	0.188	0.187	0.187	0.186
	D3 (ditch, 2nd)	0.234	0.127	0.073	0.041*	0.177	0.173	0.172	0.172
	D4 (pond, 1st)	0.982	0.978	0.976	0.975	3.750	3.732	3.723	3.718
	D4 (stream, 1st)	0.969	0.969	0.969	0.969	1.395	1.394	1.394	1.394
	D6 (ditch, 1st)	1.206	1.206	1.206	1.206	1.958	1.958	1.958	1.958
	R1 (pond, 1st)	0.334	0.325	0.320	0.317	0.744	0.723	0.712	0.706
	R1 (stream, 1st)	2.827	2.827	2.827	2.827	0.694	0.693	0.692	0.692
	R1 (pond, 2nd)	0.137	0.130	0.127	0.125	0.404	0.379	0.367	0.359
	R1 (stream, 2nd)	2.189	2.189	2.189	2.189	0.411	0.410	0.409	0.409
	R2 (stream, 1st)	1.215	1.215	1.215	1.215	0.510	0.509	0.508	0.508
	R2 (stream, 2nd)	1.044	1.044	1.044	1.044	0.243	0.242	0.242	0.241
	R3 (stream, 1st)	3.698	3.698	3.698	3.698	0.780	0.775	0.772	0.771
	R3 (stream, 2nd)	3.297	3.297	3.297	3.297	0.949	0.945	0.942	0.941
	R4 (stream, 1st)	0.830	0.830	0.830	0.830	0.239	0.238	0.238	0.238
	R4 (stream, 2nd)	2.954	2.954	2.954	2.954	0.797	0.796	0.795	0.794

Step 4		BYI 02960: Lettuce 2 , 1 x 125 g/ha (field use)							
Buffer Width & Type	FOCUS Scenario	PEC_{sw} [µg/L] Drift Reduction				PEC_{sed} [µg/kg] Drift Reduction			
		0%	50%	75%	90%	0%	50%	75%	90%
10m (drift and run-off)	D3 (ditch, 1st)	0.133	0.076	0.048*	0.031*	0.187	0.187	0.186	0.186
	D3 (ditch, 2nd)	0.133	0.077	0.048*	0.031*	0.173	0.172	0.172	0.172
	D4 (pond, 1st)	0.980	0.977	0.976	0.975	3.740	3.727	3.720	3.717
	D4 (stream, 1st)	0.969	0.969	0.969	0.969	1.394	1.394	1.394	1.394
	D6 (ditch, 1st)	1.206	1.206	1.206	1.206	1.958	1.958	1.958	1.958
	R1 (pond, 1st)	0.143	0.135	0.132	0.130	0.339	0.323	0.314	0.309
	R1 (stream, 1st)	1.270	1.270	1.270	1.270	0.294	0.293	0.293	0.292
	R1 (pond, 2nd)	0.060	0.055	0.052	0.051	0.196	0.177	0.168	0.162
	R1 (stream, 2nd)	0.954	0.954	0.954	0.954	0.193	0.193	0.192	0.192
	R2 (stream, 1st)	0.553	0.553	0.553	0.553	0.207	0.206	0.206	0.206
	R2 (stream, 2nd)	0.469	0.469	0.469	0.469	0.108	0.108	0.108	0.108
	R3 (stream, 1st)	1.683	1.683	1.683	1.683	0.367	0.365	0.363	0.362
	R3 (stream, 2nd)	1.506	1.506	1.506	1.506	0.403	0.400	0.399	0.398
	R4 (stream, 1st)	0.379	0.379	0.379	0.379	0.112	0.112	0.111	0.111
	R4 (stream, 2nd)	1.345	1.345	1.345	1.345	0.373	0.372	0.372	0.372

* reduction of the overall drift percentage is >95%, compared to STEP3, in case drift leads to PECsw max.

Table B.8.6.1-15 Maximum PEC_{sw} and PEC_{sed} values of BYI 02960 at Step 4 including buffer zones and drift reduction – Lettuce 2 (1 x 125 g/ha, field use, 15 and 20m buffer zone)

Step 4		BYI 02960: Lettuce 2 , 1 x 125 g/ha (field use)							
Buffer Width & Type	FOCUS Scenario	PEC_{sw} [µg/L] Drift Reduction				PEC_{sed} [µg/kg] Drift Reduction			
		0%	50%	75%	90%	0%	50%	75%	90%
15m (drift and run-off)	D3 (ditch, 1st)	0.097	0.058*	0.038*	0.027*	0.187	0.186	0.186	0.186
	D3 (ditch, 2nd)	0.098	0.059*	0.039*	0.028*	0.173	0.172	0.172	0.172
	D4 (pond, 1st)	0.979	0.977	0.976	0.975	3.734	3.724	3.719	3.716
	D4 (stream, 1st)	0.969	0.969	0.969	0.969	1.394	1.394	1.394	1.394
	D6 (ditch, 1st)	1.206	1.206	1.206	1.206	1.958	1.958	1.958	1.958
	R1 (pond, 1st)	0.140	0.134	0.131	0.129	0.332	0.319	0.313	0.309
	R1 (stream, 1st)	1.270	1.270	1.270	1.270	0.293	0.293	0.293	0.292
	R1 (pond, 2nd)	0.058	0.054	0.052	0.051	0.188	0.173	0.166	0.161
	R1 (stream, 2nd)	0.954	0.954	0.954	0.954	0.193	0.193	0.192	0.192
	R2 (stream, 1st)	0.553	0.553	0.553	0.553	0.207	0.206	0.206	0.206
	R2 (stream, 2nd)	0.469	0.469	0.469	0.469	0.108	0.108	0.108	0.107
	R3 (stream, 1st)	1.683	1.683	1.683	1.683	0.366	0.364	0.363	0.362
	R3 (stream, 2nd)	1.506	1.506	1.506	1.506	0.401	0.399	0.398	0.398
	R4 (stream, 1st)	0.379	0.379	0.379	0.379	0.112	0.111	0.111	0.111
	R4 (stream, 2nd)	1.345	1.345	1.345	1.345	0.373	0.372	0.372	0.372
20 m (drift and run-off)	D3 (ditch, 1st)	0.078	0.049*	0.034*	0.025*	0.187	0.186	0.186	0.186
	D3 (ditch, 2nd)	0.079	0.049*	0.035*	0.026*	0.172	0.172	0.172	0.172
	D4 (pond, 1st)	0.978	0.976	0.976	0.975	3.731	3.723	3.718	3.716
	D4 (stream, 1st)	0.969	0.969	0.969	0.969	1.394	1.394	1.394	1.394
	D6 (ditch, 1st)	1.206	1.206	1.206	1.206	1.958	1.958	1.958	1.958
	R1 (pond, 1st)	0.074	0.069	0.067	0.066	0.185	0.173	0.167	0.164
	R1 (stream, 1st)	0.662	0.662	0.662	0.662	0.156	0.155	0.155	0.155
	R1 (pond, 2nd)	0.032	0.028	0.027	0.026	0.111	0.097	0.091	0.087
	R1 (stream, 2nd)	0.500	0.500	0.500	0.500	0.104	0.104	0.104	0.104
	R2 (stream, 1st)	0.290	0.290	0.290	0.290	0.109	0.108	0.108	0.108
	R2 (stream, 2nd)	0.244	0.244	0.244	0.244	0.058	0.057	0.057	0.057
	R3 (stream, 1st)	0.882	0.882	0.882	0.882	0.199	0.198	0.197	0.196
	R3 (stream, 2nd)	0.790	0.790	0.790	0.790	0.214	0.212	0.212	0.211
	R4 (stream, 1st)	0.199	0.199	0.199	0.199	0.060	0.060	0.060	0.060
	R4 (stream, 2nd)	0.705	0.705	0.705	0.705	0.202	0.202	0.202	0.202

* reduction of the overall drift percentage is >95%, compared to STEP3, in case drift leads to PECsw max.

Remarks

The RMS agrees with the risk assessment done by the applicant and recalcultated the FOCUS surface water step 1, 2, 3 and 4 model calculations and obtained the same results as the applicant. The obtained values by the applicant were checked by the RMS with Swash version 3.1 and Swan 1.1.4.

STUDY IIIA, 9.8/01 and STUDY 9.8/02

For BYI 02960, the metabolites difluoracetic acid (DFA), 6-chloronicotinic acid (6-CNA) and BYI 02960-succinamide and BYI 02960-azabicyclosuccinamide were assessed.

Reference	:	Sur R., Ellerich C. (9.8/01) Scherr F., Ellerich C. (9.8/02)	GLP statement	:	no
type of study	:	PECsw calculations	guideline	:	FOCUS 2000, SANCO/321/2000-rev.2 FOCUS 2003, SANCO/4802/2001-rev.2 FOCUS 2006, SANCO/10058/2005 version 2.0 FOCUS 2007, SANCO 10422/2005 version 2.0
year of execution	:	2012(9.8/01) and 2013 (9.8/02)	acceptability	:	**acceptable**
test substance	:	difluoracetic acid (DFA), 6-chloronicotinic acid (6-CNA) and BYI 02960-succinamide and BYI 02960-azabicyclosuccinamide			

Materials and Methods: PECsw for the metabolites were calculated using the approach, scenarios and application rates described for the calculations for the parent compound in study IIA, 9.7/01. Input parameters for the metabolites are described in Table B.8.6.1-16.

Table B.8.6.1-16 Substance specific and model related input parameters for PECsw calculation

Parameter	Unit	DFA	6-CNA	BYI 02960-succinamide	BYI 02960-azabicyclo-succinamide
Molar Mass	g/mol	96.03	157.6	306.7	288.3
Water Solubility	mg/L	500000	1430	120000	180000
K_{oc}	mL/g	6.8	88	0	0
Degradation DT_{50}					
Soil	days	44.7	4.7	0.1*	0.1*
Total System	days	249	1000	1000	1000
Water	days	249	1000	1000	1000
Sediment	days	249	1000	1000	1000
Max Occurrence					
Water / Sediment	%	6.9	0	39.6	25.9
Soil	%	33.9	17.1	0	0

* The DT50 soil was set to 0.1 d as the model STEP2 does not accept 0 d.

Findings:

Step 1 and 2: The maximum PEC values for the metabolites of BYI 02960 at Step 1 and Step 2 are given in Table B.8.6.1-17. Time dependent PEC values or time-weighted average concentrations are not included in this summary, because they were not used in the risk assessment. However, all values are given in the report.

Table B.8.6.1-17 Maximum PECsw and PECsed values for metabolites of BYI 02960

Crop	FOCUS Step	Difluoroacetic acid		6-Chloronicotinic acid		BYI 02960-succinamide		BYI 02960-azabicyclo-succinamide	
		PECsw [µg/L]	PECsed [µg/kg]	PECsw [µg/L]	PECsed [µg/kg]	PECsw [µg/L]	PECsed [µg/kg]	PECsw [µg/L]	PECsed [µg/kg]
Hops 1 x 150 g/ha	1	5.810	0.380	4.177	3.675	4.065	<0.001	2.499	<0.001
	2 N-EU	0.743	0.050	0.232	0.204	4.065	<0.001	2.499	<0.001
	2 S-EU	1.268	0.086	0.463	0.408	4.065	<0.001	2.499	<0.001
Lettuce 1 1 x 125 g/ha (F) 1) *	1	4.683	0.317	3.481	3.063	0.484	<0.001	0.297	<0.001
	2 N-EU	0.682	0.046	0.289	0.255	0.484	<0.001	0.297	<0.001
	2 S-EU	1.339	0.091	0.579	0.509	0.484	<0.001	0.297	<0.001
Lettuce 2 1 x 125 g/ha (F) 2) *	1	4.683	0.317	3.481	3.063	0.484	<0.001	0.297	<0.001
	2 N-EU	0.551	0.037	0.193	0.170	0.484	<0.001	0.297	<0.001
	2 S-EU	1.076	0.073	0.386	0.340	0.484	<0.001	0.297	<0.001

F = Field use
1) Maximal 1 use per 24 month (minimal crop cover)
2) Maximal 1 use per 12 month (average crop cover)
* First or second cropping per year

Remarks

The RMS agrees with the risk assessment for the metabolites done by the applicant. RMS recalcultated the FOCUS PEC_{sw} and PEC_{sed} step 1 and 2 model calculations and obtained the same results as the applicant.

B.8.6.2 Predicted concentrations in sediment (Annex IIIA 9.2.3)

See B.8.6.1

B.8.6.3 Predicted concentrations in groundwater (Annex IIIA 9.2.1)

PECgw for BYI 02960 in hops

STUDY IIIA, 9.6.1/01

Tier-1:

reference	:	Sur R., Ellerich C.	GLP statement	:	no
type of study	:	PECgw calculations	guideline	:	FOCUS 2000, SANCO/321/2000, rev. 2 FOCUS 2006, SANCO/10058/2005, rev. 2 FOCUS 2009, SANCO/13144/2010, version 1 FOCUS 2010, version 2.0
year of execution	:	2012	acceptability	:	**acceptable**
test substance	:	BYI 02960			

STUDY IIIA, 9.6.1/02

Tier-2a (DFOP):

reference	:	Sur R., Ellerich C.	GLP statement	:	no
type of study	:	PECgw calculations	guideline	:	FOCUS 2000, SANCO/321/2000, rev. 2 FOCUS 2006, SANCO/10058/2005, rev. 2 FOCUS 2009, SANCO/13144/2010, version 1 FOCUS 2010, version 2.0
year of execution	:	2012	acceptability	:	**acceptable**
test substance	:	BYI 02960			

STUDY IIIA, 9.6.1/03

Tier-2b (TDS):

reference	:	Sur R., Ellerich C.	GLP statement	:	no
type of study	:	PECgw calculations	guideline	:	FOCUS 2000, SANCO/321/2000, rev. 2 FOCUS 2006, SANCO/10058/2005, rev. 2 FOCUS 2009, SANCO/13144/2010, version 1 FOCUS 2010, version 2.0
year of execution	:	2012	acceptability	:	**acceptable**
test substance	:	BYI 02960			

STUDY IIIA, 9.6.1/08

reference	:	Scherr, F., Ellerich C.	GLP statement	:	no
type of study	:	PECgw calculations	guideline	:	FOCUS 2000, SANCO/321/2000, rev. 2 FOCUS 2006, SANCO/10058/2005, rev. 2 FOCUS 2009, SANCO/13144/2010, version 1 FOCUS 2010, version 2.0
year of execution	:	2013	acceptability	:	**acceptable**
test substance	:	BYI 02960, DFA and 6-CNA			

STUDY IIIA, 9.6.1/10

reference	:	Schierver C.. et al.	GLP statement	:	no
type of study	:	Plant uptake factor	guideline	:	not available
year of execution	:	2013	acceptability	:	**not acceptable**
test substance	:	22 unknown compound			

Materials and Methods

The predicted environmental concentrations in groundwater (PEC_{gw}) for BYI 02960 and its metabolites were calculated using the simulation model FOCUS PEARL (version 4.4.4) and FOCUS PELMO (4.4.3). Detailed application data used for simulation of PEC_{gw} are compiled in Table B.8.6.3-01 and B.8.6.3-02. Simulations were conducted for annual applications as well as applications performed every second year.

Table B.8.6.3-01 Comparison of simulated and actual use pattern

Individual Crop	FOCUS Crop Used for Interception	Application				Amount Reaching the Soil per Season application [g a.s. /ha]
		Rate per Season [g a.s. /ha]	Interval [days]	Plant Interception [%]	BBCH Stage	
Hops, GAP	-	1 × 150	-	60	31 – 75	1 × 60.00
Tier 1, Tier 2b (TDS)						
Hops (every ear), Simulation 1	Vines	1 × 150	-	60	31 – 75	1 × 60.00
Hops (every 2nd year), Simulation 2	Vines	1 × 150	-	60	31 – 75	1 × 60.00
Tier 2a (DFOP)						
Hops (every ear), Simulation 1	Vines	1 × 150	-	60	31 – 75	68.4 [s] / 51.6 [f]
Hops (every 2nd year), Simulation 2	Vines	1 × 150	-	60	31 – 75	68.4 [s] / 51.6 [f]

[f] used for fast compartment
[s] used for slow compartment

Application dates for the simulation runs were defined following the crop event dates of the respective crop and scenario as given by FOCUS (2009).

Table B.8.6.3-02 First application dates and related information for BYI 02960 as used for the simulation runs (offset is relevant only for relative application dates, two sets of data are provided for crops with two seasons)

Individual crop	Hops	Hops
Repeat Interval for App. Events	Every Year	Every 2nd Year
Application Technique	Spray	Spray
Absolute / Relative to	Emergence	Emergence
Scenario	1st App. Date/(Julian day)/Offset to crop event	
	First tier/ Second tier	
Chateaudun	29 April (119) 28	29 April (119) 28
Hamburg	29 May (149) 28	29 May (149) 28
Jokioinen	-	-
Kremsmuenster	29 May (149) 28	29 May (149) 28
Okehampton	-	-
Piacenza	29 April (119) 28	29 April (119) 28
Porto	12 Apr (102) 28	12 Apr (102) 28
Sevilla	28 April (118) 28	28 April (118) 28
Thiva	12 Apr (102) 28	12 Apr (102) 28

Further input parameters for PEC_{gw} modelling of BYI 02960 are summarised in Table B.8.6.3-03 for BYI 02960 and in Table B.8.6.3-04 for the metabolites. Parameters used for degradation pathway in PEARL and PELMO are depicted in Table B.8.6.3-05.

For Tier 1, a geometric mean of half-lives derived from SFO and from the slow compartment of the DFOP model was used to obtain a conservative model input.

For Tier 2a, (DFOP) according to FOCUS (2009), DFOP degradation kinetics was considered in leaching modelling based on the procedure described in FOCUS (2006).

To obtain common DFOP parameters over all soils the following procedure was applied: Firstly, all degradation curves following SFO kinetics on Tier-1 were converted to an equivalent DFOP model where the Tier-1 SFO-DT_{50} was assigned equally (g=0.5) to the slow and fast degrading compartment. For those soils where the slow compartment of DFOP was already used for modelling at Tier-1, the corresponding shorter DT_{50} of the fast compartment and g (fraction of total amount applied to the compartment) of the DFOP fit were additionally considered. Finally, the DFOP parameters were calculated as geometic over all soils for the rate and arithmetic mean for the g value (see Table B.8.6.3-03a and Table B.8.6.3-03b).

Table B.8.6.3-03a DFOP parameters for tier-2a leaching calculations

Soil	**Labled test substance**	**Study Reference**	**Parent BYI 02960**					
			DT50 (d) Fast		**DT50 (d) Slow**		**g**	
Laacher Hof AXXa	[PYM] BYI 02960	IIA 7.2.1/07	15.8	12.9	169.1	171.3	0.38	0.37
Laacher Hof AXXa	[FUR] BYI 02960	IIA 7.2.1/08	10.7		141.5		0.33	
Laacher Hof AXXa	[ETH] BYI 02960	IIA 7.2.1/09	12.7		210.2		0.40	
Höfchen Hohenseh	[PYM] BYI 02960	IIA 7.2.1/07	12.7	33.1	90.0	54.0	0.45	0.49
Höfchen Hohenseh	[FUR] BYI 02960	IIA 7.2.1/08	40.5		40.5		0.50	
Höfchen Hohenseh	[ETH] BYI 02960	IIA 7.2.1/09	43.0		43.0		0.50	

Höfchen Hohenseh	[PYR] BYI 02960	IIA 7.2.1/10	54.4		54.4		0.50	
Hanscheiderhof	[PYM] BYI 02960	IIA 7.2.1/07	6.7	6.5	157.5	157.5	0.16	0.20
Hanscheiderhof	[FUR] BYI 02960	IIA 7.2.1/08	6.5		157.5		0.23	
Dollendorf II	[PYM] BYI 02960	IIA 7.2.1/07	60.1	50.4	60.1	50.4	0.50	0.50
Dollendorf II	[FUR] BYI 02960	IIA 7.2.1/08	55.1		55.1		0.50	
Dollendorf II	[ETH] BYI 02960	IIA 7.2.1/09	38.6		38.6		0.50	
Springfield	[FUR] BYI 02960	IIA 7.2.1/03	166.4	173.0	166.4	173.0	0.50	0.50
Springfield	[PYR] BYI 02960	IIA 7.2.1/06	179.7		179.7		0.50	
Sanger	[FUR] BYI 02960	IIA 7.2.1/03	55.5	57.2	55.5	57.2	0.50	0.50
Sanger	[PYR] BYI 02960	IIA 7.2.1/06	58.8		58.8		0.50	
Geometric mean				**33.4**		**94.8**		
Arithmetic mean								**0.43**

For leaching modelling the application rate was doubled and assigned to both compartments according to g of 0.43. Then, two separate leaching simulations were performed: one for the fast compartment using DT_{50fast} of 33.4 days (k_1 0.0207 d^{-1}) and one using DT_{50slow} of 94.8 days (k_2 0.00731 d^{-1}). Both PEC_{gw} values were summed up and divided by two to get the final result.

Tier 2b (TDS): Time dependent sorption (TDS) data of BYI 02960 on four soils were derived via curve fitting (study IIA, 7.4.1/05). These parameters constitute the prerequisite to adequately address TDS processes in regulatory exposure modelling (Beulke, S. and van Beinum, W. 2012)[3]. Experimental soil data were re-calculated to fit the TDS model, resulting in a geometric mean DT_{50_eq} of 57.9 days, an arithmetic mean K_{OM_eq} of 47.5 L/kg, an arithmetic mean f_ne of 0.5821, a geometric mean $k_{_des}$ of 0.0309 and an arithmetic mean Freundlich exponent of 0.8605 from the optimized parameters of the four data sets. The PECgw calculations given in this part are based on the average/geometric mean parameters obtained from the individual soil. At the end of this paragraph a comparison with the PECgw from each separate soil data set is given as was proposed in the draft guidance on TDS (Beulke, S. and van Beinum, W. 2012).

Table B.8.6.3-03b Substance specific and model related input parameters for PEC_{gw} calculation of BYI 02960

Parameter	Unit	BYI 02960		
		Tier 1	Tier 2a (DFOP)	Tier 2b (TDS)
Common				
Molar Mass	[g/mol]	288.7	288.7	288.7
Solubility	[mg/L]	3200	3200	3200
Vapour Pressure	[Pa]	9×10^{-7}	9×10^{-7}	9×10^{-7}
Freundlich Exponent		0.8657	0.8657	0.8605
Plant Uptake Factor *		0.5	0.5	0.5
Walker Exponent		0.7	0.7	0.7
PEARL Parameters				
Substance Code		BUTn	$BUTf^f$ / $BUTs^s$	BUTk
DT_{50}	[days]	94.8	33.4^f / 94.8^s	57.9
g		-	0.43	-
Molar Activ. Energy	[kJ/mol]	65.4	65.4	65.4

[3] Beulke, S. and van Beinum, W. (2012). Guidance on how aged sorption studies for pesticides should be conducted, analysed and used in regulatory assessments. The Food and Environment Research Agency. Sand Hutton, YO41 1LZ, UK.

K_{om}	[mL/g]	57.1	57.1	47.5
Desorp. Rate Coeff.	[1/days]	-	-	0.0309
Equ. Factor		-	-	0.5821
PELMO Parameters				
Substance Code		AS	AS	AS
Rate Constant	[1/day]	0.00730	0.0207[f] / 0.00731[s]	0.01195
Q_{10}		2.58	2.58	2.58
K_{oc}	[mL/g]	98.4	98.4	80.2

[f] DT_{50} used for the fast compartment; [s] DT_{50} used for the slow compartment

* ~~Data provided to justify that theplant uptake factor for the active substance and metabolites is 0.5~~ systemic active substance (see mode of action volume B.3.10)

Table B.8.6.3-04 Substance specific and model related input parameters for PEC_{gw} calculation of BYI 02960 metabolites

Parameter	Unit	Difluoroacetic acid	6-Chloronicotinic acid
Common			
Molar Mass	[g/mol]	96	157.6 [a)]
Solubility	[mg/L]	500000	1430
Vapour Pressure	[Pa]	1.00×10^{-10}	1.00×10^{-10}
Freundlich Exponent		0.835	0.949
Plant Uptake Factor *		~~0.5~~ 0.0	~~0.5~~ 0.0
Walker Exponent		0.7	0.7
PEARL Parameters			
Substance Code		DFA	CNA
DT_{50}	[days]	44.7	4.7
Molar Activ. Energy	[kJ/mol]	65.4	65.4
K_{om}	[mL/g]	3.9	51
Desorp. Rate Coeff.	[1/days]	0	0
Equ. Factor		0	0
PELMO Parameters			
Substance Code		A1	B1
Rate Constant	[1/day]	0.01551	0.14748
Q_{10}		2.58	2.58
K_{oc}	[mL/g]	6.8	88

[a)] The sum of formation fractions of both metabolites is > 1. In order to run the modelling with PELMO, the molar mass of 6-CNA was set to 451 g/mol, resulting from $M_{auxiliary}$ (6-CNA) = M (6-CNA) × 0.478 / (1-0.833) = 451 g/mol

* Data provided to justify that the plant uptake factor for the ~~active substance and~~ metabolites is 0.5 (Schriever et al, 2013 [4])

Table B.8.6.3-05 Degradation pathway related parameters for BYI 02960 and its metabolites

Tier 1, Tier 2a (DFOP), Tier 2b (TDS)	Degradation fraction from → to (FOCUS PEARL)	0.83 BUT -> DFA 0.48 BUT -> CNA
Tier 1, Tier 2a (DFOP, slow compartment)	Degradation rate from → to (FOCUS PELMO)	0.0060780 Active Substance → A1 0.0012180 Active Substance → B1 0.0155070 A1 → <BR/CO_2 0.1474780 B1 → <BR/CO_2
Tier 2a (DFOP, fast compartment)	Degradation rate from → to (FOCUS PELMO)	0.017287 Active Substance → A1 0.003466 Active Substance → B1 0.015507 A1 → <BR/CO_2 0.147478 B1 → <BR/CO_2
Tier 2b (TDS)	Degradation rate from → to (FOCUS PELMO)	0.0099550 Active Substance → A1 0.0019960 Active Substance → B1

[4] Schriever, C., Gottesbüren, B., Ebert, D., Sur, R. Schmitt, W. (January 2013). ECPA background paper. Plant uptake of compounds via roots sytem – A hydroponic test system to determine plant uptake as input parameter for leaching models.

This is report give the results from 22 anonymised compounds that were tested in a hydroponic system to determine the plant uptake factore (PUF). The conclusion is that the PUF, irrespective of the Kow and pKa, of the 22 tested compounds is on average 1. The applicant intends to use this fact for the PUF for the metabolites DFA and 6-CNA can be set on 0.5. The RMS cannot accept this report since used compounds are anonymized, the tests are not concducted under GLP, an adopted test guideline for the hydroponic test is not available. Additionally, the specific metabolites DFA and 6-CNA were not tested in the this test system.

		0.0155070 A1 → <BR/CO_2 0.1474780 B1 → <BR/CO_2

Findings

The 80th percentile groundwater concentrations of BYI 02960 in hops are given in Table B.8.6.3-06 (Tier 1), Table B.8.6.3-07 (Tier 2a; DFOP) and Table B.8.6.3-08 (Tier 2b; TDS).

Table B.8.6.3-06 Tier 1 - PEC_{gw} of BYI 02960 – Use in Hops

Scenario	BYI 02960			
	Hops (every year), 1 x 150 g/ha, 60 % interception		Hops (every 2nd year), 1 x 150 g/ha, 60 % interception	
	PEARL PEC_{gw} [µg/L]	PELMO PEC_{gw} [µg/L]	PEARL PEC_{gw} [µg/L]	PELMO PEC_{gw} [µg/L]
Châteaudun	0.457	0.409	0.197	0.169
Hamburg	0.567	0.591	0.271	0.230
Kremsmuenster	0.409	0.461	0.169	0.189
Piacenza	0.377	0.458	0.162	0.210
Porto	0.214	0.275	*0.091*	0.115
Sevilla	0.219	*0.060*	*0.090*	*0.022*
Thiva	0.178	0.154	*0.073*	*0.058*

In italics: values pass the trigger of 0.1 µg/L

Considering the Tier 1 simulations predicted concentrations of BYI 02960 in groundwater are below the trigger of 0.1 µg/L in the scenario Sevilla (model PELMO) when applied every year and in scenarios Sevilla and Thiva (both PEARL and PELMO) and Porto (PEARL), when applied every second year.

Higher tier calculations have additionally been performed at Tier 2a using DFOP simulations and Tier 2b using TDS.

Table B.8.6.3-07 Tier 2a (DFOP) - PEC_{gw} of BYI 02960 – Use in Hops

Scenario	BYI 02960			
	Hops (every year), 1 x 150 g/ha		Hops (every 2nd year), 1 x 150 g/ha	
	PEARL PEC_{gw} [µg/L]	PELMO PEC_{gw} [µg/L]	PEARL PEC_{gw} [µg/L]	PELMO PEC_{gw} [µg/L]
Châteaudun	0.275	0.247	0.119	0.103
Hamburg	0.338	0.358	0.164	0.140
Kremsmuenster	0.246	0.277	0.102	0.114
Piacenza	0.224	0.275	*0.097*	0.126
Porto	0.130	0.166	*0.055*	*0.069*
Sevilla	0.134	*0.037*	*0.055*	*0.014*
Thiva	0.108	*0.094*	*0.045*	*0.036*

In italics: values pass the trigger of 0.1 µg/L

When considering the use of DFOP kinetics predicted concentrations of BYI 02960 in groundwater are below the trigger of 0.1 µg/L in the scenario Sevilla and Thiva (model PELMO) when applied every year and in scenarios Porto, Sevilla and Thiva (both PEARL and PELMO) and Piacenza (PEARL), when applied every second year.

Table B.8.6.3-08 Tier 2b (TDS) - PEC_{gw} of BYI 02960 – Use in Hops

Scenario	BYI 02960			
	Hops (every year), 1 x 150 g/ha, 60 % interception		Hops (every 2nd year), 1 x 150 g/ha, 60 % interception	
	PEARL PEC_{gw} [µg/L]	PELMO PEC_{gw} [µg/L]	PEARL PEC_{gw} [µg/L]	PELMO PEC_{gw} [µg/L]
Châteaudun	0.112	0.094	*0.042*	*0.032*
Hamburg	0.156	0.144	*0.061*	*0.053*
Kremsmuenster	0.117	0.134	*0.042*	*0.047*
Piacenza	0.109	0.152	*0.040*	*0.063*
Porto	*0.048*	*0.075*	*0.017*	*0.025*
Sevilla	*0.039*	*0.006*	*0.012*	*0.002*
Thiva	*0.026*	*0.020*	*0.008*	*0.006*

In italics: values pass the trigger of 0.1µg/L

When considering TDS behaviour the predicted concentrations of BYI 02960 in groundwater are below the trigger of 0.1 µg/L in the scenario Porto, Sevilla and Thiva (models PEARL and PELMO) when applied every year and in all scenarios (both PEARL and PELMO), when applied every second year.

Conclusion

A safe use has been demonstrated as the trigger of 0.1 µg/L is met at tier 1, considering application every year, for the scenario Sevilla (PELMO). For the higher tier calculations applying DFOP the trigger was met in Sevilla and Thiva for annual applications. When considering higher tier calculations with TDS the trigger was met in all scenarios for annual uses and all scenarios for use every second year.

Remarks

The RMS agrees with the risk assessment conducted for BYI 02960 in hops. RMS recalculated the PECgw and these were all similar. The RMS also agrees with the used higher tier approach.

PECgw for BYI 02960 in lettuce

STUDY IIIA, 9.6.1/04

Tier-1:

reference	:	Sur R., Ellerich C.	GLP statement	:	no
type of study	:	PECgw calculations	guideline	:	FOCUS 2000, SANCO/321/2000, rev. 2 FOCUS 2006, SANCO/10058/2005, rev. 2 FOCUS 2009, SANCO/13144/2010, version 1 FOCUS 2010, version 2.0
year of execution	:	2012	acceptability	:	**acceptable**
test substance	:	BYI 02960			

STUDY IIIA, 9.6.1/05

Tier-2a (DFOP):

reference	:	Sur R., Ellerich C.	GLP statement	:	no
type of study	:	PECgw calculations	guideline	:	FOCUS 2000, SANCO/321/2000, rev. 2 FOCUS 2006, SANCO/10058/2005, rev. 2 FOCUS 2009, SANCO/13144/2010, version 1 FOCUS 2010, version 2.0
year of execution	:	2012	acceptability	:	**acceptable**

test substance	:	BYI 02960			

STUDY IIIA, 9.6.1/06

Tier-2b (TDS):

reference	:	Sur R., Ellerich C.	GLP statement	:	no
type of study	:	PECgw calculations	guideline	:	FOCUS 2000, SANCO/321/2000, rev. 2 FOCUS 2006, SANCO/10058/2005, rev. 2 FOCUS 2009, SANCO/13144/2010, version 1 FOCUS 2010, version 2.0
year of execution	:	2012	acceptability	:	**acceptable**
test substance	:	BYI 02960			

STUDY IIIA, 9.6.1/07

reference	:	Scherr, F.	GLP statement	:	no
type of study	:	PECgw calculations	guideline	:	FOCUS 2000, SANCO/321/2000, rev. 2 FOCUS 2006, SANCO/10058/2005, rev. 2 FOCUS 2009, SANCO/13144/2010, version 1 FOCUS 2010, version 2.0
year of execution	:	2013	acceptability	:	**acceptable**
test substance	:	BYI 02960, DFA and 6-CNA			

STUDY IIIA, 9.6.1/08

reference	:	Scherr, F., Ellerich C.	GLP statement	:	no
type of study	:	PECgw calculations	guideline	:	FOCUS 2000, SANCO/321/2000, rev. 2 FOCUS 2006, SANCO/10058/2005, rev. 2 FOCUS 2009, SANCO/13144/2010, version 1 FOCUS 2010, version 2.0
year of execution	:	2013	acceptability	:	**acceptable**
test substance	:	BYI 02960, DFA and 6-CNA			

STUDY IIIA, 9.6.1/10

reference	:	Schierver C.. et al.	GLP statement	:	no
type of study	:	Plant uptake factor	guideline	:	not available
year of execution	:	2013	acceptability	:	**not acceptable**
test substance	:	22 unknown compound			

Materials and Methods

The predicted environmental concentrations in groundwater (PEC_{gw}) for BYI 02960 were calculated using the simulation model FOCUS PEARL (version 4.4.4) and FOCUS PELMO (4.4.3). Detailed application data used for simulation of PEC_{gw} are compiled in Table B.8.6.3-09 and Table B.8.6.3-10.

Although there is no currently agreed model for simulating groundwater concentrations following the use in greenhouses as a conservative approach the simulation was performed as an outdoor application considering two applications in accordance with the GAP.

Table B.8.6.3-09 Comparison of simulated and actual use pattern (outdoor use)

Individual Crop	FOCUS Crop Used for Interception	Application				Amount Reaching the Soil per Season application [g a.s. /ha]
		Rate per Season [g a.s. /ha]	Interval [days]	Plant Interception [%]	BBCH Stage	
Lettuce 1 (GAP, every year)	-	1 × 125	-	70	41 - 49	1 × 37.5
Lettuce 1 (GAP, every 2nd year)	-	1 × 125	-	25	12 - 49	1 × 93.75
Tier 1, Tier 2b (TDS)						
Lettuce 1 (every year) (early and late), Simulation 1	Cabbage	1 × 125	-	70	41 - 49	1 × 37.5
Lettuce 1 (every 2nd year) (early and late), Simulation 2	Cabbage	1 × 125	-	25	12 - 49	1 × 93.75
Tier 2a (DFOP)						
Lettuce 1 (every year) (early and late) Simulation 1	Cabbage	1 × 125	-	70	41 - 49	42.75[s] / 32.25[f]
Lettuce 1 (every 2nd year) (early and late) Simulation 2	Cabbage	1 × 125	-	25	12 – 49	106.875[s] / 80.625[f]

F = Field use (GAP: "Lettuce 1" = 1 x 125 g/ha)
[f] used for the fast compartment
[s] used for the slow compartment

Table B.8.6.3-10 Comparison of simulated and actual use pattern (glasshouse use)

Individual Crop	FOCUS Crop Used for Interception	Application				Amount Reaching the Soil per Season application [g a.s. /ha]
		Rate per Season [g a.s. /ha]	Interval [days]	Plant Interception [%]	BBCH Stage	
Lettuce 2 (every year), GAP	-	2 × 125	10	2 x 25	12 - 49	2 × 93.75
	Tier 1, Tier 2b (TDS)					
Lettuce 2 (every year) early	Cabbage	2 × 125	10	2 x 25	12 - 49	2 × 93.75
Lettuce 2 (every year) late	Cabbage	2 × 125	10	2 x 25	12 - 49	2 × 93.75
	Tier 2a (DFOP)					
Lettuce 2 (every year) ealy	Cabbage	2 × 125	10	2 x 25	12 - 49	2×106.875[s] / 2× 80.625[f]
Lettuce 2 (every year) late	Cabbage	2 × 125	10	2 x 25	12 - 49	2×106.875[s] / 2× 80.625[f]

G = Glasshouse use (GAP: "Lettuce 2" = 2 x 125 g/ha)
[f] used for the fast compartment
[s] used for the slow compartment

Application dates for the simulation runs were defined following the crop event dates of the respective crop and scenario as given by FOCUS (2009). In this assessment the first and the second cropping per year of lettuce were calculated separately (Table B.8.6.3-11).

Table B.8.6.3-11 First application dates and related information for BYI 02960 as used for the simulation runs (two sets of data are provided for crops with two crops per season)

Individual crop	Lettuce 1, 1st cropping	Lettuce 1, 2nd cropping	Lettuce 1, 1st cropping	Lettuce 1, 2nd cropping	Lettuce 2, 1st cropping	Lettuce 2, 2nd cropping

Repeat Interval for App. Events	Every Year	Every Year	Every 2nd Year	Every 2nd Year	Every Year	Every Year
Application Technique	Spray	Spray	Spray	Spray	Spray	Spray
Absolute / Relative to	Absolute	Absolute	Emergence	Emergence	Emergence	Emergence
Scenario	1st App. Date (Julian day)	1st App. Date (Julian day)	1st App. Date (Julian day) Offset	1st App. Date (Julian day) Offset	1st App. Date (Julian day) Offset	1st App. Date (Julian day) Offset
Chateaudun	25 Jun (176)	21 Sep (264)	25 Apr (115) 5	05 Aug (217) 5	25 Apr (115) 5	05 Aug (217) 5
Hamburg	25 Jun (176)	21 Sep (264)	25 Apr (115) 5	05 Aug (217) 5	25 Apr (115) 5	05 Aug (217) 5
Jokioinen	22 Aug (234)	-	27 May (147) 7	-	27 May (147) 7	-
Kremsmuenster	25 Jun (176)	21 Sep (264)	25 Apr (115) 5	05 Aug (217) 5	25 Apr (115) 5	05 Aug (217) 5
Okehampton	-	-	-	-	-	-
Piacenza	-	-	-	-	-	-
Porto	02 Jun (64)	12 Oct (285)	07 Mar (66) 7	05 Aug (217) 5	07 Mar (66) 7	05 Aug (217) 5
Sevilla	10 May (130)	17 Aug (229)	06 Mar (65) 5	20 Jun (171) 5	06 Mar (65) 5	20 Jun (171) 5
Thiva	05 Nov (309)	-	21 Aug (233) 6	-	21 Aug (233) 6	-

Further input parameters for PEC_{gw} modelling of BYI 02960 are summarised above in Table B.8.6.3-03 for BYI 02960 and in Table B.8.6.3-04 for the metabolites. Parameters used for degradation pathway in PEARL and PELMO are depicted in Table B.8.6.3-05.

Findings

The 80th percentile concentrations of BYI 02960 for the field and glasshouse uses in lettuce are given in the following tables.

Table B.8.6.3-12 Tier 1 PEC_{gw} of BYI 02960 – Use in Lettuce 1, single application (field use), every year

Scenario	BYI 02960 (field use / every year)			
	Lettuce 1, 1st cropping, every year, 1 x 125 g/ha		Lettuce 1, 2nd cropping, every year, 1 x 125 g/ha	
	PEARL PEC_{gw} [µg/L]	PELMO PEC_{gw} [µg/L]	PEARL PEC_{gw} [µg/L]	PELMO PEC_{gw} [µg/L]
Châteaudun	0.106	*0.065*	0.135	0.089
Hamburg	0.259	0.212	0.319	0.286
Jokioinen	*0.095*	*0.080*	-	-
Kremsmuenster	0.175	0.149	0.216	0.185
Porto	0.106	0.130	0.211	0.253
Sevilla	*0.002*	*0.001*	*0.004*	*0.001*
Thiva	*0.043*	*0.031*	-	-

In italics: values pass the trigger of 0.1µg/L

Table B.8.6.3-13 Tier 1 PEC_{gw} of BYI 02960 – Use in Lettuce 1, single application (field use), every 2nd year

Scenario	BYI 02960 (field use / every 2nd year)			
	Lettuce 1, 1st cropping, every 2nd year, 1 x 125 g/ha		Lettuce 1, 2nd cropping, every 2nd year, 1 x 125 g/ha	
	PEARL PEC_{gw} [µg/L]	PELMO PEC_{gw} [µg/L]	PEARL PEC_{gw} [µg/L]	PELMO PEC_{gw} [µg/L]
Châteaudun	0.154	0.106	0.207	0.137
Hamburg	0.379	0.288	0.443	0.389

Jokioinen	0.110	*0.084*	-	-
Kremsmuenster	0.242	0.203	0.291	0.244
Porto	0.138	0.169	0.253	0.302
Sevilla	*0.006*	*0.002*	*0.009*	*0.002*
Thiva	0.111	*0.065*	-	-

In italics: values pass the trigger of 0.1µg/L

For the field use, considering the tier 1 approach the trigger value of 0. 1µg/L is met for the scenario Sevilla for both seasons and models for annual and uses every second year.

Table B.8.6.3-14 Tier 2a (DFOP) PEC_{gw} of BYI 02960 – Use in Lettuce 1, single application (field use), every year

Scenario	BYI 02960 (field use / every year / DFOP)			
	Lettuce 1, 1st cropping, every year, 1 x 125 g/ha		Lettuce 1, 2nd cropping, every year, 1 x 125 g/ha	
	PEARL PEC_{gw} [µg/L]	PELMO PEC_{gw} [µg/L]	PEARL PEC_{gw} [µg/L]	PELMO PEC_{gw} [µg/L]
Châteaudun	*0.065*	*0.040*	*0.083*	0.056
Hamburg	0.156	0.129	0.193	0.175
Jokioinen	*0.059*	*0.050*	-	-
Kremsmuenster	0.106	*0.090*	0.130	0.113
Porto	*0.064*	*0.078*	0.128	0.155
Sevilla	*0.002*	*0.001*	*0.003*	*0.001*
Thiva	*0.027*	*0.020*	-	-

In italics: values pass the trigger of 0.1µg/L

Table B.8.6.3-15 Tier 2a (DFOP) PEC_{gw} of BYI 02960 – Use in Lettuce 1, single application (field use), every 2nd year

Scenario	BYI 02960 (field use / every 2nd year / DFOP)			
	Lettuce 1, 1st cropping, every 2nd year, 1 x 125 g/ha		Lettuce 1, 2nd cropping, every 2nd year, 1 x 125 g/ha	
	PEARL PEC_{gw} [µg/L]	PELMO PEC_{gw} [µg/L]	PEARL PEC_{gw} [µg/L]	PELMO PEC_{gw} [µg/L]
Châteaudun	*0.095*	*0.065*	0.126	*0.083*
Hamburg	0.230	0.173	0.270	0.237
Jokioinen	*0.069*	*0.053*	-	-
Kremsmuenster	0.146	0.124	0.177	0.149
Porto	*0.083*	0.101	0.153	0.178
Sevilla	*0.004*	*0.001*	*0.006*	*0.002*
Thiva	*0.069*	*0.041*	-	-

In italics: values pass the trigger of 0.1µg/L

For the outdoor use in lettuce, using the Tier 2a approach (DFOP) the trigger is met for the scenario Sevilla for annual applications and the scenarios Châteaudun, Jokioinen, Porto, Sevilla and Thiva considering applications every second year.

Table B.8.6.3-16 Tier-2b (TDS) PEC_{gw} of BYI 02960 – Use in Lettuce 1, single application (field use), every year (TDS)

Scenario	BYI 02960 (field use / every year / TDS)			
	Lettuce 1, 1st cropping, every year, 1 x 125 g/ha		Lettuce 1, 2nd cropping, every year, 1 x 125 g/ha	
	PEARL	PELMO	PEARL	PELMO

	PEC_{gw} [µg/L]	PEC_{gw} [µg/L]	PEC_{gw} [µg/L]	PEC_{gw} [µg/L]
Châteaudun	*0.013*	*0.006*	*0.020*	*0.011*
Hamburg	*0.070*	*0.049*	*0.091*	*0.068*
Jokioinen	*0.010*	*0.008*	-	-
Kremsmuenster	*0.038*	*0.031*	*0.051*	*0.042*
Porto	*0.020*	*0.028*	*0.055*	*0.070*
Sevilla	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Thiva	*0.006*	*0.004*	-	-

In italics: values pass the trigger of 0.1µg/L

Table B.8.6.3-17 Tier-2b (TDS) PEC_{gw} of BYI 02960 – Use in Lettuce, single application (field use), every 2nd year (TDS)

Scenario	BYI 02960 (field use / every 2nd year / TDS)			
	Lettuce, 1st cropping, every 2nd year, 1 x 125 g/ha		Lettuce, 2nd cropping, every 2nd year, 1 x 125 g/ha	
	PEARL PEC_{gw} [µg/L]	PELMO PEC_{gw} [µg/L]	PEARL PEC_{gw} [µg/L]	PELMO PEC_{gw} [µg/L]
Châteaudun	*0.023*	*0.013*	*0.030*	*0.017*
Hamburg	*0.091*	*0.066*	0.122	*0.093*
Jokioinen	*0.013*	*0.009*	-	-
Kremsmuenster	*0.058*	*0.045*	*0.069*	*0.058*
Porto	*0.030*	*0.046*	*0.056*	*0.075*
Sevilla	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Thiva	*0.013*	*0.007*	-	-

In italics: values pass the trigger of 0.1µg/L

For the outdoor use in lettuce, using the Tier 2b approach (TDS) the trigger is met for the scenarios Châteaudun, Jokioinen, Porto, Sevilla and Thiva considering annual applications and for all scenarios except Hamburg late season for the application every second year.

Table B.8.6.3-18 Tier 1 PEC_{gw} of BYI 02960 – Use in Lettuce 2, multiple applications (glasshouse use)

Scenario	BYI 02960 (glasshouse use / every year)			
	Lettuce 2, 1st cropping, every year, 2 x 125 g/ha		Lettuce 2, 2nd cropping, every year, 2 x 125 g/ha	
	PEARL PEC_{gw} [µg/L]	PELMO PEC_{gw} [µg/L]	PEARL PEC_{gw} [µg/L]	PELMO PEC_{gw} [µg/L]
Châteaudun	1.132	0.786	1.543	1.073
Hamburg	2.179	1.879	2.843	2.540
Jokioinen	0.985	0.769	-	-
Kremsmuenster	1.503	1.330	1.819	1.654
Porto	0.877	0.980	1.691	1.787
Sevilla	*0.078*	*0.018*	0.116	*0.024*
Thiva	0.997	0.717	-	-

In italics: values pass the trigger of 0.1µg/L

For the greenhouse uses, considering the tier 1 approach the trigger value of 0.1µg/L is met for the scenario Sevilla for early season, both models and for late season one model even considering the conservative approach for the greenhouse uses.

Table B.8.6.3-19 Tier 2a (DFOP) PEC_{gw} of BYI 02960 – Use in Lettuce 2, multiple applications (glasshouse use)

Scenario	BYI 02960 (glasshouse use / every year / DFOP)			
	Lettuce 2, 1st cropping, every year, 2 x 125 g/ha		Lettuce 2, 2nd cropping, every year, 2 x 125 g/ha	
	PEARL PEC_{gw} [µg/L]	PELMO PEC_{gw} [µg/L]	PEARL PEC_{gw} [µg/L]	PELMO PEC_{gw} [µg/L]
Châteaudun	0.682	0.474	0.927	0.684
Hamburg	1.310	1.125	1.706	1.534
Jokioinen	0.598	0.473	-	-
Kremsmuenster	0.898	0.797	1.089	0.991
Porto	0.524	0.576	1.008	1.070
Sevilla	*0.050*	*0.012*	*0.076*	*0.016*
Thiva	0.606	0.438	-	-

In italics: values pass the trigger of 0.1µg/L

For the greenhouse use in lettuce, using the Tier 2a approach (DFOP) the trigger is met for the scenario Sevilla for annual application even considering the conservative use of the outdoor model.

Table B.8.6.3-20 Tier-2b (TDS) PEC_{gw} of BYI 02960 – Use in Lettuce 2, multiple applications (glasshouse use)

Scenario	BYI 02960 (glasshouse use / every year / TDS)			
	Lettuce 2, 1st cropping, every year, 2 x 125 g/ha		Lettuce 2, 2nd cropping, every year, 2 x 125 g/ha	
	PEARL PEC_{gw} [µg/L]	PELMO PEC_{gw} [µg/L]	PEARL PEC_{gw} [µg/L]	PELMO PEC_{gw} [µg/L]
Châteaudun	0.245	0.155	0.334	0.214
Hamburg	0.677	0.520	0.905	0.784
Jokioinen	0.185	0.145	-	-
Kremsmuenster	0.475	0.404	0.568	0.502
Porto	0.259	0.355	0.508	0.627
Sevilla	*0.006*	*0.001*	*0.007*	*0.001*
Thiva	0.181	0.119	-	-

In italics: values pass the trigger of 0.1µg/L

For the greenhouse use in lettuce, using the Tier 2b approach (TDS) the trigger is met for the scenario Sevilla for annual applications, even considering the conservative use of the outdoor model.

Conclusion

For all the uses considered safe use can be demonstrated in at least 1 scenario even considering tier 1 calculations. When higher tier (Tier 2) simulations are considered the calculated PEC_{gw} is less than the trigger in several scenarios.

Remarks

The RMS agrees with the risk assessment conducted for BYI 02960 in lettuce. RMS recalculated the PECgw and these were all similar. The RMS also agrees with the used tiered approach.

PECgw for BYI 02960 metabolites in hops

STUDY IIIA, 9.6.2/01

Tier-1:

reference	:	Sur R., Ellerich C.	GLP statement	:	no
type of study	:	PECgw calculations	guideline	:	FOCUS 2000, SANCO/321/2000, rev. 2 FOCUS 2006, SANCO/10058/2005, rev. 2 FOCUS 2009, SANCO/13144/2010, version 1 FOCUS 2010, version 2.0
year of execution	:	2012	acceptability	:	**acceptable**
test substance	:	BYI 02960			

STUDY IIIA, 9.6.2/02

Tier-2a (DFOP):

reference	:	Sur R., Ellerich C.	GLP statement	:	no
type of study	:	PECgw calculations	guideline	:	FOCUS 2000, SANCO/321/2000, rev. 2 FOCUS 2006, SANCO/10058/2005, rev. 2 FOCUS 2009, SANCO/13144/2010, version 1 FOCUS 2010, version 2.0
year of execution	:	2012	acceptability	:	**acceptable**
test substance	:	BYI 02960			

STUDY IIIA, 9.6.2/03

Tier-2b (TDS):

reference	:	Sur R., Ellerich C.	GLP statement	:	no
type of study	:	PECgw calculations	guideline	:	FOCUS 2000, SANCO/321/2000, rev. 2 FOCUS 2006, SANCO/10058/2005, rev. 2 FOCUS 2009, SANCO/13144/2010, version 1 FOCUS 2010, version 2.0
year of execution	:	2012	acceptability	:	**acceptable**
test substance	:	BYI 02960			

STUDY IIIA, 9.6.2/08

reference	:	Scherr, F., Ellerich C.	GLP statement	:	no
type of study	:	PECgw calculations	guideline	:	FOCUS 2000, SANCO/321/2000, rev. 2 FOCUS 2006, SANCO/10058/2005, rev. 2 FOCUS 2009, SANCO/13144/2010, version 1 FOCUS 2010, version 2.0
year of execution	:	2013	acceptability	:	**acceptable**
test substance	:	BYI 02960, DFA and 6-CNA			

STUDY IIIA, 9.6.2/09

reference	:	Scherr, F., Ellerich C.	GLP statement	:	no
type of study	:	PECgw calculations	guideline	:	FOCUS 2000, SANCO/321/2000, rev. 2 FOCUS 2006, SANCO/10058/2005, rev. 2 FOCUS 2009, SANCO/13144/2010, version 1 FOCUS 2010, version 2.0
year of execution	:	2014	acceptability	:	**acceptable**
test substance	:	BYI 02960, DFA and 6-CNA			

STUDY IIIA, 9.6.2/10

reference	:	Schierver C.. et al.	GLP statement	:	no
type of study	:	Plant uptake factor	guideline	:	not available
year of execution	:	2013	acceptability	:	**not acceptable**
test substance	:	22 unknown			

		compound			

Materials and Methods

PEC_{gw} for the metabolites were calculated using the approach, scenarios and application described for the calculations for the parent compound in studies IIIA 9.6.1. Compound specific input data for the metabolites are summarised together with the data of the parent compound in Table B.8.6.3-03b and Table B.8.6.3-04. Metabolites were simulated in the degradation schemes implemented in Pearl and Pelmo.

Findings: The 80th percentile concentrations for BYI 02960 metabolites for the different EU scenarios are presented in Table B.8.6.3-21 for Tier 1.

Table B.8.6.3-21 Tier 1 PEC_{gw} of BYI 02960 metabolites (after application of 150 g BYI 02960/ha) in hops

Scenario	Difluoroacetic acid (DFA) PEC_{gw} [µg/L]				6-Chloronicotinic acid (6-CNA) PEC_{gw} [µg/L]			
	every year		every 2nd year		every year		every 2nd year	
	PEARL	PELMO	PEARL	PELMO	PEARL	PELMO	PEARL	PELMO
Châteaudun	1.554	1.377	0.754	**0.697**	*0.010*	*0.009*	*0.005*	*0.004*
Hamburg	1.699	1.830	0.793	0.852	*0.011*	*0.012*	*0.006*	*0.005*
Kremsmuenster	1.064	1.228	**0.525**	**0.596**	*0.009*	*0.010*	*0.004*	*0.004*
Piacenza	1.059	0.957	**0.511**	**0.448**	*0.008*	*0.009*	*0.004*	*0.005*
Porto	**0.696**	0.758	**0.318**	**0.350**	*0.006*	*0.007*	*0.002*	*0.003*
Sevilla	0.976	0.776	**0.479**	**0.428**	*0.005*	*0.002*	*0.002*	*0.001*
Thiva	0.912	0.916	**0.450**	**0.426**	*0.004*	*0.004*	*0.002*	*0.002*

In italics: values pass the trigger of 0.1µg/L
In bold: values pass the trigger of 0.75 µg/L

PEC_{gw} values for the metabolite DFA are above 0.1 µg/L for all scenarios (both PEARL and PELMO), and for applications taking place every year or every 2nd year, respectively. PEC_{gw} values for the metabolite 6-CNA are below 0.1 µg/L for all scenarios (both PEARL and PELMO) for applications taking place every year or every 2nd year, respectively. There are no concerns for groundwater for this metabolite.

Additionally, for difluoroacetic acid (DFA), calculations combined with higher tier (DFOP and TDS) for parent have been performed, the results are summarized below Table B.8.6.3-22 and Table B.8.6.3-23.

Table B.8.6.3-22 Tier 2a (DFOP): PEC_{gw} of difluoroacetic acid (DFA) in hops (1 x 150 g/ha of parent)

Scenario	Difluoroacetic acid (DFA) PEC_{gw} [µg/L]			
	every year		every 2nd year	
	PEARL	PELMO	PEARL	PELMO
Châteaudun	1.459	1.299	**0.722**	**0.681**

Hamburg	1.631	1.771	0.772	0.836
Kremsmuenster	1.020	1.189	**0.515**	**0.592**
Piacenza	0.953	0.904	**0.473**	**0.439**
Porto	**0.608**	**0.691**	**0.277**	**0.315**
Sevilla	0.916	**0.715**	**0.459**	**0.424**
Thiva	0.817	0.838	**0.415**	**0.395**

In italics: values pass the trigger of 0.1µg/L
In bold: values pass the trigger of 0.75 µg/L

Table B.8.6.3-23 Tier 2b (TDS): PEC_{gw} of difluoroacetic acid (DFA) in hops (1 x 150 g/ha of parent)

Scenario	Difluoroacetic acid (DFA) PEC_{gw} [µg/L]			
	every year		every 2nd year	
	PEARL	PELMO	PEARL	PELMO
Châteaudun	1.475	1.295	**0.709**	**0.667**
Hamburg	1.637	1.777	0.756	**0.827**
Kremsmuenster	1.025	1.208	**0.498**	**0.569**
Piacenza	0.970	0.946	**0.481**	**0.429**
Porto	**0.641**	**0.723**	**0.288**	**0.330**
Sevilla	0.898	**0.732**	**0.439**	**0.408**
Thiva	0.809	0.841	**0.394**	**0.387**

In italics: values pass the trigger of 0.1µg/L
In bold: values pass the trigger of 0.75 µg/L

Conclusion

The groundwater concentration of the metabolite 6-CNA did not exceed the trigger in any scenario at Tier 1. The PEC in groundwater for the metabolite DFA may exceed the 0.1 µg/L and also the 0.75 µg/L trigger, but the concentration was < 10 µg/L in all scenarios. The relevance of the metabolite has been assessed in accordance with Sanco/221/2000 –rev.10 (2003): "Guidance Document on the Assessment of the Relevance of Metabolites in Groundwater", and the metabolite was not relevant in terms of efficacy (see IIA, 8.14.1), genotoxicity and toxicity (see IIA, 5.8/01 - 05) and has also been considered in the dietary risk assessment (see IIA, 6.9).

Please note: DFA was considered in the dietary risk assessment since it is a constituent of the plant residue definition.

Remarks

The RMS agrees with the risk assessment conducted for the metabolites of BYI 02960 in hops. RMS recalculated the PECgw and these were all similar. The RMS also agrees with the used higher tiered approach, though, based on the fact that DFA can be considered a non relevant metabolite with regard to groundwater exposure, these calculations are not required.

~~The RMS did not yet recalculated the PECgw based on the not acceptance of the Schriever et al study. Based on the expert meeting results on the discussion on aged sorption the values for PECgw will be updated and the adjusted PUF- for the metabolites will be included in these new model calculations.~~

PECgw for BYI 02960 metabolites in lettuce

STUDY IIIA, 9.6.2/04

Tier-1:

reference	:	Sur R., Ellerich C.	GLP statement	:	no
type of study	:	PECgw calculations	guideline	:	FOCUS 2000, SANCO/321/2000, rev. 2 FOCUS 2006, SANCO/10058/2005, rev. 2 FOCUS 2009, SANCO/13144/2010, version 1 FOCUS 2010, version 2.0
year of execution	:	2012	acceptability	:	**acceptable**
test substance	:	BYI 02960			

STUDY IIIA, 9.6.2/05

Tier-2a (DFOP):

reference	:	Sur R., Ellerich C.	GLP statement	:	no
type of study	:	PECgw calculations	guideline	:	FOCUS 2000, SANCO/321/2000, rev. 2 FOCUS 2006, SANCO/10058/2005, rev. 2 FOCUS 2009, SANCO/13144/2010, version 1 FOCUS 2010, version 2.0
year of execution	:	2012	acceptability	:	**acceptable**
test substance	:	BYI 02960			

STUDY IIIA, 9.6.2/06

Tier-2a (TDS):

reference	:	Sur R., Ellerich C.	GLP statement	:	no
type of study	:	PECgw calculations	guideline	:	FOCUS 2000, SANCO/321/2000, rev. 2 FOCUS 2006, SANCO/10058/2005, rev. 2 FOCUS 2009, SANCO/13144/2010, version 1 FOCUS 2010, version 2.0
year of execution	:	2012	acceptability	:	**acceptable**
test substance	:	BYI 02960			

STUDY IIIA, 9.6.2/07

reference	:	Scherr, F.	GLP statement	:	no
type of study	:	PECgw calculations	guideline	:	FOCUS 2000, SANCO/321/2000, rev. 2 FOCUS 2006, SANCO/10058/2005, rev. 2 FOCUS 2009, SANCO/13144/2010, version 1 FOCUS 2010, version 2.0
year of execution	:	2013	acceptability	:	**acceptable**
test substance	:	BYI 02960, DFA and 6-CNA			

STUDY IIIA, 9.6.2/08

reference	:	Scherr, F., Ellerich C.	GLP statement	:	no
type of study	:	PECgw calculations	guideline	:	FOCUS 2000, SANCO/321/2000, rev. 2 FOCUS 2006, SANCO/10058/2005, rev. 2 FOCUS 2009, SANCO/13144/2010, version 1 FOCUS 2010, version 2.0
year of execution	:	2013	acceptability	:	**acceptable**
test substance	:	BYI 02960, DFA and 6-CNA			

STUDY IIIA, 9.6.2/09

reference	:	Scherr, F., Ellerich C.	GLP statement	:	no
type of study	:	PECgw calculations	guideline	:	FOCUS 2000, SANCO/321/2000, rev. 2 FOCUS 2006, SANCO/10058/2005, rev. 2 FOCUS 2009, SANCO/13144/2010, version 1 FOCUS 2010, version 2.0
year of execution	:	2014	acceptability	:	**acceptable**

test substance	:	BYI 02960, DFA and 6-CNA			

STUDY IIIA, 9.6.2/10

reference type of study year of execution test substance	: : : :	Schierver C.. et al. Plant uptake factor 2013 22 unknown compound	GLP statement guideline acceptability	: : :	no not available **not acceptable**

Materials and Methods

PEC_{gw} for the metabolites were calculated using the approach, scenarios and application described for the calculations for the parent compound in Point 9.6.1. This includes the higher tier approaches for the parent substances using DFOP kinetics or TDS. Compound specific input data for the metabolites are summarised together with the data of the parent compound in Table B.8.6.3-03b and Table B.8.6.3-04.

Findings

The PEC_{gw} values for the metabolites for the different EU scenarios are presented for field and glasshouse uses in the following tables.

Table B.8.6.3-24 Tier 1 PEC_{gw} of BYI 02960 metabolites (field use in lettuce 1, 1st cropping)

Scenario	Field use Lettuce 1, 1st cropping, 1 x 125 g/ha of parent							
	Difluoroacetic acid (DFA) PEC_{gw} [µg/L]				6-chloronicotinic acid (6-CNA) PEC_{gw} [µg/L]			
	every year		every 2nd year		every year		every 2nd year	
	PEARL	PELMO	PEARL	PELMO	PEARL	PELMO	PEARL	PELMO
Châteaudun	**0.691**	**0.554**	0.847	**0.668**	*0.003*	*0.002*	*0.004*	*0.003*
Hamburg	1.165	0.990	1.393	1.169	*0.005*	*0.005*	*0.008*	*0.006*
Jokioinen	1.064	0.961	1.393	1.197	*0.003*	*0.002*	*0.003*	*0.003*
Kremsmuenster	**0.670**	**0.613**	0.852	**0.778**	*0.004*	*0.004*	*0.005*	*0.005*
Porto	**0.412**	**0.393**	**0.461**	**0.394**	*0.003*	*0.004*	*0.004*	*0.004*
Sevilla	**0.237**	**0.179**	**0.295**	**0.236**	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Thiva	**0.280**	**0.261**	**0.626**	**0.519**	*0.001*	*0.002*	*0.003*	*0.002*

In italics: values pass the trigger of 0.1 µg/L
In bold: values pass the trigger of 0.75 µg/L

Table B.8.6.3-25 Tier 1 PEC_{gw} of BYI 02960 metabolites (field use in lettuce 1, 2nd cropping)

Scenario	Field use Lettuce1 , 2nd cropping, 1 x 125 g/ha of parent							
	Difluoroacetic acid (DFA) PEC_{gw} [µg/L]				6-chloronicotinic acid (6-CNA) PEC_{gw} [µg/L]			
	every year		every 2nd year		every year		every 2nd year	
	PEARL	PELMO	PEARL	PELMO	PEARL	PELMO	PEARL	PELMO
Châteaudun	**0.691**	**0.567**	0.946	**0.748**	*0.003*	*0.003*	*0.005*	*0.003*
Hamburg	1.179	1.082	1.517	1.411	*0.007*	*0.006*	*0.009*	*0.008*
Kremsmuenster	**0.671**	**0.632**	0.892	**0.880**	*0.005*	*0.004*	*0.006*	*0.006*
Porto	**0.456**	**0.435**	**0.660**	**0.626**	*0.005*	*0.007*	*0.006*	*0.007*
Sevilla	**0.362**	**0.272**	**0.379**	**0.290**	*<0.001*	*<0.001*	*<0.001*	*<0.001*

In italics: values pass the trigger of 0.1 µg/L
In bold: values pass the trigger of 0.75 µg/L

Table B.8.6.3-26 Tier 2a (DFOP) PEC_{gw} of difluoroacetic acid (DFA) (field use in lettuce 1)

Scenario	Difluoroacetic acid (DFA) PEC_{gw} [µg/L]							
	Field use Lettuce 1, 1st cropping, 1 x 125 g/ha of parent (DFOP)				Field use Lettuce 1 , 2nd cropping, 1 x 125 g/ha of parent (DFOP)			
	every year		every 2nd year		every year		every 2nd year	
	PEARL	PELMO	PEARL	PELMO	PEARL	PELMO	PEARL	PELMO
Châteaudun	**0.657**	**0.543**	0.801	**0.651**	**0.654**	**0.558**	0.955	0.783
Hamburg	1.141	0.987	1.357	1.168	1.153	1.088	1.546	1.462
Jokioinen	1.037	0.974	1.372	1.207	-	-	-	-
Kremsmuenster	**0.650**	**0.618**	0.823	0.769	**0.651**	**0.638**	0.906	0.906
Porto	**0.373**	**0.374**	**0.397**	**0.365**	**0.479**	**0.472**	**0.688**	**0.653**
Sevilla	**0.211**	**0.164**	**0.258**	**0.219**	**0.376**	**0.291**	**0.369**	**0.293**
Thiva	**0.252**	**0.238**	**0.668**	**0.561**	-	-	-	-

In italics: values pass the trigger of 0.1 µg/L
In bold: values pass the trigger of 0.75 µg/L

Table B.8.6.3-27 Tier 2b (TDS): PEC_{gw} of BYI 02960 metabolites (field use in lettuce 1)

Scenario	Difluoroacetic acid (DFA) PEC_{gw} [µg/L]							
	Field use Lettuce 1, 1st cropping, 1 x 125 g/ha of parent (TDS)				Field use Lettuce 1, 2nd cropping, 1 x 125 g/ha of parent (TDS)			
	every year		every 2nd year		every year		every 2nd year	
	PEARL	PELMO	PEARL	PELMO	PEARL	PELMO	PEARL	PELMO
Châteaudun	**0.639**	**0.527**	**0.782**	**0.636**	**0.652**	**0.548**	**0.872**	**0.637**
Hamburg	1.133	0.970	1.344	1.169	1.148	1.068	1.475	1.116
Jokioinen	1.022	0.944	1.331	1.177	-	-	-	-
Kremsmuenster	**0.646**	**0.605**	**0.817**	**0.756**	**0.649**	**0.628**	0.855	0.758
Porto	**0.386**	**0.371**	**0.420**	**0.381**	**0.452**	**0.433**	**0.633**	**0.406**
Sevilla	**0.223**	**0.175**	**0.276**	**0.229**	**0.344**	**0.261**	**0.351**	**0.521**
Thiva	**0.250**	**0.242**	**0.584**	**0.492**	-	-	-	-

In italics: values pass the trigger of 0.1 µg/L
In bold: values pass the trigger of 0.75 µg/L

Considering the use in the field PEC_{gw} values for the metabolite DFA are above 0.1 µg/L for all scenarios (both PEARL and PELMO), and for applications taking place every year or every 2nd year, at Tier 1 and Tier 2.

PEC_{gw} values for the metabolite 6-CNA are below 0.1 µg/L for all scenarios (both PEARL and PELMO) at tier 1, for applications taking place every year or every 2nd year, respectively. There are no concerns for groundwater for this metabolite.

Table B.8.6.3-28 Tier 1 PEC_{gw} of BYI 02960 metabolites (glasshouse use in lettuce 2, 1st cropping)

Scenario	Glasshouse use Lettuce 2, 1st cropping, 2 x 125 g/ha of parent			
	Difluoroacetic acid (DFA) PEC_{gw} [µg/L]		6-chloronicotinic acid (6-CNA) PEC_{gw} [µg/L]	
	PEARL	PELMO	PEARL	PELMO
Châteaudun	3.792	3.026	*0.022*	*0.016*
Hamburg	6.542	5.246	*0.038*	*0.036*
Jokioinen	6.606	5.918	*0.021*	*0.018*
Kremsmuenster	3.748	3.411	*0.028*	*0.026*

Porto	2.145	1.863	*0.020*	*0.022*
Sevilla	1.257	0.949	*0.002*	*0.001*
Thiva	2.875	2.419	*0.019*	*0.015*

In italics: values pass the trigger of 0.1 µg/L
In bold: values pass the trigger of 0.75 µg/L

Table B.8.6.3-29 Tier 1 PEC_{gw} of BYI 02960 metabolites (glasshouse use in lettuce 2, 2nd cropping)

Scenario	Glasshouse use Lettuce 2, 2nd cropping, 2 x 125 g/ha of parent			
	Difluoroacetic acid (DFA) PEC_{gw} [µg/L]		6-chloronicotinic acid (6-CNA) PEC_{gw} [µg/L]	
	PEARL	PELMO	PEARL	PELMO
Châteaudun	4.287	3.547	*0.029*	*0.021*
Hamburg	6.976	6.277	*0.050*	*0.048*
Kremsmuenster	3.992	3.664	*0.034*	*0.031*
Porto	2.893	2.692	*0.035*	*0.038*
Sevilla	1.739	1.356	*0.003*	*0.001*

In italics: values pass the trigger of 0.1 µg/L
In bold: values pass the trigger of 0.75 µg/L

Table B.8.6.3-30 Tier 2a (DFOP) PEC_{gw} of difluoroacetic acid (DFA) (glasshouse use in lettuce 2)

Scenario	Difluoroacetic acid (DFA) PEC_{gw} [µg/L]			
	Glasshouse use Lettuce 2, 1st cropping, 2 x 125 g/ha of parent (DFOP)		Glasshouse use Lettuce 2, 2nd cropping, 2 x 125 g/ha of parent (DFOP)	
	PEARL	PELMO	PEARL	PELMO
Châteaudun	3.511	2.857	4.267	3.627
Hamburg	6.305	5.095	7.008	6.341
Jokioinen	6.390	5.751	-	-
Kremsmuenster	3.573	3.331	3.970	3.761
Porto	1.863	1.703	2.959	2.773
Sevilla	1.098	0.827	1.669	1.283
Thiva	3.020	2.649	-	-

In italics: values pass the trigger of 0.1 µg/L
In bold: values pass the trigger of 0.75 µg/L

Table B.8.6.3-31 Tier 2b (TDS): PEC_{gw} of difluoroacetic acid (DFA) (glasshouse use in lettuce 2)

Scenario	Difluoroacetic acid (DFA) PEC_{gw} [µg/L]			
	Glasshouse use Lettuce 2, 1st cropping, 2 x 125 g/ha of parent (DFOP)		Glasshouse use Lettuce 2, 2nd cropping, 2 x 125 g/ha of parent (DFOP)	
	PEARL	PELMO	PEARL	PELMO
Châteaudun	3.605	2.894	4.127	3.474
Hamburg	6.353	5.297	6.836	6.244
Jokioinen	6.230	5.829	-	-
Kremsmuenster	3.685	3.402	3.948	3.647
Porto	2.059	1.833	2.760	2.650
Sevilla	1.185	0.917	1.589	1.280
Thiva	2.727	2.416	-	-

In italics: values pass the trigger of 0.1 µg/L
In bold: values pass the trigger of 0.75 µg/L

Considering the use in the glasshouses the PEC_{gw} values for the metabolite DFA are above 0.1 µg/L for all scenarios (both PEARL and PELMO), and for applications taking place every year, at Tier 1 and Tier 2.

PEC_{gw} values for the metabolite 6-CNA are below 0.1 µg/L for all scenarios (both PEARL and PELMO) at tier 1, and for applications taking place every year, respectively. There are no concerns for groundwater for this metabolite.

Conclusion

The groundwater concentration of the metabolite 6-CNA did not exceed the trigger in any scenario. The PEC in groundwater for the metabolite DFA may exceed the 0.1 µg/L and also the 0.75 µg/L trigger, the concentration was < 10 µg/L in all scenarios The relevance of the metabolite has been assessed in accordance with Sanco/221/2000–rev.10 (2003): "Guidance Document on the Assessment of the Relevance of Metabolites in Groundwater", and the metabolite was not relevant in terms of efficacy (see IIA, 8.14.1), genotoxicity and toxicity (see IIA, 5.8/01 - 05) and has also been considered in the dietary risk assessment (see IIA, 6.9). Please note: DFA was considered in the dietary risk assessment since it is a constituent of the plant residue definition.

Remarks

The RMS agrees with the risk assessment conducted for the metabolites of BYI 02960 in lettuce. RMS recalculated the PECgw and these were all similar. The RMS also agrees with the used higher tiered approach, though, based on the fact that DFA can be considered a non relevant metabolite with regard to groundwater exposure, these calculations are not required. Since the PECgw concentration of DFA exceeds the 0.75 µg/L trigger for many scenarios the RMS conducted a consumer risk exposure with the highest PECgw for DFA of 7.008 µg/L (Hamburg scenario, Tier 2a (DFOP) second cropping). Drinking 2 L of groundwater with 7.008 µg/L DFA resulted in 0.23% of the average daily intake (ADI) for adults and 0.52% filling for toddlers thus fulfilling the criteria for consumer exposure.

~~The RMS did not yet recalculated the PECgw based on the not acceptance of the Schriever et al study. Based on the expert meeting results on the discussion on aged sorption the values for PECgw will be updated and the adjusted PUF for the metabolites will be included in these new model calculations.~~

Introduction

The previously reported tier 2b TDS results were calculated with the averages of the obtained parameter results of all the individual soils. The EU consultation indicated that the results of the results individual sets of parameters shoul be compared with the averages of the parameters of all soils. From the individual results per soil the median should calculated and compared with the results of the average parameters. This is done in the following study. The draft guidance proposal by Beulke and van Beinum (2012) was followed to present calculations of PECgw using time dependent sorption

parameters, i.e. compare median PECgw results from 4 individual soil parameter sets with PECgw results from a mean data set.

STUDY IIIA, 9.6.1/09

reference	:	Scherr, F., Ellerich C.	GLP statement	:	no
type of study	:	PECgw calculations	guideline	:	FOCUS 2000, SANCO/321/2000, rev. 2 FOCUS 2006, SANCO/10058/2005, rev. 2 FOCUS 2009, SANCO/13144/2010, version 1 FOCUS 2010, version 2.0
year of execution	:	2014	acceptability	:	**acceptable**
test substance	:	BYI 02960, DFA and 6-CNA			

Summary

Predicted environmental concentrations of the active substance flupyradifurone and its metabolites difluoroacetic acid (DFA) and 6-chloronicotinic acid (6-CNA) in groundwater recharge (PECgw) were calculated for the use in Europe, using the simulation models FOCUS PEARL 4.4.4 (Leistra et al. 2001) and FOCUS PELMO 5.5.3 (Jene 1998; Klein 1995, 1999, 2011). PECgw were evaluated as the 80th percentile of the mean annual leachate concentration at 1 m soil depth. Model parameters and scenarios consisting of weather, soil, and crop data were used as proposed by FOCUS (2009).

The use in lettuce and hops was investigated. Main substance parameters used in this assessment are given in Table B.8.6.3-32 and Table B.8.6.3-33. Parameters for Flupyradifurone represent Tier 2 parameterization using time dependent sorption. The draft guidance proposal by Beulke and van Beinum (2012) was followed to present calculations of PECgw using time dependent sorption parameters, i.e. compare results from individual parameter sets per soil with the overall average parameters.

Detailed application parameters are presented in Table B.8.6.3-01, Table B.8.6.3-02, Table B.8.6.3-09, Table B.8.6.3-10 and Table B.8.6.3-11.

Table B.8.6.3-32 Degradation and time dependent sorption data of flupyradifurone for individual soils and the overall mean parameter set.

Compound	**DT50_eq** [days]	**Koc_eq** [mL/g]	**Kom_eq** [mL/g]	**f_ne** [-]	**k_des** [1/d]	**Freundlich** exponent
Flupyradifurone						
AXXa	57.1	70.2	40.7	0.7808	0.0306	0.8445
Dollendorf	49.9	68.8	39.9	0.4738	0.0282	0.8648
HanscheiderHof	86.4	109.3	63.4	0.6865	0.0316	0.8643
Hoefchen	45.6	79.5	46.1	0.3875	0.0333	0.8682
arithmetic mean		**81.9**	**47.5**	**0.5821**		**0.8605**
geometric mean	**57.9**				**0.0309**	

Table B.8.6.3-33 Degradation and adsorption data of flupyradifurone metabolites

Compound	**DT50** [days]	**Koc** [mL/g]	**Kom** [mL/g]	**Freundlich exponent**
Difluoroacetic acid	44.7	6.8	3.9	0.8348

6-Chloronicotinic acid	4.7	88.0	51.0	0.9495

Maximum median PECgw results from 4 individual soil parameter sets are presented together with PECgw results from a mean data set in the following tables Table B.8.6.3-35 and Table B.8.6.3-36. PECgw calculations using time dependent sorption parameter sets from 4 individual soils may yield in some cases higher values, however, these are not meant to base a risk management decision upon Beulke and van Beinum, 2012.

Table B.8.6.3-35 Max. FOCUS PEARL 4.4.4 PECgw of flupyradifurone and metabolites in µg/L for the uses assessed

Use Pattern	**Flupyradifurone** µg/L	**Difluoroacetic acid** µg/L	**6-Chloronicotinic acid** µg/L
Max. median PECgw of individual soils			
Lettuce 1 first cropping, 1×125 g a.s./ha[1]	0.079	1.128	0.003
Lettuce 1 second cropping, 1×125 g a.s./ha[1]	0.108	1.154	0.004
Lettuce 1 first cropping, 1×125 g a.s./ha[2]	0.108	1.344	0.004
Lettuce 1 second cropping, 1×125 g a.s./ha[2]	0.147	1.492	0.005
Lettuce 2 first cropping, 2×125 g a.s./ha[1]	0.778	6.351	0.024
Lettuce 2 second cropping, 2×125 g a.s./ha[1]	1.092	6.883	0.032
Hops, 1×150 g a.s./ha[1]	0.185	1.641	0.007
Hops, 1×150 g a.s./ha[2]	0.076	0.763	0.003
Calculated with mean-input parameters			
Lettuce 1 first cropping, 1×125 g a.s./ha[1]	0.070	1.133	0.003
Lettuce 1 second cropping, 1×125 g a.s./ha[1]	0.091	1.148	0.003
Lettuce 1 first cropping, 1×125 g a.s./ha[2]	0.091	1.340	0.003
Lettuce 1 second cropping, 1×125 g a.s./ha[2]	0.122	1.475	0.004
Lettuce 2 first cropping, 2×125 g a.s./ha[1]	0.677	6.353	0.020
Lettuce 2 second cropping, 2×125 g a.s./ha[1]	0.905	6.836	0.027
Hops, 1×150 g a.s./ha[1]	0.156	1.637	0.005
Hops, 1×150 g a.s./ha[2]	0.061	0.756	0.002

[1] every year, [2] every 2nd year

Table B.8.6.3-36 Max. FOCUS PELMO 5.5.3 PECgw of flupyradifurone and metabolites in µg/L for the uses assessed

Use Pattern	**Flupyradifurone** µg/L	**Difluoroacetic acid** µg/L	**6-Chloronicotinic acid** µg/L
Max. median PECgw of individual soils			
Lettuce 1 first cropping, 1×125 g a.s./ha[1]	0.059	0.969	0.003
Lettuce 1 second cropping, 1×125 g a.s./ha[1]	0.089	1.075	0.004
Lettuce 1 first cropping, 1×125 g a.s./ha[2]	0.078	1.176	0.003
Lettuce 1 second cropping, 1×125 g a.s./ha[2]	0.114	1.389	0.005
Lettuce 2 first cropping, 2×125 g a.s./ha[1]	0.639	5.796	0.021
Lettuce 2 second cropping, 2×125 g a.s./ha[1]	0.920	6.269	0.029
Hops, 1×150 g a.s./ha[1]	0.178	1.781	0.007
Hops, 1×150 g a.s./ha[2]	0.072	0.830	0.003
Calculated with mean-input parameters			
Lettuce 1 first cropping, 1×125 g a.s./ha[1]	0.049	0.970	0.002
Lettuce 1 second cropping, 1×125 g a.s./ha[1]	0.070	1.068	0.003
Lettuce 1 first cropping, 1×125 g a.s./ha[2]	0.066	1.177	0.003
Lettuce 1 second cropping, 1×125 g a.s./ha[2]	0.093	1.375	0.004
Lettuce 2 first cropping, 2×125 g a.s./ha[1]	0.520	5.829	0.018
Lettuce 2 second cropping, 2×125 g a.s./ha[1]	0.748	6.244	0.024
Hops, 1×150 g a.s./ha[1]	0.152	1.777	0.005
Hops, 1×150 g a.s./ha[2]	0.063	0.827	0.002

[1] every year, [2] every 2nd year

Modelling approach

Time-dependent sorption (TDS) data of Flupyradifuroneon four soils were derived via curve fitting (Sur and Scherr, 2012). These parameters constitute the prerequisite to adequately address TDS processes in regulatory exposure modelling. TDS was evaluated according to the model of Boesten et al. (1989) implemented in the groundwater leaching models PEARL and FOCUS-PELMO (FOCUS, 2009). The determination of the TDS parameters followed the guidance of Beulke et al. (2010). This guidance was adopted later and a revised version was published in 2012 (Beulke and van Beinum, 2012). Therefore, a new evaluation was conducted to follow the new requirements by Scherr (2014).

The experimental raw data were pre-processed to calculate the concentration in the $CaCl_2$ desorption solution, and the total mass. These data were used by Sur and Scherr (2012) and by Scherr (2012) to fit the TDS model using PEARLNEQ (Boesten et al., 2007).

The evaluation showed that the experimental data was well described by the TDS model, with excellent fits and reliable parameter estimates. The inferred parameters were

- the desorption rate constant kdes,
- the ratio between the Freundlich coefficients in the non-equilibrium and in the equilibrium compartment fne,
- the degradation half-life in the equilibrium compartment DT50eq,
- the organic matter normalized distribution coefficient in the equilibrium compartment Kom,eq,
- and the initial total mass in the system Mini.

The corresponding values for all soils are given in Table B.8.6.3-37 along with the χ2-error percentage value. Shown are the parameter estimations with the lowest out of four starting value combinations. Details of the starting value dependence can be retrieved from Scherr (2014). In general the starting values for fne and kdes had virtually no impact on the parameter estimates. Low values of χ2, i.e. <15 % point towards a good description of the model to the fitted data. Reliable parameter estimates are indicated by low values for the relative standard error (RSE) of the individual parameter estimates, also given in Table B.8.6.3-37. None of the RSE values exceeds the minimum criteria of ≤ 0.4 given in Beulke and van Beinum (2012).

The kinetic desorption rate constant kdes indicates a time scale of about 22 d, calculated as "pseudo half-life" for the exchange between equilibrium and non-equilibrium domain. The criterion for an acceptable range of kdes outlined in Beulke and van Beinum (2012) accounting for less than 0.5 (1/d) is met by the present evaluation.

Values for fne in the range of 0.3875 – 0.7808 indicate that the kinetically controlled "sorption capacity" is in the range of 39 - 78% of the instantaneous sorption capacity, and accounts on average for about

58%. The fitted values of fne are well below the criterion (i.e. fne < 10) given by Beulke and van Beinum (2012).

The DT50eq represents degradation in the equilibrium domain and accounts on average (geom. mean) for 57.9 d suggesting moderate degradation during the laboratory study.

Table B.8.6.3-37 Time-dependent sorption parameters of Flupyradifurone used for Tier 2a leaching (Scherr, 2014). Numbers in brackets indicate rel. std. error RSE for the individual parameter.

Soil	M_ini [µg]	k_des [1/d]	f_ne [-]	DT50_eq [d]	Kom_eq [L/kg]	Chi^2-err [%]
AXXa	44.6 (0.0144)	0.0306 (0.1319)	0.7808 (0.1066)	57.1 (0.0397)	40.7 (0.1188)	9.68
Dollendorf	46.0 (0.0077)	0.0282 (0.0906)	0.4738 (0.0566)	49.9 (0.0181)	39.9 (0.0262)	4.90
Hanscheider Hof	46.0 (0.0090)	0.0316 (0.0906)	0.6865 (0.00610)	86.4 (0.0313)	63.4 (0.0380)	4.28
Hoefchen	47.7 (0.0071)	0.0333 (0.0995)	0.3875 (0.00654)	45.6 (0.0173)	46.1 (0.0419)	3.32
arithmetic mean			**0.5821**		**47.5**	
geometric mean		**0.0309**		**57.9**		

Method

The calculations were (where applicable) based on the maximum intended application rate together with the maximum intended number of applications per season and the minimum interval between two applications (where applicable).

Application dates for the simulation runs were defined following the crop event dates of the respective crop and scenario as given by FOCUS (2009). Crop interception was taken into account according to the BBCH growth stage, as recommended by FOCUS (2010).

Simulation Period

The simulations are carried out over 26 years for pesticides which are applied every year. The simulation length increases to 46 and 66 years for pesticides which are applied only every second and third year, respectively. The first 6 years are intended as a so called ‘warm up’ period. The following years are taken into account for the assessment of the potential leaching behaviour.

Compound Input Parameters

Substance parameters used as inputs in the simulations are summarised in Table B.8.6.3-38. Detailed information about the formation fractions and degradation rates is given in Table B.8.6.3-39.

Please note that any substance code in the following tables is just an internal identifier of the substance in the particular model and is not necessarily the same as the substance code as defined within the rest of this document.

Table B.8.6.3-38 Compound input parameters for flupyradifurone and its metabolites. sections with multiple values for individual soil parameter sets are initiated by a shaded row.

Parameter	Unit	Flupyradifurone	Difluoroacetic acid	6-Chloronicotinic acid
Common				
Molar Mass	[g/mol]	288.68	96.03	157.56 [A]
Solubility	[mg/L]	3200	500 000	1430
Vapour Pressure	[Pa]	9.10E-07	1E-10	1E-10
Q10		2.58	2.58	2.58
fne				
AXXa		0.7808		
Dollendorf		0.4738		
HanscheiderHof		0.6865		
Hoefchen		0.3875		
mean		0.5821	0	0
Desorp. Rate Coeff.	[1/days]			
AXXa		0.0306		
Dollendorf		0.0282		
HanscheiderHof		0.0316		
Hoefchen		0.0333		
mean		0.0309	0	0
Freundlich Exponent 1/n				
AXXa		0.8445		
Dollendorf		0.8648		
HanscheiderHof		0.8643		
Hoefchen		0.8682		
mean		0.8605	0.8348	0.9495
Plant Uptake Factor		0.5	0.0	0.0
Walker Exponent		0.7	0.7	0.7
PEARL Parameters				
Substance Code		BUTk	DFA	CNA
DT50eq	[days]			
AXXa		57.1		
Dollendorf		49.9		
HanscheiderHof		86.4		
Hoefchen		45.6		
mean		57.9	44.7	4.7
Molar Activ. Energy	[kJ/mol]	65.4	65.4	65.4
Kom, eq	[mL/g]			
AXXa		40.7		
Dollendorf		39.9		
HanscheiderHof		63.4		
Hoefchen		46.1		
mean		47.5	3.9	51

Table B.8.6.3-39 Degradation pathway related parameters for flupyradifurone and its metabolites

Degradation fraction from → to (FOCUS **PEARL**)	0.478 BUTk -> CNA 0.833 BUTk -> DFA
Degradation rate from → to (FOCUS **PELMO**)	
AXXa-parameter	0.010112 Active Substance -> A1 0.002027 Active Substance -> B1 0.0155070 A1 -> <BR/CO2 0.1474780 B1 -> <BR/CO2
Dollendorf-parameter	0.011571 Active Substance -> A1

	0.002320 Active Substance -> B1 0.0155070 A1 -> <BR/CO2 0.1474780 B1 -> <BR/CO2
HanscheiderHof-parameter	0.0066830 Active Substance -> A1 0.0013400 Active Substance -> B1 0.0155070 A1 -> <BR/CO2 0.1474780 B1 -> <BR/CO2
Hoefchen-parameter	0.0126620 Active Substance -> A1 0.0025380 Active Substance -> B1 0.0155070 A1 -> <BR/CO2 0.1474780 B1 -> <BR/CO2
mean-parameter	0.009972 Active Substance -> A1 0.001999 Active Substance -> B1 0.0155070 A1 -> <BR/CO2 0.1474780 B1 -> <BR/CO2

Molecule splitting with FOCUS-PELMO

The sum of molar formation fractions from the parent to both metabolites accounts for more than 1 (0.833 + 0.478), which cannot be implemented in PELMO. Therefore, with the standard approach only a single metabolite can be calculated in one PELMO run (in contrast to PEARL where individual formation fractions for metabolites are considered within the program code).

To calculate concentrations of both metabolites DFA and 6-CNA simultaneously in a single PELMO run the following procedure was applied: The molar mass of metabolite 6-CNA was artificially increased to account for the decreased formation fraction. By increasing the molar mass a heavier metabolite is formed. On a mass per volume assessment, i.e. the concentration in groundwater, this heavier metabolite equals the lighter one with a higher formation fraction.

A detailed example calculation for Tier 2a (TDS) is presented below.

DFA: The split degradation rate from Flupyradifurone (BYI 02960) to DFA is calculated as usual:

$$k_{FPF - DFA} = \ln 2 / (DT50soil\ 57.9\ d) \times 0.833 = 0.009972\ 1/d$$

6-CNA: Only a formation fraction of (1 - 0.833) is left to be used with 6-CNA, which is not sufficient, as the correct formation fraction is 0.478. Therefore, to guarantee a sufficient mass flux into this compartment, the molar mass of 6-CNA needs to be increased to obtain correct leaching concentrations of this metabolite:

$$k_{FPF - CNA} = \ln 2 / (DT50soil\ 57.9\ d) \times (1 - 0.833) = 0.001999\ d^{-1}$$

$$M_{auxiliary}\ (6\text{-}CNA) = M\ (6\text{-}CNA) \times 0.478 / (1 - 0.833) = 451\ g/mol$$

Results

A Tier 2b groundwater assessment is carried out for flupyradifurone, using time dependant sorption (TDS) parameters following the draft guidance proposal by Beulke and van Beinum (2012) to present calculations of PECgw using time dependent sorption parameters, i.e. compare median PECgw results from 4 individual soil parameter sets with PECgw results from a mean data set (Pearl 4.4.4: Table B.8.6.3-35; Pelmo 5.5.3: Table B.8.6.3-36). An overview of the maximum PECgw values obtained with

FOCUS PEARL 4.4.4 is given in Table B.8.6.3-40. Detailed FOCUS PEARL results for each scenario are listed in the following tables. Detailed results of the FOCUS PELMO 5.5.3 runs can be found in Table B.8.6.3-41. Representative examples. Detailed FOCUS PELMO results for each scenario are listed in the following tables.

Table B.8.6.3-40 Max. FOCUS PEARL 4.4.4 PECgw of flupyradifurone and its metabolites in µg/L for the uses assessed

Use Pattern	**Flupyradifurone** µg/L	**Difluoroacetic acid** µg/L	**6-Chloronicotinic acid** µg/L
Calculated with **AXXa**-parameters			
Lettuce 1 first cropping, 1×125 g a.s./ha[1]	0.075	1.131	0.003
Lettuce 1 second cropping, 1×125 g a.s./ha[1]	0.098	1.154	0.004
Lettuce 1 first cropping, 1×125 g a.s./ha[2]	0.100	1.344	0.004
Lettuce 1 second cropping, 1×125 g a.s./ha[2]	0.135	1.473	0.005
Lettuce 2 first cropping, 2×125 g a.s./ha[1]	0.812	6.392	0.024
Lettuce 2 second cropping, 2×125 g a.s./ha[1]	1.053	6.879	0.030
Hops, 1×150 g a.s./ha[1]	0.180	1.644	0.006
Hops, 1×150 g a.s./ha[2]	0.064	0.759	0.002
Calculated with **Dollendorf**-parameters			
Lettuce 1 first cropping, 1×125 g a.s./ha[1]	0.082	1.142	0.003
Lettuce 1 second cropping, 1×125 g a.s./ha[1]	0.117	1.164	0.004
Lettuce 1 first cropping, 1×125 g a.s./ha[2]	0.116	1.344	0.004
Lettuce 1 second cropping, 1×125 g a.s./ha[2]	0.159	1.511	0.005
Lettuce 2 first cropping, 2×125 g a.s./ha[1]	0.743	6.310	0.023
Lettuce 2 second cropping, 2×125 g a.s./ha[1]	1.130	6.937	0.034
Hops, 1×150 g a.s./ha[1]	0.189	1.637	0.007
Hops, 1×150 g a.s./ha[2]	0.088	0.767	0.003
Calculated with **HanscheiderHof**-parameters			
Lettuce 1 first cropping, 1×125 g a.s./ha[1]	0.129	1.119	0.003
Lettuce 1 second cropping, 1×125 g a.s./ha[1]	0.150	1.154	0.004
Lettuce 1 first cropping, 1×125 g a.s./ha[2]	0.163	1.372	0.004
Lettuce 1 second cropping, 1×125 g a.s./ha[2]	0.188	1.467	0.005
Lettuce 2 first cropping, 2×125 g a.s./ha[1]	1.232	6.490	0.028
Lettuce 2 second cropping, 2×125 g a.s./ha[1]	1.479	6.825	0.033
Hops, 1×150 g a.s./ha[1]	0.284	1.661	0.007
Hops, 1×150 g a.s./ha[2]	0.102	0.769	0.003
Calculated with **Hoefchen**-parameters			
Lettuce 1 first cropping, 1×125 g a.s./ha[1]	0.032	1.125	0.002
Lettuce 1 second cropping, 1×125 g a.s./ha[1]	0.048	1.141	0.002
Lettuce 1 first cropping, 1×125 g a.s./ha[2]	0.046	1.325	0.002
Lettuce 1 second cropping, 1×125 g a.s./ha[2]	0.064	1.512	0.003
Lettuce 2 first cropping, 2×125 g a.s./ha[1]	0.327	6.202	0.013
Lettuce 2 second cropping, 2×125 g a.s./ha[1]	0.524	6.887	0.020
Hops, 1×150 g a.s./ha[1]	0.079	1.607	0.003
Hops, 1×150 g a.s./ha[2]	0.034	0.755	0.002
Calculated with mean-parameters			
Lettuce 1 first cropping, 1×125 g a.s./ha[1]	0.070	1.133	0.003
Lettuce 1 second cropping, 1×125 g a.s./ha[1]	0.091	1.148	0.003
Lettuce 1 first cropping, 1×125 g a.s./ha[2]	0.091	1.340	0.003
Lettuce 1 second cropping, 1×125 g a.s./ha[2]	0.122	1.475	0.004
Lettuce 2 first cropping, 2×125 g a.s./ha[1]	0.677	6.353	0.020
Lettuce 2 second cropping, 2×125 g a.s./ha[1]	0.905	6.836	0.027
Hops, 1×150 g a.s./ha[1]	0.156	1.637	0.005
Hops, 1×150 g a.s./ha[2]	0.061	0.756	0.002

[1] application every year, [2] application every second year

Table B.8.6.3-41 Max. FOCUS PELMO 5.5.3 PECgw of flupyradifurone and its metabolites in µg/L for the uses assessed

Use Pattern	Flupyradifurone µg/L	Difluoroacetic acid µg/L	6-Chloronicotinic acid µg/L
Calculated with **AXXa**-parameters			
Lettuce 1 first cropping, 1×125 g a.s./ha[1]	0.053	0.971	0.002
Lettuce 1 second cropping, 1×125 g a.s./ha[1]	0.073	1.066	0.004
Lettuce 1 first cropping, 1×125 g a.s./ha[2]	0.071	1.175	0.003
Lettuce 1 second cropping, 1×125 g a.s./ha[2]	0.100	1.366	0.004
Lettuce 2 first cropping, 2×125 g a.s./ha[1]	0.627	5.807	0.020
Lettuce 2 second cropping, 2×125 g a.s./ha[1]	0.873	6.214	0.028
Hops, 1×150 g a.s./ha[1]	0.168	1.787	0.006
Hops, 1×150 g a.s./ha[2]	0.066	0.827	0.003
Calculated with **Dollendorf**-parameters			
Lettuce 1 first cropping, 1×125 g a.s./ha[1]	0.064	0.982	0.003
Lettuce 1 second cropping, 1×125 g a.s./ha[1]	0.118	1.094	0.005
Lettuce 1 first cropping, 1×125 g a.s./ha[2]	0.084	1.171	0.003
Lettuce 1 second cropping, 1×125 g a.s./ha[2]	0.127	1.412	0.005
Lettuce 2 first cropping, 2×125 g a.s./ha[1]	0.651	5.826	0.021
Lettuce 2 second cropping, 2×125 g a.s./ha[1]	0.967	6.324	0.030
Hops, 1×150 g a.s./ha[1]	0.195	1.775	0.007
Hops, 1×150 g a.s./ha[2]	0.077	0.834	0.003
Calculated with **HanscheiderHof**-parameters			
Lettuce 1 first cropping, 1×125 g a.s./ha[1]	0.091	0.983	0.003
Lettuce 1 second cropping, 1×125 g a.s./ha[1]	0.110	1.036	0.004
Lettuce 1 first cropping, 1×125 g a.s./ha[2]	0.126	1.193	0.004
Lettuce 1 second cropping, 1×125 g a.s./ha[2]	0.151	1.340	0.005
Lettuce 2 first cropping, 2×125 g a.s./ha[1]	0.927	5.515	0.023
Lettuce 2 second cropping, 2×125 g a.s./ha[1]	1.193	6.070	0.029
Hops, 1×150 g a.s./ha[1]	0.265	1.812	0.007
Hops, 1×150 g a.s./ha[2]	0.100	0.832	0.003
Calculated with **Hoefchen**-parameters			
Lettuce 1 first cropping, 1×125 g a.s./ha[1]	0.022	0.980	0.001
Lettuce 1 second cropping, 1×125 g a.s./ha[1]	0.047	1.084	0.003
Lettuce 1 first cropping, 1×125 g a.s./ha[2]	0.033	1.176	0.002
Lettuce 1 second cropping, 1×125 g a.s./ha[2]	0.050	1.411	0.003
Lettuce 2 first cropping, 2×125 g a.s./ha[1]	0.278	5.784	0.012
Lettuce 2 second cropping, 2×125 g a.s./ha[1]	0.440	6.329	0.018
Hops, 1×150 g a.s./ha[1]	0.081	1.754	0.004
Hops, 1×150 g a.s./ha[2]	0.033	0.827	0.002
Calculated with mean-parameters			
Lettuce 1 first cropping, 1×125 g a.s./ha[1]	0.049	0.970	0.002
Lettuce 1 second cropping, 1×125 g a.s./ha[1]	0.070	1.068	0.003
Lettuce 1 first cropping, 1×125 g a.s./ha[2]	0.066	1.177	0.003
Lettuce 1 second cropping, 1×125 g a.s./ha[2]	0.093	1.375	0.004
Lettuce 2 first cropping, 2×125 g a.s./ha[1]	0.520	5.829	0.018
Lettuce 2 second cropping, 2×125 g a.s./ha[1]	0.748	6.244	0.024
Hops, 1×150 g a.s./ha[1]	0.152	1.777	0.005
Hops, 1×150 g a.s./ha[2]	0.063	0.827	0.002

[1] application every year, [2] application every second year

Lettuce 1, first cropping, annual application

Table B.8.6.3-41 FOCUS PEARL 4.4.4 PECgw of flupyradifurone and its metabolites in µg/L (Lettuce 1, BBCH 41-49, 1×125 g a.s./ha, 70% interception, 1st crop, every year)

Scenario	Flupyradifurone µg/L	Difluoroacetic acid µg/L	6-Chloronicotinic acid µg/L
Calculated with **AXXa**-parameters			
Chateaudun	0.013	0.639	<0.001
Hamburg	0.075	1.131	0.003
Jokioinen	0.009	1.027	<0.001
Kremsmuenster	0.041	0.645	0.002
Porto	0.020	0.388	0.001
Sevilla	<0.001	0.227	<0.001
Thiva	0.006	0.260	<0.001
Calculated with **Dollendorf**-parameters			
Chateaudun	0.018	0.644	<0.001
Hamburg	0.082	1.142	0.003
Jokioinen	0.021	1.031	0.001
Kremsmuenster	0.049	0.656	0.002
Porto	0.025	0.378	0.001
Sevilla	<0.001	0.209	<0.001
Thiva	0.011	0.263	<0.001
Calculated with **HanscheiderHof**-parameters			
Chateaudun	0.035	0.648	0.001
Hamburg	0.129	1.119	0.003
Jokioinen	0.014	1.018	<0.001
Kremsmuenster	0.076	0.639	0.002
Porto	0.046	0.403	0.002
Sevilla	<0.001	0.246	<0.001
Thiva	0.013	0.278	<0.001
Calculated with **Hoefchen**-parameters			
Chateaudun	0.004	0.626	<0.001
Hamburg	0.032	1.125	0.002
Jokioinen	0.005	1.009	<0.001
Kremsmuenster	0.017	0.646	<0.001
Porto	0.008	0.366	<0.001
Sevilla	<0.001	0.203	<0.001
Thiva	0.002	0.240	<0.001
Median PECgw of individual soils			
Chateaudun	0.016	0.642	<0.001
Hamburg	0.079	1.128	0.003
Jokioinen	0.012	1.023	<0.001
Kremsmuenster	0.045	0.646	0.002
Porto	0.023	0.383	0.001
Sevilla	<0.001	0.218	<0.001
Thiva	0.009	0.262	<0.001
Calculated with mean-parameters			
Chateaudun	0.013	0.639	<0.001
Hamburg	**0.070**	1.133	0.003
Jokioinen	0.010	1.022	<0.001
Kremsmuenster	0.038	0.646	0.002
Porto	0.020	0.386	0.001
Sevilla	<0.001	0.223	<0.001
Thiva	0.006	0.250	<0.001

Table B.8.6.3-42 FOCUS PELMO 5.5.3 PECgw of flupyradifurone and its metabolites in µg/L (Lettuce 1, BBCH 41-49, 1×125 g a.s./ha, 70% interception, 1st crop, every year)

Scenario	**Flupyradifurone** µg/L	**Difluoroacetic acid** µg/L	**6-Chloronicotinic acid** µg/L
Calculated with **AXXa**-parameters			
Chateaudun	0.006	0.524	<0.001
Hamburg	0.053	0.971	0.002
Jokioinen	0.007	0.930	0.001
Kremsmuenster	0.032	0.603	0.002
Porto	0.029	0.371	0.002
Sevilla	<0.001	0.176	<0.001
Thiva	0.004	0.251	<0.001
Calculated with **Dollendorf**-parameters			
Chateaudun	0.009	0.532	<0.001
Hamburg	0.064	0.966	0.003
Jokioinen	0.017	0.982	0.001
Kremsmuenster	0.042	0.613	0.002
Porto	0.038	0.364	0.002
Sevilla	<0.001	0.163	<0.001
Thiva	0.007	0.247	<0.001
Calculated with **HanscheiderHof**-parameters			
Chateaudun	0.018	0.520	0.001
Hamburg	0.091	0.983	0.003
Jokioinen	0.010	0.895	0.001
Kremsmuenster	0.061	0.604	0.002
Porto	0.059	0.387	0.002
Sevilla	<0.001	0.188	<0.001
Thiva	0.009	0.268	<0.001
Calculated with **Hoefchen**-parameters			
Chateaudun	0.002	0.529	<0.001
Hamburg	0.022	0.963	0.001
Jokioinen	0.004	0.980	<0.001
Kremsmuenster	0.015	0.613	0.001
Porto	0.013	0.360	0.001
Sevilla	<0.001	0.164	<0.001
Thiva	0.002	0.231	<0.001
Median PECgw of individual soils			
Chateaudun	0.008	0.527	<0.001
Hamburg	0.059	0.969	0.003
Jokioinen	0.009	0.955	0.001
Kremsmuenster	0.037	0.609	0.002
Porto	0.034	0.368	0.002
Sevilla	<0.001	0.170	<0.001
Thiva	0.006	0.249	<0.001
Calculated with mean-parameters			
Chateaudun	0.006	0.527	<0.001
Hamburg	0.049	0.970	0.002
Jokioinen	0.008	0.944	0.001
Kremsmuenster	0.031	0.605	0.001
Porto	0.028	0.371	0.002
Sevilla	<0.001	0.175	<0.001
Thiva	0.004	0.242	<0.001

Lettuce 1, second cropping, yearly application

Table B.8.6.3-43 FOCUS PEARL 4.4.4 PECgw of flupyradifurone and its metabolites in µg/L (Lettuce 1, BBCH 41-49, 1×125 g a.s./ha, 70% interception, 2nd crop, every year)

Scenario	Flupyradifurone µg/L	Difluoroacetic acid µg/L	6-Chloronicotinic acid µg/L
Calculated with **AXXa**-parameters			
Chateaudun	0.021	0.657	<0.001
Hamburg	0.098	1.154	0.004
Kremsmuenster	0.054	0.653	0.002
Porto	0.053	0.449	0.003
Sevilla	<0.001	0.340	<0.001
Calculated with **Dollendorf**-parameters			
Chateaudun	0.029	0.662	0.001
Hamburg	0.117	1.164	0.004
Kremsmuenster	0.070	0.657	0.003
Porto	0.080	0.477	0.003
Sevilla	<0.001	0.360	<0.001
Calculated with **HanscheiderHof**-parameters			
Chateaudun	0.045	0.659	0.001
Hamburg	0.150	1.154	0.004
Kremsmuenster	0.091	0.648	0.003
Porto	0.078	0.428	0.003
Sevilla	<0.001	0.323	<0.001
Calculated with **Hoefchen**-parameters			
Chateaudun	0.008	0.636	<0.001
Hamburg	0.048	1.141	0.002
Kremsmuenster	0.026	0.641	0.001
Porto	0.033	0.472	0.002
Sevilla	<0.001	0.358	<0.001
Median PECgw of individual soils			
Chateaudun	0.025	0.658	0.001
Hamburg	0.108	1.154	0.004
Kremsmuenster	0.062	0.651	0.003
Porto	0.066	0.461	0.003
Sevilla	<0.001	0.349	<0.001
Calculated with mean-parameters			
Chateaudun	0.020	0.652	<0.001
Hamburg	**0.091**	1.148	0.003
Kremsmuenster	0.051	0.649	0.002
Porto	0.055	0.452	0.003
Sevilla	<0.001	0.344	<0.001

Table B.8.6.3-44 FOCUS PELMO 5.5.3 PECgw of flupyradifurone and its metabolites in µg/L (Lettuce 1, BBCH 41-49, 1×125 g a.s./ha, 70% interception, 2nd crop, every year)

Scenario	**Flupyradifurone** µg/L	**Difluoroacetic acid** µg/L	**6-Chloronicotinic acid** µg/L
Calculated with **AXXa**-parameters			
Chateaudun	0.011	0.547	0.001
Hamburg	0.073	1.066	0.003
Kremsmuenster	0.043	0.628	0.002
Porto	0.073	0.429	0.004
Sevilla	<0.001	0.257	<0.001
Calculated with **Dollendorf**-parameters			
Chateaudun	0.017	0.567	0.001
Hamburg	0.103	1.094	0.004
Kremsmuenster	0.060	0.640	0.002
Porto	0.118	0.464	0.005
Sevilla	<0.001	0.275	<0.001
Calculated with **HanscheiderHof**-parameters			
Chateaudun	0.025	0.530	0.001
Hamburg	0.110	1.036	0.004
Kremsmuenster	0.072	0.612	0.002
Porto	0.105	0.427	0.004
Sevilla	<0.001	0.247	<0.001
Calculated with **Hoefchen**-parameters			
Chateaudun	0.004	0.559	<0.001
Hamburg	0.039	1.084	0.002
Kremsmuenster	0.022	0.633	0.001
Porto	0.047	0.469	0.003
Sevilla	<0.001	0.279	<0.001
Median PECgw of individual soils			
Chateaudun	0.014	0.553	0.001
Hamburg	0.088	1.075	0.004
Kremsmuenster	0.052	0.631	0.002
Porto	0.089	0.447	0.004
Sevilla	<0.001	0.266	<0.001
Calculated with mean-parameters			
Chateaudun	0.011	0.548	0.001
Hamburg	0.068	1.068	0.003
Kremsmuenster	0.042	0.628	0.002
Porto	0.070	0.433	0.003
Sevilla	<0.001	0.261	<0.001

Lettuce 1, first cropping, biennial application

Table B.8.6.3-45 FOCUS PEARL 4.4.4 PECgw of flupyradifurone and its metabolites in µg/L (Lettuce 1, BBCH 12-49, 1×125 g a.s./ha, 25% interception, 1st crop, every 2nd year)

Scenario	Flupyradifurone µg/L	Difluoroacetic acid µg/L	6-Chloronicotinic acid µg/L
Calculated with **AXXa**-parameters			
Chateaudun	0.025	0.780	0.001
Hamburg	0.100	1.344	0.004
Jokioinen	0.013	1.329	<0.001
Kremsmuenster	0.061	0.819	0.002
Porto	0.032	0.435	0.002
Sevilla	<0.001	0.286	<0.001
Thiva	0.013	0.550	<0.001
Calculated with **Dollendorf**-parameters			
Chateaudun	0.029	0.804	0.001
Hamburg	0.116	1.344	0.004
Jokioinen	0.021	1.333	0.001
Kremsmuenster	0.069	0.827	0.003
Porto	0.036	0.404	0.002
Sevilla	<0.001	0.266	<0.001
Thiva	0.021	0.617	<0.001
Calculated with **HanscheiderHof**-parameters			
Chateaudun	0.052	0.781	0.002
Hamburg	0.163	1.372	0.004
Jokioinen	0.026	1.350	0.001
Kremsmuenster	0.111	0.814	0.003
Porto	0.065	0.470	0.002
Sevilla	<0.001	0.297	<0.001
Thiva	0.021	0.498	<0.001
Calculated with **Hoefchen**-parameters			
Chateaudun	0.007	0.783	<0.001
Hamburg	0.046	1.325	0.002
Jokioinen	0.004	1.313	<0.001
Kremsmuenster	0.026	0.813	0.001
Porto	0.012	0.385	<0.001
Sevilla	<0.001	0.257	<0.001
Thiva	0.006	0.618	<0.001
Median PECgw of individual soils			
Chateaudun	0.027	0.782	0.001
Hamburg	0.108	1.344	0.004
Jokioinen	0.017	1.331	0.001
Kremsmuenster	0.065	0.817	0.003
Porto	0.034	0.420	0.002
Sevilla	<0.001	0.276	<0.001
Thiva	0.017	0.584	<0.001
Calculated with mean-parameters			
Chateaudun	0.023	0.781	0.001
Hamburg	**0.091**	1.340	0.003
Jokioinen	0.013	1.327	<0.001
Kremsmuenster	0.058	0.818	0.002
Porto	0.030	0.428	0.002
Sevilla	<0.001	0.279	<0.001
Thiva	0.013	0.564	<0.001

Table B.8.6.3-46 FOCUS PELMO 5.5.3 PECgw of flupyradifurone and its metabolites in µg/L (Lettuce 1, BBCH 12-49, 1×125 g a.s./ha, 25% interception, 1st crop, every 2nd year)

Scenario	Flupyradifurone µg/L	Difluoroacetic acid µg/L	6-Chloronicotinic acid µg/L
Calculated with **AXXa**-parameters			
Chateaudun	0.014	0.635	0.001
Hamburg	0.071	1.166	0.003
Jokioinen	0.009	1.175	0.001
Kremsmuenster	0.046	0.759	0.002
Porto	0.049	0.387	0.003
Sevilla	<0.001	0.230	<0.001
Thiva	0.008	0.478	<0.001
Calculated with **Dollendorf**-parameters			
Chateaudun	0.018	0.649	0.001
Hamburg	0.084	1.154	0.003
Jokioinen	0.016	1.171	0.001
Kremsmuenster	0.055	0.758	0.002
Porto	0.053	0.365	0.002
Sevilla	<0.001	0.223	<0.001
Thiva	0.012	0.528	0.001
Calculated with **HanscheiderHof**-parameters			
Chateaudun	0.032	0.626	0.001
Hamburg	0.126	1.193	0.004
Jokioinen	0.018	1.184	0.001
Kremsmuenster	0.086	0.765	0.003
Porto	0.085	0.425	0.003
Sevilla	<0.001	0.230	<0.001
Thiva	0.014	0.435	0.001
Calculated with **Hoefchen**-parameters			
Chateaudun	0.004	0.640	<0.001
Hamburg	0.033	1.163	0.002
Jokioinen	0.004	1.176	<0.001
Kremsmuenster	0.020	0.744	0.001
Porto	0.020	0.363	0.001
Sevilla	<0.001	0.220	<0.001
Thiva	0.003	0.538	<0.001
Median PECgw of individual soils			
Chateaudun	0.016	0.638	0.001
Hamburg	0.078	1.165	0.003
Jokioinen	0.013	1.176	0.001
Kremsmuenster	0.051	0.759	0.002
Porto	0.051	0.376	0.003
Sevilla	<0.001	0.227	<0.001
Thiva	0.010	0.503	0.001
Calculated with mean-parameters			
Chateaudun	0.013	0.636	0.001
Hamburg	0.066	1.169	0.003
Jokioinen	0.009	1.177	0.001
Kremsmuenster	0.045	0.756	0.002
Porto	0.046	0.381	0.002
Sevilla	<0.001	0.229	<0.001
Thiva	0.007	0.492	<0.001

Lettuce 1, second cropping, biennial application

Table B.8.6.3-47 FOCUS PEARL 4.4.4 PECgw of flupyradifurone and its metabolites in µg/L (Lettuce 1, BBCH 12-49, 1×125 g a.s./ha, 25% interception, 2nd crop, every 2nd year)

Scenario	**Flupyradifurone** µg/L	**Difluoroacetic acid** µg/L	**6-Chloronicotinic acid** µg/L
Calculated with **AXXa**-parameters			
Chateaudun	0.034	0.870	0.001
Hamburg	0.135	1.473	0.005
Kremsmuenster	0.076	0.855	0.003
Porto	0.058	0.619	0.003
Sevilla	<0.001	0.348	<0.001
Calculated with **Dollendorf**-parameters			
Chateaudun	0.044	0.903	0.002
Hamburg	0.159	1.511	0.005
Kremsmuenster	0.086	0.877	0.003
Porto	0.080	0.659	0.003
Sevilla	0.001	0.342	<0.001
Calculated with **HanscheiderHof**-parameters			
Chateaudun	0.062	0.857	0.002
Hamburg	0.188	1.467	0.005
Kremsmuenster	0.120	0.843	0.003
Porto	0.091	0.590	0.003
Sevilla	<0.001	0.343	<0.001
Calculated with **Hoefchen**-parameters			
Chateaudun	0.013	0.891	<0.001
Hamburg	0.064	1.512	0.003
Kremsmuenster	0.032	0.871	0.002
Porto	0.030	0.658	0.002
Sevilla	<0.001	0.347	<0.001
Median PECgw of individual soils			
Chateaudun	0.039	0.881	0.002
Hamburg	0.147	1.492	0.005
Kremsmuenster	0.081	0.863	0.003
Porto	0.069	0.639	0.003
Sevilla	<0.001	0.345	<0.001
Calculated with mean-parameters			
Chateaudun	0.030	0.872	0.001
Hamburg	**0.122**	1.475	0.004
Kremsmuenster	0.069	0.855	0.003
Porto	0.056	0.633	0.003
Sevilla	<0.001	0.351	<0.001

Table B.8.6.3-48 FOCUS PELMO 5.5.3 PECgw of flupyradifurone and its metabolites in µg/L (Lettuce 1, BBCH 12-49, 1×125 g a.s./ha, 25% interception, 2nd crop, every 2nd year)

Scenario	Flupyradifurone µg/L	Difluoroacetic acid µg/L	6-Chloronicotinic acid µg/L
Calculated with **AXXa**-parameters			
Chateaudun	0.017	0.708	0.001
Hamburg	0.100	1.366	0.004
Kremsmuenster	0.062	0.860	0.003
Porto	0.078	0.594	0.004
Sevilla	<0.001	0.274	<0.001
Calculated with **Dollendorf**-parameters			
Chateaudun	0.025	0.751	0.001
Hamburg	0.127	1.412	0.005
Kremsmuenster	0.073	0.883	0.003
Porto	0.098	0.619	0.004
Sevilla	<0.001	0.289	<0.001
Calculated with **HanscheiderHof**-parameters			
Chateaudun	0.037	0.680	0.001
Hamburg	0.151	1.340	0.005
Kremsmuenster	0.096	0.837	0.003
Porto	0.120	0.573	0.004
Sevilla	<0.001	0.266	<0.001
Calculated with **Hoefchen**-parameters			
Chateaudun	0.007	0.748	<0.001
Hamburg	0.050	1.411	0.003
Kremsmuenster	0.027	0.878	0.001
Porto	0.047	0.626	0.003
Sevilla	<0.001	0.299	<0.001
Median PECgw of individual soils			
Chateaudun	0.021	0.728	0.001
Hamburg	0.114	1.389	0.005
Kremsmuenster	0.068	0.869	0.003
Porto	0.088	0.607	0.004
Sevilla	<0.001	0.282	<0.001
Calculated with mean-parameters			
Chateaudun	0.017	0.715	0.001
Hamburg	0.093	1.375	0.004
Kremsmuenster	0.058	0.864	0.002
Porto	0.075	0.601	0.004
Sevilla	<0.001	0.281	<0.001

Lettuce 2, first cropping, annual application

Table B.8.6.3-49 FOCUS PEARL 4.4.4 PECgw of flupyradifurone and its metabolites in µg/L (Lettuce 2, BBCH 12-49, 2×125 g a.s./ha, 2x25% interception, 10d app 1st crop, every year)

Scenario	Flupyradifurone µg/L	Difluoroacetic acid µg/L	6-Chloronicotinic acid µg/L
Calculated with **AXXa**-parameters			
Chateaudun	0.307	3.638	0.010
Hamburg	0.812	6.392	0.024
Jokioinen	0.227	6.238	0.009
Kremsmuenster	0.574	3.702	0.018
Porto	0.308	2.104	0.012
Sevilla	0.006	1.211	<0.001
Thiva	0.213	2.715	0.007
Calculated with **Dollendorf**-parameters			
Chateaudun	0.270	3.550	0.009
Hamburg	0.743	6.310	0.023
Jokioinen	0.255	6.271	0.009
Kremsmuenster	0.504	3.660	0.016
Porto	0.279	1.955	0.010
Sevilla	0.013	1.101	<0.001
Thiva	0.257	2.920	0.008
Calculated with **HanscheiderHof**-parameters			
Chateaudun	0.524	3.745	0.014
Hamburg	1.232	6.490	0.028
Jokioinen	0.285	6.285	0.009
Kremsmuenster	0.843	3.694	0.021
Porto	0.502	2.264	0.015
Sevilla	0.016	1.340	<0.001
Thiva	0.280	2.535	0.008
Calculated with **Hoefchen**-parameters			
Chateaudun	0.087	3.451	0.004
Hamburg	0.327	6.202	0.013
Jokioinen	0.073	6.121	0.004
Kremsmuenster	0.211	3.574	0.009
Porto	0.106	1.865	0.006
Sevilla	0.002	1.049	<0.001
Thiva	0.091	2.888	0.004
Median PECgw of individual soils			
Chateaudun	0.289	3.594	0.010
Hamburg	0.778	6.351	0.024
Jokioinen	0.241	6.255	0.009
Kremsmuenster	0.539	3.677	0.017
Porto	0.294	2.030	0.011
Sevilla	0.010	1.156	<0.001
Thiva	0.235	2.802	0.008
Calculated with mean-parameters			
Chateaudun	0.245	3.605	0.008
Hamburg	0.677	6.353	0.020
Jokioinen	0.185	6.230	0.007
Kremsmuenster	0.475	3.685	0.015
Porto	0.259	2.059	0.010
Sevilla	0.006	1.185	<0.001
Thiva	0.181	2.727	0.007

Table B.8.6.3-50 FOCUS PELMO 5.5.3 PECgw of flupyradifurone and its metabolites in µg/L (Lettuce 2, BBCH 12-49, 2×125 g a.s./ha, 2x25% interception, 10d app. 1st crop, every year)

Scenario	Flupyradifurone µg/L	Difluoroacetic acid µg/L	6-Chloronicotinic acid µg/L
Calculated with **AXXa**-parameters			
Chateaudun	0.187	2.908	0.007
Hamburg	0.627	5.313	0.020
Jokioinen	0.177	5.807	0.007
Kremsmuenster	0.487	3.405	0.015
Porto	0.410	1.862	0.016
Sevilla	0.002	0.933	<0.001
Thiva	0.140	2.370	0.006
Calculated with **Dollendorf**-parameters			
Chateaudun	0.171	2.860	0.006
Hamburg	0.651	5.160	0.021
Jokioinen	0.200	5.826	0.008
Kremsmuenster	0.435	3.400	0.014
Porto	0.374	1.725	0.013
Sevilla	0.003	0.835	<0.001
Thiva	0.179	2.546	0.006
Calculated with **HanscheiderHof**-parameters			
Chateaudun	0.330	2.943	0.009
Hamburg	0.927	5.418	0.023
Jokioinen	0.213	5.515	0.007
Kremsmuenster	0.692	3.416	0.018
Porto	0.638	2.014	0.019
Sevilla	0.004	1.048	<0.001
Thiva	0.199	2.211	0.006
Calculated with **Hoefchen**-parameters			
Chateaudun	0.053	2.800	0.003
Hamburg	0.278	5.140	0.012
Jokioinen	0.057	5.784	0.003
Kremsmuenster	0.178	3.349	0.008
Porto	0.172	1.681	0.009
Sevilla	0.001	0.812	<0.001
Thiva	0.060	2.599	0.003
Median PECgw of individual soils			
Chateaudun	0.179	2.884	0.007
Hamburg	0.639	5.237	0.021
Jokioinen	0.189	5.796	0.007
Kremsmuenster	0.461	3.403	0.015
Porto	0.392	1.794	0.015
Sevilla	0.003	0.884	<0.001
Thiva	0.160	2.458	0.006
Calculated with mean-parameters			
Chateaudun	0.155	2.894	0.006
Hamburg	0.520	5.297	0.018
Jokioinen	0.145	5.829	0.006
Kremsmuenster	0.404	3.402	0.013
Porto	0.355	1.833	0.014
Sevilla	0.001	0.917	<0.001
Thiva	0.119	2.416	0.005

Lettuce 2, second cropping, annual application

Table B.8.6.3-51 FOCUS PEARL 4.4.4 PECgw of flupyradifurone and its metabolites in µg/L (Lettuce 2, BBCH 12-49, 2×125 g a.s./ha, 2x25% interception, 10d app., 2nd crop, every year)

Scenario	**Flupyradifurone** µg/L	**Difluoroacetic acid** µg/L	**6-Chloronicotinic acid** µg/L
Calculated with **AXXa**-parameters			
Chateaudun	0.402	4.141	0.013
Hamburg	1.053	6.879	0.030
Kremsmuenster	0.675	3.961	0.021
Porto	0.574	2.771	0.021
Sevilla	0.008	1.585	<0.001
Calculated with **Dollendorf**-parameters			
Chateaudun	0.415	4.273	0.013
Hamburg	1.130	6.937	0.034
Kremsmuenster	0.637	3.994	0.020
Porto	0.630	2.828	0.021
Sevilla	0.020	1.550	<0.001
Calculated with **HanscheiderHof**-parameters			
Chateaudun	0.631	4.073	0.016
Hamburg	1.479	6.825	0.033
Kremsmuenster	0.958	3.909	0.023
Porto	0.770	2.748	0.022
Sevilla	0.018	1.654	<0.001
Calculated with **Hoefchen**-parameters			
Chateaudun	0.149	4.188	0.007
Hamburg	0.524	6.887	0.020
Kremsmuenster	0.279	3.925	0.011
Porto	0.307	2.837	0.014
Sevilla	0.003	1.540	<0.001
Median PECgw of individual soils			
Chateaudun	0.409	4.165	0.013
Hamburg	1.092	6.883	0.032
Kremsmuenster	0.656	3.943	0.021
Porto	0.602	2.800	0.021
Sevilla	0.013	1.568	<0.001
Calculated with mean-parameters			
Chateaudun	0.334	4.127	0.011
Hamburg	0.905	6.836	0.027
Kremsmuenster	0.568	3.948	0.018
Porto	0.508	2.760	0.019
Sevilla	0.007	1.589	<0.001

Table B.8.6.3-52 FOCUS PELMO 5.5.3 PECgw of flupyradifurone and its metabolites in µg/L (Lettuce 2, BBCH 12-49, 2×125 g a.s./ha, 2x25% interception, 10d app. every year)

Scenario	Flupyradifurone µg/L	Difluoroacetic acid µg/L	6-Chloronicotinic acid µg/L
Calculated with **AXXa**-parameters			
Chateaudun	0.255	3.463	0.009
Hamburg	0.873	6.214	0.028
Kremsmuenster	0.598	3.640	0.019
Porto	0.703	2.640	0.024
Sevilla	0.002	1.264	<0.001
Calculated with **Dollendorf**-parameters			
Chateaudun	0.258	3.610	0.009
Hamburg	0.967	6.324	0.030
Kremsmuenster	0.588	3.715	0.019
Porto	0.754	2.732	0.025
Sevilla	0.003	1.234	<0.001
Calculated with **HanscheiderHof**-parameters			
Chateaudun	0.401	3.298	0.011
Hamburg	1.193	6.070	0.029
Kremsmuenster	0.814	3.562	0.021
Porto	0.881	2.561	0.027
Sevilla	0.004	1.326	<0.001
Calculated with **Hoefchen**-parameters			
Chateaudun	0.086	3.579	0.004
Hamburg	0.440	6.329	0.018
Kremsmuenster	0.245	3.714	0.011
Porto	0.376	2.726	0.018
Sevilla	0.001	1.250	<0.001
Median PECgw of individual soils			
Chateaudun	0.257	3.521	0.009
Hamburg	0.920	6.269	0.029
Kremsmuenster	0.593	3.677	0.019
Porto	0.729	2.683	0.025
Sevilla	0.003	1.257	<0.001
Calculated with mean-parameters			
Chateaudun	0.214	3.474	0.008
Hamburg	0.748	6.244	0.024
Kremsmuenster	0.502	3.647	0.016
Porto	0.627	2.650	0.022
Sevilla	0.001	1.280	<0.001

Hop 1 Annual application

Table B.8.6.3-53 FOCUS PEARL 4.4.4 PECgw of flupyradifurone and its metabolites in µg/L (Hops 1, BBCH 31-75, 1×150 g a.s./ha, 60% interception, every year)

Scenario	Flupyradifurone µg/L	Difluoroacetic acid µg/L	6-Chloronicotinic acid µg/L
Calculated with **AXXa**-parameters			
Chateaudun	0.125	1.493	0.005
Hamburg	0.180	1.644	0.006
Kremsmuenster	0.131	1.031	0.005
Piacenza	0.122	0.989	0.004
Porto	0.053	0.654	0.003
Sevilla	0.042	0.903	0.002
Thiva	0.029	0.818	0.001
Calculated with **Dollendorf**-parameters			
Chateaudun	0.125	1.489	0.005
Hamburg	0.189	1.637	0.007
Kremsmuenster	0.131	1.027	0.005
Piacenza	0.117	0.957	0.004
Porto	0.056	0.620	0.002
Sevilla	0.046	0.913	0.002
Thiva	0.029	0.809	0.001
Calculated with **HanscheiderHof**-parameters			
Chateaudun	0.228	1.513	0.006
Hamburg	0.284	1.661	0.007
Kremsmuenster	0.216	1.024	0.006
Piacenza	0.216	1.058	0.006
Porto	0.108	0.703	0.004
Sevilla	0.082	0.921	0.003
Thiva	0.075	0.857	0.002
Calculated with **Hoefchen**-parameters			
Chateaudun	0.046	1.438	0.002
Hamburg	0.079	1.607	0.003
Kremsmuenster	0.054	1.002	0.003
Piacenza	0.048	0.915	0.002
Porto	0.019	0.586	0.001
Sevilla	0.014	0.877	<0.001
Thiva	0.008	0.767	<0.001
Median PECgw of individual soils			
Chateaudun	0.125	1.491	0.005
Hamburg	0.185	1.641	0.007
Kremsmuenster	0.131	1.026	0.005
Piacenza	0.120	0.973	0.004
Porto	0.055	0.637	0.003
Sevilla	0.044	0.908	0.002
Thiva	0.029	0.814	0.001
Calculated with mean-parameters			
Chateaudun	0.112	1.475	0.004
Hamburg	0.156	1.637	0.005
Kremsmuenster	0.117	1.025	0.004
Piacenza	0.109	0.970	0.004
Porto	0.048	0.641	0.002
Sevilla	0.039	0.898	0.002
Thiva	0.026	0.809	0.001

Table B.8.6.3-54 FOCUS PELMO 5.5.3 PECgw of flupyradifurone and its metabolites in µg/L (Hops 1, BBCH 31-75, 1×150 g a.s./ha, 60% interception, every year)

Scenario	**Flupyradifurone** µg/L	**Difluoroacetic acid** µg/L	**6-Chloronicotinic acid** µg/L
Calculated with **AXXa**-parameters			
Chateaudun	0.104	1.306	0.004
Hamburg	0.161	1.787	0.006
Kremsmuenster	0.150	1.213	0.005
Piacenza	0.168	0.955	0.006
Porto	0.083	0.732	0.004
Sevilla	0.006	0.736	<0.001
Thiva	0.021	0.853	0.001
Calculated with **Dollendorf**-parameters			
Chateaudun	0.113	1.320	0.004
Hamburg	0.195	1.775	0.007
Kremsmuenster	0.155	1.212	0.006
Piacenza	0.173	0.937	0.006
Porto	0.092	0.711	0.004
Sevilla	0.009	0.730	0.001
Thiva	0.026	0.847	0.001
Calculated with **HanscheiderHof**-parameters			
Chateaudun	0.189	1.313	0.005
Hamburg	0.265	1.812	0.007
Kremsmuenster	0.235	1.229	0.007
Piacenza	0.264	0.971	0.007
Porto	0.139	0.765	0.005
Sevilla	0.014	0.750	0.001
Thiva	0.047	0.878	0.002
Calculated with **Hoefchen**-parameters			
Chateaudun	0.040	1.270	0.002
Hamburg	0.081	1.754	0.004
Kremsmuenster	0.066	1.190	0.003
Piacenza	0.078	0.914	0.004
Porto	0.035	0.685	0.002
Sevilla	0.002	0.708	<0.001
Thiva	0.007	0.802	<0.001
Median PECgw of individual soils			
Chateaudun	0.109	1.310	0.004
Hamburg	0.178	1.781	0.007
Kremsmuenster	0.153	1.213	0.006
Piacenza	0.171	0.946	0.006
Porto	0.088	0.722	0.004
Sevilla	0.008	0.733	0.001
Thiva	0.024	0.850	0.001
Calculated with mean-parameters			
Chateaudun	0.094	1.295	0.004
Hamburg	0.144	1.777	0.005
Kremsmuenster	0.134	1.208	0.005
Piacenza	0.152	0.946	0.005
Porto	0.075	0.723	0.004
Sevilla	0.006	0.732	<0.001
Thiva	0.020	0.841	0.001

Hop 2 Biennial application

Table B.8.6.3-55 FOCUS PEARL 4.4.4 PECgw of flupyradifurone and its metabolites in µg/L (Hops 2, BBCH 31-75, 1×150 g a.s./ha, 60% interception, every 2nd year)

Scenario	Flupyradifurone µg/L	Difluoroacetic acid µg/L	6-Chloronicotinic acid µg/L
Calculated with **AXXa**-parameters			
Chateaudun	0.045	0.709	0.002
Hamburg	0.064	0.759	0.002
Kremsmuenster	0.044	0.499	0.002
Piacenza	0.042	0.479	0.002
Porto	0.018	0.293	<0.001
Sevilla	0.013	0.437	<0.001
Thiva	0.008	0.397	<0.001
Calculated with **Dollendorf**-parameters			
Chateaudun	0.051	0.731	0.002
Hamburg	0.088	0.767	0.003
Kremsmuenster	0.050	0.505	0.002
Piacenza	0.047	0.488	0.002
Porto	0.021	0.281	0.001
Sevilla	0.018	0.457	<0.001
Thiva	0.011	0.403	<0.001
Calculated with **HanscheiderHof**-parameters			
Chateaudun	0.086	0.708	0.003
Hamburg	0.102	0.769	0.003
Kremsmuenster	0.080	0.501	0.002
Piacenza	0.076	0.488	0.002
Porto	0.039	0.315	0.002
Sevilla	0.028	0.427	0.001
Thiva	0.025	0.411	<0.001
Calculated with **Hoefchen**-parameters			
Chateaudun	0.018	0.708	<0.001
Hamburg	0.034	0.755	0.002
Kremsmuenster	0.020	0.494	0.001
Piacenza	0.018	0.474	<0.001
Porto	0.007	0.267	<0.001
Sevilla	0.005	0.444	<0.001
Thiva	0.003	0.386	<0.001
Median PECgw of individual soils			
Chateaudun	0.048	0.709	0.002
Hamburg	0.076	0.763	0.003
Kremsmuenster	0.047	0.500	0.002
Piacenza	0.045	0.484	0.002
Porto	0.020	0.287	0.001
Sevilla	0.016	0.441	<0.001
Thiva	0.010	0.400	<0.001
Calculated with mean-parameters			
Chateaudun	0.042	0.709	0.002
Hamburg	**0.061**	0.756	0.002
Kremsmuenster	0.042	0.498	0.002
Piacenza	0.040	0.481	0.002
Porto	0.017	0.288	<0.001
Sevilla	0.012	0.439	<0.001
Thiva	0.008	0.394	<0.001

Table B.8.6.3-56 FOCUS PELMO 5.5.3 PECgw of flupyradifurone and its metabolites in µg/L (Hops 2, BBCH 31-75, 1×150 g a.s./ha, 60% interception, every 2nd year)

Scenario	Flupyradifurone µg/L	Difluoroacetic acid µg/L	6-Chloronicotinic acid µg/L
Calculated with **AXXa**-parameters			
Chateaudun	0.033	0.665	0.002
Hamburg	0.056	0.827	0.002
Kremsmuenster	0.050	0.572	0.002
Piacenza	0.066	0.431	0.003
Porto	0.027	0.335	0.002
Sevilla	0.002	0.402	<0.001
Thiva	0.006	0.389	<0.001
Calculated with **Dollendorf**-parameters			
Chateaudun	0.043	0.685	0.002
Hamburg	0.074	0.834	0.003
Kremsmuenster	0.059	0.574	0.002
Piacenza	0.077	0.434	0.003
Porto	0.032	0.320	0.002
Sevilla	0.003	0.425	<0.001
Thiva	0.009	0.394	0.001
Calculated with **HanscheiderHof**-parameters			
Chateaudun	0.068	0.655	0.002
Hamburg	0.095	0.832	0.003
Kremsmuenster	0.088	0.579	0.003
Piacenza	0.100	0.438	0.003
Porto	0.051	0.351	0.002
Sevilla	0.004	0.380	<0.001
Thiva	0.016	0.394	0.001
Calculated with **Hoefchen**-parameters			
Chateaudun	0.014	0.667	0.001
Hamburg	0.030	0.827	0.002
Kremsmuenster	0.023	0.568	0.001
Piacenza	0.033	0.429	0.002
Porto	0.012	0.305	0.001
Sevilla	0.001	0.419	<0.001
Thiva	0.002	0.376	<0.001
Median PECgw of individual soils			
Chateaudun	0.038	0.666	0.002
Hamburg	0.065	0.830	0.003
Kremsmuenster	0.055	0.573	0.002
Piacenza	0.072	0.433	0.003
Porto	0.030	0.328	0.002
Sevilla	0.003	0.411	<0.001
Thiva	0.008	0.392	0.001
Calculated with mean-parameters			
Chateaudun	0.032	0.667	0.001
Hamburg	0.053	0.827	0.002
Kremsmuenster	0.047	0.569	0.002
Piacenza	0.063	0.429	0.002
Porto	0.025	0.330	0.002
Sevilla	0.002	0.408	<0.001
Thiva	0.006	0.387	<0.001

Remarks

The calculations presented are according to the draft guidance on TDS. The results show that in some cases the median PECgw of the individual soil show higher values than PECgw obtained with the mean of the parameters.

B.8.7 Fate and behaviour in air (Annex IIA 7.2.2; Annex IIIA 9.3) OECD number 7.10

STUDY IIA, 7.10/01

Characteristics

reference	:	Hellpointer, E., 2010	purity	:	n.a.
year of execution	:	2010	test system	:	theoretical calculation
GLP statement	:	n.a.	hydroxyl rate constant	:	0.5-1.5 × 10^6 molecules (radicals)/cm^3
guideline	:	n.a.	half-life in air	:	4.4 – 13.1 hours
test substance	:	BYI 02960	acceptability	:	**acceptable**

Summary

Based on the estimation method according to structure-activity relationship (SAR) methods developed by Roger Atkinson and co-workers the chemical lifetime of the BYI 02960 in the air was assessed by the program AOPWIN™, version 1.92a (U.S. EPA, 2008). The half-life time ($t_{1/2}$) was estimated within a range of 4.4 hours (short-term scenario) to 13.1 hours (long-term scenario), depending on the mean concentration of hydroxyl radicals present in the troposphere.

In addition, BYI 02960 is susceptible to reactions with ozone, however, that attack and its resulting chemical half-life is considered to be slower by a factor of 2 to 10.

As a consequence of the short half-life in air, no long-range transport of BYI 02960 in the atmosphere is likely to occur nor an accumulation of BYI 02960 in the environmental compartment air. From the low vapor pressure of the substance it is concluded that very low, if any, quantities of BYI 02960 are expected to enter the atmosphere from volatilization.

Methods

There are different reaction mechanisms that may result in degradation of organic trace substances in the air being gaseous or bound to particles. According to the present knowledge mainly reactions with photochemical produced hydroxyl radicals, with nitrate radicals and ozone as well as direct photolysis are possibilities. The abiotic degradability and/or the reversal of that, the persistence of a substance, can be predicted if the reaction rate ki and the concentrations c(yi) of the potential reaction partners are known.

With an exception to be made for fully halogenated compounds, the abiotic degradability of an organic xenobiotic compounds can be well predicted from estimated reaction rate constants (ki) of the individual processes, and the concentrations c(yi) of the potential reaction partners, i.e. in particular the concentrations of OH radicals and ozone.

An experimental determination of the hydroxyl radical reaction rate (kOH), however, is very laborious and gas phase measurements are difficult for molecules showing a comparatively low vapor pressure. The approach of Atkinson et al. was based on a comprehensive set of experimental data to result in a quantitative structure-activity relationship (QSAR) mathematic model that allows for estimation by

calculation, starting from the molecular structure of a test compound. The calculation procedure has been transferred into the personal computer program "Atmospheric Oxidation Program" (AOP) by Meylan & Howard. The current version 1.92a (U.S. EPA, 2008) was used for the calculations. AOPWINTM requires only the chemical structure and atmospheric concentrations of the potential reaction partners as inputs.

The AOPWINTM estimation method adds up the partial reaction rates ki of the reaction of photochemically generated active species with subgroups of the test molecule (increments), resulting in the overall reaction rate. The following hydroxyl and/or ozone reactions are considered:

- hydrogen abstraction
- addition to double bonds
- addition to triple bonds
- reaction with N, S and hydroxyl groups
- addition to aromatic rings
- addition to fused rings

The listed increments (group rate constants for hydrogen abstraction, addition to double and triple bonds, ring systems, and reaction with heteroatoms) are compiled in a data base which additionally uses algorithms for consideration of adjacent groups (substituent factors) and the position of the attack to substituted aromatic rings (electrophilic substituent factors).

The reliability of the method used by AOPWINTM was examined by comparison of estimated and experimentally determined hydroxyl radical rate constants for 647 chemicals. More than 90 percent of the estimated rate constants were within a factor of two of the experiment value for these chemicals. More than 95 percent of the estimated values differed within a factor of three from measured values.

Considering the chemical structure of BYI 02960 it can be concluded that reactions with photochemical produced hydroxyl radicals will mainly determine its degradation rate (Ktotal, indirect photoreaction ≈ kOH) in the air. However, also reactions with ozone are expected to have a significant influence on the overall assessment.

Since a chemical in the troposphere is usually at a very low concentration and a steady-state concentration of OH radicals is produced by sunlight, the hydroxyl radical concentration can be treated as a constant so the reaction can be considered a pseudo first-order reaction.

As an estimate the maximum half-life in air from indirect photoreaction-oxidation processes can be derived under consideration of the concentration of hydroxyl radicals by the following formula:

The half-life in the troposphere, $t_{½} = \ln 2 / (k_{OH} \times [OH])$

where k_{OH} is the hydroxyl radical rate constant in units of cm^3/molecule-sec and [OH] is the hydroxyl radical concentration in units of molecules (or radicals) per cm^3. Thus, to calculate the half-life in the troposphere, one needs the estimated or measured rate constant of the chemical as well as some average OH radical concentration. In a U.S. EPA review by Leifer (no date), the author concluded that the diurnally and annually averaged 12-h daylight hydroxyl radical concentration of 1.5×10^6 molecules (radicals)/cm^3 should be used as the default in the AOPWIN program based upon data from Atkinson et al. of 1990. More recent reviews (Atkinson & Arey 2003) have suggested 2.0×10^6 molecules (radicals)/cm^3 12-hour daylight [OH].

Twelve hour daylight OH radical concentrations are reasonable for fast reacting chemicals but for chemicals that react more slowly (> a few days) 24 hours averages might be more appropriate. Atkinson (1985) suggested seasonally and diurnally 24 hour averaged hydroxyl radical concentrations at 298 K of 5×10^5 molecules/cm^3 in the northern hemisphere and 6×10^5 molecules/cm^3 in the southern hemisphere.

The AOPWIN program allows the user to select 12 or 24 hour time frames and any average hydroxyl radical concentrations. For the current report the default originally set at 1.5×10^6 molecules (radicals)/cm^3 per 12-h of daylight was taken for the short term, and 0.5×10^6 molecules (radicals)/cm^3 per day (24-h) for the long term estimations.

The maximum chemical life-time in air is calculated similarly by use of the formula:
$\tau = 1 / (k_{OH} \times [OH])$

The ozone rate constant estimations produced by AOPWIN are generally important when one or more functional group is attached to any olefinic or acetylenic unit. It is also important for a limited number of chemical classes (e.g., hydrazines, phenols, alkyl lead compounds, and furans). The database of experimental ozone rate constants is not nearly as extensive as the database for hydroxyl radical rate constants. Because of this smaller available database, the number of fragment values that are available for estimations is considerably smaller. Therefore, the number of compounds that can be estimated with reasonable certainty is smaller. If an "assumed value" is used to produce an estimated rate constant, it is a good idea to check the "Show Calculation" screen for ozone to see if a "default value" was used in the calculation. If a default value was used, consider the estimate questionable.
Ozone reaction half-lives for organic chemicals can also be estimated similar to the approach used with hydroxyl radical by using an average ozone concentration and equation

The half-life in the troposphere, $t½ = \ln 2 / (k_{Ozone} \times [ozone])$

Atkinson & Carter (1984) indicated that [ozone] are around 10-40 ppb (0.25 to 1×10^{-12} molecules/cm^3) at ground level and increases with altitude (30 to 100 ppb at 10 km). These authors used 7×10^{-11} molecules/cm^3 (30 ppb) per 24 hours as representative of an unpolluted lower troposphere and this is

the initial default value for AOPWIN, which was used in the current estimations. As with OH radicals, the concentration of ozone varies with amount of pollution, time of the day, climate, and zonal location.

Results

The overall reaction rate of BYI 02960 with hydroxyl radicals is estimated to be 29.3590 $\times$ 10^{-12} cm^3 $\times$ $molecule^{-1}$ $\times$ s^{-1}. This rate is derived mainly from incremental reactions like hydrogen abstraction (10.7929 $\times$ 10^{-12} cm^3 $\times$ $molecule^{-1}$ $\times$ s^{-1}) and an addition reaction to the C=C bond (18.2490 $\times$ 10^{-12} cm^3 $\times$ $molecule^{-1}$ $\times$ s^{-1}).

Based on the overall hydroxyl radical reaction rate constant in combination with the "short term" concentration of these radicals in the atmosphere during daylight (i.e. 1.5 x 10^6 OH radicals cm^{-3}) the half-life (t1/2) of BYI 02960 in air is derived to be 4.372 hours.

That estimate should be regarded as worst-case assumption as the approach does not consider the contribution of any other reactive species to the overall atmospheric degradation of BYI 02960 (i.e. by ozone; for its contribution refer to Appendix 3 and Appendix 4 of report). It should be noted that the chemical half-life of the active substance can be expected to be shorter when being applied in the early afternoon since the OH radical concentration in the troposphere may increase to 5 x 10^6 radicals cm^{-3} during the day while these values are lower in the early morning or late afternoon.

Conclusions

BYI 02960 is considered to be susceptible to reactions with hydroxyl radicals to contribute significantly to the overall degradation of the substance in the atmosphere. Various parts of the molecule were identified as potential targets for radical reactions. An attack by hydroxyl radicals and ozone should result in the formation of multiple primary radicals. Their formation may be followed by secondary oxidation products that can be eliminated from the atmosphere by wet and/or dry deposition.

In addition, BYI 02960 is susceptible to reactions with ozone, however, that attack and its resulting chemical half-life is considered to be slower by a factor of 2 to 10.

Remarks

The study is acceptable. The RMS considers the endpoint based on the 12 hour OH radical concentration, in line with FOCUSAIR, reliable. The atmospheric half life of BYI 02960 is estimated to be 4.4 hours. BYI 02960 is not considered to have potential for long range transport based on this result.

B.8.8 Predicted environmental concentrations in air (PECa) (Annex IIIA 9.3)

As a consequence of the short half-life in air, no long-range transport of BYI 02960 in the atmosphere is likely to occur nor an accumulation of BYI 02960 in the environmental compartment air. From the low vapor pressure of the substance it is concluded that very low, if any, quantities of BYI 02960 are expected to enter the atmosphere from volatilization of soil residues.

B.8.9 Definition of the residue (Annex IIA 7.3)

Detailed guidance to define the environmental residue is available in SANCO/10329/2002 rev 2. final (Oct 2002) and SANCO/3268/2001 rev 4 final (Oct 2002). All metabolites accounted for >10% in aerobic soil treated with BYI 02960.

The components of the environmental residue are therefore as follows:

Residue definition for risk assessment:

Soil:	BYI 02960, DFA, 6-CNA
Surface Water:	BYI 02960, DFA, 6-CNA, BYI 02960-succinamide, BYI 02960-azabicyclosuccinmaide
Sediment:	BYI 02960, DFA, 6-CNA
Groundwater:	BYI 02960, DFA, 6-CNA
Air:	BYI 02960

Residue definition for monitoring:

Soil:	BYI 02960
Surface Water:	BYI 02960
Groundwater:	BYI 02960
Air:	BYI 02960

B.8.10 References relied on

Literature search

A literature review was conducted for flupyradifurone and its metabolites according to the requirements of Regulation EC 1107/2009. The total number of literature findings within the last ten years before submission were 342, all referring to two metabolites. A large number of keywords, grouped by the categories “health”, “environment” and “non target species” has been used as a text mining filter. From the total of 342 references, 136 publications were identified which gave a matching overlap with one or more of the keywords used. All 342 references were reviewed by scientific experts and categorized.

The filter was merely tested, and improved, as a tool to be applied in future literature reviews for other compounds. From those references which could not be allocated to one of the categories “health”, “environment” or “non target species”, none was identified by the scientific experts to provide relevant information for regulatory purposes, i.e. no false negative findings were identified.

None of the studies was considered relevant as defined by the EFSA Guidance document on ‘submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) no. 1107/2009’, i.e. as “dealing with side-effects on health, the environment and non-target species”.

In the area of toxicology, only one publication was evaluated as “relevant”. Fortunately this publication was already known and the result was therefore already assessed and fully integrated in the supplementary dossier Therefore, no reference had to be discussed separately in the dossier, as all risk assessments remained fully valid after consideration of all references.

In conclusion, no information was identified which was considered to have an impact on an endpoint or would result in the need to adapt a risk assessment in the flupyradifurone dossier.

The applicant also provided an additional literature search for the two water metabolites. This was a data requirement set by the EFSA during the review. The search did not result in additional scientific literature since these metabolites were closely related to the active substance flupyradifurone.

Remarks RMS

A literature search and review was provided by the applicant. The search has been conducted in line with the EFSA guidance. Of all identified papers (342) the relevant ones (136) have been selected by the applicant in a transparent way using a text mining filter. The applicant provided a general justification for evaluating the relevant papers. As no study-by-study justifications were given the RMS can not properly verify whether the 136 relevant papers were correctly evaluated in terms of reliability/validity/acceptability for regulatory use. The one study that was considered relevant by the

applicant is evaluated in the DAR. RMS notes that flupyradifurone is a new active substance worldwide. Therefore, public literature is not expected to be an important new information source for the active substance itself.

References for the active substance

Annex point/ reference number	Author(s)	Year	Title, Source, Company, Report No, GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIA 7.1.1/01, IIA 7.2.1/01, IIA 7.4.1/03	Menke, U.	2011	[Pyridinylmethyl-14C] BYI 02960: Aerobic soil metabolism/degradation and time-dependent sorption in soils Bayer CropScience, Report No.: MEF-07/334, Edition Number: M-414615-01-2 Date: 2011-08-05 GLP/GEP: yes, unpublished	Y	BCS
IIA 7.1.1/02, IIA 7.2.1/02	Menke, U.; Unold, M.	2011	[Furanone-4-14C] BYI 02960: Aerobic soil metabolism/degradation Bayer CropScience, Report No.: MEF-10/804, Edition Number: M-411625-01-2 Date: 2011-07-28 GLP/GEP: yes, unpublished	Y	BCS
IIA 7.1.1/03, IIA 7.2.1/03	Ripperger, R. J.	2011	[Furanone-4-14C] BYI 02960: Aerobic soil metabolism in two US soils Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: MERVP037-2, Edition Number: M-405497-03-1 Date: 2011-01-14 ...Amended: 2012-01-05 GLP/GEP: yes, unpublished	Y	BCS
IIA 7.1.1/04, IIA 7.2.1/04	Menke, U.; Unold, M.	2011	[Ethyl-1-14C] BYI 02960: Aerobic soil metabolism Bayer CropScience, Report No.: MEF-10/858, Edition Number: M-414981-01-1 Date: 2011-09-08 GLP/GEP: yes, unpublished	Y	BCS
IIA 7.1.1/05, IIA 7.2.1/05,	Menke, U.; Unold, M.	2011	[Pyridine-2,6-14C] BYI 02960: Aerobic soil metabolism Bayer CropScience, Report No.: MEF-10/880, Edition Number: M-411693-01-2 Date: 2011-07-28 GLP/GEP: yes, unpublished	Y	BCS

Annex point/ reference number	Author(s)	Year	Title, Source, Company, Report No, GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IA 7.1.1/06, IIA 7.2.1/06, IIA 7.2.3/04	Shepherd, J. J.	2011	[Pyridine-2,6-14C] BYI 02960: Aerobic soil metabolism in two US soils Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: MERVP038-1, Edition Number: M-413425-02-1 Date: 2011-09-06 ...Amended: 2012-01-05 GLP/GEP: yes, unpublished	Y	BCS
IIA 7.1.2/01	Hellpointner, E.	2012	[Furanone-4-14C] and [Ethyl-1-14C] and [Pyridine-2,6-14C] BYI 02960: Anaerobic Soil Metabolism Bayer CropScience, Report No.: MEF-11/514, Edition Number: M-421504-01-2 Date: 2012-01-03 GLP/GEP: yes, unpublished	Y	BCS
IIA 7.1.2/02	Mislankar, S. G.; Woodard, D.	2012	[Pyridine-2,614C] BYI 02960: Anaerobic soil metabolism Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: MERVP094, Edition Number: M-421993-01-1 Date: 2012-01-10 GLP/GEP: yes, unpublished	Y	BCS
IIA 7.1.2/03	Woodard, D.	2012	[Pyridine-2,614C] BYI 02960: Anaerobic soil metabolism in Springfield, Nebraska (USA) soil Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: MERVL006, Edition Number: M-424987-01-1 Date: 2012-02-14 GLP/GEP: yes, unpublished	Y	BCS
IIA 7.1.3/01	Menke, U.; Unold, M.	2011	[Pyridinylmethyl-14C] BYI 02960 and [furanone-4-14C] BYI 02960: Phototransformation on soil Bayer CropScience, Report No.: MEF-10/351, Edition Number: M-405776-01-2 Date: 2011-03-24 GLP/GEP: yes, unpublished	Y	BCS
IIA 7.2.1 /01 IIA 7.1.1/01 IIA 7.4.1/03	Menke, U.	2011	[Pyridinylmethyl-14C] BYI 02960: Aerobic soil metabolism/degradation and time-dependent sorption in soils Bayer CropScience, Report No.: MEF-07/334, Edition Number: M-414615-01-2 Date: 2011-08-05 GLP/GEP: yes, unpublished	Y	BCS

Annex point/ reference number	Author(s)	Year	Title, Source, Company, Report No, GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIA 7.2.1/02 IIA 7.1.1/02	Menke, U.; Unold, M.	2011	[Furanone-4-14C] BYI 02960: Aerobic soil metabolism/degradation Bayer CropScience, Report No.: MEF-10/804, Edition Number: M-411625-01-2 Date: 2011-07-28 GLP/GEP: yes, unpublished	Y	BCS
IIA 7.2.1/03 IIA 7.1.1/03	Ripperger, R. J.	2011	[Furanone-4-14C] BYI 02960: Aerobic soil metabolism in two US soils Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: MERVP037-2, Edition Number: M-405497-03-1 Date: 2011-01-14 ...Amended: 2012-01-05 GLP/GEP: yes, unpublished	Y	BCS
IIA 7.2.1/04 IIA 7.1.1/04	Menke, U.; Unold, M.	2011	[Ethyl-1-14C] BYI 02960: Aerobic soil metabolism Bayer CropScience, Report No.: MEF-10/858, Edition Number: M-414981-01-1 Date: 2011-09-08 GLP/GEP: yes, unpublished	Y	BCS
IIA 7.2.1/05 IIA 7.1.1/05	Menke, U.; Unold, M.	2011	[Pyridine-2,6-14C] BYI 02960: Aerobic soil metabolism Bayer CropScience, Report No.: MEF-10/880, Edition Number: M-411693-01-2 Date: 2011-07-28 GLP/GEP: yes, unpublished	Y	BCS
IIA 7.2.1/06 IIA 7.1.1/06 IIA 7.2.3/04	Shepherd, J. J.	2011	[Pyridine-2,6-14C] BYI 02960: Aerobic soil metabolism in two US soils Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: MERVP038-1, Edition Number: M-413425-02-1 Date: 2011-09-06 ...Amended: 2012-01-05 GLP/GEP: yes, unpublished	Y	BCS
IIA 7.2.1/07	Sur, R.; Dorn, S.	2012	Kinetic evaluation of the aerobic metabolism of BYI 02960 in four soils for the determination of modelling endpoints Bayer CropScience, Report No.: MEF-11/619, Edition Number: M-423020-01-1 Date: 2012-01-19 GLP/GEP: no, unpublished	Y	BCS

Annex point/ reference number	Author(s)	Year	Title, Source, Company, Report No, GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIA 7.2.1/08	Sur, R.; Dorn, S.	2012	Kinetic evaluation of the aerobic metabolism of BYI 02960 in four soils for the determination of modelling endpoints Bayer CropScience, Report No.: MEF-11/620, Edition Number: M-423347-01-1 Date: 2012-01-19 GLP/GEP: no, unpublished	Y	BCS
IIA 7.2.1/09 IIA 7.2.3/05	Sur, R.; Dorn, S.	2012	Kinetic evaluation of the degradation rate of [Ethyl-1-14C] BYI 02960 and its metabolite difluoroacetic acid (BYI 02960-DFA) for the determination of trigger and modelling endpoints Bayer CropScience, Report No.: MEF-11/855, Edition Number: M-422874-01-1 Date: 2012-01-18 GLP/GEP: no, unpublished	Y	BCS
IIA 7.2.1/10 IIA 7.2.3/03	Sur, R.; Dorn, S.	2012	Kinetic evaluation of the degradation rate of (pyridine-2,6-14C] BYI 02960 and its metabolite 6-chloronicotinic accid (6-CNA) for the determination of trigger and modelling endpoints Bayer CropScience, Report No.: MEF-11/838, Edition Number: M-422853-01-1 Date: 2012-01-18 GLP/GEP: no, unpublished	Y	BCS
IIA 7.2.3/01	Lowden, P.; Oddy, A. M.; Jones, M. K.	1997	Rate of degradation of the acid metabolite, (14C)-IC-O in three soils NI-25 Rhone-Poulenc Agriculture Ltd., Ongar, Essex, United Kingdom Bayer CropScience, Report No.: C007660, Edition Number: M-196378-01-1 Date: 1997-08-14 GLP/GEP: yes, unpublished	N	NISSO
IIA 7.2.3/02	Sur, R.; Dorn, S.	2012	Kinetic evaluation of the degradation rate of 6-chloronicotinic acid (6-CNA) for the determination of trigger and modelling endpoints Bayer CropScience, Report No.: MEF-11/837, Edition Number: M-422843-01-1 Date: 2012-01-18 GLP/GEP: no, unpublished	Y	BCS

Annex point/ reference number	Author(s)	Year	Title, Source, Company, Report No, GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIA 7.2.3/03 IIA 7.2.1/10	Sur, R.; Dorn, S.	2012	Kinetic evaluation of the degradation rate of (pyridine-2,6-14C] BYI 02960 and its metabolite 6-chloronicotinic accid (6-CNA) for the determination of trigger and modelling endpoints Bayer CropScience, Report No.: MEF-11/838, Edition Number: M-422853-01-1 Date: 2012-01-18 GLP/GEP: no, unpublished	Y	BCS
IIA 7.2.3/04 IIA 7.1.1/06 IIA 7.2.1/06	Shepherd, J. J.	2011	[Pyridine-2,6-14C] BYI 02960: Aerobic soil metabolism in two US soils Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: MERVP038-1, Edition Number: M-413425-02-1 Date: 2011-09-06 ...Amended: 2012-01-05 GLP/GEP: yes, unpublished	Y	BCS
IIA 7.2.3/05 IIA 7.2.1/09	Sur, R.; Dorn, S.	2012	Kinetic evaluation of the degradation rate of [Ethyl-1-14C] BYI 02960 and its metabolite difluoroacetic acid (BYI 02960-DFA) for the determination of trigger and modelling endpoints Bayer CropScience, Report No.: MEF-11/855, Edition Number: M-422874-01-1 Date: 2012-01-18 GLP/GEP: no, unpublished	Y	BCS
IIA 7.3.1/01	Heinemann, O.	2011	Determination of the residues of BYI 02960 in/on soil after spraying of BYI 02960 SL 200 in the field in Germany, Italy, Spain and the United Kingdom Bayer CropScience, Report No.: 09-2702, Report includes Trial Nos.: 09-2702-01 09-2702-02 09-2702-03 09-2702-05 09-2702-06 09-2702-07 Edition Number: M-414245-01-1 Date: 2011-09-13 GLP/GEP: yes, unpublished	Y	BCS
IIA 7.4.1/01	Menke, U.; Telscher, M.	2008	[Pyridinylmethyl-14C] BYI 02960: Adsorption to and desorption from soils Bayer CropScience, Report No.: MEF-08/261, Edition Number: M-327492-01-2 Date: 2008-12-17 GLP/GEP: yes, unpublished	Y	BCS

Annex point/ reference number	Author(s)	Year	Title, Source, Company, Report No, GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIA 7.4.1/02	Stroech, K.	2010	[Pyridinylmethyl-14C] BYI 02960: Adsorption/desorption on two soils Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: MERVP017, Edition Number: M-363541-01-1 Date: 2010-01-29 GLP/GEP: yes, unpublished	Y	BCS
IIA 7.4.1/03 IIA 7.1.1/01 IIA 7.1.2/01	Menke, U.	2011	[Pyridinylmethyl-14C] BYI 02960: Aerobic soil metabolism/degradation and time-dependent sorption in soils Bayer CropScience, Report No.: MEF-07/334, Edition Number: M-414615-01-2 Date: 2011-08-05 GLP/GEP: yes, unpublished	Y	BCS
IIA 7.4.1/04	Sur, R.; Scherr, F.	2012	Evaluation of the time-dependent sorption of flupyradifurone based on laboratory time-dependent sorption experiments in four soils Bayer CropScience, Report No.: MEF-11/723, Edition Number: M-422824-01-1 Date: 2012-01-18 GLP/GEP: no, unpublished	Y	BCS
IIA 7.4.1/05	Scherr, F.	2014	Evaluation of the kinetic sorption parameters for flupyradifurone and their dependence on starting values Bayer CropScience, Report No.: EnSa-14-0804v2, Edition Number: M-491714-02-1 Date: 2014-07-10 **...Amended: 2014-07-30** GLP/GEP: no, unpublished	Y	BCS
IIA 7.4.2/01	Liu, A. C.	1997	Soil adsorption/desorption study 6-chloronicotinic acid (Acetamiprid metabolite) Rhone-Poulenc Ag Company, RTP, NC, USA Bayer CropScience, Report No.: C007666, Edition Number: M-196394-01-1 Date: 1997-09-15 GLP/GEP: yes, unpublished	N	NISSO
IIA 7.4.2/02	Menke, U.; Unold, M.	2011	[1-14C] BYI 02960-DFA (BCS-AB60481): Adsorption to and desorption from five soils Bayer CropScience, Report No.: MEF-10/538, Edition Number: M-413836-01-2 Date: 2011-08-26 GLP/GEP: yes, unpublished	Y	BCS

Annex point/ reference number	Author(s)	Year	Title, Source, Company, Report No, GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIA 7.4.3/01	de Souza, T. J. T.	2012	BYI 02960 - Mobility of [pyridine-2,6-14C]-BYI 02960 in Brazilian soils - Soil columns leaching method Bioensaios Análises e Consultoria Ambiental, Viamão, RS, Brazil Bayer CropScience, Report No.: 2301-LIX-344-11, Edition Number: M-424966-01-2 Date: 2012-02-08 GLP/GEP: yes, unpublished	Y	BCS
IIA 7.4.9/01 IIA 2.3.1/01	Smeykal, H.	2008	BYI 02960, pure substance: Vapour pressure - Final report Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20080615.01, Edition Number: M-309853-01-1 Date: 2008-10-10 GLP/GEP: yes, unpublished	Y	BCS
IIA 7.4.9/02 IIA 2.3.2/01	Bogdoll, B.; Eyrich, U.	2011	Flupyradifurone (BYI 02960): Calculation of the Henry´s law constant Bayer CropScience, Report No.: AF09/042, Edition Number: M-414341-01-1 Date: 2011-08-15 GLP/GEP: no, unpublished	Y	BCS
IIA 7.5/01 IIA 2.9.1/01	Mislankar, S.; Woodard, D.	2011	BYI-02960: Hydrolytic degradation Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: MERVP019, Edition Number: M-398952-01-1 Date: 2011-01-07 GLP/GEP: yes, unpublished	Y	BCS
IIA 7.6/01 IIA 2.9.2/01 IIA 2.9.4/01	Hall, L. R.	2011	Phototransformation of [14C] BYI 02960 in aqueous pH 7 buffer - amended report Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: MERVP042-1, Edition Number: M-418426-02-1 Date: 2011-11-28 ...Amended: 2012-03-05 GLP/GEP: yes, unpublished	Y	BCS
IIA 7.6/02	Heinemann, O.	2011	BYI 02960: Determination of the quantum yield and assessment of the environmental half-life of the direct photo-degradation in water Bayer CropScience, Report No.: MEF-11/554, Edition Number: M-414756-01-2 Date: 2011-09-26 GLP/GEP: yes, unpublished	Y	BCS

Annex point/ reference number	Author(s)	Year	Title, Source, Company, Report No, GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIA 7.6/03	Hall, L. R.	2011	Phototransformation of [14C] BYI 02960 in natural water Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: MERVP020, Edition Number: M-415368-01-1 Date: 2011-08-16 GLP/GEP: yes, unpublished	Y	BCS
IIA 7.8.2/01	Xu, T.	2012	[Pyridine-2,6-14C] BYI 02960: Anaerobic aquatic metabolism in two water/sediment systems Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: MERVP027, Edition Number: M-422616-01-1 Date: 2012-01-17 GLP/GEP: yes, unpublished	Y	BCS
IIA 7.8.3/01	Hellpointner, E.; Unold, M.	2012	[Pyridine-2,6-14C] BYI 02960: Aerobic aquatic metabolism Bayer CropScience, Report No.: MEF-11/907, Edition Number: M-422359-01-1 Date: 2012-01-12 GLP/GEP: yes, unpublished	Y	BCS
IIA 7.8.3/02	Unold, M.; Menke, U.	2012	[Furanone-4-14C] and [ethyl-1-14C] BYI 02960: Aerobic aquatic metabolism Bayer CropScience, Report No.: MEF-10/730, Edition Number: M-426504-01-1 Date: 2012-02-16 GLP/GEP: yes, unpublished	Y	BCS
IIA 7.8.3/03	Hellpointner, E.; Unold, M.	2012	[1-14C] BYI 02960-DFA (BCS-AB60481): Aerobic aquatic degradation Bayer CropScience, Report No.: M-422371-01-1, Edition Number: M-422371-01-1 Date: 2012-01-12 GLP/GEP: yes, unpublished	Y	BCS
IIA 7.8.3/04	Bruns, E.	2012	Fate of BYI 02960 (tech.) in outdoor microcosm ponds simulating actual exposure conditions in agricultural use Bayer CropScience, Report No.: EBRVP109, Edition Number: M-427167-01-1 Date: 2012-03-19 GLP/GEP: yes, unpublished	Y	BCS
IIA 7.10/01	Hellpointner; E.	2010	BYI 02960: Calculation of the chemical half-life in the troposphere Bayer CropScience, Report No.: MEF-10/896, Edition Number: M-398741-01-2 Date: 2010-12-27 GLP/GEP: no, unpublished	Y	BCS

References for the plant protection product

Annex point/ reference number	Author(s)	Year	Title, Source, Company, Report No, GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIIA 9.2.2/01	Schaefer, D.; Stroech, K.; Garside, C. M.	2014	Concentrations of flupyradifurone and its metabolites at drinking water abstraction points Bayer CropScience Bayer CropScience, Report No.: M-495332-01-1, Edition Number: M-495332-01-1 Date: 2014-09-02 GLP/GEP: n.a., unpublished	Y	BCS
IIIA1 9.4/01 IIIA1 9.5/01	Sur, R.; Ellerich, C.	2012	FPF PECsoil FOCUS EUR: Predicted environmental concentrations in soil (PECsoil) of flupyradifurone and its metabolites- Use in hops and lettuce in Europe Bayer CropScience, Report No.: EnSa-12-0117, Edition Number: M-428042-01-1 Date: 2012-03-21 GLP/GEP: no, unpublished	Y	BCS
IIIA1 9.4/02 IIIA1 9.5/02	Sur, R.; Ellerich, C.	2013	FPF PECsoil FOCUS EUR: Predicted environmental concentrations in soil (PECsoil) of flupyradifurone and its metabolites- Use in hops and lettuce in Europe Bayer CropScience, Report No.: EnSa-13-0432, Edition Number: M-461359-01-1 Date: 2013-07-09 GLP/GEP: no, unpublished	Y	BCS
IIIA1 9.4/03 IIIA1 9.5/03	Scherr, F; Ellerich, C.	2014	FPF PECsoil FOCUS EUR: Predicted environmental concentrations in soil (PECsoil) of flupyradifurone and its metabolites- Use in hops and lettuce in Europe Bayer CropScience, Report No.: EnSa-14-0903, Edition Number: M-495501-01-1 Date: 2014-08-01 GLP/GEP: no, unpublished	Y	BCS
IIIA1 9.6.1/01 IIIA1 9.6.2/01	Sur, R.; Ellerich, C.	2012	FPF PECgw FOCUS EUR: Predicted environmental concentrations in groundwater recharge based on models FOCUS PEARL and FOCUS PELMO - Use in hops in Europe Bayer CropScience, Report No.: EnSa-12-0089, Edition Number: M-427737-01-1 Date: 2012-03-16 GLP/GEP: no, unpublished	Y	BCS

Annex point/ reference number	Author(s)	Year	Title, Source, Company, Report No, GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIIA1 9.6.1/02 IIIA1 9.6.2/02	Sur, R.; Ellerich, C.	2012	Tier 2a (DFOP) FPF PECgw EU: Predicted environmental concentrations in groundwater recharge based on models Focus Pearl and Focus Pelmo - Use in hops in Europe - Flupyradifurone (BYI 02960) - Difluoroacetic acid (DFA) - 6-Chloronicotinic acid (6-CNA) Bayer CropScience, Report No.: EnSa-12-0090, Edition Number: M-427991-01-1 Date: 2012-03-21 GLP/GEP: no, unpublished	Y	BCS
IIIA1 9.6.1/03 IIIA1 9.6.2/03	Sur, R.; Ellerich, C.	2012	FPF PECgw FOCUS EUR (TDS): Predicted environmental concentrations in groundwater recharge based on models FOCUS PEARL and FOCUS PELMO - Use in hops in Europe Bayer CropScience, Report No.: EnSa-12-0091, Edition Number: M-427980-01-1 Date: 2012-03-21 GLP/GEP: no, unpublished	Y	BCS
IIIA1 9.6.1/04 IIIA1 9.6.2/04	Sur, R.; Ellerich, C.	2012	FPF PECgw FOCUS EUR: Predicted environmental concentrations in groundwater recharge based on models FOCUS PEARL and FOCUS PELMO - Use in lettuce in Europe Bayer CropScience, Report No.: EnSa-12-0097, Edition Number: M-427736-01-1 Date: 2012-03-16 GLP/GEP: no, unpublished	Y	BCS
IIIA1 9.6.1/05 IIIA1 9.6.2/05	Sur, R.; Ellerich, C.	2012	Tier 2a (DFOP) FPF PECgw EU: Predicted environmental concentrations in groundwater recharge based on models Focus Pearl and Focus Pelmo - Use in lettuce in Europe - Flupyradifurone (BYI 02960) - Difluoroacetic acid (DFA) - 6-Chloronicotinic acid (6-CNA) Bayer CropScience, Report No.: EnSa-12-0098, Edition Number: M-427987-01-1 Date: 2012-03-21 GLP/GEP: no, unpublished	Y	BCS
IIIA1 9.6.1/06 IIIA1 9.6.2/06	Sur, R.; Ellerich, C.	2012	FPF PECgw FOCUS EUR (TDS): Predicted environmental concentrations in groundwater recharge based on models FOCUS PEARL and FOCUS PELMO - Use in lettuce in Europe Bayer CropScience, Report No.: EnSa-12-0099, Edition Number: M-427981-01-1 Date: 2012-03-21 GLP/GEP: no, unpublished	Y	BCS

Annex point/ reference number	Author(s)	Year	Title, Source, Company, Report No, GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIIA1 9.6.1/07 IIIA1 9.6.2/07	Scherr, F.	2013	Flupyradifurone (FPF) and metabolites: PECgw FOCUS PEARL, PELMO EUR - Use in lettuce (glasshouse) in Europe Bayer CropScience, Report No.: EnSa-13-0468, Edition Number: M-461318-01-1 Date: 2013-07-25 GLP/GEP: no, unpublished	Y	BCS
IIIA1 9.6.1/08 IIIA1 9.6.2/08	Scherr, F.; Ellerich, C.	2013	Flupyradifurone (FPF) and metabolites: PECgw FOCUS PEARL, PELMO EUR - Use in lettuce and hops in Europe Bayer CropScience, Report No.: EnSa-13-0426, Edition Number: M-461289-01-1 Date: 2013-07-25 GLP/GEP: no, unpublished	Y	BCS
IIIA1 9.6.1/09 IIIA1 9.6.2/09	Scherr, F.; Ellerich, C.	2014	Flupyradifurone (FPF) and metabolites: PECgw FOCUS PEARL, PELMO EUR - Use in lettuce and hops in Europe Bayer CropScience, Report No.: EnSa-14-0869, Edition Number: M-495503-01-1 Date: 2014-07-15 GLP/GEP: no, unpublished	Y	BCS
IIIA1 9.7/01 IIIA1 9.8/01	Sur, R.; Ellerich, C.	2012	FPF PECsw FOCUS EUR: Predicted environmental concentrations in surface water and sediment - Use in hops and lettuce in Europe Bayer CropScience, Report No.: EnSa-12-0071, Edition Number: M-427646-01-1 Date: 2012-03-16 GLP/GEP: no, unpublished	Y	BCS
IIIA1 9.7/02 IIIA1 9.8/02	Scherr, F.; Ellerich, C.	2013	Flupyradifurone (FPF) and metabolites: PECsw,sed FOCUS EUR - Use in lettuce and hops in Europe Bayer CropScience, Report No.: EnSa-13-0431, Edition Number: M-461364-01-1 Date: 2013-07-22 GLP/GEP: no, unpublished	Y	BCS

European Commission

Flupyradifurone

VOLUME 3

ANNEX B

B.9 Ecotoxicology

Rapporteur Member State: The Netherlands

January 2014 November 2014 December 2014

Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible approval of flupyradifurone under Regulation (EC)1107/2009

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NB for the Pesticide Peer Review meeting of December 2014, an addendum was made as a discussion document. This addendum is integrated in this revised DAR of December 2014. Thus, all relevant information can be found in the document in hand.

B.9 Ecotoxicology

Introduction

Flupyradifurone (BYI 02960) is a new active substance. It is a xylem mobile insecticide with systemic activity, effective against aphids, leafhoppers, whiteflies, scales and psyllids.

The formulation BYI 02960 SL200 (Sivanto, also called BYI 02960 SL 200 G, BYI 02960 SL 200 g/L and Flupyradifurone SL 200 G) is the representative formulation for the registration of Flupyradifurone (BYI 02960) in Europe, with a nominal content of 200 g a.s./L.
The intended uses of Sivanto, which are being supported for the first inclusion of flupyradifurone in Annex I of the Council Directive 91/414/EEC, are aphid control in hops (field) and in lettuce (field and greenhouse). See table 10-1 for the intended use pattern.

Table 10- 1 Intended use pattern

Crop	F or G*	Timing of application BBCH (months)	Number of applications	Application interval [days]	Maximum label rate [L/ha]	Maximum application rate, individual treatment [g a.s./ha] BYI 02960	Remarks
Hops	F	31 – 75 (May-June)	1	-	0.750	150	
Lettuce 1)	F	12 – 49 (May-Oct)	1	-	0.625	125	Max 1 application per 24 months
Lettuce 1)	F	41 – 49 (May-Oct)	1	-	0.625	125	Max 1 application per 12 months
Lettuce 1)	G 2)	12 – 49 (Jan-Dec)	2	10	0.625	125	Max 2 applications per 12 months

*F Field use G Glasshouse use

1) Head and leafy lettuce 2) Soil bound

List of BYI 02960 metabolites included in this section

In the original study reports on BYI 02960 the metabolites are sometimes named by different synonyms, the metabolites referred to in this section are summarized below (table provided by the notifier). Full details are provided in Document N.

	Name, Structure IUPAC name CAS name, [CAS number]	Molecular formula molar mass Other names / codes	Occurrence
a.s.	BYF 02960 (parent compound)		
		C12 H11 Cl F2 N2 O2 288.68 g/mol Flupyradifurone	all matrices
M03	BYI 02960-OH		
		C12 H11 Cl F2 N2 O3 304.68 g/mol BYI 02960-hydroxy BCS-CQ74364	Animal, Plant:
M21	BYI 02960-CHMP		
		C6 H6 Cl N O 143.57 g/mol 6-CPA (6-chloro-picolylacohol) BCS-AA52175	Plant:
M27	6-CNA		
		C6 H4 Cl N O2 157.56 g/mol 6-chloronicotinic acid IC-0 (in reports from Nippon Soda Co. Ltd.) BYI 02960-6-CNA BCS-AA35572	Animal, Plant Environment Aerobic soil (major)
M34	BYI 02960-difluoroethyl-amino-furanone		
		C6 H7 F2 N O2 163.12 g/mol DFEAF	Animal, Plant
M44	DFA		

		C2 H2 F2 O2 96.03 g/mol difluroacetic acid BYI 02960-DFA BCS-AA56716 (In aquatic studies, tested as sodium difluoroacetate (Na-salt of difluoroacetic acid) (code: BCS-AB60481)	Animal, Plant: Environment Aerobic Soil (major) Aerobic water/Sediment (major)
M47	BYI 02960-azabicyclosuccinamide		
		C12H14F2N2O4 288.25 g/mol BCS-CS64875 (Tested as BYI 02960-azabicyclosuccinamide Na-Salt, BCS-CU93236)	Environment Water – aquatic photolysis (major)
M48	BYI 02960-succinamide		
		C12H13ClF2N2O3 306.69 g/mol BCS-CR74729	Environment Water – Aquatic photolysis (major)

B.9.1 Effects on birds (IIA 8.1; IIIA 10.1)

B.9.1.1 Acute oral toxicity (IIA 8.1.1)

STUDY IIA, 8.1.1/01

Characteristics of the study

reference	:	[redacted]. (2010)	species	:	Bobwhite quail (*Colinus virginianus*)
type of study	:	Acute oral toxicity study	observations	:	14 days
year of execution	:	2009	dose	:	25, 50, 100, 200 and 400 mg a.s./kg bw
GLP statement	:	Yes	dosing method	:	Gavage in gelatin capsule
guideline	:	OPPTS 850.2100, OECD 223	acceptability	:	Acceptable
test substance	:	BYI 02960; technical, batch BYI 02960-01-03 (origin batch 2009-000239)	LD50	:	232 mg a.s./kg bw
purity	:	96.2%			

Methods

In a study on the acute oral toxicity of BYI 02960 technical to bobwhite quail (*Colinus virginianus*), groups of five males and five females per dose level (14-week-old, fasted overnight) were orally dosed with BYI 02960 technical in gelatin capsules at dose levels of 25, 50, 100, 200 and 400 mg a.s./kg bw, followed by a fourteen day observation period. A control receiving empty gelatin capsules was included.

Results

The number of bird moralities during the study were: control (0), 25 (0), 50 (0), 100 (0), 200 (4), and 400 (9) mg a.s./kg body weight. The acute oral LD50 (Probit analysis) was 232 mg a.s./kg bw (95% CI 173-313 mg a.s./kg bw).

Signs of toxicity (hypo-reactivity) were observed at 100 mg a.s./kg bw (1 bird) and at 200-400 mg a.s./kg bw (4-8 birds).

Male and female body weight was statistically significantly reduced at 200 mg a.s./kg bw on day 7, and the body weight of the single female survivor at 400 mg a.s./kg bw was also reduced on day 7. Male and female body weight gain was statistically significantly reduced at 200 mg a.s./kg bw during day 0-7, and female body weight gain also during day 0-14, whereas body weight gain of the single female survivor at 400 mg a.s./kg bw was reduced on day 0-7 and 0-14.

Feed consumption was not subjected to statistical analysis, but was reduced in a dose-related manner in all treated female groups. Male feed consumption was increased relative to the control in all treated groups.

Necropsy (performed on all birds in the control and at 100-400 mg a.s./kg bw, and on 4 birds in the remaining groups) revealed no treatment-related abnormalities.

Conclusion

Acute oral LD50 for bobwhite quail 232 mg a.s./kg bw.

Guidelines and limitations

The study is acceptable.

STUDY IIA, 8.1.1/02

Characteristics of the study

reference	:	[redacted] (2011)	species	:	Canary (*Serinus canaria*)
type of study	:	Acute oral toxicity study	observations	:	14 days
year of execution	:	2010	dose	:	44, 88, 175, 350 and 700 mg a.s./kg bw
GLP statement	:	Yes	dosing method	:	Gavage in gelatin capsule
guideline	:	OPPTS 850.2100, OECD 223	acceptability	:	Acceptable
test substance	:	BYI 02960; technical, batch BYI 02960-01-03 (origin batch 2009-000239)	LD50	:	330 mg a.s./kg bw
purity	:	96.2%			

Methods

In a study on the acute oral toxicity of BYI 02960 technical to the canary (*Serinus canaria*), groups of five males and five females per dose level (in adult plumage, fasted overnight) were orally dosed with BYI 02960 technical in gelatin capsules at dose levels of 44, 88, 175, 350 and 700 mg a.s./kg bw, followed by a fourteen day observation period. A control receiving empty gelatin capsules was included.

Results

The number of bird moralities during the study were: control (0), 44 (0), 88 (0), 175 (5), 350 (5), and 700 (7) mg a.s./kg body weight. The acute oral LD50 (Probit analysis) was 330 mg a.s./kg bw (95% CI 215-625 mg a.s./kg bw).

Signs of toxicity (including loss of muscular coordination, lethargy, diminished reaction to stimuli, prostrate, unable to move) were observed at 44-700 mg a.s./kg bw (6-10 birds).

There were no treatment related effects on male and female body weight, body weight gain and feed consumption.

Post-mortem examinations were not performed.

Conclusion

Acute oral LD50 for canary 330 mg a.s./kg bw.

Guidelines and limitations

The study is acceptable.

STUDY IIA, 8.1.1/03

Characteristics of the study

reference	:	[redacted] (2011)	species	:	Hen (*Gallus gallus domesticus*)
type of study	:	Acute oral toxicity study	observations	:	28 days
year of execution	:	2011	dose	:	2000 mg a.s./kg bw
GLP statement	:	Yes	dosing method	:	Gavage in gelatin capsule
guideline	:	OPPTS 850.2100, OECD 223	acceptability	:	**Not acceptable**
test substance	:	BYI 02960 (tech.), batch BYI 02960-01-03 (origin batch 2009-000239)	LD50	:	**Not reliable**
purity	:	96.2%			

Methods

In a study on the acute oral toxicity of BYI 02960 technical to hens (*Gallus domesticus*), five hens (18 weeks old, fasted overnight) were orally dosed with BYI 02960 technical in gelatin capsules at a dose level of 2000 mg a.s./kg bw, followed by a 28-day observation period. A control consisting of 10 hens receiving empty gelatin capsules was included.

Results

No mortalities occurred in the control and the treated group during the 28-day observation period. Due to findings on day 21, the test was prolonged to 28 days. Half of the control birds were sacrificed on day 21. The report described the study findings as follows:

"*The most noticeable effect after the application was an almost complete reduction of food consumption with all dosed birds. All further observed symptoms were consequences of this starvation, e.g. excretion of uric acid, fluffed feather, or reduced vigilance. One bird started to feed in the 2nd week after application, another one in the 3rd week after application. Both birds showed soft excrements or diarrhoea as indication of digestion distress. A third bird behaved similarly in the 4th week after application.*

Two birds dosed did not resume feeding at all after application of the test substance. They were sacrificed for human reasons on day 28. These birds may be considered as treatment related mortalities. Since all other birds were free of symptoms or showed clear indications of recovery by that time point, the test was terminated on day 28.

Due to the reduced food consumption all dosed birds showed severe body weight losses. Two birds started to regain body weight in the last week of the test.

Pathological findings at necropsy: All birds were emaciated."

The two birds humanely killed on day 28 were considered as treatment related mortalities and factored into the LD50 calculation. Based on these findings, the author of the report estimated the LD50 to be >2000 mg a.s./kg bw.

Evaluation by RMS

Results on feed intake and body weight measurements are summarised in the table below. The report did not state which two dosed birds, which did not resume feeding at all after application of the test substance, were sacrificed for humane reasons on day 28. The results in the Table show that feed intake of birds 17, 18 and 19 during day 7-28 was comparable and negligible, and terminal body weights of these 3 birds were equally low, with comparable body weight loss during day 21-28. It is unclear why the author of the report stated that "*One bird started to feed in the 2nd week after application, another one in the 3rd week after application. Both birds showed soft excrements or diarrhoea as indication of digestion distress. A third bird behaved similarly in the 4th week after application.*", since 3 out of 5 birds hardly fed during day 7-28.

Post mortem examination of these 3 birds gave comparable findings (all 3 kidneys reduced and emaciated or strongly emaciated). It is unclear why the author of the report stated that on day 28 "*three birds were free of symptoms or showed clear indications of recovery*", since no clear indications of recovery of feed intake or body weight gain were apparent during the last study week. In the opinion of the RMS, not two but three birds should have been considered as treatment related mortalities and factored into the LD50 calculation. Since a limit test with three mortalities out of 5 tested is not suitable to derive an accurate LD50 value, the conclusion is that the test does not produce a reliable LD50.

Table B.9.1.1-01 Feed intake and body weight measurements in acute oral toxicity study in hen

	feed intake (g/hen/day)						
treatment	day 0	day 1	day 2	day 3-7	day 7-14	day 14-21	day 21-28
control (mean)	107	88	80	79	83	87	97
bird 16	1	0	1	11	4	12	48
bird 17	2	1	1	1	0	3	1
bird 18	2	0	1	13	0	0	4
bird 19	2	1	2	13	2	5	4
bird 20	2	1	1	15	10	27	97
	body weight (g)						
	day -1	day 3	day 7	day 14	day 21	day 28	
control (mean)	1294	1371	1403	1429	1412	1440	
bird 16	1243	1048	1004	907	847	1045	
bird 17	1265	1217	1106	974	835	748	
bird 18	1452	1336	1201	1035	882	731	
bird 19	1366	1135	1089	990	891	781	
bird 20	1170	987	951	862	879	1196	

Conclusion

The test did not produce a reliable LD50.

Guidelines and limitations

Comments were incorporated in the study summary. The study is not acceptable.

STUDY IIIA, 10.1.6/01

Characteristics of the study

reference	:	[redacted] (2012)	species	:	Bobwhite quail (*Colinus virginianus*)
type of study	:	Acute oral toxicity study	observations	:	21 days
year of execution	:	2011	dose	:	250, 500, 1000, 2000 and 4000 mg a.s./kg bw
GLP statement	:	Yes	dosing method	:	Gavage in deionised water
guideline	:	OPPTS 850.2100	acceptability	:	Acceptable
test substance	:	BYI 02960 SL 200, batch 2010-001067	LD50	:	431 mg a.s./kg bw
a.s. content	:	17.1 % w/w			

Methods

In a study on the acute oral toxicity of BYI 02960 SL 200 (17.1% BYI 02960) to bobwhite quail (*Colinus virginianus*), groups of five males and five females per dose level (16-week-old, fasted overnight) were orally dosed with BYI 02960 SL 200 in deionised water at dose levels of 250, 500, 1000, 2000 and 4000 mg a.s./kg bw, followed by a 21-day observation period. A control receiving deionised water only was included.

Results

The number of bird moralities during the study were: control (0), 250 (1), 500 (6), 1000 (10), 2000 (10), and 4000 (10) mg a.s./kg body weight. The acute oral LD50 (Probit analysis) was 431 mg a.s./kg bw (95% CI 320-576 mg a.s./kg bw).

Signs of toxicity (immobility and hypo-reactivity) were observed at 250 mg a.s./kg bw and above. Male body weights were reduced at 500 mg a.s./kg bw on day 7, and female body weight of the single female survivor at 500 mg a.s./kg bw was reduced on day 7, 14 and 21. Feed consumption of the males at 500 mg a.s./kg bw was reduced compared to the control. Necropsy (performed on all surviving birds at the end of the study) revealed no treatment-related abnormalities.

Conclusion

Acute oral LD50 for bobwhite quail 431 mg a.s./kg bw.

Guidelines and limitations

The study is acceptable.

STUDY IIIA, 10.1.6/02

Characteristics of the study

reference	:	[redacted] (2012)	species	:	Hen (*Gallus domesticus*)
type of study	:	Acute oral toxicity study	observations	:	21 days
year of execution	:	2011	dose	:	2000 mg product/kg bw

GLP statement	:	Yes	dosing method	:	Gavage in gelatin capsule
guideline	:	OPPTS 850.2100, OECD 223	acceptability	:	Acceptable
test substance	:	BYI 02960 SL 200, batch 2011-002192	LD50	:	>2000 mg product/kg bw (>342 mg a.s./kg bw)
a.s. content	:	17.1 % w/w			

Methods

In a study on the acute oral toxicity of BYI 02960 SL 200 (17.1% BYI 02960) to hens (*Gallus domesticus*), five hens (18 weeks old, fasted overnight) were orally dosed with BYI 02960 SL 200 in gelatin capsules at a dose level of 2000 mg product/kg bw, followed by a 21-day observation period. A control consisting of 10 hens receiving empty gelatin capsules was included.

Results

No mortalities occurred in the control and the treated group during the 21-day observation period. Signs of intoxiciation in the treated group included sitting, tremor, reduced vigilance, apathy, discoordinated movements, ptosis, excretion of uric acid, diarrhea and soft excrements. At test termination all birds were free of symptoms. Treated birds lost weight between day 0 and 3, but regained weight thereafter. After the treatment, the dosed birds avoided food almost completely. Four of 5 birds started to feed from day 3 onwards, the last bird from day 7 onwards. Afterwards their feed intake was comparable to control birds. Necropsy (performed on 5 birds in the control and all treated birds) revealed no treatment-related abnormalities.

Conclusion

Acute oral LD50 for hen >2000 mg product/kg bw (>342 mg a.s./kg bw).

Guidelines and limitations

The study is acceptable.

B.9.1.2 Dietary toxicity (IIA 8.1.2 & IIA 8.1.3)

STUDY IIA, 8.1.2/01

Characteristics of the study

reference	:	[redacted] (2010)	species	:	Mallard duck (*Anas platyrhynchos*)
type of study	:	Dietary LC50 study	exposure duration	:	5 days
year of execution	:	2009	nominal conc.	:	313, 625, 1250, 2500 and 5000 mg a.s./kg diet
GLP statement	:	Yes	dosing method	:	Test substance in solvent was mixed with diet using corn oil as excipient
guideline	:	OPPTS 850.2200 & OECD 205	acceptability	:	Acceptable
test substance	:	BYI 02960; technical, batch 2009-000239	LC50	:	>4741 mg a.s./kg diet ⇔ >825 mg a.s./kg bw/day
purity	:	96.2%			

Methods

In a study on the dietary toxicity of BYI 02960 technical, five groups of 10-day old mallard ducks of indeterminate sex (10/group) were fed BYI 02960 technical (dissolved in an unspecified solvent, excipient corn oil, max 1% w/w) in the diet at nominal concentrations of 313, 625, 1250, 2500 and 5000 mg a.s./kg diet, respectively, for a period of 5 days followed by a 3-day recovery period. A control group of ten 10-day old ducklings received feed containing the solvent and corn oil only. Samples of test diet were taken from all treatment groups and analysed for accuracy, using a sufficiently validated HPLC/UV method. Homogeneity and freezer and feeder stability were determined for the lowest and the highest test concentration.

Results

The analytically confirmed concentrations were 294, 581, 1175, 2238 and 4741 mg a.s./kg diet, representing 90-95% of the nominal concentrations. Based on day 0-5 mean feed consumption and body weight data, these concentrations corresponded with 66, 129, 272, 459 and 825 mg a.s./kg bw/d. Homogeneity and freezer and feeder stability were confirmed.

No birds died and no signs of toxicity were observed in any group. Body weights and body weight gain were statistically significantly (5% level) reduced at 2238 and 4741 mg a.s./kg diet on day 5, but after a 3-day recovery period a statistically significantly reduction of body weight was only apparent at 4741 mg a.s./kg diet, whilst body weight gain during day 5-8 in all treatment groups was comparable to the control. Feed consumption was not statistically analysed, but was reduced at 2238 and 4741 mg a.s./kg diet on day 5. During day 5-8 feed consumption in all treatment groups was comparable to the control.

Post-mortem examination (performed on all birds in the control and the high dose group, and on 4 birds in the remaining groups) revealed no abnormalities.

The 5-day dietary LC50 was >4741 mg a.s./kg diet, equivalent to >825 mg a.s./kg bw/day.

Conclusion

5-day dietary LC50 >4741 mg a.s./kg diet, equivalent to >825 mg a.s./kg bw/day.

Guidelines and limitations

The study is acceptable.

STUDY IIA, 8.1.2/02

Characteristics of the study

reference	:	[redacted] (2010)	species	:	Bobwhite quail (*Colinus virginianus*)
type of study	:	Dietary LC50 study	exposure duration	:	5 days
year of execution	:	2009	nominal conc.	:	313, 625, 1250, 2500 and 5000 mg a.s./kg diet
GLP statement	:	Yes	dosing method	:	Test substance in solvent was mixed with diet using corn oil as excipient
guideline	:	OPPTS 850.2200 & OECD 205	acceptability	:	Acceptable
test substance	:	BYI 02960; technical, batch 2009-000239	LC50	:	>4876 mg a.s./kg diet ⇔ >470 mg a.s./kg bw/day
purity	:	96.2%			

Methods

In a study on the dietary toxicity of BYI 02960 technical, five groups of 13-day old bobwhite quail (*Colinus virginianus*) of indeterminate sex (10/group) were fed BYI 02960 technical (dissolved in an unspecified solvent, excipient corn oil, max 1% w/w) in the diet at nominal concentrations of 313, 625, 1250, 2500 and 5000 mg a.s./kg diet, respectively, for a period of 5 days followed by a 3-day recovery period. A control group of ten 10-day old quails received feed containing the solvent and corn oil only. Samples of test diet were taken from all treatment groups and analysed for accuracy, using a sufficiently validated HPLC/UV method. Homogeneity and freezer and feeder stability were determined for the lowest and the highest test concentration.

Results

The analytically confirmed concentrations were 278, 607, 1133, 2075 and 4876 mg a.s./kg diet, representing 83-98% of the nominal concentrations. Based on day 0-5 mean feed consumption and body weight data, these concentrations corresponded with 48, 99, 170, 262 and 470 mg a.s./kg bw/d. Homogeneity and freezer and feeder stability were confirmed.

No birds died and signs of toxicity (hyporeactivity, loss of righting reflex, wing drop) were only observed in the highest treatment group. Body weights and body weight gain were statistically significantly (5% level) reduced in the three highest treatment groups on day 5, and after a 3-day recovery period a statistically significantly reduction of body weight was still apparent in the two highest treatment groups, whilst body weight gain during day 5-8 in all treatment groups was comparable to the control. Feed consumption was not statistically analysed, but was reduced in the two highest treatment groups during day 0-4. During day 5-7 feed consumption in all treatment groups was comparable to the control.

Post-mortem examination (performed on all birds in the control and the high dose group, and on 4 birds in the remaining groups) revealed no abnormalities.

The 5-day dietary LC50 was >4876 mg a.s./kg diet, equivalent to >470 mg a.s./kg bw/day.

Conclusion

5-day dietary LC50 >4876 mg a.s./kg diet, equivalent to >470 mg a.s./kg bw/day.

Guidelines and limitations

The study is acceptable.

B.9.1.3 Subchronic and reproductive toxicity (IIA 8.1.4)

STUDY IIA, 8.1.4/01

Characteristics of the study

reference	:	[redacted] (2012a)	species	:	Mallard duck (*Anas platyrhynchos*)
type of study	:	birds, reproduction	exposure duration	:	20 weeks
year of execution	:	2009/2010	nominal conc.	:	111, 333 and 1000 mg a.s./kg diet (actual measured: 91, 298 and 845 mg a.s./kg diet)
GLP statement	:	Yes	dosing method	:	Test substance in solvent was mixed with diet
guideline	:	OPPTS 850.2300; US-EPA, FIFRA Subdivision E, 71-4; OECD 206	acceptability	:	Acceptable
test substance	:	BYI 02960; technical, batch 2009-000239	NOEC	:	845 mg a.s./kg diet ⇔ 83 mg a.s./kg bw/day
purity	:	96.2%			

Introduction

Following summary and evaluation is the combination of the initial evaluation of the study of Stoughton et al (2012a) by the RMS and a more detailed evaluation provided by the notifier as response to questions posed by RMS. Also figures, tables and statistical evaluation performed by RMS are added to the text.

Description

Reproduction study with the mallard.

Methods. Birds were housed in pairs. 15 pairs were tested per concentration. Adult birds were acclimated for 7 weeks and then exposed to the test substance in the diet for 20 weeks, 8 weeks prior to egg laying and 10 weeks during egg laying and were evaluated for treatment related effects upon bird health and reproduction. Birds were 12 weeks old upon arrival. Test substance was mixed with diet, nominal concentrations 111, 333 and 1000 mg as/kg fd and a control containing an amount of solvent (unknown) equivalent to the amount in the treated diets. Fifteen replicate groups with one male and one female each. Eggs were collected twice a day during working days from the onset of egg production and set weekly for incubation. Table with the study phases is given below.

Table 9.1.3-1. Study phases test with *Anas platyrhynchos*

Study phases
Acclimation – 7 weeks
Pre-photo stimulation with adult exposure to flupyradifurone – 8 weeks
Pre-egg laying (with photo stimulation) period with adult exposure to flupyradifurone –3 weeks
Egg laying with adult exposure to flupyradifurone – approx. 9 weeks
Post-adult termination (final incubation, hatching, and 14-day offspring rearing period) –6 weeks

Observations. Birds were observed daily for mortality, abnormal behaviour and signs of toxicity. Body weight of adults was determined at test initiation, on weeks 3, 5, 7, 9 and at the adult termination. Feed consumption was measured once a week. Reproductive parameters were determined as from week 12, and number of hatchlings and number of 14-day old survivors, body weight of hatchlings and

14-day old survivors were recorded. Hatchlings were observed daily for signs of toxicity, injuries, illness and mortality. Hatchlings were sacrificed by CO_2 asphyxiation at day 14. Gross pathology of adults was conducted at test termination.

Reproductive endpoints.

Following reproductive endpoints were assessed (see Table below).

Table 9.1.3-2. Reproductive endpoints

Endpoints	Description
Adult Body Weight:	The adult body weight change from randomization to adult termination. Analyzed on a per cage basis by sex.
Adult Feed Consumption:	The means were calculated as grams/bird/day. Analyzed on a per cage basis.
Eggs Laid per Hen:	Analyzed as the total egg production. Eggs laid were analyzed on a per hen basis.
Eggs Cracked of Eggs Laid:	Cracked eggs are determined by candling prior to incubation.Cracked eggs were analyzed on a per hen basis.
Eggs Set:	Eggs placed under incubation are “set.” Analyzed as the total number during the dosing phase. Eggs set were analyzed on a per hen basis.
Viable Embryos (mean):	Fertility is determined by candling on the 14th day of incubation. Fertility analyzed as total fertile eggs. Fertility was analyzed on a per hen basis.
Live Embryos (mean):	Live embryos are determined by candling on the 21st day of incubation. Analyzed as the total number of live embryos on a per hen basis.
Eggs Not Cracked of Laid (%):	Eggs laid that were not cracked as a percentage of eggs laid on a per hen basis. Analyzed as the mean percentage during the reproductive phase.
Eggs Set of Laid (%):	Eggs set as a percentage of eggs laid on a per hen basis. Analyzed as the mean percentage during the reproductive phase.
Viable Embryos of Eggs Set (%):	Live embryos as a percentage of eggs set on a per hen basis. Analyzed as the mean percentage during the reproductive phase.
Live Embryos of Viable Embryos (%):	Live embryos as a percentage of viable embryos on a per hen basis. Analyzed as the mean percentage during the reproductive phase.
Number Hatched (mean):	Live hatchlings that had liberated themselves from their eggs by day 28 of incubation. Analyzed as the total number of normal hatchlings on a per hen basis.
14-day-old Survivors (mean):	Live chicks at 14-days post hatch. Analyzed as the total number of 14-day survivors on a per hen basis.
Number Hatched of Eggs Laid (%):	Hatchlings as a percentage of eggs laid on a per hen basis. Analyzed as the mean during the reproductive phase.
Number Hatched of Eggs Set (%):	Hatchlings as a percentage of eggs set on a per hen basis. Analyzed as the mean during the reproductive phase.
Number Hatched of Live Embryos (%):	Hatchlings as a percentage of live embryos on a per hen basis. Analyzed as the mean during the reproductive phase.
14-day-old Survivors of Eggs Set (%):	Live chicks at 14-days post hatch as a percentage of eggs set on a per hen basis. Analyzed as the mean during the reproductive phase.
14-day-old Survivors of Number Hatched (%):	Live chicks at 14-days post hatch as a percentage of hatchlings on a per hen basis. Analyzed as the mean during the reproductive phase.
Hatchling Body Weight:	Individual weights of the live hatchlings taken upon removal from the hatcher, analyzed on a per hen basis. Analyzed as the overall mean during the reproductive phase
14-day-old Survivor Body Weight:	Individual weights of the 14-day-old offspring taken at sacrifice, analyzed on a per hen basis. Analyzed as the overall mean during the reproductive phase.

Eggshell Strength:	The force needed to penetrate the shell and membrane measured at one point on the waist of the egg, analyzed on a per hen basis.
Eggshell Thickness:	The thickness of the shell plus the membrane measured at three points around the waist of the egg, analyzed on a per hen basis

Conditions. housing in pairs, 21.3 °C, 54.7% RH, 7:17 h L:D for the first 15 weeks including acclimation phase. Photoperiod was increased to 17:7 h L:D to induce egg laying (95-250 lux). Eggs were placed in an incubator (36.9°C, 59.8 % RH). On day 23 of in cubation, eggs were place into a hatcher and allowed to hatch (36.8 °C, 69.3 % RH). Hatchlings were placed in a brooding compartment at approx. 32-38°C in 14-d post hatchling phase with 14 h light per day. Feed and water *ad libitum*.

Chemical analysis.

Method validation: Samples were spiked at 10, 100, 500 and 1000 mg/kg fd, extracted and analysed.

Analysis of test diet: Actual concentrations in the diet were determined at week 1, 5, 10, 15 and 20 from samples of 111, 333 and 1000 mg as/kg feed.

Homogeneity of test diet: Samples from test concentrations 111 and 1000 mg/kg fd were taken from the top, middle and bottom of the mixing vessel of samples taken from the first week.

Stability test diet: Samples to verify the stability of the test substance under actual test conditions were taken on day 7 and after a 5-week freezing period, both with samples treated at 111 and 1000 mg/kg fd.

Extraction with acetonitrile/HPLC-grade water (1:1 v/v) by by placing on a magnetic stir plate for stirring during 2 h. Then, the feed was allowed to settle to the bottom of the jar. A 3 mL aliquot of the organic layer was transferred to a centrifuge tube and was rinsed with with acetonitrile/HPLC-grade water. Extracts were analysed by HPLC. Limit of quantitiation was 10 mg/kg fd.

Statistics. The no observed adverse effect concentration (NOAEC) and lowest observed adverse effect concentration (LOAEC) were identified for each parameter (with the exception of adult termination bodyweight) using hypothesis testing methodology. Data from flupyradifurone treatment groups were compared to controls using the Shapiro-Wilk's test for normality and Levene's test of equal variance to determine if dose groups had unequal variances. If assumption of normality ($p \leq 0.01$) and homogeneity of variance ($p \leq 0.05$) were met, then parametric analyses were conducted using analysis of variance (ANOVA) followed by Dunnett's test or William's test. If variances were unequal, then the non-parametric analyses were conducted using the Jonckheere or Mann-Whitney procedures. Statistical analyses were performed at the $\alpha = 0.05$ (95% confidence level). Analysis of adult termination bodyweight data was conducted by subjecting data to a Chi Square Test for normality and a Bartlett's Test for equal variances. The analysis of variance was conducted followed by a Dunnett's Test for unequal replicate size.

Results

Diet analysis

Analytical recovery. Overall mean procedural recovery of fortified samples was 111%, relative standard deviation (RSD) 2%.

Homogeneity*:* The average of the measured concentrations from six 111-ppm homogeneity samples was 108 ppm (97 % of nominal) with a RSD of 13 %. The average of the measured concentrations from six 1000-ppm homogeneity samples was 1001 ppm (100 % of nominal) with a RSD of 8 %.
Concentration verification*:* Freshly prepared feed contained overall average measured concentrations of 91.3, 298, 845 mg/kg fd for the 111, 333 and 1000 mg/kg fd concentrations, respectively (83, 90 and 85% recovery)..
Stability: At day 7 recovery was 78 and 93%, from 100 and 1000 mg/kg fd concentrations, respectively, confirming stability. The recovery from frozen samples averaged 81% for 106 mg/kg fd and 93% for 999 mg/kg fd spiked concentration.
Biological observations
Adult mortality: There were no treatment related mortalities in any of the treatment groups tested. No overt signs of toxicity were noted.
Body weight: No statistically significant differences in body weight (mean weights over the whole study period and termination weight) between the control group and any of the concentrations were found for either males nor females. Results are summarised in the tables below.

Table 9.1.3-3. Weight gain in males and females

Reproduction Study SAS Statistical Output Adult Male Weight Gain (g)				
Nominal Treatment (ppm)	Mean	Std. Dev.	% Reduction	Dunnett's p-value
Control	-4.3	83.3	.	.
111	-4.5	54.5	-4.6	0.747
333	9.5	78.2	318.5	0.905
1000	-21.2	47.8	-389.2	0.460

Reproduction Study SAS Statistical Output Adult Female Weight Gain (g)				
Nominal Treatment (ppm)	Mean	Std. Dev.	% Reduction	Dunnett's p-value
Control	203.1	86.3	.	.
111	236.3	95.6	-16.4	0.973
333	241.3	90.1	-18.8	0.982
1000	208.3	52.1	-2.6	0.808

The daily dietary dose for the study was calculated and presented in the table below.

Table 9.1.3-4. Mallard daily dietary dose of flupyradifurone

Mallard Reproduction Study Daily Dietary Dose					
Measured Dietary Concentration (mg ai/kg feed)	Body Weight At Randomization (kg bw)	Body Weight at Termination (kg bw)	Mean Body Weight (kg bw)	Food Consumption[a] (kg feed/bird/day)	Daily Dietary Dose (mg ai/kg bw/day)
91	1.040	1.156	1.098	0.105	9
298	1.043	1.168	1.106	0.103	28
845	1.017	1.110	1.064	0.105	83

[a]Food Consumption duration was measured for the 22-week treated feed exposure period.

$$\textit{Daily Dietary Dose} = \frac{\textit{(Concentration in Feed) * (Food Consumption)}}{\textit{(Mean Body Weight)}}$$

Where:
Concentration in Feed = measured concentration in mg ai /kg feed
Food Consumption = mean consumption during exposure period in kg feed/bird/day.
Mean Body Weight = average of initiation and termination body weight in kg body weight/bird.

Feed consumption: No treatment related effects on feed consumption were observed. Differences were not significant. Reduction fo feed consumption was -1.5%, 1.1% and -1.0%, for 111, 333 and 1000 mg/kg fd, respectively.

Necropsy results

Necropsy observations of all adult birds subjected to necropsies showed no compound related effects. Concern of RMS was that the post-mortem examination showed that the contents of crop and proventricula gizzard at 1000 ppm were similar to that of the control, while at 111 and 333 ppm crop content seemed to be lower. The number of maturing follicles and eggs in the oviduct was the same in control and 1000 ppm (n = 12 and 5, respectively), but lower at the two other doses (n = 5 and 2)
Response by the notifier: In order to avoid misinterpretation of the post-mortem examination it is important to recognize that the number of necropsied individuals is different in the control and the top dose level (all birds necropsied: 15 males and 15 females at each level), whilst necropsy in the low and mid dose level is limited to only 5 males and 5 females from each dose level (unless triggered by findings at the top dose level). Taking these unequal numbers into account, there are no differences or findings in the post-mortem investigation that could induce concerns at any dose level (see table 9.1.3-6).
Two semi-quantative evaluations were performed by the notifier.
In the first calculation the contents of the crop and proventriculus were each related to the number of females and then combined. The mean numbers were 1.70, 1.50, 1.80 and 1.30 for control, 111, 333 and 1000 mg as/kg fd.
In the second calculation the notifier combined the maturing follicles and the number of eggs in the oviduct and related this number to the number of female birds. Total eggs in production/N were 1.13, 1.40, 1.75 and 1.13 for control, 111, 333 and 1000 mg as/kg fd. According to notifier, there are no

differences of findings in the post-mortem investigation that could induce concerns at any dose level when taking the unequal numbers into account.

Evaluator agrees to this evaluation. It should be remarked that in the study results it was not possible to relate the females in necropsy to a particular pen number. It is assumed that the two outliers were not part of this necropsy.

Table 9.1.3-5 Semi-quantitative evaluation of necropsy results

Test group	Control		111 ppm		333 ppm		1000 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
N (# examined)	15	15	5	5	5	4	15	15
FC (0 / ½ / 1)	10/5 /0	15/0/0	5/0/0	5/0/0	5/0/0	4/0/1	12/2/1	12/3/0
$\sum FC_{crop}$	2.5	0	0	0	0	1	2	1.5
$\sum FC_{crop}$ / N	0.17	0.00	0.00	0.00	0.00	0.25	0.13	0.10
mean FC_{crop}	0.17		0.00		0.25		0.23	
FC (0 / ½ / 1)	0/5/10	1/7/7	0/3/2	0/2/3	0/2/3	0/4/1	3/10/2	1/10/4
$\sum FC_{proventriculus}$	12.5	10.5	3.5	4	4	3	7	9
$\sum FC_{proventriculus}$ / N	0.83	0.70	0.70	0.80	0.80	0.75	0.47	0.60
mean $FC_{proventriculus}$	1.53		1.50		1.55		1.07	
mean $\sum FC$	0.17+1.53 = 1.70		0.0 + 1.50 = 1.50		0.25+ 1.55 = 1.80		0.23 + 1.07 = 1.30	
Maturing follicles		12		5		5		12
Egg in oviduct		5		2		2		5
Total eggs in production		17		7		7		17
Total eggs in production / N		1.13		1.40		1.75		1.13

In the table below a summary is given for all reproductive parameters tested. For none of the parameters, a significant difference with the control was observed. However, some differences at 333 mg/kg fd were close to significance, with p-values close to 0.05. Only these p values were included in the table. The non-breeder in cage 301 of 1000 mg/kg fd was excluded from the analyses. Notifier claimed a lack of a consistent dose response in this study.

Table 9.1.3-6 Summary of reproductive performance from a mallard reproduction study with flupyradifurone. Including data normalized as percentages (%).

Reproductive parameter	Experimental group (mg as/kg fd)			
	Control	111	333	1000
Number of replicates	15	15	15	15
Eggs laid per hen	61.6	56.5	53.7 (p = 0.088)	61.3
Eggs cracked per hen	0.2	0.27	0.33	0.21
Eggs not cracked/eggs laid (%)	99.7	99.3	99.5	99.7
Eggs set per hen	55.9	50.3	47.5 (p = 0.082)	55.4

Eggs set/eggs laid (%)	90.4	88.0	86.5	89.9
Viable embryos per hen	51.5	48.5	42.8 (p = 0.065)	51.4
Viable embryos/eggs set (%)	92.7	96.7	87.3	93.0
Live embryos per hen	51.2	48.0	42.5 (p = 0.066)	51.1
Live embryos/viable embryos (%)	99.5	98.0	99.2	99.3
Hatchlings per hen	47.5	43.3	37.7 (p = 0.051)	46.3
Initial hatchling body weight (g)	36.3	35.3	36.3	35.0
Survivor hatchling body weight (g)	269.8	268.9	273.4	266.4
Hatchlings/eggs laid (%)	77.3	75.4	66.9	74.1
Hatchlings/live 3-week embryos (%)	93.0	90.3	88.8	89.4
Hatchlings/eggs set (%)	85.7	85.6	76.0	82.3
Hatchling survival/no. hatched (%)	99.4	99.5	99.7	99.5
Eggshell strength	2.66	2.72	2.55	2.82
Eggshell thickness	0.363	0.355	0.359	0.369

RMS was concerned about the p-values which were close to 0.05 and concluded initially that the NOAEL in this study should be set at 111 mg/kg fd. This was underpinned by the fact that several reproductive parameters reductions between 13 and 21% were present at 333 mg as/kg fd (see Table 9.1.3-6) (an effect percentage of 10% was considered to be the level of unacceptable effects) and that the improved performance at 1000 mg/kg fd could not be explained. Therefore, more information was requested from the notifier and the data were analysed in more detail.

In the table below, the reproductive parameters are presented as percentage difference to the control.

Table 9.1.3-7. Summary of reproductive performance, expressed as % difference from the control, from a mallard reproduction study with flupyradifurone. A negative sign indicates a decrease as compared to the control, a positive sign an increase.

Reproductive parameter	Experimental group (mg as/kg fd)		
	111	333	1000
Eggs laid per hen	-8.2	-12.9	-0.5
Eggs cracked per hen	+33.3	+66.7	+7.1
Eggs not cracked/eggs laid (%)	-0.4	-0.2	-0.0
Eggs set per hen	-9.9	-14.9	-0.9
Eggs set/eggs laid (%)	-2.7	-4.3	-0.5
Viable embryos per hen	-5.7	-16.8	-0.1
Viable embryos/eggs set (%)	+4.3	-5.8	+0.3
Live embryos per hen	-6.3	-17.1	-0.3
Live embryos/viable embryos (%)	-1.5	-0.3	-0.2
Hatchlings per hen	-8.9	-20.5	-2.5

Initial hatchling body weight (g)	-2.8	0.0	-3.7
Survivor hatchling body weight (g)	-0.3	+1.4	-1.2
Hatchlings/eggs laid (%)	-2.4	-13.5	-4.2
Hatchlings/live 3-week embryos (%)	-2.8	-4.5	-3.8
Hatchlings/eggs set (%)	-0.1	-11.3	-3.9
Hatchling survival/no. hatched (%)	+0.1	+0.3	+0.1
Eggshell strength	-2.08	-4.11	-0.35
Eggshell thickness	-2.06	-0.88	-1.7

In response, notifier performed an additional statistical evaluation whereby excluding another outlier that performed worse than other ducks. This was the bird in cage 213 as it only had 14 eggs laid. The number of eggs laid is considered to be the primary determinant in the determination of the outlier. In the table below the p-values are given from the initial and new analysis for those parameters which had p values close to 0.05 in the initial analysis.

Table 9.1.3-8. Summary of reproductive performance from a mallard reproduction study with flupyradifurone. P= values were obtained after using Dunnett's test.

Reproductive parameter	Experimental group (mg as/kg fd)				
	Control	111	333 Initial analysis	New analysis	1000
Number of replicates	15	15	15		15
Eggs laid per hen	61.6	56.5	53.7 (p = 0.088)	(p = 0.220)	61.3
Eggs set per hen	55.9	50.3	47.5 (p = 0.082)	(p = 0.205)	55.4
Viable embryos per hen	51.5	48.5	42.8 (p = 0.065)	(p = 0.171)	51.4
Live embryos per hen	51.2	48.0	42.5 (p = 0.066)	(p = 0.171)	51.1
Hatchlings per hen	47.5	43.3	37.7 (p = 0.051)	(p = 0.126)	46.3

Recalculation by RMS

GraphPad Prism was used to statistically reanalyze the data. Two normality tests are being employed in GraphPad Prism: D'Agostino & Pearson omnibus normality test (recommended) and Shapiro-Wilk normality test. Tables 9.1.3-8 and 9.1.3-9 show whether the data pass the normality tests according to each of these tests. Calculations were performed for three situations of data treatment:

1 = No outliers removed (a "0" for all parameters in cage 301)
2 = Outlier removed for 1000 mg as/kg fd (cage 301 with 0 eggs)
3 = Outliers removed for 333 and 1000 mg as/kg fd (cage 301 with 0 eggs and cage 213 with 14 eggs.)

Tables 9.1.3-9 and 9.1.3-10 both show that the data for the three parameters eggs laid, viable embryos/hen and hatchlings per hen, all have a normal distribution at 333 mg as/kg fd according to the recommended D'Agostino & Pearson omnibus normality test and according to Shapiro-Wilk's test that has been used by the notifier.

As the concentration level of 333 mg as/kg fd is under consideration, evaluator concludes that the use of a parametric test when considering cages 301 and 213 as being outliers is justified.

Table 9.1.3-9. Passing normality test (D'Agostino & Pearson omnibus normality test)

	control			111 mg as/kg fd			333 mg as/kg fd			1000 mg as/kg fd		
	1	2	3	1	2	3	1	2	3	1	2	3
Eggs laid per hen	y	y	y	y	y	y	n	n	y	n	y	y
Viable embryos per hen	y	y	y	n	n	n	n	n	y	n	y	y
Hatchlings per hen	y	y	y	y	y	y	y	y	y	y	y	y

1 = No outliers removed (a "0" for all parameters in cage 301)

2 = Outlier removed for 1000 mg as/kg fd (cage 301 with 0 eggs)

3 = Outliers removed for 333 and 1000 mg as/kg fd (cage 301 with 0 eggs and cage 213 with 14 eggs)

Table 9.1.3-10. Passing normality test (Shapiro-Wilk's normality test)

	control			111 mg as/kg fd			333 mg as/kg fd			1000 mg as/kg fd		
	1	2	3	1	2	3	1	2	3	1	2	3
Eggs laid per hen	y	y	y	y	y	y	y	y	y	n	y	y
Viable embryos per hen	y	y	y	y	y	y	n	y	y	y	y	y
Hatchlings per hen	y	y	y	y	y	y	y	y	y	y	y	y

1 = No outliers removed (a "0" for all parameters in cage 301)

2 = Outlier removed for 1000 mg as/kg fd (cage 301 with 0 eggs)

3 = Outliers removed for 333 and 1000 mg as/kg fd (cage 301 with 0 eggs and cage 213 with 14 eggs)

The results show that at 333 mg/kg feed no significant effects are found for the three analysed parameters when the two outliers are removed. As these three parameters had p-values closest to 0.05, it is not considered necessary to reanalyze other parameters (eggs set per hen and live embryos per hen) as well.

To confirm whether 14 eggs produced by the bird in cage 213 exposed to 333 mg/kg fd can indeed be considered to be an outlier, evaluator has further analysed the data by preparing six figures. In these figures all birds were included; also the assumed outlier of 14 eggs at 333 mg as/kg fd.and the outlier of 0 eggs at the highest concentration. Data of the control derived from the concurrent control.

1) number of eggs vs treatment
2) consumption vs eggs
3) weight t=0 vs eggs
4) weight gain vs eggs
5) weight gain vs consumption
6) consumption vs treatment

In Figure 9.1.3-11 three data points, one at each concentration (111, 333 and 1000 mg as/kg fd), fall out of the range of the other data.

Figure 9.1.3-11. Relation treatment and number of eggs laid

Figure 9.1.3-12 shows that the birds with 0 and 14 eggs are on the lower range of feed consumption as compared with other animals in their group. The bird with 26 eggs (111 mg as/kg fd) did not consume much either, but still is within the range of other birds. Another bird ate just as much but produces 45 eggs. The bird with 14 eggs shows a similar feed consumption as the bird in the 1000 mg as/kg fd batch that produces 62 eggs. It is unclear whether there is an effect of the toxicant or that the three birds did not feel well irrespective of the toxicant and therefore did not eat much and also did not produce eggs. There seems to be a dose related effect but on the other hand, it could have been a coincidence that the most mal-performing bird was allotted to the highest dose.

Figure 9.1.3-12. Relation feed consumption and number of eggs laid

From Figure 9.1.3-13 it appears that there is no obvious relation between number of eggs and weight at t=0.

Figure 9.1.3-13. Relation start weight and number of eggs laid

A relationship with weight gain is also absent, as shown in Figure 9.1.3-14. One control bird has only gained 22 g but produced 57 eggs. The birds with 0 and 14 eggs are in the middle of the range of measured weight gains (278 and 244 g gain).

Figure 9.1.3-14. Relation weight gain and number of eggs laid

Figure 9.1.3-15 shows that the birds with 0, 14 and 26 eggs (at 1000, 333 and 111 mg as/kg fd, respectively) are situated at the far left of the figure. The weight gain was related with increasing concentrations (167, 244, 278 g at 111, 333 and 1000 mg as/kg fd) but inversely related with food consumption (91, 75, 67 g at 111, 333 and 1000 mg as/kg fd; see Figure 9.1.3-12). This means that the bird that was eating less (1000 mg as/kg fd batch) had most weight gain. This may be explained by the fact that this bird had not lost weight by laying eggs. On the whole, this bird therefore had most weight gain.

Figure 9.1.3-15. Relation feed consumption and body weight gain

Figure 9.1.3-16 shows an increase in the variation in feed consumption between birds of the 1000 mg as/kg fd batch as compared to the lower levels and the control. It is not clear whether increased consumption by some birds was actually caused by increased spillage of feed as the feed might have been unpalatable. This figure indicates that there might be an effect on consumption of the active substance at the highest concentration.

Figure 9.1.3-16. Relation treatment and consumption

It can be concluded from Figures 9.1.3-11 to 9.1.3-16 that although there seems to be a dose related effect, it is probably a coincidence that the most mal-performing bird was allotted to the highest dose. It is unclear whether there is a real effect of the toxicant. Evaluator assumes that the two birds did not feel well irrespective of the toxicant and therefore did not eat much and also did not produce many eggs.

The author concluded that the NOAEL for both parental and reproduction endpoints of mallard ducks exposed to flupyradifurone over a 20-week reproduction study was 845 mg as/kg fd or 81 mg as/kg bw/d, based on actual measured concentrations.

Remarks by RMS (additional to those already incorporated in above text)

The study was conducted in line with current OECD guidelines. A question was posed by RMS whether notifier could explain the improved performance at 1000 mg/kg fd as compared to the lower doses. A reduced uptake due to e.g. repellency could not be the explanation, since consumption at this level was equal to or slightly higher than at the other dose levels. The analysis of diet also ruled out a mistake in diet preparation. This question was indirectly answered by notifier by removing an outlier at the 333 mg/kg fd level. The outcome removed the impression of a partial dose response. Thus, the impression of an improved performance at the highest level was removed.

Statistical evaluations were performed by RMS to confirm the use of a parametric test. These showed that data indeed have a normal distribution after removal of two outliers from the dataset (cage 301 and cage 213 at 1000 and 333 mg as/kg fd, respectively). When data have a normal distribution, a parametric test such as Dunnett's test can be used after ANOVA. Recalculations by RMS did not show significant effects in any of the parameters as was also demonstrated by the notifier.

Subsequently, figures were prepared by RMS in order to evaluate whether or not the two outliers were acceptable. It can be concluded that it is justified to remove data of the birds from cages 213 (333 mg/kg fd, 14 eggs) and 301 (1000 mg/kg fd, 0 eggs) from the dataset. There are no convincing reasons that could tell the opposite. These two mal performing birds are possibly birds that do not feel well enough. The observed dose related effect, as is clearly observed in Figures 9.1.3-11 to 9.13.-14, is most likely coincidental. The NOEC can therefore be set at a nominal concentrations of 1000 mg as/kg fd. This is equivalent to a mean measured concentration of 845 mg as/kg fd or daily dietary dose of 83 mg as/kg bw/day.

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Characteristics of the study

reference	:	[redacted] (2012b)	species	:	Bobwhite quail (*Colinus virginianus*)
type of study	:	birds, reproduction	exposure duration	:	23 weeks
year of execution	:	2010/2012	nominal conc.	:	111, 333 and 1000 mg a.s./kg diet (actual measured: 107, 302 and 999 mg a.s./kg diet)
GLP statement	:	Yes	dosing method	:	Test substance in solvent was mixed with diet, corn oil used as vehicle
guideline	:	OPPTS 850.2300; US-EPA, FIFRA Subdivision E, 71-4; OECD 206	acceptability	:	Acceptable
test substance	:	BYI 02960; technical, batch 2009-000239	NOEC	:	111 mg a.s./kg diet ⇔ 14 mg a.s./kg bw/day

purity	:	96.2%

Description

Reproduction study with the bobwhite quail.

Methods. Adult birds (14 weeks old upon arrival from a commercial provider and from the same hatch) were acclimated for 4 weeks and then exposed to the test substance in the diet for 23 weeks, 13 weeks prior to egg laying and 10 weeks during egg laying and were evaluated for treatment related effects upon bird health and reproduction. Birds were 18 weeks old at test initiation, male body weights ranged from 195 to 202 g, female body weights was 199 g. Test substance was prepared by mixing the test substance into a premix that was rmixed with diet, test substance was dissolved using acetone. Diet was prepared on a weekly basis. Nominal concentrations 111, 333 and 1000 mg as/kg fd and a control containing an amount of solvent (acetone) and vehicle (corn oil) equivalent to the amount in the treated diets. Eighteen pair per treatment with one male and one female each per cage. Eggs were collected twice daily on weekdays and once on weekends from the onset of egg production and set weekly for incubation. First eggs were set during week 13. Table with the study phases is given below.

Table 9.1.3-7. Study phases test with *Colinus virginianus*

Study phases
Acclimation – 4 weeks
Photo stimulation with adult exposure to flupyradifurone – 8 weeks
First egg set with adult exposure to flupyradifurone- 13 weeks
Hatchlong phase started on day 21 of incubation of the eggs
Adult termination- 23 weeks
Biological termination- 28 weeks.

Observations. Birds were observed daily for mortality, abnormal behaviour and signs of toxicity. Body weight of adults was determined the day prior to experimental start, on weeks 3, 5, 7, and 9 and at the adult termination. Feed consumption was measured once a week. Reproductive parameters: embryo viability was determined on day's 11, 12, of incubation of the eggs and on day's 18 or 19 to determine embryo survival. One egg was collected from each cage and eggshell strength and thickness was determined. Number of hatchlings and number of 14-day old survivors, body weight of hatchlings and 14-day old survivors were recorded. Hatchlings were observed daily for signs of toxicity, injuries, illness and mortality. Hatchlings were sacrificed by CO_2 asphyxiation at day 14. Gross pathology of adults was conducted at test termination.

Reproductive endpoints.

Following reproductive endpoints were assessed (see Table below).

Table 9.1.3-8. Reproductive endpoints

Endpoints	Description
Adult Body Weight:	The adult body weight change from randomization to adult termination. Analyzed on a

	per cage basis by sex.
Adult Feed Consumption:	The means were calculated as grams/bird/day. Analyzed on a per cage basis.
Eggs Laid per Hen:	Analyzed as the total egg production. Eggs laid were analyzed on a per hen basis.
Eggs Cracked of Eggs Laid:	Cracked eggs are determined by candling prior to incubation.Cracked eggs were analyzed on a per hen basis.
Eggs Set:	Eggs placed under incubation are "set." Analyzed as the total number during the dosing phase. Eggs set were analyzed on a per hen basis.
Viable Embryos (mean):	Fertility is determined by candling on the 11th or 12th day of incubation. Fertility analyzed as total fertile eggs.
Live Embryos (mean):	Live embryos are determined by candling on the 19th day of incubation.
Eggs Not Cracked of Laid (%):	Eggs laid that were not cracked as a percentage of eggs laid on a per hen basis. Analyzed as the mean percentage during the reproductive phase.
Eggs Set of Laid (%):	Eggs set as a percentage of eggs laid on a per hen basis. Analyzed as the mean percentage during the reproductive phase.
Viable Embryos of Eggs Set (%):	Live embryos as a percentage of eggs set on a per hen basis. Analyzed as the mean percentage during the reproductive phase.
Live Embryos of Viable Embryos (%):	Live embryos as a percentage of viable embryos on a per hen basis. Analyzed as the mean percentage during the reproductive phase.
Number Hatched (mean):	Live hatchlings that had liberated themselves from their eggs by day 25 of incubation. Analyzed as the total number of normal hatchlings on a per hen basis.
14-day-old Survivors (mean):	Live chicks at 14-days post hatch. Analyzed as the total number of 14-day survivors on a per hen basis.
Number Hatched of Eggs Laid (%):	Hatchlings as a percentage of eggs laid on a per hen basis. Analyzed as the mean during the reproductive phase.
Number Hatched of Eggs Set (%):	Hatchlings as a percentage of eggs set on a per hen basis. Analyzed as the mean during the reproductive phase.
Number Hatched of Live Embryos (%):	Hatchlings as a percentage of live embryos on a per hen basis. Analyzed as the mean during the reproductive phase.
14-day-old Survivors of Eggs Set (%):	Live chicks at 14-days post hatch as a percentage of eggs set on a per hen basis. Analyzed as the mean during the reproductive phase.
14-day-old Survivors of Number Hatched (%):	Live chicks at 14-days post hatch as a percentage of hatchlings on a per hen basis. Analyzed as the mean during the reproductive phase.
Hatchling Body Weight:	Individual weights of the live hatchlings taken upon removal from the hatcher, analyzed on a per hen basis. Analyzed as the overall mean during the reproductive phase
14-day-old Survivor Body Weight:	Individual weights of the 14-day-old offspring taken at sacrifice, analyzed on a per hen basis. Analyzed as the overall mean during the reproductive phase.
Eggshell Strength:	The force needed to penetrate the shell and membrane measured at one point on the waist of the egg, analyzed on a per hen basis.
Eggshell Thickness:	The thickness of the shell plus the membrane measured at three points around the waist of the egg, analyzed on a per hen basis

Conditions. Housing in pairs, 21.3 °C, 55.3% RH, 7:17 h L:D for the first 8 weeks including acclimation phase. Photoperiod was increased to 17:7 h L:D to induce egg laying (64-194 lux). Eggs were placed in an incubator (37.0°C, 58.9 % RH). On day 21 of incubation, eggs were place into a hatcher and allowed to hatch (36.8 °C, 69.3 % RH). Hatchlings were placed in a brooding compartment at approx. 32 - 38°C and 57.5% RH in a 14-d post hatchling phase with 14 h light per day. Feed and water *ad libitum*.

Chemical analysis.

Method validation: Samples were spiked at 10.1, 101, 504 and 1007 mg/kg fd, extracted and analysed.

Analysis of test diet: Actual concentrations in the diet were determined at week 1, 5, 10, 15 and 20 from samples of 111, 333 and 1000 mg as/kg feed.

Homogeneity of test diet: Samples from test concentrations 111 and 1000 mg/kg fd were taken from the top, middle and bottom of the mixing vessel following the initial feed mixing procedure..

Stability test diet: Samples to verify the stability of the test substance under actual test conditions were taken on day 7 of the freezing period, both with samples treated at 111 and 1000 mg/kg fd.

Extraction with acetonitrile/HPLC-grade water (1:1 v/v) and a stir bar for stirring, capped and placed into a microwave extractor two times during 30 seconds and 5.5 minutes at 50 ºC. Then, the feed was sonicated for 15 minutes in a water bath, filtered and dilutied when needed using acetonitrile/HPLC-grade water (1 : 1 v/v). Extracts were analysed by HPLC-UV/VIS detection at 260 nm. Limit of quantitation was 10 mg/kg.

Statistics. The no observed adverse effect concentration (NOAEC) and lowest observed adverse effect concentration (LOAEC) was identified for each parameter using hypothesis testing methodology. Data from treatment groups were compared to controls using the Shapiro-Wilk's test for normality and Levene's test of equal variance to determine if dose groups had unequal variances. If assumption of normality ($p \leq 0.01$) and homogeneity of variance ($p \leq 0.05$) were met, then parametric analyses were conducted using analysis of variance (ANOVA) followed by Dunnett's test or William's test. If variances were unequal, then the non-parametric analyses were conducted using the Jonckheere or Mann-Whitney procedures. Statistical analyses were performed using SAS program at the $\alpha = 0.05$ (95% confidence level).

Results

Diet analysis

Analytical recovery. Overall mean procedural recovery of fortified samples was 99%.

Homogeneity: The average of the measured concentrations from the 111-mg/kg homogeneity samples was 97.1 mg/kg (week 1l) with a RSD of 10 %, 112 mg/kg with 8% RSD (week 10), and 99.3 mg/kg with 3% RSD (week 20) . The average of the measured concentrations from the 1000 mg/kg homogeneity samples was 855 mg/kg with 8% RSD (week 1), 977 mg/kg with 9% RSD (week 10), and 997 with 9% RSD (week 20).

Concentration verification: Freshly prepared feed contained a mean measured concentrations of 107, 302, and 999 mg/kg fd for the 111, 333 and 1000 mg/kg fd concentrations, respectively (96, 91 and 100% of nominal).

Stability: At day 7 recovery was 114 and 106%, from 100 and 1000 mg/kg fd concentrations compared to the week 1 analysis, respectively, confirming stability. The recovery from the 8 weeks frozen samples averaged 80% for 111 mg/kg fd and 108% for 1000 mg/kg fd compared to the week 1 analysis.

Biological observations

Adult mortality: Nine birds died during the study: two birds in the control group (1 m, 1f), one bird in the 111 mg as/kg group (1 m) and six in the 1000 mg as/kg group (1 m, 5 f). No mortalities in the 333 mg /kg fd group. Treatment related effects were observed in the 1000 mg/kg dose group at necropsy. Four females were observed as being emaciated and all five females had regressed ovaries, no maturing follicles or reduced egg-laying. These results are in agreement with necropsy of surviving birds in the 1000 mg/kg level.

Clinical observations: No overt signs of intoxication were observed during the study or acclimatisation period in any adult test group.

Body weight: A statistically significant difference at the 333 mg/kg dose group as compared to the control was found for the adult male weight gain (reduction of 68.73%). A statistically significant difference at the 1000 mg/kg dose group as compared to control was found for the female weight gain (reduction of 69.43%).

Adult bird feed consumption (g bird/day): No statistically significant differences at any treatment level as compared to the control.

In the table below a summary is given for all reproductive parameters tested.

Table 9.1.3-9 . Summary of reproductive performance from a quail reproduction study with flupyradifurone, technical. Including data normalized as percentages (%).

Reproductive parameter	Experimental group (mg as/kg fd)			
	Control	111	333	1000
Number of replicates	18	18	18	18
Eggs laid per hen	55.6	51.1	60.8	30.9*
Eggs cracked per hen	0.59	1.82	2.18*	0.64
Eggs not cracked/eggs laid (%)	98.6	96.5	96.5*	98.2
Eggs set per hen	50.1	44.3	53.1	26.6*
Eggs set/eggs laid (%)	88.7	86.3	87.1*	85.5*
Viable embryos per hen	44.8	42.9	49.3	24.8*
Viable embryos/eggs set (%)	91.4	96.6	94.2	92.6
Live embryos per hen	44.3	42.5	48.9	24.7*
Live embryos/viable embryos (%)	99.0	99.0	99.0	99.8
Hatchlings per hen	42.1	39.8	45.0	21.4*
Survived hatchlings	41.8	39.6	44.8	21.2*
Initial hatchling body weight (g)	6.9	6.7	6.8	6.0*
Survivor hatchling body weight (g)	34.5	33.2	33.6	30.8*
Hatchlings/eggs laid (%)	76.5	77.5	74.5	69.4*
Hatchlings/live 3-week embryos (%)	95.9	94.0	91.9*	88.2*
Hatchlings/eggs set (%)	86.6	89.8	85.8	81.4
Hatchling survival/no. eggs set (%)	86.1	89.3	85.5	80.2
Hatchling survival/no. hatched (%)	99.5	99.5	99.7	98.8

Eggshell strength	0.84	0.90	0.79	0.79
Eggshell thickness (mm)	0.21	0.22	0.21	0.22

*Statistically significant difference between treatment and control group

In the table below, the reproductive parameters are presented as percentage difference to the control.

Table 9.1.3-10. Summary of reproductive performance, expressed as % difference from the control, from a quail study with flupyradifurone, technical. A negative sign indicates a decrease as compared to the control, a positive sign an increase.

Reproductive parameter	Experimental group (mg as/kg fd)		
	111	333	1000
Eggs laid per hen	-8.15	+9.42	-44.4*
Eggs cracked per hen	+210	+270*	+8.2
Eggs not cracked/eggs laid (%)	-2.19	-2.10*	-0.38
Eggs set per hen	-11.6	+5.87	-46.9*
Eggs set/eggs laid (%)	-2.78	-1.89*	-3.62*
Viable embryos per hen	-4.20	+10.1	-44.6*
Viable embryos/eggs set (%)	+5.65	+3.07	+1.33
Live embryos per hen	-3.98	+10.4	-44.2*
Live embryos/viable embryos (%)	-0.02	+0.05	+0.87
Hatchlings per hen	-5.45	+6.99	-49.2*
Survived hatchlings	-5.34	+7.17	-49.4*
Initial hatchling body weight (g)	-3.91	-2.21	-13.6*
Survivor hatchling body weight (g)	-3.87	-2.59	-10.8*
Hatchlings/eggs laid (%)	+1.41	-2.52	-9.25
Hatchlings/live 3-week embryos (%)	-2.05	-4.16*	-8.03*
Hatchlings/eggs set (%)	+3.72	-0.85	-5.98
Hatchling survival/no. eggs set (%)	+3.69	-0.75	-6.84
Hatchling survival/no. hatched (%)	0	+0.13	-0.70
Eggshell strength	+7.85	-5.82	-5.71
Eggshell thickness (mm)	+5.43	+2.46	+4.06

*Statistically significant difference between treatment and control group

The author concluded that the NOAEL for both parental toxicity and reproduction endpoints of the bobwhite quail exposed to flupyradifuronetechnical over a 23 week period was nominal 333 mg/kg feed, equivalent to a measured concentration of 302 mg as/kg feed or mean dose of 40 mg as/kg bw/day. The NOAEL was based on statistically and biologically significant parameters.

Remarks by RMS

Validity criteria were met. The study was conducted in line with current OECD guidelines. No significant deviations from the guidelines were noted. At 1000 mg/kg fd, several reproductive parameters showed a statistically significant reduction as compared to the control. Some significant differences were also noted at 333 mg/kg feed. At that dose level there does not seem to be a consistent effect on reproduction. The study author classified some significant differences as not being biologically relevant, but an explanation for this is not given in the study report. When asked, the notifier explained the statement. They say that at the 333 mg as/kg feed concentrations several reproduction parameters even performed better than the control. According to notifier the statistical significance in the % hatchlings of 3-w embryos is merely due to the lower fertility rate in the control. Evaluator agrees that some endpoints are secondary endpoints as they rely on the outcome of more important initial parameters. This specifically accounts for eggs not cracked/eggs laid. Notifier argues that the statistical significance in the parameter hatchling/live 3-week embryos is a secondary or incidental consequence of the lower fertility rate in the control. The lower fertility rate in the control is indeed visible in the primary parameter “eggs laid per hen” (55.6 in the control vs. 60.8 in the 333 mg as/kg fd treatment) and “viable embryos per hen” (44.8 in the control vs. 49.3 in the 333 mg as/kg fd treatment). The parameter “hatchling/live 3-week embryos” has a lower score for the 333 mg as/kg fd than in the control. However, this calculation stands on its own. In principle a 100% score could be reached if all live 3-week embryos lead to the hatchling stage, irrespective of any other primary parameter. It appears that there is a clear dose-related effect: 95.9, 94, 91.9, 88.2% at increasing concentrations. It is common practice to acknowledge that significant differences at a certain concentration lead to a NOEC in the lower concentrations. In this particular case, there is no reason to argue that this effect could be inconsistent, incidental or not meaningful. This would deny the usefulness of any statistical evaluation. Therefore, based on the reproduction parameter “hatchling/live 3-week embryos”, the NOAEL is set to 111 mg/kg feed, equivalent to a measured concentration of 107 mg as/kg feed or daily dietary dose of 14 mg as/kg bw/day (data taken from the report).

B.9.1.4 Summary

Acute, short-term and (sub-)chronic dietary toxicity data

Toxicity studies with BYI 02960 (flupyradifurone) were performed with Bobwhite quail (*Colinus virginianus*), canary (*Serinus canaria*) and mallard duck (*Anas platyrhynchos*). The acute oral toxicity of the formulated product was determined in studies on Bobwhite quail (*Colinus virginianus*) and in Chicken (*Gallus gallus domesticus*). There is no indication that the formulation is more toxic than expected based on the active substance content.

Endpoint selection was done according to the recommendations of the Guidance Document on Risk Assessment for Birds & Mammals, EFSA 2009[1]:

Acute risk assessment: where acute tests for more than one species are available the geometric mean may be used for the refined assessment, except when the endpoint for the most sensitive species is more than a factor 10 below the geometric mean of all the tested species. For BYI 02960, in a worst-case approach the lowest LD50 of 232 mg/kg bw is used for Tier 1 acute risk assessment.

Short-term risk assessment; According to the risk assessment scheme a short-term risk assessment is not required. However, the endpoint from short-term dietary studies, e.g. 5-day dietary study in birds (OECD 205) should be used in an acute risk assessment when indicating a higher toxicity via the dietary exposure route (lower LDD50). In the case of BYI 02960 there is no indication that 5-day exposure via dietary route would provoke higher toxicity than one application via gavage in acute study and therefore the short-term toxicity is not considered in the acute risk assessment.

Reproductive risk assessment: The acute oral LD50 value used in the acute avian assessment should be divided by 10 for comparison with the lowest NOAED from the reproduction study (studies) ignoring purely parental effects (e.g. changes in parental body weight and food consumption). The lower of the values should be used in the tier 1 risk assessment.

For BYI 02960 the endpoint from the reproduction study (14 mg/kg bw/d) is lower than the 1/10th of the LD50 of 232 mg/kg bw that has been proposed for use in the Tier 1 acute risk assessment.

Therefore, the endpoint of 14 mg/kg bw will be used in a Tier 1 reproductive risk assessment for birds.

An overview of accepted toxicity data for a.s. and formulation is given in Table B.9.1.4-01. Endpoints selected for first tier risk assessment are marked **bold.**

Table B.9.1.4-01 Overview of accepted toxicity data for BYI 02960 and BYI 02960 SL 200

Study	Substance	Species	Route	Criterion	Value [mg/kg fd]	Value [mg a.s./kg bw·d]
IIA 8.1.1/01	BYI 02960	*Colinus virginianus*	acute, oral intubation	LD_{50}		**232**
IIA 8.1.1/02	BYI 02960	*Serinus canaria*	acute, oral intubation	LD_{50}		330
IIIA 10.1.6/01	BYI 02960 SL 200	*Colinus virginianus*	acute, oral intubation	LD_{50}		431
IIIA 10.1.6/02	BYI 02960 SL 200	*Gallus domesticus*	acute, oral intubation	LD_{50}		>342
IIA 8.1.2/01	BYI 02960	*Anas platyrhynchos*	5-day-feeding	LC_{50}	> 4741	> 825
IIA 8.1.2/02	BYI 02960	*Colinus virginianus*	5-day-feeding	LC_{50}	> 4876	> 470
IIA 8.1.4/01	BYI 02960	*Anas platyrhynchos*	20-week feeding	NOEC	845	83

[1] EFSA (2009): Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. The EFSA Journal (2009), 7(12):1438.

IIA 8.1.4/02	BYI 02960	*Colinus virginianus*	chronic, reproduction 23-week feeding chronic, reproduction	NOEC	111	**14**

Risk to birds of metabolites of BYI 02960

In the residue section of the DAR, metabolites DFA and DFEAF are considered relevant:

Plants

Residue definition for monitoring:

Results from supervised residue trials show that flupyradifurone and difluoroacitic acid (DFA) are, by far, the most prominent components of the residues in target plants and rotational crops. The residues of metabolite difluoroethyl-amino-furanone (DFEAF) were below the LOQ of 0.01 mg/kg in virtually all samples. Considering these results as well as those from the metabolism studies, the proposed residue definition for monitoring for target plants and rotational crops is:

The sum of parent compound and DFA, expressed in flupyradifurone equivalents.

Residue definition risk assessment:

For flupyradifurone residues in target plants (primary crops) and rotational crops (succeeding crops), the proposed residue definition for data collection and risk assessment is:

The sum of parent compound flupyradifurone and its metabolites DFA and difluoroethyl-amino-furanone (DFEAF), expressed in flupyradifurone equivalents. A conversion factor is not required to cover the difference between the residue definition for monitoring and risk assessment as bith DFA and flupyradifurone are part of the residue definition for monitoring and as DFEAF is found in levels below <0.01 mg/kg in supervised residue trials and in rotational crops.

In the human toxicology risk assessment, the following is concluded:

Toxicologically relevant compounds (animals and plants)	Parent compound, DFA
Toxicologically relevant compounds (environment)	Parent compound, DFA

Based on the above, metabolite DFA is considered to be relevant for the terrestrial vertebrate risk assessment.

No avian toxicity study is conducted with DFA but mammalian toxicity data suggest no higher toxicity of the metabolite than from the parent.

Furthermore, the residue data from trials in lettuce and cereals (as surrogate for ground vegetation in hops) demonstrate little difference in the decline DT50 whether or not DFA is included in the residue concentrations (see B.9.3). The trials indicate mostly faster dissipation on foliage than the default DT50 of 10 days.

The first tier risk assessment is therefore based on initial maximum concentrations of the a.s. and the generic DT50 of 10d and this is considered to cover metabolite DFA.

B.9.1.5 Risk assessment for birds (IIIA 10.1)

Risk assessment

The risk assessment procedure follows the EFSA Guidance Document on Risk Assessment for Birds & Mammals (2009).

At Tier 1, the risk is considered acceptable, if the 'Toxicity Exposure Ratio' (TER) value pass the trigger values of ≥ 10 for acute exposure and ≥ 5 for chronic exposure.

If the TER values are below these a-priori acceptability trigger values in certain areas, a refined risk assessment based on more relevant and realistic conditions is performed for those particular areas.

Routes of exposure

The formulation is to be sprayed on hops in the field and on lettuce in glasshouses and in the field.

The following routes are considered:

- direct dietary exposure: scenarios for hop and leafy vegetables – field uses only
- direct exposure via drinking water: field and glasshouse uses
- indirect exposure by consumption of earthworms and fish containing residues of BYI 02960 field and glasshouse uses

B.9.1.5.1 Exposure via feed

Calculation of Toxicity Exposure Ratio (TER)

According to the EFSA Guidance Document on Risk Assessment for Birds & Mammals (2009), the calculation of acute and long-term Toxicity to Exposure Ratio (TER) is defined as follows:

Acute risk: TERa = LD50 [mg as/kg bw] / DDDac

Long-term risk: TERlt = [lowest of NOEL and LD50/10] [mg as/kg bw] / DDDmean

Calculation of Daily Dietary Dose (DDD)

Acute exposure DDDac:

The daily dietary dose for a single application per crop is given by the following equation:

DDDACac = application rate [kg/ha] × shortcut value (SV90)

Long-term exposure DDDmean:

For a single application the daily dietary dose is given by the following equation:

DDDmean = application rate [kg/ha] × shortcut value (SVm) × fTWA

Where

DDD Daily dietary dose

fTWA Time weighted average factor ftwa based on a default time window of 21 days and a DT50 of 10 days leading to a value of 0.53

90 90th percentile values for acute exposure

m mean values for reproductive/long-term exposure

Standard exposure scenario for Tier 1 risk assessment

The main potential exposure route for birds is expected to be consumption of contaminated feed. This is only relevant for field uses.

The Tier 1 risk assessment is based on generic focal species associated with specific crop scenarios. Default ("shortcut"-) values for the exposure estimate will be used as provided in Appendix A of the EFSA Guidance Document on Risk Assessment for Birds & Mammals (2009) representing a worst case assessment.

In the Tier 1 risk assessment it is assumed that

- animals satisfy their entire food demand in the treated area (PT = 1),
- over an acute time frame (hours) the animals feed on items containing maximum residues (90th percentile), whereas they would ingest food containing mean residues over a long-term period (days to weeks),
- long-term predicted environmental concentrations to be compared with chronic endpoints can be calculated as the time-weighted average concentration. Default assumptions are a time window of 21 days and a DT50 of 10 days leading to a time weighted average factor (= ftwa) of 0.53. This factor is equally valid for feed items consisting of vegetation and for arthropods.

Avian generic focal species for Tier 1 risk assessment

In the field, the product is intended to be used in hops at BBCH 31-75 and in lettuce at BBCH 12-49. According to the EFSA Guidance Document on Risk Assessment for Birds & Mammals (2009) the following generic focal species are addressed in Tier 1 risk assessment.

Table B.9.1.5.1-01: Relevant generic avian focal species for Tier 1 risk assessment

Crop	Growth stage (BBCH)	Generic focal species	Representative species	Shortcut value	
				For long-term RA based on RUD_m	For acute RA based on RUD_{90}
Hops	≥ 20	Small insectivorous bird "finch"	Chaffinch	**10.6**	**25.3**
	20-39	Small granivorous bird "finch"	Goldfinch	**5.7**	**12.3**
	≥ 40	Small granivorous bird "finch"	Goldfinch	3.4	7.4
Lettuce (Leafy vegetables)	10-19	Medium herbivorous/granivorous bird "pigeon"	Wood pigeon	**22.7**[1]	**55.5**[1]
	10-49	Small granivorous bird "finch"	Serin	**12.6**	**27.4**
	10-49	Small omnivorous bird “lark”	Woodlark	**10.9**	**24.0**
	10-19	Small insectivorous bird “wagtail”	Yellow wagtail	**11.3**	**26.8**
	≥ 20	Small insectivorous bird “wagtail”	Yellow wagtail	9.7	25.2

Bold values were used for Tier 1 risk assessment. Where the same focal species is representative for different BBCH stages, only the worst-case SV values were chosen for the Tier 1 risk assessment.

[1] The long-term and acute SV-values for this scenario reported in the EFSA GD are 37.0 and 90.6, respectively. However, these were mistakenly based on a FIR/bw of 1.29 (calculated for a passerine species) instead of 0.79 (for non-passerine species, which is correct for the pigeon). The corrected values are in line with those reported for the “pigeon” in early stages of legume forage, maize and oilseed rape.

Tier 1 acute toxicity exposure ratio for birds

Table B.9.1.5.1-02: Tier 1 acute DDD and TER calculation for birds

Crop (BBCH)	Generic focal species	LD50 [mg/kg bw]	DDD			DDD	TERA	Trigger
			Appl. rate [kg/ha]	SV90	MAF90			
BYI 02960								
Hops (≥ 20)	Small insectivorous bird "finch"	232	0.150	25.3	1	3.80	61	10
Hops (20-39)	Small granivorous bird "finch"			12.3		1.85	126	
Lettuce (10-19)	Medium herbiv./ granivorous bird “pigeon”	232	0.125	55.5	1	6.94	33	10
Lettuce (10-49)	Small granivorous bird “finch”			27.4		3.43	68	
Lettuce (10-49)	Small omnivorous bird “lark”			24.0		3.0	77	
Lettuce (10-19)	Small insectivorous bird “ wagtail”			26.8		3.35	69	

The TER values for both crops are above the trigger of 10 for acute exposure. Hence, no unacceptable acute risk to birds from the use of the product according to the intended use pattern is to be expected.

Tier 1 long-term toxicity exposure ratio for birds

Table B.9.1.5.1-03: Tier 1 long-term DDD and TER calculation for birds

Crop (BBCH)	Generic focal species	NO(A)EL [mg/kg bw/d]	DDD				DDD	TERLT	Trigger
			Appl. rate [kg/ha]	SVm	MAFm	fTWA			
BYI 02960									
Hops (≥ 20)	Small insectivorous bird "finch"	14	0.150	10.6	1.0	0.53	0.84	16.6	5
Hops (20-39)	Small granivorous bird "finch"			5.7			0.45	31.9	
Lettuce (10-19)	Medium herbiv./graniv. bird “pigeon”	14	0.125	22.7	1.0	0.53	1.50	9.3	5
Lettuce (10-49)	Small granivorous bird “finch”			12.6			0.83	16.8	
Lettuce (10-49)	Small omnivorous bird “lark”			10.9			0.72	19.4	
Lettuce (10-19)	Small insectivorous bird “ wagtail”			11.3			0.75	18.7	

The TER values for both crops are above the trigger of 5 for long-term exposure. It is noted that targeted residue decline data are available in the section on wild mammal risk assessment, which indicate that the TER values for (partly) herbivorous birds are even underestimated.
Hence, no unacceptable risk to birds from the use of the product according to the intended use pattern in lettuce and hops is to be expected.

B.9.1.5.2 Exposure via drinking water

An assessment of the risk potentially posed by consumption of contaminated drinking water is required according to the EFSA Guidance Document for Birds and Mammals (2009).
Two scenarios were identified as relevant for assessing the risk of pesticides via drinking water to birds and mammals:

- Leaf scenario, only relevant for birds possibly drinking water from puddles in leaf whorls after application of a pesticide to a crop and subsequent rainfall or irrigation. This scenario is only relevant for acute exposure and only for certain crop types. When the product is applied in hops, the formation of pools in leaf axils where an acute exposure possibly might occur can be

excluded. However, for lettuce, the risk of birds drinking from puddles in leaf whorls cannot be excluded. Hence, a corresponding risk assessment has been performed below.

- Puddle scenario. Birds and mammals taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil. This scenario is relevant for all types of applications that may cause contamination of soil and only for long-term exposure.

Risk assessment for the leaf scenario (field use in lettuce):

The exposure of birds to drinking water from pools in leaf whorls as outlined above cannot be excluded from the use of the product in lettuce.

The respective calculations for birds have to be performed only for acute exposure. Generic focal species is a small granivorous bird (body weight 15.3 g) with a DWR (daily drinking water rate) of 0.46 L/kg bw/d. It should be mentioned that DWR, unlike FIR in the case of food intake exposure assessment, is already related to body weight.

According to EFSA Guidance Document for Birds and Mammals (2009), the PECpool calculates as follows:

PECpool = Cspray / 5 [mg/L]

Cspray = 0.025 kg a.s./hL = 25 g a.s./hL = 0.25 g a.s./L = 250 mg a.s./L

The PECpool (= PECdw) is 250 / 5 = 50 mg a.s./L for BYI 02960. The calculation of DDD (here: daily drinking water uptake) and TER are as follows:

DDD = DWR * PECdw

TERA = LD50 [mg as/kg bw/d] / DDD [mg as/kg bw/d]

Table B.9.1.5.2-01: Tier 1 acute DDD and TER calculation for exposure via drinking water from pools in leaf axils or on leaves

Compound	**DWR [L/kg bw/d]**	**PECdw [mg/L]**	**DDD [mg/kg bw/d]**	**LD50 [mg/kg bw]**	**TER_A**	**Trigger**
BYI 02960	0.46	50	23	232	10.1	10

The TER_{AC} value for drinking water exposure exceeds the a-priori acceptability trigger of 10 in a worst case risk assessment (highest concentration in spray water volume, lowest LD50). Hence, no unacceptable risk is to be expected from the use of the product according to the intended use pattern.

Risk assessment for the puddle scenario (field use in hop and lettuce):

An “escape clause” recommended in the EFSA Guidance Document for Birds and Mammals (2009) allows for screening the need for a quantitative risk assessment by a comparison between the application rate and the toxicity of the respective substance. This escape clause specifies that “*due to*

the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals …, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (= application rate x MAF) (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≥ 500 L/kg).” [2].

Table B.9.1.5.2-02: Evaluation of potential concern for exposure of birds drinking water from puddles (escape clause)

Compound	Koc [mL/g]	Application rate * MAF [g a.i./ha]	LD50 or NO(A)EL [mg a.i./ kg bw/d]	Ratio (Application rate * MAF) / NO(A)EL	“Escape clause” No concern if ratio	Conclusion
BYI 02960	98.4 1)	150 2) x 1	232	0.64	≤ 50	No concern
			14	10.7	≤ 50	No concern

1) Arithmetic mean of ten K_{OC} values (see B.8)
2) Worst-case application rate in hops

This evaluation confirms that the risk for birds from drinking from puddles that may contain residues from the use of product is acceptable.

B.9.1.5.3 Bioaccumulation and food chain behaviour: Indirect exposure via contaminated worms or fish

Substances with a high bioaccumulation potential could theoretically bear a risk of secondary poisoning for birds if feeding on contaminated prey like fish or earthworms. For organic chemicals, a log P_{OW} > 3 is used to trigger an in-depth evaluation of the potential for bioaccumulation.

Table B.9.1.5.3-1: Log Pow values of BYI 02960 and its metabolites

Substance	Maximum Log Pow	Reference
BYI 02960	1.2	Vol.3 B.2.1.13
BYI 02960-succinamide	0.6 at pH 5	Vol.3 B.2 Table B.2.1-2 IIA 7.13b
BYI 02960-azabicyclosuccinamide NA-salt	-1.3 at pH 5	Vol.3 B.2 Table B.2.1-2 IIA 7.13b
DFA	- 3.1 at pH5	Vol.3 B.2 Table B.2.1-2 IIA 7.13b

[2] EFSA (2009): Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA, p. 66

6-CNA	1.52 at pH 1.98	Vol.3 B.2 Table B.2.1-2 IIA 7.13b

As neither the active ingredient nor any of its metabolites has a log Pow above 3, the potential of bioaccumulation is considered to be low. Hence, there is no need to evaluate potential effects of secondary poisoning of birds.

B.9.2 Effects on aquatic life (IIA 8.2-8.6; IIIA 10.2)

B.9.2.1 Acute toxicity to aquatic life (IIA 8.2; IIIA 10.2; IIA 8.3; IIA 8.4; IIA 8.5; IIA 8.6; IIA 8.11)

B.9.2.1.1 Acute toxicity of the active substance (IIA 8.2.1; IIA 8.3.1; IIA 8.5.1)

STUDY IIA, 8.2.1.1/01

Characteristics

reference	:	[redacted](2010a)	water solubility	:	3000-3200 mg/L at pH 4-9 & 20°C
type of study	:	Acute toxicity study	species	:	Rainbow trout (*Oncorhynchus mykiss*)
year of execution	:	2009	exposure duration	:	96 hours
GLP statement	:	Yes	nominal concn.	:	5, 10, 20, 40 and 80 mg a.s./L
guideline	:	FIFRA 72-1, OPPTS 850.1075, OECD 203	dosing method	:	Static; stock solution in DMF
test substance	:	BYI 02960 technical, batch 2009-000239	acceptability	:	Acceptable
purity	:	96.2%	96-h LC50	:	>74.2 mg a.s./L

Methods

A 96-hour acute toxicity test in juvenile rainbow trout (*Oncorhynchus mykiss*) (1 replicate of ten fish per concentration, not fed for 48 hours prior to testing) was conducted under static conditions with BYI 02960 technical at test concentrations of 5, 10, 20, 40 and 80 mg a.s./L. It was reported that numerous preliminary trials demonstrated that a solvent stock would be needed to achieve maximum solubility in dilution water. The test solutions were prepared by adding aliquots of a stock solution in DMF to test water (max 0.1 mL/L DMF). The test included untreated and solvent controls (0.1 mL/L DMF). The concentrations of BYI 02960 were analytically confirmed by LC-MS/MS in samples of all test solutions taken at the start and end of exposure.

Results

The measured concentrations represented 72-99% of nominals at the start and 69-92% at the end of exposure. Test endpoints were based on mean measured concentrations (3.52, 8.31, 19.0, 35.1 and 74.2 mg a.s./L). Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 203 guideline. No mortality and clinical signs were observed in any of the treatments and the controls. The 96-hour LC50 is >74.2 mg a.s./L.

Conclusion

96-hour LC50 >74.2 mg a.s./L.

Guidelines and limitations

The study is acceptable.

STUDY IIA, 8.2.1.2/01

Characteristics

reference	:	[redacted] (2010b)	water solubility	:	3000-3200 mg/L at pH 4-9 & 20°C
type of study	:	Acute toxicity study	species	:	Fathead minnow (*Pimephales promelas*)
year of execution	:	2009	exposure duration	:	96 hours
GLP statement	:	Yes	nominal concn.	:	5, 10, 20, 40 and 80 mg a.s./L
guideline	:	FIFRA 72-1, OPPTS 850.1075, OECD 203	dosing method	:	Static; stock solution in DMF
test substance	:	BYI 02960 technical, batch 2009-000239	acceptability	:	Acceptable
purity	:	96.2%	96-h LC50	:	>70.5 mg a.s./L

Methods

A 96-hour acute toxicity test in juvenile fathead minnow (*Pimephales promelas*) (1 replicate of ten fish per concentration, not fed for 48 hours prior to testing) was conducted under static conditions with BYI 02960 technical at test concentrations of 5, 10, 20, 40 and 80 mg a.s./L. It was reported that numerous preliminary trials demonstrated that a solvent stock would be needed to achieve maximum solubility in dilution water. The test solutions were prepared by adding aliquots of a stock solution in DMF to test water (max 0.1 mL/L DMF). The test included untreated and solvent controls (0.1 mL/L DMF). The concentrations of BYI 02960 were analytically confirmed by LC-MS/MS in samples of all test solutions taken at the start and end of exposure.

Results

The measured concentrations represented 80-96% of nominals at the start and 85-98% at the end of exposure. Test endpoints were based on mean measured concentrations (4.29, 9.00, 19.4, 34.3 and 70.5 mg a.s./L). Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 203 guideline. No mortality and clinical signs were observed in any of the treatments and the controls. The 96-hour LC50 is >70.5 mg a.s./L.

Conclusion

96-hour LC50 >70.5 mg a.s./L.

Guidelines and limitations

The study is acceptable.

STUDY IIA, 8.2.1.2/02

Characteristics

reference	:	[redacted](2011a)	water solubility	:	3000-3200 mg/L at pH 4-9 & 20°C
type of study	:	Acute toxicity study	species	:	Common carp (*Cyprinus carpio*)
year of execution	:	2011	exposure duration	:	96 hours
GLP statement	:	Yes	nominal concn.	:	100 mg a.s./L (limit test)
guideline	:	FIFRA 72-1, OPPTS 850.1075, EEC C.1, OECD 203	dosing method	:	Static; stock solution in DMF
test substance	:	BYI 02960 (tech), batch BYI 02960-01-03 (original batch number 2009-000239)	acceptability	:	Acceptable
purity	:	96.2%	96-h LC50	:	>100 mg a.s./L

Methods

A 96-hour acute toxicity limit test in juvenile carp (*Cyprinus carpio*) (2 replicates of 15 fish, not fed for 48 hours prior to testing) was conducted under static conditions with BYI 02960 technical at a nominal test concentration of 100 mg a.s./L. The test solutions were prepared by adding aliquots of a stock solution in DMF to test water (max 0.1 mL/L DMF). The test included untreated and solvent controls (0.1 mL/L DMF). The concentrations of BYI 02960 were analytically confirmed by LC-MS/MS in samples of all replicates taken at the start, after 48 hours and at the end of exposure.

Results

The measured concentrations represented 101-120% of nominals at the start and 97-104% after 48 hours and at the end of exposure. The value of 120% for 1 replicate was the average of two injections, one giving 147% of nominal and the other one giving 92% of nominal. The value for 147% of nominal may be considered an outlier, since the results clearly demonstrated that the test substance was stable under the test conditions, and measured concentrations in the same replicate were 101-104% of nominal after 48 and 96 hours. Test endpoints were based on nominal concentrations, which is acceptable. Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 203 guideline. No mortality and clinical signs were observed in the treated and control replicates. The 96-hour LC50 is >100 mg a.s./L.

Conclusion

96-hour LC50 >100 mg a.s./L.

Guidelines and limitations

The study is acceptable.

STUDY IIA, 8.11.1/01

Characteristics

Reference	:	[redacted]. (2009b)	water solubility	:	3000-3200 mg/L at pH 4-9 & 20°C
type of study	:	Acute toxicity study (marine species)	species	:	Sheepshead minnow (*Cyprinodon variegatus*)
year of execution	:	2009	exposure duration	:	96 hours

GLP statement	:	Yes	nominal concn.	:	5, 10, 20, 40 and 80 mg a.s./L
guideline	:	FIFRA 72-3, OPPTS 850.1075, OECD 203	dosing method	:	Static; stock solution in DMF
test substance	:	BYI 02960; technical, batch 2009-000239	acceptability	:	Acceptable
purity	:	96.2%	96-h LC50	:	>83.9 mg a.s./L

Methods

A 96-hour acute toxicity test on juvenile sheepshead minnow (*Cyprinodon variegatus*; saltwater species) (1 replicate of ten fish per concentration, not fed for 48 hours prior to testing) was conducted under static conditions with BYI 02960 technical at test concentrations of 5, 10, 20, 40 and 80 mg a.s./L. It was reported that numerous preliminary trials demonstrated that a solvent stock would be needed to achieve maximum solubility in dilution water. The test solutions were prepared by adding aliquots of a stock solution in DMF to test water (max 0.1 mL/L DMF). The test included untreated and solvent controls (0.1 mL/L DMF). The concentrations of BYI 02960 were analytically confirmed by a sufficiently validated LC-MS/MS method in samples of all test solutions taken at the start and end of exposure.

Results

The measured concentrations represented 100-113% of nominals at the start and 102-111% at the end of exposure. Test endpoints were based on mean measured concentrations (5.6, 10.4, 21.0, 40.4 and 83.9 mg a.s./L). Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 203 guideline. No mortality and clinical signs were observed in any of the treatments and the controls. The 96-hour LC50 is >83.9 mg a.s./L.

Conclusion

96-hour LC50 >83.9 mg a.s./L.

Guidelines and limitations

The study is acceptable.

STUDY IIA 8.2.1.1/02

Characteristics

reference	:	[redacted]	water solubility	:	80 mg/L (functional limit)
type of study	:	acute toxicity	species	:	frog *Xenopus laevis*
year of execution	:	2011	exposure duration	:	48 hours
GLP statement	:	Yes	nominal concn.	:	80 mg a.s./L (limit test)
guideline	:	EPA OPPTS 850.1075, FIFRA, 40 CFR, part 158, guideline 72-1, OECD 203 (1992).	dosing method	:	Static; stock solution in DMF
test substance	:	BYI 02960 technical, Batch number: 2009-000239, beige powder.	acceptability	:	Acceptable
purity	:	96.2%	96-h LC50	:	>80 mg a.s./L

Description

Methods. Acute toxicity of BYI 02960 to the tadpole *Xenopus laevis* from a commercial provider, was tested under static conditions. A limit test at a nominal concentration of 80 mg as/L (the functional limit of solubility ot the test substance in fresh water), control, and a solvent control (dimethylformamide 0.1 mL/L) was performed. A stock solution (800 g as/L) was prepared and diluted with test water to the nominal test concentration of 80 mg as/L. Test water was pring water blended with dechlorinated reverse osmosis water (hardness 180 mg/L, pH 8.4, conductivity 457 µmhos/cm). Tadpoles were acclimated for 4 days under test conditions. Tadpoles were 15.0 ± 0.97 mm at the experimental start. Three replicates with 10 tadpoles each, 7 L solution per unit. Observation on mortality, signs of toxicity and abnormal behaviour were performed 6, 24, and 48 h after test initiation.
Conditions. 21.5 – 22.0 ºC, 16:8 h L:D (739 - 1040 lux with 30 minutes transition period), no aeration, no feeding.
Chemical analysis. Samples were taken at the start and end of the study to determine actual concentrations. Stock solution was also analysed. Analysis by HPLC with MS detection, after dilution with HPLC-grade water. Method recovery of spiked solutions was 94 – 102% (mean 98%). LOQ 8.0 mg as/L.
Calculations and statistics. No statistical calculations were necessary.

Results

Measured concentration at the start was 64.7 mg as/L (81% of nominal) and after 48 hours 82.9 mg as/L (104% of nominal). Mean measured concentration was 73.8 mg as/L (92% of nominal). Measured concentration at test initiation was always lower than concentration measured at the end of the test. The test substance was stable during the experiment. Test solutions were clear, no precipitation. No mortality in control, solvent control and test concentration. All tadpoles appeared healthy and normal. LC_{50} for 48 hours exposure reported as > 80 mg as/L, based on nominal concentration.

Remarks by RMS

Water quality parameters within accepted range. The result 48-h LC_{50} > 80 mg/L, based on nominal concentrations, is used for risk assessment.

STUDY IIA, 8.3.1.1/01

Characteristics

reference	:	Banman C.S. & Lam C.V. (2009a)	water solubility	:	3000-3200 mg/L at pH 4-9 & 20°C
type of study	:	Acute toxicity study	species	:	*Daphnia magna*
year of execution	:	2009	exposure duration	:	48 hours
GLP statement	:	Yes	nominal concn.	:	80 mg a.s./L
guideline	:	FIFRA 72-2, OPPTS 850.1010, OECD 202	dosing method	:	Static; stock solution in DMF
test substance		BYI 02960 technical, batch 2009-000239	acceptability	:	Acceptable
purity	:	96.2%	48 h-EC50	:	>77.6 mg a.s./L

Methods

A 48-hour acute toxicity test in *Daphnia magna* (<24 h old, 6 replicates of five daphnia each for the treatment and each of the controls) was conducted under static conditions with BYI 02960 technical at a nominal test concentration of 80 mg a.s./L, with untreated control and solvent control (0.1 mL/L DMF). The concentrations of BYI 02960 were analytically confirmed by a sufficiently validated LC-MS/MS method in samples of all test solutions taken at the start and end of exposure.

Results

The measured concentration was 102% of nominal at the start and 92% at the end of exposure. Test endpoinst were based on the mean measured concentration (77.6 mg a.s./L). Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 202 guideline. No mortality and signs of intoxication were observed in any replicate. The 48-hour EC50 is >77.6 mg a.s./L.

Conclusion

48-hour EC50 >77.6 mg a.s./L.

Guidelines and limitations

The study is acceptable.

STUDY IIA, 8.3.1.2/01

Characteristics

reference	:	Bruns E. (2011e)	water solubility	:	3000-3200 mg/L at pH 4-9 & 20°C
type of study	:	Acute toxicity study	species	:	*Chironomus riparius*
year of execution	:	2011	exposure duration	:	48 hours
GLP statement	:	Yes	nominal concn.	:	3.125, 6.25, 12.5, 25, 50 and 100 µg a.s./L
guideline	:	OPPTS 850.1300, OECD 202	dosing method	:	Static; direct addition to test water
test substance	:	BYI 02960 (tech.), batch 2009-000239 (batch code BYI 02960-01-03)	acceptability	:	Acceptable
purity	:	96.2%	48 h-EC50	:	61.7 µg a.s./L

Methods

A 48-hour acute toxicity test in *Chironomus riparius* (2-3 day old first instar larvae, 4 replicates of 10 larvae each for the treatments and the control) was conducted under static conditions with BYI 02960 technical at nominal test concentrations of 3.125, 6.25, 12.5, 25, 50 and 100 µg a.s./L, with untreated control. The test solution of the highest concentration was prepared by adding the test substance directly to the test water. Lower concentrations were prepared by serial dilution. A small amount of feed (0.01 mL of an aqueous fish food suspension) was added immediately after addition of the larvae. The concentrations of BYI 02960 were analytically confirmed by a sufficiently validated LC-MS/MS method in samples of all test solutions taken at the start and end of exposure.

Results

The measured concentrations were 97-103% of nominal at the start and 101-107% at the end of exposure. Test endpoints were based on nominal concentrations, which is acceptable. Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 202 guideline. At 0, 3.125, 6.25, 12.5, 25, 50 and 100 µg a.s./L, respectively, immobility was 0%, 0%, 0%, 2.5%, 2.5%, 30% and 85%. The reported 48-hour EC50 (Probit analysis) was 61.7 µg a.s./L (95% CI 41.4-109 µg a.s./L. At 100 µg a.s./L, surviving larvae showed reduced immobility.

Conclusion

48-hour EC50 61.7 µg a.s./L.

Guidelines and limitations

The study is acceptable.

STUDY IIA, 8.4/01

Characteristics

reference	:	Banman C.S. & Lam C.V. (2010)	water solubility	:	3000-3200 mg a.s./L at pH 4-9 & 20°C
type of study	:	Algae growth inhibition	species	:	*Pseudokirchneriella subcapitata*
year of execution	:	2010	exposure duration	:	96 hours
GLP statement	:	Yes	nominal concn.	:	5, 10, 20, 40 and 80 mg a.s./L
guideline	:	FIFRA 123-2, OPPTS 850.5400, OECD 201 (2006)	dosing method	:	Static; stock solution in DMF
test substance	:	BYI 02960 technical, batch 2009-000239	acceptability	:	Acceptable
purity	:	96.2%	72h-ErC50 & EyC50	:	>80 mg a.s./L

Methods

A 96-hour toxicity test on green algae (*Pseudokirchneriella subcapitata*) (3 replicates for the treatment and the untreated and solvent controls, each containing 1 x 10^4 cells/mL at the start) was conducted with BYI 02960 technical at test concentrations of 5, 10, 20, 40 and 80 mg a.s./L. The solution of the highest concentration was prepared by adding an aliquot of a stock solution in DMF to test water (0.1 mL/L DMF). The lower concentrations were prepared by serial dilution of this solution. The concentrations of BYI 02960 were analytically confirmed by a sufficiently validated LC-MS/MS method in samples of all test solutions taken at the start and end of exposure.

Results

The measured concentrations were in the range 111-120% of nominal at the start and end of exposure. Test endpoints were based on nominal concentrations, which is acceptable. Physico-chemical parameters were in accordance with the OECD 201 guideline. The pH increased from 7.3-7.4 at the start to 10.1-10.3 at the end, but this is associated with CO_2 depletion due to strong cell growth in the controls and all treaments and does not invalidate the test. The mean growth factor in the controls was 144 within 72 hours. The mean CV of the section-by-section specific growth rates in the control was 13%. The CV of the average specific growth rate in the control during the 72-hour

period was 1.0%. Hence the validity criteria of OECD 201 (2006) were satisfied. The % inhibition range for specific growth rate and area under the curve (biomass), respectively, were in the range -3.3 to -1.0% and -3.5 to -17.7%. The 72-h ErC50 and EbC50 was >80 mg a.s./L. The 72-h EyC50 was not reported, but clearly also in excess of 80 mg a.s./L.

Conclusion

72-h ErC50, EbC50 and EyC50 >80 mg a.s./L.

Guidelines and limitations

The study is acceptable.

STUDY IIA, 8.6/01

Characteristics

Reference	:	Banmann C.S. *et al* (2010)	species	:	Duckweed (*Lemna gibba G3*)
type of study	:	Duckweed growth inhibition test	duration	:	7 days
year of execution	:	2010	nominal conc.	:	5, 10, 20, 40 and 80 mg a.s./L
GLP statement	:	Yes	dosing method	:	Semi-static; stock solution in DMF
guideline	:	FIFRA 123-2, OECD 221 (2006) & OPPTS 850.4400	acceptability	:	Acceptable
test substance	:	BYI 02960 technical, batch 2009-000239	Lowest NOEC	:	34.2 mg a.s./L
purity	:	96.2%	Lowest EC50	:	>67.7 mg a.s./L

Methods

A 7-day toxicity test on the growth of duckweed (*Lemna gibba G3*) (3 replicates per concentration, each containing three plants with four fronds each at test initiation) was conducted under semi-static conditions (renewal on day 3) with BYI 02960 technical at nominal test concentrations of 5, 10, 20, 40 and 80 mg a.s./L, with untreated and solvent control each tested in 3 replicates. The solution of the highest concentration was prepared by adding an aliquot of a stock solution in DMF to test water (0.1 mL/L DMF). The lower concentrations were prepared by serial dilution of this solution (including addition of solvent such that the final concentration of DMF was 0.1 mL/L in all treatments). The concentrations of BYI 02960 were analytically confirmed by a sufficiently validated LC-MS/MS method in samples of all test solutions taken at the start (fresh solutions), on day 3 (aged and fresh solutions) and at the end of exposure (aged solutions). On day 0, 3, 5 and 7, frond number and any change in plant development were recorded. Frond dry weight was recorded on day 0 and 7.

Results

The measured concentrations in the test solutions taken on day 0 (fresh), day 3 (aged and fresh) and day 7 (aged) were in the range 65-68%, 71-77%, 86-104% and 93-101%, respectively. The mean measured concentrations were in the range 80-86% of nominal: 4.02, 8.17, 16.0, 34.2 and 67.7 mg a.s./L. The test endpoints were based on mean measured concentrations.

Water quality parameters (light intensity, pH and temperature) were in accordance with the OECD 221 guideline. The doubling time in the control was 1.65 days, hence the validity criterion of OECD 221

(2006) was satisfied (doubling time <2.5 days). Phytotoxic effects were not observed in any treatment. Other test results are summarised in the Table below.

All EC50 values are >67.7 mg a.s./L as effects at the highest test concentration were well below 50% for all parameters. No statistically significant effects on yield and growth rate based on biomass were recorded, hence the corresponding NOECs are 67.7 mg a.s./L. Based on statistically significant reductions of yield and growth rate based on frond number at the highest tested concentration, the corresponding NOECs are 34.2 mg a.s./L.

The report also calculated percentage inhibition values for cumulative biomass based on frond number: 13%, 10%, 19%, 8% and 29% at 4.02, 8.17, 16.0, 34.2 and 67.7 mg a.s./L, respectively, with statistical significance (5% level) reached at 16.0 and 67.7 mg a.s./L. The author of the report stated that the statistically significant reduction of 19% at 16.0 mg a.s./L was unrelated to the treatment, since the next higher dose level was unaffected, and since in a pre-test at 16 mg a.s./L the inhibition was -1.5% for fround counts. This is acceptable. In addition, this parameter is not required by OECD 221.

Table B.9.2.1.1-01 The toxicity of BYI 02960 technical to *Lemna gibba*

Mean measured conc. (mg a.s./L)	frond number			biomass		
	mean no. of fronds (day 7)	% inhibition growth rate	% inhibition yield	mean biomass (mg d.w.) (day 7)	% inhibition growth rate	% inhibition yield
Control	238	-	-	27.2	-	-
Solvent cont	217	-	-	25.2	-	-
Pooled con	228	-	-	26.2	-	-
4.02	208	3.0	8.4	23.8	3.6	9.0
8.17	215	2.0	5.5	24.9	2.0	4.8
16.0	191	5.9	16.0	23.0	4.9	12.3
34.2	219	1.5	3.7	28.0	-1.7	-6.8
67.7	169	10.3*	25.9*	21.5	7.7	17.9

* statistically significant difference from control at 5% level.

Conclusion

Semi-static test: lowest NOEC 34.2 mg a.s./L, lowest EC50 >67.7 mg a.s./L.

Guidelines and limitations

The test was performed in agreement with OECD 221 and is acceptable.

STUDY IIA, 8.11.1/02

Characteristics

reference	:	Gallagher S.P. et al. (2009a)	water solubility	:	3000-3200 mg/L at pH 4-9 & 20°C
type of study	:	Acute toxicity study (marine organism)	species	:	Eastern oyster (*Crassostrea virginica*)
year of execution	:	2009	exposure duration	:	96 hours
GLP statement	:	Yes	nominal concn.	:	0.94, 1.9, 3.8, 7.5, 15 and 30 mg a.s./L
guideline	:	OPPTS 850.1025	dosing method	:	Flow through; stock solutions in DMF
test substance	:	BYI 02960 technical, batch 2009-000239	acceptability	:	Acceptable
purity	:	96.2%	96-h EC50	:	>29 mg a.s./L

Methods

A 96-hour shell deposition test in Eastern oyster (*Crassostrea virginica*) (1 replicate of 20 oysters per concentration) was conducted under flow-through conditions with BYI 02960 technical at test concentrations of 0.94, 1.9, 3.8, 7.5, 15 and 30 mg a.s./L. The test included untreated and solvent controls (0.1 mL/L DMF). The concentrations of BYI 02960 were analytically confirmed by a sufficiently validated HPLC/UV method in samples of all test solutions taken at the start, after 48 hours and at the end of exposure.

Results

The measured concentrations represented 94-100% of nominals. Test endpoints were based on mean measured concentrations (0.90, 1.8, 3.6, 7.3, 15 and 29 mg a.s./L). Water quality parameters (pH, oxygen concentration, temperature and salinity) were in accordance with the OPPTS 850.1025 guideline (pH 8.1-8.2, dissolved oxygen ≥6.9 mg/L(≥86% of saturation), 19.2-21.1°C, salinity 20$^{0}/_{00}$). The validity criteria of OPPTS 850.1025 were satisfied (mortality <10%, dissolved oxygen >60%, no evidence of spawning, >2 mm shell growth in controls). No mortality was observed in any of the treatments and the controls. At 0.90, 1.8, 3.6, 7.3, 15 and 29 mg a.s./L, respectively, inhibition of shell growth was 2.2, 2.9, -13, 13, -19 and 13%. None of the differences between the treated groups and the pooled control were statistically significant at the 5% uncertainty level. The 96-hour EC50 is >29 mg a.s./L.

Conclusion

96-hour EC50 >29 mg a.s./L.

Guidelines and limitations

The study is acceptable.

STUDY IIA, 8.11.1/03

Characteristics

reference	:	Gallagher S.P. et al. (2009b)	water solubility	:	3000-3200 mg/L at pH 4-9 & 20°C
type of study	:	Acute toxicity study (marine organism)	species	:	Salt water mysid (*Americamysis bahia*)
year of execution	:	2009	exposure duration	:	96 hours
GLP statement	:	Yes	nominal concn.	:	0.13, 0.22, 0.36, 0.60 and 1.0 mg a.s./L
guideline	:	OPPTS 850.1035	dosing method	:	Static; test substance dissolved in test water
test substance	:	BYI 02960 technical, batch 2009-000239	acceptability	:	Acceptable for juveniles (not tested whether these are most sensitive life-stage)
purity	:	96.2%	96-h LC50	:	0.26 mg a.s./L

Methods

A 96-hour acute toxicity test on juveniles (<24 h old) of the saltwater mysid *Americamysis bahia* (2 replicates of 10 mysids per concentration) was conducted under static conditions with BYI 02960 technical at test concentrations of 0.13, 0.22, 0.36, 0.60 and 1.0 mg a.s./L. The test included untreated controls. The concentrations of BYI 02960 were analytically confirmed by a sufficiently validated HPLC/UV method in samples of all test solutions taken at the start, after 48 hours and at the end of exposure.

Results

The measured concentrations represented 94-98% of nominals. Test endpoints were based on mean measured concentrations (0.12, 0.21, 0.35, 0.58 and 0.98 mg a.s./L). Water quality parameters (pH, oxygen concentration, temperature and salinity) were in accordance with the OPPTS 850.1035 guideline (pH 8.1-8.2, dissolved oxygen ≥6.5 mg/L(≥89% of saturation), 23.2-25.7°C, salinity 20$^{0}/_{00}$). No mortality was observed in the controls. At 0.12, 0.21, 0.35, 0.58 and 0.98 mg a.s./L, respectively, mortality was 5, 60, 50, 100 and 100%. Signs of toxicity were noted at 0.21 mg a.s./L and above. The 96-hour LC50 was 0.26 mg a.s./L (95% CI 0.12-0.58 mg a.s./L.

Conclusion

96-hour LC50 0.26 mg a.s./L.

Guidelines and limitations

The study result is acceptable for juvenile mysids. The OPPTS 850.1035 guideline states that a range-finding test should be conducted to determine which life stage (juvenile or young adult) is to be utilized in the definitive test. The definitive test should be conducted on the mysid life stage (juveniles or young adults) which is most sensitive to the test substance. The report did not state that a range-finding test was conducted with juveniles and young adults, hence it is unclear whether the juveniles tested were the most sensitive life stage.

B.9.2.1.2 Acute toxicity of metabolites (IIA 8.2.1, IIA 8.3.1)

STUDY IIA, 8.2.1.3/01

Characteristics

reference	:	[redacted](2011b)	water solubility	:	No data
type of study	:	Acute toxicity study	species	:	Rainbow trout (*Oncorhynchus mykiss*)
year of execution	:	2011	exposure duration	:	96 hours
GLP statement	:	Yes	nominal concn.	:	100 mg metabolite/L (limit test)
guideline	:	FIFRA 72-1, OPPTS 850.1075, EEC C.1, OECD 203	dosing method	:	Static; stock solution in DMF
test substance	:	BYI 02960-succinamide; batch BCS-CR74729-01-01 (original batch number BCOO 6329-2-10)	acceptability	:	Acceptable
purity	:	97.8%	96-h LC50	:	>100 mg metabolite/L

Methods

A 96-hour acute toxicity limit test in juvenile rainbow trout (*Oncorhynchus mykiss*) (2 replicates of 15 fish, not fed for 48 hours prior to testing) was conducted under static conditions with BYI 02960-succinamide (metabolite of BYI 02960) at a nominal test concentration of 100 mg metabolite/L. The test solutions were prepared by adding a solution in DMF to the test water. The test included untreated and solvent controls (0.1 mL/L DMF). The concentrations of BYI 02960-succinamide were analytically confirmed by LC-MS/MS in samples of all replicates taken at the start, after 48 hours and at the end of exposure.

Results

The measured concentrations represented 112-115% of nominals (overall range for 0-, 48- and 96-hour samples). Test endpoints were based on nominal concentrations, which is acceptable. Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 203 guideline. No mortality was observed in the treated and control replicates. Two fish died in one of the two solvent control replicates. This does not invalidate the test since the overall mortality rate in the solvent control was 6.7%, hence <10%, and in the pooled controls 3.3%. No signs of intoxication were observed in the control and treated replicates and in the surviving fish of the solvent control. The 96-hour LC50 is >100 mg metabolite/L.

Conclusion

96-hour LC50 >100 mg metabolite/L.

Guidelines and limitations

The study is acceptable.

STUDY IIA, 8.2.1.3/02

Characteristics

reference	:	[redacted] (2011c)	water solubility	:	No data
type of study	:	Acute toxicity study	species	:	Rainbow trout (*Oncorhynchus mykiss*)
year of execution	:	2010	exposure duration	:	96 hours
GLP statement	:	Yes	nominal concn.	:	10.0 mg metabolite/L (limit test)
guideline	:	FIFRA 72-1, OPPTS 850.1075, EEC C.1, OECD 203	dosing method	:	Static; direct addition to test water
test substance	:	BCS-AB60481 (tech.) = sodium difluoroacetate; batch BCS-AB60481-01-01 (original batch number BCOO 6092-3-1)	acceptability	:	Acceptable
purity	:	>99.0%	96-h LC50	:	>10.0 mg metabolite/L

Methods

A 96-hour acute toxicity limit test in juvenile rainbow trout (*Oncorhynchus mykiss*) (2 replicates of 15 fish, not fed for 48 hours prior to testing) was conducted under static conditions with BCS-AB60481 (tech.) (sodium difluoroacetate; metabolite of BYI 02960 technical) at a nominal test concentration of 10.0 mg metabolite/L. The test solutions were prepared by adding the test substance directly to the test water. The test included untreated controls. The concentrations of sodium difluoroacetate were

analytically confirmed by LC-MS/MS in samples of all replicates taken at the start, after 48 hours and at the end of exposure.

Results

The measured concentrations represented 100-105% of nominals (overall range for 0-, 48- and 96-hour samples). Test endpoints were based on nominal concentrations, which is acceptable. Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 203 guideline. No mortality and sublethal effects were observed in the treated and control replicates. The 96-hour LC50 is >10.0 mg metabolite/L.

Conclusion

96-hour LC50 >10.0 mg metabolite/L.

Guidelines and limitations

The study is acceptable.

STUDY IIA, 8.3.1.1/02

Characteristics

reference	:	Bruns E. (2011d)	water solubility	:	No data
type of study	:	Acute toxicity study	species	:	*Daphnia magna*
year of execution	:	2011	exposure duration	:	48 hours
GLP statement	:	Yes	nominal concn.	:	10 mg metabolite/L (limit test)
guideline	:	OPPTS 850.1300, EEC C.2, OECD 202	dosing method	:	Static; direct addition to test water
test substance	:	BCS-AB60481 (tech.) = sodium difluoroacetate; batch BCS-AB60841-01-01 (original batch number BCOO 6092-3-1)	acceptability	:	Acceptable
purity	:	>99.0%	48 h-EC50	:	>10 mg metabolite/L

Methods

A 48-hour acute toxicity test in *Daphnia magna* (<24 h old, 12 replicates of five daphnia each for the treatment and the control) was conducted under static conditions with BCS-AB60481 (tech.) (sodium difluoroacetate; metabolite of BYI 02960 technical) at a nominal test concentration of 10 mg metabolite/L, with untreated control. The test solutions were prepared by adding the test substance directly to the test water. The concentrations of sodium difluoroacetate were analytically confirmed by a sufficiently validated LC-MS/MS method in samples of all replicates taken at the start and end of exposure.

Results

The measured concentration was 101% of nominal at the start and 103% at the end of exposure. Test endpoints were based on the nominal concentration, which is acceptable. Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 202 guideline. No

mortality and signs of intoxication were observed in any replicate. The 48-hour EC50 is >10 mg metabolite/L.

Conclusion

48-hour EC50 >10 mg metabolite/L.

Guidelines and limitations

The study is acceptable.

STUDY IIA, 8.3.1.1/03

Characteristics

reference	:	Mc Elligott A. (1997)	water solubility	:	1.43 g/L at 20°C
type of study	:	Acute toxicity study	species	:	*Daphnia magna*
year of execution	:	1997	exposure duration	:	48 hours
GLP statement	:	Yes	nominal concn.	:	6.3, 12.5, 25, 50 and 100 mg/L
guideline	:	FIFRA 72-2, EEC C.2, OECD 202	dosing method	:	Static; direct addition to test water
test substance	:	IC-0 (metabolite of BYI 02960, batch MF-4267, elsewhere named 6-CNA)	acceptability	:	Acceptable
purity	:	99.7%	48 h-EC50	:	>95.1 mg/L

Methods

A 48-hour acute toxicity test in *Daphnia magna* (<24 h old, 2 replicates of 10 daphnia each for the treatments and the control) was conducted under semi-static conditions (renewal after 24 hours) with IC-0 (metabolite of BYI 02960) at nominal test concentrations of 6.3, 12.5, 25, 50 and 100 mg/L, with untreated control. The test solutions of the highest concentration was prepared by adding the test substance directly to the test water. Lower concentrations were prepared by serial dilution. The concentrations of IC-0 were analytically confirmed by a sufficiently validated HPLC/UV method in samples of all treatments taken at the start and end of each renewal period.

Results

The measured concentrations were 94-96% of nominal at the start and 94-97% at the end of the renewal periods. Test endpoints were based on the mean measured concentrations, which is acceptable (6.0, 11.9, 23.9, 47.2 and 95.1 mg/L). Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 202 guideline. No mortality and signs of intoxication were observed in any replicate, except for one immobile daphnid at 47.2 mg/L, but this was considered to be unrelated to the treatment. The 48-hour EC50 is >95.1 mg/L.

Conclusion

48-hour EC50 >95.1 mg/L.

Guidelines and limitations

The study is acceptable.

STUDY IIA, 8.3.1.2/02

Characteristics

reference	:	Bruns E. (2011f)	water solubility	:	No data
type of study	:	Acute toxicity study	species	:	*Chironomus riparius*
year of execution	:	2011	exposure duration	:	48 hours
GLP statement	:	Yes	nominal concn.	:	26, 36, 51, 71 and 100 mg pure metabolite/L
guideline	:	OECD 202, OPPTS 850.supp	dosing method	:	Static; direct addition to test water
test substance	:	BYI 02960-succinamide; batch BCS-CR74729 (original batch number BCOO 6329-2-10)	acceptability	:	Acceptable
purity	:	97.8%	48 h-EC50	:	>100 mg pure metabolite/L

Methods

A 48-hour acute toxicity test in *Chironomus riparius* (2-3 day old first instar larvae, 4 replicates of 10 larvae each for the treatments and the control) was conducted under static conditions with BYI 02960-succinamide (metabolite of BYI 02960) at nominal test concentrations of 26, 36, 51, 71 and 100 mg pure metabolite/L, with untreated control. The test solutions were prepared by serial dilution from a stock solution prepared in test water. A small amount of feed (0.01 mL of an aqueous fish food suspension) was added immediately after addition of the larvae. The concentrations of BYI 02960-succinamide were analytically confirmed by a sufficiently validated LC-MS/MS method in samples of all test solutions taken at the start and end of exposure.

Results

The measured concentrations were 103-106% of nominal at the start and 102-107% at the end of exposure. Test endpoints were based on nominal concentrations, which is acceptable. Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 202 guideline. At 0, 26, 36, 51, 71 and 100 mg pure metabolite/L, respectively, immobility was 0%, 0%, 0%, 2.5%, 2.5% and 15%. The 48-hour EC50 was >100 mg pure metabolite/L.

Conclusion

48-hour EC50 >100 mg pure metabolite/L.

Guidelines and limitations

The study is acceptable.

STUDY IIA, 8.3.1.2/03

Characteristics

reference	:	Bruns E. (2012a)	water solubility	:	No data
type of study	:	Acute toxicity study	species	:	*Chironomus riparius*
year of execution	:	2011	exposure duration	:	48 hours
GLP statement	:	Yes	nominal concn.	:	26, 36, 51, 71 and 100 mg pure metabolite/L
guideline	:	OECD 235	dosing method	:	Static; direct addition to test water
test substance	:	BYI 02960-azabicyclosuccinamide,	acceptability	:	Acceptable

		sodium salt; batch SES 11732-3-1			
purity	:	48.0%	48 h-EC50	:	>100 mg pure metabolite/L

Methods

A 48-hour acute toxicity test in *Chironomus riparius* (2-3 day old first instar larvae, 6 replicates of 5 larvae each for the treatments and the control) was conducted under static conditions with BYI 02960-azabicyclosuccinamide, sodium salt (metabolite of BYI 02960) at nominal test concentrations of 26, 36, 51, 71 and 100 mg pure metabolite/L, with untreated control. The test solutions were prepared by serial dilution from a stock solution prepared in test water. A few drops of an aqueous fish food suspension were added prior to insertion of the larvae. The concentrations of BYI 02960-azabicyclosuccinamide were analytically confirmed by a sufficiently validated LC-MS/MS method in samples of all test solutions taken at the start and end of exposure.

Results

The measured concentrations were 107-112% of nominal at the start and 117-120% at the end of exposure. Test endpoints were based on nominal concentrations, which is acceptable. Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 202 guideline. At 0, 26, 36, 51, 71 and 100 mg pure metabolite/L, respectively, immobility was 0%, 0%, 0%, 3.3%, 3.3% and 20%. The 48-hour EC50 was >100 mg pure metabolite/L.

The results of the most recently conducted test with the reference substance 3,5-dichlorophenol (48-h EC50 3.1 mg/L) was within the range defined by OECD 235 (0.50-3.6 mg/L).

Conclusion

48-hour EC50 >100 mg pure metabolite/L.

Guidelines and limitations

The study is acceptable.

STUDY IIA, 8.3.1.2/04

Characteristics

reference	:	Bowers L.M. & Lam C.V. (1998)	water solubility	:	2 g/L (reported value)
type of study	:	Acute toxicity study	species	:	*Chironomus tentans*
year of execution	:	1998	exposure duration	:	96 hours
GLP statement	:	Yes	nominal concn.	:	1 mg/L
guideline	:	ASTM (1987), EPA (1972, 1982, 1985)	dosing method	:	Static; direct addition to test water
test substance	:	6-chloronicotinic acid (6-CNA = IC-0); batch 9931	acceptability	:	Acceptable
purity	:	97%	96 h-EC50	:	>1 mg/L

Methods

A 96-hour acute toxicity test in *Chironomus tentans* (12-day old adult larvae, 3 replicates of 10 larvae for the treatment and 1 replicate of 10 larvae for the control) was conducted under semi-static conditions (renewal after 2 days) with 6-chloronicotinic acid (6-CNA = IC-0, metabolite of BYI 02960) at a nominal test concentration of 1 mg/L, with untreated control. The test solution was prepared by dissolving the test substance in test water. Each replicate 1-L test dish contained 800 mL (6 cm) of test solution and 5 g of fine silica sand. The test organisms were fed 0.1 mL of blended Tetramin each day except day 2, when feeding was not necessary since the blended Tetramin was still present in the test vessels.

The concentrations of 6-chloronicotinic acid were not analytically confirmed in the test solutions. However, the stability of 6-chloronicotinic acid in deionised water was confirmed by HPLC/UV after two days at room temperature. Taking this into consideration, as well as the facts that the preparation of the test solutions was well documented, and that in study 4 of section B.9.2.1.2 6-chloronicotinic acid was stable during a 48-hour acute toxicity test with *Daphnia magna*, the lack of analytical confirmation of the concentrations of 6-chloronicotinic acid in the test solutions does not invalidate the study.

Results

The test temperature was 20.5-21.9°C, the pH was consistently 8.4 and the dissolved oxygen concentrations were 55-99% of saturation. Mortality was 10% in the control and 0% in the treated solutions. The 96-hour EC50 was >1 mg/L.

Conclusion

96-hour EC50 >1 mg/L.

Guidelines and limitations

An OECD guideline is not avaliable for acute toxicity testing of *C. tentans*. The use of 12-day old (3rd instar) larvae is recommended by EPA guidelines (1995) for 10-day tests with *C. tentans* in the presence of sediment. The study was evaluated in the DAR of imidacloprid (6-chloronicotinic acid is also a metabolite of imidacloprid) and considered to be valid. The study is acceptable.

STUDY IIA, 8.4/02

Characteristics

reference	:	Bruns E. (2011j)	water solubility	:	No data
type of study	:	Algae growth inhibition	species	:	*Pseudokirchneriella subcapitata*
year of execution	:	2010-2011	exposure duration	:	72 hours
GLP statement	:	Yes	nominal concn.	:	10 mg pure metabolite/L
guideline	:	OPPTS 850.5400, OECD 201 (2006)	dosing method	:	Static (spiked water)
test substance	:	sodium difluoroacetate; batch BCS-AB60841-01-01 (original batch number BCOO 6092-3-1)	acceptability	:	Acceptable
purity	:	>99.0%	72h-ErC50 & EyC50	:	>10 mg pure metabolite/L

Methods

A 72-hour toxicity test on green algae (*Pseudokirchneriella subcapitata*) (6 replicates for the treatment and the control, each containing 1 x 10^4 cells/mL at the start) was conducted with sodium difluoroacetate (metabolite of BYI 02960 technical) at a test concentration of 10 mg pure metabolite/L. The test solution was prepared by adding the test substance directly to the test water. The concentrations of sodium difluoroacetate were analytically confirmed by a sufficiently validated LC-MS/MS method in samples taken at the start and end of exposure.

Results

The measured concentrations were in the range 100-103% of nominal at the start and end of exposure. Test endpoints were based on nominal concentrations, which is acceptable. Physico-chemical parameters were in accordance with the OECD 201 guideline. The mean growth factor in the controls was 68 within 72 hours. The mean CV of the section-by-section specific growth rates in the control was 21.4%. The CV of the average specific growth rate in the control during the 72-hour period was 1.0%. Hence the validity criteria of OECD 201 (2006) were satisfied. The % inhibition for 0-72 h specific growth rate and yield, respectively, was 4.0% and 15.8% (both statistically significantly different from the control at 5% uncertainty level). The 72-h ErC50 and EyC50 was >10 mg pure metabolite/L.

Conclusion

72-h ErC50 and EyC50 >10 mg pure metabolite/L.

Guidelines and limitations

The study is acceptable.

STUDY IIA, 8.4/03

Characteristics

reference	:	Sobczyk H. (2011)	water solubility	:	No data
type of study	:	Algae growth inhibition	species	:	*Pseudokirchneriella subcapitata*
year of execution	:	2011	exposure duration	:	72 hours
GLP statement	:	Yes	nominal concn.	:	10 mg pure metabolite/L
guideline	:	OECD 201 (2006), OPPTS 850.5400	dosing method	:	Static (stock solution in DMF)
test substance	:	BYI 02960-succinamide; batch BCS-CR74729-01-01 (original batch number BCOO 6329-2-10)	acceptability	:	Acceptable
purity	:	97.8%	72h-ErC50 & EyC50	:	>10 mg pure metabolite/L

Methods

A 72-hour toxicity test on green algae (*Pseudokirchneriella subcapitata*) (6 replicates for the treatment and the control and solvent control, each containing 1 x 10^4 cells/mL at the start) was conducted with BYI 02960-succinamide (metabolite of BYI 02960) at a test concentration of 10 mg pure metabolite/L. The test solution was prepared by adding an aliquot of a stock solution in DMF to test water (0.1 mL/L

DMF). The concentrations of BYI 02960-succinamide were analytically confirmed by a sufficiently validated HPLC-UV method in samples taken at the start and end of exposure.

Results

The measured concentrations were in the range 113-114% of nominal at the start and end of exposure. Test endpoints were based on nominal concentrations, which is acceptable. Physico-chemical parameters were in accordance with the OECD 201 guideline. The mean growth factor in the pooled controls was 75 within 72 hours. The mean CV of the section-by-section specific growth rates in the control was 18.4%. The CV of the average specific growth rate in the control during the 72-hour period was 1.2%. Hence the validity criteria of OECD 201 (2006) were satisfied. The % inhibition for 0-72 h specific growth rate and yield, respectively, was 0.1% and 0.4% (both not statistically significantly different from the control at 5% uncertainty level). The 72-h ErC50 and EyC50 was >10 mg pure metabolite/L.

Conclusion

72-h ErC50 and EyC50 >10 mg pure metabolite/L.

Guidelines and limitations

The study is acceptable.

STUDY IIA, 8.4/04

Characteristics

reference	:	Bruns E. (2012b)	water solubility	:	2 g/L (from other report)
type of study	:	Algae growth inhibition	species	:	*Pseudokirchneriella subcapitata*
year of execution	:	2011	exposure duration	:	72 hours
GLP statement	:	Yes	nominal concn.	:	6.25, 12.5, 25, 50 and 100 mg pure metabolite/L
guideline	:	OPPTS 850.5400, OECD 201 (2006)	dosing method	:	Static (spiked water)
test substance	:	6-chloronicotinic acid (6-CNA = IC-0); batch AE F161089 00 1B99 0001	acceptability	:	Acceptable
purity	:	98.8%	72h-ErC50 72-h EyC50	:	>100 mg pure metabolite/L 85 mg pure metabolite/L

Methods

A 72-hour toxicity test on green algae (*Pseudokirchneriella subcapitata*) (3 replicates for each treatment and 6 for the control, each containing 1 x 10^4 cells/mL at the start) was conducted with 6-chloronicotinic acid (6-CNA = IC-0, metabolite of BYI 02960). A stock solution of the test substance was prepared in test water. The test solutions were prepared by adding an aliqout of this stock solution to test water. The test concentrations were 6.25, 12.5, 25, 50 and 100 mg pure metabolite/L. Since the test substance is an acid, the pH of the 100 mg/L test solution was 5.1. Therefore, at this concentration the test was conducted without pH adjustment (3 replicates) and with the pH adjusted to 8.2 (8.2 was the pH of the controls) (3 replicates). The concentrations of 6-chloronicotinic acid were

analytically confirmed by a sufficiently validated LC-MS/MS method in samples taken at the start and end of exposure.

Results

The measured concentrations were in the range 99-112% of nominal at the start and end of exposure. Test endpoints were based on nominal concentrations, which is acceptable. Physico-chemical parameters were in accordance with the OECD 201 guideline. The change in pH was within 0.7 units in all treatments, except at 100 mg/L without pH adjustment (pH after 0, 24, 48 and 72 hours 5.1, 6.6, 6.2 and 5.1, respectively). The mean growth factor in the controls was 102 within 72 hours. The mean CV of the section-by-section specific growth rates in the control was 32.8%. The CV of the average specific growth rate in the control during the 72-hour period was 2.9%. Hence the validity criteria of OECD 201 (2006) were satisfied.

Effects on 0-72 h specific growth rate were in the range -9.0 to 3.7% (negative values represent increases relative to control, hence no adverse effects), except for the 100 mg/L treatment without pH adjustment (31.5% inhibition, statistically significant difference from the control at 5% uncertainty level). Effects on 0-72 h yield were in the range -51.5% to 16%, except for the 100 mg/L treatment without pH adjustment (77.4% inhibition, statistically significant difference from the control at 5% uncertainty level). The report stated that for the treatments without pH adjustment the 72-h ErC50 and EyC50 was 114 and 85 mg pure metabolite/L, respectively, but the 72-h ErC50 is an extrapolated value. Without pH adjustment, the 72-h ErC50 is >100 mg pure metabolite/L. With pH adjustment, the 72-h ErC50 and EyC50 is >100 mg pure metabolite/L.

Conclusion

Without pH adjustment: 72-h ErC50 >100 mg pure metabolite/L, 72-h EyC50 85 mg pure metabolite/L.
With pH adjustment: 72-h ErC50 and EyC50 >100 mg pure metabolite/L.

Guidelines and limitations

The study is acceptable. The relevant endpoints are taken from the test without pH adjustment, since the acidification of the test medium (responsible for the effects noted without pH adjustment at 100 mg/L) is caused by the test substance.

B.9.2.1.3 Acute toxicity of the formulated product (IIIA 10.2.2.2)

STUDY IIIA, 10.2.2.1/01

Characteristics

reference	:	[redacted]. (2010a)	species	:	Rainbow trout (*Oncorhynchus mykiss*)
type of study	:	Acute toxicity study	exposure duration	:	96 hours
year of execution	:	2010	nominal concn.	:	100 mg a.s./L
GLP statement	:	Yes	dosing method	:	Static; direct addition to test water
guideline	:	OPPTS 850.1075, EEC C.1, OECD	acceptability	:	Acceptable

		203			
test substance	:	BYI 02960 SL 200 G (200 g/L SL formulation), batch 2010-001067	96-h LC50	:	>100 mg a.s./L
a.s. content	:	17.1 % w/w (201.0 g/L) BYI 02960			

Methods

A 96-hour acute toxicity limit test in juvenile rainbow trout (*Oncorhynchus mykiss*) (2 replicates of 15 fish, not fed for 48 hours prior to testing) was conducted under static conditions with BYI 02960 SL 200 G (SL formulation, 17.1 % w/w (201.0 g/L) BYI 02960) at a nominal test concentration of 100 mg a.s./L. The test included an untreated control, tested also in 2 replicates of 15 fish each. The concentrations of BYI 02960 were analytically confirmed by a sufficiently validated LC-MS/MS method in samples of all test solutions taken at the start, after 48 hours and at the end of exposure.

Results

The measured concentrations were in the range 103-107% of nominals at the start, after 48 hours and at the end of exposure. Test endpoints were based on nominal concentrations which is acceptable. Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 203 guideline. No control fish died, and only one fish died (3% mortality) at 100 mg a.s./L. The clinical sign observed in all surviving treated fish after 96 hours of exposure was laboured respiration. The 96-hour LC50 is >100 mg a.s./L (585 mg formulation/L, based on 17.1% w/w) .

Conclusion

96-hour LC50 >100 mg a.s./L.

Guidelines and limitations

The study is acceptable.

STUDY IIIA, 10.2.2.1/02

Characteristics

reference	:	[redacted]. (2011k)	species	:	Common carp (*Cyprinus carpio*)
type of study	:	Acute toxicity study	exposure duration	:	96 hours
year of execution	:	2011	nominal concn.	:	100 mg a.s./L
GLP statement	:	Yes	dosing method	:	Static; direct addition to test water
guideline	:	OPPTS 850.1075, EEC C.1, OECD 203	acceptability	:	Acceptable
test substance	:	BYI 02960 SL 200 G (200 g/L SL formulation), batch 2010-001067	96-h LC50	:	>100 mg a.s./L
a.s. content	:	17.1 % w/w (201.0 g/L) BYI 02960			

Methods

A 96-hour acute toxicity limit test in juvenile carp (*Cyprinus carpio*) (2 replicates of 15 fish, not fed for 48 hours prior to testing) was conducted under static conditions with BYI 02960 SL 200 G (SL formulation, 17.1 % w/w (201.0 g/L) BYI 02960) at a nominal test concentration of 100 mg a.s./L. The test included an untreated control, tested also in 2 replicates of 15 fish each. The concentrations of

BYI 02960 were analytically confirmed by a sufficiently validated LC-MS/MS method in samples of all test solutions taken at the start, after 48 hours and at the end of exposure.

Results

The measured concentrations were in the range 97-106% of nominals at the start, after 48 hours and at the end of exposure. Test endpoints were based on nominal concentrations which is acceptable. Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 203 guideline. No control and treated fish died, and no clinical sign were observed. The 96-hour LC50 is >100 mg a.s./L (585 mg formulation/L, based on 17.1% w/w).

Conclusion

96-hour LC50 >100 mg a.s./L.

Guidelines and limitations

The study is acceptable.

STUDY IIIA, 10.2.2.2/01

Characteristics

reference	:	Riebschläger T. (2010)	species	:	*Daphnia magna*
type of study	:	Acute toxicity study	exposure duration	:	48 hours
year of execution	:	2010	nominal concn.	:	62.5, 125, 250, 500 and 1000 mg product/L
GLP statement	:	Yes	dosing method	:	Static; direct addition to test water
guideline	:	OPPTS 850.1010, EEC C.2, OECD 202	acceptability	:	Acceptable
test substance	:	BYI 02960 SL 200 G (200 g/L SL formulation), batch 2010-001067	48 h-EC50	:	684 mg product/L (117 mg a.s./L)
a.s. content	:	17.1 % w/w			

Methods

A 48-hour acute toxicity test in *Daphnia magna* (<24 h old, 6 replicates of five daphnia each for each of the treatments and the control) was conducted under static conditions with BYI 02960 SL 200 G (SL formulation, 17.1 % w/w BYI 02960) at nominal test concentrations of 62.5, 125, 250, 500 and 1000 mg product/L. The test included an untreated control. The concentrations of BYI 02960 were analytically confirmed by a sufficiently validated LC-MS/MS method in samples of all test solutions taken at the start and end of exposure.

Results

The measured concentrations were in the range 99-110% of nominal at the start and end of exposure. Test endpoints were based on nominal concentrations which is acceptable. Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 202 guideline. No mortality and signs of intoxication were observed in the control. At nominal test concentrations of 62.5, 125, 250, 500 and 1000 mg product/L, respectively, immobility after 48 hours was 0%, 6.7%, 30%, 33% and 63%. The 48-hour EC50 was 684 mg product/L, equivalent to 117 mg a.s./L.

Conclusion

48-hour EC50 684 mg product/L (117 mg a.s./L).

Guidelines and limitations

The study is acceptable.

STUDY IIIA, 10.2.2.3/01

Characteristics

reference	:	Bruns E. (2010b)			
type of study	:	Algae growth inhibition	species	:	*Pseudokirchneriella subcapitata*
year of execution	:	2010	exposure duration	:	72 hours
GLP statement	:	Yes	nominal concn.	:	49.4, 74.1, 111, 167 and 250 mg product/L
guideline	:	OECD 201 (2006)	dosing method	:	Static; stock solution in test water
test substance	:	BYI 02960 SL 200 G (200 g/L SL formulation), batch 2010-001067	acceptability	:	Acceptable
a.s. content	:	17.1 % w/w	72h-ErC50 & EyC50	:	>250 mg product/L (>42.8 mg a.s./L)

Methods

A 72-hour toxicity test on green algae (*Pseudokirchneriella subcapitata*) (3 replicates for each of the treatments and 6 replicates for the untreated control, each containing 1 x 10^4 cells/mL at the start) was conducted with BYI 02960 SL 200 g/L (SL formulation, 17.1 % w/w BYI 02960) at test concentrations of 49.4, 74.1, 111, 167 and 250 mg product/L. The concentrations of BYI 02960 were analytically confirmed by a sufficiently validated LC-MS/MS method in samples of all test solutions taken at the start and end of exposure.

Results

The measured concentrations were in the range 101-107% of nominal at the start and end of exposure. Test endpoints were based on nominal concentrations, which is acceptable. Physico-chemical parameters were in accordance with the OECD 201 guideline. The mean growth factor in the controls was 70 within 72 hours. The mean CV of the section-by-section specific growth rates in the control was 20%. The CV of the average specific growth rate in the control during the 72-hour period was 0.4%. Hence the validity criteria of OECD 201 (2006) were satisfied. The % inhibition range for specific growth rate and yield (% inhibition for yield not reported, but calculated by zRMS), respectively, were in the range -0.3 to 1.1% and -1.3 to 4.7%. The 72-h ErC50 and EyC50 was >250 mg product/L equivalent to >42.8 mg a.s./L.

Conclusion

72-h ErC50 and EyC50 >250 mg product/L (>42.8 mg a.s./L).

Guidelines and limitations

The study is acceptable.

B.9.2.2 Chronic toxicity

B.9.2.2.1 Laboratory testing (IIA 8.2.2; IIA 8.2.4; IIA 8.2.5; IIA 8.3.2; IIA 8.5.2; IIA 8.11)

STUDY IIA, 8.2.4/01

Characteristics

reference	:	[redacted]. (2011)	water solubility	:	3000-3200 mg/L at pH 4-9 & 20°C
type of study	:	Chronic toxicity study (ELS)	species	:	Fathead minnow (*Pimephales promelas*)
year of execution	:	2010	exposure duration	:	35 days
GLP statement	:	Yes	nominal concn.	:	0.625, 1.25, 2.5, 5 and 10 mg a.s./L
guideline	:	FIFRA 72-4, OPPTS 850.1400, OECD 210	dosing method	:	Flow-through; stock solution in DMF
test substance		BYI 02960 technical, batch 2009-000239	acceptability	:	Acceptable
purity	:	96.2%	NOEC	:	4.41 mg a.s./L

Methods

A 35-day fish early life stage flow-through study was undertaken with fathead minnow. Newly fertilised eggs (<24 hours post fertilisation, four replicate glass vessels with 35 eggs each per concentration) were exposed to BYI 02960 technical at test concentrations of 0.625, 1.25, 2.5, 5 and 10 mg a.s./L, with untreated and solvent control (DMF, 0.1 mL/L). On test day 5, when at least 90% of all viable control eggs had hatched, alevin were thinned to 20 per replicate. The total duration of the exposure period was 35 days. Samples for analytical confirmation of test concentrations using a sufficiently validated LC-MS/MS method were taken on day 0 and thereafter once weekly from selected replicates, with additional measurements on day 15 and 22. Hatching, mortality and fish behaviour was observed daily, and fish length and dry weight were measured at the end of the test.

Results

The mean measured concentrations of BYI 02960 technical were 0.619, 1.11, 2.05, 4.41 and 8.40 mg a.s./L, with limited and acceptable variation between replicates and time points. During the test, the pH was in the range 7.6-8.0, the oxygen concentration was 76-98% of the saturation value, and the temperature was in the range 24.6-25.0. In all treatments and the controls, hatch started on day 3. Other biological results are summarised in the table below. The observations of scoliosis and being at the water surface were infrequent, did not represent a dose-relationship and are considered to be unrelated to the treatment. The only adverse treatment related effect was reduced larval survival at the highest concentration. The reduced larval survival at 0.619 mg a.s./L is considered to be unrelated to the treatment in the absence of a dose-relationship. The NOEC is 4.41 mg a.s./L.

Table B.9.2.2.1-01 Results of an Early-Life-Stage test on fathead minnow (*Pimephales promelas*) with BYI 02960 technical

treatment	Observations (no. of fish)	% hatch (day 5)	larval survival (day 35)	fish length (mm)	dry weight (mg)
control	None	80.7	93.8	21.7	31.6
Solvent control	Scoliosis (1)	85.0	93.8	22.4	33.1
0.619	Scoliosis (4), at surface (1)	80.0	86.3*	22.5	34.2
1.11	None	83.6	95.0	22.2	33.8
2.05	Scoliosis (1)	85.7	97.5	22.6	34.8
4.41	None	80.7	88.8	22.0	33.7
8.40	Scoliosis (2)	83.6	87.5*	22.5	36.1

* Significantly different from control at 5% level

Conclusion

NOEC 4.41 mg a.s./L.

Guidelines and limitations

The study is acceptable.

STUDY IIA, 8.3.2.1/01

Characteristics

reference	:	Riebschläger T. (2011)	water solubility	:	3000-3200 mg/L at pH 4-9 & 20°C
type of study	:	Chronic toxicity study	species	:	*Daphnia magna*
year of execution	:	2011	exposure duration	:	21 days
GLP statement	:	Yes	nominal concn.	:	0.8, 1.6, 3.2, 6.4, 12.8 and 25.6 mg a.s./L
guideline	:	EPA 72-4, OPPTS 850.1300, EEC C.20, OECD 211	dosing method	:	Semi-static; stock solution in DMF
test substance	:	BYI 02960 technical, batch BYI02960-01-03 (original batch 2009-000239)	acceptability	:	Acceptable
purity	:	96.2%	21-day NOEC	:	3.2 mg a.s./L

Methods

A 21-day chronic reproductive toxicity test in *Daphnia magna* (<24 h old, 10 replicates with one daphnia each per concentration) was conducted according to OECD 211 under semi-static conditions (renewal every 2-3 days) with BYI 02960 technical at nominal test concentrations of 0.8, 1.6, 3.2, 6.4, 12.8 and 25.6 mg a.s./L, with untreated control and solvent control (0.1 mL/L DMF). The concentrations of BYI 02960 were analytically confirmed by a sufficiently validated HPLC-MS/MS method in samples of all fresh test solutions of day 0, 9 and 19 and in the corresponding aged solutions. Endpoints reported were parent mortality, total number of offspring per parent animal, parent age at first offspring emergence, neonates behaviour and length and dry weight of parent daphnia at the end of the test.

Results

The measured concentrations of BYI 02960 were 102-109% of nominal in fresh solutions and 102-117% in the corresponding aged solutions. Test endpoinst were based on nominal concentrations,

which is acceptable. Water quality parameters and the number of offspring in the controls were in accordance with the requirements of the OECD 211 guideline.

No sublethal effects were observed in neonates. The remaining biological results are summarised in the Table below. Treatment related statistically significant adverse effects were recorded on reproduction, as measured by the cumulative number of offspring per living female, at and above 12.8 mg a.s./L and on growth (body length) at and above 6.4 mg a.s./L, whereas body mass was statistically significantly reduced at the highest concentration of 25.6 mg a.s./L only. The NOEC is 3.2 mg a.s./L.

Table B.9.2.2.1-02 Summary of biological results of 21-day Daphnia reproduction test with BYI 02960 technical

nominal concentration (mg a.s./L)	parent mortality (%)	total offspring per living parent	parent age at first offspring emergence (d)	body length (mm)	body dry mass (mg)
Control	0	126.1	9.1	4.5	0.97
solvent control	0	126.2	9.4	4.6	0.98
0.80	0	132.8	9.0	4.5	0.97
1.6	0	122.3	9.2	4.4	0.89
3.2	0	121.2	9.4	4.4	0.83
6.4	0	107.7	9.3	4.3*	0.98
12.8	0	102.8*	9.6	4.2*	0.83
25.6	0	85.4*	9.6	3.9*	0.63*

* Statistically significant at 5% level

A study with the reference item potassium dichromate was performed about 4 months earlier and gave a 24-h EC50 of 1.0 mg/L, which is acceptable (OECD 202 (2004): 0.6-2.1 mg/L).

Conclusion

NOEC 3.2 mg a.s./L.

Guidelines and limitations

The study is acceptable.

STUDY IIA, 8.3.2.1/02

Characteristics

reference	:	Riebschläger T. (2012)	water solubility	:	No data
type of study	:	Chronic toxicity study	species	:	*Daphnia magna*
year of execution	:	2011	exposure duration	:	21 days
GLP statement	:	Yes	nominal concn.	:	3.5, 8.1, 18.7, 43.3 and 100 mg pure metabolite/L
guideline	:	EPA 72-4, OPPTS 850.1300, EEC C.20, OECD 211	dosing method	:	Semi-static; direct addition to test water
test substance	:	BYI 02960-succinamide; batch BCS-CR74729-01-01 (original batch number BCOO 6329-2-10)	acceptability	:	Acceptable
purity	:	97.8%	21-day NOEC	:	43.3 mg pure metabolite/L

Methods

A 21-day chronic reproductive toxicity test in *Daphnia magna* (<24 h old, 10 replicates with one daphnia each per concentration) was conducted according to OECD 211 under semi-static conditions (renewal every 2-3 days) with BYI 02960-succinamide (metabolite of BYI 02960) at nominal test concentrations of 3.5, 8.1, 18.7, 43.3 and 100 mg pure metabolite/L, with untreated control. The test solutions were prepared by direct addition of the test material to the test water. The concentrations of BYI 02960-succinamide were analytically confirmed by a sufficiently validated HPLC-UV method in samples of all fresh test solutions of day 0, 9 and 19 and in the corresponding aged solutions. Endpoints reported were parent mortality, total number of offspring per parent animal, parent age at first offspring emergence, neonates behaviour and length and dry weight of parent daphnia at the end of the test.

Results

The measured concentrations of BYI 02960-succinamide were 103-111% of nominal in fresh solutions and 104-109% in the corresponding aged solutions. Test endpoints were based on nominal concentrations, which is acceptable. Water quality parameters and the number of offspring in the controls were in accordance with the requirements of the OECD 211 guideline.

No sublethal effects were observed in neonates. The remaining biological results are summarised in the Table below. The only treatment related statistically significant adverse effect was an increase of parent age at first offspring emergence at the highest test concentration of 100 mg pure metabolite/L. Based on this finding, the NOEC is 43.3 mg pure metabolite/L.

Table B.9.2.2.1-02 Summary of biological results of 21-day Daphnia reproduction test with BYI 02960-succinamide

nominal concentration (mg pure metabolite/L)	parent mortality (%)	total offspring per living parent	parent age at first offspring emergence (d)	body length (mm)	body dry mass (mg)
control	0	104.9	9.0	4.3	0.97
3.5	0	101.9	9.1	4.2	0.86
8.1	0	92.7	9.2	4.2	0.90
18.7	0	90.0	9.0	4.2	0.91
43.3	0	98.5	9.0	4.3	0.83
100	0	90.6	9.7*	4.2	0.90

* Statistically significant at 5% level

The most recent study with the reference item potassium dichromate gave a 24-h EC50 of 0.87 mg/L, which is acceptable (OECD 202 (2004): 0.6-2.1 mg/L).

Conclusion

NOEC 43.3 mg pure metabolite/L.

Guidelines and limitations

The study is acceptable.

STUDY IIA, 8.3.2.2/01

Characteristics

reference	:	Bruns E. (2011g)	water solubility a.s.	:	3000-3200 mg/L at pH 4-9 & 20°C
type of study	:	Chronic toxicity study	species	:	*Chironomus riparius*
year of execution	:	2010	exposure duration	:	28 days
GLP statement	:	Yes	nominal concn.	:	1.25, 2.50, 5, 10, 20 and 40 µg a.s./L
guideline	:	OECD 219	dosing method	:	Static (spiked water)
test substance	:	BYI 02960; technical, batch 2009-000239	acceptability	:	Acceptable
a.s. content	:	96.2%	28-day NOEC	:	~~10~~ 6.81 µg a.s./L (based on mean measured concentrations).

Methods

The chronic toxicity of BYI 02960 technical to *Chironomus riparius* (2-3 days old, 1st instar larvae) was assessed in a 28-day water/sediment system under static conditions using the spiked water method. The test was performed in 0.6 L glass beakers, with four replicates for each test concentration and the solvent control. The beakers were filled with 140 g wet sediment (artificial OECD 219 soil: 75.8% quartz sand, 4% peat (measured organic carbon content 2.0%), 20% kaolinite clay, 0.2% calcium carbonate) and 380 mL (about 6 cm) of overlying water, 7 days prior to treatment. Twenty midge larvae were added to each beaker one day prior to treatment. The water was gently aerated during the test, except for 24 hours following addition of the midges. On day 0, the test water was spiked just below the surface with an aliquot of a stock solution of the test substance in DMF, to provide nominal test concentrations of 1.25, 2.50, 5, 10, 20 and 40 µg a.s./L, with solvent control (0.1 mL/L DMF). Additional replicates were set up for analytical measurements on overlying water and pore water on day 0 (1 hour after treatment), 7 and 28 after treatment. Water samples were analysed by HPLC-MS/MS using a sufficiently validated method. Behaviour, mortality and emergence of the midges were monitored until test end.

Results

The measured concentrations of BYI 02960 in the overlying water were in the range 85-110% (mean 99%), 37-83% (mean 60%) and 30-52% (mean 41%) of nominal, after 1 hour, 7 days and 28 days, respectively. The measured concentrations of BYI 02960 in the porewater were in the range 0.4-1.0% (mean 0.6%), 1.0-3.0% (mean 2.0%) and 1.4-2.9% (mean 2.2%) of nominal, after 1 hour, 7 days and 28 days, respectively. No measurements were done in the sediment. The test endpoints were based on nominal concentrations, which was considered acceptable by the RMS in the original DAR. However, during pesticide peer review meeting 124 in December 2014, it was agreed that the endpoints for chironomus should be expressed in terms of mean measured concentration in the water column when the analytical measurement concentrations are outside of the range 80-120% and when concentrations in the sediment are not available.

The biological test results are summarised in the Table below. Water quality parameters were in accordance with the OECD 219 guideline. All validity criteria of the OECD 219 guideline concerning

emergence in the control and physico-chemical conditions during the test were satisfied. No emergence occurred at 40 µg a.s./L (nom.) / 29.8 µg a.s./L (m.m.). Treatment related statistically significant adverse effects on emergence and development rate were recorded at 20 µg a.s./L (nom.) / 15.4 µg a.s./L (m.m.). The NOEC was 10 µg a.s./L (nom.) / 6.81 µg a.s./L (m.m.).

Table B.9.2.2.1-03 Summary of biological results of 28-day chronic toxicity test with BYI 02960 technical on *Chironomus riparius*

nominal conc. (µg a.s./L)	geom. mean measured conc. (µg a.s./L)	emergence rate (%)	development rate (1/d)
solvent control	solvent control	90.0	0.058
1.25	0.679	87.5	0.058
2.5	1.25	92.5	0.057
5	2.25	83.8	0.058
10	6.81	88.8	0.059
20	15.4	52.5*	0.050*
40	29.8	0	0

* Statistically significant at 5% level

Conclusion

NOEC ~~10~~ 6.81 µg a.s./L (based on mean measured concentrations).

Guidelines and limitations

The study was performed according to OECD 219 (2004) and is acceptable.

STUDY IIA, 8.3.2.2/02

Characteristics

reference	:	Bruns E. (2011h)	water solubility a.s.	:	No data
type of study	:	Chronic toxicity study	species	:	*Chironomus riparius*
year of execution	:	2011	exposure duration	:	28 days
GLP statement	:	Yes	nominal concn.	:	100 mg pure metabolite/L
guideline	:	OECD 219	dosing method	:	Static (spiked water)
test substance	:	sodium difluoroacetate; batch BCS-AB60841-01-01 (original batch number BCOO 6092-3-1)	acceptability	:	Acceptable
a.s. content	:	99.0%	28-day NOEC	:	100 mg pure metabolite/L

Methods

The chronic toxicity of sodium difluoroacetate (metabolite of BYI 02960 technical) to *Chironomus riparius* (2-3 days old, 1st instar larvae) was assessed in a 28-day water/sediment system under static conditions using the spiked water method. The test was performed in 0.6 L glass beakers, with six replicates for the test concentration and the control. The beakers were filled with 140 g wet sediment (artificial OECD 219 soil: 75.0% quartz sand, 4% peat (measured organic carbon content 2.1%), 20% kaolinite clay, 0.1% calcium carbonate) and 380 mL (about 6 cm) of overlying water, 7 days prior to treatment. Twenty midge larvae were added to each beaker one day prior to treatment. The water was gently aerated during the test, except for 24 hours following addition of the midges. On day 0, the test water was spiked just below the surface with an aliquot of a stock solution of the test substance in test

water, to provide the nominal test concentration of 100 mg pure metabolite/L, with untreated control. Additional replicates were set up for analytical measurements on overlying water and pore water on day 0 (1 hour after treatment), 7 and 28 after treatment. Water samples were analysed by HPLC-MS/MS using a sufficiently validated method. Behaviour, mortality and emergence of the midges were monitored until test end.

Results

The measured concentrations of sodium difluoroacetate in the overlying water were 105%, 95.1% and 98.3% of nominal, after 1 hour, 7 days and 28 days, respectively. The test endpoints were based on nominal concentrations, which is acceptable. The measured concentrations of sodium difluoroacetate in the porewater were 1.55%, 6.61% and 7.95% of nominal, after 1 hour, 7 days and 28 days, respectively.

The biological test results are summarised in the Table below. Water quality parameters were in accordance with the OECD 219 guideline. All validity criteria of the OECD 219 guideline concerning emergence in the control and physico-chemical conditions during the test were satisfied. No treatment related statistically significant adverse effects on emergence and development rate were recorded at 100 mg pure metabolite/L. The NOEC was 100 mg pure metabolite/L.

Table B.9.2.2.1-04 Summary of biological results of 28-day chronic toxicity test with sodium difluoroacetate on *Chironomus riparius*

nominal conc. (mg pure metabolite/L)	emergence rate (%)	development rate (1/d)
Control	95.8	0.062
100	90.0	0.063

Conclusion

NOEC 100 mg pure metabolite/L.

Guidelines and limitations

The study was performed according to OECD 219 (2004) and is acceptable.

STUDY IIA, 8.3.2.2/03

Characteristics

reference	:	Bruns E. (2011i)	water solubility a.s.	:	2 g/L (from other report)
type of study	:	Chronic toxicity study	species	:	*Chironomus riparius*
year of execution	:	2011	exposure duration	:	28 days
GLP statement	:	Yes	nominal concn.	:	100 mg pure metabolite/L
guideline	:	OECD 219	dosing method	:	Static (spiked water)
test substance	:	6-chloronicotinic acid (6-CNA = IC-0); batch AE F161089 00 1B99 0001	acceptability	:	Acceptable
a.s. content	:	98.8%	28-day NOEC	:	100 mg pure metabolite/L

Methods

The chronic toxicity of 6-chloronicotinic acid (6-CNA = IC-0, metabolite of BYI 02960) to *Chironomus riparius* (2-3 days old, 1st instar larvae) was assessed in a 28-day water/sediment system under static conditions using the spiked water method. The test was performed in 0.6 L glass beakers, with six replicates for the test concentration and the control. The beakers were filled with 140 g wet sediment (artificial OECD 219 soil: 75.0% quartz sand, 4% peat (measured organic carbon content 2.1%), 20% kaolinite clay, 1% calcium carbonate) and 380 mL (about 6 cm) of overlying water, 7 days prior to treatment. Twenty midge larvae were added to each beaker one day prior to treatment. The water was gently aerated during the test, except for 24 hours following addition of the midges. On day 0, the test water was spiked just below the surface with an aliquot of a stock solution of the test substance in test water, to provide the nominal test concentration of 100 mg pure metabolite/L, with untreated control. Additional replicates were set up for analytical measurements on overlying water and pore water on day 0 (1 hour after treatment), 7 and 28 after treatment. Water samples were analysed by HPLC-MS/MS using a sufficiently validated method. Behaviour, mortality and emergence of the midges were monitored until test end.

Results

The measured concentrations of 6-chloronicotinic acid in the overlying water were 102%, 89.6% and 90.6% of nominal, after 1 hour, 7 days and 28 days, respectively. The test endpoints were based on nominal concentrations, which is acceptable. The measured concentrations of 6-chloronicotinic acid in the porewater were in 1.29%, 6.66% and 7.29% of nominal, after 1 hour, 7 days and 28 days, respectively.

The biological test results are summarised in the Table below. Water quality parameters were in accordance with the OECD 219 guideline. All validity criteria of the OECD 219 guideline concerning emergence in the control and physico-chemical conditions during the test were satisfied. No treatment related statistically significant adverse effects on emergence and development rate were recorded at 100 mg pure metabolite/L. The NOEC was 100 mg pure metabolite/L.

Table B.9.2.2.1-05 Summary of biological results of 28-day chronic toxicity test with 6-chloronicotinic acid on *Chironomus riparius*

nominal conc. (mg pure metabolite/L)	emergence rate (%)	development rate (1/d)
Control	87.5	0.064
100	88.3	0.066

Conclusion

NOEC 100 mg pure metabolite/L.

Guidelines and limitations

The study was performed according to OECD 219 (2004) and is acceptable.

STUDY IIA, 8.11.1/04

Characteristics

reference	:	Claude M.B. *et al.* (2011)	water solubility	:	3000-3200 mg/L at pH 4-9 & 20°C
type of study	:	Life cycle toxicity study (marine organism)	species	:	Saltwater mysid (*Mysidopsis bahia*)
year of execution	:	2010-2011	exposure duration	:	28 days
GLP statement	:	Yes	nominal concn.	:	4.6, 8.0, 13.9, 24.2 and 42 µg a.s./L
guideline	:	OPPTS 850.1350	dosing method	:	Flow through; stock solutions in DMF
test substance	:	BYI 02960 technical, batch BYI 2009-02960-01-03	acceptability	:	Acceptable
purity	:	96.2%	NOEC	:	13.2 µg a.s./L

Methods

A 28-day life-cycle toxicity test on juveniles (<24 h old) of the saltwater mysid *Mysidopsis bahia* was conducted under flow-through conditions with BYI 02960 technical at test concentrations of 4.6, 8.0, 13.9, 24.2 and 42 µg a.s./L. The test included untreated and solvent controls (0.1 mL/L DMF). The test was initiated with <24-h old juveniles (4 replicate chambers with 15 neonate mysids each for each concentration and the controls). On day 14 of the test, after mysids attained sexual maturity, male and female adults were paired in each treatment and control group, with a maximum of five reproductive pairs per replicate. Reproduction of the paired mysids was monitored through termination on day 28. Observations for mortality and signs of toxicity were conducted daily throughout the test. At test termination, the total body lengths and dry weights of all surviving first-generation mysids were measured. The concentrations of BYI 02960 were analytically confirmed by a sufficiently validated HPLC/UV method in samples of all test solutions taken at the start of the test, weekly during the test and at test termination.

Results

The measured concentrations were in the range 76-103% of nominals. Test endpoints were based on mean measured concentrations (4.2, 7.8, 13.2, 23.6 and 40 µg a.s./L). Water quality parameters (pH, oxygen concentration, temperature and salinity) were in accordance with the OPPTS 850.1350 guideline (pH 7.9-8.1, dissolved oxygen ≥5.0 mg/L(≥68% of saturation), 24.5-26.4°C, salinity 19-21 $^{0}/_{00}$). No treatment related signs of intoxication were observed in any of the treatments. No treatment related mortality was observed in any treatment, and at test termination there were no biologically relevant and statistically significant differences in body length and dry weight between the treated groups and the controls. The results for reproduction are summarised in the Table below.

Table B.9.2.2.1-06 Reproductive results of 28-day life cycle toxicity test with BYI 02960 technical on *Mysidopsis bahia*

Treatment (µg a.s./L)	no. of young/reproductive day	
	mean	standard deviation
negative control	0.396	0.119
solvent control	0.450	0.167
pooled control	0.423	0.137
4.2	0.358	0.250

7.8	0.267	0.145
13.2	0.240	0.090
23.6	0.156*	0.157
40	0.173*	0.113

** Statistically significant difference from control at 5% uncertainty level.*

At 4.2, 7.8, 13.2, 23.6 and 40 µg a.s./L, respectively, reproduction was reduced by 15, 37, 43, 63 and 59%, relative to the pooled control. The difference from the pooled control was statistically significant at the two highest test concentrations. The high variability of reproduction (see standard deviations in Table above) is associated with the test design, where total reproduction per replicate of 5 pairs is determined. Based on statistical significance, the NOEC concluded by the author of the report was 13.2 µg a.s./L. However, biological relevance should also be taken into consideration. The replicate mean reproduction in the controls was in the range 0.261-0.661 young per reproductive day. At 4.2 µg a.s./L (mean reproduction reduced by 15%), replicate mean reproduction was in the range 0.102-0.600 young per reproductive day, and 2 of 4 replicate means were below the control range. At 7.8 µg a.s./L (mean reproduction reduced by 37%), replicate mean reproduction was in the range 0.107-0.415 young per reproductive day, and 2 of 4 replicate means were below the control range. At 13.2 µg a.s./L (mean reproduction reduced by 43%), replicate mean reproduction was in the range 0.169-0.369 young per reproductive day, and 3 of 4 replicate means were below the control range. Based on the fact that there is a concentration-related trend in effects on reproduction, that at 7.8 and 13.2 µg a.s./L reproduction was reduced by 37-43% relative to the pooled control, and that reproduction in 2-3 out of 4 replicates was below the control range, it was proposed by the RMS to set the NOEC at 4.2 µg a.s./L. In response to this proposal, the applicant submitted two statements (Springer T, 2013, BCS doc M-452382-01-1; Bruns *et al.*, 2013, BCS doc M-452374-01-1). These statements are summarised below.

Statement by Springer (2013)

Springer (2013) suggested that the decision to designate 4.2 µg a.i./L as the NOEC might be related to current discussions on using EC10 values as a surrogate for the NOEC. The mean reproduction rate in the pooled controls was 0.423 offspring/female/day, with 95% confidence limits of 0.128 to 0.627. Based on this confidence interval, the smallest change in reproduction that can dependably be shown to be a decrease relative to the pooled controls is approximately 30% (i.e. 100-100*0.128/0.423). When using the value of 0.128 offspring/female/day as the within-group standard deviation during power analysis (based on 4 replicates and a type I error rate of 0.05), the smallest effect size that could be detected with a power of 0.8 is approximately a 73 % difference between the control and treatment group. Springer (2013) stated that this power is far less than is desirable, and indicates that that small changes in mysid reproduction cannot be dependably assessed using the standard experimental design described in test guideline. Springer also noted that the pooled control mean was strongly influenced by a single replicate from the solvent control, where reproduction had an unusually high value of 0.66.

Statement by Bruns et al. (2013)

Bruns *et al.* (2013) stated that "*the mean value of the solvent control has been dramatically impacted by an extraordinary high reproduction of one single breeding pair. The number of 29 young per reproductive female observed for this single breeding pair in the solvent control appears to be inconsistently high considering that the next highest value is 16 young per breeding pair. The fact that this value was extraordinary was checked by BCS using the control data of the 10 last mysid studies available in the BCS electronically archiving system DART. In none of the evaluated studies a control breeding pair produced 29 young within 14 reproduction days. Hence it is reasonable to exclude this number from the evaluation.*" The results excluding this single breeding pair are summarised in the Table below.

Table B.9.2.2.1-07 Reproductive results of 28-day life cycle toxicity test with BYI 02960 technical on *Mysidopsis bahia* (excluding outlier in solvent control)

Mean Measured Concentration (µg a.i./L)	Mean Number of Young Produced Per Reproductive Day ± SD	Mean Percent of Surviving Females Producing Young[1]	Mean Number of Young Per Surviving Female ± SD[1]
Negative Control	0.396 ± 0.119	100	5.2 ± 1.54
Solvent Control[2]	0.352 ± 0.105	85.0	4.6 ± 1.36
Pooled Control[2]	0.374 ± 0.106	92.3	4.9 ± 1.38
4.2	0.358 ± 0.250	92.9	4.9 ± 3.76
7.8	0.267 ± 0.145	88.9	3.5 ± 1.89
13.2	0.240 ± 0.090	77.8	3.1 ± 1.16
23.6	0.156 ± 0.157*	47.1**	2.1 ± 2.02*
40	0.173 ± 0.113*	50.0**	2.3 ± 1.47*

* Statistically significant decrease in reproduction and mean number of young per surviving female in comparison to the pooled control using Dunnett's test ($p \leq 0.05$).
** Statistically significant decrease in percent of surviving females producing young in comparison to the pooled control using Fisher's Exact test ($p \leq 0.05$).
[1] Calculated based on the total number of surviving females present at test termination. Females that died prior to test termination and the young that they produced were excluded from the calculation of the mean percent of females producing young and the mean number of young per female.
[2] One female in compartment 3, replicate D of the Solvent Control group had an inconsistently high number of young (the female produced 29 mysids whereas the next closest amount produced from a female out of the entire test was 16 young). Therefore, the data from this female was excluded and the statistics were reanalyzed.

Bruns *et al.* (2013) further argued that the discriminatory power of the chronic mysid shrimp study especially for the endpoint 'reproduction' is hampered by the natural high biological variability of this endpoint. BCS performed additional statistics of the data set by evaluating the endpoint 'reproduction' on the basis of the single breeding pairs instead of using the mean values for the replicates. This approach is covering the entire biological variation as observed for the single breeding pairs. The data set was used to calculate EC30 values as well as the corresponding confidence limits where possible. The EC30 was used due to the known standard deviation of control data as well as taking the statement of Springer (2013) into consideration. The results are summarised in the Table below. Bruns *et al.* concluded that all calculated EC30 values are slightly above the originally reported NOEC of

13.2 µg/L, and that in all cases the NOEC of 13.2 µg/L was within the calculated confidence limits of the EC30 values.

Table B.9.2.2.1-08 EC_{30} calculations based on the 10 previous control data sets (excluding outlier in solvent control)

Data used for calculation	EC_{30}	R^2	P(F)	Lower 95% confidence limit	Upper 5% confidence limit
Historical control data of 10 previous studies - Probit	17.8 µg/L	0.74	0.061	-	-
Historical control data of 10 previous studies plus data minus concentration 40 µg/L - Probit	15.4 µg/L	0.96	0.019	10.6 µg/L	19.7 µg/L
Historical control data of 10 previous studies plus data minus concentration 40 µg/L - Logit	15.7 µg/L	0.97	0.018	10.9 µg/L	19.6 µg/L
Historical control data of 10 previous studies plus data minus concentration 40 µg/L - Weibull	16.1 µg/L	0.97	0.016	11.3 µg/L	19.5 µg/L

The coefficient of determination, r^2 ($0 <= r^2 <= 1$), gives the proportion of variance explained by the dose/response function.
if p(F) <= alpha, the selected significance level, (e.g., alpha = 0.05) the regression revealed significant results (= slope is significantly different from zero).

Bruns *et al.* presented historical control data from 10 chronic mysid studies available to the applicant (including those of the present study). Based on this control data, Bruns *et al.* concluded that 30% difference to the control mean value for the endpoint 'reproduction' is not a clear effect as the controls are varying by about ±30%. Bruns *et al.* stated that the most important points which were concluded on the basis of this data are:

- The number of young per female is extremely variable: 0 – 45 young per female within 12-17 reproductive days.
- Number of Young produced per female and reproduction day ranges from 0 to 2.65
- The system is resulting in LOEC's which are ranging between 33 to 93% inhibition compared to controls
- The NOECs are ranging between -19 to 43% difference compared to the controls
- The concentrations below and including the NOEC range between -38.7 up to 47.3% difference compared to the controls.

Bruns *et al.* (2013) stated that this evaluation underpins the observation that the endpoint 'reproduction' is highly variable.

Lastly, Bruns *et al.* (2013) gave the following additional arguments supporting their case:

- The chronic mysid shrimp study is a problematic test system. In frequent cases studies have to be performed several times before a valid test (according to guideline recommendations) is available.
- The observed NOEC, as presented in the original report, is fulfilling the validity criteria for controls according to OPPTS 850.1350. This gives further support to the reliability of the presented NOEC of 13.2 µg/L.

Evaluation of statements by RMS

It is agreed that the solvent control breeding pair with 29 young per reproductive female is an outlier and may be eliminated from the data (although there was one breeding pair in the historical control data that produced an even higher number of 45 young in 17 reproduction days). The RMS performed a Dixon's outlier test on this data of 29 young per reproductive female using the reported results for 20 breeding pairs in the solvent control, and this gave a Q-value of 0.552, which is higher than the statistical Q value of 0.401 for n=20. Hence this test confirmed that the data for this breeding pair are exceptionally high and may be removed prior to statistical analysis. The evaluation below is based on data excluding this outlier. When excluding this outlier, at 4.2, 7.8, 13.2, 23.6 and 40 µg a.s./L, reproduction (expressed as mean number of young produced per reproductive day) was reduced by -3%, 29%, 33%, 62% and 52% relative to the pooled control. Statistical significance (5% uncertainty level) was reached only at the two highest concentrations. Since the reductions at 7.8 and 13.2 µg a.s./L (29-33%) were approximately equal to the relative standard deviation in the control (30%) and the solvent control (30%), it is insufficiently certain that the reductions of 29-33% at 7.8 and 13.2 µg a.s./L are a treatment related effect. Hence the NOEC may be set at 13.2 µg a.s./L.

The submitted historical control data originated from 3 different laboratories, and only 4 studies (inlcuding the present study) were performed at Wildlife. The studies were conducted between 1996 and 2008, whereas the present study was performed in 2011. Historical control data should preferably be recent data from the same laboratory where the study is performed. Therefore the use of the combined historical control data from all ten studies in EC30 estimations is considered to be a questionable procedure. However, these control data confirm the high variability of the reproduction rate: the relative standard deviation of the reproduction rate in 14 controls (4 studies had controls and solvent controls) was in the range of 5-73%, with a mean of 25%.

Conclusion

NOEC 13.2 µg a.s./L.

Guidelines and limitations

The study is acceptable. Comments on the interpretation of the study results were incorporated in the above summary. The conclusion as stated above is acceptable.

STUDY IIIA, 10.2.6.2/01

Characteristics

Reference	:	Silke G. (2011)	species	:	*Chironomus riparius*
type of study	:	Chronic toxicity study	exposure duration	:	28 days
year of execution	:	2011	nominal concn.	:	1.50, 3.00, 6.00, 12.0, 24.0 and 48.0 µg a.s./L
GLP statement	:	Yes	dosing method	:	Static (spiked water)
Guideline	:	OECD 219	acceptability	:	Acceptable
test substance	:	BYI 02960 SL 200 G (200 g/L SL formulation), batch 2010-001067	28-day NOEC	:	~~12.0~~ 9.89 µg a.s./L (based on mean measured concentrations).
a.s. content	:	17.1 % w/w (201.0 g/L) BYI 02960			

Methods

The chronic toxicity of BYI 02960 SL 200 G (SL formulation, 17.1 % w/w (201.0 g/L) BYI 02960) to *Chironomus riparius* (2-3 days old, 1st instar larvae) was assessed in a 28-day water/sediment system under static conditions using the spiked water method. The test was performed in 0.6 L glass beakers, with four replicates for each test concentration and the untreated control. Seven days prior to treatment, the beakers were filled with 140 g wet sediment (artificial OECD 219 soil: 75.0% quartz sand, 4% peat (equivalent to 2.1% organic carbon), 20% kaolinite clay, 0.1% calcium carbonate) and 380 mL (about 6 cm) of overlying water. Twenty midge larvae were added to each beaker one day prior to treatment. The water was gently aerated during the test, except for 24 hours following addition of the midges. On day 0, the test water was spiked just below the surface with an aliquot of a stock solution of the test substance in test water, to provide nominal test concentrations of 1.50, 3.00, 6.00, 12.0, 24.0 and 48.0 µg a.s./L, with untreated control. Additional replicates were set up for analytical measurements on overlying water and pore water on day 0 (1 hour after treatment) and 7 (28 days after treatment analysis was performed on biological replicates). Water samples were analysed by HPLC-MS/MS using a sufficiently validated method. Behaviour, mortality and emergence of the midges were monitored until test end.

Results

The measured concentrations of BYI 02960 in the overlying water were in the range 101-112% (mean 109%), 78-84% (mean 81%) and 54-62% (mean 58%) of nominal, after 1 hour, 7 days and 28 days, respectively. The measured concentrations of BYI 02960 in the porewater were in the range 1.0-2.0% (mean 1.4%), 4.0-5.5% (mean 4.8%) and 3.5-4.7% (mean 4.1%) of nominal, after 1 hour, 7 days and 28 days, respectively. No measurements were done in the sediment. The test endpoints were based on nominal concentrations, which was considered acceptable by the RMS in the original DAR. However, during pesticide peer review meeting 124 in December 2014, it was agreed that the endpoints for chironomus should be expressed in terms of mean measured concentration in the water column when the analytical measurement concentrations are outside of the range 80-120% and when concentrations in the sediment are not available.

The biological test results are summarised in the Table below. Water quality parameters were in accordance with the OECD 219 guideline. All validity criteria of the OECD 219 guideline concerning emergence in the control and physico-chemical conditions during the test were satisfied. No emergence occurred at 48.0 µg a.s./L (nom.) / 38.0 µg a.s./L (m.m.). Treatment related statistically significant adverse effects on emergence and development rate were recorded at 24.0 µg a.s./L (nom.) / 20.1 µg a.s./L (m.m.). The NOEC was 12.0 µg a.s./L (nom.) / 9.89 µg a.s./L (m.m.).

Table B.9.2.2.3-01 Summary of biological results of 28-day chronic toxicity test with BYI 02960 SL 200 G on *Chironomus riparius*

Nominal conc. (µg a.s./L)	geom. mean measured conc. (µg a.s./L)	emergence rate (%)	development rate (1/d)
Control	solvent control	87.5	0.063
1.50	1.20	91.3	0.063

3.00	2.34	86.3	0.064
6.00	4.52	91.3	0.065
12.0	9.89	88.8	0.064
24.0	20.1	35.0*	0.051*
48.0	38.0	0	-

* Statistically significant at 5% level

Conclusion

~~12.0~~ 9.89 µg a.s./L (based on mean measured concentrations).

Guidelines and limitations

The study was performed according to OECD 219 (2004) and is acceptable.

B.9.2.3 Bioaccumulation (IIA 8.2.6.1)

MIII: BYI 02960 is a substance of low bioaccumulation potential due to the high water solubility (3 g/L) and low Log Pow (1.2). It can therefore be concluded that BYI 02960 is rapidly distributed in the water environment and the tendency for accumulation in biota will be low. Further testing of non-target species is therefore not justifiable.

B.9.2.4 Summary of aquatic toxicity data

Active substance and formulation

The active ingredient of BYI 02960 SL200, BYI 02960, has been tested on all laboratory indicator species for aquatic organisms representing all trophic levels of the aquatic environment. Acute data on fish toxicity are available for four different species, amongst them the sensitive cold water fish trout, and the salt water fish *Cyprinodon variegatus*. The results indicate that BYI 02960 is practically non-toxic to fish. As such, the LC50 values reported are greater than the highest concentration within the test. Using the formulated product BYI 02960 SL 200G the LC50 as related to the active substance was >100 mg a.i./L.

An early life-stage test for BYI 02960 confirms the low toxicity to fish also on chronic scale. Exposed continuously over the full developmental life-phase of fathead minnow a slightly reduced fry survival at the highest test concentration of 10 mg a.i./L was recorded. As compared to the untreated control survival was reduced by 7% resulting in an overall fry survival of 87.5% at a mean measured concentration of 8.4 mg a.i./L. Time to hatch and hatching success was not influenced, neither was any effect observed on growth as determined on length and weight.

A very selective non-target activity of BYI 02960 is further confirmed by low toxicity to representatives of three other taxonomic groups, namely amphibians, algae and aquatic plants. There were no adverse effects noted in the 48h acute frog test, and the influence of the active substance on *Pseudokirchneriella subcapitata* and *Lemna gibba* was also negligible. In a test with the formulation no effects on algal development were recorded at the highest test concentration used 250 mg

formulation/L corresponding to 42.5 mg a.i./L. For Lemna, an onset of effects was determined at a mean measured concentration of 67.7 mg a.i./L ; therefore aquatic plants are also considered to be insensitive to the active substance.
BYI 02960 is an insecticide against sucking insects. Hence, a side activity against aquatic invertebrates can be anticipated. Data were generated on all laboratory standard indicator species of aquatic arthropods: the cladoceran *Daphnia magna*, the midge *Chironomus riparius* and, the marine species *Americamysis bahia* and the mollusc *Crassostrea virginica*. The most sensitive organism for BYI 02960 was found to be *Chironomus riparius*. While acute toxicity to daphnids was negligible with a NOEC for the active substance at or greater than 77.6 mg a.i./L, the 50% effect concentration (EC50) to the midge was determined to be at a concentration of 0.062 mg a.i./L. For the saltwater species, the LC50 of the mysid shrimp was at 0.26 mg a.i./L and indicates as such a lower sensitivity than the insect. BYI 02960 was non-toxic to the marine oyster.
Chronic tests confirm the high toxicity of the active substance to *Chironomus riparius*. The lowest-observed-effect concentration (LOEC) impacting the emergence of the developed Chironomus larvae was at ~~0.02~~ 0.015 mg a.i./L, only a factor of ~~3~~ 4 below the acute EC50 values. The acute-to-chronic ratio for the most sensitive ecotoxicological endpoint, survival of Chironomus, for BYI 02960 is thus low. The immediate activity observed in the acute test appears to be reflected in the chronic test.
The 28 day chronic mysid shrimp test resulted in a LOEC of 0.0236 mg a.i./L based upon a statistically significant influence on reproductive output. The notifier considers that from the first-tier laboratory test species indicative for a potential effect of an insecticide, Chironomus is considered to be of higher relevance for the risk assessment than the marine shrimp as it reflects a real taxonomic group present in the freshwater biocoenosis. The influence of the formulation BYI 02960 SL 200 G was therefore examined on Chironomus as the most sensitive invertebrate. The lowest-observed-effect concentration was at ~~0.024~~ 0.020 mg a.i./L, the NOEC was established at ~~0.012~~ 0.00989 mg a.i./L. However, RMS does not accept to disregard the mysid shrimp data. Saltwater species are always included in the risk assessment unless a clear difference in sensitivity is observed between saltwater and freshwater species and it can be proven that exposure of saltwater environments is not relevant. In this case, the mysid shrimp NOEC is 0.0132 mg a.i./L and therefore close to the Chironomus endpoint. Hence, it is not considered acceptable to disregard the data available for the mysid shrimp. However, the Chironomus endpoint is driving the risk assessment since it is slightly lower.
In general, there was no relevant influence of the formulation on the toxicity in comparison to the toxicity with the active substance.

Accepted data on the acute toxicity of BYI 02960 (flupyradifurone) and BYI 02960 200 SL (Sivanto) are summarised in Table B.9.2.4-01. In the tables, it is indicated whether endpoints are based on nominal or actual concentrations. Data indicated in bold are the lowest effect concentrations for that species group.

Table B.9.2.4.01. The acute toxicity of BYI 02960 and BYI 02960 SL 200 to aquatic life. Bold data are the lowest effect concentrations for that specific group.

Group	Test substance	Time-scale (Test type)	End point	Toxicity[1] (mg/L)
Fish				
Rainbow trout (*Oncorhynchus mykiss)*	a.s.	96 hr (static)	Mortality, LC_{50}	>74.2 ($_{mm}$)
Fathead minnow (*Pimephales promelas*)	a.s.	96 hr (static)	Mortality, LC_{50}	**>70.5 ($_{mm}$)**
Common carp (*Cyprinus carpio)*	a.s.	96 hr (static)	Mortality, LC_{50}	>100 ($_{nom}$)
Sheepshead minnow (*Cyprinodon variegatus)* (saltwater species)	a.s.	96 hr (static)	Mortality, LC_{50}	>83.9 ($_{mm}$)
Rainbow trout (*Oncorhynchus mykiss)*	BYI 02960 SL 200[2]	96 hr (static)	Mortality, LC_{50}	>585 (form, $_{nom}$) >100 (a.s.,$_{nom}$)
Common carp (*Cyprinus carpio)*	BYI 02960 SL 200[2]	96 hr (static)	Mortality, LC_{50}	>585 (form, $_{nom}$) >100 (a.s.,$_{nom}$)
Amphibians				
Frog (*Xenopus laevis)*	a.s.	96 hr (static)	Mortality, LC_{50}	>80 ($_{nom}$)
Aquatic invertebrates				
Daphnia magna	a.s.	48 h (static)	Mortality, EC_{50}	>77.6 ($_{mm}$)
Chironomus riparius	a.s.	48 h (static)	Mortality, EC_{50}	**0.0617** ($_{nom}$)
Eastern oyster (*Crassostrea virginica)* (saltwater species)	a.s.	96 h (static)	Inhibition of shell growth, EC_{50}	>29 ($_{mm}$)
Mysid (*Americamysis bahia)* (saltwater species), juveniles*	a.s.	96 h (static)	Mortality, EC_{50}	0.26 ($_{mm}$)
Daphnia magna	BYI 02960 SL 200[2]	48 h (static)	Mortality, EC_{50}	684 (form, $_{nom}$) 117 (a.s., $_{nom}$)
Algae				
Pseudokirchneriella subcapitata	a.s.	72 h (static)	Biomass: E_bC_{50}, Growth rate: E_rC_{50} & Yield: E_yC_{50}	>80 ($_{nom}$)
Pseudokirchneriella subcapitata	BYI 02960 SL 200[2]	72 h (static)	Biomass: E_bC_{50} & Growth rate: E_rC_{50}	>250 (form, $_{nom}$) **>42.8** (a.s., $_{nom}$)

Group	Test substance	Time-scale (Test type)	End point	Toxicity[1] (mg/L)
Aquatic higher plants				
Lemna gibba	a.s.	7 d (static)	Biomass: E_bC_{50}, Growth rate: E_rC_{50} and Yield: E_yC_{50}	**>67.7** ($_{mm}$)

Metabolites

According to the LoEP from fate and behaviour, the environmental occurring metabolites requiring further assessment by other disciplines (toxicology and ecotoxicology) are:

Surface Water: 6-CNA, DFA, BYI 02960-succinamide, BYI 02960-azabicyclosuccinmaide

Sediment: 6-CNA, DFA

Accepted acute data on the acute toxicity of the relevant metabolites are summarized in Table B.9.2.4-02.

Table B.9.2.4.02 The acute toxicity of metabolites of BYI 02960 to aquatic life.

Group	Test substance	Time-scale (Test type)	End point	Toxicity[1] (mg/L)
Fish				
Rainbow trout (*Oncorhynchus mykiss)*	BYI 02960-succinamide	96 hr (static)	Mortality, EC_{50}	>100 ($_{nom}$)
Rainbow trout (*Oncorhynchus mykiss)*	DFA (BCS-AB60481 (tech.) = sodium difluoroacetate)	96 hr (static)	Mortality, EC_{50}	>10 ($_{nom}$)
Aquatic invertebrates				
Daphnia magna	DFA (BCS-AB60481 (tech.) = sodium difluoroacetate)	48 hr (static)	Mortality, EC_{50}	>10 ($_{nom}$)
Daphnia magna	6-CNA (IC-0)	48 h (static)	Mortality, EC_{50}	>95.1 ($_{mm}$)
Chironomus riparius	BYI 02960-succinamide	48 h (static)	Mortality, EC_{50}	>100 ($_{nom}$)
Chironomus riparius	BYI 02960-azabicyclosuccinamide, sodium salt	48 h (static)	Mortality, EC_{50}	>100 ($_{nom}$)
Chironomus tentans	6-CNA	96 h (static)	Mortality, EC_{50}	>1 ($_{nom}$)

Group	Test substance	Time-scale (Test type)	End point	Toxicity[1] (mg/L)
Algae				
Pseudokirchneriella subcapitata	DFA (BCS-AB60481 (tech.) = sodium difluoroacetate)	72 hr (static)	Growth rate: E_rC_{50} and Yield: E_yC_{50}	>10 ($_{nom}$)
Pseudokirchneriella subcapitata	BYI 02960-succinamide	72 hr (static)	Growth rate: E_rC_{50} and Yield: E_yC_{50}	>10 ($_{nom}$)
Pseudokirchneriella subcapitata	6-CNA (IC-0)	72 hr (static)	Growth rate: E_rC_{50} Yield: E_yC_{50}	>100 ($_{nom}$) 85 ($_{nom}$)

Chronic toxicity data

Active substance and formulation

Accepted data on the chronic toxicity of BYI 02960 (flupyradifurone) and BYI 02960 200 SL (Sivanto) are summarised in Table B.9.2.4-03. In the tables, it is indicated whether endpoints are based on nominal or actual concentrations. Data indicated in bold are the lowest effect concentrations for that species group.

Table B.9.2.4.03 The chronic toxicity of BYI 02960 and BYI 02960 SL 200 to aquatic life. Bold data are the lowest effect concentrations for that specific group.

Group	Test substance	Time-scale (Test type)	End point	Toxicity[1] (mg/L)
Laboratory tests ‡				
Fish				
Fathead minnow (*Pimephales promelas*)	a.s.	ELS, 35 d (flow-through)	Survival NOEC	**4.41** ($_{mm}$)
Aquatic invertebrate				
Daphnia magna	a.s.	21 d (semi-static)	Reproduction, NOEC	3.2 ($_{nom}$)
Mysid (*Americamysis bahia)* (saltwater species), juveniles	a.s.	28 d (semi-static)	life-cycle toxicity, NOEC	0.0132 ($_{mm}$)
Sediment dwelling organisms				
Chironomus riparius	a.s.	28 d (static; water-spiked)	NOEC	**~~0.010~~ 0.00681** (~~$_{nom}$~~ $_{mm}$)

Group	Test substance	Time-scale (Test type)	End point	Toxicity[1] (mg/L)
Chironomus riparius	BYI 02960 SL 200[2]	28 d (static; water-spiked)	NOEC	~~0.012~~ 0.00989 mg a.s./L (~~nom~~ mm)

Chronic toxicity data for the metabolites are summarised in Table B.9.2.4-03.

Table B.9.2.4.02 The chronic toxicity of metabolites of BYI 02960 to aquatic life.

Group	Test substance	Time-scale (Test type)	End point	Toxicity[1] (mg/L)
Aquatic invertebrates				
Daphnia magna	BYI 02960-succinamide	21 d (semi-static)	Reproduction, NOEC	43.3 ($_{nom}$)
Sediment dwelling organisms				
Chironomus riparius	DFA (BCS-AB60481 (tech.) = sodium difluoroacetate)	28 d (static; water-spiked)	NOEC	100 ($_{nom}$)
Chironomus riparius	6-CNA (IC-0)	28 d (static; water-spiked)	NOEC	100 ($_{nom}$)

B.9.2.5 Risk assessment

The risk assessment is based on Guidance Document on Aquatic Ecotoxicology, SANCO/3268/2001, rev 4 final, 17 October 2002. Toxicity exposure ratios (TER values) are calculated based on the most sensitive species and worst-case PEC_{SW} values.

The TER-values have been calculated based on the following equations:

TER_A = LC50 or EC50 / maximum PEC_{SW}

TER_{LT} = ErC50 / maximum PEC_{SW}

TER_{LT} = chronic NOEC /maximum PEC_{SW}

The risk is considered acceptable if the TER_A values are ≥ 100, and the TER_{LT} values ≥ 10.

Acute and chronic risk assessment was carried out in tier 1 using the initial PEC_{SW} calculated with FOCUS Surface Water, Step 2, and the lowest available toxicity endpoints for algae, *Daphnia* and other invertebrates, fish, amphibians, and *Lemna*. The resulting TER-values are presented for the different uses of BYI 02960 SL 200 for the a.s. and the metabolites. TERs that do not meet the relevant trigger are indicated in bold.

B.9.2.5.1 Tier 1 risk assessment for fish (and other aquatic vertebrates)

The acute risk assessment for the a.s. is based on the lowest LC50 for fish of >70.5 mg a.s./L; this also covers amphibians since the LC50 for *Xenopus laevis* is higher (>80 mg a.s./L).

Table B.9.2.5.1-01 TER_A calculations for fish based on FOCUS Step 2 for application in hops

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Crop: Hops					
BYI 02960	*P. promelas*	LC_{50} > 70 500	17.36	> 4 061	100
BYI 02960 – succinamide	*O. mykiss*	LC_{50} > 100 000	4.065	> 24 600	
DFA*	*O. mykiss*	LC_{50} > 10 000	1.268 (1.421*)	>7 886 (> 7 037*)	

*) In a worst-case approach, exposure concentrations for DFA are based upon groundwater calculations; however, dilution will occur during transport to surface waters.

Table B.9.2.5.1-02 TER_A calculations for fish based on FOCUS Step 2 for application in lettuce – biennial (covers annual use)

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Crop: Lettuce					
BYI 02960	*P. promelas*	LC_{50} > 70 500	11.88	> 5 934	100
BYI 02960 – succinamide	*O. mykiss*	LC_{50} > 100 000	0.484	> 206 612	
DFA*	*O. mykiss*	LC_{50} > 10 000	1.339 (5.599*)	> 7 468 (> 1 786*)	

*) In a worst-case approach, exposure concentrations for DFA are based upon groundwater calculations; however, dilution will occur during transport to surface waters.

The TERA values meet the required trigger for both uses, indicating an acceptable acute risk to fish for application of the product.

Table B.9.2.5.1-03 TER_{LT} calculations for fish based on FOCUS Step 2 for application in hops

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	TER_{LT}	Trigger
Crop: Hops					
BYI 02960	*P. promelas*	NOEC 4410	17.36	254	10

Table B.9.2.5.1-04 TER_{LT} calculations for fish based on FOCUS Step 2 for application in lettuce – biennial (covers annual use)

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_{LT}	Trigger
Crop: Lettuce						
BYI 02960	*P. promelas*	NOEC	4410	11.88	371	10

The TER_{LT} values meet the required trigger for both uses, indicating an acceptable long-term risk to fish for application of the product.

B.9.2.5.2 Tier 1 risk assessment for aquatic invertebrates (including sediment-dwelling organisms)

The acute risk assessment for the a.s. is based on the lowest LC50 for aquatic invertebrates of 0.0617 mg a.s./L, for *Chironomus*. Sediment-spiked tests with *Chironomus* are not available, therefore the risk assessment is performed with PECsw only (so PECsed is not used).

Table B.9.2.5.2-01 TER_A calculations for aquatic invertebrates based on FOCUS Step 2 for application in hops

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Crop: Hops						
BYI 02960	*C. riparius*	EC_{50}	61.7	17.36	**3.53**	100
DFA*	*D. magna*	EC_{50}	> 10 000	1.268 (1.421*)	> 7 886 (> 7 037*)	
6-CNA	*C. tentans*	EC_{50}	> 1 000	0.463	> 2 160	
BYI 02960-succinamide	*C. riparius*	EC_{50}	> 100 000	4.065	> 24 600	
BYI 02960-azabicyclosuccinamide, sodium salt	*C. riparius*	EC_{50}	> 100 000	2.499	>40 016	

*) In a worst-case approach, exposure concentrations for DFA are based upon groundwater calculations; however, dilution will occur during transport to surface waters.

Table B.9.2.5.2-02 TER_A calculations for aquatic invertebrates based on FOCUS Step 2 for application in lettuce – biennial (covers annual use)

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Crop: Lettuce						
BYI 02960	*C. riparius*	EC_{50}	61.7	11.88	**5.20**	100
DFA*	*D. magna*	EC_{50}	**>** 10 000	1.339 (5.599*)	> 7 468 (> 1 786*)	
6-CNA	*C. tentans*	EC_{50}	> 1 000	0.579	> 1 727	
BYI 02960-succinamide	*C. riparius*	EC_{50}	> 100 000	0.484	> 206 612	
BYI 02960-azabicyclosuccinamide, sodium salt	*C. riparius*	EC_{50}	> 100 000	0.297	> 336 700	

*) In a worst-case approach, exposure concentrations for DFA are based upon groundwater calculations; however, dilution will occur during transport to surface waters.

The TERA values meet the required trigger for both uses for the metabolites, indicating an acceptable acute risk to aquatic invertebrates. However, for the acute risk of the a.s., refinement is required.

The long-term risk assessment for the a.s. is based on the lowest NOEC for aquatic invertebrates of ~~0.0102~~ 0.00681 mg a.s./L, for *C.riparius*.

Table B.9.2.5.2-03 TER_{LT} calculations for aquatic invertebrates based on FOCUS Step 2 for application in hops

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_{LT}	Trigger
Crop: Hops						
BYI 02960	*C. riparius*	NOEC	~~10~~ 6.81	17.36	~~0.58~~ **0.39**	10
BYI 02960 – succinamide	*D. magna*	NOEC	43 300	4.065	10 652	
DFA*	*C. riparius*	NOEC	100 000	1.268 (1.421*)	78 864 (70 373*)	
6-CNA	*C. riparius*	NOEC	100 000	0.463	215 983	

*) In a worst-case approach, exposure concentrations for DFA are based upon groundwater calculations; however, dilution will occur during transport to surface waters.

Table B.9.2.5.2-04 TER_{LT} calculations for aquatic invertebrates based on FOCUS Step 2 for application in lettuce – biennial (covers annual use)

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_{LT}	Trigger
Crop: Lettuce						
BYI 02960	*C. riparius*	NOEC	~~10~~ 6.81	11.88	~~**0.84**~~ **0.57**	10
BYI 02960 – succinamide	*D. magna*	NOEC	43 300	0.484	89 463	
DFA*	*C. riparius*	NOEC	100 000	1.339 (5.599*)	74 683 (17 860*)	
6-CNA	*C. riparius*	NOEC	100 000	0.579	172 712	

*) In a worst-case approach, exposure concentrations for DFA are based upon groundwater calculations; however, dilution will occur during transport to surface waters.

The TERLT values meet the required trigger for both uses for the metabolites, indicating an acceptable long-term risk to aquatic invertebrates. However, for the long-term risk of the a.s., refinement is required.

B.9.2.5.3 Tier 1 risk assessment for algae

The risk assessment for the a.s. is based on the lowest EC50 for algae of >42.8 mg a.s./L, based on the study with the formulated product.

Table B.9.2.5.3-01 TER_A calculations for algae based on FOCUS Step 2 for application in hops

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Crop: Hops						
BYI 02960	*P. subcapitata*	EC50	>42 800	17.36	>2 465	10
DFA	*P. subcapitata*	EC50	>10 000	1.268	>7 886	10
BYI 02960 – succinamide	*P. subcapitata*	EC50	>10 000	4.065	>2 460	10
6-CNA	*P. subcapitata*	EC50	85 000	0.463	183 585	10

Table B.9.2.5.3-02 TER_A calculations for algae based on FOCUS Step 2 for application in lettuce – biennial (covers annual use)

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Crop: Lettuce						
BYI 02960	*P. subcapitata*	EC50	>42 800	11.88	>3 603	10
DFA	*P. subcapitata*	EC50	>10 000	1.339	>7 468	10
BYI 02960 – succinamide	*P. subcapitata*	EC50	>10 000	0.484	>20 661	10
6-CNA	*P. subcapitata*	EC50	85 000	0.579	146 805	10

The TERA values meet the required trigger for both uses for the a.s. and the metabolites, indicating an acceptable risk to algae.

B.9.2.5.4 Tier 1 risk assessment for aquatic macrophytes

Table B.9.2.5.4-01 TER_A calculations for lemna based on FOCUS Step 2 for application in hops

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Crop: Hops						
BYI 02960	*L. gibba*	EC50	>67 700	17.36	>3 890	10

Table B.9.2.5.4-02 TER_A calculations for lemna based on FOCUS Step 2 for application in lettuce – biennial (covers annual use)

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Crop: Lettuce						
BYI 02960	*L. gibba*	EC50	>67 700	11.88	>5 699	10

The TERA values meet the required trigger for both uses for the a.s., indicating an acceptable risk to algae.

B.9.2.5.5 Refined risk assessment for aquatic invertebrates

Based on the tier 1 risk assessments shown above, acceptable risk is found for the a.s. for fish and other aquatic vertebrates, algae and aquatic macrophytes.

For the relevant metabolites, sufficient toxicity data are available. Acceptable risk is found for all groups.

However, refinement is necessary for the a.s. for the acute and long-term risk to aquatic invertebrates.

The risk is refined on the exposure side, with Step 3 and Step 4 FOCUSsw calculations.

For lettuce, FOCUS Step 4 PECsw values are available both for the annual (once per 12 months) and the biennial (once per 24 months) use. The annual use is proposed at BBCH 41-49, the biennial at BBCH 12-49. Because a higher interception percentage can be taken into account at later crop stages, the PEC values from FOCUS Step 2 are higher for the biennial use. In Step 3 and 4, however, the PEC values are sometimes higher for the annual and sometimes for the biennial use, depending on the scenario. This was not presented clearly in the original DAR, but in this revised version TER values are presented both for the biennial and the annual use.

B.9.2.5.5.1 Refined acute risk assessment for aquatic invertebrates

Table B.9.2.5.5-01 TER_A calculations for *C. riparius* based on FOCUS Step 3 for application in hops

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Scenario	TER_A	Trigger
Crop: Hops						
BYI 02960	*C. riparius*	EC_{50} 61.7	0.394	R1, pond	157	100
			5.531	R1, stream	**11**	

Table B.9.2.5.5-02a TER_A calculations for *C. riparius* based on FOCUS Step 3 for application in lettuce – biennial use

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Scenario	TER_A	Trigger
Crop: Lettuce						
BYI 02960	*C. riparius*	EC_{50} 61.7	0.830	D3 (ditch, 1st)	**74.3**	100
			0.840	D3 (ditch, 2nd)	**73.5**	
			1.035	D4 (pond, 1st)	**59.6**	
			0.794	D4 (stream, 1st)	**77.7**	
			1.268	D6 (ditch, 1st)	**48.7**	
			0.060	R1 (pond, 1st)	1028	
			0.858	R1 (stream, 1st)	**71.9**	
			0.097	R1 (pond, 2nd)	636	
			1.186	R1 (stream, 2nd)	**52.0**	
			1.586	R2 (stream, 1st)	**38.9**	
			0.940	R2 (stream, 2nd)	**65.6**	
			2.226	R3 (stream, 1st)	**27.7**	
			3.570	R3 (stream, 2nd)	**17.3**	
			0.522	R4 (stream, 1st)	118.2	
			4.808	R4 (stream, 2nd)	**12.8**	

Table B.9.2.5.5-02b TER_A calculations for *C. riparius* based on FOCUS Step 3 for application in lettuce – annual use

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Scenario	TER_A	Trigger
Crop: Lettuce						
BYI 02960	*C. riparius*	EC_{50} 61.7	0.813	D3 (ditch, 1st)	**75.9**	100
			0.811	D3 (ditch, 2nd)	**76.1**	
			0.983	D4 (pond, 1st)	**62.8**	
			0.969	D4 (stream, 1st)	**63.7**	
			1.206	D6 (ditch, 1st)	**51.2**	
			0.337	R1 (pond, 1st)	183	
			2.827	R1 (stream, 1st)	**21.8**	
			0.139	R1 (pond, 2nd)	444	
			2.189	R1 (stream, 2nd)	**28.2**	
			1.215	R2 (stream, 1st)	**50.8**	
			1.044	R2 (stream, 2nd)	**59.1**	
			3.698	R3 (stream, 1st)	**16.7**	
			3.297	R3 (stream, 2nd)	**18.7**	
			0.83	R4 (stream, 1st)	**74.3**	
			2.954	R4 (stream, 2nd)	**20.9**	

For use in hops and both uses in lettuce a safe use is shown at Step 3 for at least one subscenario (TER > 100). Potential mitigation measures are investigated considering Step 4 calculations.

Table B.9.2.5.5-03 Refined TER_A calculations for *C. riparius* based on FOCUS Step 4 for application in hops (90 % drift reduction)

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Scenario	TER_A	Trigger
Crop: Hops; 90 % drift reduction (no buffer zone)						
BYI 02960	*C. riparius*	EC_{50} 61.7	0.553	R1, stream	112	100
Crop: Hops; 75 % drift reduction and 10 m buffer zone						
BYI 02960	*C. riparius*	EC_{50} 61.7	0.589	R1, stream	105	100

The above table shows that for hops, acceptable risk can be reached with different combinations of buffer zones and spray drift reduction.

Table B.9.2.5.5-04 Refined TER_A calculations for *C. riparius* based on FOCUS Step 4 for application in lettuce (drift reducing nozzles) – biennial use

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Drift reduction [%]	Scenario	TER_A	Trigger
Crop: Lettuce; 50% drift reduction (No buffer)							
BYI 02960	*C. riparius*	EC_{50} 61.7	**0.434**	50	D3 (ditch, 1st)	143	100
			0.446	50	D3 (ditch, 2nd)	139	
			1.033	90	D4 (pond, 1st)	**59.7**	
			0.721	90	D4 (stream, 1st)	**85.6**	
			1.268	90	D6 (ditch, 1st)	**48.7**	
			0.858	90	R1 (stream, 1st)	**71.9**	
			1.186	90	R1 (stream, 2nd)	**52.0**	
			1.586	90	R2 (stream, 1st)	**38.9**	
			0.940	90	R2 (stream, 2nd)	**65.6**	
			2.226	90	R3 (stream, 1st)	**27.7**	
			3.570	90	R3 (stream, 2nd)	**17.3**	
			4.808	90	R4 (stream, 2nd)	**12.8**	

Table B.9.2.5.5-11 shows that for the biennial use in lettuce, most scenario's do not meet the trigger considering drift reducton only. For the annual use in lettuce, PECsw values are not available considering drift reduction only. Therefore, further refinement is necessary for both lettuce uses. FOCUS Step 4 PECsw values are available based on buffer zones (with drift and/or run-off reduction.

Table B.9.2.5.5-05a Refined TER_A calculations for *C. riparius* based on FOCUS Step 4 for application in lettuce (5 m distance) – biennial use

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Scenario	TER_A	Trigger
Crop: Lettuce; 5 m drift buffer (no drift reduction)						
BYI 02960	*C. riparius*	EC_{50} 61.7	1.035	D4 (pond, 1st)	**59.7**	100
			0.721	D4 (stream, 1st)	**85.6**	
			1.268	D6 (ditch, 1st)	**48.7**	
			0.858	R1 (stream, 1st)	**71.9**	
			1.186	R1 (stream, 2nd)	**52.0**	
			1.586	R2 (stream, 1st)	**38.9**	
			0.940	R2 (stream, 2nd)	**65.6**	
			2.226	R3 (stream, 1st)	**27.7**	
			3.570	R3 (stream, 2nd)	**17.3**	
			4.808	R4 (stream, 2nd)	**12.8**	

Table B.9.2.5.5-05b Refined TER_A calculations for *C. riparius* based on FOCUS Step 4 for application in lettuce (5 m distance) – annual use

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Scenario	TER_A	Trigger
Crop: Lettuce; 5 m drift buffer (no drift reduction)						
BYI 02960	*C. riparius*	EC_{50} 61.7	0.234	D4 (pond, 1st)	264	100
			0.234	D4 (stream, 1st)	264	
			0.982	D4 (pond, 1st)	**59.7**	
			0.969	D4 (stream, 1st)	**85.6**	
			1.206	D6 (ditch, 1st)	**48.7**	
			2.827	R1 (stream, 1st)	**71.9**	
			2.189	R1 (stream, 2nd)	**52.0**	
			1.215	R2 (stream, 1st)	**38.9**	
			1.044	R2 (stream, 2nd)	**65.6**	
			3.698	R3 (stream, 1st)	**27.7**	
			3.297	R3 (stream, 2nd)	**17.3**	
			0.830	R4 (stream, 1st)	**74.34**	
			2.954	R4 (stream, 2nd)	**12.8**	

Table B.9.2.5.5-06a Refined TER_A calculations for *C. riparius* based on FOCUS Step 4 for application in lettuce (10 m distance) – biennial use

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Buffer type	Scenario	TER_A	Trigger
Crop: Lettuce; 10 m distance (spray drift mitigation only (S) or spray drift mitigation + VFS* (R))							
BYI 02960	*C. riparius*	EC_{50} 61.7	1.034	S	D4 (pond, 1st)	**59.7**	100
			0.721	S	D4 (stream, 1st)	**85.6**	
			1.268	S	D6 (ditch, 1st)	**48.7**	
			0.389	R	R1 (stream, 1st)	159	
			0.540	R	R1 (stream, 2nd)	114	
			0.716	R	R2 (stream, 1st)	**86.2**	
			0.422	R	R2 (stream, 2nd)	146	
			1.009	R	R3 (stream, 1st)	**61.2**	
			1.630	R	R3 (stream, 2nd)	**37.9**	
			2.184	R	R4 (stream, 2nd)	**28.3**	

*VFS: vegetated filter strip

Table B.9.2.5.5-06b Refined TER_A calculations for *C. riparius* based on FOCUS Step 4 for application in lettuce (10 m distance) – annual use

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Buffer type	Scenario	TER_A	Trigger
Crop: Lettuce; 10 m distance (spray drift mitigation only (S) or spray drift mitigation + VFS* (R))							
BYI 02960	*C. riparius*	EC_{50} 61.7	0.975	S	D4 (pond, 1st)	**63.3**	100
			0.969	S	D4 (stream, 1st)	**63.7**	
			1.206	S	D6 (ditch, 1st)	**51.2**	
			1.270	R	R1 (stream, 1st)	**48.6**	
			0.954	R	R1 (stream, 2nd)	**64.7**	
			0.553	R	R2 (stream, 1st)	112	
			0.469	R	R2 (stream, 2nd)	132	
			1.683	R	R3 (stream, 1st)	**36.7**	
			1.506	R	R3 (stream, 2nd)	**41.0**	
			1.345	R	R4 (stream, 1st)	**45.9**	
			0.705	R	R4 (stream, 2nd)	**87.5**	

*VFS: vegetated filter strip

Table B.9.2.5.5-06c Refined TER_A calculations for *C. riparius* based on FOCUS Step 4 for application in lettuce (15 m distance) – biennial use

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Buffer type	Scenario	TER_A	Trigger
Crop: Lettuce; 15 m distance (spray drift mitigation only (S) or spray drift mitigation + VFS* (R))							
BYI 02960	*C. riparius*	EC_{50} 61.7	1.034	S	D4 (pond, 1st)	**59.7**	100
			0.721	S	D4 (stream, 1st)	**85.6**	
			1.268	S	D6 (ditch, 1st)	**48.7**	
			0.375	R	R2 (stream, 1st)	165	
			0.528	R	R3 (stream, 1st)	117	
			0.856	R	R3 (stream, 2nd)	**72.1**	
			1.144	R	R4 (stream, 2nd)	**53.9**	

*VFS: vegetated filter strip

Table B.9.2.5.5-06d Refined TER_A calculations for *C. riparius* based on FOCUS Step 4 for

application in lettuce (15 m distance) – annual use

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Buffer type	Scenario	TER_A	Trigger
Crop: Lettuce; 15 m distance (spray drift mitigation only (S) or spray drift mitigation + VFS* (R))							
BYI 02960	*C. riparius*	EC_{50} 61.7	0.975	S	D4 (pond, 1st)	**63.3**	100
			0.969	S	D4 (stream, 1st)	**63.7**	
			1.206	S	D6 (ditch, 1st)	**51.2**	
			1.270	R	R1 (stream, 1st)	**48.6**	
			0.954	R	R1 (stream, 2nd)	**64.7**	
			1.683	R	R3 (stream, 1st)	**36.7**	
			1.506	R	R3 (stream, 2nd)	**41.0**	
			1.345	R	R4 (stream, 1st)	163	
			0.705	R	R4 (stream, 2nd)	**45.9**	

*VFS: vegetated filter strip

Table B.9.2.5.5-07a Refined TER_A calculations for *C. riparius* based on FOCUS Step 4 for application in lettuce (20 m distance) – biennial use

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Buffer type	Scenario	TER_A	Trigger
Crop: Lettuce; 20 m distance (spray drift mitigation only (S) or spray drift mitigation + VFS* (R))							
BYI 02960	*C. riparius*	EC_{50} 62	1.033	S	D4 (pond, 1st)	**59.7**	100
			0.721	S	D4 (stream, 1st)	**85.6**	
			1.268	S	D6 (ditch, 1st)	**48.7**	
			0.856	R	R3 (stream, 2nd)	**72.1**	
			1.144	R	R4 (stream, 2nd)	**53.9**	

*VFS: vegetated filter strip

Table B.9.2.5.5-07a Refined TER_A calculations for *C. riparius* based on FOCUS Step 4 for application in lettuce (20 m distance) – annual use

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	Buffer type	Scenario	TER_A	Trigger
Crop: Lettuce; 20 m distance (spray drift mitigation only (S) or spray drift mitigation + VFS* (R))							
BYI 02960	*C. riparius*	EC_{50} 61.7	0.975	S	D4 (pond, 1st)	**63.3**	100
			0.969	S	D4 (stream, 1st)	**63.7**	
			1.206	S	D6 (ditch, 1st)	**51.2**	
			0.662	R	R1 (stream, 1st)	**93.2**	
			0.500	R	R1 (stream, 2nd)	123	
			0.882	R	R3 (stream, 1st)	**70.0**	
			0.790	R	R3 (stream, 2nd)	**78.1**	
			0.705	R	R4 (stream, 2nd)	**87.5**	

*VFS: vegetated filter strip

Conclusion acute risk aquatic invertebrates:

A safe use has been shown for the subscenario R1 (pond) in both hops and lettuce with no risk mitigation.

For hops, acceptable risk can be reached in the one relevant complete scenario with different combinations of buffer zones and spray drift reduction.

For the biennial use in lettuce, by applying mitigation measures an acceptable use can be shown for for ten of the fifteen FOCUS subscenarios considering different mitigation options (up to 20m VFS). ~~scenario D3 with no buffer zone and drift reducing nozzles, and for some of the subscenarios R1 stream, R2 stream and R3 stream considering different buffer zones (vegetated filter strips) and drift reducing nozzles~~. Of the seven complete scenario's, three meet the trigger.

For the annual use in lettuce, by applying mitigation measures an acceptable use can be shown for for eight of the fifteen FOCUS subscenarios considering different mitigation options (up to 20m VFS). Of the seven complete scenario's, two meet the trigger.

B.9.2.5.5.2 Refined long-term risk assessment for aquatic invertebrates

Table B.9.2.5.5-08 TER_{LT} calculations for *C. riparius* based on FOCUS Step 3 for application in hops

Compound	Species	Endpoint [µg a.i./L]	$PEC_{sw,max}$ [µg/L]	Scenario	TER_{LT}	Trigger
Crop: Hops						
BYI 02960	*C. riparius*	NOEC ~~10~~ 6.81	0.394	R1, pond	~~25~~ 17.3	10
			5.531	R1, stream	~~1.81~~ **1.23**	

Table B.9.2.5.5-09a TER_{LT} calculations for *C.riparius* based on FOCUS Step 3 for application in lettuce – biennial use

Compound	Species	Endpoint [µg a.i./L]	$PEC_{sw,max}$ [µg/L]	Scenario	TER_{LT}	Trigger
Crop: Lettuce						
BYI 02960	*C. riparius*	NOEC ~~10~~ 6.81	0.830	D3 (ditch, 1st)	~~12.0~~ **8.20**	10
			0.840	D3 (ditch, 2nd)	~~11.9~~ **8.11**	
			1.035	D4 (pond, 1st)	~~9.7~~ **6.58**	
			0.794	D4 (stream, 1st)	~~12.6~~ **8.58**	
			1.268	D6 (ditch, 1st)	~~7.9~~ **5.37**	
			0.060	R1 (pond, 1st)	~~167~~ 114	
			0.858	R1 (stream, 1st)	~~11.7~~ **7.94**	
			0.097	R1 (pond, 2nd)	~~103~~ 70.2	
			1.186	R1 (stream, 2nd)	~~8.4~~ **5.74**	
			1.586	R2 (stream, 1st)	~~6.3~~ **4.29**	
			0.940	R2 (stream, 2nd)	~~10.6~~ **7.24**	
			2.226	R3 (stream, 1st)	~~4.5~~ **3.06**	
			3.570	R3 (stream, 2nd)	~~2.8~~ **1.91**	
			0.522	R4 (stream, 1st)	~~19.2~~ 13.1	
			4.808	R4 (stream, 2nd)	~~2.1~~ **1.42**	

Table B.9.2.5.5-09b TER_{LT} calculations for *C.riparius* based on FOCUS Step 3 for application in lettuce – annual use

Compound	Species	Endpoint [µg a.i./L]	$PEC_{sw,max}$ [µg/L]	Scenario	TER_{LT}	Trigger
Crop: Lettuce						
BYI 02960	*C. riparius*	NOEC ~~10~~ 6.81	0.830	D3 (ditch, 1st)	**8.38**	10
			0.811	D3 (ditch, 2nd)	**8.40**	
			0.983	D4 (pond, 1st)	**6.93**	
			0.969	D4 (stream, 1st)	**7.03**	
			1.206	D6 (ditch, 1st)	**5.65**	
			0.337	R1 (pond, 1st)	20.2	
			2.827	R1 (stream, 1st)	**2.41**	
			0.139	R1 (pond, 2nd)	49.0	
			2.189	R1 (stream, 2nd)	**3.11**	
			1.215	R2 (stream, 1st)	**5.60**	
			1.044	R2 (stream, 2nd)	**6.52**	
			3.698	R3 (stream, 1st)	**1.84**	
			3.297	R3 (stream, 2nd)	**2.07**	
			0.830	R4 (stream, 1st)	**8.20**	
			2.954	R4 (stream, 2nd)	**2.31**	

The TER_{LT} values for aquatic invertebrates do not meet the required trigger based upon Step 3 PECsw values in one of the two subscenario's in hops, in twelve of the fifteen subscenarios in the biennial use in lettuce and in thirteen of the fifteen subscenarios (all of the seven complete scenarios) in the annual use in lettuce. A refined risk assessment with FOCUS Step 4 values, considering mitigation options is required.

Table B.9.2.5.5-10 TER_{LT} calculations for *C. riparius* based on FOCUS Step 4 for application in hops with exposure mitigation measures

Compound	Species	Endpoint [µg a.i./L]	$PEC_{sw,max}$ [µg/L]	Scenario	TER_{LT}	Trigger
Crop: Hops; no buffer zone 90% drift reduction						
BYI 02960	*C.riparius*	NOEC ~~10~~ 6.81	0.553	R1, stream	~~18.1~~ 12.3	10
5 m distance (spray drift buffer) and 90 % drift reduction						
BYI 02960	*C.riparius*	NOEC ~~10~~ 6.81	0.452	R1, stream	~~22.1~~ 15.1	10
10 m distance (spray drift buffer & VFS*) and 90 % drift reduction						
BYI 02960	*C.riparius*	NOEC ~~10~~ 6.81	0.235	R1, stream	~~42.6~~ 29.0	10
15 m distance (spray drift buffer & VFS*) and 75 % drift reduction						
BYI 02960	*C.riparius*	NOEC ~~10~~ 6.81	0.388	R1, stream	~~25.8~~ 17.6	10

*VFS: vegetated filter strip

The above table shows that for hops, acceptable risk can be reached with different combinations of buffer zones and spray drift reduction.

Table B.9.2.5.5-11 TER_{LT} calculations for *C. riparius* based on FOCUS Step 4 for biennial application in lettuce (drift reducing nozzles)

Compound	Species	Endpoint [µg a.i./L]	$PEC_{sw,max}$ [µg/L]	Drift reduction [%]	Scenario	TER_{LT}	Trigger
Crop: Lettuce; drift reduction (no buffer)							
BYI 02960	*C.riparius*	NOEC ~~10~~ 6.81	0.434	50%	D3 (ditch, 1st)	15.7	10
			0.446	50%	D3 (ditch, 2nd)	15.3	
			1.033	90%	D4 (pond, 1st)	~~9.68~~ **6.59**	
			0.721	90%	D4 (stream, 1st)	~~13.9~~ **9.45**	
			1.268	90%	D6 (ditch, 1st)	~~7.89~~ **5.37**	
			0.858	90%	R1 (stream, 1st)	~~11.7~~ **7.94**	
			1.186	90%	R1 (stream, 2nd)	~~8.43~~ **5.74**	
			1.586	90%	R2 (stream, 1st)	~~6.31~~ **4.29**	
			0.940	90%	R2 (stream, 2nd)	~~10.6~~ **7.24**	
			2.226	90%	R3 (stream, 1st)	~~4.49~~ **3.06**	
			3.570	90%	R3 (stream, 2nd)	~~2.80~~ **1.91**	
			4.808	90%	R4 (stream, 2nd)	~~2.08~~ **1.42**	

Table B.9.2.5.5-11 shows that for the biennial use in lettuce, most scenario's do not meet the trigger considering drift reducton only. For the annual use in lettuce, PECsw values are not available considering drift reduction only. Therefore, further refinement is necessary for both lettuce uses. FOCUS Step 4 PECsw values are available based on buffer zones, with drift and/or run-off reduction). Since the acute endpoint for C.chironomus is driving the risk assessment, TERLT values are not presented for all possible mitigation measures, but only for the minimum required mitigation that meets the trigger, ór for the strictest mitigation measure in case this does not meet the trigger yet.

Table B.9.2.5.5-12 TER_{LT} calculations for *C.riparius* based on FOCUS Step 4 for biennial application in lettuce (per subscenario, the minimum required mitigation measure is shown to reach a TER < trigger. If this cannot be reached, the TER is shown at the strictest mitigation measure)

Compound	**Species**	**Endpoint [µg a.i./L]**	**$PEC_{sw,max}$ [µg/L]**	**% drift reduction**	**buffer zone**	**Scenario**	**TER_{LT}**	**Trigger**
Crop: Lettuce; D scenarios: buffer (spray drift only) + drift reduction (nozzles); R scenarios: buffer (spray drift + VFS*) + drift reduction)								
BYI 02960	*C.riparius*	NOEC ~~10~~ 6.81	1.033	90%	20	D4 (pond, 1st)	**6.59**	10
			0.721	90%	20	D4 (stream, 1st)	**9.45**	
			1.268	90%	20	D6 (ditch, 1st)	**5.37**	
			0.389	0%	20	R1 (stream, 1st)	17.5	
			0.540	0%	10	R1 (stream, 2nd)	12.6	
			0.375	0%	10	R2 (stream, 1st)	18.2	
			0.422	0%	10	R2 (stream, 2nd)	16.1	
			0.528	90%	15	R3 (stream, 1st)	12.9	
			0.856	90%	20	R3 (stream, 2nd)	**7.96**	
			1.144	90%	20	R4 (stream, 2nd)	**5.95**	

*VFS: vegetated filter strip

Table B.9.2.5.5-12 TER_{LT} calculations for *C.riparius* based on FOCUS Step 4 for annual application in lettuce (per subscenario, the minimum required mitigation measure is shown to reach a TER < trigger. If this cannot be reached, the TER is shown at the strictest mitigation measure)

Compound	Species	Endpoint [µg a.i./L]	$PEC_{sw,max}$ [µg/L]	% drift reduction	buffer zone	Scenario	TER_{LT}	Trigger
Crop: Lettuce; D scenarios: buffer (spray drift only) + drift reduction (nozzles); R scenarios: buffer (spray drift + VFS*) + drift reduction)								
BYI 02960	*C.riparius*	NOEC ~~10~~ 6.81	0.234	0%	5	D3 (ditch, 1st)	29.1	10
			0.234	0%	5	D3 (ditch, 2nd)	29.1	
			0.975	90%	20	D4 (pond, 1st)	**6.98**	
			0.969	90%	20	D4 (stream, 1st)	**7.03**	
			1.206	90%	20	D6 (ditch, 1st)	**5.65**	
			0.662	0%	20	R1 (stream, 1st)	10.29	
			0.500	0%	20	R1 (stream, 2nd)	13.62	
			0.553	0%	10	R2 (stream, 1st)	12.31	
			0.469	0%	10	R2 (stream, 2nd)	14.52	
			0.882	90%	20	R3 (stream, 1st)	7.72	
			0.790	90%	20	R3 (stream, 2nd)	**8.62**	
			0.379	0%	10	R4 (stream, 1st)	17.97	
			0.705	90%	20	R4 (stream, 2nd)	**9.66**	

*VFS: vegetated filter strip

Conclusion long-term risk aquatic invertebrates:

Based on the calculations presented above a safe use of the formulation in hops can be shown for all

FOCUS scenarios considering different mitigation options (up to 90% drift reducing nozzles).

Based on the calculations presented above a safe biennial use of the formulation in lettuce can be shown for ten of the ~~thir~~fifteen FOCUS subscenarios considering different mitigation options (up to 20m VFS). Of the seven complete scenario's, three meet the trigger.

A safe annual use of the formulation in lettuce can be shown for nine of the fifteen FOCUS subscenarios considering different mitigation options (up to 20m VFS). Of the seven complete scenario's, three meet the trigger.

Overall conclusion risk aquatic invertebrates:

For hops, acceptable risk can be reached in the one relevant complete scenario with different combinations of buffer zones and spray drift reduction.

For the biennial use in lettuce, an acceptable use can be shown for three of the seven complete FOCUS scenarios considering different mitigation options (up to 20m VFS).

For the annual use in lettuce, an acceptable use can be shown for two of the seven complete FOCUS scenarios considering different mitigation options (up to 20m VFS).

Expert consultation on the risk assessment for aquatic invertebrates (especially insects)

Experts to discuss if a test on mayflies should be considered needed in the aquatic risk assessment. The applicant provided a position paper with input for this discussion. The conclusion of this position paper is included below.

STUDY Q 14 /01 (relevant for KIIA 8.3.2.2

reference	:	Hager, J. (2014)
type of study	:	Position paper on the need for additional data on mayflies (Ephemeroptera)
year of execution	:	not relevant
GLP statement	:	not relevant
guideline	:	not relevant
test substance	:	not relevant

Conclusion of the position paper of the notifier:

"The risk assessment for flupyradifurone is based on tier 1 data including a high margin of safety which covers species potentially more sensitive than standard laboratory species.

There is no well-founded reason for questioning the protectiveness of the tier 1 risk assessment of flupyradifurone:

- The publication by Roessink et al. is an indication that Ephemeroptera are sensitive to imidacloprid. The paper is however **not suitable to question current data requirements and current state of the art of pesticide risk assessment**.
- The publication was presenting results for imidacloprid and not for flupyradifurone
- For the tier 1 risk assessment high assessment factors are given by the current aquatic guidance documents. These assessment factors are chosen in order to provide a margin of safety large enough to cover species that might be more sensitive than the standard laboratory species strictly defined by current regulation. The current risk assessment for flupyradifurone is driven by the acute endpoint for chironomus. Thus the corresponding assessment factor is 100, **including a sufficiently large margin of safety**.
- It is unlikely that Ephemeroptera would be more than 100 times more sensitive to flupyradifurone than chironomids.
- The acute data of the publication by Roessink et al. fit well into the picture of all acute aquatic invertebrate data available for imidacloprid. As the aquatic RAC of flupyradifurone is defined by acute data there is no reason to question its protectiveness based on the publication by Roessink et al.
- The data published by Roessink et al are derived with a compound that is **structurally different from flupyradifurone**.

Hence BCS is of the opinion that there is no concern regarding the current risk assessment for flupyradifurone."

Expert discussion in Pesticide Peer Review meeting 124 of December 2014:

Aquatic invertebrates drive the risk assessment for aquatic organisms. The lowest endpoint that was considered in the higher tier RA was from an acute water-spiked *C. riparius* study that resulted in an EC50 of 61.7 ug/L (the lowest chronic NOEC for *C. riparius* was 10 ug/L).

A comment from the commenting phase highlighted that the concerns about possible effects on mayflies (Ephemeroptera) should be investigated and discussed further because mayflies show higher sensitivity to neonicotinoids compared to Chironomides. It was pointed out that formally all data requirements are fulfilled and no additional data would be needed. Generally, the safety factor should cover such issues (sensitivity between species). On the other hand, recent concerns of imidacloprid for aquatic organisms raised in the open literature should not be omitted as it may underpin some concerns for insecticides.

The applicant provided a position paper for this issue highlighting the official data requirement and standard RA scheme that includes a safety factor, arguing on the difference of the molecule structure of flupyradifurone and imidacloprid and on the uncertainties of testing mayfly.

Discussion:

EFSA clarified that for imidacloprid the data set available at the time of the first peer review already indicated some concerns as regards Ephemeroptera. The endpoint agreed for risk assessment was based on chironomus from a mesocosm study where Ephemeroptera were not enough abundant.

The experts questioned whether potentially more sensitive insects should always be considered for insecticides. Some experts considered that the available data were not sufficient and it is needed to ask for more data because a higher sensitivity for ephemeroptera is expected for this substance. However, not enough data were available to support such a requirement.

The experts discussed in deep the available data on chironomus. Some experts considered that the chironomus endpoint should be expressed in terms of mean measured concentration in the water column since the substance does not partition quickly to sediment. Likely the substance degrades relatively fast in water. The endpoint expressed in this way would be relevant also for other organisms living in the water.

However, the concentration in the sediment was unknown; in the pore water it was relatively low. Therefore, some experts noted that there is not enough information to agree on the mean measured concentration in the whole test system (which could be more appropriate). However, for the water column the information was available therefore the experts agreed to express the chironomus endpoint based on mean measured concentration.

The current TERs with the chironomus standard endpoint expressed in terms of nominal concentrations resulted below the trigger for many scenarios. With the endpoint expressed in terms of mean measured concentrations, likely the majority of scenarios will be below the trigger. Therefore, it is likely that higher tier studies should be performed to further address the risk to aquatic insects. In performing this higher tier assessment, potentially more sensitive insects should also be considered.

B.9.2.5.5.6 Assessment of bioconcentration potential

BYI 02960 and its relevant metabolites have a log Pow of <3, therefore no study on bioaccumulation is required and there is no concern for bioconcentration.

Table B.9.2.5.6-01 Log Pow values

Substance	Maximum Log Pow	Reference
BYI 02960	1.2	Vol.3 B.2.1.13
BYI 02960-succinamide	0.6 at pH 5	Vol.3 B.2 Table B.2.1-2 IIA 7.13b
BYI 02960-azabicyclosuccinamide NA-salt	-1.3 at pH 5	Vol.3 B.2 Table B.2.1-2 IIA 7.13b
DFA	- 3.1 at pH5	Vol.3 B.2 Table B.2.1-2 IIA 7.13b
6-CNA	1.52 at pH 1.98	Vol.3 B.2 Table B.2.1-2 IIA 7.13b

B.9.3 Effects on other terrestrial vertebrates (IIIA 10.3.2)

B.9.3.1 Generic field monitoring (IIIA 10.3.3)

STUDY IIIA, 10.3.3/01

Characteristics

reference	:	Wolf (2004)	Locations/crops	:	Germany, Hop yards
type of study	:	Birds and mammals, generic field monitoring	Growth stages covered	:	not indicated; study performed in June/July
year of execution	:	2003		:	
GLP statement	:	Yes		:	
guideline	:	Monitoring designed for the purpose of this study	acceptability	:	Acceptable
test substance	:	-	Endpoint	:	See below

Description

The study presented here was conducted to find out which bird and mammal species visit hop yards, and how much time per day they spent in hop. This information is needed to identify focal species and to determine the time spent in the treated area. Furthermore, the feeding behaviour of these species within hop yards was investigated to determine the proportion of time spent foraging. The hop yards were treated according good agricultural practice (GAP) by local farmers and all their farming activities were recorded. The study aimed to define realistic species of concern and reveal behavioural information for these species.

Methods

The study was conducted in seven hop yards in the 'Hallertau' region between Ingolstadt, Regensburg and Landshut, Germany. This region is the main area of hop cultivation in Europe. Four study plots and their adjacent areas close to nature were chosen to be typical sites for hop cultivation which provided a high diversity of birds and mammals. Three of the four plots were used for the bird inventory, and three plots were used for the mammal inventory. For comparison, three study plots within an intensively cultivated arable area were chosen; results for these plots are not described in the report, but only presented in the summary table, comparing the two types of plots.

The landscape of the first four plots is very diverse, consisting of meadows, set aside hop yards, areas with shrubs and trees, woods, alder forest, ponds and some agricultural used fields.

Soil types were not described in the study.

Birds

Transect counts

Prevalence and abundance of species within six hop yards and surrounding habitats were surveyed by transect counts (three yards surrounded by nature elements, three within the intensively used agricultural area. Counts took place within the yards and in the surrounding, on average 6 times in each of the six fields in the period from 18 June to 30 July 2003. The area's covered by the transect counting are given in Table B.9.3.1-1.

Table B.9.3.1-1 Overview on size of bird census transects and the type of hop yards

No.	Name	Hop-yard [ha]	Surrounding [ha]	Tvpe
1	Giessübel	2.25	2.91	Close-to-nature
2	Buchersried	0.84	1.33	Close-to-nature
4	Axenhofen	0.93	1.49	Close-to-nature
5	Schillwitzried	1.39	2.15	Intensive-use
6	Lindach	1.46	2.13	Intensive-use
7	Unterwanoenbach	1.64	2.56	Intensive-use

The observed area from the observer was assumed to be 30 m. Additionally to species and number of individuals, also behaviour (e.g.foraging, singing/breeding/feeding young, locomotion, resting, others) and exact location (e.g. flying over, resting on wire, in vegetation, on the ground) was recorded to allow later analysis of hop yard use. Species densities were calculated for all birds observed in the corresponding habitat when not grouped as "Flying over, not related to area". To allow comparisons with other data, the size of the area, covered by the observations, was also recorded.

Bird trapping

Birds were trapped by mist-netting within three hop yards in the study site. Two mist nets were attached to each other to ensure a net height of 4.8 m. Three of these arrangements were stringed together to form a net row. On every yard, four rows of nets with a resulting net area of 148.32 m^2 each were placed. In Buchersried (site 2) and Giessübel (site 1), two net rows were located at the hop yard borders and two inside the hop yard. In Axenhofen (site 4), three net rows were placed along the hop yard border and one net row was located inside the hop yard. The nets were checked at least every 30 min. Birds were taken out of the nets and marked individually. The species and age of the bird was identified. Afterwards the bird was released. Trappings were performed on each plot (i.e. plot 1, 2 and 4) for two and a half consecutive days, then the nets were transferred to the next plot and trapping started on the next morning again for 2.5 days. There were five trapping periods on sites Buchersried and Axenhofen and four trapping periods at Giessübel.

Radio telemetry of birds.

In the three hop yards used for bird monitoring, 17 individuals, belonging to 12 species, were tracked after tagging with a radio transmitter. In Giessübel (plot 1), 1 robin, 2 chaffinches and 2 great tits were followed. In Buchersried (plot 2), 1 yellowhammer, 1 goldfinch, 1 blackbird, 1 spotted flycatcher, 1 garden warbler and 1 marsh warbler were tracked. In Axenhofen (plot 4), 2 blackcaps, 1 yellowhammer, 1 mistle thrush, 1 blackbird and 1 chiffchaff were tracked. The radio tagged birds were tracked continuously for one whole day (from dawn to dusk). During tracking each change of habitat (and behaviour if recognisable) was recorded with time and location (on an aerial map fitted with a grid or GPS position). This modified basic data, which is included in the raw data was used for the evaluation. These data allow to calculate the proportion of time the bird spent in a specific habitat type (e.g. the hop yard) and, furthermore, to calculate the size and shape of the total home range (at this day).

Home ranges

To identify the home range, it was calculated from the telemetry data. Every new position of the animals was used for the calculation. Portion of hop yard of the total home range of the individuals.

The calculation of home range sizes and habitat content according to the Minimum Convex polygon (MCP) was performed with the RANGES 6 computer package. The percentage hop yard of the home range for a species was calculated from the mean data of the individuals.

Small mammals

Small mammal trapping

Small mammal live trapping was conducted at three different hop yards in the study site Giessübel (site 1) and in Axenhofen (site 3 and 4). In each yard 45 multi-capture 'Ugglan' live traps were installed in a U-shape. A first line of fifteen traps was set inside to the hop yard parallel to the border (trap distance approximately 10 m, distance to the border approx. 40 m). In a right angle to this first trap line another fifteen traps were arranged with a trap distance of approx. 5 m as a transect line from the surrounding vegetation into the hop yard. The third trap line of 15 traps was set up in a right angle to line two and parallel to line one, and was situated outside the hop yard in the surrounding vegetation with a distance between traps of approximately 10 m (distance to border approx. 25 m). Each trap was covered by a metal roof as shelter against weather and was supplied with crushed oats and hazelnuts as bait and food for trapped animals. The trapping started on 18th of June and was carried out daily with the exception when radio tracking was conducted. Traps were set-up in the evening and checked approximately every six hours, around midnight and in the morning when they were deactivated. Each captured animal was individually marked by a Passive Integrated Transponder (PIT). The PIT was applied in the field by a specific injector. Species, sex, weight, reproductive state, animal ID (the PIT), location (trap), date and time of trapping (checking time) were noted as raw data.

Radio telemetry of mammals

During the study suitable adult individuals trapped in the live traps were supplied with radio collars. Animals were regarded as suitable if the collar weight (approx. 2 g) did not exceed more than 10% of the body weight. Those animals were tracked individually for one 24 h period. While radio tracked, each change of habitat was recorded with time and location (GPS position) of changing. Additionally a GPS position was taken approximately every 15 to 20 minutes. These data allowed to calculate the time in habitat and the potentially foraging time an animal spent in a specific habitat type (e.g. the hop yard) and, furthermore, to calculate the size and shape of the total home range (at this day) and the speed in the different habitats. The potentially foraging time of wood mouse (*Apodemus sylvaticus*) was the active period when foraging was likely. The wood mouse as nocturnal animal is resting during the daytime and is active from dusk till dawn. The potentially foraging time in the hop yard for wood mice was the time an individual spent in the hop yard during the active period.

The speed was calculated from radio tracking data, the time and the distance two consecutive locations of an animal.

Home ranges

See description for birds.

Endpoints of the study

Number and kind of bird and mammal species that can be found in and around hop yards to identify species of concern.
Home range size of birds and small mammals living in hop yards or their vicinity to assess the spatial importance of the cultivation.
Diurnal use pattern of birds and small mammals to assess the proportion of time individuals spend in hop yards.
Any available information about behaviour and diet of birds and mammals in and around hop yards.
Diet of birds could not be determined since distance to the birds was too big to observe the exact feeding.

Results

Birds

Transect counts

In total 50 bird species were found during six census counts in the six fields (see table below). Of these species, 21 were found inside of the hop yards, and all species (except the green woodpecker) were found in the surrounding areas. Most abundant species in the hop yards were chaffinch, wood pigeon, goldfinch, fieldfare, great tit, and greenfinch. Most abundant species in the surrounding areas are starling, fieldfare, chaffinch, great tit, blackbird, wagtail and chiffchaff.

Table B.9.3.1-2. Mean abundance of all species [ind./ha] observed in hop yards and surrounding during six census walks in six hop yards and adjacent areas each, sorted after the abundance inside the hop yards.

Species		Location	
English Name	Scientific Name	Surrounding	Hop Yard
Chaffinch	*Fringilla coelebs*	0,91	0,64
Wood Pigeon	*Columba palumbus*	0,38	0,61
Goldfinch	*Carduelis carduelis*	0,26	0,51
Fieldfare	*Turdus pilaris*	1,14	0,48
Great Tit	*Parus major*	0,86	0,45
Greenfinch	*Carduelis chloris*	0,50	0,40
House Martin	*Delichon urbica*	0,13	0,24
Starling	*Sturnus vulgaris*	2,00	0,23
Yellowhammer	*Emberiza citrinella*	0,41	0,21
Wagtail	*Motacilla alba*	0,58	0,19
Blackbird	*Turdus merula*	0,60	0,19
Carrion Crow	*Corvus corone*	0,47	0,17
Serin	*Serinus serinus*	0,01	0,14
Tree Sparrow	*Passer montanus*	0,31	0,10
Cuckoo	*Cuculus canorus*	0,06	0,05
Mistle Trush	*Turdus viscivorus*	0,25	0,02
Crested Tit	*Parus cristatus*	0,20	0,02
Wren	*Troglodytes troglodytes*	0,06	0,01
Jay	*Garrulus glandarius*	0,03	0,01
Spotted Flycatcher	*Muscicapa striata*	0,03	0,01
Green Woodpecker	*Picus viridis*		0,01
Chiffchaff	*Phylloscopus collybita*	0,58	
Pheasant	*Phasanius colchicus*	0,47	
Blackcap	*Sylvia atricapilla*	0,29	
Garden Warbler	*Sylvia borin*	0,28	
Song Trush	*Turdus philomelos*	0,23	
Barn Swallow	*Hirundo rustica*	0,22	
Robin	*Erithacus rubecula*	0,20	
Skylark	*Alauda arvensis*	0,18	
Goldcrest	*Regulus regulus*	0,18	
Marsh Tit	*Parus palustris*	0,18	
Mallard	*Anas platyrhynchos*	0,15	
Reed Warbler	*Acrocephalus scirpaceus*	0,13	
Blue Tit	*Parus caeruleus*	0,12	
Kestrel	*Falco tinnunculus*	0,11	
Marsh Warbler	*Acrocephalus palustris*	0,10	
Nuthatch	*Sitta europaea*	0,10	
Yellow Wagtail	*Motacilla flava*	0,09	
Great Spotted Woodpecker	*Picoides major*	0,09	
Coot	*Fulica atra*	0,06	
Crossbeak	*Loxia curvirostra*	0,04	
Tree Pipit	*Anthus trivialis*	0,04	
Grey Heron	*Ardea cinerea*	0,02	
Turtle Dove	*Streptopelia turtur*	0,02	
Whitethroat	*Sylvia communis*	0,02	
Wood Warbler	*Phylloscopus sibilatrix*	0,02	
Black Woodpecker	*Dryocopus martius*	0,02	
Bussard	*Buteo buteo*	0,01	
Coal Tit	*Parus ater*	0,01	
Goshawk	*Accipiter gentilis*	0,01	
Treecreeper spec.	*Certhia spec.*	0,01	
Number of species		50	21
Mean total bird abundance per census [ind./ha]		17,05	6,37

When the close-to-nature hop yards are compared to the hop yards in intensively use areas, it is clear that the close-to-nature hop yards have a higher number of bird species (19 species, mean density was 7.8 individuals/ha in the close-to-nature hop yards compared to 12 species, mean density 4.9 individual/ha in the intensive yards). In the surrounding environment of the close-to-nature hop yards 46 species were observed, mean density 21.4 individuals/ha compared to 22 species, mean density 12.7 in the surrounding of the intensively cultivated area. Most common species inside the close-to-nature hop yards were chaffinch, fieldfare, great tit, wood pigeon, goldfinch and greenfinch with mean abundance of more than 0.5 individuals/ha. Most common species in the hop yards with intensive use environment, were wood pigeon, starling, goldfinch, carrion crow, greenfinch and chaffinch with a mean abundance of more than 0.25 individuals per hectare. Carrion crow and starling were recorded in smaller densities or not all in close-to-nature environment. Chiffchaff, great spotted woodpecker, house martin, jay, and spotted flycatcher were not recorded in hop yards or surrounding of intensively used environment. Results are reported in the following table.

Table B.9.3.1-3 Bird abundance in close-to-nature and intensive-use environment

	Close-to-nature environment		Intensive use environment	
Species	HopYard	Surrounding	Hop Yard	Surrounding
Black woodpecker		0.04		
Blackbird	0.37	1.18		0.02
Blackcap		0.55		0.02
Blue Tit		0.21		0.03
Bussard				0.02
Carrion Crow		0.20	0.34	0.74
Chaffinch	1.02	1.58	0.26	0.24
Chiffchaff	0.02	1.20		
Coal Tit		0.02		
Coot		0.12		
Crested Tit		0.39	0.03	
Crossbeak		0.07		
Cuckoo	0.07	0.10	0.03	0.03
Fieldfare	0.95	2.09		0.20
Garden Warbler		0.54		0.02
Goldcrest		0.35		
Goldfinch	0.60	0.26	0.43	0.27
Goshawk		0.02		
Great Tit	0.83	1.69	0.07	0.03
Greenfinch	0.50	0.69	0.31	0.29
Grey Heron		0.04		
House Martin	0.48	0.27		
Jay	0.02	0.06		
Kestrel		0.13		0.09
Mallard		0.29		
Marsh Tit		0.35		
Marsh Warbler		0.21		
Mistle Trush	0.05	0.21		0.28
Nuthatch		0.21		
Pheasant		0.78		0.15
Pied Wagtail	0.27	0.14	0.11	1.01
Reed Warbler		0.27		
Robin		0.40		
Serin	0.26	0.02	0.03	
Skylark				0.37
Song Trush		0.46		
Spotted Flycatcher	0.02	0.06		
Starling	0.02	0.04	0.44	3.95
Stock Dove				
Swallow		0.44		
Tree Pipit		0.07		
Tree Sparrow	0.20	0.25		0.38
Turtle Dove		0.04		
Whitethroat		0.04		
Wood Pigeon	0.75	0.13	0.47	0.62
Wood Warbler		0.04		
Woodpecker	0.29	0.17		
Wren		0.11		
Yellow wagtail				0.18
Yellowhammer	0.29	0.56	0.14	0.25
Number of species	19	46	12	22
Mean number of individuals per census	7.78	21.42	4.95	12.73

Behaviour observed during the transect counts was deemed to be unreliable and not analysed.

Bird trapping

In the three close-to-nature sites, a total of 738 birds of 44 different species were trapped (67 individuals were trapped more than one time, total number of trapping 831, only first traps are taken into account of analyses). Results are based on 14 trapping periods at three sites, values are standardised on mean no. of individuals per net and session, incorporating the varying number of trapping sessions and varying number of mist nets in hop yard and at borders. Results are reported in the table below.

Table B.9.3.1-4 Bird species trap list for hop yard's centre and border (plots 1, 2 and 4, close to nature)

Species	Giessübel		Buchersried		Axenhofen		Mean		Mean[%]	
	Hop	Border	Hop	Border	Hop	Border	Hop	Border	Hop	Border
Chaffinch	2.3	3.3	0.2	3.5		0.4	1.2	2.4	33.9	66.1
Blackbird	0.5	3.1	0.3	2.1	1.6	4.6	0.8	3.3	19.6	80.4
Song Thrush	0.9	0.1	0.2	1.5	1.0	1.6	0.7	1.1	39.2	60.8
Fieldfare	1.0	0.1	0.3	1.1			0.7	0.6	51.5	48.5
Mistle Thrush				0.2	0.4	0.1	0.4	0.1	75.0	25.0
Pied WaQtail	0.4			0.1			0.4	0.1	78.9	21.1
Great Tit	0.3	2.0	0.4	3.4	0.4	3.1	0.4	2.8	11.0	89.0
Blackcap	0.3	1.1	0.2	0.8	0.4	1.1	0.3	1.0	21.7	78.3
Robin	0.3	1.1	0.1	0.7	0.4	2.9	0.3	1.6	13.8	86.2
Chiffchaff		1.8	0.2	1.8	0.2	1.9	0.2	1.8	10.0	90.0
Garden Warbler	0.3		0.1	0.6		0.1	0.2	0.3	34.4	65.6
Common Treecreeper	0.1			0.1	0.2	0.3	0.2	0.2	47.0	53.0
Greenfinch	0.3			0.8			0.3	0.8	23.8	76.2
Crested Tit	0.1					0.3	0.1	0.3	31.9	68.1
Wren	0.1					0.1	0.1	0.1	48.4	51.6
Reed Warbler	0.1	0.1					0.1	0.1	50.0	50.0
Black Redstart	0.1			0.1			0.1	0.1	55.6	44.4
Goldcrest	0.1		0.1			0.6	0.1	0.6	15.8	84.2
Marsh Warbler		0.1	0.1	0.4			0.1	0.3	27.6	72.4
Spotted Flycatcher		0.3	0.1				0.1	0.3	28.6	71.4
Jay			0.1	0.2			0.1	0.2	33.3	66.7
Willow Warbler			0.1			0.2	0.1	0.2	33.3	66.7
Great Spotted Woodpecker		0.1	0.1	0.3		0.1	0.1	0.2	35.0	65.0
Grey Wagtail		0.1	0.1	0.1			0.1	0.1	47.1	52.9
Whitethroat			0.1				0.1		100.0	0.0
Goldfinch				0.9				0.9	0.0	100.0
Marsh Tit		0.5		0.7		0.5		0.6	0.0	100.0
Yellowhammer		0.1		0.6		0.4		0.4	0.0	100.0
Bullfinch						0.3		0.3	0.0	100.0
Blue Tit		0.3		0.4		0.3		0.3	0.0	100.0
Cuckoo				0.3				0.3	0.0	100.0
Coal Tit				0.1		0.3		0.2	0.0	100.0
Dunnock		0.1		0.3				0.2	0.0	100.0
Nuthatch		0.3		0.2		0.1		0.2	0.0	100.0
Grasshopper Warbler						0.1		0.1	0.0	100.0
Short-toed Treecreeper				0.2		0.1		0.1	0.0	100.0
Kingfisher				0.1				0.1	0.0	100.0
Lesser Whitethroat				0.1				0.1	0.0	100.0
Tree Sparrow				0.1				0.1	0.0	100.0
Willow Tit				0.1				0.1	0.0	100.0
Firecrest						0.1		0.1	0.0	100.0
Grey Woodpecker						0.1		0.1	0.0	100.0
Hawfinch						0.1		0.1	0.0	100.0
Wood Warbler						0.1		0.1	0.0	100.0
Total	7.0	14.6	2.8	21.9	4.6	19.7	4.8	18.7	20.4	79.6
Number of species	16	18	17	31	8	27	25	43		

On average, a number of 4.8 individuals per net and session were caught inside the yards (20.4 % of the total number of birds caught in the nets), and 18.7 individuals were caught in the border areas (79.6%). 19 species were only trapped at the borders. The whitethroat (1 individual) was the only species which was caught exclusively in the centre area of a hop yard. Most common species trapped (sum of border and centre) were blackbird, great tit, chaffinch, robin, chiffchaff, song thrush and blackcap, representing up to 25 % of trapped individuals over all three sites. Overall, chaffinch, blackbird, song thrush, fieldfare, great tit, blackcap and robin were most abundant within the hop yards.

Radio telemetry of birds.

Potential foraging time of focal species

The percentage of time individual birds spent potentially foraging in different habitats is based on 17 radio-tracked individuals, the table below gives an overview on the habitats in which the birds spent their potentially foraging time (referring to known time periods).

Table B.9.3.1.5 Percentage of time individual birds spent potentially foraging in different habitats

Individual		Habitat while potentially foraging					
Species	ID	Hop	Woodland	arable land	Grassland	Reed	other
Blackbird 1	EC 10004	2.8	51.0	36.7	1.8	6.8	0.8
Blackbird 2	EC 10021	0.6	99.4	0.0	0.0	0.0	0.0
Blackcap 1	C1J6390	0.0	100.0	0.0	0.0	0.0	0.0
Blackcap 2	C1J6391	0.0	100.0	0.0	0.0	0.0	0.0
Chaffinch 1	C1J 6417	85.9	12.3	1.7	0.0	0.0	0.0
Chaffinch 2	C1J6415	14.5	14.0	58.9	10.0	2.6	0.0
Chiffchaff	B1R 4321	0.4	99.1	0.5	0.0	0.0	0.0
Garden Warbler	C1J6380	0.0	90.6	0.1	0.0	2.3	6.9
Goldfinch	B1R 5121	35.7	33.7	9.3	0.0	20.3	1.0
Great Tit 1	B1R 5049	8.8	88.8	1.3	1.0	0.0	0.0
Great Tit 2	B1R 5109	6.3	93.7	0.0	0.0	0.0	0.0
Marsh Warbler	B1R 5191	0.0	5.2	0.0	0.0	88.5	6.3
Mistle Thrush	EC10075	0.0	100.0	0.0	0.0	0.0	0.0
Robin	B1R 5044	1.5	96.7	1.4	0.3	0.0	0.0
Spotted Flycatcher	B1R5123	6.0	87.5	0.0	0.0	0.0	6.5
Yellowhammer 1	C1J 6310	4.6	4.4	57.0	19.2	11.1	3.7
Yellowhammer 2	C1J6395	0.7	76.4	20.5	0.0	0.0	2.4
all		9.0	71.1	10.1	1.7	6.7	1.4

The time periods spent potentially foraging in different habitats by 17 radio-tracked individuals is reported in the following table. The time in which no information on the birds location or behaviour was collected due to loss of signal is shown in the last column. This time period is not included in the time spent potentially foraging.

Table B.9.3.1.-6 Composition of the time spent "potentially foraging" in birds

Species	ID	Hop	Woodland	arable land	Grassland	Reed	other	Unknown
Chaffinch 1	C1J 6417	12:32:00	1:48:00	0:15:00				0:46:00
Chaffinch 2	C1J6415	2:08:00	2:04:00	8:41:00	1:28:00	0:23:00		1:03:00
Great Tit 1	B1R 5049	1:27:00	14:34:00	0:13:00	0:10:00			0:06:00
Great Tit 2	B1R 5109	0:58:00	14:18:00					0:17:00
Robin	B1R 5044	0:15:00	15:47:00	0:14:00	0:03:00			0:01:00
Blackbird 2	EC 10021	0:06:00	17:33:00					0:43:00
Garden Warbler	C1J6380		11:36:00	0:01:00		0:18:00	0:53:00	2:36:00
Goldfinch	B1R 5121	2:19:00	2:11:00	0:36:00		1:19:00	0:04:00	5:17:00
Marsh Warbler	B1R 5191		0:41:00			11:43:00	0:50:00	0:04:00
Spotted Flycatcher	B1R5123	0:41:00	9:53:00				0:44:00	5:16:00
Yellowhammer 1	C1J 6310	0:29:00	0:28:00	5:59:00	2:01:00	1:10:00	0:23:00	1:05:00
Blackbird 1	EC 10004	0:25:00	7:29:00	5:23:00	0:16:00	1:00:00	0:07:00	0:43:00
Blackcap 1	C1J6390		16:13:00					0:00:00
Blackcap 2	C1J6391		17:20:00					1:00:00
Chiffchaff	B1R 4321	0:03:00	12:34:00	0:04:00				2:40:00
Mistle Thrush	EC10075		14:54:00					1:42:00
Yellowhammer 2	C1J6395	0:06:00	10:15:00	2:45:00			0:19:00	4:41:00

For those 12 individuals that were located in hop yards during their potentially foraging time, the following table shows the percentage of the time per stratum.

Table B.9.3.1-7 Preferred stratum of individual birds while located in hop yards

Individual		Stratum within hop yard [% of time]				
Species	ID	Hop Plant	Ground	Rack	Air	Unknown
Chaffinch 1	C1J 6417	14.1	7.0	0.0	0.0	78.9
Goldfinch	B1R 5121	65.5	0.0	0.0	0.7	33.8
Chaffinch 2	C1J6415	3.9	15.6	7.0	0.0	73.4
Great Tit 1	B1R 5049	62.1	0.0	0.0	0.0	37.9
Great Tit 2	B1R 5109	46.6	0.0	0.0	0.0	53.4
Spotted Flycatcher	B1R5123	31.7	0.0	51.2	0.0	17.1
Yellowhammer 1	C1J 6310	10.3	0.0	6.9	0.0	82.8
Blackbird 1	EC 10004	0.0	0.0	0.0	0.0	100.0
Robin	B1R 5044	0.0	0.0	0.0	0.0	100.0
Yellohammer 2	C1J6395	0.0	0.0	0.0	0.0	100.0
Blackbird 2	EC 10021	0.0	83.3	16.7	0.0	0.0
Chiffchaff	B1R 4321	100.0	0.0	0.0	0.0	0.0

Mammal trapping

The live trapping revealed that 5 different species had stable populations on the study plots. All species were trapped mainly in the surrounding structure. They preferred the natural habitats instead of the hop yards. Only the wood mouse was caught in significant numbers inside the hop yard (37.5 %) and was the most abundant species inside the hop yard.

Radio telemetry of mammals.

Time spent in different habitats of individuals that were radiotracked is reported in the following table.

Table B.9.3.1-8 Individual time in habitat of small mammals

species	animal ID	site	portion of time in habitat [%]					
			hop yard	wood land	grass land	field path	hedge row	game stripe[A]
Apodemus flavicollis	7200262624	1	0.4	98.2	-	1.4	-	-
	7200263701	1	-	100.0	-	-	-	-
	7200280673	1	-	100.0	-	-	-	-
Apodemus sylvaticus	7200135163	1	-	100.0	-	-	-	-
	7200265865	1	-	100.0	-	-	-	-
	7200067991	3	2.9	1.6	78.7	0.2	-	16.6
	7200132406	3	11.3	-	69.8	-	-	18.9
	7200056132	4	7.7	89.9	-	2.7	-	-
	7200053244	4	1.3	98.7	-	-	-	-
	[C,D]7200061042	1	0.7	74.3	21.6	-	2.5	1.0[B]
	[C]7200054471	4	82.7	14.9	2.2	0.2	-	-
Clethrionomys glareolus	7200281439	1	-	100.0	-	-	-	-
	7200056911	4	-	98.6	1.4	-	-	-
	7200273341	4	-	100.0	-	-	-	-
Microtus arvalis	7200062434	3	-	-	100.0	-	-	-
	7200062722	3	-	-	19.6	-	-	80.4
	7200270869	3	-	-	69.0	-	-	31.0
	7200273592	3	-	-	10.7	-	-	89.3
	7200135998	4	100.0	-	-	-	-	-
Microtus agrestis	7200055919	4	-	96.8	-	-	3.3	-
	7200272637	4	-	97.3	2.7	-	-	-

[A] Game strip on study site 3 consisted of maize.
[B] This individual was tracked in a thin arm of a big maize field on study site 1.
[C] Due to methodological problems the individual was tagged in the morning and radio tracked directly afterwards. So the place where it spent the day might not be their nest. The wood mouse as nocturnal animal just crashed into the next possibility.
[D] The individual was tagged in the morning and directly left the hop yard and did not return. So the individual did not use the hop yard during the potentially foraging time.

The mean data per species showed that *Apodemus flavicollus, Clethrionomys glareolus* and *Microtus agrestis* spent 97% or more of the time in the wood lands. *Microtus arvalis* spent approximately 40% of its time in the game stripe, 40% in the wood lands and 20% in the hop yard and *Apodemus sylvaticus* spent approximately 60% of its time in the wood lands, just over 20% in the grassland and 13% of its time in the hop yard.

Of the time the wood mice spent in total foraging, the mean potentially foraging time in hop yard is 24.2%.

Home ranges

Because of the small number of tracked individuals the results of all individuals of one species in one area were pooled and with these data the percentage of test hop yard of the total home range on the study sites was calculated. Summarising the results of the three plots for each species: 37.6 % of the wood mice home range was situated in the test hop yards. For the yellow-necked it was 18.7 %, for the common voles 40.0 %, for the bank vole 16.3% and for the field vole 31.9 %, even if the last two species never enter the hop yards at all. This discrepancy is due to the method of the minimum convex polygon calculation.

Remarks

To identify the bird species of concern for hop cultivation, three study plots within an intensively cultivated area (plots 5, 6 and 7) were chosen for comparison with four plots close to nature areas (plots 1-4). This was done to investigate the influence of the structure of surrounding habitats on bird activity inside the hop yards. There is no description of the habitat of the plots that were intensively cultivated (sites 5, 6 and 7).

Bird trapping only took place on study plots 1, 2 and 4 at hop yards in a close-to-nature environment. Mammals were only monitored in plots 1-4.

Bird census took place in six plots. The bird census walks on the six plots showed that species diversity and abundance was considerably higher in the close-to-nature environment than in the monitored hop yards in intensive use environment.

The quality of the study is good. The techniques used in this study (transect and scan counts, trapping, radio tracking) are sound techniques to provide information on the occurrence and abundance of bird and mammals and whether these species do forage in certain crop types or eat certain crops or parts of the crop.

The MCP method was used in this study to calculate home ranges and is a non-probabilistic estimation that does not assess the probability of occurrence of the animal. This method was used by the author to calculate the home range for the mammals. For the birds only the MPC for individual species were provided but no overall assessment was presented. The MCP method was used as a standard method to make their data comparable to published data. Different core centres can be examined in this method. For this evaluation a 100% core centre was assumed so that all outliers were also included. By this means, the real size of the home range is overestimated. Using this method, often artefacts occur and results may imply that the home range includes a specific habitat, even if the individual never entered, but only surrounded it. Therefore the results of the MCP method cannot be used for risk assessment.

The hop yards are clearly not preferred by most bird species. The results of the census counts and the trapping approach showed that the chaffinch (*Fringilla coelebs*) was the most abundant species within the hop yards. The two tracked chaffinches spent on average 50.2 % of their potentially foraging time in the hop yard thus also for this aspect (percentage of time spent), this species reached the highest value.

The live trapping of mammals showed that they preferred the natural habitats instead of the hop yard. The wood mouse was the most abundant species inside the hop yard and was the only species tracked regularly inside the hop yard. The average potentially foraging time in the hop yard was 24 % for the individuals tracked inside the hop yard.

It should be noted that the results from this study (for both mammals and birds) are based on hop yards close to nature areas. It is not clear if the same focal species can be used for hop yards in an intensively cultivated area, if present at all. For intensively cultivated areas the starling, wood pigeon, goldfinch and the greenfinch (see Table 10.3.1-3) are more abundant than the chaffinch. Mammal trapping was only conducted in the hop yards close to nature, therefore it is not possible to compare species abundance between plots in intensively cultivated area and plots close to nature areas.

Furthermore, few individuals were radio-tracked per species (at most two per species for birds, two to eight per species for mammals).
Results of this study can only be used taking these remarks into account.

B.9.3.2 Residue data (IIIA 10.3.3)

STUDY IIIA, 10.3.3/02

Characteristics

reference	:	Uceda L. (2012)	Locations/crops	:	2 X Europe-N (1x wheat, 1 X barley) and 2 X Europe-S (1x wheat, 1 X barley)
type of study	:	Field residue trials (residue decline)	Growth stage at treatment	:	BBCH 25-29
year of execution	:	2010-2011	Treatment rate	:	1 x 125 g a.s./ha (spray)
GLP statement	:	Yes	Residues	:	BYI 02960, difluoroacetic acid and BYI 02960-difluoroethylaminofuranone, all calculated as BYI 02960; 6-chloronicotinic acid.
guideline	:	EU 7029/VI/95 rev.5 (1997)	acceptability	:	Acceptable for BYI 02960, difluoroacetic acid and BYI 02960-difluoroethylaminofuranone.
test substance	:	BYI 02960 SL 200 g/L (200 g/L SL formulation), batch 2010-007173	Endpoint	:	See residue values in Tables below.
a.s. content	:	198.6 g/L BYI 02960			

Methods

The magnitude of the residues of BYI 02960 in/on barley and wheat (sample material green material) after one spray application with BYI 02960 SL 200 g/L was determined in residue decline trials on two locations in Northern Europe and two in Southern Europe up to 21 days after application.

Information on the trial locations is provided in Table B.9.3-01 below. Each trial included one treated and one untreated plot. Treatment dates for trials 01, 02, 03 and 04 were 11 April 2011, 29 March 2011, 07 March 2011 and 18 March 2011, respectively. The crop of each plot was treated once with BYI 02960 SL 200 g/L at a rate of 125 g a.s./ha in 300 L water/ha. At the time of treatment, the BBCH growth stage of the crop was 29, 25, 29 and 29 for trial 01, 02, 03 and 04, respectively. Samples of green material were collected for residue analysis at 0, 1, 3, 4-5, 7, 10, 14-15 and 21 DAT (Day After Treatment). Residues of BYI 02960, difluoroacetic acid, BYI 02960-difluoroethylaminofuranone and 6-chloronicotinic acid were determined by method 01212 (extraction with acetonitrile/water/formic acid (80/20/0.22 v/v/v), addition of internal standard, purification by clean up with cationic resin, HPLC-MS/MS). Residues of BYI 02960, difluoroacetic acid and BYI 02960-difluoroethylaminofuranone were calculated as BYI 02960 (in agreement with the plant residue definition for risk assessment). The LOQ was 0.01 mg/kg for BYI 02960 and BYI 02960-difluoroethylaminofuranone calculated as BYI 02960, 0.02 mg/kg for difluoroacetic acid calculated as BYI 02960, and 0.005 mg/kg for 6-chloronicotinic acid calculated as 6-chloronicotinic acid (equivalent to 0.01 mg/kg as BYI 02960). Concurrent validation was provided (calibration, recovery and repeatability at LOQ and higher levels, chromatograms) and considered to be adequate to support the validity of the residue values. The storage period of deep-

frozen samples intended for the analysis of BYI 02960 and its metabolites ranged between 214 and 270 days. The List of Endpoints states that during a storage period of 12 months under deep-freezer conditions, the components of the relevant residues of BYI 02960 (including parent compound, BYI 02960-difluoroethyl-aminofuranone, and DFA) were stable in orange fruit (high acid content), spinach leaves and tomato fruit (high water content), wheat grain (high starch content), bean seed (high protein content), coffee bean and soybean seed (high oil content), and sugar cane. Wheat and barley green material is a watery matrix and thus considered to be covered by storage stability data for spinach leaves and tomato fruit. Therefore the residue values for parent compound, BYI 02960-difluoroethyl-aminofuranone, and DFA are valid with respect to storage stability. However, the storage stability of 6-chloronicotinic acid under frozen conditions in watery matrix for a period of 270 days (9 months) should be addressed in order for the results for this compound to be valid.

Table B.9.3.2-01 Trial locations and cropping information.

Trial number	11-2958-01	11-2958-02	11-2958-03	11-2958-04
Principal investigator	K. Merten	K.P. Brückmann	F. Leroux	G. Magli
Trial location	49377 Vechta - Langförden	51399 Burscheid	86200 Ceaux En Loudun	I-40134 Bologna
Country	Germany	Germany	France	Italy
Area of application	Field	Field	Field	Field
Plot size [m^2]	100*	216	200	225
Type of soil	Loamy sand	Sandy Loam	Silty clay	Silty sand
pH-value of soil (in water)	-	-	8.3	8.2
pH-value of soil (in $CaCl_2$)	5.8	6.3	-	-
Content of organic C [%]	1.6	1.3	1.7	1.2
Test system	Barley	Wheat	Barley	Wheat
Variety	Laverda	Hermann	Kétos	Mieti
Seed rate [kg/ha]	158	197	130	200
Date of sowing	2010-09-30	2010-10-11	2010-10-14	2010-10-15
Start of flowering	2011-05-12	2011-05-26	-	2011-05-01
End of flowering	2011-05-20	2011-06-03	-	2011-05-10
Date of commercial harvest	2011-07-05 to 2011-07-25	2011-06-30 to 2011-07-31	-	2011-07-01 to 2011-07-10

Results

Residues in samples of untreated plots were all <LOQ. The results for treated plots are summarised in the Tables below.

Table B.9.3.2-02 Residue summary in/on barley/wheat (parent compound, BYI 02960-difluoroethyl-aminofuranone and DFA)

Trial No. Country Crop	Sample material	DALT	Residues [mg/kg]			
			BYI 02960	difluoroacetic acid	BYI 02960-difluoroethylaminofuranone	total residue of BYI 02960 calc.[1]
11-2958-01 Germany Barley	green material	0	6.9	0.021	0.069	6.9
	green material	1	1.3	0.032	0.093	1.4
	green material	3	0.75	0.036	0.11	0.89
	green material	5	0.57	0.038	0.095	0.71
	green material	7	0.25	0.040	0.068	0.36
	green material	10	0.14	0.034	0.040	0.21
	green material	15	0.042	0.038	0.021	0.10
	green material	21	0.016	0.043	0.010	0.069

Trial No. Country Crop	Sample material	DALT	Residues [mg/kg]			
			BYI 02960	difluoroacetic acid	BYI 02960-difluoroethylaminofuranone	total residue of BYI 02960 calc.[1]
11-2958-02 Germany Wheat	green material	0	11	0.021	0.019	11
	green material	1	8.3	0.028	0.044	8.4
	green material	3	1.7	0.071	0.078	1.8
	green material	5	0.66	0.080	0.039	0.78
	green material	7	0.27	0.078	0.017	0.37
	green material	10	0.081	0.060	< 0.01	0.15
	green material	14	0.027	0.056	< 0.01	0.093
	green material	21	0.021	0.072	< 0.01	0.10
11-2958-03 France Barley	green material	0	16	< 0.02	0.024	16
	green material	1	9.8	0.024	0.039	9.8
	green material	3	9.7	0.089	0.10	9.9
	green material	4	8.3	0.096	0.10	8.5
	green material	7	3.8	0.11	0.087	4.0
	green material	10	1.8	0.10	0.042	1.9
	green material	14	1.1	0.11	0.033	1.2
	green material	21	0.25	0.073	< 0.01	0.33

Trial No. Country Crop	Sample material	DALT	Residues [mg/kg]			
			BYI 02960	difluoroacetic acid	BYI 02960-difluoroethylaminofuranone	total residue of BYI 02960 calc.[1]
11-2958-04 Italy Wheat	green material	0	6.9	0.034	0.016	6.9
	green material	1	4.7	0.030	0.017	4.7
	green material	3	2.9	0.031	0.055	3.0
	green material	5	2.6	0.050	0.073	2.7
	green material	7	1.5	0.047	0.056	1.6
	green material	10	0.41	0.074	0.033	0.52
	green material	14	0.17	0.065	0.017	0.25
	green material	21	0.071	0.10	< 0.01	0.18

DALT = Days after last treatment a.s. = Active substance

[1] For the calculation of the total residue of BYI 02960 calc. as it appears in the result table above, unrounded values were used. Therefore, minor deviations may occur between the total residue value shown above and when the values given for the individual analytes in the table are summed. In cases in which the residue levels for all analytes are below the respective LOQs in a given sample, the total residue of BYI 02960 calc. is reported as < 0.04 mg/kg (the sum of the individual LOQs). If a residue value is at or above the individual LOQ level for at least one analyte in a sample, all other values below the respective LOQs in that sample are set at the LOQ before the total residue is calculated (0.01 + < 0.02 + < 0.01 = 0.04 mg/kg)

Analyte:	Final determination as:	Residues calculated as:
BYI 02960	BYI 02960	BYI 02960
BYI 02960-difluoroethylaminofuranone	BYI 02960-difluoroethylaminofuranone	BYI 02960
difluoroacetic acid	difluoroacetic acid	BYI 02960
total residue of BYI 02960 calc.		BYI 02960

Table B.9.3.2-03 Residue summary in/on barley/wheat (6-chloronicotinic acid)

Trial no. Country Crop	Sample no.	Growth stage [BBCH]	DALT	Sample material	6-chloronicotinic acid [mg/kg]
11-2958-01 Germany barley	0011E	29	0	green material	0.019
	0012E	29	1	green material	0.019
	0013E	30	3	green material	0.015
	0014E	30	5	green material	0.010
	0015E	31	7	green material	0.006
	0016E	33	10	green material	< 0.005
	0017E	37	15	green material	< 0.005
	0018E	49	21	green material	< 0.005
11-2958-02 Germany wheat	0011E	25	0	green material	< 0.005
	0012E	25	1	green material	0.008
	0013E	25	3	green material	0.016
	0014E	30	5	green material	0.011
	0015E	30	7	green material	< 0.005
	0016E	30	10	green material	< 0.005
	0017E	30	14	green material	< 0.005
	0018E	30	21	green material	< 0.005
11-2958-03 France barley	0011E	29	0	green material	< 0.005
	0012E	29	1	green material	0.008
	0013E	29	3	green material	0.021
	0014E	29	4	green material	0.021
	0015E	30	7	green material	0.016
	0016E	30	10	green material	0.008
	0017E	30	14	green material	0.011
	0018E	31	21	green material	< 0.005
11-2958-04 Italy wheat	0011E	29	0	green material	< 0.005
	0012E	29	1	green material	< 0.005
	0013E	29	3	green material	0.015
	0014E	31	5	green material	0.020
	0015E	31	7	green material	0.018
	0016E	31	10	green material	0.010
	0017E	32	14	green material	0.007
	0018E	34	21	green material	< 0.005

DALT = Days after last treatment, " - " = Days before last treatment, E = Examination sample

Analyte:	Final determination as:	Residues calculated as:
6-chloronicotinic acid	6-chloronicotinic acid	6-chloronicotinic acid

Conclusion

Residues of BYI 02960, difluoroacetic acid, BYI 02960-difluoroethylaminofuranone and 6-chloronicotinic acid were determined up to 21 days after treatment, after one treatment of wheat or barley with BYI 02960 SL 200 g/L at BBCH 25-29 in March-April 2011 in Europe-N (2 trials) or Europe-S (2 trials) at 125 g a.s./ha.

Guidelines & Limitations

The study is acceptable. The storage stability of 6-chloronicotinic acid under frozen conditions in watery matrix for a period of 270 days (9 months) should be addressed in order for the results for this

compound to be valid; however, since 6-CNA is not considered relevant for bird and mammal risk assessment, this information is not needed for this DAR.

IIIA 10.3.3/03

Characteristics

reference	:	Scherr F. & Ellerich C. (2013)	study type	:	modelling study (estimation of DT50)
year of execution	:	2013	Crop	:	lettuce
GLP statement	:	not applicable	Residues	:	Flupyradifurone only and total residues of flupyradifurone
guideline	:	Focus Kinetics v 2.0 (2006)	Endpoints	:	See Tables 7-10
test substance	:	Flupyradifurone (BYI 02960)	acceptability	:	Acceptable

NB For an earlier version of the DAR, another kinetic evaluation of the lettuce trials was submitted (Sur R. and Ellerich, 2012). After discussions between the RMS and the notifier, a revised version of the modelling was submitted. This revised version is considered to be the most relevant for risk assessment and is the only one presented here.
Please note that the summaries/evaluations of the residue trials in lettuce can be found in Vol.3 B.7.

Study Design and Methods

Kinetic modelling analysis of data from field residue trials in lettuce performed in Northern and Southern Europe with flupyradifurone (BYI 02960) was performed in order to derive DT50 values for flupyradifurone only and total residues of flupyradifurone (sum of flupyradifurone, difluoroacetic acid and BYI 02960-difluoroethylaminofuranone, expressed as flupyradifurone). The residue trials originated from the following reports: Noss, G., Bauer, J. (2012), Determination of the residues of BYI 02960 in/on lettuce after spraying of BYI 02960 SL 200 in the field in the Netherlands, Belgium, France (North) and Germany, Edition Number: M-424742-01-1,Study Report Number: 10-2223; Schoening, R., Bauer, J. (2012), Determination of the residues of BYI 02960 in/on lettuce, head after spray application of BYI 02960 SL 200 in the field in France (South), Spain and Italy - Amendment no. 0001 to report no. 10-2213; Uceda, L. (2012a), Determination of the residues of BYI 02960 in/on lettuce after spray application of BYI 02960 SL 200 in the field in Spain, Italy, Southern France and Portugal, Edition Number: M-425784-01-1, Study Report Number: 11-2071; Uceda, L. (2012b), Amendment No. 1 - Determination of the residues of BYI 02960 in/on lettuce after spray application of BYI 02960 SL 200 in the field in Germany, northern France and Belgium, Number: M-425941-02-1, Study Report Number: 11-2082. In all trials lettuce was treated twice with a 9-11 day interval at rate 125 g a.s./ha. In all trials residues of flupyradifurone were determined after the second treatment, but in a few trials residues of flupyradifurone were also determined after the first application. DT50 values were estimated for each decline interval. The data used for modelling, which comprised for each set only 5 data points, are summarised in Table 1 and 2. These data were checked by the RMS and corresponded with the original study data, except in one case (trial R17), and for this reason this data set was recalculated by the RMS (see “Evaluation by RMS”).

Data handling was in agreement with the guidance provided by FOCUS Kinetics (2006). In cases where the original residue data contained the value <LOQ, the next non-detect was replaced by 0.5xLOQ. The degradation rate of was evaluated using the software package KinGUI (version 2.2011). The kinetics were evaluated in a stepwise approach, first using SFO, and if the SFO fit was not acceptable, in addition by the bi-phasic model DFOP. The goodness of fit of the kinetic models was assessed both statistically (χ2-error for fits and t-test parameter) and visually (plots of actual versus modelled residues, residuals plots), in accordance with the guidance provided by FOCUS (2006).

Table B.9.3.2-04 Results of residue trials conducted in/on lettuce after spraying with BYI 02960 SL 200 in the field (northern EU residue region)

trial code	trial no.	Country	DALT (d)	BYI 02960 (mg/kg)	difluoroacetic acid (mg/kg)	BYI 02960-difluoroethylamino furanone (mg/kg)	total residue of BYI 02960 (mg/kg)
R01	10-2223-01	NL	0	1.9	<0.02	<0.01	2.0
			1	1.8	<0.02	<0.01	1.8
			3	0.58	<0.02	<0.01	0.61
			5	0.34	<0.02	<0.01	0.37
			7	0.22	<0.02	<0.01	0.25
R02	10-2223-02	BE	0	1.7	<0.02	<0.01	1.7
			1	0.43	<0.02	<0.01	0.46
			3	0.37	<0.02	<0.01	0.4
			5	0.34	<0.02	<0.01	0.37
			7	0.21	<0.02	<0.01	0.24
R03	10-2223-03	Fr-N	0	1.5	<0.02	<0.01	1.5
			1	1.3	<0.02	<0.01	1.3
			3	0.68	<0.02	<0.01	0.71
			5	0.52	0.02	<0.01	0.55
			7	0.46	0.03	<0.01	0.5
R04	10-2223-04	DE	0	1.7	<0.02	<0.01	1.8
			1	1.1	<0.02	<0.01	1.1
			3	1.0	<0.02	0.01	1.0
			5	0.87	<0.02	<0.01	0.9
			7	0.66	<0.02	<0.01	0.69
R05	10-2223-05	DE	0	4.1	<0.02	0.02	4.1
			1	1.0	<0.02	0.01	1.1
			3	0.83	<0.02	0.01	0.87
			5	0.86	<0.02	0.01	0.86
			7	0.65	<0.02	<0.01	0.68
R06	11-2082-01	DE	0	2.7	0.02	<0.01	2.7
			3	1.5	0.023	0.017	1.6
			7	0.52	0.028	<0.01	0.56
			10	0.13	0.02	<0.01	0.16
			14	0.073	0.024	<0.01	0.11
R07	11-2082-02	DE	0	2.6	<0.02	<0.01	2.6

			3	0.11	<0.02	<0.01	0.14
			7	0.033	0.022	<0.01	0.065
			10	0.023	0.027	<0.01	0.06
			14	0.011	0.027	<0.01	0.047
R08	11-2082-04	BE	-10	5.6	<0.02	0.011	5.6
			-8	2.0	<0.02	0.015	2.0
			-5	0.63	0.028	<0.01	0.67
			-2	0.35	0.03	<0.01	0.39
			0*	0.25	0.026	<0.01	0.28
			0	2.8	0.022	<0.01	2.8
			3	0.43	0.028	<0.01	0.47
			7	0.22	0.03	<0.01	0.26
			10	0.12	0.026	<0.01	0.15
			14	0.058	0.025	<0.01	0.093
R18	11-2082-03	Fr-N	-10	2.9	<0.02	<0.01	2.9
			-8	2.6	<0.02	0.028	2.7
			-5	1.4	0.027	0.049	1.5
			-2	0.76	0.037	0.044	0.84
			0*	0.57	0.049	0.04	0.65
			0	3.8	0.048	0.053	3.9
			3	2.9	0.096	0.093	3.0
			7	1.6	0.11	0.074	1.7
			10	0.9	0.14	0.062	1.1
			14	0.75	0.2	0.082	1.0

DALT = days after last treatment
** prior to last treatment*

Table B.9.3.2-05 Results of residue trials conducted in/on lettuce after spraying with BYI 02960 SL 200 in the field (southern EU residue region)

trial code	trial no.	Country	DALT (d)	BYI 02960 (mg/kg)	difluoroacetic acid (mg/kg)	BYI 02960-difluoroethylamino furanone (mg/kg)	total residue of BYI 02960 (mg/kg)
R09	10-2213-01	Fr-S	0	2.6	<0.02	<0.01	2.6
			1	0.57	<0.02	<0.01	0.6
			3	0.4	0.02	<0.01	0.43
			5	0.29	0.02	<0.01	0.32
			7	0.15	0.02	<0.01	0.19
R10	10-2213-02	SP	0	3.8	0.02	0.02	3.8
			1	3.5	0.03	0.02	3.5
			3	2.7	0.03	0.02	2.7
			4	2.3	0.03	0.02	2.4
			7	1.3	0.03	0.01	1.4
R11	10-2213-03	IT	0	2.7	0.04	0.01	2.7
			1	2.2	0.04	0.02	2.2
			3	0.48	0.05	<0.01	0.53
			5	0.21	0.05	<0.01	0.27
			7	0.09	0.05	<0.01	0.15
R12	10-2213-04	SP	0	3.6	0.03	0.02	3.7
			1	3.0	0.04	0.02	3.1
			4	2.1	0.05	0.02	2.2
			5	2.0	0.05	0.02	2.1
			7	1.3	0.05	0.02	1.3
R13	10-2213-05	SP	0	2.9	<0.02	<0.01	2.9
			1	1.9	<0.02	0.01	2.0
			3	1.1	<0.02	0.02	1.2
			5	0.21	<0.02	<0.01	0.24
			7	0.17	0.02	<0.01	0.2
R14	11-2071-01	SP	0	2.6	0.062	0.031	2.7
			3	1.5	0.077	0.046	1.6
			7	0.77	0.097	0.034	0.9
			10	0.57	0.11	0.024	0.71
			14	0.033	0.15	<0.01	0.19
R15	11-2071-02	IT	0	7.3	0.039	0.023	7.4
			3	3.1	0.083	0.045	3.2
			7	1.4	0.13	0.025	1.5
			10	0.24	0.11	<0.01	0.37
			14	0.17	0.12	<0.01	0.3
R16	11-2071-03	Fr-S	-9	5.3	<0.02	0.015	5.4
			-7	3.4	<0.02	0.024	3.5
			-4	0.69	<0.02	0.014	0.72
			-1	0.39	<0.02	<0.01	0.42
			0*	0.29	<0.02	<0.01	0.32
			0	3.5	0.02	0.022	3.6
			3	0.72	0.035	0.02	0.78
			7	0.25	0.032	0.011	0.29

			10	0.2	0.047	0.012	0.26
			14	0.094	0.046	<0.01	0.15
R17	11-2071-04	PT	-8	1.8	0.022	0.021	1.8
			-5	0.92	0.031	0.015	0.97
			-2	0.27	0.027	<0.01	0.31
			0*	0.11	0.029	<0.01	0.15
			0	1.9	0.026	<0.01	1.9
			3	0.35	0.03	<0.01	0.39
			7	0.16	0.031	<0.01	0.2
			10	0.067	0.034	<0.01	0.11
			14	0.046	0.038	<0.01	0.094

DALT = days after last treatment
** prior to last treatment*

Results

The reported results are summarised in Table B.9.3.2-06 and -07 (flupyradifurone only) and Table -08 and -09 (total residues of flupyradifurone). All relevant raw data were provided in the report and included plots of fitted versus measured values and residuals plots. All fits and all plots were subjected to review by the RMS and the assessment by the RMS is provided following the Tables. Data sets for which additional estimations were performed by the RMS are shaded in Tables B.9.3.2-06-09 below.

Table B.9.3.2-06 Summary of DT50 values for flupyradifurone only calculated with SFO

Trial code	Trial description	evaluation after application	Crop	DT50 [days]	X^2 error [%]	t-test
R01	10-2223-01, N-EU	2nd (last)	lettuce head	2.27	14.91	0.0028
R03	10-2223-03, N-EU	2nd (last)	lettuce head	3.34	7.40	0.0025
R04	10-2223-04, N-EU	2nd (last)	lettuce head	5.45	10.41	0.0184
R06	11-2082-01, N-EU	2nd (last)	lettuce head	3.04	6.80	<0.0001
R07	11-2082-02, N-EU	2nd (last)	lettuce head	0.20	6.87	< 0.0001
R18	11-2082-03, N-EU	1st	lettuce head	4.69	9.62	0.0030
R18	11-2082-03, N-EU	2nd (last)	lettuce head	5.52	5.99	0.0008
R10	10-2213-02, S-EU	2nd (last)	lettuce head	5.25	3.70	<0.0001
R11	10-2213-03, S-EU	2nd (last)	lettuce head	1.61	15.09	0.0020
R12	10-2213-04, S-EU	2nd (last)	lettuce head	5.15	3.28	< 0.0001
R13	10-2213-05, S-EU	2nd (last)	lettuce head	1.77	8.32	0.0016
R14	11-2071-01, S-EU	2nd (last)	lettuce head	3.91	7.42	0.0010
R15	11-2071-02, S-EU	2nd (last)	lettuce head	2.56	6.07	0.0004
R16	11-2071-03, S-EU	1st	lettuce head	2.21	12.11	0.0029
R17	11-2071-04, S-EU	1st	lettuce head	1.95	2.57	0.0009
R17	11-2071-04, S-EU	2nd (last)	lettuce head	1.31	9.01	0.0005

Table B.9.3.2-07 Summary of DT50 values for flupyradifurone only calculated with DFOP

Trial code	Trial description 10-2223-02, N-EU	evaluation after application	X^2 error [%]	$DT50_{fast}$ [d]	t-test (fast)	$DT50_{slow}$ [d]	t-test (slow)	g	k_{fast} (d-1)	K_{slow} (d-1)
R02	10-2223-05, N-EU	2nd (last)	4.70	0.06	0.4370	7.09	0.1180	0.713	10.98	0.09782
R05	10-2223-05, N-EU	2nd (last)	2.14	0.16	0.1173	12.32	0.0816	0.752	4.3551	0.05625
R08	11-2082-04, N-EU	1st	0.46	1.14	0.0101	7.08	0.0848	0.883	0.61029	0.09785
R08	11-2082-04, N-EU	2nd (last)	0.55	0.23	0.1167	3.91	0.0005	0.737	2.9981	0.1774
R09	10-2213-01, S-EU	2nd (last)	2.06	0.06	0.3787	3.57	0.0061	0.730	11.17	0.1942
R16	11-2071-03, S-EU	2nd (last)	1.36	0.95	0.0049	5.99	0.0321	0.843	0.7301	0.1157

Note: values for k_{fast} and k_{slow} were added by the RMS.

Table B.9.3.2-08 Summary of DT50 values for total residue of flupyradifurone in lettuce head calculated with SFO

Trial code	Trial description	evaluation after application	DT50 [days]	X^2 error [%]	t-test
R01	10-2223-01, N-EU	2nd (last)	2.08	12.57	0.0061
R03	10-2223-03, N-EU	2nd (last)	3.58	6.56	0.0003
R04	10-2223-04, N-EU	2nd (last)	4.17	13.82	0.0055
R06	11-2082-01, N-EU	2nd (last)	3.25	7.72	0.0001
R07	11-2082-02, N-EU	2nd (last)	0.21	9.59	< 0.0001
R18	11-2082-03, N-EU	1st	5.04	10.06	0.0039
R18	11-2082-03, N-EU	2nd (last)	6.15	5.58	0.0008
R10	10-2213-02, S-EU	2nd (last)	5.55	3.16	< 0.0001
R11	10-2213-03, S-EU	2nd (last)	1.68	13.59	0.0015
R12	10-2213-04, S-EU	2nd (last)	5.22	3.80	0.0001
R13	10-2213-05, S-EU	2nd (last)	1.91	8.86	0.0020
R14	11-2071-01, S-EU	2nd (last)	4.40	5.41	< 0.0001
R15	11-2071-02, S-EU	2nd (last)	2.67	5.76	0.0004
R16	11-2071-03, S-EU	1st	2.24	12.19	0.0029
R17	11-2071-04, S-EU	1st	2.12	2.81	0.0011
R17	11-2071-04, S-EU	2nd (last)	1.44	11.88	0.0016

Table B.9.3.2-09 Summary of DT50 values for total residue of flupyradifurone in lettuce head calculated with DFOP

Trial code	Trial description	evaluation after application	X2 error [%]	$DT50_{fast}$ [days]	t-test (fast)	$DT50_{slow}$ [days]	t-test (slow)	g	k_{fast} (d-1)	K_{slow} (d-1)
R02	10-2223-02, N-EU	2nd (last)	4.45	0.06	0.4349	7.69	0.1219	0.696	10.91	0.09018
R05	10-2223-05, N-EU	2nd (last)	2.67	0.16	0.2170	12.32	0.1742	0.752	4.3545	0.05624
R08	11-2082-04, N-EU	1st	0.50	1.09	0.0119	6.51	0.0688	0.855	0.6362	0.1065

R08	11-2082-04, N-EU	2nd (last)	0.91	0.26	0.3014	4.51	0.0244	0.734	2.627	0.1536
R09	10-2213-01, S-EU	2nd (last)	1.84	0.06	0.3987	3.95	0.0341	0.723	10.75	0.1753
R16	11-2071-03, S-EU	2nd (last)	2.01	1.01	0.0420	9.11	0.1666	0.866	0.6890	0.07608

Note: values for k_{fast} and k_{slow} were added by the RMS.

Evaluation by RMS

The reported assessment is acceptable, with the following annotations/exceptions.

- In a number of cases, the k_{slow} and/or k_{fast} of DFOP fitting was not statistically significantly different from zero at the 10% level (p was >0.1). However, the data sets consisted of only 5 data points and for such a small data set statistical significance of DFOP parameters is not easily achieved. In all cases however the visual assessment of the DFOP fit was excellent, and the X2 error values were low, and the DFOP endpoints are considered acceptable, unless noted differently below.
- Trial R17, after 1st application, flupyradifurone alone and total residues of flupyradifurone: The sampling times used in the modelling report were 0, 2, 5 and 8 days, but this should be 0, 3, 6 and 8 days. Modelling was performed by the RMS using KINGUI based on the correct data. For both data sets the SFO fit was visually and statistically acceptable.
- Trial R07, flupyradifurone alone and total residues of flupyradifurone: According to the modelling report SFO fit was acceptable, however visual assessment indicates that the decline is biphasic (see Figure B.9.3.2-04). DFOP modelling was performed by the RMS and gave a fit which was visually better than SFO (see Figure 2) and had a lower X2 error value. The t-test for the k_{slow} and k_{fast} of DFOP fitting was not statistically significantly different from zero at the 10% level (p was >0.1), however this is considered to be acceptable (see first bullet).
- Trial R04, total residues of flupyradifurone: According to the modelling report SFO fit was acceptable, however visual assessment indicates that the decline is biphasic (see Figure 3). DFOP modelling was performed by the RMS and gave a fit which was visually better than SFO (see Figure 3) and had a lower X2 error value. The t-test for the k_{slow} and k_{fast} of DFOP fitting was not statistically significantly different from zero at the 10% level (p was >0.1), however this is considered to be acceptable (see first bullet).
- Trial R17, 2nd application, total residues of flupyradifurone: According to the modelling report SFO fit was acceptable, however visual assessment indicates that the decline is biphasic (see Figure 4). DFOP modelling was performed by the RMS and gave a fit which was visually better than SFO (see Figure 4) and had a lower X2 error value. The t-test for the k_{fast} of DFOP fitting was not statistically significantly different from zero at the 10% level (p was >0.1), however this is considered to be acceptable (see first bullet).
- Trial R05, total residues of flupyradifurone: The modelling report performed the DFOP fitting using the residue values of flupyradifurone instead of those of total residues of flupyradifurone. DFOP modelling was performed by the RMS using the correct values and gave an acceptable DFOP fit (see Figure 5). The t-test for the k_{slow} and k_{fast} of DFOP fitting was not statistically significantly

different from zero at the 10% level (p was >0.1), however this is considered to be acceptable (see first bullet).

A summary of the acceptable study endpoints, consisting of the acceptable endpoints from the modelling report and the endpoints recalculated by the RMS, is provided in Table B.9.3.2-10-13.

Figure 1 R07: Reported actual versus modelled residues and the residuals plots of SFO (flupyradifurone alone and total residues of flupyradifurone)

flupyradifurone alone

total residues of flupyradifurone

Figure 2 R07: actual versus modelled residues and the residuals plots of DFOP (fitted by RMS) (flupyradifurone alone and total residues of flupyradifurone)

flupyradifurone alone

total residues of flupyradifurone

Figure 3 R04: actual versus modelled residues and the residuals plots of SFO (reported fit) and DFOP (fitted by RMS) (total residues of flupyradifurone)

Reported SFO fit **DFOP fit (by RMS)**

Figure 4 R17 (2nd application): actual versus modelled residues and the residuals plots of SFO (reported fit) and DFOP (fitted by RMS) (total residues of flupyradifurone)

Reported SFO fit **DFOP fit (by RMS)**

Figure 5 R05: actual versus modelled residues and the residuals plots of DFOP (fitted by RMS) (total residues of flupyradifurone)

Table B.9.3.2-10 Summary of DT50 values for flupyradifurone only calculated with SFO after amendment by RMS

Trial code	Trial description	evaluation after application	Crop	DT50 [days]	X2 error [%]	t-test
R01	10-2223-01, N-EU	2nd (last)	lettuce head	2.27	14.91	0.0028
R03	10-2223-03, N-EU	2nd (last)	lettuce head	3.34	7.40	0.0025
R04	10-2223-04, N-EU	2nd (last)	lettuce head	5.45	10.41	0.0184
R06	11-2082-01, N-EU	2nd (last)	lettuce head	3.04	6.80	<0.0001
R18	11-2082-03, N-EU	1st	lettuce head	4.69	9.62	0.0030
R18	11-2082-03, N-EU	2nd (last)	lettuce head	5.52	5.99	0.0008
R10	10-2213-02, S-EU	2nd (last)	lettuce head	5.25	3.70	<0.0001
R11	10-2213-03, S-EU	2nd (last)	lettuce head	1.61	15.09	0.0020
R12	10-2213-04, S-EU	2nd (last)	lettuce head	5.15	3.28	< 0.0001
R13	10-2213-05, S-EU	2nd (last)	lettuce head	1.77	8.32	0.0016
R14	11-2071-01, S-EU	2nd (last)	lettuce head	3.91	7.42	0.0010
R15	11-2071-02, S-EU	2nd (last)	lettuce head	2.56	6.07	0.0004
R16	11-2071-03, S-EU	1st	lettuce head	2.21	12.11	0.0029
R17	11-2071-04, S-EU	1st	lettuce head	2.50	9.13	0.009
R17	11-2071-04, S-EU	2nd (last)	lettuce head	1.31	9.01	0.0005

Table B9.3.2-11 Summary of DT50 values for flupyradifurone only calculated with DFOP after amendment by RMS

Trial code	Trial description 10-2223-02, N-EU	evaluation after application	X^2 error [%]	$DT50_{fast}$ [d]	t-test (fast)	$DT50_{slow}$ [d]	t-test (slow)	g	k_{fast} (d-1)	K_{slow} (d-1)
R02	10-2223-05, N-EU	2nd (last)	4.70	0.06	0.4370	7.09	0.1180	0.713	10.98	0.09782

R05	10-2223-05, N-EU	2nd (last)	2.14	0.16	0.1173	12.32	0.0816	0.752	4.3551	0.05625
R07	11-2082-02, N-EU	2nd (last)	0.83	0.24	0.4844	2.71	0.1596	0.9102	2.8722	0.256
R08	11-2082-04, N-EU	1st	0.46	1.14	0.0101	7.08	0.0848	0.883	0.61029	0.09785
R08	11-2082-04, N-EU	2nd (last)	0.55	0.23	0.1167	3.91	0.0005	0.737	2.9981	0.1774
R09	10-2213-01, S-EU	2nd (last)	2.06	0.06	0.3787	3.57	0.0061	0.730	11.17	0.1942
R16	11-2071-03, S-EU	2nd (last)	1.36	0.95	0.0049	5.99	0.0321	0.843	0.7301	0.1157

Table B.9.3.2-12 Summary of DT50 values for total residue of flupyradifurone calculated with SFO after amendment by RMS

Trial code	**Trial description**	**evaluation after application**	**Crop**	**DT50 [days]**	**X^2 error [%]**	**t-test**
R01	10-2223-01, N-EU	2nd (last)	lettuce head	2.08	12.57	0.0061
R03	10-2223-03, N-EU	2nd (last)	lettuce head	3.58	6.56	0.0003
R06	11-2082-01, N-EU	2nd (last)	lettuce head	3.25	7.72	0.0001
R18	11-2082-03, N-EU	1st	lettuce head	5.04	10.06	0.0039
R18	11-2082-03, N-EU	2nd (last)	lettuce head	6.15	5.58	0.0008
R10	10-2213-02, S-EU	2nd (last)	lettuce head	5.55	3.16	< 0.0001
R11	10-2213-03, S-EU	2nd (last)	lettuce head	1.68	13.59	0.0015
R12	10-2213-04, S-EU	2nd (last)	lettuce head	5.22	3.80	0.0001
R13	10-2213-05, S-EU	2nd (last)	lettuce head	1.91	8.86	0.0020
R14	11-2071-01, S-EU	2nd (last)	lettuce head	4.40	5.41	< 0.0001
R15	11-2071-02, S-EU	2nd (last)	lettuce head	2.67	5.76	0.0004
R16	11-2071-03, S-EU	1st	lettuce head	2.24	12.19	0.0029
R17	11-2071-04, S-EU	1st	lettuce head	2.67	8.74	0.0087

Table B.9.3.2-13 Summary of DT50 values for total residue of flupyradifurone calculated with DFOP after amendment by RMS

Trial code	**Trial description**	**evaluation after application**	**X2\error [%]**	**$DT50_{fast}$ [days]**	**t-test (fast)**	**$DT50_{slow}$ [days]**	**t-test (slow)**	**g**	**k_{fast} (d-1)**	**K_{slow} (d-1)**
R02	10-2223-02, N-EU	2nd (last)	4.45	0.06	0.4349	7.69	0.1219	0.696	10.91	0.09018
R04	10-2223-04, N-EU	2nd (last)	3.61	0.10	0.4911	9.85	0.1379	0.329	6.7763	0.0704
R05	10-2223-05, N-EU	2nd (last)	2.48	0.19	0.1453	11.73	0.1618	0.738	3.5691	0.0591
R07	11-2082-02, N-EU	2nd (last)	1.94	0.22	0.4919	5.53	0.2041	0.9251	3.1075	0.1254
R08	11-2082-04, N-EU	1st	0.50	1.09	0.0119	6.51	0.0688	0.855	0.6362	0.1065
R08	11-2082-04, N-EU	2nd (last)	0.91	0.26	0.3014	4.51	0.0244	0.734	2.627	0.1536
R09	10-2213-01, S-EU	2nd (last)	1.84	0.06	0.3987	3.95	0.0341	0.723	10.75	0.1753
R16	11-2071-03, S-EU	2nd (last)	2.01	1.01	0.0420	9.11	0.1666	0.866	0.6890	0.07608
R17	11-2071-04, S-EU	2nd (last)	2.43	0.09	0.5	4.33	0.0534	0.759	7.5696	0.1601

Note: values for k_{fast} and k_{slow} were added by the RMS.

Conclusions

The acceptable endpoints (SFO and DFOP rate constants and DFOP g values) are summarised in Tables B.9.3.2-10 to -13 above.

Guidelines & Limitations

Comments were incorporated in the above summary. The conclusion as stated above is acceptable. Comment on how to use these data for risk assessment: Since the best fit was obtained from different models, (SFO for some, DFOP for others), it is not possible to derive an overall average first order DT50 for residue decline on lettuce. For the risk assessment, an f_{TWA} for each individual SFO or DFOP (set of) endpoint(s) should be calculated followed by averaging of all f_{TWA} values obtained this way.

IIIA 10.3.3/04

Characteristics

Reference	:	Sur R. and Ellerich C. (2012)	Compound	:	BYI 02960 and total residue of BYI 02960 (sum of BYI 02960, difluoroacetic acid and BYI 02960-difluoroethylaminofuranone, expressed as BYI 02960)
Type of study	:	Modelling	Matrix	:	Wheat and barley
Year of execution	:	2012	Type of fit	:	SFO and DFOP
GLP statement	:	Not relevant	Acceptability	:	Acceptable
Guideline	:	Not applicable	DT50	:	See study conclusion

Study design

The aim of this modelling study was to derive first order half-lives (DT50 values) of BYI 02960 and total residue of BYI 02960 in wheat and barley green material based on residue data from outdoor field trials at 4 locations (2 trials R01 and R02 in Europe-N, 2 trials R03 and R04 in Europe-S), performed using 1 treatment at 125 g a.s./ha. The residue trials concerned were summarised in section B.9.3 of the DAR (study IIIA 10.3.3/02) and the reader is referred to the summary of that study for the residue data that were used for estimating DT50 values.

In a first approach, the single first-order (SFO) model was used for the estimations. Single first-order kinetics (SFO) is a simple exponential equation with only two parameters

$M = M_0 e^{-kt}$ (1)

where

M = Total amount of chemical present at time t

M_0 = Total amount of chemical present at time t=0

k = Rate constant (=ln2/DT50).

Where no acceptable SFO fit was obtained, the Double-First-Order in Parallel (DFOP) model was fitted to the data. This model assumes that there are two SFO compartments with specific relative size g and 1-g, and degradation rate constants k1 and k2. The DFOP model contains three kinetic

parameters and is suitable to describe bi-phasic degradation behaviour. The integrated model equation can be written as follows:

$M = M_0 (g\, e^{-k1t} + (1-g)\, e^{-k2t})$

where

M = Total amount of chemical present at time t

M_0 = Total amount of chemical applied at time t=0

g = Fraction of M0 applied to compartment 1

k1 = Rate constant in compartment 1 (=ln2/DT50fast)

k2 = Rate constant in compartment 2 (=ln2/DT50slow).

The best fitting values of the kinetic parameters in the equation discussed above were determined by a numerical optimization process. Using non-linear least square fitting algorithms the parameter values leading to the smallest deviations between observed and calculated residues were determined. Apart from the kinetic rates *k* also the initial amount was fitted, as well as the parameter g for DFOP fitting. Dissipation half-lives (DT50) were calculated from the dissipation rates *k*, as DT50 = ln(2) / k.

Evaluation was based first and foremost on visual inspection of the fitted graph and the residuals. A statistical measure of the quality of a fit was given by the χ2–test (according to FOCUS Kinetics (2006), the χ2 error ε should preferably be <15%). A t-test was employed to identify the probability that a parameter is not significantly different from zero.

The model fit as well as the statistical evaluation of the results was carried out with the in-house developed software KinGUII.

Results

The results are summarized in the Tables below, and the reported fits are displayed in Figure B.9.3-04 (BYI 02960) and Fig B.9.3-05 (BYI 02960 total residue).

Table B.9.3.2-07 Estimated DT50 parameter values of the SFO model for **BYI 02960** in cereals and goodness-of-fit statistics

Trial code	Trial description	Crop	DT50 [days]	ε [%]	t-test
R02	11-2958-02	cereals	1.40	17.21	0.0001
R03	11-2958-03	cereals	4.08	16.98	0.0005
R04	11-2958-04	cereals	2.98	11.13	< 0.0001

Table B.9.3.2-08 Estimated DT50 parameter values of the DFOP model for **BYI 02960** in cereals and goodness-of-fit statistics

Trial code	Trial description	ε [%]	DT50fast [days]	t-test (fast)	DT50slow [days]	t-test (slow)	g
R01	11-2958-01	2.71	0.153	0.0575	2.961	0.0010	0.7725

Table B.9.3.2-09 Estimated DT50 parameter values of the SFO model for **BYI 02960 total residue** in cereals and goodness-of-fit statistics

Trial code	Trial description	Crop	DT50 [days]	ε [%]	t-test
R02	11-2958-02	cereals	1.44	16.75	0.0001
R03	11-2958-03	cereals	4.22	17.04	0.0006
R04	11-2958-04	cereals	3.15	11.02	< 0.0001

Table B.9.3.2-10 Estimated DT50 parameter values of the DFOP model for **BYI 02960 total residue** in cereals and goodness-of-fit statistics

Trial code	Trial description	ε [%]	DT50fast [days]	t-test (fast)	DT50slow [days]	t-test (slow)	g
R01	11-2958-01	2.97	0.145	0.0795	3.555	0.0007	0.7612

Fig B.9.3-04 R01 BYI02960 SFO

Fig B.9.3-04 R01 BYI02960 DFOP

Fig B.9.3-04 R02 BYI02960 SFO

Fig B.9.3-04 R03 BYI02960 SFO

Fig B.9.3-04 R04 BYI02960 SFO

Fig B.9.3-05 R01 BYI02960 total SFO

Fig B.9.3-05 R01 BYI02960 total DFOP

Fig B.9.3-05 R02 BYI02960 total SFO

Fig B.9.3-05 R03 BYI02960 total SFO

Fig B.9.3-05 R04 BYI02960 total SFO

Comments by RMS

(1) In 18 trials performed in lettuce (see previous study), there was no remarkable difference between the results from trials in Europe-N and Europe-S. The data set for cereals was too small (n=2 for Europe-N, n=2 for Europe-S) to make a robust comparison, but the available data do not suggest a remarkable difference. Hence for risk assessment the results from the trials in Europe-N and Europe-S may be combined.

(2) Since the best fit was obtained from different models, (SFO in 3 trials, DFOP in 1 trial), it is not possible to derive an overall average first order DT50 for residue decline on cereals. The approach followed in the applicant's risk assessment was calculating f_{TWA} for each individual SFO or DFOP (set of) endpoint(s) followed by averaging of all f_{TWA} values obtained this way.

Conclusions

DT50 for decline of residues of BYI 02960 and total residue of BYI 02960 in cereals based on residue data from 4 outdoor field trials (2 in Europe-N, 2 in Europe-S) were determined (see Table B.9.3-07 to

Table B.9.3-10). The best fit model was SFO in 3 trials and DFOP in 1 trial.

Guidelines & Limitations

Comments were incorporated in the above summary. The conclusion as stated above is acceptable.

B.9.3.3 Summary of toxicity endpoints for mammals

Acute and chronic toxicity data

The summary of the toxicity profile of the active substance BYI 02960 to mammals is provided in Table B.9.3.3-1. Details of the studies concerned are provided in B.6 of this DAR.

Table B.9.3.3-1 Toxicity of BYI 02960 to mammals

Test species	Test design	Ecotoxicological endpoint			Reference
Acute risk assessment endpoint:					
Rat	acute oral, toxic class method	$LD_{50, cut-off}$ [1)] (4/6 dead at 2000, 0/6 at 300)	2000	mg a.i./kg bw	[redacted] (2009) B.6.2.1 Study 1
Rat	acute oral neurotoxicity study	LD_{50} (2/24 dead at 800, 0/24 at 200 and below)	> 800	mg a.i./kg bw	[redacted] (2009) B.6.7.1 Study 1
Combined LD_{50} calculation (TOXCALC, probit): 1607 mg/kg bw					
Reproductive risk assessment endpoint:					
Rat reproduction	Dietary exposure over 2 generations	$NOAEL_{maternal}$ $NOAEL_{repro}$ $NOAEL_{offspring}$	6.4~~7.8~~ [1)] (100 ppm) 34 ~~39.2~~ [2)] (500 ppm) 6.4~~7.8~~ [1)] (100 ppm)	mg a.i./kg bw/d mg a.i./kg bw/d mg a.i./kg bw/d	[redacted] (2011) B.6.6.1 Study 2
Rat developmental toxicity	Gavage over GD 6-20 (gestation days)	$NOAEL_{maternal}$ $NOAEL_{offspring}$	50~~15~~ 50	mg a.i./kg bw/d mg a.i./kg bw/d	[redacted] (2010)
Rabbit developmental toxicity	Gavage over GD 6-28 (gestation days)	$NOAEL_{maternal}$ $NO(A)EL_{offspring}$	15 40	mg a.i./kg bw/d mg a.i./kg bw/d	[redacted] (2012)
Reproductive risk assessment endpoint proposal of notifier		**Tier 1:**	6.4~~7.8~~ **mg/kg bw/d**		
		Tier 2 (refined):	34 ~~39.2~~ **mg/kg bw/d**		

1) Mean achieved dose of P & F1 females at 100 ppm (Table 5.6-14 of KIIA 5.6.1/02, Milius, 2011) In B.6, the NOAEL is based on the lowest intake by either males or females during a particular study phase, in this case intake by males F1-gen during premating, see Table 6.6.1-8 of Vol3 B.6.

2) Mean achieved dose of P & F1 females at 500 ppm (Table 5.6-14 of KIIA 5.6.1/02, Milius, 2011) In B.6, the NOAEL is based on the lowest intake by either males or females during a particular study phase, in this case intake by males F1-gen during premating, see Table 6.6.1-8 of Vol.3 B.6.

There are no indications that the formulated product enhances the toxicity of the a.s., see the Table

below.

Table B.9.3.3-2 Mammalian toxicity data of the formulated product BYI 02960 SL 200

Test species	Test design	Ecotoxicological endpoint	Reference
Rat	acute, oral	LD50 >2000 mg/kg bw [1)]	[redacted] (2010) B.6.12.1 Study 1

1) 0 out of 6 animals died. According to OECD Guideline 423, the LD50 cut-off of BYI 02960 SL 200 is >5000 mg/kg body weight (equivalent to Category 5 (unclassified) of the GHS).

The proposed **acute endpoint of 1607 mg/kg bw** (derived by combining the mortality data from the two acute studies with oral exposure with the a.s.) is accepted.

As long-term endpoint, the notifier proposes a maternal and offspring NOAEL of 100 ppm (7.8 mg/kg bw/d) for tier 1 risk assessment. ~~As a worst-case approach is acceptable but RMS notes that in the mammalian toxicology section of the DAR the endpoints are set at a higher dose level. Also for the developmental studies the endpoints in B.6 of the DAR are higher. See Table B.9.3.3-2.~~

~~Table B.9.3.3-2 Endpoints from 2-generation study in rats and developmental studies in according to B.6.6.1~~

~~Study type~~	~~Reproductive endpoint determined in B.6 :~~	~~Dose (ppm):~~	~~Dose (mg/kg bw/d):~~	~~Based on intake during study phase:~~
~~Rat reproduction~~	~~Relevant parental NOAEL~~	~~500 ppm~~	~~32 mg/kg bw/day~~	~~premating, P-gen, males~~
~~Rat reproduction~~	~~Relevant reproductive NOAEL~~	~~500 ppm~~	~~34 mg/kg bw/day~~	~~gestation, P-gen, females~~
~~Rat reproduction~~	~~Relevant offspring NOAEL~~	~~500 ppm~~	~~34 mg/kg bw/day~~	~~gestation, P-gen, females~~
~~Rat developmental~~	~~Relevant maternal NOAEL~~		~~50 mg/kg bw/day~~	
~~Rat developmental~~	~~Relevant offspring NOAEL~~		~~50 mg/kg bw/day~~	
~~Rabbit developmental~~	~~Relevant reproductive NOAEL~~		~~40 mg/kg bw/day~~	
~~Rabbit developmental~~	~~Relevant offspring NOAEL~~		~~40 mg/kg bw/day~~	

~~Based on the above, the endpoint proposed by the notifier for the first tier is considered to be very conservative.~~ For a tier 2 assessment, the notifier reconsidered this endpoint. Their statement is presented below, with minor modifications by RMS, regarding references to Tables and dossier sections. RMS also incorporated the changes made following Pesticide Peer Review meeting 122 of mammalian toxicology.

According to the refinement options provided in the EFSA GD (Section 4.4, Module 4: Reproductive risk assessment for mammals, step 9), the Tier 1 endpoint can be refined after re-examination of the reproductive studies for endpoints relevant for reproductive performance. Effects on other endpoints can be disregarded. In this context the EFSA GD specifies a number of potentially relevant endpoints to be evaluated, including body weight, behavior and reproductive performance of adults, and observations on offspring (number, weight, development). A corresponding evaluation for BYI 02960 is presented in the table below, based on the toxicological studies summarized in more detail B.6 of this dossier. If such refinement of the Tier 1 endpoint is considered, the EFSA GD recommends that additional mammalian toxicity studies should also be examined in order to check whether they contain lower NOAELs for relevant endpoints.

Approach taken in this evaluation:

In order to ensure a high level of protection, all repeated dose oral toxicity studies conducted with BYI 02960 in rodents (rat, mouse), rabbits or dogs were evaluated for potentially relevant endpoints. This evaluation included also neurotoxicity studies, in order to account for non-lethal behavioral effects that could become relevant under field conditions.

Since the protection goal of the reproductive risk assessment is the sustainability of potentially exposed wild mammal populations, these studies were evaluated with regard to population level relevant endpoints, i.e. survival, growth, development, behavior and reproductive performance. Other endpoints (e.g. organ weights, physiology, blood chemistry etc.) were not included in the evaluation. Furthermore, neither very high doses (more than 3-fold above the proposed refined endpoint), nor chronic studies of extreme exposure duration (≥ 1 year) were included in the tables.

Since effects on females are typically more relevant for small mammal populations than effects on males, the results of this evaluation are presented in Table B.9.3.3-4 in the order of increasing achieved doses for females over all evaluated studies. In order to facilitate the comparison of effects at the different dose levels of each study, the letter code in the first column indicates the study ID (letters A-N, as presented in the following overview table), with the respective dosing group indicated by numbers 1-3 (low, mid and high dose level).

Table B.9.3.3-3 Repeated oral dosing toxicity studies evaluated for selection of the Tier 2 wild mammal reproductive risk assessment endpoint in Table B.9.3.3-4

Study ID	Study type	Report reference
A	Rat 28-d gavage	B.6.3.1 Study 1, KIIA 5.3.1/01
B	Rat 28-d dietary	B.6.3.1 Study 2, KIIA 5.3.1/02

C	RAT 90-d	B.6.3.4 Study 1, KIIA 5.3.2/01
D	RAT 90-d NT (neurotoxicity)	B.6.7.2 Study 1, KIIA 5.7.4/01
E	RAT 1-gen (one generation reproduction)	B.6.6.1 Study 1, KIIA 5.6.1/01
F	RAT 2-gen (two generation reproduction)	B.6.6.1 Study 2, KIIA 5.6.1/02
G	RAT DT (developmental toxicity)	B.6.6.2 Study 1, KIIA 5.6.10/01
H	RAT DT (suppl.)	B.6.6.2 Study 2, KIIA 5.6.10/02
I	RABBIT DT (developmental toxicity)	B.6.6.2 Study 3, KIIA 5.6.11/01
K	MOUSE 28-d	B.6.3.1 Study 3, KIIA 5.3.1/03
L	MOUSE 90-d	B.6.3.4 Study 2, KIIA 5.3.2/02
M	DOG 28-d	B.6.3.1 Study 4, KIIA 5.3.1/04
N	DOG 90d	B.6.3.4 Study 3, KIIA 5.3.3/01

Results of this evaluation:

Most prominent in the evaluation of all these repeated oral dosing studies with BYI 02960 (see Table B.9.3.3-4) were observations of moderate body weight effects. Severity of effects was seen to increase with dose and exposure time.

Within reasonable timeframes of environmental exposures (weeks to months), effects of ecological relevance on wild mammal population did not occur at dose levels of at least 30-50 mg/kg bw/d.

In contrast, pronounced reproductive effects of clear ecological relevance did occur at dose levels significantly exceeding 100 mg/kg bw/d (e.g., 14.8% litter size reduction and reduced body weight of > 10% at 120-150 mg/kg bw/d (1800 ppm) in the rat 2-generation study), and only after prolonged exposure in a second generation (F1 females or F2 offspring).

Moderate effects of bodyweight can usually be considered less relevant in wild mammal risk assessments. Moreover, the results of the 90-d rat study with BYI 02960 showed that bodyweight effects approaching 10% (e.g., 8.9% in males and 9.5% in females at 500 ppm) were reversible after cessation of treatment (recovery phase). Potential exposure of wild mammals to BYI 02960 is transient and short-lived (single application, DT50 on foliage ≤ 5 days). Therefore an effect magnitude of ≤ 10% on bodyweight can be considered ecologically acceptable. Body weight effects exceeding this magnitude were not observed at dose levels below 140 mg/kg bw/d (P females: 15.9%), even after prolonged exposure.

Reproductive effects like reduction of litter size were only moderately pronounced and were only observed after long-term exposure in F1 parents and F2 litter (note: not observed in the 1-generation rat reproduction study nor in the first generation of the 2-generation study), indicating reduced risk for reproductive effects under environmentally relevant conditions (low application rate, single application, DT50 on foliage < 5 days).

Overall, this evaluation suggests that a dose level of up to 39.2 mg as/kg bw/d (achieved dose of

females at 500 ppm in the 2-gen rat reproduction study) can be proposed as relevant and sufficiently conservative risk assessment endpoint for wild mammal populations:

- no ecologically relevant effects in the parental generation or the F1 litter
- only slight reductions of bodyweight (< 10%) in F1 parents and F2 litter
- body weight reduction by BYI 02960 of ca. 10% was reversible after cessation of dosing.

Severe effects only occurred at dose levels which were 3-4 times higher than 39.2 mg/kg bw/d.

Therefore the refined wild mammal reproductive risk assessment endpoint proposed in this dossier is 39.2 mg as/kg bw/d, the achieved dose of females at 500 ppm in the 2-gen rat reproduction study.

Table B.9.3.3-4 Potentially relevant wild mammal reproductive risk assessment effects of BYI 02960 in repeated oral dosing studies

F: females; M: males; ↑: increase (higher than control); ↓: reduction (lower than control);
P: parental generation M+F; F1: offspring produced by P M+F, F2: offspring produced by F1 M+F
#: number; bw: body weight; FC: food consumption
n.e.: not evaluated (specific study type without reproductive endpoints); empty fields: no effect observed. Blue shading indicates the NOAELs as determined by RMS in B.6 of the original DAR. Green shading indicates the NOAELs as determined in the updated version of B.6 of December 2014 following Pesticide Peer Review meeting 122.

ID	Species & study type	Dose ppm	mg/kg bw/d* males	mg/kg bw/d* females	Potentially relevant findings in adults	Potentially relevant findings on reproduction and offspring
D1	RAT 90-d NT	100	5.7	6.9		n.e.
C1	RAT 90-d	100	6.0	7.6		n.e.
I1	RABBIT DT			7.5		
F1	RAT 2-gen	100	6.4-6.6	7.0-7.8		
N1	DOG 90d	400	12	12		n.e.
I2	RABBIT DT			15		
G1	RAT DT			15		
E1	RAT 1-gen	200	14.5	15.8-17.5		
M1	DOG 28-d	500	16	18		n.e.
L1	MOUSE 90-d	100	16	19		n.e.
H1	RAT DT (suppl.)			20		n.e.
H2	RAT DT (suppl.)			30		n.e.
B1	Rat 28-d	500	33.6			n.e.
D2	RAT 90-d NT	500	29.4	34.8		n.e.
C2	RAT 90-d	500	~~30.2*~~	38.3		n.e.
F2	RAT 2-gen	500	~~32*~~-32.5	**35.5-39.8**	bw ↓ ≤ 7.8% (F1 F: D_{gest} 0)	bw ↓ ≤ 7.4% (F2 pups)
I3	RABBIT DT			40	FC ↓ ≤ 20%, bw ↓ ≤ 1.7 %	
N2	DOG 90d	1200	33	41	bw ↓ ≤ 9% (M), minimal/slight muscle atrophy	n.e.
G2	RAT DT			50	FC ↓ ≤ 8%, bw ↓ ≤ 1.6 %	
K1	MOUSE 28-d	300	50	59		n.e.
E2	RAT 1-gen	700	50.1	48.8-60.9	bw ↓ ≤ 6.7% (F D_{lact} 14)	bw ↓ ≤ 9.9 % (F1 pups)
A1	RAT 28d		75	75		n.e.
L2	MOUSE 90-d	500	81	96		n.e.
K2	MOUSE 28-d	600	98	122		n.e.
G3	RAT DT			150	FC ↓ ≤ 27%, bw ↓ ≤ 4.2%	bw ↓ 2-3%, indications of slightly delayed fetal development
F3	Rat 2-gen	1800	117.4-122.1	140.2-151.4	bw ↓ ≤ 9.6% (M), ≤ 15.9% (F)	litter size ↓ 14.8% (F2) bw ↓ ≤ 12.5% (F1 & F2 pubs) # estrous cycles ↓ (F1 F)
C3	RAT 90-d	2500	156	168	bw ↓ ≤ 8.9% (M), 9.5% (F), reversible in recovery phase	n.e.
D3	RAT 90-d NT	2500	143	173	FC ↓ ≤ 29% (F, w1); bw ↓ ≤ 10% (M), ≤ 9% (F);	n.e.
E3	RAT 1-gen	2000	147.5	164.4-182.3	bw ↓ F (≤ 12% D_{lact} 13)	bw ↓ ≤ 9.9% (F1 pups)

*NOAEL set by RMS in original DAR but not accepted in peer review.

~~RMS agrees with the notifier that the ecologically relevant endpoint for wild mammals is the mid-dose of the two-generation study in rats (500 ppm). Based on the mammalian toxicology assessment (see table B.9.3.3-2) this corresponds to **34 mg/kg bw/d,** based on intake by females of the P-generation during the gestation period. This is considered to be the relevant endpoint for the ecotoxicological risk assessment.~~

Expert consultation during Pesticide Peer Review meeting 124, ecotoxicology, December 2014:

The 1-year dog study concluded an endpoint of 7.8 mg/kg bw (mammalian toxicity section, original DAR) and it was questioned whether this should be taken into account.

Furthermore, the endpoint from the 2-generation rat study has changed to 'parental' and 'offspring' NOAEL of 6.4 mg/kg bw per day (100 ppm) and 'reproductive' NOAEL of 32 mg/kg bw per day (500 ppm). The 'parental' NOAEL of 6.4 mg/kg bw per day is based on significant decrease in body weight (<10% observed in the mid-dose of the F_1 females) and a non-significant decrease in body weight gain found in the parental animals (21% in females) and the F_1 animals (16% in females). The 'offspring' NOAEL of 6.4 mg/kg bw per day is based on reduced body weight/body weight gain in the F_2 pups. These were agreed by the meeting of experts for mammalian toxicology (PESTICIDES PEER REVIEW MEETING 122).

Discussion:

The 1y-dog study was considered as not relevant for ecotox for reproductive risk assessment. Some observed effects (degeneration of skeleton muscle and other muscle degeneration) from this study might be considered as relevant but not for the reproduction. It was noted that in general such type of study is not considered robust by tox experts (few test individuals).

The discussion was focussed on the 2-generation rat study. Three endpoints are available from this study, NOAEL of 500 ppm for reproduction and 100 ppm for offspring and parental toxicity. A dose response relationship was noted for the pup body weight decrease. At 500 ppm the decrease was statistically significant but < 10%. It was questioned if this should be considered as biologically relevant. At 1800 ppm (the next higher dose) the decrease was >10%. Some experts considered that the statistical significance in this case should not be disregarded considering also the dose response relationship and preferred the lowest NOAEL of 100 ppm.

Overall, the majority of the experts considered that a NOAEL of 100 ppm should be used for risk assessment (**6.4 mg a.s./kg bw/d**).

Risk to mammals of metabolites of BYI 02960

The notifier provided the following information: " The toxicological properties of two metabolites of BYI 02960 were investigated in a series of studies and evaluated in more detail in section KIIA 5.8 of this submission. Toxicological studies performed on two other metabolites which are also common metabolites to other agrochemicals are also reported in this section. Only a brief summary of the results of these studies is presented in this section. Overall, the results indicate that none of the metabolites is more toxic to mammals than the parent, so that the risk assessment for wild mammals can be based on the toxicity and exposure ratios calculated for BYI 02960 a.i.

The metabolite difluoroacetic acid (DFA) is a major soil, water and plant metabolite and is found in the rat ADME study at an amount of around 6%. It is considered a non-relevant metabolite in terms of the EU Guidance document Sanco/221/2000-rev.10 (25 February, 2003). This metabolite was devoid of genotoxic potential; the acute oral LD50 was between 300 and 2000 mg/kg. When DFA was

administered via the diet to Wistar rats for at least 14 days at concentrations of 500, 2000 and 8000 ppm (equating approximately to 48, 187 and 745 mg/kg body weight/day, respectively in males and 51, 201 and 800 mg/kg body weight/day, respectively in females), slight body weight decrease was observed in both sexes at 8000 ppm. In a 90-day rat study, DFA was administered in the diet administration to Wistar rats at concentrations of 200, 1000 and 6000 ppm (equating approximately to 12.7, 66.2, 380 mg/kg body weight/day, respectively in males and 15.6, 78.7, 472 mg/kg body weight/day, respectively in females). Mean body weight and food consumption were reduced at 6000 and 1000 ppm, respectively in both sexes but not at 200 ppm. When the NOAEL of 200 ppm DFA is expressed in BYI 02960 equivalents, it equates to 38 and 47 mg/kg/day in males and females, respectively. Therefore, DFA was not more toxic than BYI 02960 after subchronic administration to the rat.

BYI 02960-difluoroethyl-amino-furanone (DFEAF) is a minor plant metabolite. The acute oral LD50 in rats was higher than 2000 mg/kg, indicating that DFEAF is not more toxic than BYI 02960.

BYI 02960-CHMP ((6-chloro-3-pyridyl)methanol) is a plant metabolite. The acute oral rat LD50 was 1842 mg/kg in males and 1483 mg/kg in females. In a 90-day rat study, BYI 02960-CHMP was administered in the diet to Sprague Dawley rats (10/sex/group) at concentrations of 160, 800, 4000 and 20 000 ppm, (corresponding to 9.9, 48.9, 250.1 and 1246.6 mg/kg/day, respectively for males and 11.1, 55.9, 275.9 and 1173.7 mg/kg/day, respectively for females). Mean body weights and mean food consumption were decreased at 20000 ppm in both sexes. Based on eosinophilic intranuclear inclusions in the proximal tubular epithelium of kidneys at 20000 ppm and 4000 ppm, the no observed effects level (NOEL) was 800 ppm (48.9 mg/kg/day) in males, and 4000 ppm (275.9 mg/kg/day) in females, respectively. When the NOEL is expressed in BYI 02960 equivalents, it equates to 97.8 and 551.8 mg/kg/day, respectively. Therefore, BYI 02960-CHMP was less toxic than BYI 02960 after subchronic administration to the rat.

6-CNA (6-chloronicotinic acid) is a major soil and plant metabolite. An acute oral rat toxicity study and an Ames test were performed on this metabolite for the registration of acetamiprid. 6-CNA was not genotoxic and not acutely toxic."

RMS agrees that no separate risk assessment is required for the metabolites.

B.9.3.4 Risk assessment for terrestrial vertebrates other than birds

The risk assessment procedure follows the EFSA Guidance Document on Risk Assessment for Birds & Mammals (2009).

At Tier 1, the risk is considered acceptable, if the 'Toxicity Exposure Ratio' (TER) value pass the trigger values of ≥ 10 for acute exposure and ≥ 5 for chronic exposure.

If the TER values are below these a-priori acceptability trigger values in certain areas, a refined risk assessment based on more relevant and realistic conditions is performed for those particular areas.

Routes of exposure

The formulation is to be sprayed on hops in the field and on lettuce in glasshouses and in the field. The following routes are considered:

- direct dietary exposure: scenarios for hop and leafy vegetables – field uses only
- direct exposure via drinking water: field and glasshouse uses
- indirect exposure by consumption of earthworms and fish containing residues of BYI 02960 field and glasshouse uses

B.9.3.4.1 Exposure via feed

The first tier risk assessment calculation of is performed in the same way as for birds. The risk assessment procedure follows the EFSA Guidance Document on Risk Assessment for Birds & Mammals (2009). The methodology is described in B.9.1.5.

Mammalian generic focal species for Tier 1 risk assessment

In the field, the product is intended to be used in hop at an application rate of 1 x 0.150 kg a.i./ha, BBCH 31-75, and in lettuce at an application rate of 1 x 0.125 kg a.i./ha, BBCH 12-49. According to the EFSA Guidance Document on Risk Assessment for Birds & Mammals (2009) the following generic focal species have to be addressed in the risk assessment.

Table B.9.3.4-1 Relevant generic mammalian focal species for Tier 1 risk assessment

Crop	Growth stage (BBCH)	Generic focal species	Representative species	Shortcut value	
				For long-term RA based on RUD mean	For acute RA based on RUD 90th perc.
Hops	≥20	Small insectivorous mammal "shrew"	Common shrew	**1.9**	**5.4**
	≥ 40	Small herbivorous mammal "vole"	Common vole	**21.7**	**40.9**
	20 - 39	Small omnivorous mammal "mouse"	Wood mouse	**3.9**	**8.6**
	≥ 40	Small omnivorous mammal "mouse"	Wood mouse	2.3	5.2
Lettuce (Leafy vegetables)	10-19	Small omnivorous mammal "shrew"	Common shrew	**4.2**	**7.6**
	≥20	Small insectivorous mammal "shrew"	Common shrew	1.9	5.4
	40-49	Small herbivorous mammal "vole"	Common vole	**72.3**	**136.4**
	≥ 50	Small herbivorous mammal "vole"	Common vole	21.7	40.9
	All season	Large herbivorous mammal "lagomorph"	Rabbit	**14.3**	**35.1**
	10-49	Small omnivorous mammal "mouse"	Wood mouse	**7.8**	**17.2**
	≥ 50	Small omnivorous mammal "mouse"	Wood mouse	2.3	5.2

Bold values were used for Tier 1 risk assessment. Where the same focal species is representative for different BBCH stages, only the worst-case SV values were chosen for risk assessment.

The acute and long-term risk are calculated in the tables below.

Table B.9.3.4-2 Tier 1 acute DDD and TER calculation for mammals

Crop	Generic focal species	LD_{50} [mg/kg bw]	DDD			DDD	TER_A	Trigger
			Appl. rate [kg/ha]	SV_{90}	MAF_{90}			
BYI 02960								
Hops (≥20)	Small insectivorous mammal “shrew”	1607	0.150	5.4	1	0.81	1984	10
Hops (≥ 40)	Small herbivorous mammal "vole"			40.9		6.14	262	
Hops (20 – 39)	Small omnivorous mammal “mouse”			8.6		1.29	1246	
Lettuce (≥20)	Small insectivorous mammal “shrew”	1607	0.125	5.4	1	0.68	2381	10
Lettuce (40-49)	Small herbivorous mammal "vole"			136.4		17.05	94	
Lettuce (All season)	Large herbivorous mammal “lagomorph”			35.1		4.39	366	
Lettuce (10-49)	Small omnivorous mammal “mouse”			17.2		2.15	747	

All TER values for both crops are well above the trigger of 10 for acute exposure. Hence, no unacceptable risk is to be expected from the use of the product according to the intended use pattern.

Table B.9.3.4-3 Tier 1 long-term DDD and TER_{LT} calculation for mammals

Crop	Generic focal species	NOAEL [mg/kg bw/d]	DDD				DDD	TER_{LT}	Trigger
			Appl. rate [kg/ha]	SV_m	MAF_m	f_{TWA}			
BYI 02960									
Hops (≥20)	Small insectivorous mammal "shrew"	~~34~~ 6.4	0.150	1.9	1	0.53	0.151	~~225~~ 42	5
Hops (≥ 40)	Small herbivorous mammal "vole"			21.7			1.73	~~20~~ **3.7**	
Hops (20 – 39)	Small omnivorous mammal "mouse"			3.9			0.31	~~110~~ 21	
Lettuce (≥20)	Small insectivorous mammal "shrew"	~~34~~ 6.4	0.125	1.9	1	0.53	0.13	~~270~~ 51	5
Lettuce (40-49)	Small herbivorous mammal "vole"			72.3			4.79	~~7.1~~ **1.3**	
Lettuce (All season)	Large herbivorous mammal "lagomorph"			14.3			0.95	~~36~~ 6.8	
Lettuce (10-49)	Small omnivorous mammal "mouse"			7.8			0.52	~~66~~ 12.4	

Bold values do not meet the trigger

All TER values for both crops are above the trigger of 5 for long-term exposure, except for the generic focal species the "vole". ~~Hence, no unacceptable risk is to be expected from the use of the product according to the intended use pattern.~~

Based on the first tier endpoint of 7 mg/kg bw/d proposed originally by the notifier, a risk would be identified for small herbivorous mammals. Therefore, the notifier has proposed two further refinements:

I. measured residue data that allow refinement of the time-weighted average residue concentrations (21-d fTWA) of BYI 02960 on foliage as potential diet of small herbivorous mammals
II. a more realistic evaluation of the focal species exposure scenario (relevance of hop yards and lettuce fields for voles), based on general knowledge from literature and field study results.

<u>I) Refined DT50 of BYI 02960 on foliage</u>

According to the recommendations of the EFSA GD (2009), the Tier 1 risk assessment has been conducted with a "generic" DT50 of 10 days of BYI 02960 on all food items. However, measured residue decline data (B.7.6.2 study 1 to 4 KIIIA1 6.3.1/01-04 for lettuce, and B.9.3.2 Study IIIA

10.3.3/02 for cereals) are available that allow the calculation of compound-specific DT50 values for BYI 02960.

From the available residue decline data, it is proposed to select substrates and application types that can be considered representative for foliage as food of small herbivorous mammals in lettuce fields (i.e., spray application residue trial results) and in hop yards (spray application residue trials in young cereals as surrogate for wild undergrowth).

With these data, specific DT50 values for BYI 02960 were calculated by Scherr & Ellerich (2013),; lettuce: KIIIA 10.3.3/03; and Sur & Ellerich (2012), cereals: KIIIA 10.3.3/04) according to best practice in environmental modelling. The residue decline data were analysed with the software package KinGUI, which is a standard tool used for kinetic evaluations. In the first place, fits using the single first order (SFO) model were performed. Their quality was assessed visually and also based on statistical significance of the individual parameters. In case of clear biphasic behaviour in the respective residue data, a DFOP fit (double-first order in parallel) was also performed. Also here, visual acceptability and statistical significance were evaluated.

Residue decline data were analysed both for flupyradifurone only and for total residues of flupyradifurone including also metabolites DFA and DFEAF.

In all trials residues were determined after the second treatment, but in four trials residues were also determined after the first application and DT50 values were estimated for each decline interval.

As a result of KinGUI evaluation, 13 trials in lettuce provided SFO – DT50 values and 5 trials provided a DFOP-DT50. One trial provided an SFO-DT50 value after the first application and an DFOP-DT50 after the second application.

In young cereals, 3 trials provided SFO – DT50 values and 1 trial provided a DFOP-DT50.

Half-live (DT50) values from SFO and DFOP cannot be simply mathematically averaged in order to generate an overall DT50 to be used in time-weighted average (TWA) calculations. Since the (single) DT50 from SFO fits cannot simply be averaged with the (double) DT50 from DFOP fits, calculations of the 21-d fTWA were conducted for each of the trials, and the 17 resulting 21-d fTWA values were combined afterwards. With this approach the full set of available data can be included in the refined exposure assessment.

The TWA values themselves were thus calculated in a second step as follows. First, numerical evaluation of predicted residues in time (with timestep of 0.1 days) was performed, employing the respective kinetic type and the relevant degradation parameter(s) - DT50 value(s) and g-factor (for DFOP). These predicted residues are then used for the 21-d fTWA evaluation per data set and averaged afterwards.

Lettuce: overall, 18 residue dissipation trials with BYI 02960 were available for lettuce. In 13 of these trials, the residue dissipation could be considered according to SFO, whilst the remaining 5 trial results needed fitting with DFOP. Furthermore, one trial provided an SFO-DT50 value after the first application and an DFOP-DT50 after the second application. Since the (single) DT50 from SFO fits

cannot simply be averaged with the (double) DT50 from DFOP fits, calculations of the 21-d fTWA were conducted for each of the trials, and the 17 resulting 21-d fTWA values were combined afterwards (for each trial the value after the second application was taken). Due to the number of trials in the evaluation it is proposed to use the geometric mean 21-d fTWA = 0.17 for the refined long-term exposure assessment for small herbivorous mammals in lettuce fields (Table B.9.3.4.-4).

Table B.9.3.4-4 DT_{50} and 21-d f_{TWA} for residue dissipation of total flupyradifurone on lettuce

Trial code	**Trial description**	**SFO DT_{50}**	**DFOP DT_{50fast}**	**DFOP DT_{50slow}**	**21-d f_{TWA}**
R01	10-2223-01, N-EU	2.08	-	-	0.14
R02	10-2223-02, N-EU	-	0.06	7.69	0.14
R03	10-2223-03, N-EU	3.58	-	-	0.24
R04	10-2223-04, N-EU		0.100	9.850	0.35
R05	10-2223-05, N-EU	-	0.19	11.73	0.16
R06	11-2082-01, N-EU	3.25	-	-	0.22
R07	11-2082-02, N-EU		0.22	5.53	0.041
R18 (1st)	11-2082-03, N-EU	5.04			0.33
R18	11-2082-03, N-EU	6.15			0.38
R08 (1st)	11-2082-03, N-EU		1.09	6.51	0.12
R08	11-2082-04, N-EU	-	0.26	4.51	0.093
R09	10-2213-01, S-EU	-	0.06	3.95	0.077
R10	10-2213-02, S-EU	5.55	-	-	0.35
R11	10-2213-03, S-EU	1.68	-	-	0.12
R12	10-2213-04, S-EU	5.22	-	-	0.34
R13	10-2213-01, S-EU	1.91	-	-	0.13
R14	11-2071-01, S-EU	4.40	-	-	0.29
R15	11-2071-02, S-EU	2.67	-	-	0.18
R16 (1st)	11-2071-03, S-EU	2.24	-	-	0.15
R16	11-2071-03, S-EU	-	1.01	9.11	0.13
R17 (1st)	11-2071-04, S-EU	-	0.09	4.33	0.074
Geometric mean 21-d f_{TWA} (only ftwa values after second application included)					**0.17**

However, some uncertainty remains on whether this value is applicable to the full application period. In the GAP, application in lettuce is proposed between BBCH 12-49. The notifier states that the inclusion of the evaluation of some residue decline after the first application and the fact that there is no large difference between the decline after the first and the second application, addresses the question of potential impact of the application stage on the decline curves. To investigate this statement, RMS checked the lettuce trials to see at which BBCH-stages they were performed. See Table B.9.3.4-5.

Table B.9.3.4-5 BBCH stages at application in lettuce trials (shaded trials were analysed both after the first and after the second application)

Trial code	Trial no.	BBCH at first application	BBCH at second application
R01	10-2223-01	41	48
R02	10-2223-02	42	48
R03	10-2223-03	42	48
R04	10-2223-04	43	48

R05	10-2223-05	46	48
R06	10-2082-01	46	48
R07	10-2082-02	43	48
R18	10-2082-03	47	48
R08	10-2082-04	43	48
R09	10-2213-01	41	48
R10	10-2213-02	48	49
R11	10-2213-03	42	46
R12	10-2213-04	44	49
R13	10-2213-05	47	49
R14	10-2071-01	47	49
R15	10-2071-02	17	46
R16	10-2071-03	43	48
R17	10-2071-04	17	49

The table shows that only one of the four doubly analysed trials was performed at a relatively early BBCH stage. In the view of the RMS, this is not enough proof that the residue decline trials really cover also early application stages (from BBCH 12). If these data are used in a refined risk assessment, this issue should be addressed further.

Cereals as surrogate for undergrowth in hop yards: overall, 4 residue dissipation trials with BYI 02960 were available for young cereals. In 3 of these trials, the residue dissipation could be considered according to SFO, whilst in the remaining trial results needed fitting with DFOP. Since the (single) DT50 from SFO fits cannot simply be averaged with the (double) DT50 from DFOP fits, calculations of the 21-d fTWA were conducted for each of the trials, and the 4 resulting 21-d fTWA values were combined afterwards. Due to the number of trials in the evaluation it is proposed to select the median 21-d fTWA = 0.15 for the refined long-term exposure assessment for small herbivorous mammals feeding on undergrowth in hop yards (Table B.9.4.3-5). The trials were performed at BBCH 25-29, meaning that the cereal plants were in the tillering stage. RMS considers this reasonably representative for the grass that is expected to form the undergrowth vegetation of hop plantations.

Table B.9.3.4-5 DT_{50} and 21-d f_{TWA} for residue dissipation of total flupyradifurone on young cereals

Trial code	**Trial description**	**SFO DT_{50}**	**DFOP $DT_{50\ fast}$**	**DFOP $DT_{50\ slow}$**	**21-d f_{TWA}**
R01	11-2958-01, N-EU	-	0.145	3.555	0.0649
R02	10-2958-02, N-EU	1.44	-	-	0.10
R03	10-2958-03, N-EU	4.22	-	-	0.28
R04	10-2958-04, S-EU	3.15	-	-	0.21
Median 21-d f_{TWA}					**0.15**

The two residue data sets result in very similar 21-d f_{TWA} values (0.17 and 0.15).
~~However, the residue decline data are not used, because based on the NOEAL of 34 mg/kg bw/d, a refined risk assessment is not necessary.~~

Expert consultation during Pesticide Peer Review meeting 124, ecotoxicology, December 2014:

The approach described above, for deriving overall ftwa values from the lettuce and cereal trials, was discussed in the expert meeting. It was questioned whether the approach taken by the RMS was reasonable as a refinement. The studies performed on lettuce were questioned as food items could be weeds including monocots. The RMS highlighted that trials on cereals were also available and the difference in the fTWA was minor indicating not too dissimilar speed of degradation/dissipation. It was noted that 18 trials were available for lettuce and only 4 for cereals. It was discussed whether the scenarios in the GD cover mammals consuming the lettuce crop plants in addition to weeds. Experts noted that different FIR would be needed for a lettuce scenario. The experts agreed that, when refining the assessment, the food items taken by the focal species should be considered.

The representativeness of the studies used was discussed noting that the studies were not performed at the correct growth stage. The lettuce trials were performed at BBCH 17 to 46 whereas the GAP is BBCH 12 to 49 (lettuce). The RMS considered this in the DAR and noted that the lettuce trials do not cover the entire GAP for lettuce. Furthermore, the RMS noted during the meeting that it could be that at later growth stages the dissipation is slower due to slower growth. Some experts did not consider the BBCH stage of the crop to be an issue as the studies should represent weeds in addition to the crop. Furthermore, the DT50 in the two types of trials were not too dissimilar.

The cereal trials were performed at BBCH 25 to 29 and therefore were at a growth stage palatable to birds and mammals. The RMS considered that this was reasonable to represent weeds. The fTWA from both trials were comparable regardless of the growth stage further supporting the use of the studies.

Overall the experts agreed that the available studies were suitable to be used for the refined assessment for the representative uses.

It was discussed how the fTWA values should be used in the risk assessment (geomean, pooled, lower, two assessments). The geomean was questioned as it does not result in a worst case value but then the RMS noted that there were more than ten values indicating that the geomean was reasonable (NL approach). The RMS also noted that the studies needed to cover the entire EU. Some studies were performed in NEU and SEU, no differences were noted. Overall, the experts considered that the data were sufficient for N and SEU. The RMS suggested that the data could be pooled and then the geomean from the pooled data used. The experts noted that pooling data should be done with care and therefore it is difficult to agree on the basis of the available raw data. It was agreed that the RMS should pool the data but check statistically significant differences between the two data sets (lettuce

crop vs. cereals and N vs. SEU) whether the pooled data are ok. If so, then the geomean can be taken. If not, then the experts considered that separate risk assessments may be required.

It was agreed that the data was suitable for all weed types and lettuce crop plants given that the data cover monocots and dicots and that the DT50 may not depend on the structure of the plant.

On the basis of the above, the RMS needs to update the risk assessment using the appropriate fTWA.

It was noted that the approach in which the 21-d ftwa is calculated from measurements which started at the last application (in trials with two applications) is acceptable for the current GAP, since this has only one application per year. It was discussed that it may not cover GAPs which have multiple applications, as the 21-d exposure window may be higher at an earlier stage. Therefore, for other GAPs it needs to be carefully considered how to use these data.

The differences between the data sets were checked by the RMS (t-test, unequal variance, two-sided): lettuce N-lettuce S; cereal N- cereal S (note that only four trials were available here so this exercise is less useful); all trials lettuce-cereals; all trials N-S. Result: P>0.1 in all cases. Therefore, the data can be pooled and the overal geometric mean can be taken for all weed types and lettuce crop plants. Thus, in the refined risk assessment for the small herbivorous mammal “vole”, an ftwa of 0.164 can used for the representative uses.

Table B.9.3.4-6 Ftwa values for the different residue trials and mean values

trial no.	**N/S**	**ftwa**	
Lettuce			
R01	N	0.14	
R02	N	0.14	
R03	N	0.24	
R04	N	0.35	
R05	N	0.16	
R06	N	0.22	
R07	N	0.041	
R18	N	0.38	
R08	N	0.093	
R09	S	0.077	
R10	S	0.35	
R11	S	0.12	
R12	S	0.35	
R13	S	0.13	
R14	S	0.29	
R15	S	0.18	
R16	S	0.13	
			geometric mean lettuce trials: 0.17
Cereals			

R01	N	0.0649	
R02	N	0.1	
R03	S	0.28	
R04	S	0.21	
			median cereal trials: 0.16
			overall geometric mean: 0.164

The shortcut value which was used in the first tier calculation is calculated as SV = FIR/bw * deposition factor * RUD. In the first tier table from the EFSA GD (Appendix A), the shortcut value for the “vole” in “leafy vegetables” at BBCH 40-49 is 72.3. This is derived as follows:

FIR/bw = 1.33 (based on 100% grass intake and 25 g body weight)

Deposition factor = 1 for lettuce (no interception), 0.3 for hops (70% interception)

RUD = 54.2 (default RUD from “grass + cereal” database).

In the refined risk assessment, it needs to be considered if these values are still appropiate.

The deposition values are dependent on crop stage and therefore still relevant. Note that for lettuce, interception should not be taken into account at any stage for the part of the diet that consists of crop plants.

The “vole” foraging in lettuce fields may feed on all types of plants, not only grass. The FIR/bw for a 25 g mammal feeding 100% on ‘non-grass herbs’ is 1.61 (calculated according to Appendix G). Therefore, the first tier FIR/bw of 1.33 may slightly underestimate the risk. However, the default RUD value for the food category “non-grass herbs” is 28.7, which is almost half that of “grass”. Therefore, the calculations based only on 100% “grass” intake are clearly worst-case even though the diet composition of the “vole” in lettuce fields is not known, and as long as the default RUDs are used it is not necessary to use a different FIR/bw in the refined calculations.

Table B.9.3.4-7 Refined long-term DDD and TER_{LT} calculation for the "vole"

Crop	Generic focal species	NOAEL [mg/kg bw/d]	DDD				DDD	TER_{LT}	Trigger
			Appl. rate [kg/ha]	SV_m	MAF_m	f_{TWA}			
BYI 02960									
Hops (≥ 40)	Small herbivorous mammal "vole"	6.4	0.150	21.7	1	0.164	0.53	12	5
Lettuce (40-49)	Small herbivorous mammal "vole"	6.4	0.125	72.3	1	0.164	1.48	**4.3**	5

Bold values do not meet the trigger

The refined TER value for the use in hops is above the trigger, indicating acceptable risk. For the use in lettuce the TER is below the trigger. The applicant provided a statement to address the risk.

Relevance of the "vole" scenario in hops and lettuce fields

The notifier submitted a statement which is presented without modifications below. Please note that the TER values are based on the worst-case endpoint of 7.8 mg/kg bw/d.

"*The risk to wild mammals from the use of BYI 02960 has been evaluated according to the Tier 1 scenarios provided in the EFSA GD. These scenarios include small insectivorous mammals ("shrew"), small omnivorous mammals ("mouse"), large herbivorous mammals ("lagomorph"), and small herbivorous mammals ("vole").*

*Whilst all other scenarios demonstrated adequate margins of safety, even with the worst case settings of a Tier 1 assessment, the scenario "vole" resulted in Tier 1 TERLT values of 4.5 and 1.6 for the uses in hop and lettuce, respectively. These Tier 1 TERLT values for voles exceed a threshold of 1, indicating that some margins of safety exist even under the worst case settings applied at Tier 1. Quantitative refinement elements (refined reproductive risk assessment endpoint 39.23 mg/kg/bw/d; measured residue decline DT50 resulting in 21-d fTWA = 0.19) that have been introduced above allow a re-calculation of the Tier 2 TERLT values that include margins of safety clearly in excess of the a-priori acceptability trigger of 5 (*values not shown here*).*

Thus, a quantitative refinement at the level of individual exposure and risk can be conducted to demonstrate acceptable risk at the level of individual voles.

However, the protection goal in ecotoxicology aims ultimately at protection of populations rather than individuals. Therefore the section below is intended to additionally evaluate the risk to vole populations from exposure to BYI 02960 after application in hop or lettuce fields. This evaluation is based on the

well known biology and ecology of Common voles (Microtus arvalis) as the representative species "behind" the generic focal species scenario "vole", and complemented with a targeted field study in hop yards.

Exposure of Populations

The optimum or prime habitat of the common vole requires permanent vegetation cover and includes grassland or perennial crops such as alfalfa and clover fields. Common voles also occur in sub-optimal (secondary) habitats such as arable land. However, only prime habitats harbor permanent vole populations and are essential for the survival of common vole populations. Secondary habitats are only transiently populated, with regular population declines up to extinction during agronomic operations (e.g., ploughing or harvest), or over winter.

Wherever the species occurs; the population densities of Common voles vary seasonally, with regular mass occurrences being followed by a population break-down which can even lead to local extinction.

Spring crops like lettuce fields are not populated during winter, would not provide much cover against predation and could thus be colonized only from adjacent prime habitats at times of high population density. Spring sown vegetable fields would therefore not belong to the potential prime habitats of Common voles (e.g. Spitz, 1977[3]).

A field study conducted in hop yards in Germany (KIIIA1 10.3.3/01) also confirms that the treated area does not harbor significant proportions of the local vole populations.

Thus, any effect from exposure of voles in areas treated with BYI 02960 would be of low, if any, importance on the local population level which depends on the voles living outside of the lettuce fields or the hop yards.

Vulnerability of populations:

The failure to demonstrate TERLT > 5 for voles is not a rare event, due to the worst case combination of factors involved in the scenario. Actually, in regard of theoretical exposure of individual animals, the vole scenarios are nearly always the most critical all over the EFSA GD (2009). However, with regard of the vulnerability of vole populations, the vole scenario is probably the least critical.

The Joint Working Group on the Guidance Document on Risk Assessment for Birds & Mammals (SANCO 10997/2009) has already raised the question on the "need for the vole scenario… given the resilience of the vole populations"; i.e. well-known fact that voles are able to recover after large population breakdowns, or despite eradication programs with targeted rodenticide use (for recent evaluations see e.g.. Jacob & Tkadlec 2010[4], or Jokic et al. 2010[5]).

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[3] Spitz F (1977), Le campagnol des champs (*Microtus arvalis* (Pallas)) en Europe. EPPO Bulletin 7:156-175. BCS Edition no. M-228600-01-1

[4] Jacob & Tkadlec (2010), Rodent outbreaks in Europe: dynamics and damage. In:Singleton et al. (Eds): Rodent outbreaks: Ecology and Impacts.,p: 217-233. BCS Edition no: M-427173-01-1

[5] Jokic G, Vuksa P & Vuksa M (2010), Comparative efficacy of conventional and new rodenticides against Microtus arvalis (Pallas, 1778) in wheat and alfalfa crops. Crop Protection 29: 487-491. BCS Edition no: M-427179-01-1

This potential for recovery of Common voles is extraordinarily large, due to the ability of Common voles to achieve high population numbers within short periods of time thanks to the reproduction biology of the species (e.g. Lauenstein 1979[6], Jacob 2003[7]):

- *the potentially highest number of young per litter ranges between 12 and 13. The weight of a litter is about 53% of the females weight*
- *average number of young per litter in the field is about 7*
- *gestation period is about 20 days in the field*
- *successful mating occurs when young females are not yet weaned*
- *long reproduction period including occasional winter reproduction*

The most straightforward application of the conclusion by the Joint Working Group on the Guidance Document on Risk Assessment for Birds & Mammals (SANCO 10997/2009) (questioning the "need for the vole scenario… given the resilience of the vole populations)" would be that the failure to meet the a-priori acceptability trigger for long-term exposure of individual voles is not to be considered as problematic as long as the TER trigger for the other wild mammal scenarios reach the respective TER trigger.

This is clearly the case for exposure of wild mammals to BYI 02960.

Small herbivorous mammals in hops

A targeted field study has been conducted in hop yards in Germany (KIIIA1 10.3.3/01).

Live trapping revealed that hop yards do not harbor significant proportions of small herbivorous mammals (Common vole, Field vole or Bank vole), as only 0.09% (3 of 3272) of the individuals of the local vole populations was recorded in-field. Radio-tracking confirmed that voles make very little use of hop yards as feeding habitat. Only one of 10 voles (1 of 5 common voles) potentially spent foraging time in a hop yard. This individual was only trapped once, thus considered a disperser rather than part of a local in-field population.

Overall the results of the study do not confirm that the generic focal species scenario of "small herbivorous mammals (vole)" provided in the EFSA GD 2009 would be relevant for hop yards. To the contrary, a very low number of individual voles (< 0.1%) would be exposed on hop yards and the risk on local populations from effects on these individuals would be negligible.

Small herbivorous mammals in leafy vegetables

The prime habitat of voles is grassland but the species can also be found in cultivated areas. Stable populations can be developed in multi-annual crops, particularly clover/alfalfa fields which perfectly match the species ecological needs. Whilst autumn sown fields might serve as overwintering habitat, spring sown fields would only be colonized later in the season (summer) and only during periods of high population density, then representing rather a "sink" than a "source" habitat for the local populations). "

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6 Lauenstein G (1979), Zur Problematik der Bekämpfung von Feldmäusen (Microtus arvalis Pall.)) auf Grünland. Zeitschrift für angewandte Zoologie 66: 35-59. BCS Edition no M-419441-01-1

7 Jacob J (2003), Short-term effects of farming practices on populations of common voles. Agriculture, Ecosystems and Environment 95: 321-325. BCS Edition no. M-415511-01-1

Response RMS: The first tier risk assessment based on the ecologically relevant endpoint of 34 mg/kg bw/d was acceptable in the original DAR, so the public literature referred to was not evaluated. The monitoring trial in hop fields was however evaluated above (Study IIIA, 10.3.3/01).
The live trapping of mammals showed that they preferred the natural habitats instead of the hop yard. The wood mouse was the most abundant species inside the hop yard and was the only species tracked regularly inside the hop yard. Five common voles were radiotracked; one of those spent all its time in the hop yard. This individual, a fertile male, used the habitat hop yard and spent 100% of the 24 h telemetry in it. This individual seemed to be a so-called disperser trapped on study site 4, it was the only common vole trapped on this site. In late July the undersown crops Raphanus sativa, Galinsoga spec, and Chenopodiaceae were already high and therefore the predation risk inside the hop yard decreased. The population densities of common voles in the surroundings in late July was high and the need for new territories increased. These factors indicate that this individual was migrating and used the hop yard not as a sustainable habitat. However, this finding means that common voles can use hop fields as feeding habitat, at least occasionally. Therefore, RMS does not agree with the notifier that the study shows that the common vole is not a relevant species in hop fields.
It should also be noted that the results from this study (for both mammals and birds) are based on hop yards close to nature areas. It is not clear if the same focal species can be used for hop yards in an intensively cultivated area, if present at all. Mammal trapping was only conducted in the hop yards close to nature, therefore it is not possible to compare species abundance between plots in intensively cultivated area and plots close to nature areas.
If the study is used in a refined risk assessment, these remarks should be taken into account.

Furthermore, in case of higher tier, EFSA (2009) requires an uncertainty analysis of the refinements (see 6.8 and 6.9).This is however not available in the dossier. Should a refined risk assessment become necessary (e.g. by change of the endpoint after peer review), an uncertainty analysis should be provided, in the tabular form recommended by EFSA.

Based on the new long-term endpoint for mammals, the TER for the small herbivorous mammal “vole” from the use of the a.s. in lettuce is 4.3, which is below the trigger of 5. The notifier questions the relevance of the “vole” scenario in general. This issue should be considered by a dedicated EFSA working group or the PPR panel. As long as the guidance document includes the vole scenario, it has to be taken into account. Non-relevance of a species (group) can be shown with field monitoring data, but these are not available for mammals in lettuce fields. Population effects can be investigated with field effect data and/or population modelling, but these are not available either. Based on the available data, a risk to the “vole” cannot be excluded. It is noted that the TER is not far below the trigger, that the only refinement included is one on residue dissipation, that the TER is calculated with the lowest

available long-term endpoint which is based on <10% lower bodyweight at the next level, and that the diet composition use in the calculations is worst-case.

B.9.3.4.2 Exposure via drinking water

An assessment of the risk potentially posed by consumption of contaminated drinking water is required according to the EFSA Guidance Document for Birds and Mammals (2009).
Two scenarios were identified as relevant for assessing the risk of pesticides via drinking water to birds and mammals:

- Leaf scenario, only relevant for birds possibly drinking water from puddles in leaf whorls after application of a pesticide to a crop and subsequent rainfall or irrigation; not relevant for mammals.
- Puddle scenario. Birds and mammals taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil. This scenario is relevant for all types of applications that may cause contamination of soil and only for long-term exposure. A risk assessment is performed below.

Risk assessment for the puddle scenario (field use in hop and lettuce):

An "escape clause" recommended in the EFSA Guidance Document for Birds and Mammals (2009) allows for screening the need for a quantitative risk assessment by a comparison between the application rate and the toxicity of the respective substance. This escape clause specifies that "*due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals …, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (= application rate x MAF) (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≥ 500 L/kg).*" [8].

Table B.3.4-~~6~~8 Evaluation of potential concern for exposure of mammals drinking water from puddles (escape clause)

Compound	Koc [mL/g]	Application rate * MAF [g a.i./ha]	LD50 or NO(A)EL [mg a.i./ kg bw/d]	Ratio (Application rate * MAF) / NO(A)EL	"Escape clause" No concern if ratio	Conclusion
BYI 02960	98.4 [1)]	150 [2)] x 1	1607	0.09	≤ 50	No concern
			~~34~~ 6.4	~~4.4~~ 23	≤ 50	No concern

[8] EFSA (2009): Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA, p. 66

1) Arithmetic mean of ten K_{OC} values (see B.8)
2) Worst-case application rate in hops

This evaluation confirms that the risk for mammals from drinking from puddles that may contain residues from the use of product is acceptable.

B.9.3.4.3 Bioaccumulation and food chain behaviour: Indirect exposure via contaminated worms or fish

Substances with a high bioaccumulation potential could theoretically bear a risk of secondary poisoning for mammals if feeding on contaminated prey like fish or earthworms. For organic chemicals, a log POW > 3 is used to trigger an in-depth evaluation of the potential for bioaccumulation. As shown in B.9.1.5.3, neither the active ingredient nor any of its metabolites has a log Pow above 3 and the potential of bioaccumulation is considered to be very low. Hence, there is no need to evaluate potential effects of secondary poisoning of mammals.

B.9.4 Effects on bees (IIA 8.7, IIIA 10.4)

B.9.4.1 Toxicity

B.9.4.1.1 Toxicity of the active substance and metabolites (IIA 8.7.1 and IIA 8.7.2)

STUDY IIA, 8.7.1/01

Characteristics

reference	:	Schmitzer S. (2012)	nominal dose		Contact: 12.5, 25, 50, 100 and 200 µg a.s./bee; Oral: 0.16, 0.31, 0.63, 1.3, 2.5 and 5.0 µg a.s./bee
type of study	:	Acute oral and contact toxicity	species	:	Honey bee (*apis mellifera*)
year of execution	:	2008	exposure duration	:	48 (oral) or 96 (contact) hours
GLP statement	:	Yes	dosing method	:	Contact: Stock solution in acetone; Oral: Stock solution in acetone added to sucrose solution
guideline	:	OECD 213 and OECD 214	acceptability	:	Acceptable
test substance	:	BYI 02960; batch code BYI 0296-01-01*; original batch no. NLL 7780-44-6;	LD50 (oral)	:	1.2 µg a.s./bee
a.s. content	:	99.5%	LD50 (contact)	:	122.8 µg a.s./bee

*The batch used was originally not reported in Document J (confidential information). The applicant submitted additional information (which is included in the revised Volume 4) which shows that this batch is equivalent to the technical specification of flupyradifurone.

Methods

A 48-hour acute oral and contact toxicity test on honeybees (*Apis mellifera*) was conducted with BYI 02960. In the oral toxicity test 3 replicates of ten bees each per concentration were exposed to a nominal dose of 0.16, 0.31, 0.63, 1.3, 2.5 and 5.0 µg a.s./bee, with untreated (50% sugar syrup in water), solvent (50% sugar syrup, 5% acetone, 45% water) and positive control (dimethoate, 0.05, 0.08, 0.15 and 0.30 µg a.s./bee). In the contact toxicity test, 3 replicates of ten bees each were exposed to a dose of 12.5, 25, 50, 100 and 200 µg a.s./bee, with water treated control (tap water with 0.5% Adhäsit), solvent control (acetone) and positive control (dimethoate, 0.10, 0.15, 0.20 and 0.30 µg a.s./bee). Due to increasing mortality between 24 and 48 hours, and between 48 and 72 hours, the contact test was prolonged to 96 hours.

Results

In the oral toxicity test, the actual consumed dose was 0.17, 0.34, 0.68, 1.3, 2.1 and 2.8 µg a.s./bee. In the oral toxicity test, discoordinated movements and apathy occurred at 0.68, 1.3, 2.1 and 2.8 µg a.s./bee. In the contact toxicity test, discoordinated movements occurred at 50, 100 and 200 µg a.s./bee. The positive control gave the expected response in both tests (24-h oral and contact LD50 0.13 and 0.17 µg a.s./bee, respectively). The remaining results are summarised in the Table below.

Table B.9.4.1.1-01 The acute oral and contact toxicity of BYI 02960 to honeybees (*Apis mellifera*)

Contact toxicity test					Oral toxicity test		
dosage	Mortality [%]				dosage	Mortality [%]	
[µg a.i./ bee]	24 h	48 h	72 h	96 h	[µg a.i./ bee]	24 h	48 h
test item					test item		
200.0	0.0	36.7	60.0	73.3	2.8	100.0	100.0
100.0	3.3	23.3	33.3	36.7	2.1	90.0	90.0
50.0	6.7	6.7	20.0	23.3	1.3	53.3	53.3
25.0	0.0	6.7	13.3	13.3	0.68	6.7	6.7
12.5	0.0	0.0	0.0	6.7	0.34	0.0	0.0
					0.17	0.0	3.3
LD_{50} [µg/bee]	> 200	> 200	158.4	122.8	LD_{50} [µg/bee]	1.3	1.2
NOED [µg/bee]	> 200	50.0	25.0	50.0	NOED [µg/bee]	0.68	0.68
water-treated control	0.0	0.0	6.7	10.0	water-treated control	0.0	0.0
solvent control	0.0	0.0	0.0	3.3	solvent control	0.0	0.0

The NOED was estimated using Fisher Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$).

Conclusion

48-hour oral LD50: 1.2 µg a.s./bee; 96-h contact LD50 122.8 µg a.s./bee.

Guidelines and limitations

The test is acceptable.

STUDY IIA, 8.7.1/02

Characteristics

reference	:	Schmitzer S. (2010a)	nominal dose		Contact and oral: 6.3, 12.5, 25, 50 and 100 µg pure metabolite/bee
type of study	:	Acute oral and contact toxicity	species	:	Honey bee (*apis mellifera*)
year of execution	:	2010	exposure duration	:	48 hours
GLP statement	:	Yes	dosing method	:	Contact: Stock solution in acetone; Oral: Stock solution in acetone added to sucrose solution
guideline	:	OECD 213 and OECD 214	acceptability	:	Acceptable
test substance	:	BYI02960-difluoroethyl-amino-furanone; batch BCS-CC98193-PU-01	LD50 (oral)	:	>81.5 µg pure metabolite/bee
a.s. content	:	99.2%	LD50 (contact)	:	>100 µg pure metabolite/bee

Methods

A 48-hour acute oral and contact toxicity test on honeybees (*Apis mellifera*) was conducted with BYI02960-difluoroethyl-amino-furanone (plant metabolite of BYI 02960). In the oral toxicity test 3 replicates of ten bees each per concentration were exposed to a nominal dose of 6.3, 12.5, 25, 50 and 100 µg pure metabolite/bee, with untreated (50% sugar syrup in water), solvent (50% sugar syrup, 5% acetone, 45% water) and positive control (dimethoate, 0.05, 0.08, 0.15 and 0.30 µg a.s./bee). In the contact toxicity test, 3 replicates of ten bees each were exposed to a dose of 6.3, 12.5, 25, 50 and 100 µg pure metabolite/bee, with water treated control (tap water with 0.5% Adhäsit), solvent control (acetone) and positive control (dimethoate, 0.10, 0.15, 0.20 and 0.30 µg a.s./bee).

Results

In the oral toxicity test, the actual consumed dose was 6.7, 13.7, 26.8, 54.3 and 81.5 µg pure metabolite/bee. In the oral and contact toxicity test, no behavioural effects were noted in any treatment and the controls. The positive control gave the expected response in both tests (24-h oral and contact LD50 0.13 and 0.19 µg a.s./bee, respectively). In both tests, no bee mortality occurred in any treatment and the controls, except for 1 bee (3.3%) in the contact test in the 50 µg a.s./bee treatment and the solvent control. The 48-hour oral LD50 is >81.5 µg pure metabolite/bee, the 48-h contact LD50 >100 pure metabolite/bee.

Conclusion

48-hour oral LD50 >81.5 µg pure metabolite/bee, 48-h contact LD50 >100 pure metabolite/bee.

Guidelines and limitations

The test is acceptable.

STUDY IIA, 8.7.1/03

Characteristics

reference	:	Schmitzer S. (2011)	nominal dose		Contact and oral: 100 µg pure metabolite/bee
type of study	:	Acute oral and contact toxicity	species	:	Honey bee (*apis mellifera*)
year of execution	:	2011	exposure duration	:	48 hours
GLP statement	:	Yes	dosing method	:	Contact: Stock solution in acetone; Oral: Stock solution in acetone added to sucrose solution
guideline	:	OECD 213 and OECD 214	acceptability	:	Acceptable
test substance	:	BYI02960 - hydroxy; batch BCS-CQ74364-PU-01	LD50 (oral)	:	>105.3 µg pure metabolite/bee
a.s. content	:	95.5%	LD50 (contact)	:	>100 µg pure metabolite/bee

Methods

A 48-hour acute oral and contact toxicity limit test on honeybees (*Apis mellifera*) was conducted with BYI02960 – hydroxy (plant metabolite of BYI 02960). In the oral toxicity test 5 replicates of ten bees each were exposed to a nominal dose of 100 µg pure metabolite/bee, with untreated (50% sugar syrup in water), solvent (50% sugar syrup, 5% acetone, 45% water) and positive control (dimethoate, 0.05, 0.08, 0.15 and 0.30 µg a.s./bee). In the contact toxicity test, 5 replicates of ten bees each were exposed to a dose of 100 µg pure metabolite/bee, with water treated control (tap water with 0.5% Adhäsit), solvent control (acetone) and positive control (dimethoate, 0.10, 0.15, 0.20 and 0.30 µg a.s./bee).

Results

In the oral toxicity test, the actual consumed dose was 105.3 µg pure metabolite/bee. In the oral and contact toxicity test, no behavioural effects were noted in any treatment and the controls, except for 1 treated bee in the oral test (discoordinated movement before dying). The positive control gave the

expected response in both tests (24-h oral and contact LD50 0.12 and 0.17 µg a.s./bee, respectively). In both tests, no bee mortality occurred, except for 2 treated bees (4%) in the oral test and 1 water control bee (2%) in the contact test. The 48-hour oral LD50 is >105.3 µg pure metabolite/bee, the 48-h contact LD50 >100 pure metabolite/bee.

Conclusion

48-hour oral LD50 >105.3 µg pure metabolite/bee, 48-h contact LD50 >100 pure metabolite/bee.

Guidelines and limitations

The tests is acceptable.

STUDY IIA, 8.7.1/04

Characteristics

Reference	:	Schmitzer S. (2010b)	nominal dose		Contact and oral: 100 µg pure metabolite/bee
type of study	:	Acute oral and contact toxicity	species	:	Honey bee (*apis mellifera*)
year of execution	:	2010	exposure duration	:	48 hours
GLP statement	:	Yes	dosing method	:	Contact: Stock solution in acetone; Oral: Stock solution in acetone added to sucrose solution
Guideline	:	OECD 213 and OECD 214	acceptability	:	Acceptable
test substance	:	Difluoroacetic acid; batch BCS-AA56716-01-01 (original batch number BCOO 5984-1)	LD50 (oral)	:	>107.9 µg pure metabolite/bee
a.s. content	:	95.8%	LD50 (contact)	:	>100 µg pure metabolite/bee

Methods

A 48-hour acute oral and contact toxicity limit test on honeybees (*Apis mellifera*) was conducted with difluoroacetic acid (plant metabolite of BYI 02960). In the oral toxicity test 5 replicates of ten bees each were exposed to a nominal dose of 100 µg pure metabolite/bee, with untreated (50% sugar syrup in water), solvent (50% sugar syrup, 5% acetone, 45% water) and positive control (dimethoate, 0.05, 0.08, 0.15 and 0.30 µg a.s./bee). In the contact toxicity test, 5 replicates of ten bees each were exposed to a dose of 100 µg pure metabolite/bee, with water treated control (tap water with 0.5% Adhäsit), solvent control (acetone) and positive control (dimethoate, 0.10, 0.15, 0.20 and 0.30 µg a.s./bee).

Results

In the oral toxicity test, the actual consumed dose was 107.9 µg pure metabolite/bee. In the oral and contact toxicity test, no behavioural effects were noted in any treatment and the controls. The positive control gave the expected response in both tests (24-h oral and contact LD50 0.13 and 0.15 µg a.s./bee, respectively). In both tests, no bee mortality occurred, except for 2 treated bees (4%) in the contact test and 1 water control bee (2%) in the oral test. The 48-hour oral LD50 is >107.9 µg pure metabolite/bee, the 48-h contact LD50 >100 pure metabolite/bee.

Conclusion

48-hour oral LD50 >107.9 µg pure metabolite/bee, 48-h contact LD50 >100 pure metabolite/bee.

Guidelines and limitations

The tests is acceptable.

STUDY IIA, 8.7.1/05

Characteristics

Reference	:	Schmitzer S. (2010c)	nominal dose		Contact and oral: 100 µg pure metabolite/bee
type of study	:	Acute oral and contact toxicity	species	:	Honey bee (*apis mellifera*)
year of execution	:	2010	exposure duration	:	48 hours
GLP statement	:	Yes	dosing method	:	Contact: Stock solution in acetone; Oral: Stock solution in acetone added to sucrose solution
Guideline	:	OECD 213 and OECD 214	acceptability	:	Acceptable
test substance	:	6-chloronicotinic acid (6-CNA = IC-0); batch AE F161089 00 1B99 0001	LD50 (oral)	:	>107.1 µg pure metabolite/bee
a.s. content	:	98.8%	LD50 (contact)	:	>100 µg pure metabolite/bee

Methods

A 48-hour acute oral and contact toxicity limit test on honeybees (*Apis mellifera*) was conducted with 6-chloronicotinic acid (6-CNA = IC-0, plant metabolite of BYI 02960). In the oral toxicity test 5 replicates of ten bees each were exposed to a nominal dose of 100 µg pure metabolite/bee, with untreated (50% sugar syrup in water), solvent (50% sugar syrup, 5% acetone, 45% water) and positive control (dimethoate, 0.05, 0.08, 0.15 and 0.30 µg a.s./bee). In the contact toxicity test, 5 replicates of ten bees each were exposed to a dose of 100 µg pure metabolite/bee, with water treated control (tap water with 0.5% Adhäsit), solvent control (acetone) and positive control (dimethoate, 0.10, 0.15, 0.20 and 0.30 µg a.s./bee).

Results

In the oral toxicity test, the actual consumed dose was 107.1 µg pure metabolite/bee. In the oral and contact toxicity test, no behavioural effects were noted in any treatment and the controls. The positive control gave the expected response in both tests (24-h oral and contact LD50 0.13 and 0.20 µg a.s./bee, respectively). In both tests, no bee mortality occurred, except for 1 solvent control bee (2%) in the contact test. The 48-hour oral LD50 is >107.1 µg pure metabolite/bee, the 48-h contact LD50 >100 pure metabolite/bee.

Conclusion

48-hour oral LD50 >107.1 µg pure metabolite/bee, 48-h contact LD50 >100 pure metabolite/bee.

Guidelines and limitations

The tests is acceptable.

STUDY IIA, 8.7.1/06

Characteristics

Reference	:	Schmitzer S. (2010d)	nominal dose		Contact and oral: 100 µg pure metabolite/bee
type of study	:	Acute oral and contact toxicity	species	:	Honey bee (*apis mellifera*)
year of execution	:	2009	exposure duration	:	48 hours
GLP statement	:	Yes	dosing method	:	Contact: solution in water with 0.5% Adhäsit; Oral: solution in 50% sugar syrup
Guideline	:	OECD 213 and OECD 214	acceptability	:	Acceptable
test substance	:	6-chloro-picolylalcohol, batch M06773	LD50 (oral)	:	>106.7 µg pure metabolite/bee
a.s. content	:	98.9%	LD50 (contact)	:	>100 µg pure metabolite/bee

Methods

A 48-hour acute oral and contact toxicity limit test on honeybees (*Apis mellifera*) was conducted with 6-chloro-picolylalcohol (plant metabolite of BYI 02960). In the oral toxicity test 5 replicates of ten bees each were exposed to a nominal dose of 100 µg pure metabolite/bee, with solvent control (50% sugar syrup in water) and positive control (dimethoate, 0.05, 0.08, 0.15 and 0.30 µg a.s./bee). In the contact toxicity test, 5 replicates of ten bees each were exposed to a dose of 100 µg pure metabolite/bee, with solvent control (tap water with 0.5% Adhäsit) and positive control (dimethoate, 0.10, 0.15, 0.20 and 0.30 µg a.s./bee).

Results

In the oral toxicity test, the actual consumed dose was 106.7 µg pure metabolite/bee. In the oral and contact toxicity test, no behavioural effects were noted in any treatment and the controls. The positive control gave the expected response in both tests (24-h oral and contact LD50 0.10 and 0.17 µg a.s./bee, respectively). In both tests, no bee mortality occurred, except for 1 treated bee (2%) in the oral test. The 48-hour oral LD50 is >106.7 µg pure metabolite/bee, the 48-h contact LD50 >100 pure metabolite/bee.

Conclusion

48-hour oral LD50 >106.7 µg pure metabolite/bee, 48-h contact LD50 >100 pure metabolite/bee.

Guidelines and limitations

The tests is acceptable.

B.9.4.1.2 Acute toxicity of the formulated product (IIIA 10.4.2.1 and 10.4.2.2)

STUDY IIIA, 10.4.2.1/01

Characteristics

reference	:	Schmitzer S. (2009)	nominal dose		Contact: 6.3, 12.5, 25, 50, 100 and 200 µg a.s./bee; Oral: 0.31, 0.63, 1.3, 2.5, 5.0 and 10 µg a.s./bee
type of study	:	Acute oral and contact toxicity	species	:	Honey bee (*apis mellifera*)
year of execution	:	2009	exposure duration	:	48 (oral) or 72 (contact) hours
GLP statement	:	Yes	dosing method	:	Contact: application solution in 0.5%

					Adhäsit in water; Oral: solution in 50% sucrose solution
guideline	:	OECD 213 and OECD 214	acceptability	:	Acceptable
test substance	:	BYI 02960 SL 200 G (200 g/L SL formulation), batch 2009-001253	LD50 (oral)	:	3.2 µg a.s./bee
a.s. content	:	17.0 % w/w (199.8 g/L) BYI 02960	LD50 (contact)	:	15.7 µg a.s./bee

Methods

A 48-hour acute oral and contact toxicity test on honeybees (*Apis mellifera*) was conducted with BYI 02960 SL 200 G (200 g/L SL formulation, 17.0 % w/w (199.8 g/L) BYI 02960). In the oral toxicity test 3 replicates of ten bees each per concentration were exposed to a nominal dose of 0.31, 0.63, 1.3, 2.5, 5.0 and 10 µg a.s./bee, with control (50% sugar syrup in water) and positive control (dimethoate, 0.05, 0.08, 0.15 and 0.30 µg product/bee). In the contact toxicity test, 3 replicates of ten bees each were exposed to a dose of 6.3, 12.5, 25, 50, 100 and 200 µg a.s./bee, with control (tap water with 0.5% Adhäsit) and positive control (dimethoate, 0.10, 0.15, 0.20 and 0.30 µg product/bee). Due to increasing mortality between 24 and 48 hours, the contact test was prolonged to 72 hours.

Results

The positive control gave the expected response in both tests (24-h oral and contact LD50 0.13 and 0.20 µg a.s./bee, respectively).

In the oral toxicity test, the actual consumed dose was 0.34, 0.70, 1.3, 2.6, 4.0 and 5.6 µg a.s./bee. Mortality in the control of both the oral and contact toxicity test was 0%. In the oral toxicity test, coordination problems and/or apathy occurred at 2.6-5.6 µg a.s./bee. In the contact toxicity test, a few bees at 25-200 µg a.s./bee showed abnormal behaviour (movement coordination problems and/or intensive cleaning).

In the oral toxicity test, at 0.34, 0.70, 1.3, 2.6, 4.0 and 5.6 µg a.s./bee, respectively, mortality was 0%, 0%, 0%, 16.7%, 93.3% and 93.3% after 48 hours. The 48-h oral LD50 was 3.2 (95% CI 2.3-3.9) µg a.s./bee. In the contact toxicity test, at 6.3, 12.5, 25, 50, 100 and 200 µg a.s./bee, respectively, mortality was 10.0%, 36.7%, 76.7%, 93.3%, 100% and 100% after 72 hours. The 72-h contact LD50 was 15.7 (95% CI 12.7-19.0) µg a.s./bee. NOEDs were estimated using Fisher's exact test (one-sided greater, α = 0.05): 2.6 and 6.3 µg a.s./bee in the oral and contact test, respectively.

Conclusion

48-h oral LD50 3.2 µg a.s./bee; 72-h contact LD50 15.7 µg a.s./bee.

Guidelines and limitations

The test is acceptable.

STUDY IIA, 8.7.1 /07

Characteristics

Reference	:	Vergé 2012	nominal dose		6.25, 12.5, 25, 50 and 100 µg a.s./bee
type of study	:	Acute contact toxicity	species	:	*Bombus terrestris* L.
year of execution	:	2012	duration	:	2 days
GLP statement	:	Yes	dosing method	:	solution in water
Guideline	:	OEPP/EPPO 170 (4) 2010, OECD 214 and Van der Steen (2001)	acceptability	:	Acceptable
test substance	:	Flupyradifurone SL 200 G, containing flupyradifurone 199.8 g/L analysed, density of the formulation 1.175 g/cm^3. Batch/Lot No. 2012-001530	LD50 (contact)	:	>100 µg a.s./bee
a.s. content	:	199.8 g/L			

Methods

Bombus terrestris L., young adult worker bumblebees derived from a healthy colony were exposed to test concentrations by contact exposure. Mortality and behaviour were assessed after 24 and 48 hours.

The contact test was carried out with five doses of Flupyradifurone SL 200 G. The doses were 6.25, 12.5, 25, 50 and 100 µg as/bumblebee. Bumblebees were treated by dorsal application to the thorax with an aliquot of 2 µL/bee. Test item was mixed with tap water to obtain the required doses. Three replicates per treatment with 10 bees each for contact, control and reference substance tests. Reference substance was Perfekthion (dimethoate, 411.7 g/L analysed) at 0.30 µg product/bee. Control was tap water.

Environmental conditions were: 24.4 – 25.6 ºC, 55.3 – 66.4% RH, darkness. Feeding with 50% aqueous sucrose solution. No statistics were applied.

Results

Mortality did not occur in the control nor in any of the dose levels up to and including 50 µg as/bumblebee. A mortality of 6.7% was observed at the dose level of 100 µg as/bumblebee, 24 and 48 h after application. No behavioural abnormalities or sub-lethal effects were recorded during the entire 48 hours observation period. The reference substance showed a mortality of 96.7% 24 h after application and of 100% 48 h after application.

Remarks

An LD50 value > 100 µg as/bumblebee (contact 48 h) can be used for risk assessment.

Guidelines and limitations

The test is acceptable.

B.9.4.1.3 Chronic toxicity of the active substance and metabolites (IIA 8.16.1)

B.9.4.1.3.1 Chronic toxicity on adult honeybees

STUDY IIA, 8.16.1/01

Characteristics

Reference	:	Kling, A. (2011)	nominal dose		100, 300, 1000, 3000 and 10000 µg as/L
type of study	:	honeybees, 10 days feeding test	species	:	Honey bee (*Apis mellifera*)
year of execution	:	2010	exposure duration	:	10 d
GLP statement	:	Yes	dosing method	:	chronic oral toxicity – continuous feeding test
Guideline	:	No specific guideline available	acceptability	:	**Not acceptable**
test substance	:	BYI 02960, Batch no: BYI 02960-01-03.	NOEC	:	[≥ 10000 µg as/L / ≥ 4.64 µg/bee/day]*
a.s. content	:	96.2%	LC50		[> 10000 µg as/L / > 4.64 µg/bee/day]*

**Endpoint not accepted by RMS*

Description

The chronic effects of the test item BYI 02960 on the honey bee, *Apis mellifera* L., were assessed in a 10 days continuous feeding test in the laboratory. The test was carried out with young adult worker bees derived from a healthy colony. The bees were brushed off the brood combs of one colony in order to receive young, viable adult worker bees (e.g. nurse bees). The bees were collected and distributed into the test cages one day before start of the test and were kept under test conditions over night. Bees that died during this acclimatisation period were replaced by reserve bees which were collected at the same time. During the entire test period, the bees were kept in cages made of stainless steel (base: 8.2 cm x 4 cm; height: 6 cm). The front side of the cages was equipped with a transparent pane so that the bees could be observed. The bottom of the cages consisted of a perforated board, which guaranteed sufficient air supply for the test animals. The test cages were lined with filter paper. The feeding solutions were offered to the bees in syringes fitted into the test cages. Over a period of 10 days, honey bees were exposed to 50 % (w/v) sucrose solution, containing (nominally) 100, 300, 1000, 3000 and 10000 µg as/L of the test item BYI 02960 by continuous and ad libitum feeding. All test item feeding solutions contained additionally 1% acetone. The control group was exposed to an untreated 50 % (w/v) sucrose feeding solution, also containing 1 % acetone. Mortality, sublethal effects and behavioural observations were assessed every day throughout the 10 days exposure period.

At each test item treatment level, 10 replicates of 10 honey bees each were tested (100 honey bees per test item concentration). The control group comprised 30 replicates of 10 honey bees each (300 honey bees in total). A toxic standard was not included in this test.

Samples of the stock solution and all daily prepared feeding solutions were taken directly after preparation, before start of the test. Samples were analysed by reversed-phase HPLC with Turbolon spray MS/MS-detection.

The number of dead bees in the individual test cages was recorded every day at about the same time of day during the 10 days test period. Abnormal behavioural effects and behavioural differences were assessed.

Test conditions: 24 – 25°C, 60 - 68% RH, 24 h darkness, except during observations.

The mortality of the test item treatments was corrected for control mortality, according to ABBOTT (1925), modified by SCHNEIDER-ORELLI (1947).

The consumption of feeding solution per bee/day was calculated by dividing the total daily consumption per cage by the mean number of living bees at the corresponding time interval. The mean value per treatment group as well as the daily mean food consumption per bee was calculated.

The NOEC was determined by using Fisher's Exact Test (Bonferroni-Holms corrected, one-sided, p ≤ 0.05). Data of food consumption were analysed by using the t-Test (with Bonferroni-Holms correction, one-sided, p ≤ 0.05). Statistical calculations were performed using the statistical program SAS release Version 9.2.

Results

The actual concentration of flupyradifurone in the feeding solutions, determined for each preparation day, was in the range from 90 to 95% of the nominal concentration.

A summary of endpoints is given in the table below. Table was compiled by RMS from 10 individual tables from the report. T-test was performed on cumulative mortality (%). In the table significant effects were marked for the corresponding corrected cumulative mortality.

Table 9.4.1.3-01 Mean consumption of feeding solution, mean intake of test item, mean intake accumulated over test days (mg/bee) and cumulative mortality in the BYI 02960 treatment groups and the control group (day 1-10). A negative sign indicates an increase as compared to the control.

			Test item (µg as/L) [1]				
day	**Treatment level**	**control**	**100**	**300**	**1000**	**3000**	**10000**
1	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 3.3%)*	61	50 *-2.4*	52 *-2.4*	54 *-1.3*	64 *-1.3*	57 *-3.4*
2	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 4.7%)*	59	48 *-3.9*	51 *-2.8*	53 *-1.8*	59 *-0.7*	61 *-4.9*
3	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 5.7%)*	64	53 *-3.9*	54 *-2.9*	56 *-2.9*	66 *-2.4*	58 *-5.0*
4	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 6.3%)*	56	51 *-3.5*	53 *-3.5*	54 *-1.4*	64 *-1.8*	59 *-4.6*
5	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 8.3%)*	58	51 *-4.7*	55 *-5.8*	53 *-1.4*	60 *-2.9*	55 *-4.7*
6	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 9.7%)*	58	57 -6.3	52 -6.3	56 -1.9	61 1.4	55 -5.2
7	Mean consumption of feeding solution (mg/bee) Corr. cum. mortality *(%) (control mortality 11.0%)*	59	58 -5.6	48 -5.6	59 -3.4	61 3.4	54 -6.7
8	Mean consumption of feeding solution (mg/bee) Corr. cum. mortality *(%) (control mortality 12.7%)*	51	58 -7.9	50 -4.2	61 -5.4	53 3.8	53 -8.8
9	Mean consumption of feeding solution (mg/bee) Corr. cum. mortality *(%) (control mortality 14.7%)*	54	50 -7.9	47 -5.5	57 -6.7	49 7.4	51 -6.7
10	Mean consumption of feeding solution (mg/bee) Corr. cum. mortality *(%) (control mortality 18.0%)*	53	48 - 11.1	47 -4.9	62 -8.5	51 7.3	47 -9.8 [3]
	Overall mean consumption of sucrose solution [mg/bee/d] [2]	**57.4**	**52.4**	**50.8****	**56.4**	**58.8**	**54.9**
	Mean intake accumulated over 10 test days [µg as/bee]	**-**	**0.04**	**0.13**	**0.48**	**1.49**	**4.64**

1. The control group was fed with untreated 50 % (w/v) sucrose solution mixed with 1 % acetone; all test item treatment groups were fed with BYI 02960 treated 50 % (w/v) sucrose solution mixed with 1 % acetone
2. The mean values per cage over the test period were used as basis for the calculation of the mean consumption of feeding solution per treatment over the test period [mg/bee]. Density of the sucrose solution was in the range of 1.179-1.190 g/cm3.
3. Determined to be the NOEC (not significantly different compared to the control) (Fisher's Exact Test (Bonferroni-Holms corrected, one-sided, p ≤ 0.05))

** Significantly reduced compared to the control group (t-Test with Bonferroni-Holms correction, one-sided, p ≤ 0.05)

After 10 days of continuous exposure, mortality at all test item treatment levels was not significantly increased compared to the control group.

According to the author, at all test item treatment levels, no significant sublethal effects or behavioural abnormalities were observed throughout the entire test period. The highest test item treatment level of 10000 µg as/L was determined to be the NOEC (Fisher's Exact Test; Bonferroni-Holms corrected, one-sided, $p \leq 0.05$).

At the 300 µg as/L test item treatment level, the mean food consumption per bee was slightly, although statistically significantly reduced when compared to the food consumption of the control group (t-Test with Bonferroni-Holms correction, one-sided, $p \leq 0.05$). However, at all other dose levels, higher and lower than 300 µg as/L (i.e. 100, 1000, 3000 and 10000 µg as/L), the food consumption was not statistically significantly different when compared to the food consumption of the control group.

The accumulated nominal intake of the test item via BYI 02960-treated sucrose solution was 0.04, 0.13, 0.48, 1.49 and 4.64 µg as/bee after 10 days of continuous exposure.

Remarks by RMS

The author states that a toxic standard was not included in this test, since a toxic reference substance had not been defined or validated for this type of study. The mortality in the control is very high (18%) compared to the control mortality in studies 8.16.1/02 – 8.16.1/06 with the metabolites (2% in studies 02 and 03, and 4 % in studies 04, 05 and 06). Both treatment and control mortality in this study are clearly higher than in the metabolite studies. It is difficult to compare the control results of the metabolite studies with the treatment results of the a.s. since the studies are performed in different years (2010 for the a.s., 2011 for the metabolites).

It is acknowledged that no harmonised guideline is as yet available for this type of study. However, recent descriptions of this study (e.g. in Appendix M of the EFSA GD on bees and Appendix O of the final version of the EFSA GD on bees (published July 2013), recommendations on the 10-d oral feeding study on adult honeybees include a control mortality of <15%.

Furthermore, in the treatment of this study, there are a number of bees that are affected (a, (bees still upright and attempting to walk but showing signs of reduced coordination) or show signs of apathy (ap, bees show only low or delayed reactions to stimulation [i.e. light, air blow] bees are either sitting motionless in the cage or walk but not correctly). These effect appear mostly from day 7 onwards but otherwise there is no clear pattern. Few bees were affected, affected bees usually show no symtoms anymore on the next day, and there seems to be no dose-respons.

The table shows the number of bees with sublethal effects. Moribund bees were also recorded but it has to be taken into account that the moribund bees usually die in the following days.

Table 9.4.1.3-02 Sublethal effects during the test

Test item (µg as/L)	Total number of bees (affected+apathic) – (moribund) per tested concentration									
	d1	d2	d3	d4	d5	d6	d7	d8	d9	d10
0	0 - 0	0 - 1	0 - 1	0 - 2	0 - 1	0 - 1	0 - 0	0 - 0	0 - 1	0 - 0
100	0 - 0	0 - 0	0 - 0	0 - 0	0 - 0	0 - 0	0 - 0	0 - 1	1 - 0	1 - 0

300	0 - 0	0 - 0	0 - 0	0 - 0	0 - 0	0 - 0	1 - 2	1 - 1	1 - 0	1 - 0
1000	0 - 0	0 - 0	0 - 0	0 - 0	0 - 0	0 - 0	0 - 0	0 - 0	0 - 0	3 - 1
3000	0 - 0	0 - 0	0 - 0	0 - 1	0 - 0	0 - 0	0 - 0	1 - 0	1 - 1	3 - 0
10000	0 - 0	0 - 0	0 - 0	1 - 0	0 - 0	0 - 0	0 - 0	2 - 0	0 - 0	2 - 0

In the control affected or apathetic bees were never seen. It can therefore not be concluded that there are no effects on behaviour (it is acknowledged that it is not yet clear what the impact of the sublethal effects on behaviour is on bee colonies).

Based on the high control mortality and the uncertainty about the sublethal effects, this study is not accepted. Therefore, the applicant performed a new 10-d oral study with adult honey bees, see below.

Study IIA 8.16.1/09

Characteristics

Reference	:	Gladbach, Theis et al., 2013	nominal dose	:	10, 16, 26, 41 and 66 mg as/kg
Type of study	:	honey bees, 10 days feeding test	species	:	Honey bee (*Apis mellifera*)
Year of execution	:	2013	exposure duration	:	10 d
GLP statement	:	yes	dosing method	:	chronic oral toxicity – continuous feeding test
Guideline	:	no specific guideline available	Acceptability	:	Acceptable
Test substance	:	BYI 02960, Batch no: BYI 02960-02-04	NOEC	:	26 mg/kg diet / 0.79 µg/bee/day
a.s. content	:	98.0%	LC50		61.1 mg/kg diet / 1.83 µg/bee/day

Description

The chronic effects of the test item BYI 02960 on the honey bee, Apis mellifera L., were assessed in a 10 days continuous feeding test in the laboratory. The test was carried out with young adult worker bees derived from a healthy colony. Four days prior to test start, three brood combs containing capped cells with an expected hatch on the same day were collected from three honey bee colonies and transferred into a ventilated plexi-glass containment, placed inside a climatic chamber in the laboratory. The brood combs contained some honey as food for the freshly hatched bees. One day prior to test start of the test, the 0 - 3 days old bees were collected from the plexi-glass containment and transferred to the test cages and kept under test conditions until test start.

During the entire test period, the bees were kept in cages made of stainless steel (85 x 45 x 65 mm [length x width x height]). The front side of the cages was equipped with a transparent pane so that the bees could be observed. The bottom of the cages consisted of a perforated board, which guaranteed sufficient air supply for the test animals. The test cages were lined with filter paper. The feeding solutions were offered to the bees in syringes fitted into the test cages.

Over a period of 10 days, honey bees were exposed to 50 % (w/v) aqueous sugar solution, containing five concentrations (nominally) of the test item BYI 02960: 10, 16, 26, 41 and 66 mg as/kg, by continuous and ad libitum feeding. All test item feeding solutions contained additionally 1% acetone. The control group was exposed to an untreated 50 % (w/v) aqueous sugar feeding solution, also containing 1 % acetone. Mortality, sublethal effects and behaviour were assessed every day

throughout the 10 days exposure period. Test conditions: 31.8 – 33.1°C (average: 32.9°C), 37.3 – 76.1 % (average 72.3 %), 24 h darkness, except during observations.

Chemical analysis

At each test item treatment level and the control, 10 replicates of 10 honey bees each were tested (100 honey bees per test item concentration). A toxic standard was not included in this test.
Per individual feeding day and per individual treatment level and control, samples of approximately 10 mL and retain samples of about 5 mL were taken directly after the preparation of the respective treatment-specific feeding solutions (i.e. per test item treatment level and control, respectively, at D1 – D10). Samples were analysed by HPLC coupled with MS/MS-detection and electrospray ionizations (ESI).

Calculations and statistics

The mortality of the test item treatments was corrected for control mortality, according to ABBOTT (1925), modified by SCHNEIDER-ORELLI (1947). The consumption of feeding solution per bee/day was calculated by dividing the total daily consumption per cage by the mean number of living bees at the corresponding time interval. The mean value per treatment group as well as the daily mean food consumption per bee was calculated. Actual daily intake and cumulative intake of the test substance per bee were calculated from the consumption data and chemical analysis. The NOEC was determined by using Fisher's Exact Test (Bonferroni corrected, one-sided, $p \leq 0.05$) . The LC50 / LDD50 was determined by using Probit analysis with linear, maximum likelihood regression. Data on food consumption were statistically analysed by using the Williams Multiple Sequential t-test Procedure, $p \leq 0.05$. All statistical calculations were made by using the statistical program "ToxRat Professional", Version 2.10.

Results

The actual concentration of flupyradifurone in the feeding solutions, determined for each preparation day, was in the range from 80 to 114% of the nominal concentration (mean 103%).
A summary of endpoints is given in the table below. Table was compiled by RMS from individual tables from the report.

Table B.9.4.1.3-03 Mean consumption of feeding solution, mean intake of test item, mean intake accumulated over test days and cumulative mortality in the BYI 02960 treatment groups and the control group (day 1-10). A negative sign indicates an increase as compared to the control.

day	Parameter	Test item treatment (mg as/kg diet)[1]					
		control	10	16	26	41	66
1	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 0.0%)*	34.9	37.3 *0*	33.8 *0*	35.3 *0*	36.3 *0*	30.5 *0*
2	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 0.0%)*	27.1	30.1 *0*	29.1 *0*	27.6 *0*	26.1 *-1.0*	24.1 *-1.0*
3	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 0.0%)*	33.8	27.6 *0*	28.1 *0*	25.2 *0*	22.9 *-4.0*	24.9 *-8.9*
4	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 0.0%)*	28.2	28.2 *0*	30.4 *0*	28.8 *0*	25.3 *-4.0*	28.0 *-11.9*
5	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 0.0%)*	36.1	36.6 *0*	29.8 *0*	28.7 *0*	27.2 *-4.0*	31.1 *-17.8*
6	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 0.0%)*	31.3	28.8 *0*	31.9 *0*	26.1 *0*	28.1 -6.0	28.1 -22.8
7	Mean consumption of feeding solution (mg/bee) Corr. cum. mortality *(%) (control mortality 0.0%)*	32.3	32.6 *0*	33.1 *0*	32.7 *0*	25.6 -6.0	30.6 -32.7
8	Mean consumption of feeding solution (mg/bee) Corr. cum. mortality *(%) (control mortality 0.0%)*	36.5	33.5 *0*	28.3 *0*	34.5 *0*	25.3 -8.0	38.6 -41.6
9	Mean consumption of feeding solution (mg/bee) Corr. cum. mortality *(%) (control mortality 0.0%)*	29.6	36.9 *0*	33.7 *0*	31.6 *0*	28.2 -10.0	28.4 -47.5
10	Mean consumption of feeding solution (mg/bee) Corr. cum. mortality *(%) (control mortality 0.0%)*	36.1	36.1 *0*	35.1 *0*	35.1 *0*	26.5 -13.0	39.7 -58.4
	Overall mean consumption of sucrose solution [mg/bee/d] [2]	32.6	32.8	31.3	30.6	27.1*	30.4*
	Mean daily dose per bee over 10 test days [µg as/bee/day]	-	0.33	0.50	0.79	1.11	2.01
	Cumulative mortality after ten test days [%]	0.0	0.0	0.0	0.0	13.0**	58.4**

1: The control group was fed with untreated 50 % (w/v) sucrose solution mixed with 1 % acetone; all test item treatment groups were fed with BYI 02960 treated 50 % (w/v) sucrose solution mixed with 1 % acetone

2 The daily consumption per cage throughout the test period was used as basis for the calculation of the mean consumption of feeding solution per treatment level over the test period

* Statistically significantly lower compared to control (Williams Multiple Sequential t-test, α = 0.05, one-sided smaller)

** Statistically significantly higher compared to control (Fisher's Exact Test, Bonferroni corrected, one-sided, p ≤ 0.05)

After 10 days of continuous exposure, mortality at the two highest test item treatment levels was significantly increased as compared to the mortality in the control group.

At the two highest test item treatment levels, sublethal effects or behavioural abnormalities were observed. The NOEC was determined to be 26 mg as/kg diet (Fisher's Exact Test; Bonferroni corrected, one-sided, p ≤ 0.05).

At the two highest test item treatment levels, the mean food consumption per bee over ten days was statistically significantly reduced when compared to the food consumption of the control group (Williams Multiple Sequential t-test, α = 0.05, one-sided smaller). At all other dose levels the food consumption was not statistically significantly different when compared to the food consumption of the control group.

The nominal intake of the test item via BYI 02960-treated sucrose solution was 0.0, 0.33, 0.50, 0.79, 1.11 and 2.01 µg as/bee per day over 10 days of continuous exposure. The total accumulated dose over the 10-days period was 3.28, 5.01, 7.94, 11.13 and 20.10 µg as/bee.

Remarks by RMS

The author states that a toxic standard was not included in this test, since a toxic reference substance had not been defined or validated for this type of study. This is acceptable.

In the control and the three lowest test concentrations (10, 16 and 26 mg as/kg diet), no mortality occurred (0.0%) after ten days of continuous exposure. In the control and the three lowest test concentrations there were no affected/apathic animals with the exception of one animal (at 26 mg as/kg diet) that showed no symptoms the day after. It can therefore be concluded that the NOEC for mortality, food intake and behaviour is 26 mg as/kg diet. The results, a NOEC of 26 mg as/kg diet and an LC50 value of 61.1 mg as/kg diet, can be used for risk assessment. The NOEC-level corresponds with an average daily intake of 0.79 µg as/bee/day and an accumulated dose over 10 days of 7.94 µg/bee, the LD50 with an average daily intake of 1.83 µg as/bee/day.

Study IIA 8.16.1 /02

Characteristics

Reference	:	Kling, A. (2012)	nominal dose		10000 µg as/L (limit test)
type of study	:	honeybees, 10 days feeding test	species	:	Honey bee (*Apis mellifera*)
year of execution	:	2011	exposure duration	:	10 d
GLP statement	:	Yes	dosing method	:	chronic oral toxicity – continuous feeding test
Guideline	:	No specific guideline available	acceptability	:	Acceptable
test substance	:	BYI 02960-DFEAF, Batch no. BCS-CC98193-01-03	NOEC	:	≥ 10000 µg as/L / ≥ 0.435 µg/bee/day
a.s. content	:	98.9%	LC50		> 10000 µg as/L / > 0.435 µg/bee/day

Description

The chronic effects of the test item BYI 02960-DFEAF, a metabolite of flupyradifurone, on the honey bee, *Apis mellifera* L., were assessed in a 10 days continuous feeding test in the laboratory. The test was carried out with young adult worker bees derived from a healthy colony. The bees were brushed off the brood combs of one colony in order to receive young, viable adult worker bees (e.g. nurse bees). The bees were collected and distributed into the test cages one day before start of the test and were kept under test conditions over night. Bees which died during this acclimatisation period were replaced by reserve bees which were collected at the same time. During the entire test period, the bees were kept in cages made of stainless steel (base: 8.2 cm x 4 cm; height: 6 cm). The front side of the cages was equipped with a transparent pane so that the bees could be observed. The bottom of the cages consisted of a perforated board, which guaranteed sufficient air supply for the test animals. The test cages were lined with filter paper. The feeding solutions were offered to the bees in syringes fitted into the test cages.

Ten replicates of 10 honey bees each were tested (in total 100 honey bees per test item concentration). The control group comprised 30 replicates of 10 honey bees each (in total 300 honey bees). A toxic standard was not included.

Samples of the stock solution and all feeding solutions were taken directly after preparation for subsequent residue analysis. Samples were analysed by reversed-phase HPLC with Turbolon spray MS/MS-detection.

The number of dead bees in the individual test cages was recorded every day at about the same time of day during the 10 days test period. Abnormal behavioural effects and behavioural differences were assessed.

Test conditions: 25.1 – 25.8°C, 51.2 – 69.4% RH, 24 h darkness, except during observations.

The mortality of the test item treatments was corrected for control mortality, according to ABBOTT (1925), modified by SCHNEIDER-ORELLI (1947).

The consumption of feeding solution per bee/day was calculated by dividing the total daily consumption per cage by the mean number of living bees at the corresponding time interval. The mean value per treatment group as well as the daily mean food consumption per bee was calculated.

The NOEC was determined by using Fisher's Exact Test (Bonferroni-Holms corrected, one-sided, $p \leq 0.05$). Data of food consumption were analysed by using the t-Test (with Bonferroni-Holms correction, one-sided, $p \leq 0.05$). If the data deviated from the normal distribution, statistical analysis was performed by using the Mann Whitney – U test. Statistical calculations were made by using the statistical program SAS release Version 9.2.

Results

The actual concentration of BYI 02960-DFEAF in the feeding solutions, determined for each preparation day, was in the range from 97 to 110% of the nominal concentration (mean 103%).

A summary of endpoints is given in the table below. Table was compiled by RMS from 10 individual tables from the report. T-test was performed on cumulative mortality (%). In the table significant effects were marked for the corresponding corrected cumulative mortality.

Table B.9.4.1.3-04 Mean consumption of feeding solution, mean intake of test item, mean intake accumulated over test days and cumulative mortality in the BYI 02960-DFEAF treatment groups and the control group (day 1-10). A negative sign indicates a decrease as compared to the control.

			Test item (µg as/L) [1]
day	**Treatment level**	**control**	**10000**
1	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 0.0%)*	59	59 *0.00*
2	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 0.0%)*	57	60 *0.00*
3	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 0.0%)*	60	59 *0.00*
4	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 0.33%)*	61	66 *-0.33*
5	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 1.0%)*	43	47 *-1.01*
6	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 1.33%)*	53	53 -1.35
7	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 1.67%)*	39	36 -1.70
8	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 1.67%)*	49	48 -1.70
9	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 2.00%)*	49	42 ** -2.04
10	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 3.67%)*	45	46 -3.81 [3]

	Overall mean consumption of sucrose solution [mg/bee/d] [2]	**51.4**	**51.5**
	Mean intake accumulated over 10 test days [µg as/bee]	**-**	**4.348**

1. The control group was fed with untreated 50 % (w/v) sucrose solution mixed with 1 % acetone; all test item treatment groups were fed with BYI 02960 treated 50 % (w/v) sucrose solution mixed with 1 % acetone
2. The mean values per cage over the test period were used as basis for the calculation of the mean consumption of feeding solution per treatment over the test period [mg/bee] Density of the sucrose solution was in the range of 1.180-1.189 g/cm3.
3. Determined to be the NOEC (not significantly different compared to the control; Fisher's Exact Test (Bonferroni-Holms corrected, one-sided, p≤ 0.05))

* Food consumption significantly lower compared to the control group (t-test; one-sided, p<0.05)

After 10 days of continuous exposure, mortality in the test item treatment group was not significantly different compared to the control group. The cumulative control mortality accounted to 3.67 %, as determined at the final assessment (day 10). In the test item treatment group at 10000 µg as/L, the cumulative mortality at the final assessment (day 10) accounted to 0.00 % (corrected -3.81 %).

Furthermore, neither sublethal effects nor behavioural abnormalities were observed throughout the entire testing period in the test item treatment group. The test item treatment level of 10000 µg as/L was determined to be the NOEC, Fisher's Exact Test (Bonferroni-Holms corrected, one-sided, p ≤ 0.05).

The overall mean daily consumption of the aqueous sucrose feeding solution (i.e. the average value over 10 days) in the test item treatment group was virtually identical to the untreated control group (51.5 mg/bee in the test item treatment compared to 51.4 mg/bee in the control group).

The mean daily consumption of the aqueous sucrose feeding solution was statistically significantly reduced in the test item treatment group (42 mg/bee) compared to the control group (49 mg/bee) only on day 9, (t-test; one-sided, p ≤ 0.05). On all other test days, the mean daily consumption of the aqueous sucrose feeding solution was almost identical in the test item treatment group compared to the control group (day-by-day comparison).

The accumulated nominal intake of the test item BYI 02960-DFEAF via BYI 02960-DFEAF - treated aqueous sucrose feeding solution was 4.35 µg as/bee after 10 days of continuous exposure.

Remarks by RMS

A toxic standard was not included in this test, since a toxic reference substance had not been defined or validated for this type of study. The NOEC was determined to be ≥ 10000 µg as/L at the end of the test period. The result can be used for risk assessment.

Study IIA 8.16.1/03

Characteristics

Reference	:	Kling, A. (2012)	nominal dose		10000 µg as/L (limit test)
type of study	:	honeybees, 10 days feeding test	species	:	Honey bee (*Apis mellifera*)
year of execution	:	2011	exposure duration	:	10 d
GLP statement	:	Yes	dosing method	:	chronic oral toxicity – continuous feeding test
Guideline	:	No specific guideline available	acceptability	:	Acceptable

test substance	:	BYI 02960-hydroxy, Batch no. BCS-CQ74364-PU-01	NOEC	:	≥ 10000 µg as/L / ≥ 0.420 µg/bee/day
a.s. content	:	95.5%	LC50		> 10000 µg as/L / > 0.420 µg/bee/day

Description

The chronic effects of the test item BYI 02960-hydroxy, a metabolite of flupyradifurone, on the honey bee, *Apis mellifera* L., were assessed in a 10 days continuous feeding test in the laboratory. The test was carried out with young adult worker bees derived from a healthy colony. The bees were brushed off the brood combs of one colony in order to receive young, viable adult worker bees (e.g. nurse bees). The bees were collected and distributed into the test cages one day before start of the test and were kept under test conditions over night. Bees that died during this acclimatisation period were replaced by reserve bees that were collected at the same time. During the entire test period, the bees were kept in cages made of stainless steel (base: 8.2 cm x 4 cm; height: 6 cm). The front side of the cages was equipped with a transparent pane so that the bees could be observed. The bottom of the cages consisted of a perforated board, which guaranteed sufficient air supply for the test animals. The test cages were lined with filter paper. The feeding solutions were offered to the bees in syringes fitted into the test cages.

Ten replicates of 10 honey bees each were tested (in total 100 honey bees per test item concentration). The control group comprised 30 replicates of 10 honey bees each (in total 300 honey bees). A toxic standard was not included.

Samples of the stock solution and all feeding solutions were taken directly after preparation for subsequent residue analysis. Samples were analysed by reversed-phase HPLC with Turbolon spray MS/MS-detection.

The number of dead bees in the individual test cages was recorded every day at about the same time of day during the 10 days test period. Abnormal behavioural effects and behavioural differences were assessed.

Test conditions: 25.1 – 25.8°C, 51.2 – 69.4% RH, 24 h darkness, except during observations.

The mortality of the test item treatments was corrected for control mortality, according to ABBOTT (1925), modified by SCHNEIDER-ORELLI (1947).

The consumption of feeding solution per bee/day was calculated by dividing the total daily consumption per cage by the mean number of living bees at the corresponding time interval. The mean value per treatment group as well as the daily mean food consumption per bee was calculated.

The NOEC was determined by using Fisher's Exact Test (Bonferroni-Holms corrected, one-sided, $p \leq 0.05$). Data of food consumption were analysed by using the t-Test (with Bonferroni-Holms correction, one-sided, $p \leq 0.05$). Statistical calculations were made by using the statistical program SAS release Version 9.2.

Results

The actual concentration of BYI 02960-hydroxy in the feeding solutions, determined for each preparation day, was in the range from 92 to 103% of the nominal concentration (mean 99%).

A summary of endpoints is given in the table below. Table was compiled by RMS from 10 individual tables from the report. T-test was performed on cumulative mortality (%). In the table significant effects were marked for the corresponding corrected cumulative mortality.

Table 9.4.1.3-05. Mean consumption of feeding solution, mean intake of test item, mean intake accumulated over test days and cumulative mortality in the BYI 02960-hydroxy treatment groups and the control group. A negative sign indicates a decrease as compared to the control.

			Test item (µg as/L) [1]
day	**Treatment level**	**control**	**10000**
1	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 0.0%)*	59	59 *0.00*
2	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 0.0%)*	57	60 *0.00*
3	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 0.0%)*	60	59 *0.00*
4	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 0.33%)*	61	63 *0.67*
5	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 1.0%)*	43	40 *0.01*
6	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 1.33%)*	53	52 -0.32
7	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 1.67%)*	39	37 -0.67
8	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 1.67%)*	49	46 0.36
9	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 2.0%)*	49	43 1.05
10	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 3.67%)*	45	39 -0.66 [3]
	Overall mean consumption of sucrose solution [mg/bee/d] [2]	**51.4**	**49.6**
	Mean intake accumulated over 10 test days [µg as/bee]	**-**	**4.198**

1. The control group was fed with untreated 50 % (w/v) sucrose solution mixed with 1 % acetone; all test item treatment groups were fed with BYI 02960 treated 50 % (w/v) sucrose solution mixed with 1 % acetone
2. The mean values per cage over the test period were used as basis for the calculation of the mean consumption of feeding solution per treatment over the test period [mg/bee]. Density of the sucrose solution was in the range of 1.180-1.189 g/cm3.
3. Determined to be the NOEC (not significantly different compared to the control; Fisher's Exact Test (Bonferroni-Holms corrected, one-sided, p≤ 0.05)).

After 10 days of continuous exposure, mortality in the test item treatment group was not significantly different compared to the control group. The cumulative control mortality accounted to 3.67 %, as determined at the final assessment (day 10). In the test item treatment group at 10000 µg as/L, the cumulative mortality at the final assessment (day 10) accounted to 3.03 % (corrected -0.66 %).

Furthermore, neither sublethal effects nor behavioural abnormalities were observed throughout the entire testing period in the test item treatment group. The test item treatment level of 10000 µg as/L was determined to be the NOEC (Fisher's Exact Test (Bonferroni-Holms corrected, one-sided, p ≤ 0.05)).

The overall mean daily consumption of the aqueous sucrose feeding solution (i.e. the average value over 10 days) in the test item treatment group was almost identical to the untreated control group (49.6 mg/bee in the test item treatment compared to 51.4 mg/bee in the control group).

The mean daily consumption of the aqueous sucrose feeding solution was not significantly different between the control group and the test item treatment group throughout the entire testing period (day-by-day comparison).

The accumulated nominal intake of the test item via BYI 02960-hydroxy - treated aqueous sucrose feeding solution was 4.20 µg as/bee after 10 days of continuous exposure.

Remarks by RMS

A toxic standard was not included in this test, since a toxic reference substance had not been defined or validated for this type of study. The NOEC was determined to be ≥ 10000 µg as/L at the end of the test period. The result can be used for risk assessment.

Study IIA 8.16.1 /04

Characteristics

Reference	:	Kling, A. (2012)	nominal dose		10000 µg as//L (limit test)
type of study	:	honeybees, 10 days feeding test	species	:	Honey bee (*Apis mellifera*)
year of execution	:	2011	exposure duration	:	10 d
GLP statement	:	Yes	dosing method	:	chronic oral toxicity – continuous feeding test
Guideline	:	No specific guideline available	acceptability	:	Acceptable
test substance	:	Difluoroacetic acid, Batch no. BCS-AA56716-01-01	NOEC	:	≥ 10000 µg as/L / ≥ 0.379 µg/bee/day
a.s. content	:	95.8%	LC50		> 10000 µg as/L / > 0.379 µg/bee/day

Description

The chronic effects of the test item difluoroacetic acid, a metabolite of flupyradifurone, on the honey bee, *Apis mellifera* L., were assessed in a 10 days continuous feeding test in the laboratory. The test was carried out with young adult worker bees derived from a healthy colony. The bees were brushed off the brood combs of one colony in order to receive young, viable adult worker bees (e.g. nurse bees). The bees were collected and distributed into the test cages one day before start of the test and were kept under test conditions over night. Bees that died during this acclimatisation period were replaced by reserve bees that were collected at the same time. During the entire test period, the bees were kept in cages made of stainless steel (base: 8.2 cm x 4 cm; height: 6 cm). The front side of the cages was equipped with a transparent pane so that the bees could be observed. The bottom of the cages consisted of a perforated board, which guaranteed sufficient air supply for the test animals. The test cages were lined with filter paper. The feeding solutions were offered to the bees in syringes fitted into the test cages.

Ten replicates of 10 honey bees each were tested (in total 100 honey bees per test item concentration). The control group comprised 30 replicates of 10 honey bees each (in total 300 honey bees). A toxic standard was not included.

Samples of the stock solution and of all feeding solutions were taken directly after preparation for subsequent residue analysis. Samples were analysed by reversed-phase HPLC with Turbolon spray MS/MS-detection.

The number of dead bees in the individual test cages was recorded every day at about the same time of day during the 10 days test period. Abnormal behavioural effects and behavioural differences were assessed.

Test conditions: 25.1 – 25.4°C, 64.0 – 68.8% RH, 24 h darkness, except during observations.

The mortality of the test item treatments was corrected for control mortality, according to ABBOTT (1925), modified by SCHNEIDER-ORELLI (1947).

The consumption of feeding solution per bee/day was calculated by dividing the total daily consumption per cage by the mean number of living bees at the corresponding time interval. The mean value per treatment group as well as the daily mean food consumption per bee was calculated.

The NOEC was determined by using Fisher's Exact Test (Bonferroni-Holms corrected, one-sided, $p \leq 0.05$). Data of food consumption were analysed by using the t-Test (one-sided, $p \leq 0.05$). If the data deviated from the normal distribution, statistical analysis was performed by using the Mann Whitney – U test. Statistical calculations were performed using the statistical program SAS release Version 9.2.

Results

The actual concentration of difluoroacetic acid in the feeding solutions, determined for each preparation day, was in the range from 71 to 88% of the nominal concentration (mean 82%).

A summary of endpoints is given in the table below. Table was compiled by RMS from 10 individual tables from the report. T-test was performed on cumulative mortality (%). In the table significant effects were marked for the corresponding corrected cumulative mortality.

Table 9.4.1.3-06 Mean consumption of feeding solution, mean intake of test item, mean intake accumulated over test days and cumulative mortality in the difluoroacetic acid treatment groups and the control group (day 1-10). A negative sign indicates an increase as compared to the control.

			Test item (µg as/L) [1]
day	**Treatment level**	**control**	**10000**
1	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 0.0%)*	55	56 *0.00*
2	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 0.33%)*	48	49 *-0.33*
3	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 0.33%)*	47	49 *-0.33*
4	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 0.67%)*	41	43 *-0.67*
5	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 0.67%)*	51	45 *-0.67*
6	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 1.0%)*	38	38 -1.01
7	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 1.33%)*	45	43 -1.35
8	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 1.67%)*	49	45 -1.69
9	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 1.67%)*	46	44 -0.68
10	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 2.33%)*	42	38 -1.37 [3]
	Overall mean consumption	**46.2**	**45.0**

	of sucrose solution [mg/bee/d] [2]		
	Mean intake accumulated over 10 test days [µg as/bee]	**-**	**3.79**

1. The control group was fed with untreated 50 % (w/v) sucrose solution mixed with 1 % acetone; all test item treatment groups were fed with BYI 02960 treated 50 % (w/v) sucrose solution mixed with 1 % acetone
2. The mean values per cage over the test period were used as basis for the calculation of the mean consumption of feeding solution per treatment over the test period [mg/bee]. Density of the sucrose solution was in the range of 1.185-1.193 g/cm3.
3. Determined to be the NOEC (not significantly different compared to the control; Fisher's Exact Test (Bonferroni-Holms corrected, one-sided, p≤ 0.05)).

After 10 days of continuous exposure, mortality in the test item treatment group was not significantly different compared to the control group. The cumulative control mortality accounted to 2.33 %, as determined at the final assessment (day 10). In the test item treatment group at 10000 µg as/L, the cumulative mortality at the final assessment (day 10) accounted to 1.00 % (corrected -1.37 %). Furthermore, neither sublethal effects nor behavioural abnormalities were observed throughout the entire testing period in the test item treatment group. The test item treatment level of 10000 µg as/L was determined to be the NOEC (Fisher's Exact Test (Bonferroni-Holms corrected, one-sided, p ≤ 0.05)).

The overall mean daily consumption of the aqueous sucrose feeding solution (i.e. the average value over 10 days) in the test item treatment group was almost identical to the untreated control group (45.0 mg/bee in the test item treatment compared to 46.2 mg/bee in the control group).

The mean daily consumption of the aqueous sucrose feeding solution was not significantly different between the control group and the test item treatment group throughout the entire testing period (day-by-day comparison).

The accumulated nominal intake of the test item difluoroacetic acid via difluoroacetic acid - treated aqueous sucrose feeding solution was 3.79 µg as/bee after 10 days of continuous exposure.

Remarks by RMS

A toxic standard was not included in this test, since a toxic reference substance had not been defined or validated for this type of study. The NOEC (No Observed Effect Concentration) was determined to be ≥ 10000 µg as/L at the end of the test period. The result can be used for risk assessment.

Study IIA 8.16.1 /05

Characteristics

Reference	:	Kling, A. (2012)	nominal dose		10000 µg as/L (limit test)
type of study	:	honeybees, 10 days feeding test	species	:	Honey bee (*Apis mellifera*)
year of execution	:	2011	exposure duration	:	10 d
GLP statement	:	Yes	dosing method	:	chronic oral toxicity – continuous feeding test
Guideline	:	No specific guideline available	acceptability	:	Acceptable
test substance	:	6-chloronicotinic acid, Batch no. AE F161089 00 1B99 0001	NOEC	:	≥ 10000 µg as/L / ≥ 0.418 µg/bee/day
a.s. content	:	98.8%	LC50		> 10000 µg as/L / > 0.418 µg/bee/day

Description

The chronic effects of the test item 6-chloronicotinic acid, a metabolite of flupyradifurone, on the honey bee, *Apis mellifera* L., were assessed in a 10 days continuous feeding limit test in the laboratory. The test was carried out with young adult worker bees derived from a healthy colony. The bees were brushed off the brood combs of one colony in order to receive young, viable adult worker bees (e.g. nurse bees). The bees were collected and distributed into the test cages one day before start of the test and were kept under test conditions over night. Bees that died during this acclimatisation period were replaced by reserve bees that were collected at the same time. During the entire test period, the bees were kept in cages made of stainless steel (base: 8.2 cm x 4 cm; height: 6 cm). The front side of the cages was equipped with a transparent pane so that the bees could be observed. The bottom of the cages consisted of a perforated board, which guaranteed sufficient air supply for the test animals. The test cages were lined with filter paper. The feeding solutions were offered to the bees in syringes fitted into the test cages.

Ten replicates of 10 honey bees each were tested for the test item (i.e. in total 100 honey bees). The control group comprised 30 replicates of 10 honey bees each (i.e. in total 300 honey bees). A toxic standard was not included.

Samples of the stock solution and of all feeding solutions were taken directly after preparation for subsequent residue analysis. Samples were analysed by reversed-phase HPLC with Turbolon spray MS/MS-detection.

The number of dead bees in the individual test cages was recorded every day at about the same time of day during the 10 days test period. Abnormal behavioural effects and behavioural differences were assessed.

Test conditions: 25.1 – 25.4°C, 64.0 – 68.8% RH, 24 h darkness, except during observations.

The mortality of the test item treatments was corrected for control mortality, according to ABBOTT (1925), modified by SCHNEIDER-ORELLI (1947).

The consumption of feeding solution per bee/day was calculated by dividing the total daily consumption per cage by the mean number of living bees at the corresponding time interval. The mean value per treatment group as well as the daily mean food consumption per bee was calculated.

The NOEC was determined by using Fisher's Exact Test (Bonferroni-Holms corrected, one-sided, $p \leq 0.05$). Data of food consumption were analysed by using the t-Test (one-sided, $p \leq 0.05$). If the data deviated from the normal distribution, statistical analysis was performed by using the Mann Whitney – U test. Statistical calculations were made by using the statistical program SAS release Version 9.2.

Results

The actual concentration of 6-chloronicotinic acid in the feeding solutions, determined for each preparation day, was in the range from 92 to 102% of the nominal concentration (mean 98%).

A summary of endpoints is given in the table below. Table was compiled by RMS from 10 individual tables from the report. T-test was performed on cumulative mortality (%). In the table significant effects were marked for the corresponding corrected cumulative mortality.

Table 9.4.1.3-07 Mean consumption of feeding solution, mean intake of test item, mean intake accumulated over test days and cumulative mortality in the 6-chloronicotinic acid treatment groups and the control group (day 1-10).

			Test item (µg as/L) [1]
day	**Treatment level**	**control**	**10000**
1	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 0.0%)*	55	56 *0.00*
2	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 0.33%)*	48	52 *0.67*
3	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 0.33%)*	47	53 *0.67*
4	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 0.67%)*	41	51 *0.33*
5	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 0.67%)*	51	50 *1.34*
6	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 1.0%)*	38	42 1.01
7	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 1.33%)*	45	50 0.68
8	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 1.67%)*	49	47 0.34
9	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 1.67%)*	46	51 0.34
10	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 2.33%)*	42	45 0.69 [3]
	Overall mean consumption of sucrose solution [mg/bee/d] [2]	**46.2**	**49.7**
	Mean intake accumulated over 10 test days [µg as/bee]	**-**	**4.18**

1. The control group was fed with untreated 50 % (w/v) sucrose solution mixed with 1 % acetone; all test item treatment groups were fed with BYI 02960 treated 50 % (w/v) sucrose solution mixed with 1 % acetone
2. The mean values per cage over the test period were used as basis for the calculation of the mean consumption of feeding solution per treatment over the test period [mg/bee]. Density of the sucrose solution was in the range of 1.185-1.193 g/cm3.
3. Determined to be the NOEC (not significantly different compared to the control; Fisher's Exact Test (Bonferroni-Holms corrected, one-sided, p≤ 0.05)).

After 10 days of continuous exposure, mortality in the test item treatment group was not significantly different compared to the control group. The cumulative control mortality accounted to 2.33 %, as determined at the final assessment (day 10). In the test item treatment group at 10000 µg as/L, the cumulative mortality at the final assessment (day 10) accounted to 3.00 % (corrected 0.69 %).
Except for one moribund bee at the assessment after 4 days (d4) and one affected bee at the assessment after 5 days (d5), neither sublethal effects nor behavioural abnormalities were observed throughout the entire testing period in the test item treatment group. The test item treatment level of 10000 µg as/L was determined to be the NOEC (Fisher's Exact Test (Bonferroni-Holms corrected, one-sided, p ≤ 0.05)).
The overall mean daily consumption of the aqueous sucrose feeding solution (i.e. the average value over 10 days) in the test item treatment group was comparable to the untreated control group (49.7 mg/bee in the test item treatment compared to 46.2 mg/bee in the control group).
The mean daily consumption of the aqueous sucrose feeding solution was not significantly different between the control group and the test item treatment group throughout the entire testing period (day-by-day comparison).

The accumulated nominal intake of the test item 6-chloronicotinic acid via 6-chloronicotinic acid - treated aqueous sucrose solution was 4.18 µg as/bee after 10 days of continuous exposure.

Remarks by RMS

A toxic standard was not included in this test, since a toxic reference substance had not been defined or validated for this type of study. The NOEC was determined to be ≥ 10000 µg as/L at the end of the test period. The result can be used for risk assessment.

Study IIA 8.16.1 /06

Characteristics

Reference	:	Kling, A. (2012)	nominal dose		10000 µg as/L (limit test)
type of study	:	honeybees, 10 days feeding test	species	:	Honey bee (*Apis mellifera*)
year of execution	:	2011	exposure duration	:	10 d
GLP statement	:	Yes	dosing method	:	chronic oral toxicity – continuous feeding test
Guideline	:	No specific guideline available	acceptability	:	Acceptable
test substance	:	6-chloropicolyl alcohol, Batch no. AE F157983-PU-01	NOEC	:	≥ 10000 µg as/L / ≥ 0.413 µg/bee/day
a.s. content	:	99.4%	LC50		> 10000 µg as/L / > 0.413 µg/bee/day

Description

The chronic effects of the test item 6-chloropicolyl alcohol, a metabolite of flupyradifurone, on the honey bee, *Apis mellifera* L., were assessed in a 10 days continuous feeding limit test in the laboratory. The test was carried out with young adult worker bees derived from a healthy colony. The bees were brushed off the brood combs of one colony in order to receive young, viable adult worker bees (e.g. nurse bees). The bees were collected and distributed into the test cages one day before start of the test and were kept under test conditions over night. Bees that died during this acclimatisation period were replaced by reserve bees that were collected at the same time. During the entire test period, the bees were kept in cages made of stainless steel (base: 8.2 cm x 4 cm; height: 6 cm). The front side of the cages was equipped with a transparent pane so that the bees could be observed. The bottom of the cages consisted of a perforated board, which guaranteed sufficient air supply for the test animals. The test cages were lined with filter paper. The feeding solutions were offered to the bees in syringes fitted into the test cages.

Ten replicates of 10 honey bees each were tested for the test item (in total 100 honey bees). The control group comprised 30 replicates of 10 honey bees each (in total 300 honey bees). A toxic standard was not included.

Samples of the stock solution and of all feeding solutions were taken directly after preparation for subsequent residue analysis. Samples were analysed by reversed-phase HPLC with Turbolon spray MS/MS-detection.

The number of dead bees in the individual test cages was recorded every day at about the same time of day during the 10 days test period. Abnormal behavioural effects and behavioural differences were assessed.

Test conditions: 25.1 – 25.4°C, 64.0 – 68.8% RH, 24 h darkness, except during observations.

The mortality of the test item treatments was corrected for control mortality, according to ABBOTT (1925), modified by SCHNEIDER-ORELLI (1947).

The consumption of feeding solution per bee/day was calculated by dividing the total daily consumption per cage by the mean number of living bees at the corresponding time interval. The mean value per treatment group as well as the daily mean food consumption per bee was calculated. The NOEC was determined by using Fisher's Exact Test (Bonferroni-Holms corrected, one-sided, $p \leq 0.05$). Data of food consumption were analysed by using the t-Test (one-sided, $p \leq 0.05$). If the data deviated from the normal distribution, statistical analysis was performed by using the Mann Whitney – U test. Statistical calculations were made by using the statistical program SAS release Version 9.2.

Results

The actual concentration of 6-chloropicolyl alcohol in the feeding solutions, determined for each preparation day, was in the range from 79 to 101% of the nominal concentration (mean 96%).

A summary of endpoints is given in the table below. Table was compiled by RMS from 10 individual tables from the report. T-test was performed on cumulative mortality (%). In the table significant effects were marked for the corresponding corrected cumulative mortality.

Table 9.4.1.3-08 Mean consumption of feeding solution, mean intake of test item, mean intake accumulated over test days and cumulative mortality in the 6-chloropicolyl alcohol treatment groups and the control group (day 1-10). A negative sign indicates an increase as compared to the control.

			Test item (µg as/L) [1]
day	**Treatment level**	**control**	**10000**
1	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 0.0%)*	55	46 * *0.00*
2	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 0.33%)*	48	48 *0.67*
3	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 0.33%)*	47	48 *0.67*
4	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 0.67%)*	41	47 *0.33*
5	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 0.67%)*	51	57 *0.33*
6	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 1.0%)*	38	37 0.00
7	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 1.33%)*	45	55 -0.33
8	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 1.67%)*	49	46 -0.68
9	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 1.67%)*	46	55 1.35
10	Mean consumption of feeding solution (mg/bee) *Corr. cum. mortality (%) (control mortality 2.33%)*	42	52 2.73 [3]
	Overall mean consumption of sucrose solution [mg/bee/d] [2]	**46.2**	**49.1**
	Mean intake accumulated over 10 test days [µg as/bee]	**-**	**4.132**

1. The control group was fed with untreated 50 % (w/v) sucrose solution mixed with 1 % acetone; all test item treatment groups were fed with BYI 02960 treated 50 % (w/v) sucrose solution mixed with 1 % acetone
2. The mean values per cage over the test period were used as basis for the calculation of the mean consumption of feeding solution per treatment over the test period [mg/bee]. Density of the sucrose solution was in the range of 1.185-1.193 g/cm3.

3. Determined to be the NOEC (not significantly different compared to the control; Fisher's Exact Test (Bonferroni-Holms corrected, one-sided, p≤ 0.05)).

* Food consumption significantly lower compared to the control group (t- test pooled; one-sided, p □ 0.05)

After 10 days of continuous exposure, mortality in the test item treatment group was not significantly different compared to the control group. The cumulative control mortality accounted to 2.33 %, as determined at the final assessment (day 10). In the test item treatment group at 10000 µg as/L, the cumulative mortality at the final assessment (day 10) accounted to 5.00 % (corrected 2.73 %). Furthermore, neither sublethal effects nor behavioural abnormalities were observed throughout the entire testing period in the test item treatment group. The test item treatment level of 10000 µg as/L was determined to be the NOE, Fisher's Exact Test (Bonferroni-Holms corrected, one-sided, p ≤ 0.05)).

The overall mean daily consumption of the aqueous sucrose feeding solution (i.e. the average value over 10 days) in the test item treatment group was comparable to the untreated control group (49.1 mg/bee in the test item treatment compared to 46.2 mg/bee in the control group).

The mean daily consumption of the aqueous sucrose feeding solution was statistically significantly reduced in the test item treatment group (46 mg/bee) compared to the control group (55 mg/bee) only on day 1 (t- test pooled; one-sided, p ≤ 0.05). On all other test days, the mean daily consumption of the aqueous sucrose feeding solution was almost identical or even higher in the test item treatment group compared to the control group (day-by-day comparison).

The accumulated nominal intake of the test item 6-chloropicolyl alcohol via 6-chloropicolyl alcohol - treated aqueous sucrose solution was 4.13 µg as/bee after 10 days of continuous exposure. The accumulated nominal intake of the test item 6-chloropicolyl alcohol via 6-chloropicolyl alcohol - treated aqueous sucrose solution was 4.18 µg as/bee after 10 days of continuous exposure.

Remarks by RMS

A toxic standard was not included in this test, since a toxic reference substance had not been defined or validated for this type of study. The NOEC was determined to be ≥ 10000 µg as/L at the end of the test period. The result can be used for risk assessment.

B.9.4.1.3.1 Chronic toxicity on honeybee larvae

Study KIIA 8.16.1/07 (also submitted as Study KIIIA 10.4.6.1/01)

Characteristics

Reference	:	Nikolakis, A. et al. (2011)	nominal dose		150, 600, 2500 and 10000 µg as/kg
type of study	:	Honeybee larvae, spiked diet	species	:	Honey bee larvae (*Apis mellifera*)
year of execution	:	2011	test duration	:	22 d
GLP statement	:	Yes	exposure duration	:	treated diet was given on days 4, 5 and 6 of the larval stage
Guideline	:	No specific guideline available - Study design according to the recommendations of the INRA (Institut National de la Recherche Agronomique) - method for testing pesticide toxicity to honeybee brood in laboratory conditions	dosing method	:	larval oral toxicity – *in-vitro* test

		(January, 2008) and the recommendations of the honeybee larvae laboratory ring-test group, organized by ICPBR (AUPINEL ET AL., 2009)			
test substance	:	BYI 02960, Batch no. BYI 02960-01-03	acceptability	:	Acceptable
a.s. content	:	96.2%	NOEC	:	≥ 10000 µg as/diet

Description

First instar larvae of *Apis mellifera carnica* were collected preferably from a single bee colony (test runs No. 3, 4 and 5). Due to an insufficient number of healthy bee larvae on day +1, the use of two colonies was necessary for test run No. 1 and 2. Larvae originating from each colony were distributed equally to each test plate. The colonies were obtained from a local bee keeper, in Wermelskirchen, North-Rhine Westphalia, Germany, and were free of obvious symptoms of bee diseases and were located close to the laboratory in Monheim am Rhein, North-Rhine Westphalia, Germany. To obtain first instar larvae, the queen of the selected colony was confined on day -3 in her colony in a queen excluder cage, containing a comb with empty cells, for approximately 24 hours. The excluder cage was placed into the centre of the hive, so that the queen and the larvae were well-tended by nurse bees. On day -2, about 24 hours after encaging, the queen was removed from the cage after checking the presence of freshly laid eggs. The comb containing the eggs was kept inside the cage during the incubation stage and until grafting of the larvae on day +1. At day +1, the comb, containing small first instar larvae, was carried from the bee hive to the laboratory of the test facility for grafting of the larvae into the testing units. For the transport of the comb, a wooden container was used in order to keep the ambient temperature for the bee larvae as stable as possible.The bee larvae were fed with standardised amounts of untreated artificial diet at day +1 and day +3. On day +4, +5 and +6, the bee larvae in the test item treatment groups were fed with standardised amounts of test item spiked artificial exposure diet. On day +4, the bee larvae in the reference item treatment group were fed with standardised amounts of reference item spiked artificial exposure diet. Concurrently, the bee larvae in the control group (on day +4, +5 and +6) and in the reference group (on day +5 and +6) received untreated artificial exposure diet, respectively.

Larvae were maintained during the test in 48-well culture plates with commercially obtained grafting cells of an internal diameter of 9 mm. Before using the grafting cells for inoculating bee larvae, the cells were disinfected. After the disinfection one grafting cell was placed into each well of a 48-well culture plate, respectively, which was previously half-filled with a piece of a dental roll (cellulose), wetted with a solution of 15.5% glycerol in 0.4% MBC solution. The grafting cells were inserted on top of these dental rolls. These so prepared 48-well culture plates were thereafter closed with a lid and stored at approximately +5°C in a refrigerator until use. During the biological phase of the test, no lid was used on the 48-well culture plates.

Applications

The test item BYI 02960 (tech.) was incorporated into the artificial exposure diet at the following nominal test concentrations: 150, 600, 2500 and 10000 µg as/kg diet. Dimethoate (tech.) was used as

toxic reference at a nominal test concentration of 3.0 µg as/L. Emerging bees were fed with untreated syrup and pollen powder ad libitum.

Observations

On day +22, the number of hatched adult bees was determined. Mortality was determined on day +5, +6, +7, +8, +11, +13, +15 and +22. Dead test animals were discarded for sanitary reasons.

Analysis concentrations in diet

The actual concentration of BYI 02960 in the test item spiked exposure diet was determined according to analytical method 01206 (MR-10/046, 2010-07-12, R. Schöning, P. Köster), by using High Performance Liquid Chromatography, coupled with tandem mass spectrometry.

Calculations and statistics

Mortalities are expressed in percentage and in a corrected percentage according to Abbott (ABBOTT, 1925).

The data of the test item treatment groups were statistically evaluated and compared to the control data by using a Chi^2 Test.

Results

Climatic conditions.

During the development of the honeybee larvae, the larvae were incubated at about +35°C. From day +1 to +8, the relative humidity inside the incubator was on average about 95 ± 5% and from day +8 to +22 the mean relative humidity was about 80 ± 5%.

Actual concentrations

Actual concentrations ranged from 87 to 118% of nominal.

Validity criteria

In total, five independent test runs were conducted. In all test runs, the validity criteria as stated in the INRA - method for testing pesticide toxicity to honeybee brood in laboratory conditions (January 2008) and proposed by the recommendations of the honeybee larvae laboratory ring-test group (AUPINEL et al., 2009) were met (i.e. mortality in the control group ≤ 15% and in the reference group ≥ 50% until day +7). In addition to the validity criteria as proposed by the ring-test group, an additional self-set validity criterion was employed (i.e. mortality in the control group ≤ 30% until day +22). This self-set validity criterion was applied in order to exclude test runs from which it is difficult to derive biologically meaningful information due to elevated mortality levels.

Results

Results of the five runs are reported in the following table (copied from the report).

Table 9.4.1.3-09 Control and test item performance and statistical evaluation

Test object	Honeybee larvae *(Apis mellifera carnica)*					
	Control (untreated exposure diet)	Test item (BYI 02960 - spiked exposure diet)				Reference item (dimethoate - spiked exposure diet)
Test concentration (µg a.s./kg diet)	---	150	600	2500	10000	3.0 (µg a.s./larva)
Test run No. 1 [a]						
Mortality until day +22 [%]	31.3 [a]	39.6	29.2	41.7	45.8	89.6
Abbott-corrected mortality until day +22 [%]	0.0	12.1	-3.0	15.2	21.2	84.8
Test run No. 2						
Mortality until day +22 [%]	16.7	31.0	38.1	21.4	16.7	100
Abbott-corrected mortality until day +22 [%]	0.0	17.1	25.7	5.7	0.0	100
Statistical comparison to the control [b]	---	n.s.	n.s.	n.s.	n.s.	---
NOEC [b]	≥ 10000 µg a.s./kg diet					---
LOEC [b]	> 10000 µg a.s./kg diet					---
Test run No. 3 [a]						
Mortality until day +22 [%]	32.4 [a]	40.5	35.1	40.5	48.6	91.9
Abbott-corrected mortality until day +22 [%]	0.0	12.0	4.0	12.0	24.0	88.0
Test run No. 4						
Mortality until day +22 [%]	18.8	16.7	10.4	16.7	10.4	95.8
Abbott-corrected mortality until day +22 [%]	0.0	-2.6	-10.3	-2.6	-10.3	94.9
Statistical comparison to the control [b]	---	n.s.	n.s.	n.s.	n.s.	---
NOEC [b]	≥10000 µg a.s./kg diet					---
LOEC [b]	>10000 µg a.s./kg diet					---
Test run No. 5						
Mortality until day +22 [%]	28.6	31.0	26.2	31.0	40.5	100
Abbott-corrected mortality until day +22 [%]	0.0	3.3	-3.3	3.3	16.7	100
Statistical comparison to the control [b]	---	n.s.	n.s.	n.s.	n.s.	---
NOEC [b]	≥10000 µg a.s./kg diet					---
LOEC [b]	>10000 µg a.s./kg diet					---

[a] Although control performance met the validity criteria as stated in the INRA - method for testing pesticide toxicity to honeybee brood in laboratory conditions (January 2008), the self-set validity criterion for control performance at the end of the test (i.e. ≤ 30%) was not met; no distinct differences in larval mortality can be observed at concentrations of up to and including 10000 µg BYI 02960 a.s./kg diet (as the self-set validity criterion was not met, no detailed statistical evaluation is presented; however, when subjecting the data to statistical analysis, there is no statistical significance up to and including 10000 µg a.s./kg diet; Chi^2 Test [Bonferroni-Holms corrected, one-sided, α = 0.05])

[b] Chi^2 Test, (Bonferroni-Holms corrected, one-sided, α = 0.05)
n.s.: mean value not statistically significantly different compared to the control

Overall, three test runs (2, 4 and 5) fulfilled both the validity criteria as proposed by the INRA-method (January, 2008) for testing pesticide toxicity to honeybee brood in laboratory conditions and the self-set validity-criterion of ≤ 30% mortality in the control group until day +22. The statistical processing of the data as obtained in the test runs 2, 4 and 5 consistently revealed no statistically significant effects on mortality of exposed honeybee larvae until day +22 (end of the test, emergence) at concentrations of up to and including 10000 µg BYI 02960 as/kg diet (Chi2 Test, Bonferroni-Holms corrected, one-sided, α = 0.05). This conclusion is supported by the findings of the test runs 1 and 3.

Remarks by RMS

Validity criteria as stated in the INRA – method (control mortality ≤ 15%, reference mortality ≥ 50% until day +7) were met for all five test runs. Larvae from test runs 3, 4 and 5 were collected from the same single bee colony. The larvae from test runs 1 and 2 were collected from another colony. The five runs can therefore not be regarded as true replicas (from the same colony). However, since this study was performed, the OECD has published a guidance document on acute larval tests (OECD 237) and a draft guidance document on repeated exposure larval tests (latest version 25th Nov 2013) and in both, larvae of three colonies are used. Since the results of the positive control show clear effects and the test concentrations show no effect, the NOEC as determined in this in-vitro honeybee larvae study of ≥ 10000 µg BYI 02960 as/kg diet (fed on days 4, 5 and 6 of the larval stage) can be used for risk assessment.

From Table 5 of the study report, which contains the nominal feeding rates of the larvae in µg a.s./larvae, it can be calculated that the NOEC level of 10.000 µg a.s./kg diet corresponds to an accumulated (from exposure on day +4, +5 and +6 after grafting of the larvae) test item intake of 1.32 µg a.s./larvae (nominal).

B.9.4.1.4 Foliage residue toxicity in the laboratory (no EU data requirement)

There are currently no harmonised and ring tested test guidelines in Europe to assess the residual foliage toxicity to honey bees. Nonetheless, in North America, there are test guidelines available investigating the toxicity of foliar residues on honey bees under laboratory conditions. To meet USA requirements, the applicant has performed this study. As BYI 02960 is applied to the foliage via spray, the SL 200 straight formulation was selected for this study. RMS did not evaluate the study since it is not an EU data requirement and it is unclear how it can be used in the risk assessment. The endpoint as determined by the notifier is shown in Table B.9.4.1.4-01.

Table B.9.4.1.4-01 Chronic toxicity of BYI 02960 honey bee larvae in the laboratory

Test substance	Ecotoxicological Endpoint: NOEC / NOED	Reference	Remark

BYI 02960 SL 200	No treatment-related adverse effects on behaviour and on survival of honey bees were observed when the bees were exposed for 24 hours to alfalfa foliage, collected after 3, 8 and 24 hours after treatment with BYI 02960 200 SL at a rate corresponding to 205 g a.i./ha.	Porch & Krueger 2011 KIIIA1 10.4.3/01	Study not evaluated by RMS. PMRA Canada deems this study 'reliable with restrictions' [1.]

[1] The following deviations from the test guideline were indicated in PMRA-Canada's summary of 2013/04/26 (text copied from the reporting table of the OECD Joint Review of flupyradifurone, containing comments from US-EPA and Australia):

- Test plots used are not considered to be sufficiently large.
- A formulation blank (plot) was not used, only water control and treatment plots.
- Foliage was coarsely chopped and mixed by hand prior to transferring to the laboratory for analysis. It is not known whether chopping prior to transport to the lab had an effect on residues and if so, to what extent.
- Despite collection of samples for residue analysis, residue analysis was not performed, only nominal application rates were provided.

The validity/acceptance criteria were met. The study is considered reliable with restrictions.

B.9.4.2 Semi-field studies (IIIA 10.4.7)

Study KIIIA 10.4.7/01

Characteristics

Reference	:	Schnorbach, H. (2012a)	nominal dose		0.75 and 1.5 L/ha = 75 and 150 g a.s./ha
type of study	:	Honeybee semi-field tunnel test	species	:	Honey bee (*Apis mellifera*)
year of execution	:	2008	observation duration	:	29 d
GLP statement	:	No	exposure duration	:	7 d
Guideline	:	OEPP/EPPO guideline No. 170 (3), 2001	dosing method	:	Tunnel test in flowering *Phacelia tanacetifolia*, 1x foliar spray during flowering and active foraging of bees
test substance	:	BYI 02960 SL 100, Batch no. 2008-000707	acceptability	:	Less reliable due to non-GLP
a.s. content	:	100 g flupyradifurone 100 g/L (analysed)	Result	:	No adverse effect on mortality, flight intensity, brood development; Effects on behaviour not reported

Description

The study was conducted on test fields that were located within the local subdistrict “Billiger Wald”, close to 53881 Euskirchen-Billig, North-Rhine Westphalia, Germany.

The overall size of the test field was approximately 1000 m² (100 m x 10 m). The crop *Phacelia tanacetifolia* was drilled on 21 April 2008 by using a sowing machine. The *Phacelia*-seeds were not seed-treated and the crop has not received any maintenance treatment with plant protection products.

Three replicates (= three gauze tunnels) were set up for each experimental group, consisting of an untreated control, a toxic reference (Insegar with a.s. fenoxycarb, 0.6 kg product/ha, corresponding to 150 g as/ha) and two concentrations of the test item (75 g as/ha, 150 g as/ha), respectively. In addition, two replicates (=two gauze tunnels) were set up for an extra toxic reference Actara (thiamethoxam 25% w/w), application rate 0.2 kg product/ha, corresponding to 50 g thiamethoxam as/ha. The tunnels were covered with gauze, preventing the bees from escaping but allowing for sufficient ventilation (mesh-size approx. 1.5 mm). The floor space of each tunnel was approximately 50 m^2 (5 m width x 10 m length). The distance between the tunnels was approx. 3 - 5 m.

Honey bees of the race *Apis mellifera carnica* L. were obtained from a beekeeper.

On 16 June 2008, one nucleus was prepared by taking 30 brood combs (size: 370 mm x 223 mm) from different bee colonies plus 10 food combs and honey bees, and placed into a multiple-storey hive with 4 supers. One queen was added.

On 20 June 2008, a sufficient quantity of 3-comb-hives were prepared by combining two brood combs from the nucleus (plus honey bees) with one empty frame, respectively. Finally, one queen was placed into each 3-comb-hive, containing approx. 2500 honey bees. All queens of the employed experimental colonies (3-comb-hives, prepared as outlined above) used in this test were sisters. The assignment of the bee colonies to the tunnels (test units) followed a randomized complete block design.

The honey bees were placed inside the tunnels after the beginning of flowering on 22 June 2008, 4 days before the respective application (DAA-4), in the morning. During the applications, honey bees

were actively foraging on the crop but care was taken not to directly overspray the colonies. In front of the bee hives, dead bee traps and linen sheets (1 m x 1 m) were installed. In addition, at the tunnel edges, linen sheets of 40 - 50 cm width x 30 m total length (2 x 10 m (left and right tunnel length, respectively) + 2 x 5 m (tunnel width at tunnel ends, respectively)) were spread out on the ground

Observations

The small honey bee colonies in all experimental groups were examined daily during the confined exposure period for effects on mortality, behaviour and foraging activity (10 days in succession, from DAA-3 until DAA6) and hive weight. Honey bee colonies were relocated to a monitoring site on DAA8, where the bees were allowed to forage freely. Colony assessments, including an assessment of colony strength, eggs, larvae, pupae, nectar- and pollen stores as well as hive weight, were performed in regular intervals during and after the confined exposure period until DAA27 (last colony assessment).

The following parameters were expressed as percentage values per comb area on the study days DAA-1, DAA4, and DAA6 (during confinement) as well as on the study days DAA 14, DAA 21 and on DAA 27 (on the monitoring site).

- Nectar stores: The amount of sampled nectar was assessed by the estimation of the percentage of total comb area, on both sides of the comb, containing cells filled with nectar. The nectar storage was compared between control, test item and toxic references.
- Pollen stores: The amount of sampled pollen was assessed by the estimation of the percentage of total comb area, on both sides of the comb, containing cells with pollen. The pollen storage was compared between control, test item and toxic references.
- Egg-laying activity: The egg-laying activity of the queens was assessed by inspection of the brood combs. During each inspection, the percentage of total comb area was estimated on both sides of the comb, containing cells with eggs. The egg deposition was compared between control, test item and toxic references.
- Breeding success: During each inspection, the percentage of total comb area was estimated on both sides of the comb, containing honey bee larvae and pupae (capped brood). Abundance of larvae and pupae (capped brood) was compared between control, test item and toxic references.
- Colony strength: During each inspection, the percentage of total comb area was estimated on both sides of the comb, which was covered by adult honey bees. This endpoint integrates potential impacts of the test item on breeding success, longevity and mortality of honey bees. Abundance of adult honey bees found on the combs was compared between control, test item and toxic references. A proportion of the adult bees of the hives could have been foraging at the moments of the assessment, but as the assessment of all experimental groups was conducted within a short period of time, it is assumed that this proportion is equal for all hives.

Any behavioural anomalies, including e.g. exaggerated motility, aggressiveness, discoordinated movements (trembling, flight incapability, etc.), apathy, lethargic behaviour, clustering at the hive

entrance, clustering on the gauze netting of the tunnel, obvious symptoms of intoxication (cramping, agony, etc.), etc., whenever observed, were recorded with the date and daytime of observation. Temperature and relative humidity was recorded during the confinement of the honey bees in the tunnels using a calibrated data logger inside one control tunnel from 24 June 2008 (DAA-2) until 4 July 2008 (DAA8), while rainfall was measured outside the control tunnel by means of a portable rain-gauge.

Results

Climatic conditions

Measured minimum temperatures ranged between 8.8 and 16.7°C and maximum temperatures between 20.2 and 46.0°C, the minimum relative humidity (RH) ranged between 0.3 and 35.6% and the maximum RH between 86.7 and 100.5%.

Mortality and foraging activity

Results on foraging activity and mortality are reported in the following table (copied from the report).

Table 9.4.2-1. Mortality and foraging activity after application.

Time Interval	Untreated	Insegar	BYI 02960 (75 g a.s./ha)	BYI 02960 (150 g a.s./ha)	Actara
Foraging activity (Daily average number of flower visits on 1 m² per experimental group)					
	three replicates				two replicates
DAA-3 – DAA-1	29.4	28.7	29.6	29.2	28.7
DAA0ba	24.3	26.2	25.5	28.3	26.5
DAA0aa	40.2	40.8	39.6	37.2	0.4
DAA1	22.8	23.3	22.4	22.8	0.3
DAA2 – DAA3	39.3	35.5	36.8	37.5	0.3
DAA4 – DAA6	39.1	38.1	37.8	37.3	6.7
Mortality (Daily average number of dead worker bees, drones and pupae in front of the hive per experimental group)					
	three replicates				two replicates
Worker bees, DAA-3 – DAA-1	1.4	2.1	0.7	1.3	1.3
Worker bees, DAA0ba	1.7	2.0	1.7	2.3	0.5
Worker bees, DAA0aa	1.3	0.0	0.7	1.7	465.5
Worker bees, DAA1	0.0	1.3	0.0	0.0	153.0
Worker bees, DAA2 – DAA3	0.3	0.3	0.3	0.0	139.8
Worker bees, DAA4 – DAA6	1.9	2.1	2.8	1.4	301.3
Worker bees, DAA7 – DAA11	0.0	0.2	0.0	0.3	3.3
Worker bees, DAA12 – DAA25	0.1	0.3	0.5	0.4	0.4
Drones, DAA-3 – DAA0ba	0.0	0.0	0.0	0.0	0.0
Drones, DAA0aa – DAA25	0.0	0.0	0.0	0.0	0.0
Pupae, DAA-3 – DAA-1	0.1	0.0	0.0	0.0	0.0
Pupae, DAA0ba	0.0	0.0	0.0	0.0	0.0
Pupae, DAA0aa	0.0	0.0	0.0	0.0	0.0
Pupae, DAA1	0.0	0.0	0.0	0.0	0.0
Pupae, DAA2 – DAA3	0.3	0.0	0.2	0.0	0.0
Pupae, DAA4 – DAA6	0.0	0.0	0.0	0.0	0.2
Pupae, DAA7 – DAA11	0.0	1.8	0.1	0.0	0.0
Pupae, DAA12 – DAA25	0.0	3.2	0.0	0.0	0.1

DAA = Day after application; DAA0ba = Day of application, before application; DAA0aa = Day of application, after application; a.s. = active substance

Foraging activity after application was comparable in the control group, in the test item group (as BYI 02960, at 75 g as/ha and 150 g as/ha, respectively) as well as in the Insegar (as fenoxycarb) group, while foraging activity decreased strongly to very low levels in the Actara (as thiamethoxam) group. Foraging activity in the Actara (as thiamethoxam) group has not recovered during the entire confinement period. In the test item group at 150 g as/ha, foraging activity was slightly reduced immediately after the treatment when compared to control, but recovered fully within a couple of hours after treatment and followed the control group at any point in time thereafter.
While worker bee mortality after application was comparably low in the control-, in the test item (as BYI 02960 75 g as/ha and 150 g as/ha)- and in the Insegar (as fenoxycarb) group at any point in time during the study, worker bee mortality was strongly increased in the Actara (as thiamethoxam) group. Mortality of worker bees in the Actara (as thiamethoxam) group remained on an elevated level compared to control at least until the end of the confinement period (DAA6).
In none of the experimental groups there was any conspicuous drone mortality.
Except for the Insegar group (as fenoxycarb), where the number of dead pupae was considerably increased from DAA11 until at least DAA14 (potentially from about DAA7 until DAA18), only some single dead pupae were found in any of the other experimental groups. In the Actara (as thiamethoxam) group, the treatment resulted at least in transient effects on hive weight development, the extent of nectar- and pollen stores, on larval- and pupal abundance as well as on population development. In the Insegar (as fenoxycarb) group, the treatment resulted at least in transient effects on larval- and pupal abundance as well as on population development.

Weight development of the bee hives

Results for weight development are reported in the following figure (copied from the report).

Figure 9.4.2-1. Weight development of the bee hives (average value per experimental group).

During confinement, the hive weights in all experimental groups decreased due to the tunnel-specific limited foraging conditions. After the end of the confined exposure period (DAA8), hive weight in all experimental groups was very similar and mirrored the relative proportions as before the treatment, except for the Actara group, where hive weights had decreased in a more pronounced manner. At the first assessment after confinement (DAA14), hive weights in all experimental groups had further decreased proportionally and reached their overall minimum, with the Actara group not having recovered when compared to control. Thereafter, at the following assessment days, hive weights had increased with no distinct differences between the different experimental groups, but with the typical fluctuations for this variable endpoint under field conditions, once the bees were allowed to forage freely after confinement; the hive weights at the last assessment day (DAA27) had still not recovered in the Actara group when compared to control. No adverse effects of the test item were found on the hive weight development.

Number of honey bees on the tent wall

The average number of honey bees at the tent walls shortly before the actual treatment (DAA0ba) was low and comparable in all experimental groups. Immediately after the treatment (DAA0aa), the average number of honey bees at the tent walls increased in all experimental groups, but more pronounced in the test item group at 150 g as/ha and in the Actara group, respectively. At the day following the treatment (DAA1), the average number of honey bees at the tent walls was in all experimental groups again low, comparable and even below the corresponding level shortly before the respective treatment (DAA0ba). Thereafter, until the end of the confinement period, the average number of honey bees at the tent walls remained on a comparable level in all experimental groups, showing the typical fluctuations of this parameter. No adverse effects of the test item were found.

Nectar stores

The total average comb area with cells containing nectar in % of the total comb area per experimental group, over time is reported in the following figure (copied from the report).

Figure 9.4.2-2. Nectar stores in % of the total comb area per experimental group (total average)

No adverse effects of the test item were found on nectar storage.

Pollen stores

The quantity of pollen stores found in the combs was recorded at regular intervals throughout the study period, starting on the day before application. The data are summarised in the following figure (copied from the report).

Figure 9.4.2-3. Pollen stores in % of the total comb area per experimental group (total average)

No adverse effects of the test item were found on pollen storage.

Population development and breeding performance

Egg-laying activity was assessed and recorded at regular intervals throughout the study

period, starting on the day before application. The data are presented in the following figure (copied from the report).

Figure 9.4.2-4. Comb area with cells containing eggs in % of the total comb area per experimental group (total average)

No adverse effects of the test item were found on egg-laying activity.

<u>Abundance of honey bee larvae</u>

Abundance of honey bee larvae was assessed and recorded at regular intervals throughout the study period, starting on the day before application. The data are presented in the following figure (copied from the report).

Figure 9.4.2-5. Comb area with cells containing larvae in % of the total comb area per experimental group (total average)

No adverse effects of the test item were found on honey bee larvae.

Abundance of honey bee pupae

Abundance of honey bee pupae (capped brood) was assessed and recorded at regular intervals throughout the study period, starting on the day before application. The data are presented in the following figure (copied from the report).

Figure 9.4.2-6. Comb area with cells containing pupae in % of the total comb area per experimental group (total average)

No adverse effects of the test item were found on honey bee pupae.

Total abundance of honey bee brood

The total abundance of honey bee brood was assessed and recorded at regular intervals throughout the study period, starting on the day before application. The data are presented in the following figure (copied from the report).

Figure 9.4.2-7. Comb area with cells containing brood in % of the total comb area per experimental group (total average)

No adverse effects of the test item were found on total brood abundance.

Population development

The size of the comb area covered with adult honey bees was assessed at regular intervals throughout the study period, starting on the day before application. The data are presented in the following figure (copied from the report).

Figure 9.4.2-8. Comb area covered with adult honey bees in % of the total comb area per experimental group (total average)

No adverse effects of the test item were found on population development.

Conclusions of the author

The results of the study revealed that BYI 02960 can be applied via foliar application at a rate corresponding to 75 g as/ha and 150 g as/ha, respectively, into a full-flowering and highly bee attractive crop, as represented here by *Phacelia tanacetifolia*, during honey bees actively foraging on the crop, without adverse effects on mortality, foraging activity, behaviour, brood-, food-, and population development as well as on overall hive vitality.

Remarks by RMS

A positive control was included in the test and showed that the test system was behaving as expected. Author concluded that behaviour was not affected. This could not be evaluated by RMS as observations on behaviour were not reported.

According to the EFSA Opinion on the Science behind the development of a risk assessment of plant protection products for bees (EFSA Journal 2012, 10: 2668, section 3.5.1), the main exposure route for bee brood is via pollen. Since *Phacelia tanacetifolia* is a major source of pollen, and pollen from uncontaminated sources was not present in the tunnels, the present test is suitable to show potential effect of flupyradifurone on brood related parameters, mortality and flight intensity.

The results of the study show that there is no risk to honeybees by foraging on *Phacelia tanacetifolia* sprayed once with BYI 02960 (at both application rates; 75 and 150 g as/ha) in a tunnel test. There were no test item related effects in any endpoint (except for behaviour as these data were not reported). This study can be considered for risk assessment for BYI 02960 but should be considered with caution as the study was not performed under GLP.

Expert discussion in Pesticide Peer Review meeting 124 (December 2014):

The discussion on this first study by Schnorbach 2012a includes general discussions on the design and usefulness of semi-field studies.

(Please note that the word ‘effect’ in the text below might be interpreted as different from the control (i.e. may or may not include effects from the treatment), pending on the context.)

The weight development of the bee hives in the treated groups was slightly lower at later sampling points. Similar picture in egg laying.

Some experts considered that these studies were mostly well performed and useful (except for the non-GLP status). The size of frames were relatively small and the colonies were quite small <3000 bees (noting that the EFSA GD recommends >6000 bees). When young bees are primarily used, the biological variability can be large. The adaption of young bees may be an additional issue, there was 6 days between when the queen was added and the test substance was applied. This resulted in many stressful steps for the colony just prior to application.

The conditions of a semi-field study are very artificial limiting their usefulness when comparing to the field i.e. the colony results are of limited use as there is not sufficient food so a downward trend will always be observed – limiting the ability of the study to detect treatment related effects on the colony. The large advantage is to ensure that there is no alternative foraging area other than the treated crop. The assessment methodology of the comb was discussed and the experts agreed that taking a digital image of the comb is less stressful for the bees and therefore is an advantage over making an assessment by eye.

Overall, the experts agreed with the RMS's assessment of this study.

Study KIIIA 10.4.7/02

Characteristics

Reference	:	Schnorbach, H. (2012b)	nominal dose		0.75 L/ha = 150 g a.s./ha
type of study	:	Honeybee semi-field tunnel test	species	:	Honey bee (*Apis mellifera*)
year of execution	:	2008	test duration	:	29 d
GLP statement	:	No	exposure duration	:	14 d
Guideline	:	OEPP/EPPO guideline No. 170 (3), 2001	dosing method	:	Tunnel test in flowering *Phacelia tanacetifolia*, 1x foliar spray during flowering and active foraging of bees
test substance	:	BYI 02960 SL 200, Batch no. 2009-001162 Combined with Surfactant Antarox B/848, 150 g/ha	acceptability	:	Less reliable due to non-GLP
a.s. content	:	201.9 g flupyradifurone/L	Result	:	No adverse effect on mortality, flight intensity, brood development; Effects on behaviour not reported

Description

The study was conducted on the premises of Bayer CropScience AG's Experimental Station Höfchen, 51399 Burscheid, North Rhine-Westphalia, Germany.

The overall size of the test field was approximately 1000 m² (100 m x 10 m). The crop *Phacelia tanacetifolia* was drilled on 30 May 2009 at a sowing rate of 6 kg seeds/ha, by using a sowing machine. The *Phacelia*-seeds were not seed-treated and the crop has not received any maintenance treatment with plant protection products.

Two replicates (= two gauze tunnels) were set up for each experimental group (untreated control, two toxic references (Insegar with a.s. fenoxycarb and Actara with a.s. thiamethoxam) and test item), respectively. The tunnels were covered with gauze, preventing the bees from escaping but allowing for sufficient ventilation (mesh-size approx. 1.5 mm). The floor space of each tunnel was approximately 50 m^2 (5 m width x 10 m length). The distance between the tunnels was approx. 3 - 5 m. For each experimental group (control, test item and toxic references), two tunnels were set up in the field.

Honey bees of the race *Apis mellifera carnica* L. were obtained from a beekeeper and showed no obvious signs of Varroa- (last anti-Varroa treatment in November 2008) or Nosema infestation.

On 03 July 2009, one nucleus was prepared by taking 30 brood combs (size: 370 mm x 223 mm) from different bee colonies plus 10 food combs and honey bees, and placed into a multiple-storey hive with 4 supers. One queen was added.

On 6 July 2009, a sufficient quantity of 3-comb-hives were prepared by combining two brood combs from the nucleus (plus honey bees) with one empty frame, respectively. Finally, one queen was placed into each 3-comb-hive, containing approx. 2500 - 3000 honey bees. All queens of the employed experimental colonies (3-comb-hives, prepared as outlined above) used in this test were sisters. The assignment of the bee colonies to the tunnels (test units) followed a randomized complete block design.

The bee colonies were placed inside the tunnels after beginning of flowering on 7 July 2009 (DAA-2). During the applications care was taken not to directly overspray the colonies. The colonies were taken out of the tunnels on 22 July 2009 (DAA13, end of confinement period), at a growth stage, where the crop could not longer provide a sufficient food supply for the colonies (end of flowering). The colonies were transferred at the same day (DAA13) to a monitoring site, where the honey bees were free to forage. Final colony assessments were conducted on 6 August 2009 (DAA28).

Observations

The small honey bee colonies in all experimental groups were examined daily during the confined exposure period for effects on mortality, behaviour and foraging activity (14 days in succession, from DAA-1 until DAA12) and hive weight.

Honey bee colonies were relocated to a monitoring site on DAA13, where the bees were allowed to forage freely. Colony assessments included an assessment of colony strength, eggs, larvae, pupae, nectar- and pollen stores as well as hive weight were performed in regular intervals during and after the confined exposure period until DAA28 (last colony assessment).

Foraging activity was assessed by recording the number of honey bees foraging in two 1 m x 1 m observation squares marked in the crop inside each tunnel during the daily observation periods (duration: 2 min/square). In total, three assessments were carried out before and 28 assessments after the application.

The following parameters were expressed as percentage values per comb area on the study days DAA-0, DAA4, and DAA7 (during confinement), on DAA 14 (just after confinement, on the monitoring site) and on DAA 21 and DAA 28 (on the monitoring site).

- Nectar stores: The amount of sampled nectar was assessed by the estimation of the percentage of total comb area, on both sides of the comb, containing cells filled with nectar. The nectar storage was compared between control, test item and toxic references.
- Pollen stores: The amount of sampled pollen was assessed by the estimation of the percentage of total comb area, on both sides of the comb, containing cells with pollen. The pollen storage was compared between control, test item and toxic references.
- Egg-laying activity: The egg-laying activity of the queens was assessed by inspection of the brood combs. During each inspection, the percentage of total comb area was estimated on

both sides of the comb, containing cells with eggs. The egg deposition was compared between control, test item and toxic references.

- Breeding success: During each inspection, the percentage of total comb area was estimated on both sides of the comb, containing honey bee larvae and pupae (capped brood). Abundance of larvae and pupae (capped brood) was compared between control, test item and toxic references.
- Colony strength: During each inspection, the percentage of total comb area was estimated on both sides of the comb, which was covered by adult honey bees. This endpoint integrates potential impacts of the test item on breeding success, longevity and mortality of honey bees.
- Abundance of adult honey bees found on the combs was compared between control, test item and toxic references. A proportion of the adult bees of the hives could have been foraging at the moments of the assessment, but as the assessment of all experimental groups was conducted within a short period of time, it is assumed that this proportion is equal for all hives.
- Any behavioural anomalies, including e.g. exaggerated motility, aggressiveness, discoordinated movements (trembling, flight incapability, etc.), apathy, lethargic behaviour, clustering at the hive entrance, clustering on the gauze netting of the tunnel, obvious symptoms of intoxication (cramping, agony, etc.), whenever observed, were recorded with the date and daytime of observation.

Temperature and relative humidity was recorded during the confinement of the honey bees in the tunnels using a calibrated data logger inside one control tunnel from 07 July 2009 (DAA-2) until 21 July 2009 (DAA12), while rainfall was measured outside the control tunnel by means of a portable rain-gauge.

Results

Climatic conditions

Measured minimum temperatures (data logger) ranged between 10.4 and 16.3°C and maximum temperatures between 14.9 and 34.7°C, the minimum relative humidity (RH) ranged between 33.2 and 85.5% and the maximum RH between 93.2 and 105.6%.

<u>Mortality</u>

No adverse effects of the test item were found on worker bee-, drone- and pupal mortality, respectively. Results on mortality are reported in the following tables (copied from the report).

Table 9.4.2-2. Mortality (in front of the hives) after application of BYI 02960 SL 200 in combination with Surfactant Antarox B/848

	Daily average number of dead worker bees, drones and pupae in front of the hive per experimental group			
Category, Time Interval	**Untreated**	**Insegar**	**BYI 02960**	**Actara**
Worker bees, DAA-1	1.5	1.5	0.5	2.0
Worker bees, DAA0ba	3.0	4.5	3.0	3.5
Worker bees, DAA0aa – DAA1	0.0	1.0	4.5	1440
Worker bees, DAA2 – DAA3	1.0	5.5	2.5	159.0
Worker bees, DAA4 – DAA6	2.5	3.2	4.3	58.5
Worker bees, DAA7 – DAA12	1.1	0.5	1.8	22.1
Worker bees, DAA13 – DAA21	0.4	0.4	0.6	0.2
Worker bees, DAA22 – DAA28	0.8	1.0	1.0	0.9
Drones, DAA-1 – DAA0ba	0.0	0.0	0.0	0.0
Drones, DAA0aa – DAA28	0.0	0.0	0.0	0.0
Pupae, DAA-1	0.0	0.0	0.0	0.0
Pupae, DAA0ba	0.0	0.0	0.0	0.0
Pupae, DAA0aa – DAA1	0.0	0.5	0.0	0.0
Pupae, DAA2 – DAA3	0.0	0.5	0.0	0.0
Pupae, DAA4 – DAA6	0.0	2.3	0.2	0.0
Pupae, DAA7 – DAA12	0.3	15.8	0.0	1.0
Pupae, DAA13 – DAA21	0.0	6.4	0.0	0.0
Pupae, DAA22 – DAA28	0.0	4.2	0.0	0.1

DAA = Day after application; DAA0ba = Day of application, before application; DAA0aa = Day of application, after application

Table 9.4.2-3. Mortality (at the tunnel edges) after application of BYI 02960 SL 200 in combination with Surfactant Antarox

B/848

	Daily average number of dead worker bees and drones at the tunnel edges per experimental group			
Category, Time Interval	**Untreated**	**Insegar**	**BYI 02960**	**Actara**
Worker bees, DAA-1	10.5	8.0	10.0	15.5
Worker bees, DAA0ba	14.0	16.0	12.5	11.5
Worker bees, DAA0aa – DAA1	18.0	32.0	22.0	59.5
Worker bees, DAA2 – DAA3	21.5	21.0	19.3	19.5
Worker bees, DAA4 – DAA6	20.5	29.5	17.0	44.2
Worker bees, DAA7 – DAA12	13.4	19.0	17.0	11.1
Drones, DAA-1 – DAA0ba	0.0	0.0	0.0	0.0
Drones, DAA0aa – DAA12	0.0	0.0	0.0	0.0

DAA = Day after application; DAA0ba = Day of application, before application; DAA0aa = Day of application, after application

EFSA asked RMS to provide more detailed data (i.e. for each repetition for each day) at least for adult and larvae/pupa mortality. The mortality data for adults and pupae for the individual hives are copied below from the study report (Tables 35, 37 and 38). Drone mortality is not presented since it was always 0.

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Table 35: Mortality of worker bees in front of the bee hives (individual value)

DAA (day after application)	Individual number of dead worker bees per replicate, found in front of the hive							
	Untreated		Insegar		BYI 02960		Actara	
	Plot		Plot		Plot		Plot	
d	101	203	102	201	103	205	104	204
-1	2	1	3	0	0	1	3	1
0ba	3	3	3	6	4	2	5	2
1	0	0	1	1	7	2	1368	1512
2	2	0	0	4	1	1	83	97
3	2	0	1	6	1	2	63	75
4	4	9	3	13	12	2	94	67
5	2	0	0	3	6	1	47	53
6	0	0	0	0	0	5	34	56
7	1	0	0	0	0	0	46	31
8	1	6	0	2	5	5	48	41
9	2	0	1	1	0	0	10	20
10	0	0	0	0	0	1	2	1
11	1	2	2	0	2	7	49	15
12	0	0	0	0	0	1	2	0
14	0	0	1	0	0	0	0	0
21	6	2	3	4	6	5	3	1
28	4	7	9	5	11	3	9	4

DAA0ba = Day of application, before application

Table 37: Mortality of pupae in front of the bee hives (individual value)

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DAA (day after application)	Individual number of dead pupae per replicate, found in front of the hive							
	Untreated		Insegar		BYI 02960		Actara	
	Plot		Plot		Plot		Plot	
d	101	203	102	201	103	205	104	204
-1	0	0	0	0	0	0	0	0
0ba	0	0	0	0	0	0	0	0
1	0	0	0	1	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	1	0	0	0	0
4	0	0	0	0	0	0	0	0
5	0	0	6	2	1	0	0	0
6	0	0	0	6	0	0	0	0
7	0	0	2	0	0	0	0	0
8	0	0	3	1	0	0	0	0
9	0	0	26	38	0	0	0	2
10	0	0	34	16	0	0	0	0
11	0	3	17	15	0	0	9	0
12	0	0	23	14	0	0	0	1
14	0	0	18	37	0	0	0	0
21	0	0	19	41	0	0	0	0
28	0	0	16	43	0	0	0	1

DAA0ba = Day of application, before application

Table 38: Mortality of worker bees found on the sheets at the tunnel edges (individual value)

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DAA (day after application)	Individual number of dead worker bees per replicate, found at the tunnel edges							
	Untreated		Insegar		BYI 02960		Actara	
	Plot		Plot		Plot		Plot	
d	101	203	102	201	103	205	104	204
-1	14	7	3	13	5	15	22	9
0ba	13	15	18	14	12	13	9	14
1	12	24	27	37	23	21	61	58
2	16	23	33	19	25	14	36	11
3	19	28	17	15	20	18	12	19
4	31	28	32	39	18	17	29	25
5	21	19	20	31	14	23	41	40
6	13	11	31	24	16	14	111	19
7	16	5	21	18	30	17	2	5
8	24	22	35	33	21	16	13	13
9	15	6	11	14	19	19	2	5
10	12	7	21	18	13	10	15	16
11	10	20	17	18	12	16	17	10
12	10	14	5	17	21	10	22	13

DAA0ba = Day of application, before application

Foraging activity

Foraging activity is reported in the following table (copied from the report).

Table 9.4.2-4 Foraging activity of bees after application.

Time Interval	Untreated	Insegar	BYI 02960	Actara
Foraging activity (Daily average number of flower visits on 1 m² per experimental group)				
DAA-1	10.6	12.1	11.9	12.8
DAA0ba	12.8	12.8	16.3	19.0
DAA0aa	23.6	20.7	20.7	0.5
DAA1	14.8	13.0	15.3	1.8
DAA2 – DAA3	26.1	23.1	24.9	4.8
DAA4 – DAA6	52.3	49.7	53.5	3.3
DAA7 – DAA12	43.5	39.5	40.5	2.1

Foraging activity after application was comparable in the control, test item (as BYI 02960) and the toxic reference Insegar (as fenoxycarb), while foraging activity decreased strongly to very low levels in Actara (as thiamethoxam) group, respectively. Foraging activity in the Actara (as thiamethoxam) group had not recovered during the entire confinement period. In the test item group, foraging activity was slightly reduced immediately after the treatment when compared to control, but recovered fully within 1 – 2 hours after treatment and followed the control group at any point in time thereafter.

Weight development of the bee hives

Results for weight development are reported in the following figure (copied from the report).

Figure 9.4.2-9. Weight development of the bee hives (average value per experimental group).

During confinement, the hive weights in all experimental groups decreased due to the tunnel-specific limited foraging conditions. Just after the end of the confined exposure period (DAA14), hive weight in

all experimental groups was very similar. Except for the Actara group, hive weights increased in all other experimental groups thereafter, with the typical fluctuations for this variable endpoint under field condtions, once the bees were allowed to forage freely after confinement. At the last assessment on DAA28, hive weights in the Actara group had not recovered. No adverse effects of the test item were found on the hive weight development.

Number of honey bees on the tent wall

The average number of honey bees at the tent walls shortly before the actual treatment (DAA0ba) was low and comparable in all experimental groups. Immediately after the treatment (DAA0aa), the average number of honey bees at the tent walls remained comparable and low in all experimental groups. From DAA2 until DAA4, the average number of honey bees at the tent walls increased in all experimental groups, the highest average number was recorded in the afternoon of DAA4 and accounted to about 70 honey bees in the test item treatment and in the control group, respectively, with the overall highest recorded individual number in the test item treatment group. From DAA5 onwards, the average number of honey bees at the tent walls had decreased and remained until the end of the confinement period on a rather low and comparable level in all experimental groups, by showing the typical fluctuations for this parameter, with sometimes slightly higher numbers in the test item treatment group. In the Actara group, with a few exceptions only, consistently the lowest numbers of honey bees sitting at the tent walls were recorded throughout the entire confined post-application period.

No adverse effects of the test item were found.

Nectar stores

The total average comb area with cells containing nectar in % of the total comb area per experimental group, over time is reported in the following figure (copied from the report).

Figure 9.4.2-10. Nectar stores in % of the total comb area per experimental group (total average)

During confinement, the nectar stores in all experimental groups decreased due to the tunnel-specific limited foraging conditions. Just after the end of the confined exposure period (DAA14), nectar stores in all experimental groups were very similar. Except for the Actara group, nectar stores increased in all other experimental groups thereafter, with the typical fluctuations for this variable endpoint under field conditions, once the bees were allowed to forage freely after confinement. At the last assessment on DAA28, nectar stores in the Actara group had still not recovered. No adverse effects of the test item were found on nectar storage.

Pollen stores

The quantity of pollen stores found in the combs was recorded at regular intervals throughout the study period, starting on the day of application. The data are summarised in the following table (copied from the report).

Figure 9.4.2-11. Pollen stores in % of the total comb area per experimental group (total average)

Honey bee colonies in all experimental groups started with small amounts of pollen (DAA0ba), which motivated the bees to collect pollen also under the limited foraging condition during confined exposure. Throughout and just after the end of the confined exposure period (DAA14), pollen stores had increased in all experimental groups, except for the Actara group. Pollen stores increased in all experimental groups with the typical fluctuations for this variable endpoint under field conditions, once the bees were allowed to forage freely after confinement, however, on the last assessment day (DAA28), pollen stores in the Actara group had still not recovered compared to control. No adverse effects of the test item were found on pollen storage.

Population development and breeding performance

Egg-laying activity was assessed and recorded at regular intervals throughout the study

period, starting on the day of application. The data are presented in the following figure (copied from the report).

Figure 9.4.2-12. Comb area with cells containing eggs in % of the total comb area per experimental group (total average)

No adverse effects of the test item were found on egg-laying activity.

<u>Abundance of honey bee larvae</u>

Abundance of honey bee larvae was assessed and recorded at regular intervals throughout the study period, starting on the day of application. The data are presented in the following figure (copied from the report).

Figure 9.4.2-13. Comb area with cells containing larvae in % of the total comb area per experimental group (total average)

No adverse effects of the test item were found on honey bee larvae.

Abundance of honey bee pupae

Abundance of honey bee pupae (capped brood) was assessed and recorded at regular intervals throughout the study period, starting on the day of application. The data are presented in the following figure (copied from the report).

Figure 9.4.2-14. Comb area with cells containing pupae in % of the total comb area per experimental group (total average)

No adverse effects of the test item were found on honey bee pupae.

Total abundance of honey bee brood

The total abundance of honey bee brood was assessed and recorded at regular intervals throughout the study period, starting on the day of application. The data are presented in the following figure (copied from the report).

Figure 9.4.2-15. Comb area with cells containing brood in % of the total comb area per experimental group (total average)

No adverse effects of the test item were found on total brood abundance.

<u>Population development</u>

The size of the comb area covered with adult honey bees was assessed at regular intervals throughout the study period, starting on the day of application. The data are presented in the following figure (copied from the report).

Figure 9.4.2-16. Comb area covered with adult honey bees in % of the total comb area per experimental group (total average)

No adverse effects of the test item were found on population development.

Conclusions of the author

The results of the study revealed that BYI 02960 can be applied via foliar application at a rate corresponding to 150 g as/ha into a full-flowering and highly bee attractive crop, as represented here by *Phacelia tanacetifolia*, during honey bees actively foraging on the crop, without adverse effects on mortality, foraging activity, behaviour, brood-, food-, and population development as well as on overall hive vitality.

Remarks by RMS

A positive control was included in the test and showed that the test system was behaving as expected. Author concluded that behaviour was not affected. This could not be evaluated by RMS as observations on behaviour were not reported.

According to the EFSA Opinion on the Science behind the development of a risk assessment of plant protection products for bees (EFSA Journal 2012, 10: 2668, section 3.5.1), the main exposure route for bee brood is via pollen. Since *Phacelia tanacetifolia* is a major source of pollen, and pollen from uncontaminated sources was not present in the tunnels, the present test is suitable to show potential effect of flupyradifurone on brood related parameters, mortality and flight intensity.

The results of the study show that there were no test item related effects on mortality, foraging activity, brood-, food-, and population development as well as on overall hive vitality of honeybees after foraging on *Phacelia tanacetifolia* sprayed once with BYI 02960 SL200 (at an application rate corresponding to 150 g as/ha) together with a surfactant in a tunnel test. Behaviour effects cannot be evaluated as these data were not reported.

The results of this study can be considered for risk assessment but should be considered with caution as the study was not performed under GLP.

Expert discussion in Pesticide Peer Review meeting 124 (December 2014):

(Please note that the word ‘effect’ in the text below might be interpreted as different from the control (i.e. may or may not include effects from the treatment), pending on the context.)

Some possible effects on workers and larvae were observed. The second toxic reference (Insegar), normally used in bee brood studies, did not have consistent effect on the larvae in this study. This questions the results of the larvae assessments.

The experts agreed that the toxic references are only for demonstrating that the conditions of the study were suitable to detect effects. The results of the test items should not be compared to the results of the toxic reference.

The possible effect on workers was discussed in detail given that the increase in mortality was very low and could be only the natural variation. However, it was stressed that any increase should be considered. The effects were only apparent only just after application and not at later time points.

These apparent effects were only evident in front of the hive and not at the tunnel edge. The experts agreed that the level of effect on mortality was not of concern and did not affect the colony.

Study KIIIA 10.4.7/03

Characteristics

Reference	:	Rentschler,S. (2012a)	nominal dose		2x 1 L/ha = 200 g a.s./ha
type of study	:	Honeybee semi-field tunnel test	species	:	Honey bee (*Apis mellifera*)
year of execution	:	2009	observation duration	:	29 d
GLP statement	:	Yes	exposure duration	:	16 d
Guideline	:	OEPP/EPPO guideline No. 170 (3), 2001	dosing method	:	Tunnel test in flowering *Phacelia tanacetifolia*, 2x foliar spray. 1st 6 d before hive set-up (flowers in bud), second 10 d after 1st application during flowering and active foraging of bees
test substance	:	BYI 02960 SL 200 G, Batch no. 2009-001253.	acceptability	:	Acceptable
a.s. content	:	199.8 g flupyradifurone/L	Result	:	No adverse effect on mortality, behaviour , brood development. Adverse effect on flight intensity.

Description

The study was conducted in Niefern-Öschelbronn, federal state of Baden-Wuerttemberg, Germany on a field of flowering *Phacelia tanacetifolia*. The experimental design of this study comprised three experimental groups with three replicates each, 9 tunnel tents were set-up before moving the hives to the experimental field. The individual treatment groups, the respective application rates per ha and the respective spray volumes per ha are described in the following table (copied from the report).

Table 9.4.2-5. Treatment groups, application rates and spray volume.

Treatment Group	Code	Time of application	Application rate		Spray Volume
			[g a.s./ha]*	[g product/ha]	
Control (tap water)	C	DAA0, BBCH 65	None	None	200 L/ha
Test item (BYI 02960 SL 200 G)	T	DAA-10, BBCH 58	200	1 L	200 L/ha
		DAA0, BBCH 65	200	1 L	200 L/ha
Reference item	R	DAA0, BBCH 65	400	1 L	200 L/ha

DAA = days after 2nd test item application
* calculation based on the nominal content of a. s.

The reference item was Perfekthion EC 400 (a.s. dimethoate). The area covered per tunnel tent was 72 m^2. The dimensions of each tunnel tent (covered plot) were 12 m long, 6 m wide and 2.5 m high in

the centre. The tunnel tent frames were covered with light plastic gauze (mesh size: 1.5 mm). The distance between the tunnel tents was approximately 3 m. Before start of the test, alleys were created in each tunnel tent by removal of the plants and smoothing the ground. Subsequently, the alleys were covered with linen sheets of 0.6 m width (area covered approx. 13.7 m^2). About 0.5 m *Phacelia tanacetifolia* plants were removed along the tents to get the required size of the crop area that have to applied (size of boom sprayer: 2.2 m).

The 1st and the 2nd test item application were carried out inside the tunnel tents. One small bee colony was introduced per tunnel, 4 days before the 2nd application in T and the concurrent applications in C and R, respectively. After the end of the confined exposure period, the colonies were relocated to a monitoring site without intensive agriculture.

For the test, small healthy colonies with five combs were used. The colony size at set-up was in the range of approximately 4000 – 7000 bees. All nuclei were produced at the same time. The colonies were from one breeding line in order to guarantee uniform bee material in all treatments. Furthermore the following criteria for the nuclei were guaranteed:

- at least two brood combs containing eggs, larvae and capped cells;
- at least one comb containing honey and pollen;
- bees were free of Nosema and Varroa disease symptoms and other bee diseases

Wooden bee traps with gauze on bottom and on top were attached to the entrance of the nuclei in order to register those dead bees which were carried out of the hives.

The test item solution was prepared shortly before each application. The applications were carried out with a portable boom sprayer that simulates a commercial application. The following conditions were met for both applications:

- crop at BBCH 58, at 1st application in T; flowers still in bud
- crop at full bloom (BBCH 65), at 2nd application in T and the concurrent applications in C and R, respectively
- bees were actively foraging at 2nd application in T and the concurrent applications in C and R, respectively
- air temperature did not exceed 30°C (both application times)
- wind speed was less than 3 m/s at 1st application in T
- wind speed inside the tunnel tent was less than 2 m/s at 2nd application in T and the concurrent applications in C and R, respectively

During the 2nd application in T and the concurrent applications in C and R, respectively, the bee colonies were covered with plastic sheets and the water supply was moved out of the tunnels during the application to avoid direct contamination.

Bee traps with gauze on bottom and on top were attached to the entrance of the hives in order to register those dead bees which were carried out of the hives. The mortality assessments started 2 days before set up of the colonies inside the tunnels and lasted until the re-location of the hives to the monitoring location

Furthermore, mortality was recorded in the crop. To facilitate and standardise the mortality assessment in the crop, *Phacelia tanacetifolia* plants were removed prior to the set-up of the hives from the areas, moreover, water-permeable linen sheets were spread out on the ground. At each assessment, the dead bees found on the sheets and in the bee traps were counted and removed. The mortality of honeybees of the different treatment groups in the dead bee trap at the entrance of the hive and on the linen sheets were assessed prior to set up (DAA -6 to -4), prior to 2nd test item application (DAA -3 to -1), on the day of the 2nd test item application (before and after the application: 0ba and 0aa) and after the 2nd test item application (DAA 1 to 11).

Flight Intensity

At each assessment during the exposure period, the number of forager bees that were both foraging on flowering *Phacelia tanacetifolia* and flying over the crop were counted on a square of 1 m² for a short period of time (approximately 10-15 seconds). At each assessment time per plot, the square observed was chosen by chance.

Condition of the colonies, development of the bee brood.

The condition of the colonies and the development of the bee brood was checked five times during the test. The first colony assessment took place on day before set-up of the colonies inside the tunnels (DAA-5). The second colony assessment was done on DAA7. Three further colony assessments took place after re-location of the colonies to the monitoring site at weekly intervals (±1 day).

In order to record possible effects of the test item, the following parameters were assessed:

- Strength of the colony (number of bees estimated according to IMDORF & GERIG, 1999 and IMDORF et al., 1987)
- Presence of a healthy queen
- Visual assessment of the brood (estimation of the number of cells filled with eggs/larvae/pupae, according to IMDORF & GERIG, 1999 and IMDORF et al., 1987)

At each colony assessment, the comb area containing bees and cells with nectar, pollen, eggs, larvae and capped cells was estimated per comb side and the total number of bees and cells containing the brood stages, pollen and nectar on the comb were calculated. This was done for all combs per hive. Afterwards, the total values were calculated for each hive and assessment date.

Behaviour of the Bees

The behaviour of the bees on the crop and around the hive was assessed during the exposure period. The assessments were performed during the flight intensity assessments.

At least the following symptoms were checked in comparison to the control bees:

- Aggressiveness
- Intensive flying activity without landing on the crop
- Clustering at the bee hive entrance
- Intoxication symptoms

Recording of the Meteorological Data

For the entire exposure period the following climatic data were recorded by a weather station (non-GLP record; distance from the field site less than 5 km):

- air temperature (daily min/max)
- relative air humidity (daily min/max)

During applications and assessments, the following data were recorded at the field site (GLP record):

- cloud cover (estimated during application and assessments of flight intensity)
- air temperature, relative air humidity (only during the application)
- wind speed (only during the application)
- rainfall (rain gauge placed tunnel C2)

Collection of Flowers

On DAA0aa1 and DAA7, flowers of *Phacelia tanacetifolia* were collected. Samples from the control tunnels were taken first, followed by the samples from the test item tunnels. No samples were taken in the reference item tunnels.

The terminal inflorescences with open flowers was cut at the end of the stipe and bagged. A pooled sample comprising ≥ 10 g flowers per treatment was collected from at least 12 different locations across the tunnel. Each sample was divided into two sub-samples (A and B).

Each sample was labelled twice with the following information: study code, treatment, number of replicate, type of sample and date of sampling.

Collection of Pollen from Combs

On DAA7 one pollen sample per tunnel (excluding the reference item tunnel) was taken from the hives. Samples from the control tunnels were taken before those of the test item tunnels.

Each sample was divided into two sub-samples in order to get one A (residue analysis) and one B sample (retained).

Each sub-sample contained enough *Phacelia tanacetifolia* pollen (dark purple) for analysis procedure. The pollen was collected with a pollen lifter which takes the pollen directly from the cell.

Each sample was labelled twice with the following information: study code, treatment, number of replicate, type of sample and date of sampling.

Collection of Nectar from Combs

One nectar sample per tunnel (excluding the reference item tunnel) was taken from the hives on DAA7. Samples from the control tunnels were taken before those of the test item tunnels.

Each sample was divided into two sub-samples in order to get one A (residue analysis) and one B sample (retained).

Each sub-sample contained enough *Phacelia tanacetifolia* nectar for analysis procedure. The nectar was collected by means of a table spoon which was pushed into the wax covered nectar cells.

Statistical analysis

The data of mortality and flight intensity of the test item and the reference item group were compared to the control in two separate approaches. The data were tested for normality and homoscedasticity using Shapiro-Wilk test ($p > 0.05$) and folded F-test ($p > 0.05$), respectively. Data were statistically compared using t-test (method pooled, $p \leq 0.5$) in case of normality and homoscedasticity. In case of no homoscedasticity but proven normality t-test was conducted with method Satterthwaite (same as Welch's t-test, $p \leq 0.5$). In case of no normality and no homoscedasticity Wilcoxon test ($p \leq 0.5$) was

used. Log-transformation was conducted if necessary to achieve better normatlity and homoscedascity of data.

Results

Climatic conditions

Temperature during 2rd application (July 19) was between 12.9 and 20.8.°C, 56.1-85.9 RH, 0.3 mm rainfall; Temperature during observation ranged from 10.5 to 31.7 °C. Rainfall 1.5 mm 1 day after 2rd application and 10 mm 5 days after 2rd application. Maximally 3 mm per day afterwards.

Mortality

Mortality of the different test groups are reported in the following figure (copied from the report). Significant increase of mortality was observed at DAA1. At most other days between DAA0aa and DAA11 mortality was increased although not significantly.

* = statistically significant (higher, R versus C; t-test, method pooled, two-sided, α = 0.05).
\# = statistically significant (higher, T versus C; t-test, method pooled, two-sided, α = 0.05).
0ba = before application
0aa = after application

Figure 9.4.2-17. Mean mortality values of the control group C, the test item group T and the reference group R prior to and after the 2nd application in T and concurrent applications in C and R.

EFSA asked RMS to provide more detailed data for adult and larvae/pupa mortality. The mortality data for dead bees for the individual hives are copied below from the study report (Tables 14, 15 and 16). The study report does not provide separate data for adults and pupae.

A02: Individual Results

Table 14: Bee mortality of the control group C

Date	DAA	C1 Time	C1 LS	C1 BT	C1 ∑	C2 Time	C2 LS	C2 BT	C2 ∑	C3 Time	C3 LS	C3 BT	C3 ∑	Ø (∑)	STD
13 Jul 09	-6	13:07	n. a.	20	20	13:14	n. a.	34	34	13:11	n. a.	5	5	**19.7**	14.5
14 Jul 09	-5	10:59	n. a.	3	3	11:01	n. a.	1	1	11:00	n. a.	5	5	**3.0**	2.0
15 Jul 09	-4	7:15	n. a.	12	12	7:15	n. a.	9	9	7:15	n. a.	4	4	**8.3**	4.0
Ø DAA-6 – -4				**11.7**	**11.7**			**14.7**	**14.7**			**4.7**	**4.7**	**10.3**	10.6[2)]
STD				8.5	8.5			17.2	17.2			0.6	0.6	8.5[1)]	--
16 Jul 09	-3	11:20	3	3	6	11:14	3	7	10	11:10	1	12	13	**9.7**	3.5
17 Jul 09	-2	12:13	5	1	6	12:01	4	1	5	12:04	1	2	3	**4.7**	1.5
18 Jul 09	-1	11:19	4	1	5	11:15	6	0	6	11:12	4	1	5	**5.3**	0.6
19 Jul 09	0ba	12:46	9	2	11	12:50	16	1	17	12:55	11	2	13	**13.7**	3.1
Ø DAA-3 – 0ba			**5.3**	**1.8**	**7.0**		**7.3**	**2.3**	**9.5**		**4.3**	**4.3**	**8.5**	**8.3**	4.3[2)]
STD			2.6	1.0	2.7		6.0	3.2	5.4		4.7	5.2	5.3	4.2[1)]	--
Ø DAA-6 – 0ba			**5.3**	**6.0**	**9.0**		**7.3**	**7.6**	**11.7**		**4.3**	**4.4**	**6.9**	**9.2**	7.5[2)]
STD			2.6	7.3	5.8		6.0	12.2	11.0		4.7	3.7	4.3	5.9[1)]	--
19 Jul 09	0aa	14:40	1	0	1	14:47	0	0	0	14:53	0	0	0	0.3	0.6
19 Jul 09	0aa	15:40	0	1	1	15:47	0	0	0	15:53	0	0	0	0.3	0.6
19 Jul 09	0aa	17:47	1	0	1	17:47	1	0	1	17:53	0	1	1	1.0	0.0
19 Jul 09	0aa	19:47	1	0	1	19:47	1	0	1	19:53	1	0	1	1.0	0.0
19 Jul 09	0aa	∑	3	1	4	∑	2	0	2	∑	1	1	2	**2.7**	1.2
20 Jul 09	1	12:03	5	2	7	11:55	4	0	4	11:52	1	0	1	**4.0**	3.0
21 Jul 09	2	10:20	7	1	8	9:57	4	1	5	10:02	2	1	3	**5.3**	2.5
22 Jul 09	3	10:48	17	3	20	10:42	9	3	12	10:38	1	0	1	**11.0**	9.5
23 Jul 09	4	10:54	17	1	18	10:22	10	2	12	10:27	3	4	7	**12.3**	5.5
24 Jul 09	5	11:46	6	1	7	11:18	4	0	4	11:23	3	1	4	**5.0**	1.7
25 Jul 09	6	12:02	7	6	13	11:37	7	3	10	11:43	3	2	5	**9.3**	4.0
26 Jul 09	7	9:55	17	4	21	9:39	14	0	14	9:21	6	2	8	**14.3**	6.5
27 Jul 09	8	10:31	11	9	20	10:18	5	10	15	10:22	0	5	5	**13.3**	7.6
28 Jul 09	9	9:49	14	7	21	9:26	12	2	14	9:31	4	0	4	**13.0**	8.5
29 Jul 09	10	11:35	16	12	28	11:00	21	4	25	11:50	6	4	10	**21.0**	9.6
30 Jul 09	11	11:00	13	2	15	11:05	15	6	21	11:07	2	2	4	**13.3**	8.6
Ø DAA0aa – 9			**10.4**	**3.5**	**13.9**		**7,1**	**2,1**	**9.2**		**2,3**	**1,7**	**4.0**	**9.0**	6.4[2)]
STD			5.5	2.9	6.8		4,0	3,0	4.9		1,9	1,6	2.4	4.4[1)]	
Ø DAA0aa – 11			**11.1**	**4.1**	**15.2**		**8.9**	**2.6**	**11.5**		**2.7**	**1.8**	**4.5**	**10.4**	7.4[2)]
STD			5.2	3.7	7.4		5.7	3.0	7.0		1.9	1.7	2.7	5.3[1)]	--
Q_M [3)]		2.2				1.2				0.5				--	
$Q_{M(Daa)}$ [3)]		--				--				--				0.3	
$Q_{M(mean)}$ [3)]		--				--				--				1.3	

DAA = days after 2nd application in T
n. a. = not assessed (no linen sheet at the monitoring site)
ba = before application aa = after application
BT = Dead bee trap LS = Linen sheet
STD = Standard deviation ∑ = sum
Ø = mean value
1) = calculated for the daily mean values
2) = calculated over cumulative values (∑)/day
3) = for details concerning the calculation of the quotient of mortality (QM) see chapter 6.9

Table 15: Bee mortality of the test item group T

		T1				T2				T3					
Date	DAA	Time	LS	BT	∑	Time	LS	BT	∑	Time	LS	BT	∑	Ø (∑)	STD
13 Jul 09	-6	13:12	n. a.	27	27	13:16	n. a.	38	38	13:19	n. a.	13	13	**26.0**	12.5
14 Jul 09	-5	11:00	n. a.	1	1	11:01	n. a.	9	9	11:02	n. a.	6	6	**5.3**	4.0
15 Jul 09	-4	7:15	n. a.	12	12	7:15	n. a.	23	23	7:15	n. a.	15	15	**16.7**	5.7
Ø DAA-6 – -4				**13.3**	**13.3**			**23.3**	**23.3**			**11.3**	**11.3**	**16.0**	11.5[2)]
STD				13.1	13.1			14.5	14.5			4.7	4.7	10.4[1)]	--
16 Jul 09	-3	11:07	1	5	6	11:25	2	8	10	11:17	3	12	15	**10.3**	4.5
17 Jul 09	-2	11:50	3	2	5	12:10	4	2	6	11:58	0	3	3	**4.7**	1.5
18 Jul 09	-1	11:09	0	2	2	11:21	5	1	6	11:17	4	3	7	**5.0**	2.6
19 Jul 09	0ba	13:00	8	1	9	12:56	10	0	10	12:53	9	7	16	**11.7**	3.8
Ø DAA-3 – 0ba			**3.0**	**2.5**	**5.5**		**5.3**	**2.8**	**8.0**		**4.0**	**6.3**	**10.3**	**7.9**	4.3[2)]
STD			3.6	1.7	2.9		3.4	3.6	2.3		3.7	4.3	6.3	3.6[1)]	--
Ø DAA-6 – 0ba			**3.0**	**7.1**	**8.9**		**5.3**	**11.6**	**14.6**		**4.0**	**8.4**	**10.7**	**11.4**	8.9 [2)]
STD			3.6	9.6	8.9		3.4	14.1	11.8		3.7	4.9	5.3	7.8 [1)]	--
19 Jul 09	0aa	15:10	0	0	0	15:20	0	0	0	15:39	4	3	7	2.3	4.0
19 Jul 09	0aa	16:10	2	0	2	16:20	3	0	3	16:39	6	0	6	3.7	2.1
19 Jul 09	0aa	18:10	2	0	2	18:20	2	0	2	18:39	0	3	3	2.3	0.6
19 Jul 09	0aa	20:10	0	0	0	20:20	0	0	0	20:39	0	0	0	0	0.0
19 Jul 09	0aa	∑	4	0	4	∑	5	0	5	∑	10	6	16	**8.3**	6.7
20 Jul 09	1	11:47	16	1	17	12:08	13	0	13	11:59	16	8	24	**18.0***	5.6
21 Jul 09	2	9:30	2	7	9	10:16	0	4	4	9:53	4	4	8	**7.0**	2.6
22 Jul 09	3	11:01	17	6	23	10:52	3	1	4	10:56	7	2	9	**12.0**	9.8
23 Jul 09	4	9:51	8	8	16	10:47	6	4	10	10:15	5	1	6	**10.7**	5.0
24 Jul 09	5	10:52	3	4	7	11:41	3	4	7	11:12	5	5	10	**8.0**	1.7
25 Jul 09	6	11:10	15	6	21	11:56	4	3	7	11:32	4	4	8	**12.0**	7.8
26 Jul 09	7	8:51	11	7	18	9:49	7	8	15	9:14	18	12	30	**21.0**	7.9
27 Jul 09	8	10:11	0	11	11	10:28	1	8	9	10:16	5	9	14	**11.3**	2.5
28 Jul 09	9	8:54	30	8	38	9:43	7	6	13	9:20	7	2	9	**20.0**	15.7
29 Jul 09	10	11:45	31	9	40	11:53	7	4	11	11:05	19	10	29	**26.7**	14.6
30 Jul 09	11	11:10	9	7	16	10:57	19	3	22	11:03	9	3	12	**16.7**	5.0
Ø DAA0aa – 11			**12.2**	**6.2**	**18.3**		**6.3**	**3.8**	**10**		**9.1**	**5.5**	**14.6**	**14.3**	9.1[2)]
STD			10.2	3.2	11.2		5.3	2.7	5.3		5.5	3.5	8.5	6.1[1)]	--
Q_M [3)]		3.3				1.3				1.4				--	
$Q_{M(0aa)}$ [3)]		--				--				--				1.1	
$Q_{M(mean)}$ [3)]		--				--				--				1.8	

DAA = days after 2nd application in T
n. a. = not assessed (no linen sheet at the monitoring site)
LS = Linen sheet
aa = after application
ba = before application
BT = Dead bee trap
STD = Standard deviation
∑ = sum
Ø = mean value
1) = calculated for the daily mean values
2) = calculated over cumulative values (∑)/day
3) = for details concerning the calculation of the quotient of mortality (QM) see chapter 6.9
* = statistically significant

Table 16: Bee mortality of the reference item group R

Date	DAA	Time	R1 LS	R1 BT	R1 ∑	Time	R2 LS	R2 BT	R2 ∑	Time	R3 LS	R3 BT	R3 ∑	Ø (∑)	STD
13 Jul 09	-6	13:00	n. a.	5	5	13:03	n. a.	8	8	13:10	n. a.	12	12	**8.3**	3.5
14 Jul 09	-5	10:58	n. a.	2	2	10:58	n. a.	1	1	10:58	n. a.	3	3	**2.0**	1.0
15 Jul 09	-4	7:15	n. a.	4	4	7:15	n. a.	3	3	7:15	n. a.	2	2	**3.0**	1.0
Ø DAA-6 – -4				**3.7**	**3.7**			**4.0**	**4.0**			**5.7**	**5.7**	**4.4**	3.5[2]
STD				1.5	1.5			3.6	3.6			5.5	5.5	3.4[1]	--
16 Jul 09	-3	11:00	3	11	14	11:04	1	3	4	11:32	2	4	6	**8.0**	5.3
17 Jul 09	-2	11:55	6	6	12	11:53	1	0	1	12:07	10	1	11	**8.0**	6.1
18 Jul 09	-1	11:05	4	3	7	11:07	0	1	1	11:25	7	9	16	**8.0**	7.5
19 Jul 09	0ba	13:10	8	4	12	13:08	0	0	0	13:14	12	5	17	**9.7**	8.7
Ø DAA-3 – 0ba			**5.3**	**6.0**	**11.3**		**0.5**	**1.0**	**1.5**		**7.8**	**4.8**	**12.5**	**8.4**	6.1[2]
STD			2.2	3.6	3.0		0.6	1.4	1.7		4.3	3.3	5.1	0.9[1]	--
Ø DAA-6 – 0ba			**5.3**	**5.0**	**8.0**		**0.5**	**2.3**	**2.6**		**7.8**	**5.1**	**9.6**	**6.7**	5.4 [2]
STD			2.2	2.9	4.7		0.6	2.8	2.8		4.3	4.0	6.0	3.0 [1]	--
19 Jul 09	0aa	15:57	15	52	67	16:06	0	98	98	16:15	8	66	74	79.7	16.3
19 Jul 09	0aa	16:57	13	150	163	17:06	0	120	120	17:15	60	90	150	144.3	22.1
19 Jul 09	0aa	18:57	19	200	219	19:06	4	250	254	19:15	80	200	280	251.0	30.6
19 Jul 09	0aa	20:57	18	80	98	21:06	3	100	103	21:15	65	150	215	138.7	66.2
19 Jul 09	0aa	∑	65	482	547	∑	7	568	575	∑	213	506	719	**613.7***	92.3
20 Jul 09	1	11:11	49	286	335	11:35	5	56	61	12:13	203	273	476	**290.7***	211.
21 Jul 09	2	9:44	36	114	150	9:36	3	51	54	10:05	84	91	175	**126.3***	63.9
22 Jul 09	3	11:11	41	213	254	11:05	4	40	44	12:09	55	122	177	**158.3***	106.
23 Jul 09	4	10:07	13	42	55	9:59	0	30	30	10:33	57	106	163	**82.7***	70.7
24 Jul 09	5	11:05	14	17	31	10:58	2	24	26	11:30	13	181	194	**83.7***	95.6
25 Jul 09	6	11:25	6	8	14	11:18	3	62	65	11:50	5	32	37	**38.7***	25.5
26 Jul 09	7	9:06	9	9	18	8:58	4	29	33	9:29	15	18	33	**28.0**	8.7
27 Jul 09	8	10:04	5	20	25	10:07	0	46	46	10:24	10	36	46	**39.0***	12.1
28 Jul 09	9	9:12	4	21	25	9:04	6	71	77	9:37	15	35	50	**50.7***	26.0
Ø DAA0aa – 9			**24.2**	**121.2**	**145.4**		**3.4**	**97.7**	**101.1**		**67.0**	**140.0**	**207.0**	**151.2**	190.
STD			21.8	159.4	180.4		2.3	165.9	167.3		78.7	151.1	223.2	180.7[1]	
Q_M [3]		12.9				67.4				16.6				--	
$Q_{M(0aa)}$ [3]		--				--				–				73.1	
$Q_{M(mean)}$ [3]		--				--				–				18.0	

DAA = days after 2nd application in T
n. a. = not assessed (no linen sheet at the monitoring site)
LS = Linen sheet
aa = after application
ba = before application
BT = Dead bee trap
STD = Standard deviation
∑ = sum
Ø = mean value
1) = calculated for the daily mean values
2) = calculated over cumulative values (∑)/day
3) = for details concerning the calculation of the quotient of mortality (QM) see chapter 6.9
* = statistically significant

Flight intensity

Flight intensity was significantly reduced at days DAA0 and DAA2. Asterisks were however forgotten in the figure below. A reduced flight activity in T was observed from DAA0aa up to and including DAA3.

* = statistically significant (lower, R versus C; t-test, method pooled, two-sided, α = 0.05).
\# = statistically significant (lower, T versus C; t-test, method pooled, two-sided, α = 0.05).
0ba = before application
0aa = after application

Figure 9.4.2-18. Mean flight intensity values of the control group C, the test item group T and the reference group R prior to and after the 2nd application in T and concurrent applications in C and R.

Strength of the colonies

The mean number of bees assessed before set-up of the hives (first colony assessment) in the tunnels revealed a comparable colony strength in treatment groups C T and R. At the second colony assessment, during the confined exposure period, on DAA7, the mean number of bees was higher in the treatment groups C and T, whereas the mean number of bees in the reference item group was reduced (C: 6438, T: 5854, R: 3188). At the subsequent colony assessment, after the end of the confined exposure period outside the tunnels on DAA13 (third colony assessment), the mean number of bees further increased to 6896 bees/hive for C and 6750 bees/hive for T. At the two following colony assessments on DAA21 (fourth colony assessment) and DAA29 (fifth colony assessment), the mean number of bees for C and T was slightly decreased compared to the third colony assessment (C: 5771 bees/colony on DAA21 and 5229 bees/colony on DAA29; T: 5167 bees/colony on DAA21 and 6000 bees/colony on DAA29).

The development of colony strength was comparable between C and T throughout the study period and showed the fluctuations which are typical for this endpoint. Overall, no test item related adverse effects on colony strength were observed.

Figure 9.4.2-19. Mean number of bees in the treatment groups C, T and R at 5 colony assessment dates. Figure compiled by RMS from three tables in the report.

Figure 9.4.2-20. Mean number of brood and food cells in the treatment groups C, T and R at 5 colony assessment dates. Figure compiled by RMS from three tables in the report.

Development of Brood

The brood development was comparable between the control and the test item treatment colonies, the differences were within the range of natural variation. No test item related adverse effects on brood development were observed.

EFSA asked RMS to provide more detailed data on the larvae/pupae abundance.

Numbers of larvae on the five subsequent colony assessment days were 2267, 2267, 3000, 3333, 1333 in the control; 3067, 2200, 1733, 3067, 1933 in the treatment; and 1867, 467 in the reference group.

The mean abundance of brood (sum of cells containing eggs, larvae, and pupae) assessed before set-up of the hives (first colony assessment) was 10933 cells/hive for C, 13267 cells/hive for T and 10733 cells/hive for R. At the second colony assessment, during the confined exposure period, 7 days after application (DAA7), the mean abundance of brood in C and T decreased slightly, but synchronously (9000 cells/hive for C and 10133 cells/hive for T), whereas the mean abundance of brood in R decreased strongly to 3200 cells/hive. On the third colony assessment day (DAA13, thirteen days after the second application), the mean abundance of brood in C and T was 9000 cells/hive for C with STD 917 and 7600 cells/hive with STD 4303 for T. After one honey bee brood cycle, i.e. 3 weeks after the 2nd test item application (fourth colony assessment, DAA21), the mean abundance of brood in C and T was almost identical (10799 cells/hive for C and 10400 cells/hive for T), the same holds true for the last colony assessment (fifth colony assessment, DAA29: 12733 cells/hive for C and 14000 cells/hive for T). Overall, the brood development was comparable between the control and the test item treatment colonies, the differences were within the range of natural variation. No test item related adverse effects on brood development were observed.

Based on these numbers and because on the first, second, fourth and fifth colony assessment day, there is clearly no effect on total number of brood cells (numbers either equal or higher in T), RMS considers that there is no adverse effect on brood development.

The brood assessment data for the individual hives are shown below, in tables copied from the study report (Tables 20, 21 and 22).

Table 20: Results of colony assessments in the control group C

Colony	C1	C2	C3	Mean	STD
1st colony assessment: 14 Jul 2009 (DAA-5)					
Total number of combs	5	5	5	5	0
Total number of bees[1)]	4188	5188	6250	5209	1031
No. of combs containing brood	3	3	3	3.00	0.00
No. of cells containing eggs[2)]	1200	2000	1600	1600	400
No. of cells containing larvae[2)]	2000	1400	3400	2267	1026
No. of cells containing capped brood[2)]	5400	10200	5600	7067	2715
Sum of cells containing brood	8600	13600	10600	10933	2517
No. of combs containing food	5	4	5	4.67	0.58
No. of cells containing nectar[2)]	9800	8600	6800	8400	1510
No. of cells containing pollen[2)]	200	0	400	200	200
Sum of cells containing food	10000	8600	7200	8600	1400
No. of empty cells	13400	9800	14200	12467	2344
2nd colony assessment: 26 Jul 2009 (DAA7)					
Total number of combs	5	5	5	5	0
Total number of bees[1)]	5063	7375	6875	6438	1216
No. of combs containing brood	3	3	3	3.00	0.00
No. of cells containing eggs[2)]	1400	2600	2000	2000	600
No. of cells containing larvae[2)]	2200	1800	2800	2267	503
No. of cells containing capped brood[2)]	4600	4400	5200	4733	416
Sum of cells containing brood	8200	8800	10000	9000	917
No. of combs containing food	5	5	4	4.67	0.58
No. of cells containing nectar[2)]	6000	5800	3800	5200	1217
No. of cells containing pollen[2)]	800	800	200	600	346
Sum of cells containing food	6800	6600	4000	5800	1562
No. of empty cells	17000	16600	18000	17200	721
3rd colony assessment: 01 Aug 2009 (DAA13)					
Total number of combs	5	5	5	5	0
Total number of bees[1)]	4438	8500	7750	6896	2161
No. of combs containing brood	3	3	3	3.00	0.00
No. of cells containing eggs[2)]	2000	1800	3400	2400	872
No. of cells containing larvae[2)]	2800	3600	2600	3000	529
No. of cells containing capped brood[2)]	3600	4000	4400	4000	400
Sum of cells containing brood	8400	9400	10400	9400	1000
No. of combs containing food	5	4	5	4.67	0.58
No. of cells containing nectar[2)]	6800	3200	2000	4000	2498
No. of cells containing pollen[2)]	1200	600	400	733	416
Sum of cells containing food	8000	3800	2400	4733	2914
No. of empty cells	15600	18800	19200	17867	1973

STD = Standard deviation
1) = calculated as sum of assessed areas of $1dm^2$ *125
2) = calculated as sum of assessed areas of $1dm^2$ *400

Table 20: Results of colony assessments in the control group C (continued)

Colony	C1	C2	C3	Mean	STD
4th colony assessment: 09 Aug 2009 (DAA21)					
Total number of combs	5	5	5	5	0
Total number of bees[1)]	4438	6625	6250	5771	1170
No. of combs containing brood	3	3	3	3.00	0.00
No. of cells containing eggs[2)]	2000	2200	1000	1733	643
No. of cells containing larvae[2)]	3000	3200	3800	3333	416
No. of cells containing capped brood[2)]	5600	5600	6000	5733	231
Sum of cells containing brood	10600	11000	10800	10800	200
No. of combs containing food	5	5	5	5.00	0.00
No. of cells containing nectar[2)]	6200	4200	2400	4267	1901
No. of cells containing pollen[2)]	2600	2400	2000	2333	306
Sum of cells containing food	8800	6600	4400	6600	2200
No. of empty cells	12600	14400	16800	14600	2107
5th colony assessment: 17 Aug 2009 (DAA29)					
Total number of combs	5	5	5	5	0
Total number of bees[1)]	4438	6000	5250	5229	781
No. of combs containing brood	4	4	4	4.00	0.00
No. of cells containing eggs[2)]	600	1400	1200	1067	416
No. of cells containing larvae[2)]	1000	1800	1200	1333	416
No. of cells containing capped brood[2)]	9400	10600	11000	10333	833
Sum of cells containing brood	11000	13800	13400	12733	1514
No. of combs containing food	5	5	5	5.00	0.00
No. of cells containing nectar[2)]	3800	1800	2000	2533	1102
No. of cells containing pollen[2)]	2000	4000	2800	2933	1007
Sum of cells containing food	5800	5800	4800	5467	577
No. of empty cells	15200	12400	13800	13800	1400

STD = Standard deviation
1) = calculated as sum of assessed areas of $1dm^2$ *125
2) = calculated as sum of assessed areas of $1dm^2$ *400

Table 21: Results of colony assessments in the treatment group T

Colony	T1	T2	T3	Mean	STD
1st colony assessment: 14 Jul 2009 (DAA-5)					
Total number of combs	5	5	5	5	0
Total number of bees[1)]	3938	3938	7313	5063	1949
No. of combs containing brood	4	3	4	3.67	0.58
No. of cells containing eggs[2)]	800	2200	2400	1800	872
No. of cells containing larvae[2)]	2400	3200	3600	3067	611
No. of cells containing capped brood[2)]	10200	6000	9000	8400	2163
Sum of cells containing brood	13400	11400	15000	13267	1804
No. of combs containing food	5	5	4	4.67	0.58
No. of cells containing nectar[2)]	8600	12000	7600	9400	2307
No. of cells containing pollen[2)]	400	200	800	467	306
Sum of cells containing food	9000	12200	8400	9867	2043
No. of empty cells	9600	8400	8600	8867	643
2nd colony assessment: 26 Jul 2009 (DAA7)					
Total number of combs	5	5	5	5	0
Total number of bees[1)]	6000	4563	7000	5854	1225
No. of combs containing brood	4	3	4	3.67	0.58
No. of cells containing eggs[2)]	3000	2200	2400	2533	416
No. of cells containing larvae[2)]	3200	1800	1600	2200	872
No. of cells containing capped brood[2)]	7800	5200	3200	5400	2307
Sum of cells containing brood	14000	9200	7200	10133	3495
No. of combs containing food	5	5	4	4.67	0.58
No. of cells containing nectar[2)]	6600	6800	3400	5600	1908
No. of cells containing pollen[2)]	800	800	600	733	115
Sum of cells containing food	7400	7600	4000	6333	2023
No. of empty cells	10600	15200	20800	15533	5108
3rd colony assessment: 01 Aug 2009 (DAA13)					
Total number of combs	5	5	5	5	0
Total number of bees[1)]	6500	6125	7625	6750	781
No. of combs containing brood	4	3	3	3.33	0.58
No. of cells containing eggs[2)]	2000	1600	1200	1600	400
No. of cells containing larvae[2)]	2800	1800	600	1733	1102
No. of cells containing capped brood[2)]	7000	4400	1400	4267	2802
Sum of cells containing brood	11800	7800	3200	7600	4303
No. of combs containing food	5	5	4	4.67	0.58
No. of cells containing nectar[2)]	3400	5000	2600	3667	1222
No. of cells containing pollen[2)]	200	800	400	467	306
Sum of cells containing food	3600	5800	3000	4133	1474
No. of empty cells	16600	18400	25800	20267	4876

STD = Standard deviation
1) = calculated as sum of assessed areas of $1dm^2$ *125
2) = calculated as sum of assessed areas of $1dm^2$ *400

Table 21: Results of colony assessments in the treatment group T (continued)

Colony	T1	T2	T3	Mean	STD
4th colony assessment: 09 Aug 2009 (DAA21)					
Total number of combs	5	5	5	5	0
Total number of bees[1)]	5688	4188	5625	5167	848
No. of combs containing brood	4	4	3	3.67	0.58
No. of cells containing eggs[2)]	1800	3400	2000	2400	872
No. of cells containing larvae[2)]	3600	2800	2800	3067	462
No. of cells containing capped brood[2)]	5800	6400	2600	4933	2043
Sum of cells containing brood	11200	12600	7400	10400	2691
No. of combs containing food	5	5	5	5.00	0.00
No. of cells containing nectar[2)]	5000	4600	3400	4333	833
No. of cells containing pollen[2)]	3600	3000	2200	2933	702
Sum of cells containing food	8600	7600	5600	7267	1528
No. of empty cells	12200	11800	19000	14333	4046
5th colony assessment: 17 Aug 2009 (DAA29)					
Total number of combs	5	5	5	5	0
Total number of bees[1)]	7375	5313	5313	6000	1190
No. of combs containing brood	5	4	4	4.33	0.58
No. of cells containing eggs[2)]	1200	2000	1400	1533	416
No. of cells containing larvae[2)]	1600	2600	1600	1933	577
No. of cells containing capped brood[2)]	13200	9400	9000	10533	2318
Sum of cells containing brood	16000	14000	12000	14000	2000
No. of combs containing food	4	5	4	4.33	0.58
No. of cells containing nectar[2)]	2200	3200	1600	2333	808
No. of cells containing pollen[2)]	2800	2400	2400	2533	231
Sum of cells containing food	5000	5600	4000	4867	808
No. of empty cells	11000	12400	16000	13133	2579

STD = Standard deviation
1) = calculated as sum of assessed areas of $1dm^2$ *125
2) = calculated as sum of assessed areas of $1dm^2$ *400

Table 22: Results of colony assessments in the treatment group R

Colony	R1	R2	R3	Mean	STD
1st colony assessment: 14 Jul 2009 (DAA-5)					
Total number of combs	5	5	5	5	0
Total number of bees[1)]	6250	4000	5063	5104	1126
No. of combs containing brood	3	3	3	3.00	0.00
No. of cells containing eggs[2)]	3400	1400	1400	2067	1155
No. of cells containing larvae[2)]	2800	600	2200	1867	1137
No. of cells containing capped brood[2)]	7000	3600	9800	6800	3105
Sum of cells containing brood	13200	5600	13400	10733	4447
No. of combs containing food	4	5	4	4.33	0.58
No. of cells containing nectar[2)]	5600	11800	8600	8667	3101
No. of cells containing pollen[2)]	800	0	400	400	400
Sum of cells containing food	6400	11800	9000	9067	2701
No. of empty cells	12400	14600	9600	12200	2506
2nd colony assessment: 26 Jul 2009 (DAA7)					
Total number of combs	5	5	5	5	0
Total number of bees[1)]	3625	2313	3625	3188	757
No. of combs containing brood	2	2	2	2.00	0.00
No. of cells containing eggs[2)]	1400	400	0	600	721
No. of cells containing larvae[2)]	1400	0	0	467	808
No. of cells containing capped brood[2)]	1800	1800	2800	2133	577
Sum of cells containing brood	4600	2200	2800	3200	1249
No. of combs containing food	4	5	4	4.33	0.58
No. of cells containing nectar[2)]	2600	5600	4400	4200	1510
No. of cells containing pollen[2)]	800	800	0	533	462
Sum of cells containing food	3400	6400	4400	4733	1528
No. of empty cells	24000	23400	24800	24067	702

STD = Standard deviation
1) = calculated as sum of assessed areas of 1dm² *125
2) = calculated as sum of assessed areas of 1dm² *400

Development of the Food Storage Area

The observed decrease in food stores in both, test item treatment and control during confinement as well as the subsequent increase can be considered as typical for this type of study. The decrease of the mean extent of food stores on the last assessment day (DAA29) can be explained by the advanced season. No test-item related adverse effects on the development of the food storage area were observed.

Behaviour of the Bees

Clustering at the hive entrance on DAA1 in hive C3 and on DAA10 in hive T1 was observed. No further abnormal behaviour was recorded in the control and in the test item treatment group, respectively. After the application of the reference item, a strong repellence effect (no or low flight activity on the crop) as well as several bees with obvious signs of intoxication were observed in the reference item group.

Residues in flowers

Residues in flowers are given in Table 9.4.2-6.

Table 9.4.2-6. Residues of BYI02960 and BYI 02960-DFEAF in flowers after application of BYI 02960 SL 200 at 200g as/ha

Sample material	Sample Name	Sample ID	Sub-sample (Replicate)	Time of sampling	Control/ Treatment	Date of sampling	Residue BYI 02960 [µg/kg]	Residue BYI 02960-DFEAF [µg/kg]
Flower	1C	100424	A	0DAA2	Control	2010-07-19	<LOQ	<LOD
	2C	100426		0DAA2		2010-07-19		
	3C	100428		0DAA2		2010-07-19		
	1C	100436		7(±1)DAA2		2010-07-26		
	2C	100438		7(±1)DAA2		2010-07-26		
	3C	100440		7(±1)DAA2		2010-07-26		
Flower	1C	100425	B	0DAA2	Control	2010-07-19	<LOD	<LOD
	2C	100427		0DAA2		2010-07-19		
	3C	100429		0DAA2		2010-07-19		
	1C	100437		7(±1)DAA2		2010-07-26	<LOQ	
	2C	100439		7(±1)DAA2		2010-07-26	<LOD	
	3C	100441		7(±1)DAA2		2010-07-26	<LOQ	
Flower	1T	100430	A	0DAA2	Treated	2010-07-19	7534	7
	2T	100432		0DAA2		2010-07-19	16308	12
	3T	100434		0DAA2		2010-07-19	13098	11
	1T	100442		7(±1)DAA2		2010-07-26	327	8
	2T	100444		7(±1)DAA2		2010-07-26	3141	44
	3T	100446		7(±1)DAA2		2010-07-26	729	22
Flower	1T	100431	B	0DAA2	Treated	2010-07-19	9925	9
	2T	100433		0DAA2		2010-07-19	17310	16
	3T	100435		0DAA2		2010-07-19	12199	13
	1T	100443		7(±1)DAA2		2010-07-26	238	12
	2T	100445		7(±1)DAA2		2010-07-26	1381	52
	3T	100447		7(±1)DAA2		2010-07-26	143	13

Conclusions of the author

A pre-flowering foliar application of the test item, corresponding to 200 g BYI 02960 as/ha to a highly bee-attractive crop (*Phacelia tanacetifolia*), was followed by a further foliar application of the test item, corresponding to 200 g BYI 02960 as/ha, during full-flowering with honey bees actively foraging on the crop.

This application scenario did not cause treatment-related adverse effects on mortality, on brood and food development as well as on colony vitality under forced exposure conditions.

A slight repellent effect of the test item was indicated by a reduced flight intensity on the day of the 2nd test item application (DAA0aa) as well as on DAA1 and DAA2. From DAA3 onwards, the flight intensity in the test item treatment was comparable to the control.

Remarks by RMS

A positive control was included in the test and showed that the test system was behaving as expected. On DAA1, the mean mortality in the test item treatment group was slightly, but statistically significantly higher than in the control group (t-test, method pooled, one-sided, α = 0.05; number of dead bees: 4.0, 18.0, and 290.7 for C, T, and R, respectively). In the total post application period however, the mean daily mortality was recorded to be 10.4, 14.3, and 151.2 for C, T, and R, respectively and there is no significant difference between the treatment and the control. Since the total mortality in the treatment on day 21(18 out of a mean of 5063 bees (mean), corresponds to a mortality < 0.4%, the RMS considers this effect to be negligible.

According to the EFSA Opinion on the Science behind the development of a risk assessment of plant protection products for bees (EFSA Journal 2012, 10: 2668, section 3.5.1), the main exposure route for bee brood is via pollen. Since *Phacelia tanacetifolia* is a major source of pollen, and pollen from uncontaminated sources was not present in the tunnels, the present test is suitable to show potential effect of flupyradifurone on brood related parameters, mortality and flight intensity.
The results of the study show that there is no (or a negligible) adverse effect on mortality, on brood and food development as well as on colony vitality of the honeybees after foraging on *Phacelia tanacetifolia* sprayed once with BYI 02960 during pre-flowering and once during full-flowering at an application rate corresponding to 200 g as/ha in a tunnel test except for a reduced flight intensity on the day of the second application and the two following days. The results of this study, including the residue measurements, can be used in the risk assessment.

Expert discussion in Pesticide Peer Review meeting 124 (December 2014):
(Please note that the word 'effect' in the text below might be interpreted as different from the control (i.e. may or may not include effects from the treatment), pending on the context.)

The experts noted that the colony size at the study initiation was reasonable (approx. 7000 bees). Only visual inspections were used to assess the combs.

Fewer larvae were observed in the treated groups on assessment day 3. Fig. B.4.2-20 (DAR). These effects could be within natural variation but effects of the test item could not be excluded also. In addition, there was also an increase in forager mortality, as noted by the RMS, just after application (day 1 and day 0).

It was noted that it was difficult to interpret the results of the study from visual inspection of the figures alone. The actual numbers (in the tables) need to be considered also.

Overall, there was no disagreement with the RMS's assessment.

Study KIIIA 10.4.7/04

Characteristics

Reference	:	Pröbsting, A. (2012a)	nominal dose		1x 1.5 L/ha = 300 g a.s./ha 2x 1 L/ha = 200 g a.s./ha
type of study	:	Honeybee semi-field tunnel test	species	:	Honey bee (*Apis mellifera*)
year of execution	:	2010	observation duration	:	32 d
GLP statement	:	Yes	exposure duration	:	10 d
Guideline	:	OEPP/EPPO guideline No. 170 (3), 2001	dosing method	:	Tunnel test in flowering *Phacelia tanacetifolia*, 1x soil application, 2x foliar spray: 1st foliar application 4 d before hive set-up and 2nd foliar application 7 d after 1st foliar application (3 d after hive set-up) during flowering and active foraging of

					bees
test substance	:	BYI 02960 SL 200 G, 201.0 g flupyradifurone /L Batch no. 2010-001067	acceptability	:	**Not acceptable for bee endpoints** Acceptable for residue measurements
a.s. content	:	201.0 g flupyradifurone/L	Result	:	Effects on mortality, flight intensity and brood development cannot be evaluated. No adverse effect on behaviour.

Description

The study was conducted in Kværs, near Sønderborg, Region Syddanmark, Denmark on a field of flowering *Phacelia tanacetifolia*. The experimental design of this study comprised three experimental groups with three replicates each, 9 tunnel tents were set-up before the 3rd application in T and the concurrent applications in C and R, respectively. The individual treatment groups, the respective application rates per ha and the respective spray volumes per ha are described in the following table (copied from the report).

Table 9.4.2-7. Treatment groups, application rates and spray volume.

Treatment group	Code	Time of application	Application rate		Spray volume
			[g a.s./ha]*	[g product/ha]	
Control (tap water)	C	DAA0, BBCH 65	None	None	200 L/ha
Test item (BYI 02960 SL 200 G)	T	On the day of sowing, before sowing, BBCH 00	300	1.5 L	200 L/ha
		DAA -7, BBCH 60	200	1 L	200 L/ha
		DAA0, BBCH 65	200	1 L	200 L/ha
Reference item	R	DAA0, BBCH 65	400	1 L	200 L/ha

DAA = days after 3rd test item application in T
* calculation based on the nominal content of a. s.

The reference item was Perfekthion EC 400 (a.s. dimethoate). The total area covered per tunnel tent was 60 m^2. The dimensions of each tunnel tent (covered area) was 12 m long, 5 m wide; the height of the tunnel in its centre was 3.5 m.

The 1st and the 2nd test item application were conducted on three replicate plots with 12 m width and 20 m length. The 3rd test item application was carried out inside the tunnel tents. One small bee colony was introduced per tunnel, 3 days before the 3rd application in T and the concurrent applications in C and R, respectively. After the end of the confined exposure period (10 d; 7 days after 3rd application), the colonies were relocated to a monitoring site without intensive agriculture.

For the test, small healthy colonies with ten combs were used. The colony size at set-up was in the range of approximately 1000 – 9000 bees (on DAA-3: control mean size 1337 bees, treatment mean size 4274 bees, reference group mean size 3065 bees). All nuclei were produced at the same time. The colonies were from one breeding line and as similar to one another as possible. Furthermore the following criteria for the nuclei were guaranteed:

- 3 - 6 brood combs containing eggs, larvae and capped cells;
- 5 - 10 combs containing honey and pollen;
- bees were free of Nosema and Varroa disease symptoms and other bee diseases

Wooden bee traps with gauze on bottom and on top were attached to the entrance of the nuclei in order to register those dead bees which were carried out of the hives. Set-up of the hives was three days before the 3rd application in T and the concurrent applications in C and R, respectively.

The test item solution was prepared shortly before each application. The applications were carried out with a portable boom sprayer that simulates a commercial application. Immediately after the 1st test item application, the test item was incorporated into the soil of the treatment plots to a depth of approximately 15 cm. At the same day - after application and incorporation of the test item and collection of the soil samples - *Phacelia tanacetifolia*-seeds were sown. The following conditions were met for application:

- cropless soil at 1st application in T (43 d before 2nd application)
- crop at BBCH 60, at 2nd application in T (7 d before 3rd application)
- crop at full bloom (BBCH 65), at 3rd application in T and the concurrent applications in C and R, respectively
- bees were actively foraging at 3rd application in T and the concurrent applications in C and R, respectively
- air temperature did not exceed 30°C (all applications)
- wind speed was 0.5 m/s at 1st application in T
- wind speed was 0.0 m/s at 2nd application in T
- wind speed inside the tunnel tents was 1-2 m/s at 3rd application in T and the concurrent applications in C and R, respectively (2-3 m/s during application in R).

During the 3rd test item application as well as during the concurrent application of tap water and the reference item, the bee colonies were covered with plastic sheets and the water supply was moved out of the tunnels until the end of application to avoid direct contamination. After the application of each plot the volume applied was determined by measuring the remaining spray solution.

Bee traps with gauze on bottom and on top were attached to the entrance of the hives in order to register those dead bees which were carried out of the hives. The mortality assessments started 3 days before set up of the colonies inside the tunnels and lasted until the re-location of the hives to the monitoring location.

Furthermore, mortality was recorded in the crop. To facilitate and standardise the mortality assessment in the crop, *Phacelia tanacetifolia* plants were removed prior to the set-up of the hives from the areas, moreover, water-permeable linen sheets were spread out on the ground. At each assessment, the dead bees found on the sheets and in the bee traps were counted and removed. The mortality of honeybees of the different treatment groups in the dead bee trap at the entrance of the hive and on the linen sheets were assessed prior to set up (DAA -6 to -3), prior to the 3rd test item application (DAA -2 to -1), on the day of the 3rd test item application (before the application (Oba) and 1 h, 2h, 4h and 6h after the application (0aa)) and after the 3rd test item application DAA 1 to 3 (2 times a day at approximately the same time) and DAA 4 to 7 (once a day about the same time of the day in the morning up to noon).

Flight Intensity

At each assessment during the exposure period, the number of forager bees that were both foraging on flowering *Phacelia tanacetifolia* and flying over the crop were counted on a square of 1 m² for a short period of time (approximately 10-15 seconds). At each assessment time per plot, the square observed was chosen by chance.

Condition of the colonies, development of the bee brood.

The condition of the colonies and the development of the bee brood were checked five times during the test. The first colony assessment took place shortly before set-up of the colonies inside the tunnels (DAA-3). The second colony assessment was done on DAA7. Three further colony assessments took place after re-location of the colonies to the monitoring site at weekly intervals (±1 day).

In order to record possible effects of the test item, the following parameters were assessed:

- Strength of the colony (number of bees estimated according to IMDORF & GERIG, 1999 and IMDORF et al., 1987)
- Presence of a healthy queen
- Visual assessment of the brood (estimation of the number of cells filled with eggs/larvae/pupae, according to IMDORF & GERIG, 1999 and IMDORF et al., 1987)
- Visual assessment of the food (estimation of the number of cells filled with pollen/nectar, according to IMDORF & GERIG, 1999 and IMDORF et al., 1987)

At each colony assessment, the comb area containing bees and cells with nectar, pollen, eggs, larvae and capped cells was estimated per comb side and the total number of bees and cells containing the brood stages, pollen and nectar on the comb were calculated. This was done for all combs per hive. Afterwards, the total values were calculated for each hive and assessment date.

Behaviour of the Bees

The behaviour of the bees on the crop and around the hive was assessed during the exposure period. The assessments were performed during the flight intensity assessments.

At least the following symptoms were checked in comparison to the control bees:

- Aggressiveness
- Intensive flying activity without landing on the crop
- Clustering at the bee hive entrance

- Intoxication symptoms

Recording of the Meteorological Data

For the entire exposure period the following climatic data were recorded by a weather station (GLP record):

- air temperature (daily min/max)
- relative air humidity (daily min/max)

During applications and assessments, the following data were recorded at the field site (GLP record):

- cloud cover (estimated during application and assessments of flight intensity)
- air temperature, relative air humidity (only during the application)
- wind speed (only during the application)
- rainfall (rain gauge placed in one tunnel)

Soil Sample for Residue Analysis

2 x 10 soil cores were taken on each of the with BYI 02960 SL 200G treated plots, after the 1st test item application and immediately after incorporation of the test item into the soil (i.e. before sowing *Phacelia tanacetifolia* seeds). Subsequent to the sampling, *Phacelia tanacetifolia* seeds were sown. Soil samples consisted of soil cores with a diameter of 50 mm that have been taken to a depth of 20 cm. The soil cores per individual plot (in total there were three BYI 02960 SL 200G treated plots) and sub-sample were pooled; 10 cores were used and pooled for residue analysis (i.e. sub-sample A) and 10 cores were used and pooled as a retained sample (sub-sample B). No soil samples were taken on the control and reference plots.

Collection of Flowers

On DAA0aa1 and DAA7, flowers of *Phacelia tanacetifolia* were collected. Samples from the control tunnels were taken first, followed by the samples from the test item tunnels. No samples were taken in the reference item tunnels.

The terminal inflorescences with open flowers was cut at the end of the stipe and bagged. A pooled sample comprising ≥ 10 g flowers per treatment was collected from at least 12 different locations across each tunnel. Each sample was divided into two sub-samples (A and B, each sub-sample containing ≥ 5 g).

Collection of Pollen from Combs

One pollen sample per tunnel (excluding the reference item tunnel) was taken from the hives on DAA7. Samples from the control tunnels were taken before those of the test item tunnels. The pollen was collected with a pollen lifter which takes the pollen directly from the cell. Each sample was divided into two sub-samples in order to get one A (residue analysis) and one B sample (retained).

Collection of Nectar from Combs

One nectar sample per tunnel (excluding the reference item tunnel) was taken from the hives on DAA7. Samples from the control tunnels were taken before those of the test item tunnels.

The nectar was collected by means of a table spoon which was pushed into the wax covered nectar cells.

Residue analysis in honeybee products and flowers

Residue analysis of BYI 02960 and its relevant metabolite(s) in flower, nectar, pollen and soil was performed by using High Performance Liquid Chromatography (HPLC), coupled with electrospray and tandem mass spectrometry (MS/MS) detection.

Statistical analysis

The data of mortality and flight intensity of the test item and the reference item group were compared to the control in two separate approaches. The data were tested for normality and homoscedasticity using Shapiro-Wilk test ($p > 0.05$) and folded F-test ($p > 0.05$), respectively. Data were statistically compared using t-test (method pooled, $p \leq 0.5$) in case of normality and homoscedasticity. In case of no homoscedasticity but proven normality t-test was conducted with method Satterthwaite (same as Welch's t-test, $p \leq 0.5$). In case of no normality and no homoscedasticity Wilcoxon test ($p \leq 0.5$) was used

Results

Climatic conditions

Temperature during 3rd application was between 11.6 and 34.3.°C, 31-100% RH, no rainfall; Temperature during observation ranged from 9.2 to 36.6 °C. Rainfall 6.5 mm 1 day after 3rd application and 3.5 mm 6 days after 3rd application.

<u>Mortality</u>

Mortality of the different test groups is reported in the following figure (copied from the report).

F

igure 9.4.2-21. Mean number of dead bees (worker bees, larvae and pupae)/treatment group/day counted in the dead bee traps and on the linen sheets prior to and after the 3rd test item application.

* = statistically significant (lower);

0ba = day of application;

0aa = after 3rd test application in T and the concurrent applications in C and R, respectively.

Mortality in the treatment was higher throughout the entire assessment period, but was only significant at DAA6. The high number of dead bees in R during the post application period shows the sensitivity of the test system and was statistically significantly increased from DAA0aa to DAA5. According to author, the higher number of bees in the test item treatment group was the result of a higher turnover of the more stronger colonies in terms of mortality rates (at set-up, control colonies comprised on average 1337 bees [range: 816 - 2254], treatment colonies comprised on average 4274 bees [range: 1503 - 9192]).

Flight intensity

Figure 9.4.2.22 presents the mean flight intensity in the treatments groups C, T and R. On the day of the 3rd application (DAA0aa), on the day after this application (DAA1) and from DAA3 to 5 slight reductions in flight intensity were found in the test item group T when compared to the control group (T). Only on DAA5, the flight intensity was statistically significantly lower in T when compared to C.

Figure 9.4.2.22. Mean number of forager bees/m^2 inside the tunnels for C (control), T (treatment) and R (reference) prior to and after the 3rd application in T.

* = statistically significant (lower);

0ba = day of application;

0aa = after 3rd test application in T and the concurrent applications in C and R, respectively.

Strength of the colonies

Results concerning the condition of the colonies are reported in the following two figures. Figures are compiled from the original tables in the report. The 1st and 2nd colony assessment were made during confined tunnel situation.

Increases in number of bees were observed at the 3rd colony assessment. According to the author, the observed decrease in the average number of bees in T during the confined exposure conditions can be explained by limited forage. The limited forage was, however, sufficient for the smaller colonies in C. They showed an increase of colony strength at the 2nd colony assessment.

Figure 9.4.2-23. Mean number of bees in the treatment groups C, T and R at 5 colony assessment dates (DAA-3, DAA7, DAA14, DAA20 and DAA28 , resp.). Figure compiled by RMS from three tables in the report.

Figure 9.4.2-24. Mean number of brood and food cells in the treatment groups C, T and R at 5 colony assessment dates (DAA-3, DAA7, DAA14, DAA20 and DAA28 , resp.). Figure compiled by RMS from three tables in the report.

Mean abundance of brood (eggs, larvae and capped brood) showed an increase for C and T during the whole study period. In hives of R a decrease was observed at the second assessment date, followed by an increase at the fourth and fifth assessment day (7 and 13 days, respectively, after re-location of the hives to the monitoring site).

The mean extent of food stores in the colonies (sum of cells containing nectar and pollen) assessed before set-up of the hives (first colony assessment) was 19933 cells/hive for C, 18867 cells/hive for T and 15000 cells/hive for R. At the second colony assessment, during the confined exposure period, on

DAA7, the mean extent of food stores in the colonies C, T and R decreased synchronously (14667 cells/hive for C, 14440 cells/hive for T and 13600 cells/hive for R). At the subsequent colony assessment, after the end of the confined exposure period outside the tunnels (third colony assessment), the mean extent of food stores in the colonies C and T increased again to 15600 cells/hive for C and 16667 cells/hive for T. Increases at the fourth and fifth assessment days were comparable between C and T.

Accoring to the author, the observed decrease in food stores in both, treatment and control, during confinement as well as the subsequent increase can be considered as typical for this type of study. No test-item related adverse effects on the development of the food storage area were observed.

Behaviour of the Bees

No abnormal behaviour was recorded in the control group. Bees of one of the hives in the test item treatment group were clustering at the hive entrance on the day of the 3rd test item application during the two last assessments. From DAA1 until the end of the confinement period on DAA7, no abnormal behaviour of the bees was observed in all three replicates of T. After the application of the reference item, a strong repellence effect (no or low flight activity on the crop) as well as several bees with obvious signs of intoxication were observed in the reference item group.

Residue Analysis

The residue levels of BYI02960 and BYI02960-DFEAF in all control flower, control pollen and control nectar samples were below the Limit of Quantitation (LOQ) of 1 µg/kg.

Residues in flower, pollen and nectar of treated samples are given in the Table below.

Table 9.4.2-8. Residues of BYI 02960 and its metabolite BYI 02960-DFEAF in flower, pollen, nectar and soil.

Sample material	Sample interval	Residue BYI 02960 [µg/kg]	Residue BYI 02960-DFEAF [µg/kg]
Flower	0 DAA3	37592	78
	7 DAA3	199	9
Pollen (combs) (3 samples)	7 DAA3	3025, 6109, 8144	7, 13, 20
Nectar (combs) (3 samples)	7 DAA3	15, 18, 31	< LOQ
Soil (3 samples)	0 DAA1	36, 51, 67	< LOQ*

* Analysis was not conducted under GLP

Conclusions of the author

The application scenario used in this study did not cause treatment-related adverse effects on mortality, no effects on brood and food development as well as on colony vitality under forced exposure conditions.

A slight repellent effect of the test item was observed on the day of the 3rd test item application, after the treatment, as well as on DAA1 and from DAA3 to 5.

Remarks by RMS

A positive control was included in the test and showed that the test system was behaving as expected. According to the EFSA Opinion on the Science behind the development of a risk assessment of plant protection products for bees (EFSA Journal 2012, 10: 2668, section 3.5.1), the main exposure route for bee brood is via pollen. Since *Phacelia tanacetifolia* is a major source of pollen, and pollen from uncontaminated sources was not present in the tunnels, the present test is suitable to show potential effect of flupyradifurone on brood related parameters, mortality and flight intensity.
A draw-back of the study is that smaller colonies were set up in the control treatment. According to the author these smaller colonies were able to grow during confinement in the tunnel, whereas the larger colonies seemingly were restricted in their growth by limited forage. As the hives were not assigned to the three treatments correctly, it is not possible to evaluate whether reduction of growth in the flupyradifurone treatment was caused by the active substance or by restriction of forage.
The incorrect assignment of hives to the three treatments also causes problems with the interpretation of flight behaviour and mortality. The first two colony assessment dates are at DAA-3 and DAA7. Within this period the mean number of bees increases rapidly in the control hives (Figure 9.4.2-23). In Figure 9.4.2.22 it shows that at DAA0aa and DAA1 (in between the first two colony assessment dates) a lower flight intensity is present in the treatment. It is, however, not possible to judge the relation between flight intensity at DAA0aa and DAA1 and the numbers of bees at these dates. If numbers of bees in the control hives are still much lower than in the treatment, the data on flight intensity in Figure 9.4.2-22 are based on unequal bee numbers. It is concluded that the statistical analysis is not reliable. It is possible that Figure 9.4.2-22 is an underestimation of the effect on mortality in the treatment.
As the effects on bees of this study cannot be evaluated correctly, they cannot be used in risk assessment. The residue measurements can be used in the risk assessment.

Expert discussion in Pesticide Peer Review meeting 124 (December 2014):
(Please note that the word ‘effect’ in the text below might be interpreted as different from the control (i.e. may or may not include effects from the treatment), pending on the context.)

The RMS did not consider this study as valid for risk assessment. The reason for this was that the size of the control colonies differed from the test item colonies at the study initiation.

The experts agreed with the assessment of the RMS.

Study KIIIA 10.4.7/05

Characteristics

Reference	:	Pröbsting, A. (2012b)	nominal dose		1x 1.5 L/ha = 300 g a.s./ha 2x 1 L/ha = 200 g a.s./ha
type of study	:	Honeybee semi-field tunnel test	species	:	Honey bee (*Apis mellifera*)
year of execution	:	2011	observation duration	:	33 d
GLP statement	:	Yes	exposure duration	:	12 d
Guideline	:	OEPP/EPPO guideline No. 170 (4), 2001	dosing method	:	Tunnel test in flowering *Phacelia tanacetifolia*, 1x soil application, 2x

					foliar spray: 1st foliar application 14 d before hive set-up and 2nd foliar application 4 d after hive set-up during flowering and active foraging of bees
test substance	:	BYI 02960 SL 200 G, Batch no. 2010-007173	acceptability	:	Acceptable
a.s. content	:	198.6 g flupyradifurone /L	Result	:	No adverse effect on mortality and brood development. Adverse effect on flight intensity and behaviour.

Description

The study was conducted in Poggio Renatico, province of Ferrara, in the region Emilia Romagna, Italy on a field of flowering *Phacelia tanacetifolia*. The experimental design of this study comprised three experimental groups with three replicates each, 9 tunnel tents were set-up 4 days before the 3rd application in T and the concurrent applications in C and R, respectively. The individual treatment groups, the respective application rates per ha and the respective spray volumes per ha are described in the following table (copied from the report).

Table 9.4.2-9. Treatment groups, application rates and spray volume.

Treatment group	Code	Time of application	Application rate		Spray volume
			[g a.s./ha]*	[g product/ha]	
Control (tap water)	C	DAA0, BBCH 63-68	None	None	200 L/ha
Test item (BYI 02960 SL 200 G)	T	On the day of sowing, before sowing, BBCH 00	300	1.5 L	200 L/ha
		DAA-18, BBCH 58-61	200	1 L	200 L/ha
		DAA0, BBCH 63-68	200	1 L	200 L/ha
Reference item	R	DAA0, BBCH 63-68	400	1 L	200 L/ha

DAA = days after 3rd test item application
* calculation based on the nominal content of a. s.

Reference item was Perfekthion (a.s. dimethoate). The total area covered per tunnel tent was 60 m^2. The 1st and the 2nd test item application were conducted on three replicate plots with 10 m width and 20 m length. The 3rd test item application was carried out inside the tunnel tents. One small bee colony was introduced per tunnel, 4 days before the 3rd application in T and the concurrent applications in C and R, respectively. After the end of the 12-d confined exposure period (7 days after the 3rd test item application), the colonies were relocated to a monitoring site without intensive agriculture.

For the test, small healthy colonies with 6 combs were used. The colony size at set-up was in the range of approximately 7000-13800 bees. All nuclei were produced at the same time. The colonies were from one breeding line in order to guarantee uniform bee material in all treatments. Furthermore the following criteria for the nuclei were guaranteed:

- 3 - 6 brood combs containing eggs, larvae and capped cells;
- 5 - 6 combs containing honey and pollen;
- bees were free of Nosema and Varroa disease symptoms and other bee diseases

The test item solution was prepared shortly before each application. The applications were carried out with a portable boom sprayer that simulates a commercial application.

Immediately after the 1st test item application, the test item was incorporated into the soil of the treatment plots to a depth of approximately 8-10 cm. At the same day - after application and incorporation of the test item and collection of the soil samples - *Phacelia tanacetifolia*-seeds were sown. The following conditions were met for application:

- cropless soil at 1st application in T (unknown number of days before 2nd application)
- crop at BBCH 58-61, at 2nd application in T (18 d before 3rd application)
- crop at full bloom (BBCH 63-68), at 3rd application in T and the concurrent applications in C and R, respectively
- bees were actively foraging at 3rd application in T and the concurrent applications in C and R, respectively
- air temperature did not exceed 30°C (all applications)
- wind speed was 0 m/s at 2nd application in T
- wind speed inside the tunnel tents was 0 m/s at 3_{rd} application in T and the concurrent applications in C and R, respectively.

During the 3rd application in T and the concurrent applications in C and R, respectively, the bee colonies were covered with plastic sheets. After the application of each plot, the volume applied was determined by measuring the remaining spray solution.

Mortality

Mortality was recorded in the crop. At each assessment, the dead bees found on the sheets and in the bee traps were counted and removed. The mortality of honeybees of the different treatment groups in the dead bee trap at the entrance of the hive and on the linen sheets were assessed prior to set up (DAA -19 to -5), prior to the 3rd test item application (DAA -4 to -1), on the day of the 3rd test item application (before the application (Oba) and 1 h, 2h, 4h and 6h after the application (0aa)) and after the 3rd test item application DAA 1 to 3 (2 times a day at approximately the same time) and DAA 4 to 7 (once a day about the same time of the day in the morning up to noon).

Flight Intensity

At each assessment during the exposure period, the number of forager bees that were both foraging on flowering *Phacelia tanacetifolia* and flying over the crop were counted on a square of 1 m² for a short period of time (approximately 10-15 seconds). At each assessment time per plot, the square observed was chosen by chance.

Condition of the colonies, development of the bee brood.

The condition of the colonies and the development of the bee brood was checked five times during the test. The first colony assessment took place shortly before set-up of the colonies inside the tunnels

(DAA-5). The second colony assessment was done on DAA7. Three further colony assessments took place after re-location of the colonies to the monitoring site at weekly intervals.

In order to record possible effects of the test item, the following parameters were assessed:

- Strength of the colony
- Presence of a healthy queen
- Visual assessment of the brood (estimation of the number of cells filled with eggs/larvae/pupae)
- Visual assessment of the food (estimation of the number of cells filled with pollen/nectar)

At each colony assessment, the comb area containing bees and cells with nectar, pollen, eggs, larvae and capped cells was estimated per comb side and the total number of bees and cells containing the brood stages, pollen and nectar on the comb were calculated. This was done for all combs per hive. Afterwards, the total values were calculated for each hive and assessment date.

Behaviour of the Bees

The behaviour of the bees on the crop and around the hive was assessed during the exposure period. The assessments were performed during the flight intensity assessments.

At least the following symptoms were checked in comparison to the control bees:

- Aggressiveness
- Intensive flying activity without landing on the crop
- Clustering at the bee hive entrance
- Landing on the covering net
- Intoxication symptoms

Recording of the Meteorological Data

For the entire exposure period the following climatic data were recorded by a weather station (non-GLP record, distance from the field site 3 km):

- air temperature (daily min/max)
- relative air humidity (daily min/max)
- rainfall

During applications and assessments, the following data were recorded at the field site (GLP record):

- cloud cover (estimated during application and assessments of flight intensity)
- air temperature, relative air humidity (only during the application)
- wind speed (only during the application)

Soil Sample for Residue Analysis

2 x 10 soil cores were taken on each of the with BYI 02960 SL 200G treated plots, after the 1st test item application and immediately after incorporation of the test item into the soil (i.e. before sowing *Phacelia tanacetifolia* seeds). Subsequent to the sampling, *Phacelia tanacetifolia* seeds were sown. Soil samples consisted of soil cores with a diameter of 50 mm that have been taken to a depth of 20 cm. The soil cores per individual plot (in total there were three BYI 02960 SL 200G treated plots) and sub-sample were pooled; 10 cores were used and pooled for residue analysis (i.e. sub-sample A) and

10 cores were used and pooled as a retained sample (sub-sample B). No soil samples were taken on the control and reference plots.

Collection of Flowers

On DAA0aa1 and DAA7, flowers of *Phacelia tanacetifolia* were collected. Samples from the control tunnels were taken first, followed by the samples from the test item tunnels. No samples were taken in the reference item tunnels.

The terminal inflorescences with open flowers was cut at the end of the stipe and bagged. A pooled sample comprising ≥ 10 g flowers per treatment was collected from at least 12 different locations across each tunnel. Each sample was divided into two sub-samples (A and B).

Collection of Pollen from Combs

On DAA 7 no pollen samples could be taken from the hives since no pollen was available. This can be explained by the restricted foraging area during the confinement in the tunnel tents (DAA-4 to7), which did not allow for the storage of excess *Phacelia*-pollen, concurrently to the on-going feeding of the brood.

Collection of Nectar from Combs

One nectar sample per tunnel (excluding the reference item tunnel) was taken from the hives on DAA7. Samples from the control tunnels were taken before those of the test item tunnels.

The nectar was collected by means of a table spoon which was pushed into the wax covered nectar cells. Each sample was divided into two sub-samples in order to get one A (residue analysis) and one B sample (retained).

Collection of Wax from Combs

One bee wax sample per tunnel (excluding the reference item tunnel) was taken from the hives once on DAA7. Samples from the control tunnels were taken before those of the test item tunnels. Knifes were used for sampling. Each sample was divided into two sub-samples in order to get one A (residue analysis) and one B sample (retained).

Residue analysis in honeybee products and flowers

Residue levels of BYI02960 and its metabolite BYI02960-difluoroethylaminofuranone (BYI02960-DFEAF) in pollen, nectar and beewax from comb as well as in flowers of *Phacelia tanacetifolia*. In addition, also the soil concentration of BYI02960 after application and immediate soil in corporation on the test plot of the test item treatment group has been determined.

Calculations and statistical analysis

For the evaluation of the number of dead bees, quotients of mortality (Q_M) were included. The formulas which were used for the calculation of the quotients are as follows:

QM = post-application mortality (DAA0aa to 7) ÷ pre-(3rd)-application mortality (DAA-4 to 0ba; per replicate)

QM(0aa) = mortality on the day of 3rd application (DAA0aa) ± pre-(3rd)-application mortality (DAA-4 to 0ba; per treatment group)

QM(mean) = post-application mortality (DAA0aa to 7) ± pre-(3rd)-application mortality (DAA-4 to 0ba; per treatment group).

The data of mortality and flight intensity of the test item and the reference item group were compared to the control in two separate approaches. The data were tested for normality and homoscedasticity using Shapiro-Wilk test ($p > 0.05$) and folded F-test ($p > 0.05$), respectively. Data were statistically compared using t-test (method pooled, $p \leq 0.5$) in case of normality and homoscedasticity. In case of no homoscedasticity but proven normality t-test was conducted with method Satterthwaite (same as Welch's t-test, $p \leq 0.5$). In case of no normality and no homoscedasticity Wilcoxon test ($p \leq 0.5$) was used

Results

Climatic conditions

Temperature during 3rd application was between 16.0 and 30.7.°C, 42-93% RH, no rainfall;
Temperature during observation ranged from 16.0 to 33.1 °C. No rainfall.

Mortality

Mortality of the different test groups is ported in the following figure (copied from the report).

Figure 9.4.2-25. Mean number of dead bees (worker bees, larvae and pupae)/treatment group/day counted in the dead bee traps and on the linen sheets prior to and after the 3rd test item application.

* = statistically significant (lower);

0ba = day of application;

0aa = after 3rd test application in T and the concurrent applications in C and R, respectively.

No statistically significant differences between the daily mortality values in the control and the test item treatment group were detected throughout the entire assessment period. The high number of dead bees in R during the post application period shows the sensitivity of the test system and was statistically significantly increased from DAA0aa to DAA3.

EFSA asked RMS to provide more detailed data for adult mortality. The mortality data for dead bees for the individual hives are copied below from the study report (Tables 14, 15 and 16). The study report does not provide separate data for adults and pupae.

Table 14: Results of the mortality assessments of the control group C

Date	DAA	Time	Mortality (number of dead worker bees, larvae and pupae)										
			1C			2C			3C			Ø (∑)	STD
			LS	BT	∑	LS	BT	∑	LS	BT	∑		
12 Jun 2011	-9	15:30	-	48	48	-	3	3	-	36	36	**29.0**	23.3
13 Jun 2011	-8	13:45	-	19	19	-	0	0	-	25	25	**14.7**	13.1
14 Jun 2011	-7	14:45	-	38	38	-	1	1	-	25	25	**21.3**	18.8
15 Jun 2011	-6	11:00	-	7	7	-	0	0	-	37	37	**14.7**	19.7
16 Jun 2011	-5	14:00	-	4	4	-	1	1	-	15	15	**6.7**	7.4
Ø DAA-9 to -5			**-**	**23.2**	**23.2**	**-**	**1.0**	**1.0**	**-**	**27.6**	**27.6**	**17.3**	**-**
STD			-	19.3	19.3	-	1.2	1.2	-	9.1	9.1	-	16.6
17 Jun 2011	-4	15:25 - 16:10	16	11	27	39	3	42	22	29	51	**40.0**	12.1
18 Jun 2011	-3	9:25 - 11:00	80	5	85	33	2	35	81	23	104	**74.7**	35.6
19 Jun 2011	-2	18:15 - 19:20	176	20	196	42	23	65	178	13	191	**150.7**	74.2
20 Jun 2011	-1	15:15 - 16:15	36	4	40	34	3	37	181	17	198	**91.7**	92.1
21 Jun 2011	0ba	7:40 - 7:55	17	1	18	31	1	32	40	3	43	**31.0**	12.5
Ø DAA-4 to 0ba			**65.0**	**8.2**	**73.2**	**35.8**	**6.4**	**42.2**	**100.4**	**17.0**	**117.4**	**77.6**	**-**
STD			67.3	7.5	73.3	4.5	9.3	13.3	75.3	9.9	74.2	-	64.7
Ø DAA-9 to 0ba			**-**	**15.7**	**48.2**	**-**	**3.7**	**21.6**	**-**	**22.3**	**72.5**	**47.4**	**-**
STD			-	15.9	57.0	-	6.9	23.5	-	10.6	68.7	-	55.6
21 Jun 2011	0aa	9:05 - 9:20	7	2	9	3	0	3	6	3	9	**7.0**	3.5
21 Jun 2011	0aa	10:05 - 10:20	3	0	3	2	0	2	6	0	6	**3.7**	2.1
21 Jun 2011	0aa	12:05 - 12:20	2	1	3	8	2	10	4	3	7	**6.7**	3.5
21 Jun 2011	0aa	14:05 - 14:20	5	1	6	3	0	3	7	10	17	**8.7**	7.4
∑ DAA0aa			**17**	**4**	**21**	**16**	**2**	**18**	**23**	**16**	**39**	**26.0**	**11.4**

DAA = Days after 3rd test item application
ba = Before test item application
aa = After test item application
LS = Linen sheets
BT = Bee trap
STD = Standard deviation
- = Not applicable
∑ = Sum
Ø = Mean value
Ø (∑) = Mean of sum per replicate

Table 14: Results of the mortality assessments of the control group C (continued)

Date	DAA	Time	Mortality (number of dead worker bees, larvae and pupae)										
			1C			2C			3C			Ø (∑)	STD
			LS	BT	∑	LS	BT	∑	LS	BT	∑		
22 Jun 2011	1	9:00 - 9:55	22	4	26	36	1	37	44	15	59	**40.7**	16.8
22 Jun 2011	1	18:00 - 19:02	42	0	42	22	6	28	46	34	80	**50.0**	26.9
∑ DAA1			**64**	**4**	**68**	**58**	**7**	**65**	**90**	**49**	**139**	**90.7**	41.9
23 Jun 2011	2	10:18 - 10:48	16	10	26	21	7	28	20	13	33	**31.0**	7.0
23 Jun 2011	2	15:55 - 16:50	20	4	24	26	5	31	47	64	111	**55.3**	48.3
∑ DAA2			**36**	**14**	**50**	**47**	**12**	**59**	**67**	**77**	**144**	**86.3**	55.3
24 Jun 2011	3	11:10 - 11:45	27	10	37	21	15	36	39	15	54	**42.3**	10.1
24 Jun 2011	3	16:20 - 16:50	15	5	20	13	5	18	18	24	42	**26.7**	13.3
∑ DAA3			**42**	**15**	**57**	**34**	**20**	**54**	**57**	**39**	**96**	**69.0**	23.4
25 Jun 2011	4	10:05 - 10:30	33	7	40	8	5	13	40	11	51	**34.7**	19.6
26 Jun 2011	5	8:12 - 9:05	57	13	70	19	14	33	79	92	171	**91.3**	71.4
27 Jun 2011	6	14:35 - 15:10	107	16	123	15	15	30	79	139	218	**123.7**	94.0
28 Jun 2011	7	10:40 - 11:30	64	9	73	15	6	21	92	45	137	**77.0**	58.1
Ø DAA0aa to 7			**52.5**	**10.3**	**62.8**	**26.5**	**10.1**	**36.6**	**66.6**	**58.5**	**124.4**	**74.4**	-
STD			27.5	4.9	29.9	17.9	6.1	20.0	24.6	42.5	60.0	-	54.4
Q_M			0.9			0.9			1.1			--	
$Q_{M(0aa)}$			--			--			--			0.3	
$Q_{M(mean)}$			--			--			--			1.0	

DAA = Days after 3rd test item application
aa = After test item application
LS = Linen sheets
BT = Bee trap
STD = Standard deviation
- = Not applicable
∑ = Sum
Ø = Mean value
Ø (∑) = Mean of sum per replicate
Q_M = ∅ post-application mortality (DAA0aa to 7) ÷ ∅ pre-(3rd)-application mortality (DAA-4 to 0ba, per replicate)
$Q_{M(DAA0aa)}$ = ∅ mortality on the day of 3rd application (DAA0aa) ÷ ∅ pre-(3rd)-application mortality (DAA-4 to 0ba, per treatment group)
$Q_{M(mean)}$ = ∅ post-application mortality (DAA0aa to 7) ÷ ∅ pre-(3rd)-application mortality (DAA-4 to 0ba, per treatment group)

Table 15: Results of the mortality assessments of the test item group T

Date	DAA	Time	Mortality (number of dead worker bees, larvae and pupae)										
			1T			2T			3T			Ø (∑)	STD
			LS	BT	∑	LS	BT	∑	LS	BT	∑		
12 Jun 2011	-9	15:30	-	5	5	-	29	29	-	15	15	16.3	12.1
13 Jun 2011	-8	13:45	-	11	11	-	7	7	-	26	26	14.7	10.0
14 Jun 2011	-7	14:45	-	2	2	-	7	7	-	24	24	11.0	11.5
15 Jun 2011	-6	11:00	-	1	1	-	13	13	-	10	10	8.0	6.2
16 Jun 2011	-5	14:00	-	11	11	-	2	2	-	41	41	18.0	20.4
Ø DAA-9 to -5			**-**	**6.0**	**6.0**	**-**	**11.6**	**11.6**	**-**	**23.2**	**23.2**	**13.6**	**-**
STD			-	4.8	4.8	-	10.5	10.5	-	11.9	11.9	-	11.5
17 Jun 2011	-4	15:15-15:55	6	4	10	10	6	16	26	5	31	19.0	10.8
18 Jun 2011	-3	9:00-10:30	23	0	23	29	15	44	27	15	42	36.3	11.6
19 Jun 2011	-2	18:55-20:20	11	22	33	21	23	44	90	7	97	58.0	34.2
20 Jun 2011	-1	15:00-16:10	17	6	23	21	24	45	76	7	83	50.3	30.4
21 Jun 2011	0ba	8:00-8:40	12	5	17	12	1	13	41	2	43	24.3	16.3
Ø DAA-4 to 0ba			**13.8**	**7.4**	**21.2**	**18.6**	**13.8**	**32.4**	**52.0**	**7.2**	**59.2**	**37.6**	**-**
STD			6.5	8.5	8.5	7.7	10.2	16.4	29.3	4.8	28.9	-	24.7
Ø DAA-9 to 0ba			**-**	**6.7**	**13.6**	**-**	**12.7**	**22.0**	**-**	**15.2**	**41.2**	**25.6**	**-**
STD			-	6.5	10.3	-	9.8	17.0	-	12.0	28.2	-	22.5
21 Jun 2011	0aa	9:30-9:50	3	5	8	9	3	12	7	1	8	9.3	2.3
21 Jun 2011	0aa	10:30-10:50	3	2	5	1	4	5	2	0	2	4.0	1.7
21 Jun 2011	0aa	12:30-12:50	0	5	5	3	5	8	3	1	4	5.7	2.1
21 Jun 2011	0aa	14:30-14:50	7	7	14	4	7	11	10	2	12	12.3	1.5
∑ DAA0aa			**13**	**19**	**32**	**17**	**19**	**36**	**22**	**4**	**26**	**31.3**	**5.0**

DAA = Days after 3rd test item application
ba = Before test item application
aa = After test item application
LS = Linen sheets
BT = Bee trap
STD = Standard deviation
- = Not applicable
∑ = Sum
Ø = Mean value
Ø (∑) = Mean of sum per replicate

Table 15: Results of the mortality assessments of the test item group T (continued)

Date	DAA	Time	Mortality (number of dead worker bees, larvae and pupae)										
			1T			2T			3T			Ø (∑)	STD
			LS	BT	∑	LS	BT	∑	LS	BT	∑		
22 Jun 2011	1	9:00-9:49	5	6	11	15	11	26	34	3	37	24.7	13.1
22 Jun 2011	1	17:50-18:50	9	7	16	29	14	43	19	10	29	29.3	13.5
∑ DAA1			**14**	**13**	**27**	**44**	**25**	**69**	**53**	**13**	**66**	**54.0**	**23.4**
23 Jun 2011	2	10:15-10:40	9	13	22	20	9	29	24	5	29	26.7	4.0
23 Jun 2011	2	15:50-16:15	11	18	29	23	33	56	37	5	42	42.3	13.5
∑ DAA2			**20**	**31**	**51**	**43**	**42**	**85**	**61**	**10**	**71**	**69.0**	**17.1**
24 Jun 2011	3	11:05-11:35	25	10	35	19	12	31	43	9	52	39.3	11.2
24 Jun 2011	3	16:13-16:45	7	5	12	7	14	21	24	6	30	21.0	9.0
∑ DAA3			**32**	**15**	**47**	**26**	**26**	**52**	**67**	**15**	**82**	**60.3**	**18.9**
25 Jun 2011	4	10:00-10:25	5	3	8	15	8	23	35	2	37	22.7	14.5
26 Jun 2011	5	8:20-9:10	12	11	23	30	38	68	39	19	58	49.7	23.6
27 Jun 2011	6	14:30-14:45	14	19	33	25	20	45	58	29	87	55.0	28.4
28 Jun 2011	7	10:30-11:40	15	13	28	60	15	75	52	12	64	55.7	24.6
Ø DAA0aa to 7			**15.6**	**15.5**	**31.1**	**32.5**	**24.1**	**56.6**	**48.4**	**13.0**	**61.4**	**49.2**	**-**
STD			7.8	8.1	13.5	15.4	11.4	21.2	15.1	8.5	20.9	-	23.3
Q_M			1.5			1.7			1.0			--	
$Q_{M(0aa)}$			--			--			--			0.8	
$Q_{M(mean)}$			--			--			--			1.3	

DAA = Days after 3rd test item application
aa = After test item application
LS = Linen sheets
BT = Bee trap
STD = Standard deviation
- = Not applicable
∑ = Sum
Ø = Mean value
Ø (∑) = Mean of sum per replicate
Q_M = ∅ post-application mortality (DAA0aa to 7) ÷ ∅ pre-(3rd)-application mortality (DAA-4 to 0ba, per replicate)
$Q_{M(DAA0aa)}$ = ∅ mortality on the day of 3rd application (DAA0aa) ÷ ∅ pre-(3rd)-application mortality (DAA-4 to 0ba, per treatment group)
$Q_{M(mean)}$ = ∅ post-application mortality (DAA0aa to 7) ÷ ∅ pre-(3rd)-application mortality (DAA-4 to 0ba, per treatment group)

Table 16: Results of the mortality assessments of the reference group R

Date	DAA	Time	Mortality (number of dead worker bees, larvae and pupae)										
			1R			2R			3R			Ø (∑)	STD
			LS	BT	∑	LS	BT	∑	LS	BT	∑		
12 Jun 2011	-9	15:30	-	34	34	-	30	30	-	5	5	23.0	15.7
13 Jun 2011	-8	13:45	-	13	13	-	1	1	-	3	3	5.7	6.4
14 Jun 2011	-7	14:45	-	37	37	-	6	6	-	3	3	15.3	18.8
15 Jun 2011	-6	11:00	-	18	18	-	0	0	-	10	10	9.3	9.0
16 Jun 2011	-5	14:00	-	52	52	-	4	4	-	5	5	20.3	27.4
Ø DAA-9 to -5			**-**	**30.8**	**30.8**	**-**	**8.2**	**8.2**	**-**	**5.2**	**5.2**	**14.7**	**-**
STD			-	15.6	15.6	-	12.4	12.4	-	2.9	2.9	-	16.0
17 Jun 2011	-4	15:40 – 16:20	53	7	60	32	18	50	9	5	14	41.3	24.2
18 Jun 2011	-3	9:10 – 10:45	81	33	114	95	44	139	9	1	10	87.7	68.4
19 Jun 2011	-2	19:10 – 20:10	240	35	275	154	58	212	64	4	68	185.0	106.1
20 Jun 2011	-1	15:30 – 16:20	69	13	82	270	14	284	19	8	27	131.0	135.3
21 Jun 2011	0ba	8:10 – 8:25	37	8	45	57	25	82	9	7	16	47.7	33.1
Ø DAA-4 to 0ba			**96.0**	**19.2**	**115.2**	**121.6**	**31.8**	**153.4**	**22.0**	**5.0**	**27.0**	**98.5**	**-**
STD			82.2	13.7	93.0	94.8	18.6	95.5	23.9	2.7	23.8	-	90.8
Ø DAA-9 to 0ba			**-**	**25.0**	**73.0**	**-**	**20.0**	**80.8**	**-**	**5.1**	**16.1**	**56.6**	**-**
STD			-	15.2	77.0	-	19.4	99.9	-	2.6	19.7	-	76.9
21 Jun 2011	0aa	10:00 – 10:25	45	36	81	71	42	113	46	83	129	107.7	24.4
21 Jun 2011	0aa	11:00 – 11:25	73	368	441	213	231	444	27	126	153	346.0	167.1
21 Jun 2011	0aa	13:00 – 13:25	124	364	488	115	147	262	39	158	197	315.7	152.7
21 Jun 2011	0aa	15:00 – 15:25	51	464	515	122	220	344	79	133	212	356.3	152.0
∑ DAA0aa			**293**	**1232**	**1525**	**521**	**640**	**1161**	**191**	**500**	**691**	**1125.7***	**418.1**

DAA = Days after 3rd test item application
ba = Before test item application
aa = After test item application
LS = Linen sheets
BT = Bee trap
STD = Standard deviation
- = Not applicable
∑ = Sum
Ø = Mean value
Ø (∑) = Mean of sum per replicate
* statistically significant (higher)

Table 16: Results of the mortality assessments of the reference group R (continued)

Date	DAA	Time	Mortality (number of dead worker bees, larvae and pupae)										
			1R			2R			3R			Ø (∑)	STD
			LS	BT	∑	LS	BT	∑	LS	BT	∑		
22 Jun 2011	1	9:10 – 9:40	143	708	851	205	303	508	126	220	346	568.3	257.8
22 Jun 2011	1	18:10 – 19:10	53	94	147	145	139	284	61	71	132	187.7	83.8
∑ DAA1			**196**	**802**	**998**	**350**	**442**	**792**	**187**	**291**	**478**	**756.0***	**261.9**
23 Jun 2011	2	10:22 – 10:50	34	126	160	126	47	173	65	47	112	148.3	32.1
23 Jun 2011	2	16:00 – 16:55	35	50	85	41	14	55	38	51	89	76.3	18.6
∑ DAA2			**69**	**176**	**245**	**167**	**61**	**228**	**103**	**98**	**201**	**224.7***	**22.2**
24 Jun 2011	3	11:15 – 11:50	55	61	116	121	37	158	40	54	94	122.7	32.5
24 Jun 2011	3	16:25 – 16:55	14	29	43	25	14	39	25	12	37	39.7	3.1
∑ DAA3			**69**	**90**	**159**	**146**	**51**	**197**	**65**	**66**	**131**	**162.3***	**33.1**
25 Jun 2011	4	10:10 – 10:35	16	21	37	51	5	56	48	6	54	49.0	10.4
26 Jun 2011	5	8:15 – 8:55	29	47	76	80	28	108	59	24	83	89.0	16.8
27 Jun 2011	6	14:40 – 15:15	32	36	68	55	24	79	49	7	56	67.7	11.5
28 Jun 2011	7	10:50 – 11:50	49	14	63	80	10	90	60	2	62	71.7	15.9
Ø DAA0aa to 7			**94.1**	**302.3**	**396.4**	**181.3**	**157.6**	**338.9**	**95.3**	**124.3**	**219.5**	**318.3***	**-**
STD			98.1	458.8	556.2	168.5	243.2	410.0	60.3	179.6	237.5	-	410.1
Q_M			3.4			2.2			8.1			--	
$Q_{M(0aa)}$			--			--			--			11.4	
$Q_{M(mean)}$			--			--			--			3.2	

DAA = Days after 3rd test item application
aa = After test item application
LS = Linen sheets
BT = Bee trap
STD = Standard deviation
- = Not applicable
∑ = Sum
Ø = Mean value
Ø (∑) = Mean of sum per replicate
Q_M = Ø post-application mortality (DAA0aa to 7) ÷ Ø pre-(3rd)-application mortality (DAA-4 to 0ba, per replicate)
$Q_{M(DAA0aa)}$ = Ø mortality on the day of 3rd application (DAA0aa) ÷ Ø pre-(3rd)-application mortality (DAA-4 to 0ba, per treatment group)
$Q_{M(mean)}$ = Ø post-application mortality (DAA0aa to 7) ÷ Ø pre-(3rd)-application mortality (DAA-4 to 0ba, per treatment group)
* statistically significant (higher)

Flight intensity

Figure 9.4.2-26 presents the mean flight intensity in the treatments groups C, T and R. On the day of the 3rd application (DAA0aa) until DAA7 reductions in flight intensity were found in the test item group T when compared to the control group (T). On DAA0aa and DAA5, the flight intensity was statistically significantly lower in T when compared to C.

Figure 9.4.2-26. Mean number of forager bees/m2 inside the tunnels for C (control), T (treatment) and R (reference) prior to and after the 3rd application in T.

* = statistically significant (lower);

0ba = day of application;

0aa = after 3rd test application in T and the concurrent applications in C and R, respectively.

Strength of the colonies

Results concerning the strength of the colonies are reported in the following figures. Figures were compiled with data from the original tables from the report.

Number of bees

The 1st and 2nd colony assessment were made during confined tunnel situation. Increases in colony strength were observed at the 3rd colony assessment. According to the author, the observed decrease in the average number of bees in T during the confined exposure conditions can be explained by limited forage. The limited forage was, on the other hand, sufficient for the smaller colonies in C (Individual colonies with less than 9000 bees were able to grow under confined tunnel situation (2C: 7130 to 8970 bees; 1T: 7130 to 8280 bees). These smaller colonies showed an increase of colony strength at the 2nd colony assessment.

Development of colony strength up to the last colony assessment was comparable between C and T. No test-item related adverse effect on colony strength were observed.

When comparing the mean mortality before application (DAA-4 to DAA0ba) to the day of application, Q_M(0aa) values were calculated to be 0.3, 0.8 and 11.4 for the treatment groups C, T and R, respectively. The Q_M(mean) values were calculated to be 1.0 for C, 1.3 for T and 3.2 for R,

respectively. The high mean number of dead bees in R during the post application period shows the sensitivity of the test system and was statistically significantly increased from DAA0aa to DAA3.

Figure 9.4.2-27. Mean number of bees in the treatment groups C, T and R at 5 colony assessment dates. Figure compiled by RMS from three tables in the report.

Abundance of brood and food stores

A decrease of abundance of brood (eggs, larvae and pupae) was observed in all treatments during confinement at the second assessment date (see figure below). According to the author this decrease can be explained by the unfavourable weather conditions before hive set-up and by the confined conditions inside the tunnel. Increases of brood abundance were found in all three treatments at fourth and fifth assessment day.

Decreases of food stores were observed in food stores in both, treatment and control, during confinement. The period after confinement showed increases in food stores. According to the author the decreases can be explained by the limited foraging area. No test item related adverse effects on the development of the food storage area were observed.

Figure 9.4.2-28. Mean number of brood and food cells in the treatment groups C, T and R at 5 colony assessment dates. Figure compiled by RMS from three tables in the report.

EFSA asked RMS to provide more detailed data on the adult and brood abundance.

The colony assessment data for the individual hives are shown below, in tables copied from the study report (Tables 20, 21 and 22).

Table 20: Results of the colony assessments in the control group C

Colony	1C	2C	3C	Mean	± STD
1st colony assessment: 16 Jun 2011 (DAA-5)					
Total number of combs	6	6	6	6	0
Total number of bees[1)]	9200	7130	8510	8280	1054
Number of combs containing brood	5	6	5	5.33	0.58
Number of cells containing eggs[2)]	9680	11000	7480	9387	1778
Number of cells containing larvae[2)]	2200	5500	5720	4473	1972
Number of cells containing capped brood[2)]	17600	15400	18480	17160	1586
Sum of cells containing brood	29480	31900	31680	31020	1338
Number of combs containing food	6	5	6	5.67	0.58
Number of cells containing nectar	11440	9020	8580	9680	1540
Number of cells containing pollen	1760	3080	1980	2273	707
Sum of cells containing food	13200	12100	10560	11953	1326
Number of empty cells	10120	8800	10560	9827	916
2nd colony assessment: 28 Jun 2011 (DAA7)					
Total number of combs	6	6	6	6	0
Total number of bees[1)]	8280	8970	7590	8280	690
Number of combs containing brood	4	6	4	4.67	1.15
Number of cells containing eggs[2)]	4840	3520	3520	3960	762
Number of cells containing larvae[2)]	0	1760	880	880	880
Number of cells containing capped brood[2)]	12320	10120	13640	12027	1778
Sum of cells containing brood	17160	15400	18040	16867	1344
Number of combs containing food	6	5	6	5.67	0.58
Number of cells containing nectar	13640	9460	9900	11000	2297
Number of cells containing pollen	0	0	0	0	0
Sum of cells containing food	13640	9460	9900	11000	2297
Number of empty cells	22000	27940	24860	24933	2971
3rd colony assessment: 05 Jul 2011 (DAA14)					
Total number of combs	7	7	7	7	0
Total number of bees[1)]	9660	9890	8740	9430	609
Number of combs containing brood	3	4	3	3.33	0.58
Number of cells containing eggs[2)]	7480	10560	3960	7333	3302
Number of cells containing larvae[2)]	4400	3080	4840	4107	916
Number of cells containing capped brood[2)]	3960	3300	5280	4180	1008
Sum of cells containing brood	15840	16940	14080	15620	1443
Number of combs containing food	5	6	6	5.67	0.58
Number of cells containing nectar	8800	11880	9680	10120	1586
Number of cells containing pollen	7260	6160	5500	6307	889
Sum of cells containing food	16060	18040	15180	16427	1465
Number of empty cells	29700	26620	32340	29553	2863

[1)] calculated as sum of Liebefeld units * 125
[2)] calculated as sum of Liebefeld units * 400
DAA = Days after 3rd test item application
STD = Standard deviation

Table 20: Results of the colony assessments in the control group C (continued)

Colony	1C	2C	3C	Mean	± STD
4th colony assessment: 12 Jul 2011 (DAA21)					
Total number of combs	8	8	8	8	0
Total number of bees[1)]	10350	12880	9890	11040	1610
Number of combs containing brood	5	6	7	6.00	1.00
Number of cells containing eggs[2)]	7040	7480	10560	8360	1918
Number of cells containing larvae[2)]	9240	8360	7480	8360	880
Number of cells containing capped brood[2)]	11880	13200	11440	12173	916
Sum of cells containing brood	28160	29040	29480	28893	672
Number of combs containing food	8	8	7	7.67	0.58
Number of cells containing nectar[2)]	19580	21560	18920	20020	1374
Number of cells containing pollen[2)]	7260	8580	5720	7187	1431
Sum of cells containing food	26840	30140	24640	27207	2768
Number of empty cells	15400	11220	16280	14300	2703
5th colony assessment: 19 Jul 2011 (DAA28)					
Total number of combs	9	9	9	9	0
Total number of bees[1)]	9890	11270	9430	10197	958
Number of combs containing brood	7	8	7	7.33	0.58
Number of cells containing eggs[2)]	8360	11220	5720	8433	2751
Number of cells containing larvae[2)]	5500	8140	4400	6013	1922
Number of cells containing capped brood[2)]	20240	21560	22880	21560	1320
Sum of cells containing brood	34100	40920	33000	36007	4290
Number of combs containing food	9	9	7	8.33	1.15
Number of cells containing nectar[2)]	22440	24420	18260	21707	3145
Number of cells containing pollen[2)]	5280	6160	4400	5280	880
Sum of cells containing food	27720	30580	22660	26987	4011
Number of empty cells	17380	7700	23540	16207	7985

[1)] calculated as sum of Liebefeld units * 125
[2)] calculated as sum of Liebefeld units * 400
DAA = days after 3rd test item application
STD = Standard deviation

Table 21: Results of the colony assessments in the test item group T

Colony	1T	2T	3T	Mean	± STD
1st colony assessment: 16 Jun 2011 (DAA-5)					
Total number of combs	6	6	6	6	0
Total number of bees[1)]	7130	9660	9430	8740	1399
Number of combs containing brood	4	5	5	4.67	0.58
Number of cells containing eggs[2)]	3520	9900	7920	7113	3266
Number of cells containing larvae[2)]	3740	3080	4180	3667	554
Number of cells containing capped brood[2)]	22440	16720	16280	18480	3437
Sum of cells containing brood	29700	29700	28380	29260	521
Number of combs containing food	6	6	6	6	0
Number of cells containing nectar[2)]	12760	12980	15180	13640	1338
Number of cells containing pollen[2)]	2640	1100	1760	1833	773
Sum of cells containing food	15400	14080	16940	15473	2026
Number of empty cells	7700	9020	7480	8067	833
2nd colony assessment: 28 Jun 2011 (DAA7)					
Total number of combs	6	6	6	6	0
Total number of bees[1)]	8280	7590	6900	7590	690
Number of combs containing brood	4	5	5	4.67	0.58
Number of cells containing eggs[2)]	4840	3960	3960	4253	508
Number of cells containing larvae[2)]	0	0	0	0	0
Number of cells containing capped brood[2)]	10120	11660	7920	9900	1880
Sum of cells containing brood	14960	15620	11880	14153	1996
Number of combs containing food	6	6	6	6	0
Number of cells containing nectar[2)]	11880	9020	15400	12100	3196
Number of cells containing pollen[2)]	0	0	0	0	0
Sum of cells containing food	11880	9020	15400	12100	3196
Number of empty cells	25960	28160	25520	26547	1414
3rd colony assessment: 05 Jul 2011 (DAA14)					
Total number of combs	7	7	7	7	0
Total number of bees[1)]	11730	9200	11730	10887	1461
Number of combs containing brood	3	4	4	3.67	0.58
Number of cells containing eggs[2)]	7040	5720	6160	6307	672
Number of cells containing larvae[2)]	2640	6160	2200	3667	2170
Number of cells containing capped brood[2)]	6600	8140	4840	6527	1651
Sum of cells containing brood	16280	20020	13200	16500	3415
Number of combs containing food	6	6	7	6.33	0.58
Number of cells containing nectar[2)]	13200	7700	15840	12247	4153
Number of cells containing pollen[2)]	9900	4180	5940	6673	2930
Sum of cells containing food	23100	11880	21780	18920	6132
Number of empty cells	22220	29700	26620	26180	3759

[1)] calculated as sum of Liebefeld units * 125
[2)] calculated as sum of Liebefeld units * 400
DAA = days after 3rd test item application
STD = Standard deviation

Table 21: Results of the colony assessments in the test item group T (continued)

Colony	1T	2T	3T	Mean	± STD
4th colony assessment: 12 Jul 2011 (DAA21)					
Total number of combs	8	8	8	8	0
Total number of bees[1)]	7130	11040	10580	9583	2137
Number of combs containing brood	5	6	5	5.33	0.58
Number of cells containing eggs[2)]	5940	5720	7480	6380	959
Number of cells containing larvae[2)]	7920	12320	10120	10120	2200
Number of cells containing capped brood[2)]	11880	17160	10560	13200	3492
Sum of cells containing brood	25740	35200	28160	29700	4914
Number of combs containing food	7	8	8	7.67	0.58
Number of cells containing nectar[2)]	20680	14300	25080	20020	5420
Number of cells containing pollen[2)]	5280	4620	4620	4840	381
Sum of cells containing food	25960	18920	29700	24860	5474
Number of empty cells	18700	16280	12540	15840	3103
5th colony assessment: 19 Jul 2011 (DAA28)					
Total number of combs	9	9	9	9	0
Total number of bees[1)]	8740	10810	9660	9737	1037
Number of combs containing brood	5	8	7	6.67	1.53
Number of cells containing eggs[2)]	2640	6600	1540	3593	2661
Number of cells containing larvae[2)]	1980	7260	4840	4693	2643
Number of cells containing capped brood[2)]	19360	26400	20240	22000	3836
Sum of cells containing brood	23980	40260	26620	30287	8737
Number of combs containing food	8	9	9	8.67	0.58
Number of cells containing nectar[2)]	20240	17820	24640	20900	3458
Number of cells containing pollen[2)]	4620	3960	5720	4767	889
Sum of cells containing food	24860	21780	30360	25667	4347
Number of empty cells	30360	17160	22220	23247	6660

[1)] calculated as sum of Liebefeld units * 125
[2)] calculated as sum of Liebefeld units * 400
DAA = days after 3rd test item application
STD = Standard deviation

Table 22: Results of the colony assessments in the reference group R

Colony	1R	2R	3R	Mean	± STD
1st colony assessment: 16 Jun 2011 (DAA-5)					
Total number of combs	6	6	6	6	0
Total number of bees[1)]	9660	13800	7590	10350	3162
Number of combs containing brood	6	3	4	4.33	1.53
Number of cells containing eggs[2)]	5280	880	7920	4693	3556
Number of cells containing larvae[2)]	8580	2420	5060	5353	3090
Number of cells containing capped brood[2)]	21560	15840	12100	16500	4764
Sum of cells containing brood	35420	19140	25080	26547	8239
Number of combs containing food	6	6	6	6.00	0.00
Number of cells containing nectar[2)]	11220	21120	11000	14447	5780
Number of cells containing pollen[2)]	3300	3960	1760	3007	1129
Sum of cells containing food	14520	25080	12760	17453	6989
Number of empty cells	2860	8580	14960	8800	6053
2nd colony assessment: 28 Jun 2011 (DAA7)					
Total number of combs	6	6	6	6	0
Total number of bees[1)]	6440	9660	4255	6785	2719
Number of combs containing brood	5	4	4	4.33	0.58
Number of cells containing eggs[2)]	8800	5720	4400	6307	2258
Number of cells containing larvae[2)]	880	220	0	367	458
Number of cells containing capped brood[2)]	9680	7480	4620	7260	2537
Sum of cells containing brood	19360	13420	9020	13933	5189
Number of combs containing food	6	6	6	6.00	0.00
Number of cells containing nectar[2)]	9020	17160	7920	11367	5047
Number of cells containing pollen[2)]	220	0	0	73	127
Sum of cells containing food	9240	17160	7920	11440	4997
Number of empty cells	24200	22220	35860	27427	7370
3rd colony assessment: 05 Jul 2011 (DAA15)					
Total number of combs	7	7	7	7	0
Total number of bees[1)]	10120	13110	6900	10043	3106
Number of combs containing brood	4	3	4	3.67	0.58
Number of cells containing eggs[2)]	10120	5720	6160	7333	2423
Number of cells containing larvae[2)]	3960	4840	5280	4693	672
Number of cells containing capped brood[2)]	3960	5940	3960	4620	1143
Sum of cells containing brood	18040	16500	15400	16647	1326
Number of combs containing food	6	7	6	6.33	0.58
Number of cells containing nectar[2)]	11000	16060	7920	11660	4110
Number of cells containing pollen[2)]	9680	7040	3960	6893	2863
Sum of cells containing food	20680	23100	11880	18407	5830
Number of empty cells	22880	22000	34320	26400	6873

[1)] calculated as sum of Liebefeld units * 125
[2)] calculated as sum of Liebefeld units * 400
DAA = days after 3rd test item application
STD = Standard deviation

Table 22: Results of the colony assessments in the reference group R (continued)

Colony	1R	2R	3R	Mean	± STD
4th colony assessment: 12 Jul 2011 (DAA21)					
Total number of combs	8	9	8	8.33	0.58
Total number of bees[1)]	9890	14030	5750	9890	4140
Number of combs containing brood	5	4	5	4.67	0.58
Number of cells containing eggs[2)]	9900	3960	6160	6673	3003
Number of cells containing larvae[2)]	8360	4840	9680	7627	2502
Number of cells containing capped brood[2)]	12760	12540	10560	11953	1212
Sum of cells containing brood	31020	21340	26400	26253	4842
Number of combs containing food	8	9	7	8	1
Number of cells containing nectar[2)]	17600	39600	19360	25520	12225
Number of cells containing pollen[2)]	7260	6160	2420	5280	2537
Sum of cells containing food	24860	45760	21780	30800	13047
Number of empty cells	14520	12100	22220	16280	5285
5th colony assessment: 19 Jul 2011 (DAA28)					
Total number of combs	9	9	9	9	0
Total number of bees[1)]	9430	12190	7360	9660	2423
Number of combs containing brood	7	4	5	5.33	1.53
Number of cells containing eggs[2)]	7480	0	3300	3593	3749
Number of cells containing larvae[2)]	3520	1100	2200	2273	1212
Number of cells containing capped brood[2)]	21780	17600	17160	18847	2550
Sum of cells containing brood	32780	18700	22660	24713	7261
Number of combs containing food	8	9	7	8	1
Number of cells containing nectar[2)]	18480	36960	21120	25520	9995
Number of cells containing pollen[2)]	8140	7040	1980	5720	3285
Sum of cells containing food	26620	44000	23100	31240	11190
Number of empty cells	19800	16500	33440	23247	8981

[1)] calculated as sum of Liebefeld units * 125
[2)] calculated as sum of Liebefeld units * 400
DAA = days after 3rd test item application
STD = Standard deviation

Behaviour of the Bees

In the control group C, unusual behaviour was observed on the day of application approximately one hour after tap water treatment (approximately 100 bees sitting at the hive entrance, some cleaning, some shivering). On the following two days and on DAA5 and 6, clustering at the hive entrance was observed (alternately in 1C, 2C and 3C) and once aggressiveness at hive 1C.

In the test item treatment group T a repellent effect was observed. According to the author this repellent effect was only slight and was caused by an imbalance between an intensive flying activity and an infrequent landing on the crop (DAA0aa). Also clustering at the hive entrance was observed at hive 2T. On the following day, intensive flying over the crop coupled with an infrequent landing on the crop was still observed for colony 1T and 3T, clustering at the hive entrance at colony 2T and 3T. In the reference item treatment group R, clear symptoms of intoxication were observed on the day of application, after the treatment (2R); during the following days, clustering at the hive entrance (DAA1; 1R, 2R) and intensive flight activity coupled with a considerably reduced frequency of landing on the crop (DAA5; 1R) was described.

Since clustering at the hive entrance was observed in all treatment groups it did not happen in relation to the test item application.

Residue Analysis

Details on residue analysis are given in the table below.

Table 9.4.2-10. Residues of BYI02960 and BYI02960-difluoroethylaminofuranonein in samples of flower, nectar/honey, bee wax and soil, as obtained in the test item group.

Sample material	Test item	Sub-sample	Time of sampling	Residue [µg/kg]	
				BYI 02960	BYI 02960-DFEAF
Flower	BYI 02960 SL 200 G	A	0 DAA3	9533 – 20083	18 – 37
			7 DAA3	701 – 3590	9 – 35
Nectar (combs)	BYI 02960 SL 200 G	A	7 DAA3	LOQ – 2	<LOD – LOQ
Wax (combs)	BYI 02960 SL 200 G	A	7 DAA3	LOQ – 7*	LOQ*
Soil	BYI 02960 SL 200 G	A	0 DAA1	40 - 56	**

BYI 02960-DFEAF = BYI 02960-difluoroethylaminofuranone (BCS-CC98193)
DAA3 = Days after the 3rd test item application in the test item group and the corresponding tap water application in the control group
DAA1 = Days after the 1st test item application on bare soil, followed by immediate soil incorporation and sowing of *Phacelia tanacetifolia*-seeds
LOQ = Limit of Quantitation = 1 µg/kg in flower, nectar and bee wax (BYI 02960 and BYI 02960-DFEAF); = 5 µg/kg in soil (BYI 02960 and BYI 02960-DFEAF)
LOD = Limit of Detection = 0.1 µg/kg in flower, nectar and bee wax (BYI 02960 and BYI 02960-DFEAF); = 1.5 µg/kg in soil (BYI 02960 and BYI 02960-DFEAF)
* Cross-contminated with adherent/integrated remnants of other comb matrices (e.g. pollen, larval cocoon material, etc.)
** An analysis of BYI 02960-DFEAF in soil was not conducted

Conclusions of the author

The application scenario used in this study did not cause treatment-related adverse effects on mortality, brood and food development as well as on colony vitality. A slight repellent effect of the test item was observed on the day of the 3rd test item application, after the treatment.

Remarks by RMS

A positive control was included in the test and showed that the test system was behaving as expected. According to the EFSA Opinion on the Science behind the development of a risk assessment of plant protection products for bees (EFSA Journal 2012, 10: 2668, section 3.5.1), the main exposure route for bee brood is via pollen. Since *Phacelia tanacetifolia* is a major source of pollen, and pollen from uncontaminated sources was not present in the tunnels, the present test is suitable to show potential effect of flupyradifurone on brood related parameters, mortality and flight intensity.

The results of the study show that there is no adverse effect on mortality, on brood and food development as well as on colony vitality of the honeybees after foraging on *Phacelia tanacetifolia* after 3 test item applications. Several significant effects were present on flight intensity between

DAA0aa and DAA5. Mortality was not clearly higher in the treatment than in the control. A relation between decrease of flight intensity and mortality outside the hive may exist. When fewer bees are actively foraging the percentage of mortalities outside the hive may also decrease. From the results of the study it can be concluded that one soil application with 300 g as/ha and two foliar applications with 200 g as/ha have no effect on mortality, brood-, food-, and population development as well as on overall hive vitality. There are effects on flight intensity and behaviour. The results of this study, including the residue measurements, can be used for risk assessment.

Expert discussion in Pesticide Peer Review meeting 124 (December 2014):
(Please note that the word 'effect' in the text below might be interpreted as different from the control (i.e. may or may not include effects from the treatment), pending on the context.)

Some effects on forager mortality could not be excluded. Furthermore, at the second assessment there were virtually no larvae in T2 (T2 = assessment day 2). This may have been related to the shortage of pollen or test item related. It was noted that the larvae in the control also decreased indicating something affecting the colonies. However, the test item decreased to a greater extent. Due to the decreasing control it is difficult to be able to detect test-item related effects. It was considered that these types of effects were worth noting and then can be considered as a whole taking account the entire data set. The RMS noted that when they state that write that the numbers in the control and the test item were similar they mean the values were comparable (but not necessarily equal). Other experts consider that it is more useful for evaluation if it is stated that there is a difference and then the difference is not an issue. This is a minor point but helps when considering the data set as a whole i.e. looking for trends within the available data. In this case the experts agreed with the conclusion of the RMS.

There was statistical analysis of the forager mortality performed and some statistical effects on the treatment groups were observed. The RMS noted that it is difficult to interpret results by comparison of numbers without taking account of the statistical analysis.

Overall, the experts agreed with the RMS's assessment that the very slight increase on forager mortality were likely not due to the test item. This was because the level of effect was very slight and not statistically significant.

Study KIIIA 10.4.7/06

Characteristics

Reference	:	Rentschler,S. (2012b)	nominal dose		2x 1 L/ha = 200 g a.s./ha
type of study	:	Honeybee semi-field tunnel test	species	:	Honey bee (*Apis mellifera*)
year of execution	:	2011	observation duration	:	35 d
GLP statement	:	Yes	exposure duration	:	12 d
Guideline	:	OECD Guidance document No. 75	dosing method	:	Tunnel test in flowering *Phacelia*

		(2007)			*tanacetifolia*, 2x foliar spray: 1st application 5 d before hive set-up and 2nd application 5 d after set-up during flowering and active foraging of bees
test substance	:	BYI 02960 SL 200 G, Batch no. 2010-007173.	acceptability	:	Acceptable
a.s. content	:	198.6 g flupyradifurone /L	Result	:	No adverse effect on mortality and brood development. Adverse effect on flight intensity and behaviour.

Description

The study was conducted in Pinache, federal state of Baden-Württemberg, Germany on a field of flowering *Phacelia tanacetifolia*. The experimental design of this study comprised three experimental groups with three replicates each, 9 tunnel tents were set-up before moving the hives to the experimental field. The individual treatment groups, the respective application rates per ha and the respective spray volumes per ha are described in the following table (copied from the report).

Table 9.4.2-11. Treatment groups, application rates and spray volume.

Treatment Group	Code	Time of application	Target application rate [g a.s./ha]*	Target application rate [g product/ha]	Spray Volume
Control (tap water)	C	DAA0, BBCH 63-65	None	None	400 L/ha
Test item (BYI 02960 SL 200 G)	T	DAA-10, BBCH 59-61	200	1 L	200 L/ha
		DAA0, BBCH 63-65	200	1 L	200 L/ha
Reference item (Insegar® 25 WG)	R	DAA0, BBCH 63-65	150	600 g	400 L/ha

DAA = days after 2nd test item application
* calculation based on the nominal content of a. s.

Reference item is Insegar (a.s. fenoxycarb). For the test, small healthy colonies with ten combs were used. The colony size at set-up was in the range 5313 – 8750 bees. The bee colonies were obtained from two beekeepers but were distributed
equally among the replicates in order to guarantee uniform bee material in all treatments. Furthermore the following criteria for each colony were guaranteed:

- 5 – 7 brood combs containing eggs, larvae and capped cells;
- 7 – 10 combs containing honey and pollen;
- bees are free of Nosema and Varroa disease symptoms and other bee diseases

The area covered per tunnel tent was 80 m². The bee colonies (one small bee colony per tunnel) were installed in the tunnel tents 5 days before the 2nd test item application and the concurrent application

in C and R, respectively, at early flowering of the crop. The colonies remained in the tunnels for 12 days. After the last assessment of mortality, foraging activity and behaviour during the confined exposure period on DAA7, the colonies were re-located from the tunnels and installed at a remote monitoring location for further observation. Colonies from the control, test item treatment and reference item treatment groups were installed at the same monitoring location. The test item solution was prepared shortly before each application. The applications were carried out with a portable boom sprayer that simulates a commercial application. The following conditions were met for both applications:

- crop at start of flowering (BBCH 59-61, closed flower buds) at 1st application in T
- crop at full flowering (BBCH 63-65) at 2nd application in T and the concurrent applications in C and R, respectively
- bees were actively foraging (≥ 5 bees/m²) at 2nd application in T and the concurrent applications in C and R, respectively
- wind speed was 0 m/s at 1st application in T, wind speed inside the tunnel tent was less than 2 m/s at the 2nd
- application in T and the concurrent applications in C and R, respectively
- air temperature did not exceed 30°C (both application times)
- there was no rainfall within at least two hours after the applications

During the 2nd test item application inside the tunnels, as well as during the concurrent application of tap water and the reference item, the bee colonies were covered with plastic sheets.

Mortality

Mortality was recorded in the crop. At each assessment, the dead bees found on the sheets and in the bee traps were counted and removed. Dead bees were differentiated between adult worker bees, males (drones), freshly emerged bees, pupae and larvae during each assessment.

The mortality of honeybees of the different treatment groups in the dead bee trap at the entrance of the hive and on the linen sheets were assessed prior to the 2nd test item application (DAA -4 to -1), on the day of the 2nd test item application (before and after the application: 0ba and 0aa, 1h, 2h, 4h and 6h after application) and after the 2nd test item application inside the tunnels (DAA 1 to 7) and on the monitoring site on DAA8 to 27.

Flight Intensity and behaviour

At each assessment, the number of bees that were both foraging on the flowers and flying over the crop were counted for 15 seconds. During the assessments of flight intensity, also the behaviour of the honeybees in the crop and around the hive were observed. with respect to at least the following criteria:

- aggressiveness towards the observer,
- guard bees attacking and/or preventing returning bees from entering the hive,
- intensive flying activity in front of the hives without entering the hive,
- intoxication symptoms (e.g. cramping, locomotion problems),
- clustering of large numbers of bees at the hive entrance

Condition of the colonies, development of the bee brood.

Development of Honey Bee Brood in Individual Cells

The development of the bee brood was assessed in individually marked cells. The development of each individually marked cell was determined throughout the duration of the study (the pre-imaginal development period of worker honeybees typically averages 21 days).

Condition of the Colonies

The condition of all colonies was checked during the experimental phase and the following parameters were assessed:

- Colony strength (number of bees
- Presence of a healthy queen (e.g. presence of eggs)
- Pollen storage area and area with nectar or honey
- Area containing cells with eggs, larvae and capped cells

Assessments of the colony condition were made once before placing the hives in the tunnels, once before the applications during full flowering and honeybees actively foraging on the crop, followed by five further colony assessments.

Recording of the Meteorological Data

For the entire exposure period and monitoring period, the following climatic data were recorded by an Eurofins Agroscience Services EcoChem GmbH weather station at a distance of approx. 5.66 km to the field site and approx. 5.95 km to the monitoring site (non-GLP record):

- air temperature (daily min/max)
- relative air humidity (daily min/max)
- daily precipitation

Additionally, precipitation during the exposure period was recorded by a rain gauge placed inside one the tunnel tents (GLP record). During applications and assessments, the following data were recorded at the field site (GLP record):

- cloud cover (estimated during application and assessments of flight intensity)
- air temperature, relative air humidity (only during the application)
- wind speed (only during the application)

Collection of samples

Samples of pollen, nectar/honey and wax were taken from different locations of the hives in the test item treatment group at the end of the study (DAA27).

Calculations and Statistical analysis

For the evaluation of the number of dead bees, quotients of mortality (Q_M) were included. The formulas which were used for the calculation of the quotients are as follows:

QM(DAA0aa) = mortality on the day of application (0aa) ÷ pre-(2nd)-application mortality (Q_{Mb}) (per treatment group).

QM(DAA0aa to 7) = post-application mortality exposure period ± pre-(2nd)-application mortality (Q_{Mb}) (per treatment group).

In addition, the author has calculated brood indices, compensation indices and termination rates (see following three figures). The indices and rates are calculated for several 'Brood Area Fixing Days' (BFD). At these days specific areas on the combs were evaluated for specific stages using a digital camera.

The data of mortality and flight intensity of the test item and the reference item group were compared to the control in a separate approach but in the same way. The data were tested for normality and homoscedasticity using Shapiro-Wilk test ($p > 0.05$) and folded F-test ($p > 0.05$), respectively. Data were statistically compared using t-test (method pooled, $p \leq 0.5$) in case of normality and homoscedasticity. In case of no homoscedasticity but proven normality t-test was conducted with method Satterthwaite (same as Welch's t-test, $p \leq 0.5$). In case of no normality and no homoscedasticity Wilcoxon test ($p \leq 0.5$) was used. Log-transformation was conducted if necessary to achieve better normality and homoscedasticity of data.

Results

Climatic conditions

Temperature during 2nd application was between 10.0 and 19.9.°C, 37.7-76.3% RH, no rainfall; Temperature during observation ranged from 8.6 to 27.8 °C. Rainfall from DAA19 between 0.2 and 33.3 mm per day.

<u>Mortality</u>

Mortality and flight intensity of the different test groups are reported in the following figures (copied from the report). Only on two single days, DAA9 and DAA21, the mortality of the test item treatment was statistically significantly higher compared to the control.

When comparing the mean mortality before application (DAA-4 to DAA0ba) until the day of the 2nd test item application, the QM(DAA0aa) values were calculated to be 0.9, 1.1 and 0.5 for the treatment groups C, T and R, respectively. The QM(DAA0aa to 7) values were calculated to be 1.4, 1.0 and 1.0 for C, T and R, respectively.

Figure 9.4.2-29. Mean number of dead bees/replicate/day counted in the dead bee traps and on the linen sheets inside the tunnels of the control group C, the test item group T and the reference group R prior to and after the 2nd application in T and the concurrent applications in C and R, respectively

0ba = day of application, before application

0aa = 2nd application in T and the concurrent applications in C and R, respectively

* statistically significantly higher than in the control group

Figure 9.4.2-29 shows the dead bees on the linen sheets and in the bee traps combined. EFSA asked RMS to provide more detailed data for adult and larvae/pupa mortality separately, so that it can be seen if the contribution of pupae mortality was negligible throughout the study.

Even though the report methods state that the dead bees found on the sheets and in the bee traps were differentiated between adult worker bees, males (drones), freshly emerged bees, pupae and larvae during each assessment, the results are only presented as total dead bees. However, numbers of dead bees found on the linen sheet and in the bee traps separately are available, for all replicates, treatments and assessment dates. They are shown below in Tables 18, 19 and 20, which are copied from the study report. Based on these tables, the following overview of numbers of dead bees on the linen sheets (LS) and in the dead bee traps (BT) in the three treatment groups in the different test periods can be made (numbers are the mean from three hives):

	C		T		R	
DAA	LS	BT	LS	BT	LS	BT
-4 to 0ba	40.3	10.2	74.5	12.3	69.5	24.2
0aa	34.7	11.7	74.7	16.7	31.7	12.0
0aa to 7	36.0	35.1	52.5	38.5	46.7	49.8

The relative amount of dead bees in the dead bee traps (which will contain dead pupae) is higher in the control than in the treatment group. Thus, there is no indication that the pupae mortality is higher in the treatment group than in the control.

A02: Individual Results

Table 18: Bee mortality of the control group C

Date	DAA	Mortality (number of dead worker bees, larvae and pupae)									
		C1		C2		C3		Mean		Mean per replicate and day[a]	STD[a]
		LS	BT	LS	BT	LS	BT	LS	BT		
11 Jul 2011	-4	19	2	24	6	21	7	21.3	5.0	26.3	4.7
12 Jul 2011	-3	83	9	34	10	33	4	50.0	7.7	57.7	29.9
13 Jul 2011	-2	40	3	35	23	19	6	31.3	10.7	42.0	16.5
14 Jul 2011	-1	51	6	46	13	39	11	45.3	10.0	55.3	4.7
15 Jul 2011	0ba	39	8	81	32	40	13	53.3	17.7	71.0	36.5
Mean DAA-4 to 0ba		**46.4**	**5.6**	**44.0**	**16.8**	**30.4**	**8.2**	**40.3**	**10.2**	**50.5**	--
STD		**23.5**	**3.0**	**22.1**	**10.6**	**9.9**	**3.7**	**19.5**	**7.9**	**–**	**24.7**
15 Jul 2011	0aa+1h	7	0	5	5	6	0	6.0	1.7	7.7	2.1
15 Jul 2011	0aa+2h	5	1	4	6	3	5	4.0	4.0	8.0	2.0
15 Jul 2011	0aa+4h	5	0	8	9	5	0	6.0	3.0	9.0	6.9
15 Jul 2011	0aa+6h	14	2	18	4	24	3	18.7	3.0	21.7	5.5
Sum DAA0aa		31	3	35	24	38	8	34.7	11.7	46.3	12.5
16 Jul 2011	1	10	7	33	35	31	8	24.7	16.7	41.3	25.6
17 Jul 2011	2	9	12	35	16	19	11	21.0	13.0	34.0	15.4
18 Jul 2011	3	32	4	56	54	71	8	53.0	22.0	75.0	37.2
19 Jul 2011	4	55	6	59	98	64	13	59.3	39.0	98.3	51.4
20 Jul 2011	5	44	12	29	40	32	27	35.0	26.3	61.3	6.8
21 Jul 2011	6	27	13	21	44	32	17	26.7	24.7	51.3	12.7
22 Jul 2011	7	14	31	52	318	34	33	33.3	127.3	160.7	181.6
Mean DAA0aa to 7		**27.8**	**11.0**	**40.0**	**78.6**	**40.1**	**15.6**	**36.0**	**35.1**	**71.0**	--
STD		**16.4**	**8.9**	**13.8**	**99.8**	**17.8**	**9.5**	**16.5**	**63.9**	**–**	**70.1**

DAA = Days after application
STD = Standard deviation
LS = Dead bees on linen sheet
BT = Dead bees in bee trap
0ba = before application
0aa = after application
-- = not applicable
[a] = For calculation of means and STD (standard deviation) the numbers of dead bees on the linen were added to the dead bees assessed for each hive

Table 18: Bee mortality of the control group C (continued)

Date	DAA	Mortality (number of dead worker bees, larvae and pupae)									
		C1		C2		C3		Mean		Mean per replicate and day[a]	STD[a]
		LS	BT	LS	BT	LS	BT	LS	BT		
23 Jul 2011	8	--	21	--	130	--	17	--	56.0	56.0	64.1
24 Jul 2011	9	--	14	--	61	--	26	--	33.7	33.7	24.4
25 Jul 2011	10	--	11	--	63	--	49	--	41.0	41.0	26.9
26 Jul 2011	11	--	11	--	119	--	56	--	62.0	62.0	54.2
27 Jul 2011	12	--	39	--	187	--	102	--	109.3	109.3	74.3
28 Jul 2011	13	--	17	--	124	--	38	--	59.7	59.7	56.7
29 Jul 2011	14	--	14	--	28	--	22	--	21.3	21.3	7.0
30 Jul 2011	15	--	28	--	66	--	56	--	50.0	50.0	19.7
31 Jul 2011	16	--	33	--	85	--	50	--	56.0	56.0	26.5
1 Aug 2011	17	--	15	--	77	--	30	--	40.7	40.7	32.3
2 Aug 2011	18	--	18	--	34	--	35	--	29.0	29.0	9.5
3 Aug 2011	19	--	8	--	19	--	20	--	15.7	15.7	6.7
4 Aug 2011	20	--	8	--	11	--	25	--	14.7	14.7	9.1
5 Aug 2011	21	--	9	--	8	--	16	--	11.0	11.0	4.4
6 Aug 2011	22	--	14	--	10	--	26	--	16.7	16.7	8.3
7 Aug 2011	23	--	8	--	13	--	17	--	12.7	12.7	4.5
8 Aug 2011	24	--	44	--	15	--	20	--	26.3	26.3	15.5
9 Aug 2011	25	--	19	--	21	--	9	--	16.3	16.3	6.4
10 Aug 2011	26	--	10	--	4	--	10	--	8.0	8.0	3.5
11 Aug 2011	27	--	9	--	12	--	3	--	8.0	8.0	4.6
Mean DAA0aa to 27		**23.6**		**72.7**		**38.3**		**44.9**		**44.9**	**–**
STD		**15.3**		**75.9**		**23.9**		**50.6**		**--**	**50.6**
Q_M (DAA0aa)		--		--		--		--		0.9	--
Q_M (DAA0aa to 7)		0.7		2.0		1.4		--		1.4	--

DAA = Days after application
STD = Standard deviation
LS = Dead bees on linen sheet
BT = Dead bees in bee trap
0ba = before application
0aa = after application
-- = not applicable
[a] = For calculation of means and STD (standard deviation) the numbers of dead bees on the linen were added to the dead bees assessed for each hive
$Q_{M(DAA0aa)}$ = ∅ post-application mortality on DAA0aa ÷ ∅ pre-application mortality (per treatment group)
$Q_{M(DAA0aa\ to\ 7)}$ = ∅ post-application mortality exposure period ÷ ∅ pre-application mortality (per treatment group and replicate)

Table 19: Bee mortality of the test item group T

Date	DAA	Mortality (number of dead worker bees, larvae and pupae)									
		T1		T2		T3		Mean		Mean per replicate and day[a]	STD[a]
		LS	BT	LS	BT	LS	BT	LS	BT		
11 Jul 2011	-4	10	0	55	13	36	6	33.7	6.3	40.0	29.1
12 Jul 2011	-3	43	2	108	33	95	9	82.0	14.7	96.7	48.4
13 Jul 2011	-2	23	3	69	14	58	11	50.0	9.3	59.3	29.7
14 Jul 2011	-1	36	2	116	16	35	14	62.3	10.7	73.0	51.4
15 Jul 2011	0ba	63	11	268	23	103	27	144.7	20.3	165.0	112.7
Mean DAA-4 to 0ba		**35.0**	**3.6**	**123.2**	**19.8**	**65.4**	**13.4**	**74.5**	**12.3**	**86.8**	--
STD		**20.1**	**4.3**	**84.9**	**8.3**	**32.1**	**8.1**	**62.5**	**9.6**	**–**	**69.1**
15 Jul 2011	0aa+1h	21	0	27	3	8	5	18.7	2.7	21.3	8.5
15 Jul 2011	0aa+2h	1	0	45	4	0	5	15.3	3.0	18.3	26.6
15 Jul 2011	0aa+4h	15	1	58	2	7	9	26.7	4.0	30.7	25.4
15 Jul 2011	0aa+6h	3	0	34	12	5	9	14.0	7.0	21.0	22.3
Sum DAA0aa		40	1	164	21	20	28	74.7	16.7	91.3	81.2
16 Jul 2011	1	26	7	73	15	25	17	41.3	13.0	54.3	29.5
17 Jul 2011	2	12	5	27	19	10	21	16.3	15.0	31.3	14.5
18 Jul 2011	3	60	5	104	30	92	22	85.3	19.0	104.3	35.5
19 Jul 2011	4	24	6	152	45	34	73	70.0	41.3	111.3	83.6
20 Jul 2011	5	27	15	77	98	66	73	56.7	62.0	118.7	68.8
21 Jul 2011	6	13	10	39	58	38	39	30.0	35.7	65.7	38.3
22 Jul 2011	7	23	12	72	191	42	114	45.7	105.7	151.3	114.1
Mean DAA0aa to 7		**28.1**	**7.6**	**88.5**	**59.6**	**40.9**	**48.4**	**52.5**	**38.5**	**91.0**	--
STD		**15.6**	**4.5**	**49.1**	**59.7**	**26.6**	**34.7**	**41.5**	**44.5**	**–**	**66.6**

DAA = Days after application
STD = Standard deviation
LS = Dead bees on linen sheet
BT = Dead bees in bee trap
0ba = before application
0aa = after application
-- = not applicable
[a] = For calculation of means and STD (standard deviation) the numbers of dead bees on the linen were added to the dead bees assessed for each hive

Table 19: Bee mortality of the test item group T (continued)

Date	DAA	Mortality (number of dead worker bees, larvae and pupae)									
		T1		T2		T3		Mean		Mean per replicate and day[a]	STD[a]
		LS	BT	LS	BT	LS	BT	LS	BT		
23 Jul 2011	8	--	10	--	84	--	89	--	61.0	61.0	44.2
24 Jul 2011	9	--	60	--	100	--	65	--	75.0	75.0 [1)]	21.8
25 Jul 2011	10	--	79	--	84	--	36	--	66.3	66.3	26.4
26 Jul 2011	11	--	53	--	75	--	64	--	64.0	64.0	11.0
27 Jul 2011	12	--	49	--	126	--	70	--	81.7	81.7	39.8
28 Jul 2011	13	--	17	--	65	--	43	--	41.7	41.7	24.0
29 Jul 2011	14	--	28	--	24	--	40	--	30.7	30.7	8.3
30 Jul 2011	15	--	73	--	55	--	41	--	56.3	56.3	16.0
31 Jul 2011	16	--	33	--	41	--	21	--	31.7	31.7	10.1
1 Aug 2011	17	--	14	--	23	--	14	--	17.0	17.0	5.2
2 Aug 2011	18	--	14	--	16	--	26	--	18.7	18.7	6.4
3 Aug 2011	19	--	22	--	13	--	24	--	19.7	19.7	5.9
4 Aug 2011	20	--	26	--	16	--	37	--	26.3	26.3	10.5
5 Aug 2011	21	--	36	--	19	--	43	--	32.7	32.7 [1)]	12.3
6 Aug 2011	22	--	31	--	12	--	24	--	22.3	22.3	9.6
7 Aug 2011	23	--	8	--	5	--	7	--	6.7	6.7	1.5
8 Aug 2011	24	--	30	--	26	--	18	--	24.7	24.7	6.1
9 Aug 2011	25	--	19	--	11	--	11	--	13.7	13.7	4.6
10 Aug 2011	26	--	17	--	8	--	5	--	10.0	10.0	6.2
11 Aug 2011	27	--	12	--	8	--	8	--	9.3	9.3	2.3
Mean DAA0aa to 27		**32.8**		**71.3**		**50.0**		**51.3**		**51.3**	**–**
STD		**19.1**		**68.2**		**39.9**		**49.0**		**–**	**49.0**
Q_M (DAA0aa)		--		--		--		--		1.1	--
Q_M (DAA0aa to 7)		0.9		1.0		1.1		--		1.0	--

DAA = Days after application
STD = Standard deviation
LS = Dead bees on linen sheet
BT = Dead bees in bee trap
0ba = before application
0aa = after application
-- = not applicable
[a] = For calculation of means and STD (standard deviation) the numbers of dead bees on the linen were added to the dead bees assessed for each hive
[1)] = Statistically significantly higher than control
$Q_{M(DAA0aa)}$ = ∅ post-application mortality on DAA0aa ÷ ∅ pre-application mortality (per treatment group)
$Q_{M(DAA0aa\ to\ 7)}$ = ∅ post-application mortality exposure period ÷ ∅ pre-application mortality (per treatment group and replicate)

Table 20: Bee mortality of the reference item group R

Date	DAA	Mortality (number of dead worker bees, larvae and pupae)									
		R1		R2		R3		Mean		Mean per replicate and day[a]	STD[a]
		LS	BT	LS	BT	LS	BT	LS	BT		
11 Jul 2011	-4	10	12	39	14	50	17	33.0	14.3	47.3	23.0
12 Jul 2011	-3	15	10	132	66	238	29	128.3	35.0	163.3	124.7
13 Jul 2011	-2	21	5	56	25	111	20	62.7	16.7	79.3	52.5
14 Jul 2011	-1	11	8	39	15	58	29	36.0	17.3	53.3	34.0
15 Jul 2011	0ba[#]	57	23	95	34	110	56	87.3	37.7	125.0	43.1
Mean DAA-4 to 0ba		**22.8**	**11.6**	**72.2**	**30.8**	**113.4**	**30.2**	**69.5**	**24.2**	**93.7**	--
STD		**19.6**	**6.9**	**40.5**	**21.3**	**75.2**	**15.4**	**60.5**	**17.2**	--	**72.3**
15 Jul 2011	0aa+1h	8	2	12	1	15	12	11.7	5.0	16.7	9.1
15 Jul 2011	0aa+2h	4	2	5	0	6	1	5.0	1.0	6.0	1.0
15 Jul 2011	0aa+4h	5	1	5	0	17	2	9.0	1.0	10.0	7.8
15 Jul 2011	0aa+6h	6	0	11	6	1	9	6.0	5.0	11.0	5.6
Sum DAA0aa		23	5	33	7	39	24	31.7	12.0	43.7	17.8
16 Jul 2011	1	17	6	35	18	16	42	22.7	22.0	44.7	18.9
17 Jul 2011	2	13	8	19	12	12	28	14.7	16.0	30.7	9.5
18 Jul 2011	3	50	5	85	94	113	78	82.7	59.0	141.7	75.3
19 Jul 2011	4	42	17	59	100	81	85	60.7	67.3	128.0	59.9
20 Jul 2011	5	28	6	90	49	103	52	73.7	35.7	109.3	65.7
21 Jul 2011	6	20	21	48	45	69	87	45.7	51.0	96.7	57.6
22 Jul 2011	7	18	39	62	170	46	197	42.0	135.3	177.3	104.4
Mean DAA0aa to 7		**26.4**	**13.4**	**53.9**	**61.9**	**59.9**	**74.1**	**46.7**	**49.8**	**96.5**	--
STD		**13.1**	**12.0**	**25.1**	**56.1**	**37.9**	**55.5**	**30.0**	**51.6**	--	**70.9**

DAA = Days after application
STD = Standard deviation
LS = Dead bees on linen sheet
BT = Dead bees in bee trap
0ba = before application
0aa = after application
-- = not applicable
a = For calculation of means and STD (standard deviation) the numbers of dead bees on the linen were added to the dead bees assessed for each hive

[#] = Summarized results of two assessments before start of the application in R

Table 20: Bee mortality of the reference item group R (continued)

Date	DAA	Mortality (number of dead worker bees, larvae and pupae)									
		R1		R2		R3		Mean		Mean per replicate and day[a]	STD[a]
		LS	BT	LS	BT	LS	BT	LS	BT		
23 Jul 2011	8	--	34	--	118	--	136	--	96.0	96.0	54.4
24 Jul 2011	9	--	29	--	37	--	39	--	35.0	35.0	5.3
25 Jul 2011	10	--	43	--	31	--	64	--	46.0	46.0	16.7
26 Jul 2011	11	--	32	--	45	--	48	--	41.7	41.7	8.5
27 Jul 2011	12	--	93	--	64	--	78	--	78.3	78.3	14.5
28 Jul 2011	13	--	37	--	39	--	32	--	36.0	36.0	3.6
29 Jul 2011	14	--	29	--	4	--	23	--	18.7	18.7	13.1
30 Jul 2011	15	--	61	--	34	--	24	--	39.7	39.7	19.1
31 Jul 2011	16	--	18	--	34	--	8	--	20.0	20.0	13.1
1 Aug 2011	17	--	5	--	10	--	10	--	8.3	8.3	2.9
2 Aug 2011	18	--	3	--	5	--	1	--	3.0	3.0	2.0
3 Aug 2011	19	--	7	--	7	--	2	--	5.3	5.3	2.9
4 Aug 2011	20	--	3	--	3	--	2	--	2.7	2.7	0.6
5 Aug 2011	21	--	15	--	12	--	5	--	10.7	10.7	5.1
6 Aug 2011	22	--	7	--	7	--	42	--	18.7	18.7	20.2
7 Aug 2011	23	--	7	--	3	--	6	--	5.3	5.3	2.1
8 Aug 2011	24	--	13	--	13	--	15	--	13.7	13.7	1.2
9 Aug 2011	25	--	11	--	12	--	9	--	10.7	10.7	1.5
10 Aug 2011	26	--	3	--	1	--	5	--	3.0	3.0	2.0
11 Aug 2011	27	--	0	--	4	--	3	--	2.3	2.3	2.1
Mean DAA0aa to 27		**27.4**		**50.3**		**58.0**		**45.3**		**45.3**	**--**
STD		**22.6**		**61.0**		**67.5**		**55.1**		**--**	**55.1**
Q_M (DAA0aa)		--		--		--		--		0.5	--
Q_M (DAA0aa to 7)		1.2		1.1		0.9		--		1.0	--

DAA = Days after application
STD = Standard deviation
LS = Dead bees on linen sheet
BT = Dead bees in bee trap
0ba = before application
0aa = after application
-- = not applicable
[a] = For calculation of means and STD (standard deviation) the numbers of dead bees on the linen were added to the dead bees assessed for each hive
$Q_{M(DAA0aa)}$ = ∅ post-application mortality on DAA0aa ÷ ∅ pre-application mortality (per treatment group)
$Q_{M(DAA0aa\ to\ 7)}$ = ∅ post-application mortality exposure period ÷ ∅ pre-application mortality (per treatment group and replicate)

Figure 9.4.2-30. Mean number of forager bees /m2 inside tunnels prior to and after the 2nd application in T and the concurrent application in C and R, respectively.

0ba = day of application, before application;

0aa = 2nd application in T and the concurrent applications in C and R, resp.

* statistically significantly lower than control group

X = no flight activity in any of the treatments due to adverse weather conditions

Significant effects on flight activity on the day of the 2nd test item application as well as on some further days during the confined exposure period were present.

<u>Strength of the colonies</u>

Mean numbers of bees are given in the figure below.

Figure 9.4.2-31. Strength of the colonies (mean number of the honeybees per hive) in the treatment groups C, T and R.

The development of colony strength was comparable between C and T throughout the study period and showed the fluctuations which are typical of this endpoint. As such, no test-item related adverse effects on colony strength were observed.

<u>Development of Brood</u>

Figure 9.4.2-32. Mean number of brood and food cells in the treatment groups C, T and R.

Development of brood

The mean abundance of brood (sum of cells containing eggs, larvae, and pupae) assessed before set-up of the hives (first colony assessment, DAA-7/-5) was comparable in all treatment groups with 20400 cells/hive for C,19667 cells/hive for T and 19600 cells/hive for R. At the second colony assessment (DAA-1), the mean abundance of brood in C, T and R had decreased slightly (19267 cells/hive for C, 17867 cells/hive for T and 19533 cells/hive for R). At the third colony assessment, during the confined exposure period, on DAA4, the mean abundance of brood in C and T was on an almost identical level (C: 17533 cells/hive, T: 17933 cells/hive), whereas the mean abundance of brood in R decreased strongly to 13400 cells/hive. On the fifth colony assessment (DAA14), no appreciable changes were observed in the mean abundance of brood in C and T, whereas a strong decrease in R to 8333 cells/hive occurred. This refers to a clearly detectable effect of the reference item, which is typical for this point in time. Brood of all stages (eggs, larvae, capped brood) was present in all colonies at all assessments during the study, with the exception of colony T1, which was found queenless from the fourth to the last colony assessment. The number of brood cells consequently decreased from this time on (fifth - seventh colony assessment) in colony T1 and as such, from the fifth colony assessment onwards, also the mean values in T were lower than in C (sixth colony assessment, DAA21: 20200 cells/hive for C, 13667 cells/hive for T, seventh colony assessment, DAA27: 18600 cells/hive for C, 12667 cells/hive for T). Except for the colony T1, the fluctuations of all brood stages were within the range of natural variation and typical for this kind of study. Overall, honey bee brood development and colony conditions in test item treatment T were comparable to control treatment during the whole assessment period. No test-item related adverse effects on brood development were observed.

Development of the Food Storage Area

The mean extent of food stores in the colonies (sum of cells containing nectar and pollen) assessed before set-up of the hives (first colony assessment, DAA-5) was 14067 cells/hive for C, 17333 cells/hive for T and 14600 cells/hive for R. At the second colony assessment (DAA-1), the mean extent of food stores decrease slightly in C and increased slightly in T and R (C: 13067 cells/hive, T: 17600 cells/hive, R: 15733 cells/hive). At the third colony assessment, during the confined exposure period, on DAA4, the mean extent of food stores in the colonies C, T and R had decreased (C: 9067 cells/hive, T: 16333 cells/hive, R: 11933 cells/hive). At the two subsequent assessments on the remote monitoring location at DAA10 and DAA14 (fourth and fifth colony assessment), the mean extent of food stores in C and T had decreased in parallel, increased thereafter in parallel on DAA21 and decreased again in parallel to 4400 cells/hive in C and 5133 cells/hive in T at DAA27. The mean extent of food stores in the R hives from DAA10 to DAA27 was an alternate de- and increase with 6133 cells/hive on the last colony assessment.

The observed parallel decrease in food stores in both, treatment and control, during confinement as well as the subsequent alternate, but again parallel de- and increase in C and T, respectively, can be

considered as typical for this type of study. No test-item related adverse effects on the development of the food storage area were observed.

Brood and compensations indices and termation rates are given in the three figures below. These calculations show that the control and treatment are very similar.

Figure 9.4.2-33. Course of the brood index in the treatment groups C, T and R.

Figure 9.4.2-34. Course of the compensation index in the treatment groups C, T and R.

Figure 9.4.2-35. Termination rates at the last assessment (BFD22) in the treatment groups C, T and R.

Behaviour of the Bees

No abnormal behaviour was recorded in the control, in the test item and in the reference item treatment before the 2nd test item application and the concurrent application in C and R, respectively (DAA-4 to DAA0ba). In the test item treatment T after the 2nd test item application on DAA0aa, a few bees were observed with intoxication symptoms (cramping, locomotion problems, coordination problems), moreover, an intensive flying over the crop coupled with an infrequent landing on the crop could be observed together with a slight repellent effect. On the following days (DAA1 to 7), normal behaviour was observed in all three tunnels of the test item treatment. In the reference item treatment R, after application on DAA0aa, a strong repellent effect (no or low flight activity on the crop) as well as several bees with obvious signs of intoxication were observed. In the control group, normal behaviour was recorded throughout the observation period except for the evening of DAA0aa, were about 200 to 500 bees were observed motionless on the covering net in C3 as well as in T1 - T3, respectively. In the test item treatment group, during the last assessment in the evening of DAA0aa(+6h), no bees were found motionless on the covering net any more. Except for DAA0aa, honey bee behaviour in test item treatment T was comparable to the control treatment throughout the entire assessment period.

Residue analysis

A summary of the analytical results, as obtained by analysing samples of pollen, nectar/honey and bee wax, collected from the bee hives of the test item group at the end of the study (27 days after the last test item application), is reported in the following table.

Table 9.4.2-12. Residues of BYI 02960 and BYI 02960-difluoroethylaminofluranone in samples of pollen, nectar/honey and bee wax, as obtained in the test item group

Sample	Test Item	Residue [µg/kg]	
		BYI 02960	BYI 02960-DFEAF
Pollen	BYI 02960 SL 200 G	2 - 4	< LOQ
Nectar/Honey	BYI 02960 SL 200 G	< LOQ - 17	< LOQ - 1
Bee wax	BYI 02960 SL 200 G	1 - 62	< LOQ

BYI 02960-DFEAF = BYI 02960-difluoroethylaminofluranone (BCS-CC98193),
LOQ = Limit of Quantitation = 1 µg/kg in nectar, pollen and bee wax, respectively (BYI 02960 and BYI 02960-DFEAF)

Conclusions of the author:

A pre-flowering foliar application of the test item, corresponding to 200 g BYI 02960 as/ha to a highly bee-attractive crop (*Phacelia tanacetifolia*), was followed by a further foliar application of the test item, again corresponding to 200 g BYI 02960 as/ha, during full-flowering with honeybees actively foraging on the crop.

This application scenario did not cause treatment-related adverse effects on mortality, on flight intensity, on honeybee behaviour, on brood- and food development as well as on colony vitality under forced exposure conditions. A slight repellent effect of the test item was indicated by a reduced flight intensity on the day of the 2nd test item application as well as on some further days during the confined exposure period.

Remarks by RMS

A positive control was included in the test and showed that the test system was behaving as expected. According to the EFSA Opinion on the Science behind the development of a risk assessment of plant protection products for bees (EFSA Journal 2012, 10: 2668, section 3.5.1), the main exposure route for bee brood is via pollen. Since *Phacelia tanacetifolia* is a major source of pollen, and pollen from uncontaminated sources was not present in the tunnels, the present test is suitable to show potential effect of flupyradifurone on brood related parameters, mortality and flight intensity.

A reduced flight intensity on the day of the second application and on DAA0aa, 4, 6 and during DAA0aa to DAA7 (mean) the two following days. A relation between decrease of flight intensity and mortality outside the hive may exist. When fewer bees are actively foraging the percentage of mortalities outside the hive may also decrease. Behavioural effects were observed at the day of second application (intoxication symptoms and coordination problems). From the results of the study it can be concluded that two foliar applications on *Phacelia tanacetifolia* sprayed once with BYI 02960 pre-flowering and once full-flowering at an application rate corresponding to 200 g as/ha have no effect on mortality, brood-, food-, and population development as well as on overall hive vitality. There are effects on flight intensity and behaviour. The results of the study, including the residue measurements, can be used in the risk assessment.

Expert discussion in Pesticide Peer Review meeting 124 (December 2014):

(Please note that the word 'effect' in the text below might be interpreted as different from the control (i.e. may or may not include effects from the treatment), pending on the context.)

The RMS considered that this study resulted in the greatest level of exposure. The experts noted that there was a clear indication of a potential, slight effect due to the test item. Some indications of sublethal intoxication were noted. The flower visits were also lower for the test item. These effects were noted by the RMS.

Statistical analysis on forager mortality was performed. A statistically significant effect was only observed on a few times points. The level of mortality was not consistently statistically significant compared to the control. The experts noted that there was an application prior to the introduction of the hives. Therefore, it is not possible to compare with pre-application data.

The pooling of the dead adult bees and pupae was discussed. Ideally the data would be presented separately.

There was no agreement on whether this study indicates a slight test item related effect under the exposure conditions in the study. This differs to the RMS who believes that the study indicates no clear effect on forager mortality. The majority of the experts agreed with the assessment of the RMS and did not consider that the results indicated a clear effect on mortality.

Study IIA 8.16.2/08

Characteristics

Reference	:	Rexer 2013	nominal dose	:	2x 200 g a.s./ha, 10 d interval
Type of study	:	Residues in nectar and honey under semi-field conditions in *Phacelia tanacetifolia*	species	:	Honey bee (*Apis mellifera*)
Year of execution	:	2012	exposure duration	:	14 d
GLP statement	:	yes	dosing method	:	Tunnel test in *Phacelia tanacetifolia*, 2x foliar spray: 1st application before onset of flowering and 2nd application during flowering
Guideline	:	OEPP/EPPO guideline No. 170 (4), 2010, SANCO/3029/99 rev. 4.	Acceptability	:	Acceptable
Test substance	:	BYI 02960 FS 200 G, Batch no. 2011-002192	Results	:	Residue levels in pollen and nectar were determined, see below
a.s. content	:	201.4 g flupyradifurone /L			

Description

Residues of BYI 02960 in Phacelia nectar and Phacelia pollen after two spray applications of BYI 02960 SL 200 to flowering Phacelia tanacetifolia. Nectar and pollen was collected from forager bees and combs.

Location

Field experiment was conducted from July 2012 to August 2012 in Niefern-Öschelbronn, federal state of Baden-Württemberg, Germany.

Sizes field and crop, test condition (tunnel or field)

The size of each field crop (each replicate) was 70.4 m2 (test item treatments and control plots). Fields were separated by 7.0 km. The crop was Phacelia tanacetifolia. Previous crop was maize. Three herbicides, nicosulfuron, prosulfuron and mesotrione were in the early spring (April, 27, 2011), before sowing of Phacelia.

Active substance

BYI 02960 SL 200 G; Specification No.: 102000021884 - 02; Material No.: 79718845; Batch-ID: 2011-002192; content: BYI 02960: 201.4 g/L (content of as analysed).

Treatments

Three treatment groups (see Table below):

- T1 (BYI 02960; for sampling of forager bees; three replicates), application before the onset of flowering (BBCH 58-60).
- T2 (BYI 02960; for sampling of nectar and pollen from combs; three replicates), application during full flowering (BBCH 65) and
- an untreated control C (one replicate).

Table 9.4.2-13 Application schedule

Trial S12-00038-01					
Appl' Code	Plot	Timing	Application Rate (priority to active ingredient)		Water Volume
-	C	-	-	-	-
A1	T1a-T1c, T2a-T2c	BBCH 58-60 (= 0DAA1)	993 mL product/ha [1)]	200 g a.i./ha	200 L/ha
A2	T1a-T1c, T2a-T2c	BBCH 64-65 (= 0DAA2)	993 mL product/ha [1)]	200 g a.i./ha	200 L/ha

[1)] based on analysed content of a.i.

Test concentrations

Two applications with BYI 02960 SL 200 G at a target rate of 200 g as/ha respectively. The first test item application was performed on 24 Jul 2012, 10 days before the second test item application

(10DBA2) and 7 days before set-up of the hives in the tunnels. Applications were performed in 200 L water/ha.

Species tested

Honey bees of the race *Apis mellifera*.

Population size

For the test, healthy colonies and queen-right with twenty combs containing approx. 13,000 bees in the control (1 hive), 15,500 to 28,000 bees in treatment group T1 (3 hives. Replicate T1c had 30 combs and therefore high numbers of bees (28,000). Treatment group T2 (3 hives) had 15,500 to 27,000 bees.

Timing placement hives under test conditions

The set-up of the colonies in the tunnels was performed at BBCH 63 (beginning of flowering) on 31 July 2012 (3DBA2). Three days after set-up of the colonies, the 2nd test item application was performed on 3 August 2012 (0DAA2) during full flowering (BBCH 65) and during honeybee foraging activity. One commercial honey bee colony was used per tunnel. Tunnel measured 5 x 16 m.
At the end of the confined exposure phase on 13 August 2012 (10DAA2; BBCH 67-69), the honeybee colonies were relocated to a monitoring site at a distance of approximately 0.69 km to the field site with the tunnels. The area around the monitoring site was without flowering main crops attractive to bees. Hives were placed at the monitoring site from 14 August 2012 to 31 August 2012.

Parameters observed and timing thereof

The following parameters were observed during the exposure phase:
1. Colony assessment: Four days before the 2nd test item application (4DBA2), a colony assessment was performed in all treatment groups (C, T1 and T2) which included an assessment of the colony strength as well as the brood and food status.
2. Samplings of pollen and nectar from forager bees: Sampling in T1 and C started one day before the 2nd test item application (1DBA2). Thereafter, forager bee samplings inside the tunnels were performed daily in T1 up to 10DAA2 (3 times on 0DAA2, once per day from 1DAA2 to 10DAA2) and four times in C (0DAA2, 1DAA2, 4DAA2, 7DAA2). Bees were frozen, pollen loads were collected with tweezers and honey bulbs were removed. All material was kept frozen until analysis.
3. Phacelia nectar and pollen from combs in T2 were sampled once per day from 0DAA2 to 10DAA2 inside the tunnels and on 4 occasions from 12DAA2 to 28DAA2 at the monitoring site.

Residue analysis

Pollen (sampled from combs or prepared from forager bees) and nectar (sampled from combs or prepared from forager bees) samples were analysed for residues of BYI 02960 and its metabolite BYI 02960-DFEAF (BYI 02960-difluoroethylaminofuranone). Residue analysis was performed by using high Performance Liquid Chromotography (HPLC) coupled with electrospray and tandem mass spectrometry (MS/MS) detection. Analysis of nectar and pollen followed the provisions of method 01206/M001.

Statistical evaluation

Not performed.

Results

Actually applied concentrations

Actually applied spray applications in the three replicates of T1 were between 198.9 and 203.1 g as/ha in the first spray (deviation of -0.57 to +1.56%) and between 209.5 and 211.6 g as/ha in the second spray (deviation of +4.76 to + 5.82%).

Actually applied spray applications in the three replicates of T2 were between 198.2 and 201.0 g as/ha in the first spray (deviation of -0.92 to +0.50%) and between 205 and 210 g as/ha in the second spray (deviation of +2.27 to + 5.11%).

Climatic conditions

Temperatures, humidity and precipitation were recorded at the field site during the exposure phase and at the nearest official weather station at Niefern, 0.91 km away from the monitoring site during the post exposure period. No abnormal weather data were obtained.

Residue analysis

Method validation: Mean recovery of BYI 02960 in pollen and nectar/honey were 98 and 89% resp. with corresponding relative standard deviations of 8.8 and 4.4%, resp. Mean recovery of BYI 02960-DFEAF in pollen and nectar/honey were 85 and 84%, resp., with corresponding relative standard deviations of 7.9 and 7.9%, resp. For both parent and metabolite LOQ was 1 µg/kg and LOD was 0.1 µg/kg for both pollen sampled from forager bees as from nectar/honey from combs.

In the control, no residues were detected in nectar sampled from forager bees. All residues were <LOD.

Condition of the colonies

In the figure below the condition of the colonies is presented (graph prepared by evaluator) as was assessed four days before the second test item application, before set-up of the hives.

Figure 9.4.2-36 Condition of the colonies four days before 2nd application (prepared by evaluator)

Table 9.4.2-14 Residues of BYI02960 and BYI02960-difluoroethylaminofuranone (BYI02960-DFEAF) in samples forager bees and combs as obtained from the test item treatment groups

Sample	Test Item	Residue [µg/kg]	
		BYI 02960	BYI 02960-DFEAF
Pollen from comb	BYI 02960 SL 200 G	139 - 22529	1.6 - 46
Pollen from forager bees	BYI 02960 SL 200 G	138 - 70667	1.3 - 135
Nectar from combs	BYI 02960 SL 200 G	< LOQ - 186	< LOD - 9.4
Nectar from forager bees	BYI 02960 SL 200 G	4.8 - 3304	< LOQ - 122

BYI 02960-DFEAF = BYI 02960-difluoroethylaminofuranone (BCS-CC98193),
LOQ = Limit of Quantitation = 1 µg/kg in nectar and pollen, respectively (BYI 02960 and BYI 02960-DFEAF)
LOD = Limit of Detection = 0.1 µg/kg in nectar and pollen, respectively (BYI 02960 and BYI 02960-DFEAF)

The residue data as collected in pollen and nectar from forager bees and combs are shown in tables 9.4.2-14 (ranges) and -15 (in detail). Second test item application was performed at August 3. Colonies were placed in the tunnels at July 31, 3 days before the second application. First application had taken place 7 days before set up of the hives. First samplings were performed at August 2. Residues were present in all instances but were very low. At August 3, samplings for residues in pollen and nectar sampled from forager bees were performed at three time points (1 h, 2,5 h and 4 h after application).

Table 9.4.2-15 Residues in pollen and nectar.

Nectar from combs						
day August	**day after 2nd appl**	**rep 1**	**rep 2**	**rep 3**	**mean**	**SD**
2	-1	0	0	0	0,0	0,0
3	0	12	0		6,0	8,5
4	1	186	68		127,0	83,4
5	2	33	84	16	44,3	35,4
6	3	80	108	144	110,7	32,1
7	4	12	48	46	35,3	20,2
8	5	69	117		93,0	33,9
9	6	56	108		82,0	36,8
10	7	107			107,0	0,0
11	8	95			95,0	0,0
12	9	28	31	90	49,7	35,0
13	10	38	77		57,5	27,6
15	12	40	49		44,5	6,4
21	18	30	45		37,5	10,6
27	24	19	44		31,5	17,7
Residues nectar forager bees						
day August	**day after 2nd appl**	**rep 1**	**rep 2**	**rep 3**	**mean**	**SD**
2	-1	7	59	48	38,0	27,4
3,1	0 (+1h)	2777	3304	3064	3048,3	263,8
3,25	0 (+2.5h)	2038	2031	2726	2265,0	399,3
3,4	0 (+4h)	1235	1709	1360	1434,7	245,7
4	1	699	871	687	752,3	102,9
5	2	301	331	379	337,0	39,3
6	3	150	148	175	157,7	15,0
7	4	191	223	254	222,7	31,5
8	5	102	119	132	117,7	15,0
9	6	54	57	60	57,0	3,0
10	7	37	39	46	40,7	4,7
11	8	20	21	29	23,3	4,9
12	9	12	16	17	15,0	2,6
13	10	83	10	15	36,0	40,8
Residues pollen from combs						
day August	**day after 2nd appl**	**rep 1**	**rep 2**	**rep 3**	**mean**	**SD**

2	-1	240	175	166	193,7	40,4
3	0	6691	1545	9495	5910,3	4032,1
4	1	11260	5021	3019	6433,3	4298,2
5	2	4356	7009	3142	4835,7	1977,6
6	3	13783	4451	5503	7912,3	5111,3
7	4	7381	22529	603	10171,0	11226,1
8	5	6194	5171	985	4116,7	2759,9
9	6	1807	6017	1301	3041,7	2589,1
10	7	2943	5249	1640	3277,3	1827,6
11	8	1915	4491	1026	2477,3	1799,6
12	9	3634	4495	2566	3565,0	966,3
13	10	1580	2115	1719	1804,7	277,6
15	12	5791	4843	139	3591,0	3026,9
21	18	2671	3115		2893,0	314,0
27	24	2273	1145		1709,0	797,6
Residues pollen from forager bees						
day August	**day after 2nd appl**	**rep 1**	**rep 2**	**rep 3**	**mean**	**SD**
2	-1	138	146	162	148,7	12,2
3,1	0 (+1h)	59696	69911	64622	64743,0	5108,6
3,25	0 (+2.5h)	53137	70667	66009	63271,0	9080,1
3,4	0 (+4h)	34628	31623	42126	36125,7	5409,3
4	1	6469	8481	16113	10354,3	5087,6
5	2					
6	3	779	410	522	570,3	189,2
7	4	855	662	803	773,3	99,9
8	5	941	714	976	877,0	142,2
9	6	605	1041	1128	924,7	280,2
10	7	410	795	1092	765,7	341,9
11	8	572	728	763	687,7	101,7
12	9	174	460	469	367,7	167,8
13	10	636	377	647	553,3	152,8

Figures 9.4.2-37 to 9.4.2-40 were prepared by the evaluator using the data from Table 9.4.2-15.

Figure 9.4.2-37 Residues in nectar collected from forager bees in µg/kg (T1). Day of 2nd application is August 3. Figure prepared by evaluator

Figure 9.4.2-38. Residues in pollen collected from forager bees in µg/kg (T1). Day of 2nd application is August 3. Figure prepared by evaluator

Figure 9.4.2-39 Residues in nectar from combs in µg/kg (T2). Day of 2nd application is August 3. Figure prepared by evaluator

Figure 9.4.2-40. Residues in pollen from combs in µg/kg (T2). Day of 2nd application is August 3. Figure prepared by evaluator

Conclusions of the author

The distance between the field and the monitoring site was 0.69 km instead of the anticipated 3 km. The author states that this had no impact on the study as the crop area inside the tunnels remained confined until the end of flowering and was therefore not accessible from the outside. Relocation occurred at BBCH 69 (= End of flowering: fruit set visible) and foraging of re-located bees in treated Phacelia is therefore indeed not likely and evaluator agrees that shorter distance has no impact on the result.

Apart from a short summary and the results given in Table 9.4.2-14, no evaluation or discussion of the results was made by the author.

Remarks by evaluator

One of the colonies in test item group T1 had 30 combs in order to achieve the required colony strength. As this particular hive had very high numbers of bees (28,000) it is unclear why the control hive with very low number of bees (13,000) was found to be acceptable. The possible reason might be that the control hive is only used to confirm that no residues of flupyradifurone were present in the nectar and pollen. Development of the colonies was not the main issue in the study.

Residues in pollen and nectar from combs and pollen and nectar from forager bees should be measured not only in the treatments but also in the controls. Controls were only included in the experiment for pollen of the forager bees. The reason of omission of the other controls was not mentioned by the author. However, as no residues were found in the pollen of the control forager bees, absence of residues in nectar of the forager bees can be assumed, as fewer residues are present in nectar than in pollen. Moreover, if no residues are present in the pollen of the forager bees, no residues can be expected in the combs.

Table 9.4.2-14 and figures 9.4.2-37 to 9.4.2-40 show that fewer residues are present in the nectar than in the pollen. Also, much higher residue levels of both parent and metabolite (Figures not prepared for the metabolite) can be found in the pollen and nectar of the forager bees than in the combs. At the same time, the decline of concentrations in forager material is faster than in comb material. Figures 9.4.2-37 and -38 show that residues in the pollen and nectar collected by the foragers decrease to very low values within 3 days. Figures -39 and -40 show larger standard deviations than Figures -37 and -38. This can be explained by the fact that in Figures -37 and -38 the nectar and pollen was directly sampled from forager bees and during one sampling not much variation between residue levels of collected pollen and nectar of individual bees is expected. Residue levels in the nectar and pollen sampled from the combs, on the contrary, largely depend on the date of filling of the individual cells and the choice of the cells to be collected and the degradation rate of flupyradifurone. It is not described which cells were chosen for collection of the material and how to discriminate between recently filled cells (little degradation) and older cells (more degradation).
Following results can be used for risk assessment:

1. highest mean residue level (N=3)
   - in pollen from forager bees of 64,743 µg/kg (SD 5,109) at August 3 (= day of 2nd application)
   - in nectar from forager bees of 3,048 µg/kg (SD 264) at August 3 (= day of 2nd application)
   - in pollen from combs of 10,171 µg/kg (SD 11,226) at August 7 (= 4 days after 2nd application)
   - in nectar from combs of 127 µg/kg (SD 83) at August 5 (= 2 days after 2nd application)
2. residues in pollen and nectar collected by forager bees are much higher than residues in combs;
3. residues in pollen are higher than residues in nectar;
4. residues in pollen and nectar collected by forager bees decrease to very low values within a few days.
5. residues in the comb remain present for a longer time (24 days after 2nd application, residue level in pollen from combs is 1709 µg/kg and 32 µg/kg in nectar from combs).
6. condition of the colonies was similar for T1 and T2 colonies (Figure 2). Only one control replicate with a low number of bees (13,000) was used in the study. Normally this would be a problem. But since this study was not performed to monitor the effects of the pesticide this is not considered to be a problem. The control was only necessary to confirm that residues of pesticide were not present in pollen and nectar.

IIA 8.16.2/04

Characteristics

reference	:	Gould, TJ, 2012 Report No.: RARVP019	GLP statement	:	yes (certified laboratory); Deviations: none
Type of study	:	Supervised residue trial	Guideline	:	US EPA Ref.: OPPTS 850.SUPP (Ecological Effects)
Year of execution	:	2010	Dose	:	0.410 kg as/ha (drench) 0.204-0.408 kg as/ha (foliar spray)
test substance	:	BYI 02960 SL 200 formulation Batch no: 2010-001187	LOQ	:	BYI 02960 : 0.01 mg/kg DFEAF : 0.01 mg/kg
Species	:		storage	:	≤ 255 d at -18°C
			Acceptability	:	Y
			Results	:	Residues in blossoms, nectar and pollen were determined, see below

Study design

A semi-field trial was conducted in North-Carolina (USA) to determine the residues of BYI 02960 and the metabolite difluoroethylamino furanone (DFEAF) in blossoms, nectar, and pollen collected from melon plants treated with flupyradifurone in a 200 g SL formulation.

The target applications of the formulation were two different regimes; The first as one drench application at a dose rate of 0.410 kg as/ha (treatment 1) and treatment 2 as two foliar treatments at a rate of 0.205 kg as/ha a spray interval of 7-10 days. No control plot was used. Samples were collected at intervals of 17, 19, 21, 23, 26, 28, 30, 32, and 34 days after the drench application in the T1 plots. Samples were collected at intervals of -1, +1, +3, and +5 DAA(B) and at intervals of -1, +1, +3, +5, and +7 DAA(C) in the T2 plots.

Four bee tunnels were erected (replicate tunnels for each treatment regimen) and one normally developed, apparently healthy and queen-right honey bee colony (*Apis mellifera* L. (Hymenoptera, Apidae) was placed in each tunnel four days prior to the first foliar application to the T2 plots (14 days following application to the T1 plots).

Forager bees were used to collect nectar (honey stomach) samples from all plots at intervals of +1DAA1, +3DAA1, +5DAA1, -1DAA2, +1DAA2, +3DAA2, and +7DAA2 in the foliar (T2) plots, corresponding to DAAs of 19, 21, 23, 26, 28, 30, and 34 days following the soil drench application in the T1 plots. The bees were aspirated into a box of dry ice as they returned to the colony and frozen until later dissection to obtain composite honey stomach samples.

Pollen, which was available in smaller quantities than anticipated, was collected from pollen traps affixed to the hive at +1DAA1, +3DAA1, and +5DAA1 and at +3DAA2 in the T2 plots. These corresponded to DAAs of 19, 21, 23, and 30 days following the soil drench application in the T1 plots. Additional pollen samples were obtained from the T1 plot forager bees (without honey stomachs) at the 23- and 28-day intervals by removal of pollen grains adhered to their legs with a tweezers.

The residues of BYI 02960 and DFEAF were quantified by high performance liquid chromatography/triple stage quadrupole mass spectrometry (LC/MS/MS) using stable isotopically labeled internal standards. The individual analyte residues (in parent equivalents) were summed to give a total BYI 02960 residue. The limit of quantitation was 0.010 ppm for each analyte and 0.020 ppm for the total BYI 02960 residue.

Results

Details of the trial are presented in table B.9.4.2-16.

Conclusion

In study RARVP019 an acceptable residue trial in melon matrices was performed to establish residues in pollen, honey stomachs and blossoms.

The study revealed that a drench application of BYI 02960 SL 200, corresponding to 400 g a.s/ha, to melon plants at a growth stage typical for early transplanting (BBCH 15), resulted in maximum BYI

02960 residue levels in melon blossoms, pollen, and honey stomachs of 0.363, 0.314 and 0.695 mg/kg, respectively. The corresponding maximum residue level in melon blossoms, pollen, and honey stomachs of the metabolite BYI 02960-DFEAF was determined to be 0.017, <0.010 and 0.060 mg/kg, respectively.

Two foliar applications of 200 g as/ha and 400 g as/ha at BBCH 69-70, resulted in maximum BYI

02960 residue levels in melon blossoms, pollen, and honey stomachs of 2.778, 1.441 and 0.339 mg/kg, respectively. The corresponding maximum residue level in melon blossoms, pollen, and honey stomachs of the metabolite BYI 02960-DFEAF was determined to be 0.025, 0.022, 0.029 mg/kg, respectively.

IIA 8.16.2/05

Characteristics

reference	:	Bocksch,S 2012a Report No.: S09-01391	GLP statement	:	yes (certified laboratory); Deviations: none
Type of study	:	Supervised residue trial	Guideline	:	EIVA (1992), 7029/VI/95 (EU, 1997), 1607/VI/97 (EU,1999), 1107/2009 (EU, 2009), 544/2011 (EU, 2011a) and 545/2011 (EU, 2011b) US OCSPP Guideline Number 860.1520
Year of execution	:	2009-2011	Dose	:	0.150 kg as/ha
test substance	:	BYI 02960 SL 200 formulation Batch no: 2009-001253	LOQ	:	BYI 02960 : 1 µg/kg DFEAF : 1 µg/kg
Species	:	watermelon (*Citrullus lanatus*)	storage	:	≤ 698 d at -18°C
			Acceptability	:	Y
			Results	:	Residues in plants, flowers, nectar and pollen were determined, see below

Study design

A semi-field trial was conducted in Spain to determine the residues of BYI 02960 and the metabolite difluoroethylamino furanone (DFEAF) in plants, flowers, nectar, and pollen collected from watermelon plants treated with flupyradifurone in a 200 g SL formulation.

The target application rate was three sequential drench applications of 150 g as/ha with a seven-day spray interval at a water rate of 75 mL/plant. One control plot was used. Applications were made after transplanting (BBCH 12-13).

Commercial bee colonies were placed in the tunnels, just when enough flowers were present to allow foraging of the bees (BBCH 66), 28 days after the last application. Samples of young plants were taken at the time of transplanting from the greenhouse to the field (non-GLP). More plant samples were taken in regular intervals after the applications. Additionally, flowers as well as nectar and pollen from combs were collected at several dates, starting at the beginning of flowering and continuing during the flowering period of the crop for subsequent residue analysis.

Residue analysis was performed by using High Performance Liquid Chromatography (HPLC), coupled with electrospray and tandem mass spectrometry (MS/MS) detection, according to the provisions of method 01206/M001 (BYI 02960 and its relevant metabolite BYI 02960-difluoroethylaminofuranone). The Limit of Quantitation (LOQ) of BYI 02960 and BYI 02960-difluoroethylaminofuranone, defined as the lowest validated fortification level, was 1 µg/kg in green plant material, flower, nectar and pollen, respectively. The Limit of Detection (LOD) of BYI 02960 and BYI 02960-difluoroethylaminofuranone was 0.1 µg/kg in green plant material, flower, nectar and pollen, respectively.

All results of the method validation were in accordance with the general requirements for residue analytical methods; therefore, the method was validated successfully.

The residue levels of BYI 02960 and BYI 02960-difluoroethylaminofuranone (BYI02960-DFEAF) in samples of green plant material, flower, pollen and nectar, as obtained in the untreated control group, were always below LOQ.

Results

No rainfall was recorded on the days of application, except for 0.2 mm on the day of second application, which is considered irrelevant. Details of the trial are presented in table B.9.4.2-17.

Conclusion

The study revealed that three sequential drench applications of flupyradifurone at a 7 days interval, corresponding to 150 g a.s./ha, respectively, to watermelon plants, with the first treatment at a growth stage typical for early transplanting (BBCH 15), resulted in maximum BYI 02960 residue levels of 1.184 mg/kg in watermelon flowers, 6 µg/kg in watermelon pollen and ≤ 1 µg/kg in watermelon nectar. The corresponding maximum residue level of the metabolite BYI 02960-DFEAF in watermelon flowers was 6 µg/kg and <1 µg/kg in watermelon pollen and -nectar, respectively.

IIA 8.16.2/06

Characteristics

reference	:	Bocksch,S 2012b Report No.: S10-03818	GLP statement	:	yes (certified laboratory); Deviations: none
Type of study	:	Supervised residue trial	Guideline	:	EIVA (1992), 7029/VI/95 (EU, 1997), 1607/VI/97 (EU,1999), 1107/2009 (EU, 2009), 544/2011 (EU, 2011a) and 545/2011 (EU, 2011b) US OCSPP Guideline Number 860.1520
Year of execution	:	2011	Dose	:	0.150 kg as/ha
test substance	:	BYI 02960 SL 200 formulation Batch no: 2011-001324	LOQ	:	BYI 02960 : 1 µg/kg DFEAF : 1 µg/kg
Species	:	tomato (*Lycopersicon esculentum*)	storage	:	≤ 162 d at -18°C
			Acceptability	:	Y
			Results	:	Residues in flowers and pollen were determined, see below

Study design

A semi-field trial was conducted in Spain to determine the residues of BYI 02960 and the metabolite difluoroethylamino furanone (DFEAF) in flowers and pollen collected from tomato plants treated with flupyradifurone in a 200 g SL formulation.

The target application rate was one drench applications of 200 g as/ha. One control plot was used. Applications were made after transplanting (BBCH 13-14).

Commercial bumble bee colonies were placed in the tunnels, as soon as enough flowers were present to allow foraging of the bees (BBCH 62-63), 41 days after the application.

Samples of soil characterisation were taken once. Samples of tomato flowers as well as tomato pollen collected by forager bumble bees were taken at several dates for subsequent BYI 02960-residue analysis, starting at the first day of exposure and continuing during flowering of the crop.

For determination of residues of BYI 02960 in pollen, pollen loads from forager bumble bees were sampled ten times during exposure of the bumble bees inside the respective tunnels.

Residue analysis was performed by using High Performance Liquid Chromatography (HPLC), coupled with electrospray and tandem mass spectrometry (MS/MS) detection, according to the provisions of method 01206/M001 (BYI 02960 and its relevant metabolite BYI 02960-difluoroethylaminofuranone). The Limit of Quantitation (LOQ) of BYI 02960 and BYI 02960-difluoroethylaminofuranone, defined as the lowest validated fortification level, was 1 µg/kg in flower and pollen,

respectively. The Limit of Detection (LOD) of BYI 02960 and BYI 02960-difluoroethylaminofuranone was 0.1 µg/kg in green plant material, flower, nectar and pollen, respectively.

All results of the method validation were in accordance with the general requirements for residue analytical methods; therefore, the method was validated successfully.

The residue levels of BYI 02960 and BYI 02960-difluoroethylaminofuranone (BYI02960-DFEAF) in samples of flower and nectar, as obtained in the untreated control group, were always below LOQ.

Results

Details of the trials are presented in table B.9.4.2-17.

Conclusion

The study revealed that a drench application of BYI 02960 SL 200, corresponding to 200 g a.s/ha, to tomato plants at a growth stage typical for early transplanting (BBCH 13-14), resulted in maximum BYI 02960 residue levels in tomato pollen of 107 µg/kg. The corresponding maximum residue level in tomato pollen of the metabolite BYI 02960-DFEAF was determined to be 4 µg/kg. The highest BYI 02960-residues were found during the first week after flower emergence and the residues declined thereafter.

IIA 8.16.2/07

Characteristics

reference	:	Bocksch,S 2012c Report No.: S10-0382	GLP statement	:	yes (certified laboratory); Deviations: none
Type of study	:	Supervised residue trial	Guideline	:	IVA (1992), 7029/VI/95 (EU, 1997), 1607/VI/97 (EU,1999), 1107/2009 (EU, 2009), 544/2011 (EU, 2011a) and 545/2011 (EU, 2011b) OPPTS 860.1520
Year of execution	:	2011	Dose	:	0.150 kg as/ha
test substance	:	BYI 02960 SL 200 formulation Batch no: 2011-001324	LOQ	:	BYI 02960 : 1 µg/kg DFEAF : 1 µg/kg
Species	:	watermelon (*Citrullus lanatus*)	storage	:	≤ 152 d at -18°C
			Acceptability	:	Acceptable
			Results	:	Residues in plants, flowers, nectar and pollen were determined, see below

Study design

A semi-field trial was conducted in Spain to determine the residues of BYI 02960 and the metabolite difluoroethylamino furanone (DFEAF) in plants, flowers, nectar, and pollen collected from watermelon plants treated with flupyradifurone in a 200 g SL formulation.

The target application rate was one drench applications of 200 g as/ha. One control plot was used. Applications were made after transplanting (BBCH 13). The application was performed by using a water rate of 75 mL/plant

Commercial bee colonies were placed in the tunnels, as soon as enough flowers were present to allow foraging of the bees (BBCH 65), 54 days after the application.

Samples of young plants were taken once before the application. More plant samples were taken in regular intervals after the applications. Additionally, flowers as well as nectar and pollen from combs were collected at several dates, starting at the beginning of flowering and continuing during the flowering period of the crop for subsequent residue analysis.

Residue analysis was performed by using High Performance Liquid Chromatography (HPLC), coupled with electrospray and tandem mass spectrometry (MS/MS) detection, according to the provisions of method 01206/M001 (BYI 02960 and its relevant metabolite BYI 02960-difluoroethylaminofuranone). The Limit of Quantitation (LOQ) of BYI 02960 and BYI 02960-difluoroethylaminofuranone, defined as the lowest validated fortification level, was 1 µg/kg in green plant material, flower, nectar and pollen, respectively. The Limit of Detection (LOD) of BYI 02960 and BYI 02960-difluoroethylaminofuranone was 0.1 µg/kg in green plant material, flower, nectar and pollen, respectively.

All results of the method validation were in accordance with the general requirements for residue analytical methods; therefore, the method was validated successfully.

The residue levels of BYI 02960 and BYI 02960-difluoroethylaminofuranone (BYI02960-DFEAF) in samples of green plant material, flower, pollen and nectar, as obtained in the untreated control group, were always below LOQ.

Results

Details of the trials are presented in table B.9.4.2-17.

Conclusion

The study revealed that a drench application of BYI 02960 SL 200 G, corresponding to 200 g a.s/ha, to watermelon plants at a growth stage typical for early transplanting (BBCH 13), resulted in maximum BYI 02960 residue levels of 56 µg/kg in watermelon flowers, 2 µg/kg in watermelon pollen and <1 µg/kg in watermelon nectar. The corresponding maximum residue level of the metabolite BYI 02960-DFEAF was always <1 µg/kg.

Table B.9.4.2-16 Summary of supervised residue trials with blossoms, nectar and pollen of melons under semi-field conditions in USA

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Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (**mg**/kg)			DAA	Remarks
			kg a.s./ha	Water l/ha	kg a.s./hl				BYI 02960	DFEAF	Total		
RARVP019; Mebance, NC, region 3, 2010 IIA 8.16.2/06	Melon, Ambrosia hybrid	Not reported	0.410	375		1 (drench) 16-07-2010	BBCH 15	Blossoms	0.187	0.012	0.198	17	Plot name; T1 Blossoms, : Each result a mean of two analyses *pollen from pollen traps **pollen from grains from the legs ***no sample collected due to the unavailability of bees
									0.105	<0.010	0.107	19	
									0.089	<0.010	0.092	21	
									0.178	<0.010	0.182	23	
									0.211	0.011	0.220	26	
									0.218	0.012	0.226	28	
									0.112	<0.010	0.116	30	
									0.363	0.017	0.380	32	
									0.094	<0.010	0.096	34	
								Pollen	0.109	<0.010	0.112	19	
									0.220	<0.010	0.223	21	
									0.200	<0.010	0.202	23*	
									0.293	<0.010	0.296	23**	
									0.314	<0.010	0.322	28	
									0.129	<0.010	0.132	30	
								Honey stomachs	0.055	<0.010	0.059	19	
									0.071	<0.010	0.075	21	
									0.508	0.017	0.525	23	
									0.695	0.060	0.755	26	
									0.045	<0.010	0.047	28	
									0.013	<0.010	<0.020	30	
									NA	NA	NA	32***	
									0.018	<0.010	0.020	34	
			0.204 0.408	392		2 (foliar) 03-08-2010 12-08-2010	BBCH 69 BBCH 70	Blossoms	<0.010	<0.010	<0.020	-1 DAA1	Plot name; T2 Each result a mean of two analyses
									2.496	0.022	2.518	+1 DAA1	
									0.530	0.014	0.539	+3 DAA1	
									0.229	0.011	0.235	+5 DAA1	
									0.075	<0.010	0.077	-1 DAA2	
									2.778	0.023	2.801	+1 DAA2	
									1.252	0.025	1.277	+3 DAA2	
									0.381	0.013	0.390	+5 DAA2	
									0.048	<0.010	0.050	+7 DAA2	

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Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (**mg**/kg)			DAA	Remarks
			kg a.s./ha	Water l/ha	kg a.s./hl				BYI 02960	DFEAF	Total		
								pollen	1.441 0.626 0.571 0.577	0.014 0.022 0.016 <0.010	1.454 0.644 0.583 0.580	+1 DAA1 +3 DAA1 +5 DAA1 +3 DAA2	
								Honey stomachs	0.209 0.117 0.110 0.339 0.267 0.148 NA 0.028	0.019 0.012 <0.010 0.029 0.029 <0.010 NA <0.010	0.228 0.127 0.119 0.362 0.295 0.151 NA 0.031	+1 DAA1 +3 DAA1 +5 DAA1 -1 DAA2 +1 DAA2 +3 DAA2 +5 DAA2 +7 DAA2	

Table B.9.4.2-17 Summary of supervised residue trials with blossoms, nectar and pollen of tomato and watermelon under semi-field conditions in Southern Europe

Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (**µg**/kg)			DAA	Remarks
			kg a.s./ha	Water l/ha	mL/plant				BYI 02960	DFEAF	Total		
S09-01391; Picassent; 46220; Valencia; Spain 2009	Watermelon; Dulce Maravita	1) 30-04-2009 (transplanting) 2) not reported 3) not reported	0.151 0.153 0.151		75.50 76.34 75.60	3 (drench); 08-05-2009 15-05-2009 22-05-2009	BBCH 15 BBCH 22 BBCH 60	Flowers	1184 267 102 135 80 55	6 2 <1 <1 <1 <1	1190 269 103 136 81 56	13 20 27 34 41 48	T1 and T2: Automatic syringe / dispenser / applicator gun T3: Metric / graduated cylinder LOQ = 1 µg/kg, LOD=
								Pollen	<1 <1	<1 <1	<2 <2	34 41	

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Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (**µg**/kg)			DAA	Remarks
			kg a.s./ha	Water l/ha	mL/plant				BYI 02960	DFEAF	Total		
									<1	<1	<2	48	0.1 µg/kg
								Nectar	<1 <1 <1	<1 <1 <1	<2 <2 <2	34 41 48	Flowers: mean of five replicate analyses
								Green plant material	<1 19614 54313 3200	<1 55 351 17	<2 19669 54664 3217	-8 DAA1 3 DAA1 3 DAA2 3 DAA3	
			0.149 0.148 0.172		74.27 74.01 85.96			Flowers	1026 296 155 149 91 57	5 2 <1 <1 <1 <1	1031 298 157 151 93 59	13 20 27 34 41 48	
								Pollen from combs	<1 2 <1	<1 <1 <1	<2 <2 <2	34 41 48	
								Nectar from combs	<1 <1 <1	<1 <1 <1	<2 <2 <2	34 41 48	
								Green plant material	<1 46920 7015 5207	<1 191 67 33	<2 47111 7082 5240	-8 DAA1 3 DAA1 3 DAA2 3 DAA3	
			0.148 0.152 0.146		73.75 76.02 73.07			Flowers	865 232 80 521 76 56	4 2 2 <1 <1 <1	869 234 82 522 77 57	13 20 27 34 41 48	
								Pollen from combs	<1 6 <1	<1 <1 <1	<2 2 <2	34 41 48	

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Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (**µg**/kg)			DAA	Remarks
			kg a.s./ha	Water l/ha	mL/plant				BYI 02960	DFEAF	Total		
								Nectar from combs	<1 1 <1	<1 <1 <1	<2 <2 <2	34 41 48	
								Green plant material	<1 6530 23756 13335	<1 23 112 80	<2 6553 23868 13415	-8 DAA1 3 DAA1 3 DAA2 3 DAA3	
S10-03818; Alpera; 02690; Albacete; Spain	Tomato; Diamond	1) 25-05-2011 (transplanting) 2) not reported 3) not reported	191.6		319	1 (drench; 26-05-2011	BBCH 13-14	Pollen from forager bumble bees	106 84 46 54 37 32 26 28 21 17	2 2 1 1 1 1 1 1 1 1	108 86 47 55 38 33 27 29 22 18	42 46-47 48-49 50 54-55 57 62-63 68-69 75-76 83-84	Applications with a volumetric cylinder LOQ = 1 µg/kg, LOD= 0.1 µg/kg
								Flowers	315 200 201 145 73 66 61 68 50 70	14 8 10 8 4 3 4 4 3 3	329 208 211 153 77 69 65 72 53 73	42 46 48 50 54 57 62 68 75 83	
			196.5		327			Pollen from forager bumble bees	59 44 46 33 41 29	2 1 1 1 1 1	61 45 47 34 42 30	46-47 48-49 50 54-55 57 62-63	

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Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (**µg**/kg)			DAA	Remarks
			kg a.s./ha	Water l/ha	mL/plant				BYI 02960	DFEAF	Total		
									23	1	24	68-69	
									16	1	17	75-76	
									21	1	22	83-84	
								Flowers	167	8	175	42	
									212	10	222	46	
									95	4	99	48	
									99	5	104	50	
									81	5	86	54	
									64	4	68	57	
									80	4	84	62	
									49	2	51	68	
									28	1	29	75	
									32	2	34	83	
			211.4		352			Pollen from forager bumble bees	64	1	65	42	
									107	4	111	46-47	
									59	2	61	48-49	
									77	2	79	50	
									46	1	47	54-55	
									51	1	52	57	
									34	1	35	62-63	
									29	1	30	68-69	
									29	1	30	75-76	
									24	1	25	83-84	
								Flowers	303	13	316	42	
									281	11	292	46	
									173	9	182	48	
									77	4	81	50	
									103	6	109	54	
									84	5	89	57	
									65	4	69	62	
									54	3	57	68	
									57	3	60	75	
									41	2	43	83	

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Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (**µg**/kg)			DAA	Remarks
			kg a.s./ha	Water l/ha	mL/plant				BYI 02960	DFEAF	Total		
S10-03825; Carlet; 46240; Valencia; Spain 2011	Watermelon; Dulce Maravita	1) 27-04-2011 (transplanting) 2) not reported 3) not reported	238.9		89.6	1; 28-04-2011	BBCH 13	Flowers	18 11 9 10 12	<1 <1 <1 <1 <1	19 12 10 11 13	48 54 61 68 71	T1: Automatic syringe / dispenser / applicator gun T2 and T3: volumetric cylinder LOQ = 1 µg/kg, LOD= 0.1 µg/kg Flowers: mean of three replicate analyses
								Pollen from combs	<1 <1 <1 <1 <1	<1 <1 <1 <1 <1	<2 <2 <2 <2 <2	57 61 64 68 71	
								Nectar from combs	<1 <1 <1 <1 <1	<1 <1 <1 <1 <1	<2 <2 <2 <2 <2	57 61 64 68 71	
								Young plants	<1	<1	<2	-1 DAA	
			199.9		75.0			Flowers	19 14 19 20 21	<1 <1 <1 <1 <1	20 15 20 21 22	48 54 61 68 71	
								Pollen from combs	<1 2 <1 <1 <1	<1 <1 <1 <1 <1	<2 3 <2 <2 <2	57 61 64 68 71	
								Nectar from combs	<1 <1 <1 <1 <1	<1 <1 <1 <1 <1	<2 <2 <2 <2 <2	57 61 64 68 71	
								Young	<1	<1	<2	-1 DAA	

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Report-No. Location incl. Postal code and date	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (**µg**/kg)			DAA	Remarks
			kg a.s./ha	Water l/ha	mL/plant				BYI 02960	DFEAF	Total		
								plants					
			201.3		75.5			Flowers	34	<1	35	48	
									22	<1	23	54	
									15	<1	16	61	
									12	<1	13	68	
									15	<1	16	71	
								Pollen from combs	<1	<1	<2	57	
									<1	<1	<2	61	
									<1	<1	<2	64	
									<1	<1	<2	68	
									<1	<1	<2	71	
								Nectar from combs	<1	<1	<2	57	
									<1	<1	<2	61	
									<1	<1	<2	64	
									<1	<1	<2	68	
									<1	<1	<2	71	
								Young plants	<1	<1	<2	-1 DAA	

DFEAF = Difluoroethyl-amino-furanone
Total residu of BYI 02960 + DFEAF
-1 DAA1: samples taken prior to application 1, +1 DAA1: samples taken after to application 1
-1 DAA2: samples taken prior to application 2, +1 DAA2: samples taken after to application 2

B.9.4.3 Field studies (IIIA 10.4.7)

Study KIIIA 10.4.5/01

Abbreviation used:

DAS = days after set-up of hives (mind: not after sowing!)

Characteristics

Reference/notifier	:	Rexer 2012a	GLP statement	:	yes
Type of study	:	Honeybee field test, seed treatment plus soil and foliage treatment	Guideline	:	OEPP/EPPO guideline No. 170 (4), 2010 with modifications recommendations of the IVA Guideline (Beutel *et al.*, 1992).
Year of execution	:	2010	Acceptability	:	Acceptable
Test substance	:	BYI 02960 FS 480 G, Batch no. 2010-005474 and BYI 02960 SL 200 G, Batch no. 2010-007173			

Substance	***Species***	***Method***	***Duration [d]***	***Application rate***	***application rate [kg seed/ha]***	***Parameter***	***Adverse effect***
BYI 02960 FS 480 G [1] and BYI 02960 SL 200 G[1]	*Apis mellifera*	Seed drilling winter OSR, 1 soil application, 2 foliage applications	Exposure 24 days Total duration 346 days from hive set-up	9.9 g flupyradifurone/ kg seed (analysed) 300 g as/ha (soil appl), 205 and 205 g as/ha (2 foliage applications)	5.98	Mortality	N
						Flight intensity	N
						Brood development	N
						Food area storage	N
						Behaviour	N

1: active substance flupyradifurone

Description

The long-term effects of exposure of honeybees (*Apis mellifera* L.) to oil-seed rape, grown from seeds treated with BYI 02960 FS 480 G, and sprayed three times with BYI 02960 SL 200 G (1x bare soil before sowing and 2 x foliage spray) was tested under field conditions.

Location

Field experiment was conducted on a test field which was located in the north of Germany near the village Celle. No flowering crops were present in the surrounding area (2 km radius) of the test item field. Winter OSR was present at a distance of 1.7 and 0.9 km from the control fields.

Sizes field and crop, test condition (tunnel or field)

The overall size of the test field was 4.18 ha for the test item treatment plot and 5.08 ha for the control plot. Fields were separated by 7.0 km. The crop was oil-seed rape (OSR). Previous crops were potato

and grassland in the test item field, and maize and winter wheat in the control field. Several herbicides, fungicides and insecticides (no neonicotinoid insecticides) were used in both control and test item field in the previous crops.

Test substance

BYI 02960 FS 480G (480 g flupyradifurone/L nominal, 474.7 g/L actual, density 1.191 g/mL)
BYI 02960 SL 200 G (200 g flupyradifurone /L nominal, 198.6 g/L actual, 16.9% w/w, density 1.175 g/mL)

Treatments

Two treatment groups:

1) A seed treatment product BYI 02960 FS 480G and additional treatments with product BYI 02960 SL 200 G: one treatment on the bare soil (target rate 300 g as/ha, immediately incorporated into the upper 20 cm of the soil just before sowing) and two foliar applications (at a target rate of 205 g as/ha during immediate pre-/early flowering and full flowering) and
2) a control.

Application scheme is given in the table below.

Table 9.4.3-1 Treatments and number of colonies per field (1st spray September 7, 2010, 2nd spray at April 26, 2011 and 3rd spray at May 2, 2011)

Treatment Group	Sowing of winter OSR-seeds	Time of spray application	Application rate [g a.s./ha]	Application rate [L product/ ha]	Spray Volume	No. of colonies per field plot
Control (C)	OSR seeds, variety "Titan", only fungicide treated, September 2010, 6 kg seeds/ha (target)	-	-	-	-	6 + 2
BYI 02960 (T)	OSR seeds, variety "Titan", fungicide and BYI 02960 FS 480 G treated (10 g BYI 02960 a.s./kg OSR seeds, nominal), September 2010, 6 kg seeds/ha (target)	1st spray application at the day of sowing, before seed sowing, September 2010, (BBCH 00)	300 1)	1.500 1) BYI 02960 SL 200 G	200 L/ha	6 + 2
		2nd spray application at BBCH 59-60*, spring 2011	205 2)	1.032 2) BYI 02960 SL 200 G	200 L/ha	
		3rd spray application at BBCH 64-65*, spring 2011	205 2)	1.032 2) BYI 02960 SL 200 G	200 L/ha	

* Majority of plants
1) Referring to nominal content of a.s. (200 g/L)
2) Referring analysed content of a.s. (198.6 g/L)

Test concentrations

OSR seeds, dressed with the seed treatment BYI 02960 FS 480G, were drilled in on September 7, 2010, at a target rate of 6 kg OSR seeds/ha. Analysed seed treatment rate of 9.9 g flupyradifurone/kg seeds and a 1000 grain-weight of 8.6 g. The treated field plot was matched with a control field plot drilled at the same time with untreated OSR seeds. Three applications with BYI 02960 SL 200 G as specified in Table 1 (the first on the bare soil (September 7, 2010), second and third on the foliage (April 26 and May 2).

Species tested

Honey bees of the race *Apis mellifera*.

Population size

For the test, healthy colonies were used in hives with one brood box and one honey box including 11 combs each. The colonies contained approx. 6,000 to 12,000 bees. Just before set-up, control

colonies contained on average 8562 bees (6190-11028, n=6) and treatment colonies contained 9387 bees (8273-10184, n=6).

At the end of the exposure phase the colonies were split to reduce the size of the colonies (May 30, 2011 at DAS40). This is a common and necessary manipulation in order to restrict the urge of swarming. Only the original hives were used for assessments.

Timing placement hives under test conditions

At each field (control and treatment) eight bee colonies were placed in the plots before the second spray application of BYI 02960 SL 200 G at BBCH 59-60. They remained at the exposure location until the end of flowering (= exposure phase). Afterwards, the test colonies were relocated to a remote site, without extensive agricultural crops attractive to bees (= monitoring phase).

Parameters observed and timing thereof

The following parameters were observed during the exposure phase:

- Adult bee mortality
  - Number of dead bees in dead bee trap, 5 times in the pre-exposure period.
  - Number of dead bees in dead bee trap, linen sheets in front of the hives and linen sheets during exposure
- Flight intensity (mean number of bees/1 m^2/10-15 sec) measured in five marked areas, three times a day.
- Behaviour of the bees in the crop and around the hive was measured at least once a day for 1 minute, during flight intensity assessments.
- Condition of the colonies and development of the bee brood (number of bees (strength), mean abundances of the different brood stages per colony and assessment date, measured 0-2 days before moving the bees to the exposure site, no assessments during exposure phase, then assessments every three weeks at the monitoring site (first assessments within 2 days after set-up)
- Bee diseases and viruses, including *Varroa destructor* and *Nosema* sp., were checked at start and end of exposure phase and at start and end of overwintering phase.
- Residues. Soil samples were taken once directly after application to bare soil but before sowing. During exposure period continuous collection of OSR flowers and forager bees. Two of the eight hives per field were assigned for monitoring residues. Pollen, nectar and bee wax were collected once at the end of the exposure phase. Pollen and nectar were collected during the monitoring phase. Method for residue analysis:
  - Soil: analytical method 01074/M001 for BYI 02960 and its metabolite metabolites 6-chloronicotinic acid and difluoroacetic acid.
  - Bee wax: Modification M002 of the analytical method 01206 for the determination of residues of BYI 02960 and BYI 02960-Difluoroethylaminofuranone (=BYI 02960-DFEAF) by HPLC with electrospray and MS/MS-detection

- Pollen, nectar and flowers: Modification M001 of the analytical method 01206 for the determination of residues of BYI 02960 and BYI 02960-DFEAF by HPLC with electrospray and MS/MS-detection.

- Pollen source identification: Twice during the exposure phase at the test fields, pollen was collected from pollen traps. First collection of pollen between onset of flowering and full flowering. Second collection between full flowering and re-location to monitoring site.

Virus analysis

Bees were analysed for the bee viruses deformed wing virus (DWV), sacbrood virus (SBV), acute bee paralysis virus (ABPV), chronic bee paralysis virus (CBPV), Kashmir bee virus (KBV), Israel acute paralysis virus (IAPV) and black queen cell virus (BQCV) at start of exposure phase, end of exposure phase, and start and end of overwintering. Ten bees per hive were analysed.

Bee diseases

The presence of different pathogens (*Nosema* sp., *Malphigamoeba mellificae*, *Varroa destructor*, *Paenibacillus* larvae) in bee samples was determined at different time points during the observation period.

Statistical evaluation

Not performed.

Mortality quotients were calculated as the quotient of mortality in a particular period relative to mortality in the pre-treatment period. For the calculation mean mortality/colony/day is used. If Qm <1 then mortality after treatment is lower than before treatment.

Results

Actually applied concentrations

Amounts of seeds actually drilled deviated from anticipated by -0.4 and 0% in the test item and control. Actually applied spray applications were concentrations were 310.38 g as/ha in the first spray (deviation of 4.19%) and 204.05 g as/ha in the second spray (deviation 0.24%). The third spray contained 205.03 g as/ha (deviation 0.72%).

Climatic conditions

Temperatures, humidity and precipitation were recorded at the nearest official weather station at Celle, 15 and 13 km away from the exposure sites. No abnormal weather data were obtained.

Residue analysis

Method validation:

Mean recovery of BYI 02960 in flowers, pollen, nectar, beewax and soil were 91, 88, 97, 77 and 107% with corresponding relative standard deviations of 11.3, 7.2, 5.0, 10.0 and 1.9%.

Mean recovery of BYI 02960-DFEAF in flowers, pollen, nectar, and beewax were 89, 88, 93, 85% with corresponding relative standard deviations of 3.5, 5.5, 4.0 and 8.9%. For both parent and metabolite LOQ was 1 µg/kg and LOD was 0.1 µg/kg for flower, pollen and nectar. LOQ was 1 µg/kg and LOD was 0.3 µg/kg in beewax. For soil LOQ was 5 µg/kg and LOD was 1.5 µg/kg.

.

In the control, no residues were found in the flowers. No residues of parent nor metabolite was detected in pollen from combs, pollen from forager bees, nectar from combs, nectar from forager bees, beewax. All residues were <LOD. Residues in soil were not analysed in the control field. In the table below residues in the treated field are given.

Table 9.4.3-2 Residues of BYI02960 and BYI02960-difluoroethylaminofuranone (BYI02960-DFEAF) in samples of flowers, pollen, nectar, bee wax and soil, as obtained from the test item treatment group

Sample	Test Item	Subsample	Residue [µg/kg]	
			BYI 02960	BYI 02960-DFEAF
Flower	BYI 02960 FS 480 G + BYI 02960 SL 200 G	A	1917 - 30379	9 - 80
Pollen (combs)		A	305 - 3905	3 - 12
Pollen (bee bread)		A	4 - 1958	< LOD - 7
Pollen (forager bees)		A	246 - 14259	2 - 26
Nectar (combs)		A	428 - 1347	5 - 13
Nectar/ Honey		A	45 - 1409	< LOD - 30
Nectar (forager bees)		A	269 - 4108	< LOQ - 38
Beewax (combs)		A	6 - 148	< LOD - 2
Beewax		A	5 - 17	< LOD
Soil		A	75	-

BYI 02960-DFEAF = BYI 02960-difluoroethylaminofuranone (BCS-CC98193)
LOQ = Limit of Quantitation = 1 µg/kg in flower, nectar, pollen and beewax, respectively (BYI 02960 and BYI 02960-DFEAF); 5 µg/kg in soil (BYI 02960)
LOD = Limit of Detetction = 0.1 µg/kg in flower, nectar, pollen as well as 0.3 µg/kg in beewax, respectively (BYI 02960 and BYI 02960-DFEAF); 1.5 µg/kg in soil (BYI 02960)

Pollen source identification

In the control field **the percentage of OSR pollen was <1-31% on 10 days after set-up (DAS10) and 5-28% on DAS16 in colonies C1-C6. In colonies C7-C8 (for residues) percentages were 19 and 30% and 31 and 70% respectively.** ***Cotoneaster*** **was almost always the preferred pollen source.** ***Pyrus*** **spp. was sometimes more preferred, sometimes less preferred.** ***Acer*** **was the least preferred.**

In the test item treated field **the percentage of OSR pollen was 18-50% on DAS10 and 32-38% on DAS16 in colonies C1-C6. In colonies C7-C8 (for residues) percentages were 25-52% and 69-70% respectively.** ***Cotoneaster*** **was almost always the preferred pollen source.** ***Acer*** **and** ***Pyrus*** **spp. the least preferred. In the figure below the data on pollen sources are given.**

Figure 9.4.3-1 Pollen source identification in treatment (T) and control group (C) at DAS 10 (upper figure) and DAS 16 (lower figure). Colonies 7 and 8 were assigned to the residue analyses. Figure prepared by evaluator using data from tables 49-52.

Bee viruses

SBV, ABPV, CBPV, KBV and IAPV were not detected in any of the samples. DWV, BQCV and SBV were detected in some of the control hives, while the test item colonies did not contain any of the analysed bee viruses except BQCV in two colonies. Author concluded that no test item-related adverse effects on colony health in terms of virus infestation were observed.

Bee diseases

The results of the disease analysis revealed that the test item treated colonies were as healthy as the control colonies and as such, no bee disease-related adverse effects on colony health were observed.

Biological observations

Mortality

Results on mortality are reported in the following figures (copied from the report).

Results were based on dead bee traps in period -5 to -1DAS, and based on both dead bee traps and linen sheets in the period DAS0-24. Applications were performed at DAS6 and DAS12.

Figure 9.4.3-2 Mean number of dead bees per colony observed at the monitoring site before set-up at the field site (DAS-5 to DAS-1) and after set-up at the field sites before the 2nd spray application (DAS1 to DAS6).

Figure 9.4.3-3 Mean number of dead bees per colony observed at the field sites after the 2nd and before the 3rd spray application (DAS7 to DAS12).

Figure 9.4.3-4 Mean number of dead bees per colony observed at the field sites after the 3rd spray application until relocation of the colonies to the monitoring site (DAS13 to DAS24).

The mortality quotients during the entire exposure period were calculated to be 1.3 in C and 0.8 T, respectively. For the separate period these quotients were for the first period 1.5 in C and 0.5 in T, respectively, for the second period 1.5 in C and 0.8 in T, respectively and for the last period 1.2 in C and 0.9 in T, respectively. Only at DAS 13-15 (*i.e.* the three days directly following the second foliar

application) an elevation of mortality in the test item treatment was visible compared to the control. The author concluded that no test item-related adverse effects on mortality were observed.

Flight intensity

The mean flight activity was low in the period until DAS6 as flowers were in pre-flowering state. In the period from DAS6 to DAS12 just before 2nd foliarapplication, mean flight intensity amounted to 0.4 bees/m2/day in C compared to 0.9 bees/m2/day in T. After the 2nd foliar application (DAS12) mean flight intensity over the DAS12 to DAS 23 amounted to 1.0 bees/m2/day in C compared to 1.0 bees/m2/day in T. Total daily flight intensity was 0.6 bees/m2/day in C compared to 0.8 bees/m2/day in T. Flight intensity was very low in both C and T on the day of the 2nd foliar application. A clearly lower flight activity was observed at DAS13 and DAS14 after the 2nd foliar application. According to author, overall, no test item-related adverse effects on flight intensity were observed.

Figure 9.4.3-5 Mean number of forager bees per m2 assessed at the field sites after set-up of the colonies until shortly before the 3rd spray application (DAS0 to DAS12ba).

STD: standard deviation; ba: before application, aa: after application

Figure 9.4.3-6 Mean number of forager bees per m2 assessed at the field sites after the 3rd spray application (DAS12aa to DAS23).

STD: standard deviation; aa: after application

Behaviour

At the entrance of the treatment hives several test item-related adverse effects were observed such as clustering at the hive entrance (DAS6, DAS14), intensive cleaning behaviour (DAS10, DAS13 and DAS16), locomotion problems (DAS12, DAS13). No effect on behaviour was observed in the field. Anomalies were most visible on the day after 2nd foliar spray. The observations after the 2nd foliar spray application in T and the corresponding absence of this behaviour in the control indicate a possible correlation between the application of the test item during full flowering of the crop and during bee flight and the occurrence of intensive cleaning behaviour (10-30/hive) and locomotion problems (10-20/hive) in a small fraction of honeybees in the test item treatment group. According to author, these observed differences to the control were not adverse.

In the field, one single bee was noticed with behavioural problems at DAS12 (intensive cleaning behaviour) and one at DAS13 (suspended on plant), and two bees at DAS14 (intensive cleaning behaviour). No abnormal behaviour in the control was observed apart from one honeybee suspended on plant on DAS10.

Survey of flowering crops

In the surrounding area of the test item treatment field, no flowering crops were present within a 2 km radius during exposure of bees. Winter OSR was present at a distance of 0.9 and 1.7 km from the control field. According to pollen analysis wild trees and shrubs were present in the near surroundings of both treated as control field.

Abundance, brood development and food storage

Numbers of bees, brood development and food storage area are given the two figures below.

Assessments were made two days before set-up (April 19), after the end of the exposure period (May 16) and 8 observations in the monitoring period. Six hives situated in the control field and six hives in the treated field.

Numbers of bees per colony in control and treatment showed the same seasonal tendency in increases and decreases until the end of overwintering in March 2012. No test item-related differences in colony strength were noticed. It should be noticed that the decrease observed at June 6 is caused by splitting the colonies at May 30 into two colonies to reduce the natural urge of the colonies to swarm. Some brood and store combs were removed from the original colony and placed into a new hive (= nucleus). The empty spaces in both colonies (original and new) were filled with empty combs. The old queen was left in the original colony; the bees in the new colony were encouraged to rear a new healthy, egg-laying queen. The new colonies served as a back-up colony but were not further used for data sampling in this experiment.

Figure 9.4.3-7 Mean number of bees per colony in the control and test item treatment colonies.
STD: standard deviation

The author prepared figures for brood development and food storage area for each hive separately. The mean % comb coverage in the colonies in the control and the test item treatment group showed the same development, i.e. the same increase and decrease of brood cells (eggs, larvae and pupae) and food stores (nectar and pollen). No test item related adverse effects on brood development were observed.

Figure 9.4.3-8 Brood and food development of the colonies in the treatment and control group

Figure 9.4.3-9 Mean weight per colony of the control and test item treatment colonies.

The weight development of the colonies with its increases and decreases, measured from April 14 (DAS-6) to October 2011 (before overwintering) was very similar between the test item treatment and the control group.
Sudden weight changes to a larger extent in both exposure groups were observed at
14 May 2011: removal of hive material during relocation of the colonies to the monitoring site
30 May 2011, 27 July 2011: the removal of honey from the colonies
30 May 2011: bee colony splitting.
According to author, no test item related adverse effects on colony weight development were observed.

Conclusions of the author

The author concluded that exposure of honeybee colonies to flowering OSR, grown from seeds, seed-treated with flupyradifurone, one soil treatment and two foliage treatments with flupyradifurone (target rate of 300 g as/ha in the soil and a target rate of 205 g as/ha during immediate pre-/early flowering and full flowering), do neither cause acute, short-term nor long-term effects in exposed colonies, including colony health, colony vitality and overwintering performance. A possible correlation between the application of the test item during full flowering of the crop and during bee flight (3rd spray application) in terms of intensive cleaning behaviour and locomotion problems was observed in a small fraction of bees in the test item treatment group.

Remarks by evaluator

Splitting of the colonies occurred at May 30. This was done to reduce the urge of the colony to swarm. This urge to swarm is a normal phenomenon that occurs in healthy colonies by this period of the year. The author mentions that the colonies are reduced by removing some brood and store combs. It is not documented how many combs were removed. The methods bee keepers employ to split colonies depend on the region/country and may vary. In the Netherlands it is normal practice to take one comb with food (in which brood is not present). The box is further filled with artificial combs (template on which the bees can build new cells). The new colonies are further started with the introduction of a new queen and a certain number of bees (around 1000). A comb with brood is not introduced in order to prevent further Varroa infection. Sometimes a comb with open male brood is introduced. Any Varroa mites present on the bees will eagerly move into the male brood as the male larvae appear to be most attractive. Care must be taken to destroy the male brood before the male bees emerge.
The food combs that are removed from the hive contain not only nectar and pollen with residues of flupyradifurone (collected during exposure period until splitting of the colony), but also contain nectar and pollen collected before exposure. A logical question would be whether a particular comb would be taken out that could have a much lower or higher concentration of residues. From the study report it is not possible to reconstruct whether older or newly filled food combs were chosen. This also depends on the bee keepers methods. However, since only one or at the most a few combs are removed, the effect will be minimal, if present at all. Assumedly, the same type of food combs (fully or half filled) will have been chosen, so possible effects will be the same for all hives.

This necessary manipulation caused a drop in the bee numbers. Data of June 6 show a drop of bees that was more pronounce in the treatment than in the control. The growth of the colonies after this date was more or less similar (Figures 7 and 8). It can be concluded that the manipulation was necessary. Development of the colony is best evaluated starting from June 6.

No statistics were applied to data on mortality and flight intensity. However, as SD values of test item treatments and control were overlapping, significant differences are very unlikely. Concerning flight activity, less activity in the test item treatment is observed at DAS13-14, immediately after the 3rd application at DAS12. Thereafter higher flight intensity was observed in the test item treatments. This might be caused be a repellent effect of the product. This effect coincides with a higher mortality at DAS 13-15 and some behavioural problems at DAS13, 14 and 16. At other days mortality in the test item treatment was always lower than in the control in the period DAS0-24.

Figure 8 on mean weights of the colonies show that during the exposure period (2-14 May) the control colonies show an increase in weight that is stronger than in the parallel treated colonies. This difference might be caused by the treatments with flupyradifurone. However, this is difficult to evaluate as the control and treated fields were separated from each other. Therefore, foraging bees may have collected different quantities of nectar and pollen sources, depending on the availability of flowering plants. The pollen analyses showed that bees not only forage on the OSR crop but also forage on other crops/wild plants, preferably Cotoneaster. As bees in the treated colonies also foraged on other crops the exposure to flupyradifurone was less than 100%.

Colonies in the control field had two sources of OSR within 2 km distance. There is a high chance that these crops have been treated with neonicotinoids or other insecticides. Pesticide use in these crops has not been verified by the authors. Also, pollen source analysis revealed that a substantial part of the pollen had been derived from other plants such as Cotoneaster, Acer and Pyrus. Cotoneaster and Acer are probably plants/trees occurring in the wild that have not been treated with pesticides. Pyrus, however, may have been treated with several insecticides. This crop was however, less preferred. Thus, it is uncertain whether the control colonies were not contaminated by other insecticides/fungicides/herbicides. The residue analyses however showed that residues of flupyradifurone and its metabolite were not found in the flowers, pollen, nectar and beewax in the control. Given the agricultural landscape in Germany, there is not a better set-up of a field study possible. Higher exposure to flupyradifurone in the treated field is difficult to realize and exposure to pesticides in general in the control cannot be avoided.

In conclusion, no clear effects on mortality, flight intensity, brood and food storage area, and colony development were detected. Some possible short-term effects on mortality, flight intensity, behaviour and colony weight might have occurred after the 3rd application. There was however, a clear recovery from these effects. No effects on the capacity of the colony to overwinter are expected.

Expert discussion in Pesticide Peer Review meeting 124 (December 2014):
(Please note that the word 'effect' in the text below might be interpreted as different from the control (i.e. may or may not include effects from the treatment), pending on the context.)

The treatment regime was treated seed, soil spray and two foliar applications to the same crop. This study may be useful for the assessment for the risk to bees from residues in succeeding crops.

The evaluation of the RMS was agreed. Some potential effects were noted including some sublethal effects. Overall there was a suggestion that the observed effects do indicate some test item related effect. The RMS reflected that the treatment hives showed a slower increase in weight compared with the control colonies. The RMS stated that the reason for this was unknown i.e. it could be due to difference in hive location. The RMS noted that this data was difficult to interpret and it was questionable as to whether this was due to natural variation or was treatment related.

The study included an assessment of overwintering success. The final assessment was made in March the following year. There appeared to be no effects on the over wintering success.

A concern was raised during the commenting of the low flight intensity observed. The RMS noted they have discussed foraging intensity with experts and they consider that the criteria in the EPPO (2 - 3 bees per m^2 for OSR) is very difficult to achieve.

It was noted that the study included a statement to indicate that there were no major flowering crops within 2 km of the field. The new EFSA Guidance recommends that a survey is performed and to minimise alternative foraging opportunities (i.e. also for weeds, trees, bushes). The RMS considered that the conditions of this study were reasonable and realistic. It was acknowledged that the requirements of the EFSA GD are difficult to adhere to but is there to maximise 'worst-case conditions'. The question was raised as to whether multiple studies address the concern of replicating 'worst-case conditions'. Multiple studies increases the probability that 'worst-case conditions' are achieved but it would still need to be demonstrated that this has been achieved.

Overall, the experts in the meeting agreed with the RMS's conclusions on this study.

Study KIIIA 10.4.5/02

Characteristics

Abbreviation used:

DAS = days after set-up of hives (mind: not after sowing!)

Reference	:	Rexer 2012b	GLP statement	:	yes
Type of study	:	Honeybee field test, seed treatment plus soil	Guideline	:	OEPP/EPPO Guideline No. 170 (4) (2010) with

		and foliage			modifications, the EU Directive 91/414/EEC (1997), and, in part, the recommendations of the IVA Guideline (Beutel *et al.*, 1992)
Year of execution	:	2010/2011	Acceptability	:	Acceptable
Test substance	:	BYI 02960 FS 480 G, Batch no. 2010-001457 BYI 02960 SL 200 G, Batch no. 2010-004097			

Substance	***Species***	***Method***	***Duration [d]***	***Application rate***	***application rate [kg seed/ha]***	***Parameter***	***Adverse effect***
BYI 02960 FS 480 G [1] and BYI 02960 SL 200 G[1]	*Apis mellifera*	1 soil application incorporated, drilling pre-treated oil-seed rape, 2 foliage applications	Exposure 30 days Total duration 351 days from hive set-up	10.1 g flupyradifurone/ kg seed; 300 g as/ha (soil appl), 205 and 205 g as/ha (2 foliage applications)	6.89	Mortality	N
						Flight intensity	N
						Brood development	N
						Food area storage	N
						Behaviour	N

1: active substance flupyradifurone

Description

The long-term effects of exposure of honeybees (*Apis mellifera* L.) to oil-seed rape, grown from seeds treated with BYI 02960 FS 480 G, and sprayed three times with BYI 02960 SL 200 G (1x bare soil before sowing and 2 x foliage spray) was tested under field conditions.

Location

Field experiment was conducted on a test field which was located in Alsace, France.

Sizes field and crop, test condition (tunnel or field)

The size of the field plots was approximately 4.54 ha (treated field plot) and approximately 4.11 ha (control field plot), respectively. The distance between the plots was approximately 8.0 km. The crop was oil-seed rape (OSR). Previous crops were maize and winter wheat in the test item field, and mustard and winter wheat in the control field. Several herbicides, fungicides, insecticides (no neonicotinoid insecticides) and a molluscicide were used in both control and test item field in the previous crops.

Active substance

BYI 02960 FS 480G (480 g flupyradifurone/L nominal, 474.7 g/L actual, density 1.191 g/mL)
BYI 02960 SL 200 G (200 g flupyradifurone /L nominal, 198.6 g/L actual, 16.9% w/w, density 1.175 g/mL)

Treatments

Two treatment groups:

1) A seed treatment product BYI 02960 FS 480G and additional treatments with product BYI 02960 SL 200 G: one treatment on the bare soil (target rate 300 g as/ha, immediately incorporated into the upper 20 cm of the soil just before sowing, based on nominal content of 200 g as/L) and two foliar applications (at a target rate of 205 g as/ha during immediate pre-/early flowering and full flowering, based on analysed content of 198.6 g as/L) and

2) a control.

Application scheme is given in the table below.

Table 9.4.3-2 Treatments and number of colonies per field (1st spray September 14, 2010, 2nd spray at April 9, 2011 and 3rd spray at April 18, 2011)

Treatment Group	Sowing of winter OSR-seeds	Time of spray application	Application rate [g a.s./ ha]	Application rate [L product/ ha]	Spray Volume	No. of colonies per field plot
Control (C)	OSR-seeds, variety "PR45D03", only fungicide treated, September 2010, 6 kg seeds/ha (target)	-	-	-	-	6 + 2
BYI 02960 (T)	OSR-seeds, variety "PR45D03", fungicide and BYI 02960 FS 480 G treated (10 g BYI 02960 a.s./kg OSR seeds, nominal), September 2010, 6 kg seeds/ha (target)	1st spray application at the day of sowing, before seed sowing, September 2010, (BBCH 00)	300 1)	1.500 1) BYI 02960 SL 200 G	200 L/ha	6 + 2
		2nd spray application at BBCH 59-60*, spring 2011	205 2)	1.032 2) BYI 02960 SL 200 G	200 L/ha	
		3rd spray application at BBCH 64-65, spring 2011	205 2)	1.032 2) BYI 02960 SL 200 G	200 L/ha	

* Majority of plants
1) Referring to nominal content of a.s. (200 g/L)
2) Referring analysed content of a.s. (198.6 g/L)

Test concentrations

OSR seeds, dressed with the seed treatment BYI 02960 FS 480G, were drilled in on September 14, 2010, at a rate of 6.89 kg OSR seeds/ha (target rate: 6 kg seeds/ha). Analysed seed treatment rate of 10.1 g flupyradifurone/kg seeds and a 1000 grain-weight of 9.0 g. The treated field plot was matched with a control field plot drilled at the same time with untreated OSR seeds. Three applications with BYI 02960 SL 200 G as specified in Table 1 (the first on the bare soil, on September 14, 2010, second and third on the foliage on April 9 and April 18, 2011).

Species tested

Honey bees of the race *Apis mellifera*.

Population size

For the test, healthy colonies were used with young bees (1 queen and approx. 10,000 to 20,000 bees per colony) in hives with one brood body and one honey body including 10 combs each. Just before set-up (DAS-1), control colonies contained on average 12063 bees (6000-16625, n=6) and treatment colonies 12917 bees (7875-17875, n=6).

At the end of the exposure phase the colonies were split to reduce the size of the colonies (June 01, 2011 at DAS54). This is a common and necessary manipulation in order to restrict the urge of swarming.

Timing placement hives under test conditions

At each field (control and treatment) eight bee colonies were placed in the plots one day before the second spray application of BYI 02960 SL 200 G at BBCH 55-61. They remained at the exposure location until the end of flowering (= exposure phase). Afterwards, the test colonies were relocated to a shared monitoring site (at least BBCH 69), without extensive agricultural crops attractive to bees.

Parameters observed and timing thereof

In order to determine the pre-exposure level of mortality, the number of dead bees in the dead bee traps was counted over a period of 4 days before the start of exposure (i.e. first day of contact of the honeybees with the crop) at the monitoring site.

The following parameters were observed during the exposure phase:

- Adult bee mortality
  - Number of dead bees in dead bee trap, over a period of 4 days in the pre-exposure period.
  - Number of dead bees in dead bee trap, linen sheets in front of the hives and linen sheets during exposure
- Flight intensity (mean number of bees/1 m^2/10-15 sec) measured in five marked areas, three times a day.
- Behavioural observations of the bees were carried out in the flight assessment areas once a day during 30 sec after one of the flight intensity assessments when bees were observed.
- Behavioural observations of the bees returning to the entrance of the hives was observed (over approximately 1 minute) once a day during flight activity in front of the hives and on the same days as the mortality and flight intensity assessments (in both, treatment and control).

- On DAS-1, just before relocation of the colonies from the monitoring site to the exposure site, the assessment of colony health, strength and brood development was conducted for each of the colonies (including the hives that were used as residue monitoring colonies). On DAS33, the bee colonies were transported to the monitoring site where the colony health and strength as well as the brood development was assessed on DAS35.
  Thereafter and for the entire duration of the study, a time interval of approximately 3 weeks was followed for further colony assessments. Until the end of the swarming season (beginning of July), five additional, less invasive colony beekeeper checks were performed between the three-weekly colony assessments on DAS21, DAS41 (only T1-T6 and C1-C6), DAS61, DAS69 and DAS90. No assessments were carried out during overwintering (between 06 October 2011 and 21 March 2012) and during the exposure phase (except for measures to prevent swarming of the colonies). At each colony assessment, the comb area containing bees and cells with nectar, pollen, eggs, larvae and capped cells were estimated per comb side and the total number of bees and cells containing the brood stages, pollen and nectar per hive were calculated.
- The colony health and strength as well as the brood development were assessed as follows (= invasive colony assessments):
  - Strength of the colony (estimation adapted to IMDORF & GERIG, 1999, and IMDORF et al., 1987)
  - Presence of a healthy queen; verified by the presence of freshly laid (one day old) eggs
  - Estimate of the pollen storage area and area with nectar stores (Estimation adapted to Imdorf & Gerig, 1999, and Imdorf et al., 1987)
  - Estimate of the area containing egg, larval and capped cells (Estimation adapted to IMDORF & GERIG, 1999, and IMDORF et al., 1987)
  - Weight of the colony
  - Colonies were assessed for bee diseases according to standard beekeeping practice
- Residues. Soil samples were taken once directly after the application to bare soil just before sowing. During exposure period continuous collection of OSR flowers and forager bees. Two of the eight colonies per field were assigned for monitoring residues. Pollen, nectar and bee wax were collected once at the end of the exposure phase. Pollen and nectar from the residue monitoring colonies were collected during the exposure phase and after relocation to the monitoring site.
  Method for residue analysis:
  - Soil: analytical method 01074/M001 for BYI 02960 and its metabolite metabolites 6-chloronicotinic acid and difluoroacetic acid, by HPLC-MS/MS
  - Bee wax: Modification M002 of the analytical method 01206 for the determination of residues of BYI 02960 and BYI 02960-Difluoroethylaminofuranone (=BYI 02960-DFEAF) by HPLC with electrospray and MS/MS-detection

- Pollen, nectar and flowers: Modification M001 of the analytical method 01206 for the determination of residues of BYI 02960 and BYI 02960-DFEAF by HPLC with electrospray and MS/MS-detection.

- Pollen source identification: Twice during the exposure phase at the test fields, pollen was collected from pollen traps (DAS3, DAS12). First collection of pollen between onset of flowering and full flowering. Second collection between full flowering and re-location to monitoring site.

Virus analysis

Bees were analysed for the bee viruses deformed wing virus (DWV), sacbrood virus (SBV), acute bee paralysis virus (ABPV), chronic bee paralysis virus (CBPV), Kashmir bee virus (KBV), Israel acute paralysis virus (IAPV) and black queen cell virus (BQCV) at start of exposure phase, end of exposure phase, and start and end of overwintering. At least ten bees per hive were analysed.

Bee diseases

The presence of different pathogens (*Nosema* sp., *Malphigamoeba mellificae*, *Varroa destructor*, *Paenibacillus* larvae) in bee samples was determined at different time points during the observation period.

Statistical evaluation

Not performed.

Mortality quotients (QM) were calculated as the quotient of mortality in a particular period relative to mortality in the pre-treatment period. For the calculation mean mortality/colony/day is used. If Qm <1 then mortality after treatment is lower than before treatment

Results

Actual applied concentrations

Amounts of seeds actually drilled deviated from anticipated by 14.8 and 7.5% in the test item treatment and control. Actually applied spray applications were 310.5 g as/ha in the first spray (deviation of 3.5%) and 208 g as/ha in the second spray (deviation 1.5%). The third spray contained 212 mg as/ha (deviation 3.3%).

Climatic conditions

Temperatures, humidity and precipitation were recorded at the nearest weather station in Sélestat (approximately 17 km away from the test item treatment field). No abnormal weather data were obtained.

Residue analysis

Method validation:

Mean recoveries of BYI 02960 in flowers, pollen, nectar, beewax and soil were 97, 97, 99, 83 and 107%, respectively, with corresponding relative standard deviations of 5.5, 5.9, 8.6,14.1 and 1.9%.

Mean recoveries of BYI 02960-DFEAF in flowers, pollen, nectar, and beewax were 91, 95, 100 and 82%, respectively, with corresponding relative standard deviations of 3.3, 5.5, 6.1 and 9.9%.

For both parent and metabolite LOQ was 1 µg/kg and LOD was 0.1 µg/kg for flower, pollen and nectar. LOD for beewax was 0.3 µg/kg. For soil LOQ was 5 µg/kg and LOD 1.5 µg/kg.
In the control, BYI02960 or BYI02960-DFEAF were found above the LOQ in one single sample out of 121 analysed samples in total; 6 µg/kg in pollen from combs, hive residue monitoring . No residues of parent nor metabolite > LOQ were detected in pollen from forager bees, nectar from combs, nectar from forager bees, beewax. Residues in soil were not analysed in the control field. In the table below residues in the treated field are reported.

Table 9.4.3-3 Residues of BYI02960 and BYI02960-difluoroethylaminofuranone (BYI02960-DFEAF) in samples of flowers, pollen, nectar, bee wax and soil, as obtained from the test item treatment group

Sample	Test Item	Subsample	Residue [µg/kg]	
			BYI 02960	BYI 02960-DFEAF
Flower	BYI 02960 FS 480 G + BYI 02960 SL 200 G	A	84 – 36000	< LOQ – 47
Pollen (combs)		A	4 – 1200	< LOD – 6
Nectar (combs)		A	12 – 920	< LOD – 5
Beewax (combs)		A	< LOQ – 39	< LOD
Pollen (forager bees)		A	170 – 21000	LOQ – 21
Nectar (forager bees)		A and B	< LOD – 4300	< LOD – 26
Soil		A	61	-

BYI 02960-DFEAF = BYI 02960-difluoroethylaminofuranone (BCS-CC98193)
LOQ = Limit of Quantitation = 1 µg/kg in flower, nectar, pollen and beewax, respectively (BYI 02960 and BYI 02960-DFEAF); 5 µg/kg in soil (BYI 02960)
LOD = Limit of Detetction = 0.1 µg/kg in flower, nectar, pollen as well as 0.3 µg/kg in beewax, respectively (BYI 02960 and BYI 02960-DFEAF); 1.5 µg/kg in soil (BYI 02960)

Pollen source identification

In the control field, the percentage of OSR pollen collected per hive was 23-48 % on DAS3 and 16-33 % on DAS12 in the colonies C1 to C6. In the two residue monitoring colonies of the control, the percentage of OSR pollen collected per hive was 36-39 % on DAS3 and 13-16 % on DAS12. In the test item treatment field, the percentage of OSR pollen collected per hive was 28-43 % on DAS3 and 38-69 % on DAS12 in the colonies T1 to T6. In the two residue monitoring colonies of the test item treatment group, the percentage of OSR pollen collected per hive was 32-52 % on DAS3 and 42-50 % on DAS12. In the figure below the data on pollen sources are given.

Figure 9.4.3-10 Pollen source identification in treatment (T) and control group (C) at DAS 3 (upper figure) and DAS 12 (lower figure). Figure prepared by evaluator using data from tables 49-52.

Bee viruses

ABPV, CBPV, KBV and IAPV were not detected in any of the samples. DWV was detected in some control and treatment hives. BQCV and SBV were detected in some of the control and treatment hives.

Before start of exposure, no differences in bee virus infestation were observed between the control and the test item treatment colonies. After end of exposure, SBV and BQCV was detected in three of the test item treatment colonies, whereas no viruses were found in any of the control colonies. However, before start of overwintering, no differences in bee virus infestation between the control and the test item treatment colonies were observed anymore. At the end of overwintering, none of the colonies of both exposure groups did contain any of the analysed bee viruses. Author concluded that no test item-related adverse effects on colony health in terms of virus infestation were observed.

Bee diseases

The results of the disease analysis revealed that the test item treated colonies were as healthy as the control colonies and as such, no bee disease-related adverse effects on colony health were observed.

Biological observations

Mortality

Results on mortality are reported in the following figures (copied from the report).

Figure 9.4.3-11 Mean number of dead bees per colony observed at the monitoring site before set-up at the field site (DAS-3 to DAS0) and at the field sites before the 2nd spray application (DAS1).

Figure 9.4.3-12 Mean number of dead bees per colony observed at the field sites after the 2nd and before the 3rd spray application (DAS2 to DAS10).

Figure 9.4.3-13 Mean number of dead bees per colony observed at the field sites after the 3rd spray application until end of flowering (DAS11 to DAS31).

The mortality quotients during the entire exposure period (DAS1-DAS31) were calculated to be 1.1 for both T and C. For the separate period these quotients were 1.3 in C and 2.0 in T for the first period (DAS1), 1.5 in C and 1.4 in T for the second period (DAS2 – DAS10), and 0.9 in both C and T for the last period (DAS11-DAS31). From DAS11 to DAS23 the mean number of dead bees per colony per day in the control was higher than the mortality in the treatment. From DAS24 to DAS31 the mean

number of dead bees per colony per day in the treatment was higher than in the control (with the exception of DAS26) (remark evaluator).

The author concluded that no test item-related adverse effects on mortality were observed.

Flight intensity

The mean flight activity in the period until DAS1ba was low, as flowers were in pre-flowering state. In the period from DAS1 after 2nd application to DAS10 just before 3rd application, mean flight intensity amounted to 0.8 bees/m2/day in C compared to 1.1 bees/m2/day in T. Immediately before the 3rd spray application of the test item (DAS10ba), the mean flight intensity was 1.6 bees/m2/day in the control compared to 6.6 bees/m2/day in T. After the 3rd spray application (DAS10) mean flight intensity over the DAS10aa to DAS 31 amounted to 1.5 bees/m2/day in C compared to 1.8 bees/m2/day in T. Total daily flight intensity during exposure from DAS0 to DAS31 was 1.3 bees/m2/day in C compared to 1.6 bees/m2/day in T. A clearly lower flight activity was observed at DAS14 (2 days after the 3rd application; remark evaluator). According to author, overall, no test item-related adverse effects on flight intensity were observed.

Figure 9.4.3-14 Mean number of forager bees per m2 assessed at the field sites after set-up of the colonies until shortly before the 3rd spray application (DAS0 to DAS10ba).

STD: standard deviation; ba: before application, aa: after application

Figure 9.4.3-15 Mean number of forager bees per m2 assessed at the field sites after the 3rd spray application (DAS10aa to DAS23).
STD: standard deviation; aa: after application

Behaviour

In general, only single or a low number of bees with abnormal behaviour (locomotion problems, cramping en trembling) was observed. No data were reported on behaviour at the entrance of the hives for DAS3 and DAS4. In both treatment and control there were several days at which a larger number of bees (up to 246) were motionless. On DAS9 in both the control and the treatment group 5 bees were cramping. Cramping took place in both control and treatment (max. 7 bees).
Trembling was reported taking place both in the control (max. 3 bees) and in the treatment (max. 10 bees on DAS14 in the treatment). On DAS13, 10 bees showed intensive cleaning behaviour in the control.
Locomotion problems were found in the control (max.10) and treatment group (max. 5 bees).
In those cases where larger numbers were observed (246 motionless bees on DAS22 and approximately 85 motionless bees on DAS25 in C), this could be attributed to rainfall events in the area. At the entrance of the hives several test item-related adverse effects were observed such as locomotion problems, cramping and trembling but with only a few bees per day. In both C and T there were days that a number of bees was motionless. On DAS12, clustering at the entrance was found in the control group.
In the test field no unusual foraging behaviour was observed.

Abundance, brood development and food storage

Numbers of bees, brood development and food storage area are given the two figures below.

Assessments were made just before relocation of the colonies to the exposure site (DAS-1) for all eight colonies, including those assigned for residue analysis, and after the end of the exposure period (DAS35) for the remaining six colonies, 10 observations in total.

Almost all colonies of the study showed all brood stages at all assessment dates during the observation period except in late summer and autumn, when the natural period of breeding activity of the colonies ended. Where exceptions were noticed (e.g. short-term loss of an egg-laying queen), control and test item treatment group, were equally affected.

After overwintering, all colonies of the control and the test item treatment group were found to be alive and having resumed breeding activity, except colony T2. The missing brood activity in T2 in early spring was most likely caused by experimental activities resulting in the death of the queen of T2 immediately before start of overwintering and is therefore not considered to be a test item-related effect.

Numbers of bees per colony showed the same seasonal tendency in increases and decreases until the end of overwintering in March 2012. No test item-related differences in colony strength were noticed.

Figure 9.4.3-16 Mean number of bees per colony in the control and test item treatment colonies.
STD: standard deviation

Figure 9.4.3-17 Brood and food development of the colonies in the treatment and control group

Figure 9.4.3-18 Mean weight per colony of the control and test item treatment colonies.

The weight development of the colonies with its increases and decreases, measured from April 5 (DAS-3) to October the 5th 2011 (before overwintering) was very similar for the test item treatment and the control group.

Sudden weight changes to a larger extent in both exposure groups were observed at
23 May 2011 and 4 August 2011: removal of honey bodies
June 1, colony splitting.
14 September 2011: feeding of the colonies
According to author, no test item related adverse effects on colony weight development were observed.

Survey of flowering crops

In the surrounding area of the test item field a few flowering cherry trees were present during exposure of the bees. In the surrounding area of the control field no flowering crops were present during exposure of the bees.
According to pollen analysis wild trees and shrubs were present in the near surroundings of both treated as control field.

Conclusions of the author

The author stated that treatment as performed in this study, did neither cause acute, short-term nor long-term adverse effects in the exposed colonies.

Remarks by evaluator

Splitting of the colonies occurred at June 1. This was done to reduce the urge of the colony to swarm. This urge to swarm is a normal phenomenon that occurs in healthy colonies by this period of the year. The author mentions that the colonies are reduced by removing some brood and store combs. It is not documented how many combs were removed. The methods bee keepers employ to split colonies depend on the region/country and may vary. In the Netherlands it is normal practice to take one comb with food (in which brood is not present). The box is further filled with artificial combs (template on which the bees can build new cells). The new colonies are further started with the introduction of a new queen and a certain number of bees (around 1000). A comb with brood is not introduced in order to prevent further Varroa infection. Sometimes a comb with open male brood is introduced. Any Varroa mites present on the bees will eagerly move into the male brood as the male larvae appear to be most attractive. Care must be taken to destroy the male brood before the male bees emerge.
The food combs that are removed from the hive contain not only nectar and pollen with residues of flupyradifurone (collected during exposure period until splitting of the colony), but also contain nectar and pollen collected before exposure. A logical question would be whether a particular comb would be taken out that could have a much lower or higher concentration of residues. It is not possible to reconstruct whether older or newly filled food combs were chosen. This also depends on the bee keepers methods. However, since only one or at the most a few combs are removed, the effect will be minimal, if present at all. Assumedly, the same type of food combs (fully or half filled) will have been chosen, so possible effects will be the same for all hives.
This necessary manipulation caused a drop in the bee numbers. It was not documented how many brood and store combs and worker bees/forager bees were removed. Data of June 21 show a clear drop of bees. The growth of bee numbers after this date was more or less similar in control and treatment (Figure 7). It can be concluded, the manipulation of the colonies was necessary.

Development of the colony is best evaluated starting from June 21. The reduction of mean number of brood and food cell between at June 1 as compared with May 13 (Figure 17) cannot be explained by reviewer as colony separation only took place after colony assessment.

No statistics were applied to data on mortality and flight intensity. Although the SD values of test item treatments and control were overlapping in most cases, and significant differences are very unlikely, the mean number of dead bees from DAS 24 to DAS 31 was higher in the treatment than in the control for every single day in that period (with the exception of DAS26), Furthermore, the mean number of forager bees per m2 assessed at the field sites after the 3rd spray application was higher in the treatment than in the control on every single day from DAS20 to DAS23. No data were available from DAS24 to DAS31.
Regarding the flight activity: on DAS1aa and DAS10ba the mean number of forager bees per m2 assessed at the field sites in the treatment was higher than the control values, SD values were not overlapping.

Data on behaviour of the honeybees at the entrance of the hives on the second (DAS3) and third day (DAS4) after the 2nd application are not reported. Therefore it is not possible to assess the behaviour in the days following the 2nd application. However, the potential impact on behaviour is expected to have been greatest after the 3rd application (foliar application during full flowering and active foraging), and then, no treatment-related effects on behaviour were seen.

Although no flowering crops were found within 2 km of the field sites, the pollen analyses showed that bees not only foraged on the OSR crop but also on other crops/wild plants, preferably Pyrus and Brassica. Pesticide use in these crops has not been verified by the authors. Both Pyrus and Brassica, may have been treated with several insecticides. Thus, it is still uncertain whether the control colonies were not contaminated by other insecticides/fungicides/herbicides. The residue analyses however showed that no residues of flupyradifurone and its metabolite were found in the flowers, pollen, nectar and beewax of the control with the exception of one sample (13 September 2011) of pollen that contained 6 µg/kg residue BYI 02960.

In conclusion, no clear effects on mortality, flight intensity, brood and food storage area, colony development, behaviour and colony weight were detected. Some possible effects on mortality and flight intensity might have occurred after the 3rd application. If so, these did not result in effects on colony development before and after overwintering.

Expert discussion in Pesticide Peer Review meeting 124 (December 2014):
(Please note that the word ‘effect’ in the text below might be interpreted as different from the control (i.e. may or may not include effects from the treatment), pending on the context.)

The treatment regime was treated seed, soil spray and two foliar applications to the same crop (performed in France).

Some potential effects were observed on forager mortality and bee brood. The hive weight in the treated groups was consistently higher than the control. How worst case the conditions were, was also questioned with respect to the dilution of alternative food etc. There was 60% dilution in the treated group pollen being brought back to the hive. The nectar data (residues) also indicted dilution from non-contaminated food. It was noted that one pollen sample in the control contained residues of the test item indicating contamination. This was surprising because the treatment and control hives were 8 km apart. The RMS noted that this was only 1 sample out of 121 samples and therefore the RMS did not consider this to be an issue. It was suggested that it may have been due to a mistake in the residue analysis in the laboratory.

Flight intensity was also noted to be low in relation to the criteria given in the EPPO 170 Test Guideline. The RMS reflected on this in the reporting table in the same way as for the field study above.

The experts noted that, in general, the studies were well designed. The conclusion of the RMS on this field study was agreed.

Study KIIA 8.16.2/03

Characteristics

Reference	:	Nikolakis, Krieg et al. 2012	GLP statement	:	yes
Type of study	:	Honeybee colony feeding study semi-field, + post-exposure field, fortified diet	Guideline	:	no test guideline available, special design study
Year of execution	:	2011	Acceptability	:	Acceptable
Test substance	:	BYI 02960 (tech.), Batch-code: BYI 02960-01-03			

Substance	***Species***	***Method***	***Duration [d]***	***Treatment rate***	***Parameter***	***Adverse effect***

Substance	*Species*	*Method*	*Duration [d]*	*Treatment rate*	*Parameter*	*Adverse effect*
BYI 02960 tech.	*Apis mellifera*	fortified diet in tunnels followed by relocation under field conditions	Exposure 42 days Total duration 326 days from hive set-up	600 – 2500 - 10000 ug as/kg diet	Mortality	N
					Flight intensity	N
					Brood development	N
					Food area storage	N
					Behaviour	N

Description

The short- and long-term effects of exposure of honeybees (Apis mellifera L.) to BYI 02960-fortified carbohydrate and protein diet was tested under semi-field conditions in gauze tunnels, followed by relocation of the hives to the monitoring location where the honey bees were allowed to forage freely for the remainder of the study.

Location

The confined exposure part of the study was conducted close to Burscheid, North Rhine-Westphalia, Germany and the post-exposure period took place at Monheim, Germany, approx. 20 km away from the exposure location.

Sizes field and crop, test condition (tunnel or field)

Exposure location

The experiment comprised five blocks, each consisting of four differently treated plots - one untreated control and three test-item treatments, resulting in 20 plots in total. Each plot consisted of a pre-installed gauze tunnel, which harboured one randomly assigned honey bee colony at the beginning of the confinement period, resulting in 5 replicates per exposure group (i.e. C, T1, T2, T3). The gauze tunnels were erected on perennial ryegrass, cut short regularly inside the tunnels, which prevented the presence of flowering weeds. Size of the tunnels: approximately 20 m long, 5 m wide and 3 m high (center). Total field size in the confined exposure period: 9920 m2.

Monitoring location

Colonies were set-up corresponding to their positions during the confined exposure period at a distance of 5 m between colonies to minimize drifting of bees from one colony to another.

Active substance

BYI 02960 tech., purity 96.2% w/w, batch-code: BYI 02960-01-03.

Treatments

One untreated control and three test-item treatments: 600 (T1), 2500 (T2) and 10000 (T3) µg BYI 02960 as/kg diet. Throughout the acclimation and exposure period, the honey bee colonies received ad libitum "sugar diet" (i.e. sugar + syrup, for carbohydrate supply) and "pollen diet" (i.e. pollen +

syrup, for protein supply, see below) and had further ad libitum access to tap water, provided via a drinking trough.

The pollen diet was provided inside the respective hives, whereas the sugar diet was offered in Petri-dishes outside the hive, placed about 1 m above the ground, in two feeders per hive (the exact location of the feeders in the tunnels was not reported, but based on a picture in the report it seems that one feeder was placed relatively close to the hive and another more on the other side of the tunnel).

Sugar diet and pollen diet were replenished three times a week (Monday, Wednesday and Friday) and the remainder of the respective diet was reweighed to determine the actual food consumption of the colonies.

During periods where honey bees did not sufficiently forage on the sugar diet and/or during periods of elevated carbohydrate demand (e.g. spells of cold or rainy weather, etc.) – treatment specific supplemental syrup was placed inside the colonies (in case supplemental syrup was provided, all colonies received the identical quantity). The increments of supplemental syrup were always completely taken up by the colonies within a short period of time.

Preparation of the diet

The test item was first dissolved in an Ethanol/Milli-Q-Water (50/50, v/v) and further diluted with unfortified Ethanol/Milli-Q-Water (50/50, v/v), so that three differently concentrated spiking solutions were prepared, which were used to spike all the different diets (i.e. sugar-, syrup- and pollen diet) at the three different BYI 02960-treatment levels.

Control and test item spiked sugar diet consisted of 66.67% w/w solid Apipuder® + 33.33% w/w liquid Apiinvert®). The pollen diet was prepared as dough, made up with finely-ground, natural, bee-collected, fresh pollen of multi-floral origin, mixed with concentrated, commercial sugar syrup (Apiinvert®). The appropriate amount of the spiking solutions or control solutions was added to the liquid Apiinvert®, the mixture was stirred for several minutes and increments of solid Apipuder® were added under continuous stirring until the final mixing ratio was obtained for each individual batch. Once a homogenous sugar dough had been formed, the stirring continued for a further several minutes to assure a homogenous distribution of BYI 02960 within the sugar dough.

The sugar diet was prepared as a dough, made up with finely-ground, commercial sucrose powder (Apipuder®), mixed with concentrated, commercial sugar syrup (Apiinvert®), and control and test item spiked supplemental syrup was added.

Side experiment

A comparison of the overall mean actual concentration of BYI 02960 in the sugar diet before and after the actual exposure to confined honey bee colonies during the biological course of the study revealed approx. 25% lower concentrations after honey bee feeding when compared to the concentrations before feeding. In contrast, virtually no difference in diet concentrations was observed in the corresponding pollen diet before and after feeding.

Species tested

Honey bees of the race Apis mellifera.

Population size

For the test, healthy, queen-right colonies were used. All test colonies were prepared at the same time and equalised for adult worker bees, brood and food stores as reasonably possible. At the beginning of the test, each colony contained one laying sister queen and consistently four occupied frames with nominally 2500 honey bees, in a hive with one brood chamber; three of the four occupied frames were brood frames, one frame contained bee-bread (pollen) and honey. For the confinement period of the study, three empty frames were added to each hive to allow an expansion of the colony. The quantity of the bee-bread (pollen)- and honey stores was adjusted to allow a thriving of the colonies for about one week, just enough, to maintain colony vitality throughout the acclimation period under confined conditions.

Timing placement hives under test conditions

The colonies were first trained (training period) to find and accept the feeders (located in the tunnel outside of the hive, which harboured the sugar diet (i.e. sugar + syrup)), and were then allowed to further acclimate to the confined conditions, during which all colonies received untreated (control) diet (acclimation period). At the end of the acclimation period, gauze-tunnels/bee colonies were randomly assigned to one of the four exposure groups (i.e. C, T1, T2, T3), respectively. The total confined pre-exposure period lasted for about 1 1/2 weeks, with one week on control diet at the routine feeding regime. One colony per individual tunnel, set-up close to one end of the tunnel.

The acclimation period was followed by a 6 week exposure period (end of April 2011 until mid of June 2011). After the exposure period, the test colonies were relocated to a monitoring site where the colonies were maintained according to apicultural practice until overwintering.

Parameters observed and timing thereof

The following parameters were observed during the exposure phase:

- Honey bee mortality (i.e. dead worker bees, drones, larvae and pupae) was measured on a daily basis for a period of 49 days in succession, i.e. for 7 days during the confined acclimation period and for 42 days during the confined exposure period:
    - Number of dead bees in dead bee trap
    - Number of dead worker bees, drones, larvae and pupae on a piece of cloth with a surface of appr. 50 x 50 cm directly in front of the hives
    - dead worker bees and drones (as well as larvae and pupae, if any) were collected and counted on water-permeable polyethylene-mesh-sheets (width approximately 30 - 50 cm), which were installed along the walls of every gauze tunnel
    - mortality on the perennial ryegrass, if any was also recorded, but no formal counts were made
- Foraging activity (flight activity) during confinement was assessed daily; the number of bees foraging on the sugar diets was recorded during two 3-minute observation periods per feeder and tunnel/colony, respectively
- The colony health and strength as well as the brood development were assessed during the acclimation and exposure period at weekly intervals:

  - Estimation of number of adult honey bees covering the comb cell area per comb side (estimation adapted to IMDORF & GERIG, 1999, and IMDORF et al., 1987)
  - Estimation of the area containing egg, larval and capped cells by means of an estimation frame
  - Presence of a healthy queen; verified by the presence of freshly laid (one day old) eggs
  - Estimate of the pollen storage area and area with nectar stores
  - Colonies were assessed for bee diseases according to standard beekeeping practice
  - Varroa infestation was monitored just before set-up inside the tunnels, at the end of the acclimation period, in the middle of the exposure period and at the very end of the exposure period/just at the beginning of the post-exposure observation period
- Food consumption was measured every Monday, Wednesday and Friday during the acclimation and exposure period; the amount of consumed sugar- and pollen diet was determined by re-weighing the respective diets
- Behavioural anomalies were recorded whenever observed:
  - Exaggerated motility, aggressiveness
  - Discoordinated movements (trembling, flight incapability, etc.),
  - Apathy, lethargic behaviour,
  - Clustering at the bee hive entrance,
  - Clustering on the gauze netting of the tunnel,
  - Obvious symptoms of intoxication (cramping, agony, etc.)

Bee diseases and virus analysis

Honey bee and nectar/honey samples were taken at the start of acclimation phase, start of exposure phase, before and after overwintering and were analysed:

In total,

- 76 honey bee samples for disease/pathogen analysis (Nosema sp., Malpighamoeba mellificae and Varroa destructor),

- 76 nectar/honey samples for the analysis of AFB (American foul brood; i.e. presence of Paenibacillus larvae spores)

- 76 honey bee samples for the analysis of EFB (European foul brood; i.e. presence of Melissococcus plutonius),

- and 76 honey bee samples for virus analysis (DWV [deformed wing virus], SBV [sacbrood virus], ABPV [acute bee paralysis virus], CBPV [chronic bee paralysis virus], KBV [Kashmir bee virus], IAPV [Israel acute paralysis virus] and BQCV [black queen cell virus]) for the bee viruses deformed wing virus (DWV), sacbrood virus (SBV), acute bee paralysis virus (ABPV), chronic bee paralysis virus (CBPV), Kashmir bee virus (KBV), Israel acute paralysis virus (IAPV) and black queen cell virus (BQCV).

The presence of different pathogens (Nosema sp., Malphigamoeba mellificae, Varroa destructor, Paenibacillus larvae spores, Melissococcus plutonius) in bee samples was determined at different time points during the observation period.

Chemical analysis

Actual BYI 02960-concentration in samples of spiked sugar- and pollen diet were determined by reversed phase High Performance Liquid Chromatography (HPLC), coupled with electrospray and tandem mass spectrometry (MS/MS) detection, according to method 01206/M001.

Climatic conditions

Climatic conditions (such as daily min./max. temperature, air humidity, rainfall, sunshine hours, average wind speed, etc.) were obtained from the weather station on the premises of Bayer CropScience AG's experimental station Höfchen (< 1 km distance to the gauze tunnels) throughout the confined exposure period of the study. From the beginning of the post-observation field monitoring period (including the overwintering period) to the end of the study climatic data data were obtained from the weather station on the premises of Bayer CropScience AG's Headquarters in Monheim am Rhein (approx. 5 km distance to the Laacher Hof post-exposure field monitoring location).
Throughout the confined exposure period of the study, the actual temperature inside the gauze tunnels was recorded by means of calibrated data loggers, placed evenly spaced in the different tunnels across the study site.

Statistical evaluation

Single factor ANOVA was employed for testing for significant differences of the endpoints described above between any of the exposure groups. Normal distribution of data was tested with the Shapiro-Wilk test; homogeneity of residuals was tested by applying the Brown-Forsythe test (i.e. the Levene test, by using the median instead of the mean) to the fitted model. In case homogeneity of residuals was rejected, the non-parametric Kruskal-Wallis test was performed. In case of a rejected H0, the control response was compared with the response of the different test item treatment levels via a Kruskal test, corrected for multiple comparisons (Siegel and Castellan, 1988).

Results

During the exposure period, two out of the 20 colonies differed in food uptake and/or mortality from the other colonies, and infections/infestation could not be excluded. One colony in control (colony 6) which showed little foraging activity, little food consumption and which had practically collapsed at the end of the confinement period and one colony in test item treatment group T1 (colony 8) which showed an increased mortality at the end of the confined exposure period with bees showing disease-like symptoms. In order to prevent any spread of potential bee diseases on the monitoring location, these two colonies (colony 6-tunnel 17, colony 8-tunnel 13, T1) were excluded. All statistical analyses of data were conducted without control colony 6 in order to avoid any potential bias due to this non-performing control colony. The bee data from colony 8 in tunnel 13 (T1) were not excluded from the statistical analyses when relating to the confinement period. This procedure was selected to apply a conservative approach to the data evaluation and because colony 8 - unlike colony 6, performed most of the time (during confinement) as the other T1 -colonies and was still vital.

Chemical analysis

In the fresh pollen (used for the preparation of the control and treated pollen diet), no residues above the Limit of Detection (LOD, 0.3 ug/kg) were found for all neonicotinoid insecticides under investigation (i.e. acetamiprid, thiacloprid, clothianidin, thiamethoxam and imidacloprid) in any of the pollen samples.

Actual applied BYI 02960-concentration in the diet

Residues of BYI 02960 in the control sugar- and pollen diet and the batches of the control sugar syrup were always below LOD, i.e.<0.1 ug/kg.

The mean actual BYI 02960-concentration in the pollen diet sampled before exposure to the honey bees colonies ranged from 80 to 94%, with RSD values below 6.1%; the corresponding mean actual concentration sampled after exposure ranged from 80 to 92%, with RSD values below 8.5%.

The mean actual BYI 02960-concentration in the sugar diet sampled ranged from 76 to 91% of nominal before exposure of the bees, with RSD values below 7.8%; the corresponding mean actual BYI 02960-concentration in the sugar diet sampled after exposure ranged from 47 to 68% of nominal, with RSD values below 13.4%. Moreover, during sampling and handling of the sugar diet, it was regularly noticed that samples of the sugar diet after being exposed to honey bees were less doughy and more crystalline in comparison to the samples before exposure to honey bees. This phenomenon was not observed for the pollen diet. To assess whether the lower actual concentrations of BYI 02960 in the sugar diet after foraging by honey bees was the result of selective foraging on the two components of the sugar diet (i.e. crystalline sucrose powder and sugar syrup), a side experiment was performed. In this experiment, an aliquot of a representative BYI 02960-spiked sugar diet was centrifuged and samples from the more liquid (upper layers) and the more solid part (lower layers) were analysed. The analytical data revealed actual BYI 02960 concentrations in the upper layer of the centrifuged BYI 02960-spiked sugar diet in a range from 208 to 233% of nominal, whereas the actual BYI 02960 concentrations in the middle and lower layer of the centrifuged BYI 02960-spiked sugar diet ranged from 58 to 63% of nominal. It was further investigated whether bees forage selectively on the more liquid part of the sugar diet, which in turn would result in lower actual concentrations of BYI 02960 in the sugar diet remainders after bee exposure. For this, BYI 02960-spiked sugar diet was placed in feeders inside gauze tunnels placed side by side in the field, either with or without honey bees actively foraging on the spiked sugar diet and the remainders were analysed.

Actual concentrations of BYI 02960 in the sugar diet before the exposure in the side experiment were within the range of 82 to 92% of nominal (total average: 88%; n=24). The actual BYI 02960-concentration in the sugar diet after foraging ranged from 23 to 63% of nominal (total average: 45%; n=12), whereas in the concurrently exposed spiked sugar diet without foraging, concentrations ranged from 72 to 94% of nominal (total average: 85%; n=12). The side experiment thus indicates that the lower actual concentration in the spiked sugar diet after exposure does not originate from dissipation or decomposition of BYI 02960, but originates from the selective uptake of the more liquid phase of the sugar diet by honey bees. This is further supported by the fact that BYI 02960 was found to be mainly located in the more liquid phase of the sugar diet and that the sugar diet was generally found to

be more crystalline after bee exposure. Furthermore, no decline of concentrations was found in the pollen diet after honey bee exposure.

Consumption of sugar- and pollen diet during confinement

The total average consumption of sugar- and pollen diet during the confined exposure period was not statistically significant different in any of the three test item treatment groups (i.e. T1, T2 and T3) as compared to the control,except for one single occasion (feeding interval) in exposure group T2 with a higher consumption. Consumption of the sugar diet was lowest in all groups during the confined acclimation phase. According to the author, this observation can be explained by the fact that the colonies during this period needed to adapt their feeding regime to the new situation inside the tunnels and still relied during this period on their in-hive resources, which they concurrently used-up.

Table 9.4.3-4 (copied from the original report). Consumption of sugar diet was measured on every feeding-/diet exchange event from 2011-05-04 onwards. The values are the mean sugar diet consumption from both feeders averaged over all tunnels per exposure group (NB: Colony 6-tunnel 17 (control) is not included in the presented data).

Date of reweigh (of respective feeding interval) [YYYY-MM-DD]	Control		T1		T2		T3	
	mean sugar diet consumption [g]	±sd	mean sugar diet consumption [g]	±sd	mean sugar diet consumption [g]	±sd	mean sugar diet consumption [g]	±sd
2011-05-04	7.9	17.0	0.2	1.0	3.9	7.3	2.1	4.0
2011-05-06	52.3	81.3	18.2	17.0	71.0	90.0	53.1	28.2
2011-05-09	201.5	17.6	189.6	57.3	223.9	13.7	220.6	20.0
2011-05-10	171.8	54.3	138.1	38.1	227.4	24.0	153.3	19.2
2011-05-11	177.1	48.5	147.3	45.7	225.3	32.2	139.6	17.5
2011-05-13	259.7	61.6	213.1	76.3	320.5	44.6	184.1	57.6
2011-05-16	243.7	64.8	171.1	77.3	295.2	61.7	144.0	59.9
2011-05-18	182.6	40.1	134.3	69.1	214.8	55.8	105.4	28.8
2011-05-20	398.2	117.5	314.1	98.2	433.9	31.9	329.8	80.1
2011-05-23	450.4	96.8	382.5	66.5	478.1	30.7	391.0	80.7
2011-05-25	310.3	84.7	217.4	77.1	289.3	35.6	215.6	56.6
2011-05-27	256.2	84.8	195.1	68.9	251.9	42.1	194.5	43.4
2011-05-30	342.6	132.0	311.0	96.4	420.0	63.8	240.1	50.5
2011-06-01	248.1	40.7	210.6	52.4	263.4	30.0	194.1	35.8
2011-06-03	297.9	89.8	239.0	94.3	340.9	68.2	252.9	70.1
2011-06-06	432.4	114.4	386.7	88.2	499.2	50.2	411.0	90.9
2011-06-08	276.4	60.4	218.7	51.7	321.1	73.9	221.6	40.3
2011-06-10	177.1	76.8	135.0	66.9	190.1	48.1	100.7	36.6
2011-06-13	284.1	103.6	222.7	96.3	345.1	54.8	208.7	81.9
2011-06-15	228.7	78.1	174.7	51.5	309.1	69.2	208.3	51.2
2011-06-17	253.7	81.9	232.5	75.3	406.0	67.0	231.9	49.5
2011-06-20	239.0	57.1	238.0	98.0	319.8	24.3	190.7	50.6
∑2011-05-10 - 2011-06-20	5492	1270	4490	1189	6445	565	4393	831

Table 9.4.3-5 (copied from the original report). Differences in sugar diet consumption between control and any test item treatment (ANOVA, Ho rejected). An asterisk (*) indicates the test item treatment that differs significantly from the control (NB: Colony 6-tunnel 17 (control) is not included in the presented data)

Date [YYYY-MM-DD]	Control		T1		T2		T3		Statistical Test
	mean	± SD	mean	± SD	mean	± SD	mean	± SD	
2011-05-10	171.8	54.3	138.1	38.1	227.4	24.0	153.3	19.2	ANOVA, p=0.005; Dunnett, non-significant
2011-05-11	177.1	48.5	147.3	45.7	225.3	32.2	139.6	17.5	ANOVA, p=0.007; Dunnett, non-significant
2011-05-13	259.7	61.6	213.1	76.3	320.5	44.6	184.1	57.6	ANOVA, p=0.013; Dunnett, non-significant
2011-05-16	243.7	64.8	171.1	77.3	295.2	61.7	144.0	59.9	ANOVA, p=0.008; Dunnett, non-significant
2011-05-18	182.6	40.1	134.3	69.1	214.8	55.8	105.4	28.8	ANOVA, p=0.017; Dunnett, non-significant
2011-05-30	342.6	132.0	311.0	96.4	420.0	63.8	240.1	50.5	ANOVA, p=0.03; Dunnett, non-significant
2011-06-08	276.4	60.4	218.7	51.7	321.1	73.9	221.6	40.3	ANOVA, p=0.035; Dunnett, non-significant
2011-06-15	228.7	78.1	174.7	51.5	309.1	69.2	208.3	51.2	ANOVA, p=0.025; Dunnett, non-significant
2011-06-17	253.7	81.9	232.5	75.3	406.0*	67.0	231.9	49.5	ANOVA*, p=0.003; Dunnett*, p=0.01
2011-06-20	239.0	57.1	238.0	98.0	319.8	24.3	190.7	50.6	ANOVA, p=0.04; Dunnett, non-significant
∑2011-05-10 - 2011-06-20	5492	1270	4490	1189	6445	565	4393	831	ANOVA, p=0.02; Dunnett, non-significant

The consumption of pollen diet showed a regular pattern with a peak every third feeding interval. This peak is caused by the employed feeding regime which included an extra consumption day between Friday and Monday. For those instances where H0 had to be rejected, post-hoc testing did not reveal any statistically significant difference between the control and any of the test item treatments. There was no significant difference between the total average pollen diet consumption during the confined exposure period in each of the three test item treatment groups (i.e. T1, T2 and T3) when compared to the control.

Table 9.4.3-6 (copied from the original report). Consumption of pollen diet as measured on every feeding-/diet exchange event from 2011-05-04 onwards. The values are the mean pollen diet consumption averaged over all tunnels

per exposure group

Date of reweigh [YYYY-MM-DD]	Control		T1		T2		T3	
	mean pollen consumption [g]	± SD	mean pollen consumption [g]	± SD	mean pollen consumption [g]	± SD	mean pollen consumption [g]	± SD
2011-05-04	91.9	31.1	77.1	27.0	91.1	35.8	60.3	91.9
2011-05-06	139.4	13.8	109.8	38.1	124.7	35.2	99.1	139.4
2011-05-09	206.6	14.6	195.7	37.6	202.6	27.2	185.0	206.6
2011-05-11	161.1	23.7	166.4	26.6	165.0	22.6	152.0	161.1
2011-05-13	130.4	15.1	147.3	21.8	141.0	22.7	127.8	130.4
2011-05-16	188.2	22.8	201.0	20.9	200.0	22.9	165.3	188.2
2011-05-18	128.4	24.6	126.1	26.7	134.8	19.8	96.8	128.4
2011-05-20	160.1	18.6	156.6	25.4	173.0	28.9	139.3	160.1
2011-05-23	195.2	21.5	200.7	23.4	201.2	22.3	180.0	195.2
2011-05-25	131.0	11.8	139.5	17.3	156.4	20.0	122.0	131.0
2011-05-27	137.8	9.7	147.8	19.3	148.3	14.9	134.7	137.8
2011-05-30	198.4	27.7	210.7	13.0	219.7	8.0	192.1	198.4
2011-06-01	126.1	20.3	146.3	24.6	156.3	16.9	117.5	126.1
2011-06-03	160.1	13.0	177.7	18.7	191.1	20.7	152.3	160.1
2011-06-06	202.5	16.1	221.2	10.2	223.4	7.6	206.2	202.5
2011-06-08	157.2	17.1	174.3	17.3	191.9	25.1	143.5	157.2
2011-06-10	139.2	16.2	159.7	24.8	181.2	24.4	133.0	139.2
2011-06-13	199.0	24.4	214.5	15.7	218.8	11.7	187.4	199.0
2011-06-15	151.2	20.0	165.3	20.5	177.1	10.5	146.3	151.2
2011-06-17	140.7	18.1	173.3	22.8	173.3	26.5	136.1	140.7
2011-06-20	178.7	26.0	199.6	14.4	218.5	8.1	173.0	178.7
∑2011-05-11 - 2011-06-20	2885	325	3128	372	3271	301	2075	362

Table 9.4.3-7 (copied from the original report). Differences in pollen diet consumption between control and test item treatments (ANOVA, Ho rejected). An asterisk (*) indicates the test item treatment that differs significantly from the control (NB: Colony 6-tunnel 17 (control) is not included in the presented data)

Date [YYYY-MM-DD]	Control		T1		T2		T3		Statistical Test
	mean	± SD	mean	± SD	mean	± SD	mean	± SD	
2011-05-25	131.0	11.8	139.5	17.3	156.4	20.0	122.0	131.0	ANOVA, p=0.048; Dunnett, non-significant
2011-06-03	160.1	13.0	177.7	18.7	191.1	20.7	152.3	160.1	ANOVA, p=0.04; Dunnett, non-significant
2011-06-08	157.2	17.1	174.3	17.3	191.9	25.1	143.5	157.2	ANOVA, p=0.036; Dunnett, non-significant
2011-06-20	178.7	26.0	199.6	14.4	218.5	8.1	173.0	178.7	ANOVA, p=0.041; Dunnett, non-significant

According to the author an aggravated foraging situation (cold night temperatures and exposure to rather strong and cold easterly winds during daytime) in combination with the still on-going adaptation to obtain carbohydrates from the feeder, is in line with the low consumption of the sugar diet during the acclimation period.

Nectar/Honey stores

The average number of cells containing nectar or honey is presented in the following figure.

Figure 9.4.3-19 Nectar/Honey stores, determined as the average number of cells containing nectar or honey per exposure group as evaluated during each colony assessment throughout the course of the study.

At the end of the exposure period (2011-06-20) the average number of nectar/honey cells was to some extent lower in T1 than in the other groups, but not statistically significantly different. This was at least partially caused by colony 8 (2800 nectar/honey cells), which was no longer continued after the confinement period.

According to the author, the lower number of cells at the first colony assessment at the monitoring location can be explained by the re-adaptation of colonies from their feeding habits during the confined conditions.

Figure 9.4.3-20 Consumed honey (kg) during overwintering as calculated by subtracting the honey stores before overwintering (2011-10-24) from those after overwintering (2012-03-21), under the assumption that one cell contains 0.3125 g honey. Values are given as the average amount per exposure group.

Pollen stores

The average number of cells containing pollen or bee bread is reported in the following figure.

Figure 9.4.3-21 Pollen/Bee bread stores, determined as the average number of cells containing pollen or bee bread per exposure group as evaluated during each colony assessment throughout the course of the study.

The data of 2nd colony assessment revealed that the originally available pollen stores were depleted or almost depleted in all bee hives at the end of the acclimation period. Throughout the entire exposure period, none of the cells within the control or treated colonies contained any pollen/bee bread.

At the first colony assessment on the monitoring location after relocation (9th colony assessment), the average number of cells containing pollen/bee bread indicates that colonies had re-adapted to their routine flower/pollen foraging and pollen storage. The higher average number of cells containing pollen/bee bread at this date in the control was caused by colony 1 (11960 cells with pollen/bee bread), which became the home of a swarm of bees (so the number of bees in the colony was greatly increased). The higher colony strength caused higher pollen collection and the bees from the swarm did not have to re-adapt to routine flower/pollen foraging as did the other colonies of the study. To prevent biased conclusion during the further course of the study, the swarm was conservatively extracted from colony 1 on 01 AUG 2011, on the basis of the available brood data of the current and the preceding colony assessment under consideration of the number of adult bees at the development of the other C-colonies during this period. An elevated number of cells with pollen/bee bread in colony 1 was still observed at the next colony assessment, after correction for the swarm, thereafter, the number of cells with pollen/bee bread in colony 1 had returned to the range of other C-colonies. Statistical analyses did not detect any significant differences in pollen/bee bread stores between the different exposure groups during the entire confinement period, during the entire post-exposure monitoring period as well as after overwintering, respectively (ANOVA analysis: Ho accepted on each of the 15 colony assessment dates).

Honey bee disease and virus analysis

The occurrence of pathogens and viruses occurred across all exposure groups, but was not linked to the absence or presence of the test item in the respective exposure group. Results are reported in the following figure.

Figure 9.4.3-22 Disease- and virus occurrence, determined as the average number of occurrences* per exposure group and evaluated at four distinct dates during the course of the study. (* disease occurrence was coded in a binary system for each disease and hive - i.e. if a particular disease was present in a colony, the colony received a "1", if this particular disease was not present it received a "0". From this coding, average values were calculated per treatment group)

Overall, no test-item related adverse effects on colony health in terms of pathogen/bee disease and virus infestation were observed during the entire course of the study. Throughout the 17 consecutive measurements, there was only one date (19 OCT 2011) where the ANOVA analysis of the data regarding the Varroa infestation resulted in a rejection of Ho and were subsequent post-hoc testing revealed a significant difference between the control treatment and treatment T2. However, the author states that, as the difference occurred on a very low absolute level (0.5 mites in C, 3 mites in T2), only at one single measurement out of 17, about 1 month after a successful anti-Varroa treatment with formic acid, the observed isolated statistical difference is neither considered test-item treatment related nor of any apidological or apicultural relevance.

Table 9.4.3-8 Differences in the average number of number of Varroa-mites, dropped and counted on Varroaboards underneath each hive, between control and any test item treatment (ANOVA, Ho rejected). An asterisk (*) indicates the test item treatment that differs significantly from the control (NB: Colony 6-tunnel 17 (control) is not included in the presented data; colony 8-tunnel 13 of T1 was not continued after the end of confinement)

Date	Control		T1		T2		T3		Statistical Test
	mean	± SD	mean	± SD	mean	± SD	mean	± SD	
2011-10-19	0.45	0.38	1.00	0.43	2.96*	1.50	1.12	0.83	ANOVA, p=0.007; Dunnett, p=0.004

Honey bee mortality in front of the hive and at the tunnel walls during confinement

No significant differences were observed for the total sum of dead individuals, except for worker bee mortality at the tunnel edges, which was significantly lower in exposure T1 as compared to control. Mortality in front of the hives did not differ significantly between the control group and exposure groups T1 and T3, respectively, throughout the entire confinement period; post-hoc testing revealed a significantly higher worker bee mortality in treatment group T2 than in the control on 2 out of 49 days of confinement. However, the author reports that these two occasions are isolated in time and the

difference in relation to control performance on these two days is well within the range of natural variability and is apidologically not relevant.

Bee mortality at the tunnel walls in T1 was significantly lower than in the three days during the confinement period, for T2 this was the case on two days For T3, no significant differences were observed regarding the daily mean values of dead worker bees at the tunnel walls. Since there was no consistent pattern in the mortality figures and because mortality was lower in the respective test item treatment groups than in control, the authors conclude that differences can be attributed to the natural variability. This is supported by the fact that the differences between trial days were larger than the differences between treatments.

Drone mortality as well as mortality of immature life stages (larvae and pupae) was low in all exposure groups throughout the entire confinement period.

Table 9.4.3-9 (copied from original report). Total number of dead honey bees in front of the hive as well as on the sheets at the tunnel walls, separated in dead worker bees, drones and immature stages (i.e. larvae and pupae). All values recorded during the confined acclimation and exposure period (i.e. 2011-05-02 - 2011-06-20, 49 days) were summed for each tunnel per exposure group.

Exposure group	Tunnel	Sum of dead worker bees		Sum of dead drones		Sum of dead immature stages		Sum of dead overall
		Front of Hive	Tunnel wall	Front of Hive	Tunnel wall	Front of Hive	Tunnel wall	
Control	1	128	2605	23	34	4	0	2794
	6	289	2603	18	11	13	3	2937
	10	62	3033	6	25	4	0	3130
	14	115	2991	13	25	19	2	3165
	17	178	1463	5	22	12	2	1682
	∑ Control	772	12695	65	117	52	7	13708
T1	8	105	1792	17	19	12	1	1946
	11	85	1747	3	22	5	2	1864
	13	1922	2448	18	47	4	1	4440
	16	268	2302	5	9	4	2	2590
	20	158	1786	5	8	2	1	1960
	∑ T1	2538	10075	48	105	27	7	12800
T2	3	237	3274	6	4	2	0	3523
	5	122	2280	8	14	35	2	2461
	9	276	2698	6	13	6	0	2999
	12	132	2689	21	60	20	0	2922
	18	167	2179	4	9	30	2	2391
	∑ T2	934	13120	45	100	93	4	14296
T3	2	111	2062	9	10	5	0	2197
	4	233	3141	8	12	9	2	3405
	7	121	1775	14	59	11	1	1981
	15	154	3044	24	15	14	2	3253
	19	87	1874	4	6	2	2	1975
	∑ T3	706	11896	59	102	41	7	12811

Table 9.4.3-10 (copied from the original report). Differences in the average number of dead honey bees (during the entire study period) in front of the hive as well as at the tunnel walls, separated in dead worker bees, drones and immature life stages (i.e. larvae and pupae) between control and any test item treatments (ANOVA, Ho rejected)

Sum of mean	Control		T1		T2		T3		Statistical Test
	mean	± SD	mean	± SD	mean	± SD	mean	± SD	
Dead bees front of hive	148.5	97.9	507.6	793.9	186.8	67.2	141.2	56.7	ANOVA, p=0.48; Dunnett, non-significant
Dead bees tunnel walls	2808.0	236.2	2015.0*	333.1	2624.0	432.6	2379.2	660.2	ANOVA, p=0.088; Dunnett, p=0.049
Dead drones front of hive	15.0	7.3	9.6	7.3	9.0	6.9	11.8	7.7	ANOVA, p=0.62 Dunnett, non-significant
Dead drones tunnel walls	23.8	9.5	21.0	15.8	20.0	22.7	20.4	21.8	ANOVA, p=0.99 Dunnett, non-significant
Dead immatures front of hive	10.0	7.3	5.4	3.8	18.6	14.4	8.2	4.8	Kruskal-Wallis, p=0.36; kruskalmc, non-significant
Dead immatures tunnel walls	1.3	1.5	1.4	0.5	0.8	1.1	1.4	0.9	ANOVA, p=0.77 Dunnett, non-significant

Table 9.4.3-11 (copied from the original report): Average number of dead honey bees (weekly and for the total study period) in front of the hive as well as on the sheets at the tunnel walls, separated in dead worker bees, drones and juvenile stages (i.e. larvae and pupae). All values recorded during the confined acclimation and exposure period (i.e. 2011-05-02 - 2011-06-20, 49 days) were averaged for each tunnel and week, respectively for total confinement period, per exposure group.

Time of confined exposure	Exposure group	Average No. of dead worker bees		Average No. of dead drones		Average No. of dead immature stages	
		Front of Hive	Tunnel wall	Front of Hive	Tunnel wall	Front of Hive	Tunnel wall
Acclimation period, Week 1 of confinement, 02 - 09 MAY 2011	C	1.17	15.26	1.69	1.86	1.36	0.14
	T1	1.37	10.40	1.20	2.51	0.46	0.14
	T2	0.83	8.80	1.11	1.49	2.43	0.11
	T3	1.17	13.37	1.54	2.06	1.09	0.14
Exposure period, Week 2 of confinement, Week 1 of exposure, 10 - 16 MAY 2011	C	1.06	21.57	0.06	0.57	0.04	0.00
	T1	0.97	12.03	0.09	0.06	0.11	0.03
	T2	1.17	18.97	0.06	0.11	0.06	0.00
	T3	0.97	23.54	0.09	0.09	0.00	0.06
Exposure period, Week 3 of confinement, Week 2 of exposure, 17 - 23 MAY 2011	C	3.37	62.71	0.06	0.37	0.04	0.00
	T1	4.74	39.97	0.06	0.43	0.06	0.00
	T2	4.54	59.03	0.09	0.29	0.03	0.00
	T3	4.06	56.20	0.03	0.14	0.06	0.00
Exposure period, Week 4 of confinement, Week 3 of exposure, 24 - 30 MAY 2011	C	2.77	71.77	0.06	0.40	0.00	0.00
	T1	4.69	52.34	0.00	0.00	0.09	0.00
	T2	4.26	73.37	0.00	0.63	0.09	0.00
	T3	2.29	66.63	0.03	0.51	0.00	0.00
Exposure period, Week 5 of confinement, Week 4 of exposure, 31 MAY - 06 JUN 2011	C	3.29	77.09	0.00	0.14	0.00	0.00
	T1	13.54	68.23	0.03	0.00	0.03	0.00
	T2	4.89	75.37	0.00	0.34	0.03	0.00
	T3	2.26	64.26	0.00	0.11	0.03	0.00
Exposure period, Week 6 of confinement, Week 5 of exposure, 07 - 13 JUN 2011	C	5.31	62.91	0.00	0.00	0.00	0.04
	T1	18.97	55.37	0.00	0.00	0.03	0.03
	T2	6.20	75.43	0.00	0.00	0.00	0.00
	T3	5.23	63.20	0.00	0.00	0.00	0.00
Exposure period, Week 7 of confinement, Week 6 of exposure, 14 - 20 JUN 2011	C	5.09	51.40	0.00	0.00	0.00	0.00
	T1	28.23	49.51	0.00	0.00	0.00	0.00
	T2	4.80	63.89	0.03	0.00	0.03	0.00
	T3	4.20	52.69	0.00	0.00	0.00	0.00
Total exposure period, Week 2-7 of confinement, Week 1-6 of exposure, 10 MAY - 20 JUN 2011	C	3.48	57.91	0.03	0.25	0.01	0.01
	T1	11.86	46.24	0.03	0.08	0.05	0.01
	T2	4.31	61.01	0.03	0.23	0.04	0.00
	T3	3.17	54.42	0.02	0.14	0.01	0.01

Behaviour

Except for colony 8 in tunnel 13 of T1, which exhibited disease like symptoms towards the end of the confined exposure period, sometimes associated with increased motility, no abnormal behaviour was recorded in the tunnel tents or around and in the hives during the confinement period or throughout the post-exposure monitoring period.

Foraging activity (flight activity) during confinement

Presented numbers are the number of bees observed on the two feeders during a total of 12 minutes counting time (2x 3 min per feeder, 2 feeders/hive). Additionally, the total number of honey bees on the gauze tunnel roof and walls was estimated in order to detect any indication of possible repellent/anti-feedant phenomena (no such indication was found; results not shown). Flight intensity increased from a total average of 5 honey bees visiting the feeder at the second day of confinement (i.e. during the acclimation period), to a total average of 97 feeder visiting honey bees at the first day of exposure, indicating a continuously increasing acceptance of the feeders during the acclimation period. At the start of the exposure period, the total average of honey bees visiting the feeder was within the range observed during the remaining confinement period (23 - 246). Foraging activity on each single day throughout the confinement period was similar in all exposure groups.

The statistical analysis of the daily foraging activity data revealed significant differences between one of the three exposure groups and the control on 5 occasions (days) throughout the 49 days confinement period; On three out of these 5 occasions, exposure group T3 exhibited a significantly lower foraging activity than the control, on the other two occasions, foraging activity in exposure group T1 was significantly lower as compared to control.

No significant difference was observed for the total average number of foraging honey bees in the test item treatments when compared to control performance. The total average consumption of sugar diet during was not statistically significantly different between the test item treatment groups and the control. ,There was also no statistical significant difference between the consumption of the sugar diet within two subsequent feeding events (“feeding interval”) in the exposure groups when compared to control.

Colony strength

Table 9.4.3-12 Average number of honey bees per exposure group

Colony assessment	C	T1	T2	T3
1. just before set-up of colonies	**3063**	**2975**	**3125**	**3113**
2. end of acclimation, begin of exposure period	**6038**	**5998**	**6410**	**6102**
3. end of exposure period	**9368**	**10502**	**10402**	**9891**
4. middle of September 2011	**18021**	**16663**	**18010**	**16015**
5. end of October 2011	**9083**	**8484**	**11083**	**7866**
6. middle of March 2012	**6079**	**4053**	**4752**	**4174**

Overall, the development of the colonies was homogenous throughout the confinement period of 7 weeks. No statistically significant difference in the average colony strength was observed at any of the colony assessments conducted throughout the course of the study, when comparing the performance of each test item treatment group with control performance.

The somewhat higher colony strength in C at the end of the experiment was caused by colony 1 (12498 bees). The author states: this elevated number of adult honey bees could potentially have been recruited/bred by the colony itself, but could also be the result of bees that entered the colony from outside (i.e. acceptance of
another swarm during winter/early springtime). However, the latter seems more realistic. The position of colony 1 at the edge of the bee stand increased the probability of catching swarms, colony 1 already accepted a swarm before and appeared to be particularly inclined to accept swarms and the amount of brood cells in colony 1 did not vary accordingly from the other colonies.

Colony brood status/brood development

Table 9.4.3-13 Average number of brood cells (i.e. eggs + worker bee larvae + worker bee pupae) per exposure group

Colony assessment	C	T1	T2	T3
1. just before set-up of colonies	**8855**	**8384**	**8176**	**8360**
2. end of acclimation, begin of exposure period	**6290**	**5968**	**6480**	**6352**
3. end of exposure period	**9160**	**10112**	**11968**	**9240**
4. beginning of August	**19800**	**24400**	**27000**	**25416**
5. end of August	**27240**	**24280**	**28520**	**20328**
6. end of October	**1830**	**2670**	**1056**	**1920**
7. middle of March 2012	**6270**	**3770**	**6560**	**5328**

Throughout the post-exposure observation period, there were no distinct differences when comparing the average colony brood status amongst C, T1, T2 and T3, respectively. The statistical analysis revealed a significant difference in 1 out of 15 colony assessments, where in the average number of total brood cells in T2 was significantly higher than in the control.

Colony strength after confinement period

Once the colonies were allowed to forage freely, intra-group variability increased, irrespective of the exposure group. Throughout the entire post-exposure observation period, however, there were no distinct differences when comparing the average colony strength amongst C, T1, T2 and T3, respectively. The average number of honey bees per exposure group at the last colony assessment accounted to 6079, 4053, 4752 and 4174 in C, T1, T2 and T3, respectively.

Conclusions of the author

The author concludes that the continuous exposure of honey bee colonies under confined conditions to BYI 02960 concentrations of up to and including 10000 µg as/kg diet has not resulted in adverse acute, short-term and long-term effects on mortality, colony strength and -development, brood development, food storage, honey bee behaviour, queen survival, overall hive vitality and colony health, as well as on overwintering performance.

Remarks by evaluator

The test item was unequally distributed in the sugar diet. However, based on the side-experiment which looked at the feed intake and the distribution of the a.s. in the feeders, it can be concluded that the intake achieved in the study was at least as high as the nominal concentrations.

Field history of the monitoring site (previous crops and use of pesticides) is not reported. The first colony assessment after set-up of the colonies under field conditions took place three weeks after relocation. The author states that one control colony (no. 6 tunnel no. 17) out of the five colonies was not included in the results since the colony practically collapsed at the end of confinement. Furthermore, another control colony (colony 1, 31500 bees), became the home of a swarm of bees. The authors applied a correction in numbers based on the available brood data of the consecutive colony assessments and the number of adult bees of the other C-colonies during this period. The evaluator does not agree with the inclusion of the corrected data for this colony; the colony should not be included in the control data. The low number of replicates (especially in the control, with only three remaining replicates) and the apparent variation within the treatment groups in mortality of bees/drones/immature stages make it difficult to draw conclusions on the effect of the treatment. What can be concluded is that, under the specific circumstances of this study, the different treatments of the test substance do not lead to an adverse effect in one of the observed parameters (or the combination of adverse effects of the different parameters) to such an extent, that it led to the collapse of one or more colonies.

Expert discussion in Pesticide Peer Review meeting 124 (December 2014):
(Please note that the word 'effect' in the text below might be interpreted as different from the control (i.e. may or may not include effects from the treatment), pending on the context.)

It was noted that this study design was novel and may be useful to address the uncertainties of field studies (discussed above and in the EFSA GD). The experts noted that this study ensure adequate exposure to the test item but may be unrealistic and too high. The use of this type of study could be problematic in risk assessment as the conditions are too severe to be considered as a field-type study. The study could be considered as an extended lab-type study.

This study only covers oral exposure and not contact.

The RMS noted that only 3 of the 5 control colonies survived making it not possible to detect all types of effects. The RMS considered that the study was sufficient to assess overwintering success. This was questioned since all three treatment groups were less in weight than the control (up to 33%). The RMS noted that this was explained in the study report and DAR. The apparent difference could be explained that one of the control colonies was abnormally high which was hypothesised may have been due to swarming bees coming in to the hive. This cannot be confirmed with the available data. This is problematic to consider and may affect the interpretation of the results. The RMS noted that their assessment concluded only that the treatment hives survived. No conclusion regarding the health of the treated hives could be reached given the insufficient number of controls.

The experts agreed with the assessment of the RMS indicating that the study was useful for assessing overwintering success only.

IIA 8.16.2/09

Characteristics

reference	:	Rexer, 2012c	GLP statement	:	yes (except weather data from oficial weather station and farmer information)
Type of study	:	Field Study to Determine Residues of BYI 02960 in Guttation Liquid from Winter Oil-Seed Rape (OSR) Plants in Northern Germany	Guideline	:	IVA (Beutel et al., 1992); Commission Directive 96/68/EC (1996), Working document 1607/VI/97 rev.1 (1997); OCSPP 850.SUPP
Year of execution	:	2010-2011	Dose	:	9.9 g as/kg OSR seeds; 60 g a.s./ha; sown in soil previously treated with 310 g a.s./ha
test substance	:	BYI 02960 FS 480 G formulation Batch no: 0107692-001	LOQ	:	BYI 02960 : 0.010 mg/L DFEAF : 0.010 mg/L
Species	:	Winter oil-seed rape	Acceptability Results	: :	Y Residues in guttation liquid were determined, see below

Since the formulation used in this study (seed treatment formulation BYI02960 FS 480 g) is not proposed in the EU GAP, the executive summary and the full evaluation of the study were taken from the Canadian Pesticide Management Regulatory Agency (PMRA) review of flupyradifurone (Tier 2 summary of the ecotoxicologicval studies of the plant protection product Sivanto 200 SL dd. 26/04/2013). .

EXECUTIVE SUMMARY

This study was designed to determine residues of BYI 02960 in guttation liquid, collected from winter oil-seed rape (winter OSR), grown in BYI 02960-treated soil from seeds, seed-treated with BYI 02960 FS 480 G (nominal content of a.s., 480 g BYI 02960/L). The study was carried out under field conditions in Lower-Saxony, Germany, from autumn 2010 to spring 2011. The residue analysis was an integral part of this study.

Winter oil-seed rape (OSR) seeds, seed-treated with the insecticidal seed treatment product BYI 02960 FS 480 G at a rate of actually 9.9 g BYI 02960 a.i./kg OSR seeds (nominally: 10 g a.i./kg OSR seeds), were sown on a field plot in Northern Germany, in September 2010 at a rate of 5.98 kg seeds/ha (nominally: 6 kg seeds/ha; equivalent to 60 g a.i./ha) on 07 Sep 2010. The BYI 02960-treated winter OSR seeds were additionally dressed with a standard fungicide (Thiram = TMDT). The size of the field plot was 4.2 ha.

Before the actual sowing of the BYI 02960 FS 480 G-treated OSR-seeds, a spray application with the test item BYI 02960 SL 200 G was carried out at the same day on the bare soil, followed by immediate mechanical soil incorporation into the upper 20 cm of the soil. The application rate of this spray application was 1.5 L product/ha (actually: 310 g a.i./ha). The total application is estimated to be 370 g a.i./ha when considering both application methods as the total. The last sampling day was 230 days after the application of treated seed plus soil application.

In the BYI 02960-treated field plot, droplets of guttation liquid were collected from the surface of the treated winter OSR plants. The collection of guttation liquid started as soon as the plants had emerged (BBCH 9-10) and guttation occurred. Guttation liquid was sampled in autumn 2010 on 20 dates during a period of approximately 5 weeks after emergence, and in spring 2011 on 10 dates during a period of 6 weeks before the start of flowering. The collected guttation liquid samples were analysed for residues of BYI 02960 and its metabolite BYI 02960-difluoroethylaminofuranone.

Residues of BYI 02960 above the limit of quantitation (LOQ; 0.010 mg/L) were found in 20 out of 20 analysed samples during the sampling period in autumn 2010 and ranged between 11 mg/L on the third sampling date in 2010 (20 Sep 2010; highest value during the autumn sampling period) and 0.039 mg/L on the last sampling date (19 Oct 2010; lowest value during the autumn sampling period); at the first sampling date in autumn 2010 (18 Sep 2010), the BYI 02960 concentration in guttation liquid accounted to 9.0 mg/L. Generally, residues of BYI 02960 declined by about two orders of magnitude over the 5 week autumn sampling period (2010).

Residues of BYI 02960-difluoroethylaminofuranone (BYI 02960-DFEAF) above the limit of quantitation (LOQ; 0.010 mg/L) were found in 2 out of 20 analysed samples during the sampling period in autumn 2010. The two occasions with BYI 02960-DFEAF residues >LOQ were the first and the third day of sampling (18 and 20 Sep 2010) and BYI 02960-DFEAF residues ranged between 0.020 mg/L (20 Sep 2010; highest value during the autumn sampling period) and 0.013 mg/L (18 Sep 2010; lowest value during the autumn sampling period). In all other samples from 2010, residues of BYI 02960-DFEAF were < LOQ (i.e. < 0.010 mg/L).

Residues of BYI 02960 above the limit of quantitation (LOQ; 0.010 mg/L) were found in 10 out of 10 analysed samples during the sampling period in spring 2011, and ranged between 0.21 mg/L on 05 Apr 2011 (highest value during the spring sampling period) and 0.014 mg/L on 22 Mar 2011 (lowest value during the spring sampling period).

Residues of BYI 02960-difluoroethylaminofuranone (BYI 02960-DFEAF) during the sampling period in spring 2011 were always (i.e. in 10 of 10 analysed samples) below the limit of quantitation (i.e. < LOQ; < 0.010 mg/L).

MATERIAL AND METHODS	
A. Material 1. Test materials	
Test item 1 (seed treatment): Specification number: Type: Chemical state and description: Batch No.: Master recipe ID: Tox No.: Material No.: Nominal content of active ingredient Analytical content of active ingredient: Density: Stability of test compound:	BYI 02960 FS 480 G 102000022677-01 Formulated product (seed treatment product (FS) Beige suspension 2010-001457 0107692-001 TOX 08953-00 79911750 BYI 02960: 480 g/L BYI 02960: 39.9% w/w, 474.7 g/L according to certificate of analysis 1.191 g/mL at 20 °C Expiry date: 10.03.2012
Test item 2 (spray application onto bare soil): Specification number: Type: Chemical state and description: Batch No.: Master recipe ID: Tox No.: Material No.: Nominal content of active ingredient: Analytical content of active ingredient: Density: Stability of test compound:	BYI 02960 SL 200 G 102000021884-01 Formulated product (soluble (liquid) concentrate) Clear, orange-brown liquid 2010-004097 0102142-001 TOX 09123-00 79718845 BYI 02960: 200 g/L BYI 02960: 16.9% w/w, 198.6 g/L according to certificate of analysis 1.175 g/mL at 20 °C Expiry date: 22.07.2012
Treated winter oil-seed rape seeds: Seed treatment product: Name of variety: 1000 grain weight: Tox No. of treated seeds: Batch No. of treated seeds: Nominal seed treatment rate: Analytical seed treatment rate: Chemical state and description:	BYI 02960 FS 480 G (TOX 08953-00) Titan (Hybrid) 8.6 g TOX 09124-00 2010-005474 10.0 g a.s./kg seeds 9.9 g a.s./kg seeds Red-dressed OSR seeds
2. Test organisms Species: Common name:	Not applicable Not applicable

B. Study design and methods

1. Experimental treatments

Location: The field site was located in the region Lower-Saxony, Germany.

Crop: Winter OSR seeds, seed-treated with the insecticidal seed treatment BYI 02960 FS 480 G, were sown on a field plot in Northern Germany, in autumn 2010. The size of the test field plot was 4.2 ha.

Before the actual sowing of the BYI 02960 FS 480 G-treated winter OSR-seeds, a spray application with the test item BYI 02960 SL 200 G was carried out at the same day on the bare soil of the field plot, followed by immediate mechanical soil incorporation into the upper 20 cm of the soil. The application rate of this spray application was 1.5 L product/ha (actually: 310 g a.s./ha).

The sowing rate was 5.98 kg seeds/ha.

After the end of winter OSR flowering in 2011, the whole winter OSR was destroyed.

Samples of guttation liquid were taken from the surface of several winter OSR plants and from at least 10 different locations across the test field. Droplets were collected with glass capillaries, and the guttation liquid was subsequently transferred to a micro test tube. On each sampling day, the sampling was conducted approximately at the same time of the day (in the morning).

Sampling of guttation liquid started on 18 Sep 2010 after the winter OSR plants had emerged and as soon as guttation was observed (BBCH 9-10). After the emergence of the plants and start of guttation, sampling was conducted daily on the first 7 days. After this period, three to four samplings were performed per week. In total, 20 samplings were conducted during autumn 2010.

In 2011, sampling of guttation liquid was continued. The samplings started approximately 6 weeks before flowering (full flowering was reached at the end of April). During this period, samplings followed a sporadic pattern, with 1 or 2 samplings per week. Sampling stopped when the winter OSR reached BBCH 59-60 (yellow bud stage, first flowers open). In total, 10 samplings were conducted during spring 2011.

No minimum amount of guttation liquid to be collected per sampling date was specified. The time which was spent to collect guttation droplets from the surface of the plants was less than two hours per sampling date. If more than 0.5 g guttation liquid could be sampled on one sampling date, the sample was split in two sub-samples (A and B) with at least 0.5 g remaining in sub-sample A.

Verification of sowing rates: Quantities of the sown seeds were verified by comparison of the weight of the seeds in the seed hoppers at the start and end of sowing to calculate the actually sown quantities of seeds.

Spray application technique: The application of the spray solution was carried out with spraying equipment typical for commercial applications. The test item (BYI 02960 SL 200 G) was weighed in the

laboratory. An appropriate amount of spray-tank solution was prepared immediately before each application and transported to the field site in the spraying device. After the spray application, the actually applied volume/amount of spray solution was determined by weighing the remaining spray solution.

The spray application was performed under the following conditions:

cropless soil (followed by immediate mechanical soil incorporation), just before sowing of BYI 02960-treated winter OSR seeds (BBCH 00) on the same day

air temperature less than 25 °C

average wind speed less than 3 m/s

Storage and Shipment: All collected samples were stored deep frozen (≤ -18°C) as soon as possible after sampling until residue analysis. All samples were shipped deep frozen from Niefern to the principal investigator for residue analysis Ralf Schoening, Bayer CropScience AG, Monheim, Germany. Storage and shipment conditions were recorded by use of a datalogger or min/max thermometer. The principal investigator at Bayer CropScience AG in Monheim was responsible for determining the levels of residues of BYI 02960 and BYI 02960-difluoroethylaminofuranone (BYI 02960-DFEAF) in guttation liquid samples collected from winter OSR plants.

2. Observation and measurements

Samples of guttation liquid were taken from the surface of several winter OSR plants and from at least 10 different locations across the test field. Droplets were collected with glass capillaries, and the guttation liquid was subsequently transferred to a micro test tube. On each sampling day, the sampling was conducted approximately at the same time of the day (in the morning).

The growth stage (BBCH code) of the winter OSR plants was recorded on each day of sampling.

For each day of the sampling periods in autumn 2010 and spring 2011, weather data (temperature, relative air humidity and rainfall) was collected by a nearby weather station (non-GLP records).

All collected samples were stored deep frozen (≤ -18°C) as soon as possible after sampling until residue analysis.

Residue analysis of guttation liquid from winter OSR plants was performed by using High Performance Liquid Chromatography (HPLC), coupled with electrospray and tandem mass spectrometry (MS/MS) detection according to method 01206/M001 (BCS report number MR-10/037, R. Schöning, P. Köster, 2011-03-28). The Limit of Quantitation (LOQ) of BYI 02960 and BYI 02960-DFEAF, defined as the lowest validated fortification level, was 1 µg/kg for all matrices tested in the method (bees, pollen, honey/nectar, green plant material and flowers/blossoms), respectively. The Limit of Detection (LOD)

of BYI 02960 and BYI 02960-DFEAF, for the matrices tested within the method, ranged from 0.1 to 0.2 µg/kg, respectively.

For the matrix guttation liquid, a full validation set was included in this study. The Limit of Quantitation (LOQ) of BYI 02960 and BYI 02960-DFEAF defined as the lowest validated fortification level was 10 µg/L for guttation liquid, respectively. The Limit of Detection (LOD) of BYI 02960 and BYI 02960-DFEAF was 1 µg/L for guttation liquid, respectively.

RESULTS AND DISCUSSION

Residues of BYI 02960 above the limit of quantitation (LOQ; 0.010 mg/L) were found in 20 out of 20 analysed samples during the sampling period in autumn 2010 and ranged between 11 mg/L on the third sampling date in 2010 (20 Sep 2010; highest value during the autumn sampling period) and 0.039 mg/L on the last sampling date (19 Oct 2010; lowest value during the autumn sampling period); at the first sampling date in autumn 2010 (18 Sep 2010), the BYI 02960 concentration in guttation liquid accounted to 9.0 mg/L. Generally, residues of BYI 02960 declined by about two orders of magnitude during the approximately 5 weeks lasting autumn sampling period.

Residues of BYI 02960-difluoroethylaminofuranone (BYI 02960-DFEAF) above the limit of quantitation (LOQ; 0.010 mg/L) were found in 2 out of 20 analysed samples during the sampling period in autumn 2010. The two occasions with BYI 02960-DFEAF residues >LOQ were the first and the third day of sampling (18 and 20 Sep 2010) and BYI 02960-DFEAF residues ranged between 0.020 mg/L (20 Sep 2010; highest value during the autumn sampling period) and 0.013 mg/L (18 Sep 2010; lowest value during the autumn sampling period). In all other samples from 2010, residues of BYI 02960-DFEAF were < LOQ (i.e. < 0.010 mg/L).

Residues of BYI 02960 above the limit of quantitation (LOQ; 0.010 mg/L) were found in 10 out of 10 analysed samples during the sampling period in spring 2011, and ranged between 0.21 mg/L on 05 Apr 2011 (highest value during the spring sampling period) and 0.014 mg/L on 22 Mar 2011 (lowest value during the spring sampling period).

Residues of BYI 02960-difluoroethylaminofuranone (BYI 02960-DFEAF) during the sampling period in spring 2011 were always (i.e. in 10 of 10 analysed samples) below the limit of quantitation (i.e. < LOQ; < 0.010 mg/L).

Table 10.4.5.3-1: Residues of BYI 02960 and BYI 02960-DFEAF in samples of guttation liquid

Sample	Origin	Date of sampling	Residue [mg/L]	
			BYI 02960 (min - max)	BYI 02960-DFEAF (min - max)
Guttation liquid	Winter-OSR seedlings/ plants, grown from BYI 02960960 dressed	18 September 2010 - 19 October 2010	0.039 - 11.0	< LOQ - 0.020

	seeds, sown in BYI 02960 treated soil	15 March 2011 -25 April 2011	0.014 - 0.21	< LOQ

Residues of BYI 02960and its metabolite BYI 02960-DFEAF are given as a range of the minimum and maximum values as obtained throughout different time periods (several weeks in autumn / spring, respectively) during which the samples had been collected
BYI 02960-DFEAF = BYI 02960-difluoroethylaminofuranone (BCS-CC98193),
LOQ = Limit of Quantitation = 10 µg/L in guttation liquid (BYI 02960 and BYI 02960-DFEAF)

Study Reliability and Discussion of Deficiencies

This study is considered fully reliable. No deficiency is noted.

CONCLUSION

A field study was conducted to determine residues of BYI 02960 in guttation liquid, collected from winter oil-seed rape (winter OSR), grown in BYI 02960-treated soil from seeds, seed-treated with BYI 02960 FS 480 G.

Residues of BYI 02960 above the limit of quantitation (LOQ; 0.010 mg/L) were found in 20 out of 20 analysed samples in autumn 2010 (quantifiable residues ranging from 11 mg/L to 0.039 mg/L). Generally, residues of BYI 02960 declined by about two orders of magnitude during the approximately 5 weeks lasting autumn sampling period.

Residues of BYI 02960 above the limit of quantitation (LOQ; 0.010 mg/L) were found in 10 out of 10 analysed samples in spring 2011; (quantifiable residues ranging from 0.21 mg/L to 0.014 mg/L).

Residues of the metabolite BYI 02960-DFEAF were < LOQ (i.e. < 0.010 mg/L) except for 2 out of 10 analysed samples (collected in the first and third day of sampling; quantifiable residues ranging from 0.020 mg/L to 0.013 mg/L) in autumn 2010 and for all samples (10 of 10) collected in spring 2011, respectively.

IIA 8.16.2/10

Characteristics

reference	:	Rexer, 2012d	GLP statement	:	yes
Type of study	:	Field Study to Determine Residues of BYI 02960 in Guttation Liquid from Winter Oil-Seed Rape (OSR) Plants in France	Guideline	:	IVA (Beutel et al., 1992); Commission Directive 96/68/EC (1996), Working document 1607/VI/97 rev.1 (1997); OCSPP 850.SUPP
Year of execution	:	2010-2011	Dose	:	10.11 g as/kg OSR seeds; 69.7 g a.s./ha; sown in soil previously treated with 311 g a.s./ha
test substance	:	BYI 02960 FS 480 G formulation Batch no: 2010-001457	LOQ	:	BYI 02960 : 0.010 mg/L DFEAF : 0.010 mg/L
Species	:	Winter oil-seed rape	Acceptability Results	: :	Y Residues in guttation liquid were determined, see below

Since the formulation used in this study (seed treatment formulation BYI02960 FS 480 g) is not proposed in the EU GAP, the executive summary and the full evaluation of the study were taken from the Canadian Pesticide Management Regulatory Agency (PMRA) review of flupyradifurone (Tier 2 summary of the ecotoxicological studies of the plant protection product Sivanto 200 SL dd. 26/04/2013).

EXECUTIVE SUMMARY

This study was designed to determine residues of BYI 02960 in guttation liquid, collected from winter oil-seed rape (winter OSR), grown in BYI 02960-treated soil from seeds, seed-treated with BYI 02960 FS 480 G (nominal content of a.s., 480 g BYI 02960/L). The study was carried out under field conditions in Alsace, France, from autumn 2010 to spring 2011. The residue analysis was an integral part of this study.

Winter oil-seed rape (OSR) seeds, seed-treated with the insecticidal seed treatment product BYI 02960 FS 480 G at a rate of actually 10.11 g BYI 02960 a.s./kg OSR seeds (nominally: 10 g a.s./kg OSR seeds), were sown on a field plot in Alsace, France, in September 2010 at a rate of actually 6.89 kg seeds/ha (nominally 6 kg seeds/ha) on 14 Sep 2010. The BYI 02960-treated winter OSR seeds were additionally dressed with a standard fungicide (Thiram = TMDT). The size of the field plot was approximately 4.5 ha.

Before the actual sowing of the BYI 02960 FS 480 G-treated OSR-seeds, a spray application with the test item BYI 02960 SL 200 G was carried out at the same day on the bare soil, followed by immediate mechanical soil incorporation into the upper 15-20 cm of the soil. The application rate of this spray application was 1.5 L product/ha (actually: 311 g a.s./ha).

In the BYI 02960-treated field plot, droplets of guttation liquid were collected from the surface of the treated winter OSR plants. The collection of guttation liquid started as soon as the plants had emerged (BBCH 9-11) and guttation occurred. Guttation liquid was sampled in autumn 2010 on 20 dates during a period of approximately 5 weeks after emergence, and in spring 2011 on 8 dates during a period of approximately 3 weeks before the start of flowering. The collected guttation liquid samples were analysed for residues of BYI 02960 and its metabolite BYI 02960-difluoroethylaminofuranone (BYI 02960-DFEAF) using HPLC with electrospray and MS/MS-detection.

Residues of BYI 02960 above the limit of quantitation (LOQ; 0.010 mg/L) were found in 20 out of 20 analysed samples during the sampling period in autumn 2010 and ranged between 21 mg/L on the first sampling date in 2010 (26 Sep 2010; highest value during the autumn sampling period) and 0.087 mg/L on 20 Oct 2010 (lowest value during the autumn sampling period); at the last sampling date in autumn 2010 (29 Oct 2010), the BYI 02960 concentration in guttation liquid accounted to 0.26 mg/L. Generally, residues of BYI 02960 declined by about two orders of magnitude during the approximately 5 weeks lasting autumn sampling period.

Residues of BYI 02960-difluoroethylaminofuranone (BYI 02960-DFEAF) above the limit of quantitation (LOQ; 0.010 mg/L) were found in 3 out of 20 analysed samples during the sampling period in autumn 2010. The three occasions with BYI 02960-DFEAF residues >LOQ were the first three days of sampling (26 - 28 Sep 2010) and BYI 02960-DFEAF residues ranged between 0.023 mg/L (28 Sep 2010; highest value during the autumn sampling period) and 0.014 mg/L (27 Sep 2010; lowest value during the autumn sampling period). In all other samples from 2010, residues of BYI 02960-DFEAF were < LOQ (i.e. < 0.010 mg/L). Residues of BYI 02960 above the limit of quantitation (LOQ; 0.010 mg/L) were found in 7 out of 8 analysed samples during the sampling period in spring 2011, and the quantifiable residues ranged between 0.15 mg/L on the last sampling date in 2011 (09 Apr 2011; highest value during the spring sampling period) and 0.062 mg/L on 18 Mar 2011. Residues of BYI 02960-difluoroethylaminofuranone (BYI 02960-DFEAF), during the sampling period in spring 2011, were always (i.e. in 8 of 8 analysed samples) below the limit of quantitation (i.e. <LOQ; <0.010 mg/L).

MATERIAL AND METHODS	
A. Material 1. Test materials	
Test item 1 (seed treatment): Specification number: Type: Chemical state and description: Batch No.: Master recipe ID: Tox No.: Material No.: Nominal content of active ingredient Analytical content of active ingredient: Density: Stability of test compound:	BYI 02960 FS 480 G 102000022677-01 Formulated product (seed treatment product (FS) Beige suspension 2010-001457 0107692-001 TOX 08953-00 79911750 BYI 02960: 480 g/L BYI 02960: 39.9% w/w, 474.7 g/L according to certificate of analysis 1.191 g/mL at 20 °C Expiry date: 10.03.2012
Test item 2 (spray application onto bare soil): Specification number: Type: Chemical state and description: Batch No.: Master recipe ID: Tox No.: Material No.: Nominal content of active ingredient: Analytical content of active ingredient: Density: Stability of test compound:	BYI 02960 SL 200 G 102000021884-01 Formulated product (soluble (liquid) concentrate) Clear, orange-brown liquid 2010-004097 0102142-001 TOX 09123-00 79718845 BYI 02960: 200 g/L BYI 02960: 16.9% w/w, 198.6 g/L according to certificate of analysis 1.175 g/mL at 20 °C Expiry date: 22.07.2012

Treated winter oil-seed rape seeds: Seed treatment product: Name of variety: 1000 grain weight: Tox No. of treated seeds: Batch No. of treated seeds: Nominal seed treatment rate: Analytical seed treatment rate: Chemical state and description:	BYI 02960 FS 480 G (TOX 08953-00) PR45D03 (Hybrid) 9.0 g TOX 09125-00 2010-005473 10.0 g a.s./kg seeds 10.1 g a.s./kg seeds Blue violet-dressed OSR seeds
2. Test organisms Species: Common name:	Not applicable Not applicable

B. Study design and methods

1. Experimental treatments

Location: The field site was located in the region Bas-Rhin, Alsace, France.

Crop: Winter OSR seeds, seed-treated with the insecticidal seed treatment BYI 02960 FS 480 G, were sown on a field plot in Alsace, France, in autumn 2010. The size of the test field plot was approximately 4.5 ha.

Before the actual sowing of the BYI 02960 FS 480 G-treated winter OSR-seeds, a spray application with the test item BYI 02960 SL 200 G was carried out at the same day on the bare soil of the field plot, followed by immediate mechanical soil incorporation into the upper 15-20 cm of the soil. The application rate of this spray application was 1.5 L product/ha (actually: 311 g a.s./ha).

The sowing rate was 6.89 kg seeds/ha.

After the end of winter OSR flowering in 2011, the winter OSR was destroyed.

Samples of guttation liquid were taken from the surface of several winter OSR plants and from at least 10 different locations across the test field. Droplets were collected with glass capillaries, and the guttation liquid was subsequently transferred to a micro test tube. On each sampling day, the sampling was conducted approximately at the same time of the day (in the morning).

Sampling of guttation liquid started on 26 Sep 2010 after the winter OSR plants had emerged and as soon as guttation was observed (BBCH 9-11). After the emergence of the plants and start of guttation, sampling was conducted daily on the first 6 days. After this period, approximately three samplings were performed per week. In total, 20 samplings were conducted during autumn 2010.

In 2011, sampling of guttation liquid was continued. The samplings started approximately 4 weeks before flowering (full flowering was reached in the middle of April). During this period, samplings followed a sporadic pattern, with 1 to 2 samplings per week. The last 4 samplings were conducted daily. Sampling stopped when the winter OSR reached BBCH 59-60 (yellow bud stage, first flowers open). In total, 8 samplings were conducted during spring 2011.

No minimum amount of guttation liquid to be collected per sampling date was specified. The time which was spent to collect guttation droplets from the surface of the plants did not exceed two hours per sampling date. If more than 0.5 g guttation liquid could be sampled on one sampling date, the sample was split in two sub-samples (A and B) with at least 0.5 g remaining in sub-sample A.

Verification of sowing rates: Quantities of the sown seeds were verified by comparison of the weight of the seeds in the seed hoppers at the start and end of sowing to calculate the actually sown quantities of seeds.

Spray application technique: The application of the spray solution was carried out with spraying equipment typical for commercial applications. The test item (BYI 02960 SL 200 G) was weighed in the laboratory. An appropriate amount of spray-tank solution was prepared immediately before each application and transported to the field site in the spraying device. After the spray application, the actually applied volume/amount of spray solution was determined by weighing the remaining spray solution.

The spray application was performed under the following conditions:

cropless soil (followed by immediate mechanical soil incorporation), just before sowing of BYI 02960-treated winter OSR seeds (BBCH 00) on the same day
air temperature less than 25 °C

average wind speed less than 3 m/s

Storage and Shipment: All collected samples were stored deep frozen (≤ -18°C) as soon as possible after sampling until residue analysis. All samples were shipped deep frozen from Niefern to the principal investigator for residue analysis Ralf Schoening, Bayer CropScience AG, Monheim, Germany. Storage and shipment conditions were recorded by use of a datalogger or min/max thermometer.
The principal investigator at Bayer CropScience AG in Monheim was responsible for determining the levels of residues of BYI 02960 and BYI 02960-difluoroethylaminofuranone (BYI 02960-DFEAF) in guttation liquid samples collected from winter OSR plants.

2. Observation and measurements
Samples of guttation liquid were taken from the surface of several winter OSR plants and from at least 10 different locations across the test field. Droplets were collected with glass capillaries, and the guttation liquid was subsequently transferred to a micro test tube. On each sampling day, the sampling was conducted approximately at the same time of the day (in the morning).
The growth stage (BBCH code) of the winter OSR plants was recorded on each day of sampling.
For each day of the sampling periods in autumn 2010 and spring 2011, weather data (temperature, relative air humidity and rainfall) was collected by a nearby weather station (non-GLP records).

All collected samples were stored deep frozen (≤ -18°C) as soon as possible after sampling until residue analysis.
Residue analysis of guttation liquid from winter OSR plants was performed by using High Performance Liquid Chromatography (HPLC), coupled with electrospray and tandem mass spectrometry (MS/MS) detection according to method 01206/M001 (BCS report number MR-10/037, R. Schöning, P. Köster, 2011-03-28). The Limit of Quantitation (LOQ) of BYI 02960 and BYI 02960-DFEAF, defined as the lowest validated fortification level, was 1 µg/kg for all matrices tested in the method (bees, pollen, honey/nectar, green plant material and flowers/blossoms), respectively. The Limit of Detection (LOD) of BYI 02960 and BYI 02960-DFEAF, for the matrices tested within the method, ranged from 0.1 to 0.2 µg/kg, respectively.
For the matrix guttation liquid, a full validation set was included in this study. The Limit of Quantitation (LOQ) of BYI 02960 and BYI 02960-DFEAF defined as the lowest validated fortification level was 10 µg/L for guttation liquid, respectively. The Limit of Detection (LOD) of BYI 02960 and BYI 02960-DFEAF was 1 µg/L for guttation liquid, respectively.

RESULTS AND DISCUSSION

Residues of BYI 02960 above the limit of quantitation (LOQ; 0.010 mg/L) were found in 20 out of 20 analysed samples during the sampling period in autumn 2010 and ranged between 21 mg/L on the first sampling date in 2010 (26 Sep 2010; highest value during the autumn sampling period) and 0.087 mg/L on 20 Oct 2010 (lowest value during the autumn sampling period); at the last sampling date in autumn 2010 (29 Oct 2010), the BYI 02960 concentration in guttation liquid accounted to 0.26 mg/L. Generally, residues of BYI 02960 from the spring declined by about two orders of magnitude relative to the fall sampling period.
Residues of BYI 02960-difluoroethylaminofuranone (BYI 02960-DFEAF) above the limit of quantitation (LOQ; 0.010 mg/L) were found in 3 out of 20 analysed samples during the sampling period in autumn 2010. The three occasions with BYI 02960-DFEAF residues >LOQ were the first three days of sampling (26 - 28 Sep 2010) and BYI 02960-DFEAF residues ranged between 0.023 mg/L (28 Sep 2010; highest value during the autumn sampling period) and 0.014 mg/L (27 Sep 2010; lowest value during the autumn sampling period). In all other samples from 2010, residues of BYI 02960-DFEAF were < LOQ (i.e. < 0.010 mg/L).
Residues of BYI 02960 above the limit of quantitation (LOQ; 0.010 mg/L) were found in 7 out of 8 analysed samples during the sampling period in spring 2011, and the quantifiable residues ranged between 0.15 mg/L on the last sampling date in 2011 (09 Apr 2011; highest value during the spring sampling period) and 0.062 mg/L on 18 Mar 2011.
Residues of BYI 02960-difluoroethylaminofuranone (BYI 02960-DFEAF), during the sampling period in spring 2011, were always (i.e. in 8 of 8 analysed samples) below the limit of quantitation (i.e. <LOQ; <0.010 mg/L).

Details of the analytical results as obtained by analysing samples of OSR guttation fluid are given in an Analytical Phase Report (Appendix A9 of the study report).

Table 10.4.5.5-1: Residues of BYI 02960 and BYI 02960-DFEAF in samples of guttation liquid

Sample		Origin	Date of sampling	Residue [mg/L]	
				BYI 02960 (min - max)	BYI 02960-DFEAF (min - max)
Guttation liquid	Winter-OSR seedlings/ plants, grown from BYI 02960 dressed seeds, sown in BYI 02960 treated soil		26 September 2010 - 29 October 2010	0.087 - 21.0	< LOQ - 0.023
			18 March 2011 -09 April 2011	< LOQ - 0.15	< LOQ

Residues of BYI 02960and its metabolite BYI 02960-DFEAF are given as a range of the minimum and maximum values as obtained throughout different time periods (several weeks in autumn / spring, respectively) during which the samples had been collected
BYI 02960-DFEAF = BYI 02960-difluoroethylaminofuranone (BCS-CC98193),

LOQ = Limit of Quantitation = 10 µg/L in guttation liquid (BYI 02960 and BYI 02960-DFEAF

B. Study Reliability and Discussion of Deficiencies

This study is considered fully reliable. No deficiency was noted.

Conclusion

A field study was conducted to determine residues of BYI 02960 in guttation liquid, collected from winter oil-seed rape (winter OSR), grown in BYI 02960-treated soil from seeds, seed-treated with BYI 02960 FS 480 G.

Residues of BYI 02960 above the limit of quantitation (LOQ; 0.010 mg/L) were found in 20 out of 20 analysed samples in autumn 2010 (quantifiable residues ranging from 21 mg/L to 0.087 mg/L). Generally, residues of BYI 02960 declined by about two orders of magnitude during the approximately 5 weeks lasting autumn sampling period.

Residues of BYI 02960 above the limit of quantitation (LOQ; 0.010 mg/L) were found in 7 out of 8 analysed samples in spring 2011; (quantifiable residues ranging from 0.15 mg/L to 0.062 mg/L).

Residues of the metabolite BYI 02960-DFEAF were < LOQ (i.e. < 0.010 mg/L) except for 3 out of 8 analysed samples (collected in the first three days of sampling) in autumn 2010 and for all samples (8 of 8) collected in spring 2011, respectively.

B.9.4.4 Risk assessment (IIIA 10.4.1)

Procedures for risk assessment were in agreement with the recommendations in the Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (Working Document Sanco/10329/2002 rev 2 final, 17 October 2002, referring to EPPO (EPPO 170 and EPPO 2002). EPPO 2002 was updated with EPPO 2010[9], an update focussing especially on systemic substances.

Exposure to honeybees is not relevant for the glasshouse use in lettuce (lettuce for food production needs no pollination therefore honeybees are not expected to be introduced in or be attracted to lettuce production in glasshouse).

Exposure is relevant for the field uses in hops and lettuce. The crops themselves are not attractive to honeybees for nectar and/or pollen collection. However, honey bees may be present in the field to forage on flowering weeds; bees foraging off-field may be exposed via spray drift. Furthermore, potential exposure routes are via honeydew, succeeding crops and guttation and other drinking water sources.

B.9.4.4.1 Acute toxicity data and first-tier acute risk assessment

Below the results of laboratory testing with honeybees are summarised.

Table B.9.4.4-01 Acute toxicity of technical BYI 02960 to honey bees in the laboratory

Test substance	Ecotoxicological Endpoint: LD_{50}		Reference
BYI 02960 (tech.)	oral 24 h oral 48 h contact 24 h contact 48 h contact 72 h contact 96 h	1.3 µg a.i./bee **1.2 µg a.i./bee** >200 µg a.i./bee >200 µg a.i./bee 158.4 µg a.i./bee **122.8 µg a.i./bee**	Schmitzer & Sekine (2008) KIIA 8.7.1/01
	Ecotoxicological Endpoint: NOED		
	oral contact	0.68 µg a.i./bee* 25 µg a.i./bee	

Bold values: Endpoints considered relevant for risk assessment

*The NOED for mortality is 0.68 µg a.i./bee. However, discoordinated movements and aparthy occurred at this and all higher dose rates. The $NOED_{oral}$ for sublethal effects is therefore set at 0.34 µg a.i./bee. The $NOED_{contact}$ is 25 µg a.i./bee also for sublethal effects.

[9] EPPO 2010: Environmental risk assessment scheme for plant protection products. Chapter 10: Honeybees. OEPP/EPPO, Bulletin OEPP/EPPO Bulletin 40, 323–331

Table B.9.4.4-02 Acute toxicity data of the formulated product BYI 02960 SL 200 G to honey bees in the laboratory

Test substance	Ecotoxicological Endpoint: LD_{50}		Reference
BYI 02960 SL 200 G	oral 24 h oral 48 h contact 24 h contact 48 h contact 72 h	3.2 µg a.i./bee **3.2 µg a.i./bee** 21.5 µg a.i./bee 17.1 µg a.i./bee **15.7 µg a.i../bee**	Schmitzer (2009) KIIIA1 10.4.2.1/01
	Ecotoxicological Endpoint: NOED		
	oral contact	2.6 µg a.i./bee 6.3 µg a.i./bee	

Bold values: Endpoints considered relevant for risk assessment

The acute contact toxicity of the formulaton to bumble bees was also tested, see the table below.

Table B.9.4.4-03 Acute toxicity of of the formulated product BYI 02960 SL 200 G to bumble bees in the laboratory

Test substance	Ecotoxicological Endpoint: LD_{50}		Reference
Flupyradifurone SL 200 G	contact 48 h	>100 µg a.i./bee	Vergé (2012) KIIA 8.7.1/07
	Ecotoxicological Endpoint: NOED		
	contact	≥100 µg a.i./bee	

In the plant metabolism studies, a range of metabolites have been measured in the reproductive plant organs (flowers). The observed metabolites were tested in acute and chronic toxicity studies for potential effects on honey bees. Table B.9.4.4-04 provides an overview of the investigated metabolites, including the parent compound. Results of the acute toxicity studies are provided in Table B.9.4.4-05.

Table B.9.4.4-04 Overview table on names and synonyms of compounds addressed within the bee chapter

Abbreviation / Chemical code	Alternative name
BYI 02960	Flupyradifurone
BYI 02960-DFEAF	BYI 02960-difluoroethyl-amino-furanone
BYI 02960-OH	BYI 02960-hydroxy
DFA	Difluoroacetic acid
6-CNA	6-chloronicotinic acid
BYI 02960 -CHMP	6-chloropicolyl alcohol, 6-CPA

Table B.9.4.3-05 Acute toxicity of BYI 02960 metabolites to honey bees in the laboratory

Test substance	Ecotoxicological Endpoint	Value	Reference
BYI 02960-DFEAF	LD_{50} - oral 48 h LD_{50} - contact 48 h NOED - oral 48 h NOED - contact 48 h	**>81.5 µg/bee** **>100 µg/bee** ≥81.5 µg/bee ≥100 µg/bee	Schmitzer, 2010 KIIA 8.7.1/02
BYI 02960-OH	LD_{50} - oral 48 h LD_{50} - contact 48 h NOED - oral 48 h NOED - contact 48 h	**>105.3 µg/bee** **>100 µg/bee** ≥105.3 µg/bee ≥100 µg/bee	Schmitzer, 2011 KIIA 8.7.1/03
DFA	LD_{50} - oral 48 h LD_{50} - contact 48 h NOED - oral 48 h NOED - contact 48 h	**>107.9 µg/bee** **>100 µg/bee** ≥107.9 µg/bee ≥100 µg/bee	Schmitzer, 2010 KIIA 8.7.1/04
6-CNA	LD_{50} - oral 48 h LD_{50} - contact 48 h NOED - oral 48 h NOED - contact 48 h	**>107.1 µg/bee** **>100 µg/bee** ≥107.1 µg/bee ≥100 µg/bee	Schmitzer, 2010 KIIA 8.7.1/05
BYI 02960 -CHMP	LD_{50} - oral 48 h LD_{50} - contact 48 h NOED - oral 48 h NOED - contact 48 h	**>106.7 µg/bee** **>100 µg/bee** ≥106.7 µg/bee ≥100 µg/bee	Schmitzer, 2010 KIIA 8.7.1/06

Bold values: Endpoints considered relevant for risk assessment

An indication of hazard (Hazard Quotient or HQ) can be derived according to the EPPO risk assessment scheme, by calculating the ratio between the single application rate (expressed in g/ha) and the lowest laboratory contact and oral LD50 (expressed in µg/bee).

HQ_O and HQ_C resp. = Application rate [g/ha] / LD50 oral or LD50 contact [µg/bee]

HQ values are calculated using data from the studies performed with technical material and the corresponding formulated product. HQ values higher than 50 indicate the need of higher tiered tests to clarify the actual risk to honey bees.

Table B.9.4.4-06 Hazard quotients for bees – oral exposure

Crop	Exposure route	LD_{50} [µg a.i./bee]	Application rate [g/ha]	Hazard quotient HQ_O	Trigger	Refined risk assessment
BYI 02960 SL 200						
Hops	oral	3.2	150	47	50	No
Lettuce	oral	3.2	125	39	50	No
off-field						
Hops	oral	3.2	150*0.1933	9.1	50	No
Lettuce	oral	3.2	125*0.0277	1.1	50	No
BYI 02960 (technical)						
Hops	oral	1.2	150	**125**	50	**Yes**
Lettuce	oral	1.2	125	**104**	50	**Yes**
off-field						
Hops	oral	1.2	150*0.1933	24	50	No
Lettuce	oral	1.2	125*0.0277	3.5	50	No

The hazard quotients for oral exposure calculated for BYI 02960 technical exceed the empirical trigger value for higher tier testing (HQ_O > 50) for both uses. Therefore, a refined risk assessment will be conducted, see below (higher tier semi-field and field studies).
Because a risk is indicated based on the full in-field dose, the off-field risk is also calculated. The default drift rates of the next section (non-target arthropods other than bees) are used to calculate the exposure that may occur on the off-field area. Based on these default drift rates and the trigger of 50, an off-field acute risk is not indicated.

Table B.9.4.4-07 Hazard quotients for bees – contact exposure

Crop	Exposure route	LD_{50} [µg a.i./bee]	Application rate [g/ha]	Hazard quotient HQ_O	Trigger	Refined risk assessment
BYI 02960 SL 200						
Hops	contact	15.7	150	9.6	50	No
Lettuce	contact	15.7	125	8.0	50	No
BYI 02960 (technical)						
Hops	contact	122.8	150	1.22	50	No
Lettuce	contact	122.8	125	1.02	50	No

The hazard quotient for contact exposure is below the empirical trigger value for higher tier testing (HQ_C <50) for both envisaged uses. Therefore, no unacceptable acute risk to bees is to be expected via the contact route of exposure.

Table B.9.4.4-04 shows that all relevant plant metabolites have a very low acute toxicity to honeybees (all LD50 values >100 µg a.i./bee). Therefore, the acute risk of these metabolites is low.

The bumble bee contact toxicity test does not indicate concern about higher sensitivity of bumble bees than honey bees.

B.9.4.4.2 Chronic toxicity data

There are currently no harmonised and ring tested test guidelines available to assess the chronic risk to adult honey bees. Nonetheless, there is some experience within the European honey bee testing community on conducting chronic studies in adult honey bees, by exposing honey bees orally to a treated 50% (w/v) sucrose solution as an exclusive food source for a period of 10 consecutive days by continuous and ad libitum feeding. Since the originally submitted chronic study with the a.s. was deemed unacceptable die to high control mortality and uncertainty about sublethal effects, the notifier performed a new chronic study with the a.s. Table B.9.4.4-08 provides an overview of the results obtained with parent BYI 02960 and its metabolites.

Table B.9.4.4-08 Chronic toxicity to adult honey bees in the laboratory

Test substance	Ecotoxicological Endpoint: NOEC / NOED		Reference
BYI 02960	NOEC (nominal) NOED (nominal)	26000 µg a.i./L 0.79 µg a.i./bee/day	Gladbach, Theis et al., 2013 KIIA 8.16.1/09
BYI 02960-DFEAF	NOEC (nominal) NOED (nominal)	≥ 10000 µg a.i./L ≥ 0.435 µg a.i./bee/day	Kling, 2012, KIIA 8.16.1/02
BYI 02960-OH	NOEC (nominal) NOED (nominal)	≥ 10000 µg a.i./L ≥ 0.420 µg a.i./bee/day	Kling, 2012, KIIA 8.16.1/03
DFA	NOEC (nominal) NOED (nominal)	≥ 10000 µg a.i./L ≥ 0.379 µg a.i./bee/day	Kling, 2012, KIIA 8.16.1/04
6-CNA	NOEC (nominal) NOED (nominal)	≥ 10000 µg a.i./L ≥ 0.418 µg a.i./bee/day	Kling, 2012, KIIA 8.16.1/05
BYI 02960-CHMP	NOEC (nominal) NOED (nominal)	≥ 10000 µg a.i./L ≥ 0.413 µg a.i./bee/day	Kling, 2012, KIIA 8.16.1/06

The table shows that all relevant plant metabolites show very low chronic toxicity to honeybees.

The a.s. has chronic mortality endpoints very close to the acute mortality endpoints, see Table B.9.4.4-09. Thus, the toxicity of the a.s. is not increased when the substance is taken in for several consecutive days; there is no concern about chronic/delayed toxicity with regard to mortality.

Table B.9.4.4-09 Comparison of acute and chronic endpoints for mortality in adult honey bees

	NOEDoral	$LD(D)_{50}$
Studies with technical grade active substance	[µg a.s./bee(/day)]	[µg a.s./bee(/day)]
Acute adult toxicitv studv (48 hours)	0.68	1.2
Chronic adult toxicitv studv (10 davs)	0.79	1.83

As explained above, the foliage residue toxicity study KIIIA 10.4.3/01 was not evaluated by RMS because guidance on how to perform, evaluate and use this study in the risk assessment is not available in EU.

Chronic toxicity of BYI 02960 to honey bee larvae
There are currently no harmonised and ring tested test guidelines available to assess the chronic risk to honey bee larvae. Nonetheless, there is experience in Europe within some laboratories on conducting chronic studies in honey bee larvae. A study has been performed with the active substance.

Table B.9.4.4-10 Chronic toxicity of BYI 02960 honey bee larvae in the laboratory

Test substance	Ecotoxicological Endpoint: NOEC / NOED		Reference
BYI 02960	NOEC (nominal) NOED (nominal)	≥ 10000 µg a.i./kg larval diet ≥ 0.44 µg a.i./bee larvae/day	Nikolakis *et al.*, 2010 KIIIA1 10.4.6.1/01

The chronic toxicity studies with the metabolites show no effects to adult honeybees at the highest tested dose of 10 mg/L diet. The chronic toxicity study with the a.s. shows no effects to honeybee larvae at the highest tested dose of 10 mg/kg diet. Only in the chronic toxicity study on adult honeybees with the a.s., which was performed at higher doses than the other studies, effects were seen. From this study, a NOEC of 26 mg a.i./L/ 0.79 µg a.i./bee/day could be derived. These endpoints could be used in risk calculations based on daily intake values for different castes of honeybees. This is discussed below.

B.9.4.4.3 Residue measurements in nectar and pollen and other matrices

B.9.4.4.3.1 Residue measurements in *Phacelia*

An overview of the measured residues of the a.s. and metabolite DFEAF in the semi-field trials on *Phacelia* in the different matrices was compiled by RMS in Tables B.9.4.4-11-16. In these studies, the first foliar spray applications was done just before flowering and the second foliar spray application was done during full flowering.

Table 9.4.4-11 Residues of BYI02960 and metabolite in **flowers**

Study	Application rate	DALA	Residue BYI 02960 (µg/kg) mean (individual samples)	Residue BYI 02960-DFEAF (µg/kg) mean (individual

				samples)
KIIIA 10.4.7/03	2x200 g a.s./ha (foliar spray)	0	12729 (Subsample A: 7534-16308-13098; Subsample B: 9925-17310-12199)	11 (Subsample A: 7 – 12 - 11; Subsample B: 9-16-13)
		7	993 (Subsample A: 327-3141-729; Subsample B: 238-1381-143)	25 (Subsample A: 8-44-22; Subsample B: 12-52-13)
KIIIA 10.4.7/04	1x 300 (soil appl.) and 2x 200 g a.s./ha (foliar spray)	0	37592	78
		7	199	9
KIIIA 10.4.7/05	1x 300 (soil appl.) and 2x 200 g a.s./ha (foliar spray)	0	14907 (20083-9533-15110)	60 (37-18-126)
		7	2356 (701-3590-2778)	23 (9-35-26)

Table 9.4.4-12 Residues of BYI02960 and metabolite in **pollen from forager bees**

Study	Application rate	DALA	Residue BYI 02960 (µg/kg) mean (individual samples)	Residue BYI 02960-DFEAF (µg/kg) mean (individual samples)
IIA 8.16.2/08	2x 200 g a.s./ha (foliar spray)	-1	148 (138-146-162)	2.2 (2.2-2.0-2.5)
		0 (+1h)	64743 (59696-69911-64622)	76 (68-86-74)
		0 (+2.5h)	63271 (53137-70667-66009)	101 (92-110-102)
		0 (+4h)	36126 (34628-31623-42126)	120 (116-135-110)
		1	10354 (6469-8481-16113)	57 (36-43-92)
		3	570 (779-410-522)	8.2 (9.4-6.7-8.4)
		4	773 (855-662-803)	9.7 (7.9-9.3-12)
		5	877 (941-714-976)	9.1 (7.8-7.4-12)
		6	925 (605-1041-1128)	7.7 (5.3-8.0-9.7)
		7	766 (410-795-1092)	6.0 (3.2-6.0-8.9)
		8	688 (572-728-763)	6.0 (5.1-6.3-6.7)
		9	368 (174-460-469)	3.0 (1.3-3.3-4.5)
		10	553 (636-377-647)	6.2 (11-2.8-4.9)

Table 9.4.4-13 Residues of BYI02960 and metabolite in **pollen from combs**

Study	Application rate	DALA	Residue BYI 02960 (µg/kg) mean (individual samples)	Residue BYI 02960-DFEAF (µg/kg) mean (individual samples)
KIIIA 10.4.7/04	1x 300 (soil appl.) and 2x 200 g a.s./ha (foliar spray)	7	5759 (3025-6109-8144)	13 (7-13-20)
KIIIA 10.4.7/06	2x 200 g a.s./ha (foliar spray)	27	3.5 (3-4-4-2-4-4)	<1 (6 samples)
KIIA 8.16.2/08	2x 200 g a.s./ha (foliar spray)	-1	194 (240-175-166)	2.1(2.9-1.9-1.7)
		0	5910 (6691-1545-9495)	4.7 (4.8-3.1-6.1)
		1	6433 (11260-5021-3019)	7.3 (12-6.1-3.9)
		2	4836 (4356-7009-3142)	15 (13-20-13)

		3	7912 (13783-4451-5503)	19 (26-16-14)
		4	10171 (7381-22529-603)	29 (22-46-19)
		5	4117 (6194-5171-985)	18.6 (20-28-7.7)
		6	3042 (1807-6017-1301)	15 (13-20-12)
		7	3277 (2943-5249-1640)	14 (12-18-11)
		8	2477 (1915-4491-1026)	14 (14-18-11)
		9	3565 (3634-4495-2566)	14 (14-16-11)
		10	1805 (1580-2115-1719)	11 (12-10-10)
		12	3591 (5791-4843-139)	9.3 (14-12-1.9)
		18	2893 (2671-3115)	7.7 (6.8-8.6)
		24	1709 (2273-1145)	5.6 (9.1-2.1)

Table 9.4.4-14 Residues of BYI02960 and metabolite in **nectar from forager bees**

Study	Application rate	DALA	Residue BYI 02960 (µg/kg) mean (individual samples)	Residue BYI 02960-DFEAF (µg/kg) mean (individual samples)
IIA 8.16.2/08	2x 200 g a.s./ha (foliar spray)	-1	38 (7-59-48)	<LOQ (3 samples)
		0 (+1h)	3048 (2777-3304-3064)	65 (62-65-69)
		0 (+2.5h)	2265 (2038-2031-2726)	89 (87-85-96)
		0 (+4h)	1435 (1235-1709-1360)	104 (92-122-98)
		1	752 (699-871-687)	77 (58-79-95)
		2	337 (301-331-379)	13 (11.2-17-12)
		3	158 (150-148-175)	9.4 (8.6-9.7-10)
		4	223 (191-223-254)	12 (9.7-12-13)
		5	118 (102-119-132)	8.1 (6.9-8.2-9.3)
		6	57 (54-57-60)	4.4 (4.1-4.5-4.5)
		7	41 (37-39-46)	2.9 (2.8-2.7-3.2)
		8	23 (20-21-29)	2.0 (1.8-1.9-2.2)
		9	15 (12-16-17)	1.3 (1.1-1.4-1.4)
		10	36 (83-10-15)	<1 (<LOQ-<LOQ-1.1)

Table 9.4.4-15 Residues of BYI02960 in **nectar from combs**

Study	Application rate	DALA	Residue BYI 02960 (µg/kg) mean (individual samples)	Residue BYI 02960-DFEAF (µg/kg) mean (individual samples)
KIIIA 10.4.7/04	1x 300 (soil appl.) and 2x 200 g a.s./ha (foliar spray)	7	21 (15-18-31)	<1 µg/kg (3 samples)
KIIIA 10.4.7/05	1x 300 (soil appl.) and 2x 200 g a.s./ha (foliar spray)	7	<1.3 (2-<0.1-<1)	<0.4 (<1 - <0.1 - <0.1)
KIIIA 10.4.7/06	2x 200 g a.s./ha (foliar spray)	27	<6.9 (17-8-6-2-<1-6-6-10-6)	<1 (1 sample of 1 and 8 samples of <1)
IIA 8.16.2/08	2x 200 g a.s./ha (foliar spray)	-1	<LOQ (3 samples)	<LOD (3 samples)
		0	<1 (1.2-<LOQ-1.2)	<LOD (3 samples)
		1	127 (186-68)	5.6 (9.4-1.7)
		2	44 (33-84-16)	<1.8 (<LOQ-3.3-<LOQ)
		3	111 (80-108-144)	5.5 (5.0-4.6-6.8)
		4	35 (12-48-46)	<1.7 (<LOQ-1.8-2.2)
		5	93 (69-117)	5.1 (4.8-5.4)
		6	82 (56-108)	4.5 (3.4-5.5)

		7	107 (107)	5.2 (5.2)
		8	95 (95)	4.8 (4.8)
		9	50 (28-31-90)	2.3 (1.2-1.4-4.4)
		10	57.5 (38-77)	3.2 (2.6-3.8)
		12	45 (40-49)	2.6 (2.6-2.5)
		18	38 (30-45)	1.7 (1.7-1.7)
		24	32 (19-44)	1.3 (1.1-1.4)

Table 9.4.4-16 Residues of BYI02960 in **bee wax from combs**

Study	Application rate	DALA	Residue BYI 02960 (µg/kg) mean (individual samples)	Residue BYI 02960-DFEAF (µg/kg) mean (individual samples)
KIIIA 10.4.7/05	1x 300 (soil appl.) and 2x 200 g a.s./ha	7	3.7 (7-3-1)	<1 (3 samples)
KIIIA 10.4.7/06	2x 200 g a.s./ha	27	15 (2-1-21-7-3-1-62-24-10)	<1 (9 samples)

B.9.4.4.3.2 Residue measurements in oilseed rape

An overview of the measured residues of the a.s. and metabolite DFEAF in the honey bee field trials on oil seed rape in the different matrices was compiled by RMS in Tables B.9.4.4-17-24. In these studies, the first foliar spray application was done just before flowering and the second foliar spray application was done during full flowering.

Table 9.4.4-17 Residues of BYI02960 and metabolite in **flowers**

Study	Application rate	DALA	Residue BYI 02960 (µg/kg)	Residue BYI 02960-DFEAF (µg/kg)
KIIIA 10.4.5/01	10 g a.s./kg OSR seeds (seed treatment), 1x 300 g a.s./ha (soil application). 2x205 g a.s./ha (foliar spray)	-6	14155	9
		-5	9289	15
		-4	6939	20
		-3	5244	16
		-2	2887	10
		-1	2577	10
		0	30379	28
		1	22071	28
		2	14071	45
		3	18308	50
		4	16068	67
		5	12251	48
		6	11230	44
		7	15786	80
		8	12972	61
		9	4294	20
		10	3306	15

		11	1917	10
KIIIA 10.4.5/02	10.1 g a.s./kg OSR seeds (seed treatment), 1x 300 g a.s./ha (soil application). 2x205 g a.s./ha (foliar spray)	-9	7700	5
		-8	7900	20
		-7	5900	14
		-6	5500	17
		-5	3400	13
		-4	3900	14
		-3	4100	18
		-2	2200	9
		-1	2100	8
		0	36000	35
		1	24000	47
		2	17000	40
		3	3400	20
		4	3000	7
		5	3400	9
		6	2100	5
		7	1600	4
		8	1800	7
		9	1500	6
		10	1300	6
		11	660	3
		12	830	4
		13	440	3
		14	440	4
		15	290	3
		16	170	2
		17	150	2
		18	100	1
		19	96	<1
		20	84	<1
		21	89	<1

Table 9.4.4-18 Residues of BYI02960 and metabolite in **pollen from forager bees**

Study	Application rate	DALA	Residue BYI 02960 (µg/kg)	Residue BYI 02960-DFEAF (µg/kg)
KIIIA 10.4.5/01	10 g a.s./kg OSR seeds (seed treatment), 1x 300 g a.s./ha (soil application). 2x205 g a.s./ha (foliar spray)	-4	1523	10
		-3	246	2
		-2	706	4
		-1	760	5
		0	nd	nd
		1	14259	21
		2	6147	19
		3	3252	26
		4	2941	22
		5	2721	20
		6	1814	12
		7	1475	8

		8	2034	10
		9	2318	12
		10	2001	9
		11	1788	7
KIIIA 10.4.5/02	10.1 g a.s./kg OSR seeds (seed treatment), 1x 300 g a.s./ha (soil application). 2x205 g a.s./ha (foliar spray)	-9	3200	6
		-8	nd	nd
		-7	1200	6
		-6	810	5
		-5	1300	12
		-4	1200	12
		-3	1100	8
		-2	1100	6
		-1	1000	5
		0	21000	21
		1	4300	16
		2	1800	9
		3	1600	9
		5	1500	6
		6	1400	5
		7	1400	6
		8	1200	6
		9	950	4
		10	900	4
		11	800	3
		12	670	3
		13	590	2
		14	630	3
		15	990	6
		16	220	1
		17	380	2
		18	nd	nd
		19	220	1
		20	170	1

Table 9.4.4-19 Residues of BYI02960 in **pollen from combs**

Study	Application rate	DALA	Residue BYI 02960 (µg/kg) mean (individual samples)	Residue BYI 02960-DFEAF (µg/kg) mean (individual samples)
KIIIA 10.4.5/01	10 g a.s./kg OSR seeds (seed treatment), 1x 300 g a.s./ha (soil application). 2x205 g a.s./ha (foliar spray)	10	1347 (305- 966- 950-743-1694-594-3905-1617)	7 (3-7-6-6-9-4-12-9)
KIIIA 10.4.5/02	10.1 g a.s./kg OSR seeds (seed treatment), 1x 300 g a.s./ha (soil application). 2x205 g a.s./ha (foliar spray)	23	349 (23-460-1200-140-340-130-150)	<1.9 (6-2-1-<1-1-<1-<1)

Table 9.4.4-20 Residues of BYI02960 in **beebread**

Study	Application rate	DALA	Residue BYI 02960 (µg/kg)	Residue BYI 02960-DFEAF (µg/kg)
KIIIA 10.4.5/01	10 g a.s./kg OSR seeds (seed treatment), 1x 300 g a.s./ha (soil application). 2x205 g a.s./ha (foliar spray)	14	1958	7
		35	6	<0.1
		56	113	2
		77	81	1
		99	38	<1
		18	4	<0.1
		140	11	<0.1
		155	7	<0.1
KIIIA 10.4.5/02	10.1 g a.s./kg OSR seeds (seed treatment), 1x 300 g a.s./ha (soil application). 2x205 g a.s./ha (foliar spray)	44	25	<1
		64	8	<1
		94	9	<1
		116	13	<1
		136	5	<0.1
		157	4	<0.1
		180	4	<0.1

Table 9.4.4-21 Residues of BYI02960 in **nectar from forager bees**

Study	Application rate	DALA	Residue BYI 02960 (µg/kg)	Residue BYI 02960-DFEAF (µg/kg)
KIIIA 10.4.5/01	10 g a.s./kg OSR seeds (seed treatment), 1x 300 g a.s./ha (soil application). 2x205 g a.s./ha (foliar spray)	-6	747	<1
		-5	nd	nd
		-4	2365	15
		-3	363	4
		-2	272	3
		-1	269	3
		0	2356	4
		1	2908	10
		2	nd	nd
		3	4108	37
		4	2729	38
		5	1467	16
		6	304	4
		7	341	3
		8	2823	3
		9	nd	nd
		10	2851	4
		11	3617	4

KIIIA 10.4.5/02	10.1 g a.s./kg OSR seeds (seed treatment), 1x 300 g a.s./ha (soil application). 2x205 g a.s./ha (foliar spray)	-8	4300	26
		-7	1500	11
		-6	990	6
		-5	990	15
		-4	730	16
		-3	670	9
		-2	450	6
		-1	490	4
		0	3000	21
		1	3500	20
		2	1600	11
		3	1000	6
		4	590	4
		5	470	3
		6	370	3
		7	370	4
		8	190	3
		9	180	2
		10	150	2
		11	100	1
		12	110	1
		13	50	<1
		14	58	<1
		15	3	<0.1
		16	nd	nd
		17	nd	nd
		18	nd	nd
		19	<0.1	<0.1

Table 9.4.4-22 Residues of BYI02960 in **nectar from combs**

Study	Application rate	DALA	Residue BYI 02960 (µg/kg) mean (individual samples)	Residue BYI 02960-DFEAF (µg/kg) mean (individual samples)
KIIIA 10.4.5/01	10 g a.s./kg OSR seeds (seed treatment), 1x 300 g a.s./ha (soil application). 2x205 g a.s./ha (foliar spray)	10	898 (658-870-991-428-996-758-1347-1137)	9 (7-8-11-5-9-8-13-12)
KIIIA 10.4.5/02	10.1 g a.s./kg OSR seeds (seed treatment), 1x 300 g a.s./ha (soil application). 2x205 g a.s./ha (foliar spray)	23	227 (140-78-490-12-140-110-620)	<1.6 (<1-<1-3-<0.1-1-<1-4)

Table 9.4.4-23 Residues of BYI02960 in **nectar/honey from the hive**

Study	Application rate	DALA	Residue BYI 02960 (µg/kg)	Residue BYI 02960-DFEAF (µg/kg)

KIIIA 10.4.5/01	10 g a.s./kg OSR seeds (seed treatment), 1x 300 g a.s./ha (soil application). 2x205 g a.s./ha (foliar spray)	14	1409	30
		35	45	<1
		56	575	4
		77	223	<1
		99	144	1
		18	380	5
		140	61	<0.1
		155	317	5
KIIIA 10.4.5/02	10.1 g a.s./kg OSR seeds (seed treatment), 1x 300 g a.s./ha (soil application). 2x205 g a.s./ha (foliar spray)	44	920	5
		64	260	1
		94	500	2
		116	170	<1
		136	300	<1
		157	840	2
		180	670	2

Table 9.4.4-24 Residues of BYI02960 in **beewax from combs**

Study	Application rate	DALA	Residue BYI 02960 (µg/kg) mean (individual samples)	Residue BYI 02960-DFEAF (µg/kg) mean (individual samples)
KIIIA 10.4.5/01	10 g a.s./kg OSR seeds (seed treatment), 1x 300 g a.s./ha (soil application). 2x205 g a.s./ha (foliar spray)	10	43 (6-14-25-6-148-23-94-31)	<0.3 (I sample of 2 and 7 samples of <0.1)
KIIIA 10.4.5/02	10.1 g a.s./kg OSR seeds (seed treatment), 1x 300 g a.s./ha (soil application). 2x205 g a.s./ha (foliar spray)	23	3.6 (6-2-7-2-2-2-4)	<0.1 (7 samples <0.1)
		64	32	<0.1
		94	11	<0.1
		116	39	<0.1
		136	<1	<0.1
		157	<1	<0.1
		180	6	<0.1

B.9.4.4.3.3 Residue measurements in nectar and pollen of other crops

Four supervised residue trials under semi-field conditions (studies KIIA 8.16.2/04 to /07) were submitted in which the residue levels in melon and tomato flowers, nectar (from combs or honey

stomachs) and pollen (from combs or forager honey or bumble bees) were studied. One trial in melon was performed in North-Carolina and studied either a drench or a double foliar application. Two trials in watermelon and one in tomato were performed in Spain and all studied a drench application. The location (for the trial in North Carolina) or the application method (for the drench trials) are less relevant for the GAP under evaluation. Therefore, the residue levels are not summarised in this section nor used in the risk assessment, but all results can be found in Tables B.9.4.2-16 and -17.

B.9.4.4.3.4 Residue measurements in guttation water

The flupyradifurone residue situation in guttation water was investigated in two field studies in winter oil seed rape (W-OSR) (KIIA 8.16.2/09 and /10, Rexer 2012c and d). The treated seeds in these studies were treated at a rate of nominally 10 g flupyradifurone a.s./kg and were sown at actual rates of 59 and 70 g flupyradifurone a.s./ha. The seeds were sown on field plots which were treated at the day of W-OSR-planting with nominally 300 g flupyradifurone a.s./ha (actually 310 and 311 g flupyradifurone a.s./ha), which was immediately incorporated down to a depth of 15 - 20 cm. Guttation fluid from the W-OSR seedlings were collected from the very first day onwards, at which the first guttation droplets occurred; this started at BBCH 9 - 11, which means at a crop stage with minimal dilution of residues due to plant growth and plant biomass.

The maximum residue level in guttation fluid in the two studies was measured at the very first days where guttation droplets occurred after emergence (actually 12 and 13 days after seed planting); thereafter, the concentrations of flupyradifurone decreased continuously.
In autumn, residues of BYI 02960 above the limit of quantitation (LOQ; 0.010 mg/L) were found in 20 out of 20 analysed samples in autumn 2010 (quantifiable residues ranging from 11 mg/L to 0.039 mg/L in one study and from 21 mg/L to 0.087 mg/L in the other study). Generally, residues of BYI 02960 declined by about two orders of magnitude during the approximately 5 weeks lasting autumn sampling period. In the following spring, residues of BYI 02960 above the LOQ were found in all or the majority of the analysed samples (quantifiable residues ranging from 0.21 mg/L to 0.014 mg/L or 0.15 mg/L to 0.062 mg/L).

In autumn, residues of the metabolite BYI 02960-DFEAF were < LOQ (i.e. < 0.010 mg/L) except for 2 out of 10 or 3 out of 8 analysed samples (collected in the first, (second) and third day of sampling; maximum residue 0.023 mg/L. In spring, all residues of the DFEAF-metabolite were < LOQ.

B.9.4.4.3.5 Residue measurements in soil

Residue measurements in soil were performed in some of the semi-field and field studies, to confirm the artificially incorporated background concentration in soil.

Table 9.4.4-25 Residues of BYI02960 in **soil**

Study	Application rate	DAA1	Residue BYI 02960	Residue BYI 02960-

			(µg/kg) mean (individual samples)	DFEAF (µg/kg) mean (individual samples)
KIIIA 10.4.7/04	1x 300 (soil appl.) and 2x 200 g a.s./ha (foliar spray)	0	51 (36-51-67)	<1 µg/kg (3 samples)
KIIIA 10.4.7/05	1x 300 (soil appl.) and 2x 200 g a.s./ha (foliar spray)	0	49 (40-56-50)	not done
KIIIA 10.4.5/01	1x 300 g a.s./ha (soil application). 10 g a.s./kg OSR seeds (seed treatment), 2x205 g a.s./ha (foliar spray)	0	75 (75)	not done
KIIIA 10.4.5/02	1x 300 g a.s./ha (soil application). 10.1 g a.s./kg OSR seeds (seed treatment), 2x205 g a.s./ha (foliar spray)	0	61 (61)	not done

B.9.4.4.4 Risk calculations for exposure via nectar and pollen

The envisaged use of BYI 02960 in hops is an application rate of 150 g a.i./ha, also during the flowering period of the crop. However, hop is a dioecious plant, with male and female flowers developing on separate plants. The harvested commodity in commercial hop cultivation is the female flower cluster. Male hop plants, which could theoretically offer honey bees pollen, are not cultivated in hop plantations – on the contrary: as any pollination of the female flowers (by male pollen) would significantly reduce hop quality, would significantly reduce the harvesting window and would significantly hamper the processing of the hop in breweries, hop plantations are entirely cultivated with female plants. Female flowers, however, do not offer honey bees nectar and pollen. As such, the commercial hop plantations themselves are not attractive to honey bees at any growth stage.

The envisaged use of BYI 02960 in lettuce accounts for an application rate of 125 g a.i./ha, during the development of harvestable, vegetative plant parts. Lettuce does not flower in commercial cultivation (except in seed production, which is not proposed here) and as such, honey bees are not attracted by the crop. Moreover, spray applications of BYI 02960 during flowering are excluded by the envisaged application window.

In conclusion, the crops themselves are not attractive to honeybees for nectar and/or pollen collection. However, weeds may be present in the field and these may flower. In general, the occurrence of weeds and especially flowering weeds in crop fields is not beneficial for growers, a.o. because these weeds will compete with the crop plants for resources. However, weed occurrence will depend on the crop and the individual farmer and may be MS specific. The presence of flowering weeds can

therefore not be excluded a priori. For hops especially, undergrowth vegetation is expected to be present. Therefore, the risk via nectar and pollen of flowering weeds should be addressed for the in-field situation.

A large dataset is available with residue measurements.
One semi-field study in Phacelia studied the residue levels after a twofold spray application of 200 g a.s./ha, one just before and one during flowering. Residue levels on pollen and nectar taken from foraging bees directly after the second spray application are high, but decrease quickly:
Phacelia pollen from forager bees one hour after spraying: ca. 65 mg/kg, decreasing within the first day to ca. 36 mg/kg (four hours after spraying). One day after spraying, a level of ca. 10 mg/kg is reached and this decreases within ten days to ca. 0.5 mg/kg.
Phacelia nectar from forager bees one hour after spraying: ca. 3 mg/kg, decreasing within the first day to ca. 1.4 mg/kg (four hours after spraying). One day after spraying, a level of ca. 0.8 mg/kg is reached and this decreases within ten days to ca. 0.04 mg/kg.
The residue levels in pollen and nectar in the hive (maximum ca. 10 mg/kg and 0.1 mg/kg, respectively) are much lower than the residue levels in pollen and nectar collected by forager bees, but they remain present for a longer time (24 days after spraying, the residue levels in pollen and nectar in the hive are ca. 2 mg/kg and 0.03 mg/kg, respectively). In two other semi-field trials, in which next to two foliar applications, also a soil application was used during sowing, residue levels were only measured on one timepoint but results are more or less in accordance with the results of the study with only foliar applications.

Two field trials in oilseed rape also studied residue levels in pollen and nectar taken from foraging bees. These study included two foliar applications and a seed treatment and furthermore had a background soil concentration.
The highest residue level in oilseed rape pollen from forager bees was found on the day of spraying: 21 mg/kg. The level decreased less rapidly than in the above Phacelia study (without seed treatment), reaching a level of ca. 1-2 mg/kg within ten days.
The highest residue level in oilseed rape nectar from forager bees was found three days after spraying: ca. 4 mg/kg. The level decreased to ca. 2 mg/kg within ten days.
The residue levels in pollen and nectar in the hive (maximum values of individual samples ca. 4 mg/kg and 1 mg/kg, respectively) are lower than the residue levels in pollen and nectar collected by forager bees. Hive residues were only measured on day 10 or day 23 after the last application and not earlier, so a direct comparison is difficult. Hive residues remain present for a longer time (23 days after spraying, the residue levels in pollen and nectar in the hive are maximum 1 mg/kg and 0.6 mg/kg, respectively; after 180 days, still 0.004 mg/kg is found in beebread).

In all the above studies, residue levels of metabolite DFEAF are much lower than those of the a.s. and decrease in a similar way.

It is difficult to determine a generic RUD value from these residue measurements, as the trials were performed with different application regimes and each trial had multiple applications. However, a general picture can be derived: directly after spraying, high levels of the a.s. can be found in nectar and pollen; residues in pollen are higher than those in nectar; residue levels in pollen and nectar in the hive seem to be lower than the residue levels in pollen and nectar collected by forager bees, but they remain present for a longer time; after six months, still traces of the a.s. can be found in the beebread; the residue levels of metabolite DFEAF are much lower than those of the a.s.

These residue levels could be used in exposure calculations, using daily intake values for nectar and pollen for different castes of bees. The exposure values could then be compared to the toxicity values derived above. If such an exercise is undertaken, it should be noted that the residue levels were determined in studies which used at least two foliar sprays with 200 g a.s./ha each, while the proposed uses in hops and lettuce have only a single application, at 150 and 125 g a.s./ha, respectively, and furthermore are not attractive to bees themselves. However, at the moment there is no harmonised method available to calculate these exposure levels and especially the corresponding risk. Furthermore, it is not necessary to perform these lower tier calculations, as a large number of higher tier effect studies is available. Please see the section below (higher tier toxicity data and risk assessment).

B.9.4.4.5 Higher tier toxicity data and risk assessment for exposure via nectar and pollen

Six independent semi-field (gauze tunnel) studies were submitted, where BYI 02960 formulations were applied to the highly bee attractive surrogate crop Phacelia tanacetifolia with honey bees actively foraging on the crop (i.e. during bee flight). The studies are summarised in Table B.9.4.4-26. One of these studies was not accepted (except for residue analysis) because of an unequal colony set-up.

The studies were all performed by two different laboratories. Differences between these laboratories were reflected in the report for the use of statistics and preparation of tables or figures. RMS has prepared stacked figures in those cases that figures were missing. In that way the interpretation of the results was more convenient as comparisons between studies are made easier and more transparent.

Table B.9.4.4-26 Overview of semi-field studies

Specifications and endpoint of the studies	**Number of the semi-field study (grey shading means unacceptable for bee endpoints)**					
	10.4.7/01	**10.4.7/02**	**10.4.7/03**	**10.4.7/04**	**10.4.7/05**	**10.4.7/06**
Authors	Schnorbach, 2012a	Schnorbach, 20102b	Rentschler, 2012a	Pröbsting, 2012a	Pröbsting, 2012b	Rentschler, 2012b
Guideline	OEPP/EPPO	OEPP/EPPO	OEPP/EPPO	OEPP/EPPO	OEPP/EPPO	OECD

	guideline No. 170 (3), 2001	guideline No. 170 (3), 2001	guideline No. 170 (3), 2001	guideline No. 170 (3), 2001	guideline No. 170 (4), 2010	guideline No. 75 (2007)
Test facility	Bayer CropScience AG, Monheim	Bayer CropScience AG, Monheim	Eurofins Agroscience Services, Niefern-Öschelbronn	Eurofins Agroscience Services, Niefern-Öschelbronn	Eurofins Agroscience Services, Niefern-Öschelbronn	Eurofins Agroscience Services, Niefern-Öschelbronn
Nr of applications	1	1	2	3	3	2
Type applications	1x foliar spray	1x foliar spray	2x foliar spray (10 d apart)	1x soil application, 2x foliar spray (7 d apart)	1x soil application, 2x foliar spray (18 d apart)	2x foliar spray (10 d apart)
Dose rate	75 and 150 g a.s./ha	150 g a.s./ha, including surfactant	2x 200 g a.s./ha	1 x 300 and 2 x 200 g a.s./ha	1 x 300 and 2 x 200 g a.s./ha	2x 200 g a.s./ha
Number of hives in control / treatment / reference	3 / 3 / 3	2 / 2 / 2	3 / 3 / 3	3 / 3 / 3	3 / 3 / 3	3 / 3 / 3
Hive size (initial nrs of bees)	2500	2500-3000	4000-7000 bees	1000-9000 bees	7000-13800 bees	5313-8570 bees
Tunnel size	50 m2	50 m2	72 m2	60 m2	60 m2	80 m2
Timing application	during flowering and active foraging of bees	during flowering and active foraging of bees	1s foliar application 6 d before hive set-up, 2nd foliar application 10 d after 1st application during flowering and active foraging of bees	1st foliar application 4 d before hive set-up 2nd foliar application 7 d after 1st foliar application (3 d after hive set-up) during flowering and active foraging of bees	1st foliar application 14 d before hive set-up, 2nd foliar application 4 d after hive set-up during flowering and active foraging of bees	1st foliar application 5 d before hive set-up and 2nd foliar application 5 d after set-up during flowering and active foraging of bees
Exposure duration	7 d	12 d	16 d	10 d	12 d	12 d
Observation duration	29 d	29 d	29 d	32 d	33 d	35 d
Ranking worst case exposure	6	5	3	1 – however unequal colony setup	4	2
Flight intensity	Similar to control	Similar to control	Reduction on DALA0-3,	Cannot be evaluated	Strong reduction on	Strong reduction on

			significant at DALA0 and DALA2		DALA0-7, significant on DALA0aa and DALA5	DALA0-7, significant on DALA0aa, DALA3 and DALA6
Bee mortality in bee traps linen sheets	Similar to control	Similar to control	No apparent increase of mortality	cannot be evaluated	No apparent increase of mortality	No apparent increase of mortality
Number of bees	Similar to control, Decrease caused by limited forage	Similar to control, Decrease caused by limited forage	More or less equal to control	cannot be evaluated	Small decrease explained limited foraging area. Further comparable to the control	Small decrease explained limited foraging area. Further comparable to the control
Development of brood	Similar to control, same level during confinement, increase afterwards	Similar to control, same level during confinement, increase afterwards	Decreases during confinement and increases afterwards, similar to control	cannot be evaluated	Decreases during confinement and increases afterwards, similar to control	Decreases during confinement and increases afterwards, similar to control
Development of food storage aria	Increase pollen and nectar stores similar to control	Increase pollen and nectar stores similar to control	Decreases during confinement similar to the control, increases after confinement	cannot be evaluated	Decreases during confinement and increases afterwards, similar to control	Decreases during confinement and increases afterwards, similar to control
Presentation condition of colonies	Figures for nectar, pollen etc. individually	Figures for nectar, pollen etc. individually	Tables Stack figures prepared by RMS	Tables Stack figures prepared by RMS	Tables Stack figures prepared by RMS	Stacked figures
Behaviour	Not reported	Not reported	normal	Clustering but further normal	Repellence effect on DAA0aa and DAA1.	Intoxication symptoms, coordination problems and repellence effect on DAA0aa
remark	Non-GLP	Non-GLP	No obvious higher mortality outside the hive can be related to decrease of	Unequal colony set-up, therefore most endpoints cannot be evaluated.	No obvious higher mortality outside the hive can be related to decrease of	No obvious higher mortality outside the hive can be related to decrease of

			flight intensity		flight intensity	flight intensity
Statistics for mortality and flight intensity	no	no	yes	yes	yes	yes
Statistics for brood and food storage	no	no	no	no	no	no

According to the notifier, " the results of all of the six semi-field (tunnel) studies under forced and as such worst-case exposure conditions consistently did not show any adverse effects on mortality, foraging activity, behaviour, brood-, food- and population development or on overall colony vitality". They state that "overall, the laboratory database shows that BYI 02960 does not exhibit delayed or chronic effects, either in adult bees or in honey bee larvae. BYI 02960 metabolites are virtually non-toxic to honey bees and there is no indication that BYI 02960 metabolites are of any higher toxicity regarding potentially delayed or chronic effects than the parent compound. These laboratory findings have been consistently confirmed by in total six independent semi-field tunnel studies in the highly bee attractive surrogate crop Phacelia tanacetifolia. As such, it can be concluded that BYI 02960 can be applied at foliar application rates of up to and including 200 g a.i./ha, even to bee-attractive, full-flowering crops during honey bees actively foraging, without adverse effects on honey bees, honey bee brood and honey bee colonies."

RMS however notes the following:

- Study 01 and /02 were not performed under GLP and are therefore less reliable. However, they represent the two lowest exposure situations in the dataset and are therefore less important for the overall conclusion.

- Endpoints in this type of semi-field study are interrelated. For instance mortality of bees outside the hive will depend on flight intensity. When flight intensity is reduced, mortality in the tunnel might be reduced as well as fewer bees are foraging. This was indeed the case in the studies where reduction of flight intensity was observed. Flight intensity was significantly reduced in studies 10.4.7/03-06; reduction was observed for three to seven days.

- Significant effects on flight intensity are present in studies 10.4.6/3 to 10.4.7/6. In studies 10.4.6/1 and /2 however, flight intensity was not evaluated statistically. Differences between studies may therefore be biased by the performing laboratory. However, based on visual inspection of the data RMS agrees that studies 10.4.6/1 and/2 do not suggest an effect on foraging activity.

- Repellence of flupyradifurone leads to decreased flight intensity. In that way bees will minimize exposure of the hive to flupyradifurone. In the studies in which reduced flight intensity was seen, effects on other endpoints were not clearly visible.

- Of course, a short-term reduction of flight intensity can be an effective way to reduce exposure and possibly related risk for honey bee colonies. It can only be seen as an acceptable effect if it does not lead to effects on bee colonies in the longer term from a shortage of food. This was not apparent in the semi-field studies (brood development, food storage area and colony strength comparable to control in all studies) but effects on these parameters can potentially be masked by the small colony size and confined conditions..

- Exposure in the tunnel was on average 12 days. Longer exposure is not feasible as the foraging opportunities within a tunnel are too limited. It was evident in most studies that there was a reduction of the colony strength during confinement in the tunnel. After placement of the hives in the open field, colonies started to grow again. Any effects of the active substance can therefore be masked by the stronger effect of confinement to the tunnel. Moreover, due to natural variability between the hives it is extremely difficult to evaluate differences between treatment and control.

- Reduction of flight intensity and effects on behaviour were observed in those studies that were worst case for number of applications and timing of the applications (those ranked by RMS as 1, 2, 3 and 4).

- So far guidance has not been developed for the interpretation of changes of endpoints on brood development. Since the endpoints are interrelated, possible effects are difficult to disentangle. Evaluation of these endpoints is difficult without a clear perspective on normal brood development.

- It is not yet clear if and how results can be assessed statistically. Indeed, in these 6 studies, statistical assessment has not been performed on endpoints of brood development.

- Confined conditions may result in possible stress on bees which may result in a natural reduction of brood activity when bees are kept inside tunnels for longer periods. In these studies reductions of brood activity are indeed often observed during confinement to the tunnels.

- According to the EFSA opinion on bees, it is not possible to assess pollen and nectar storage and hive weight development due to the small size of the colony; therefore, it is difficult to assess potential effects on honey production (i.e. a potential protection goal) when adverse

effects are observed on other parameters. As smaller sizes of colonies are used in semi-field studies, the interpretation of impacts on full size colonies will be difficult and the possibility to translate adverse effects on a small colony to a full size colony seems limited. Due to confined conditions, the small size of the colony and the high turnover rate of nectar and pollen in the tunnels, it is not possible to assess hive weight development and direct potential effects on honey production.

Based on the above, the semi-field studies alone are not considered sufficient proof that the risk to bees is acceptable. However, also two field studies and one long-term colony feeding study were submitted. These are summarised in Table B.9.4.4-27.

Table B.9.4.4-27 Overview of field and long-term feeding studies

Specifications and endpoint of the studies			
	10.4.5/01	10.4.5/02	8.16.2/03
Authors	Rexer 2012a	Rexer 2012b	Nikolakis, Krieg et al. 2012
Guideline	OEPP/EPPO guideline No. 170 (4), 2010 with modifications recommendations of the IVA Guideline (Beutel et al., 1992).	OEPP/EPPO guideline No. 170 (4), 2010 with modifications recommendations of the IVA Guideline (Beutel et al., 1992).	OEPP/EPPO Guideline No. 170 (4) (2010) with modifications, the EU Directive 91/414/EEC (1997), and in part, the recommendations of the IVA Guideline (Beutel et al., 1992)
Test facility	Eurofins Agroscience Services EcoChem, Niefern-Öschelbronn	Eurofins Agroscience Services EcoChem, Niefern-Öschelbronn	Bayer CropScience AG, Monheim
Nr of applications	4	4	Not applicable, exposure by feeding
Type applications	1x bare soil 1 seed treatment 2x foliar spray	1x bare soil 1 seed treatment 2x foliar spray	Fortified diet in tunnels
Treatment rate	9.9 g flupyradifurone/kg seed (analysed). 300 g as/ha (soil appl), 205 and 205 g as/ha (2 foliage applications)	10.1 g flupyradifurone/ kg seed (analysed). 300 g as/ha (soil appl), 205 and 205 g as/ha (2 foliage applications)	600 – 2500 - 10000 µg as/kg diet
Crop	Oil seed rape	Oil seed rape	Not applicable, exposure by feeding
Number of hives in control / treatment /	8 / 8 / n.a.	8 / 8 / n.a.	5 (3 remaining) / 5 / n.a.

reference			
Hive size (mean initial nrs of bees)	9,000	12,000	6000
Timing application	first foliar application immediately pre/early flowering; second foliar application 6 d later during full flowering and active foraging of bees	first foliar application immediately pre/early flowering; second foliar application 9 d later during full flowering and active foraging of bees	No flowering crop in the tunnel, forced diet feeding
Exposure duration	24 d	30 d	42 d
Observation duration	346 d	351 d	326 d
Flight intensity	Similar to control, except slightly lower for 2 d after 2nd foliar application	Similar to control, except clearly lower 2 and 3 d after 2nd foliar application	Similar to control
Bee mortality in bee traps, linen sheets	Similar to control	Similar to control	Similar to control
Number of bees	Similar to control	Similar to control	Similar to control
Weight of colony	Similar to control	Similar to control	Similar to control
Development of brood	Similar to control	Similar to control	Similar to control
Development of food storage aria	Increase pollen and nectar stores similar to control	Increase pollen and nectar stores similar to control	Increase pollen and nectar stores similar to control
Behaviour	intensive cleaning behaviour and locomotion problems in small fraction of bees of treated colonies on day after 2nd foliar application	no treatment-related effects	
Remark			The low number of replicates (especially in the control, with only three remaining replicates) and the apparent variation within the treatment groups in mortality of bees/drones/immature stages make it difficult to draw conclusions on the effect of the treatment. What can be concluded is that, under the specific circumstances of this study, the different treatments of the test substance do not lead to an adverse effect in one of the observed parameters (or the combination

			of adverse effects of the different parameters) to such an extent, that it led to the collapse of one or more colonies.
Statistics for mortality and flight intensity	no	no	yes
Statistics for brood and food storage	no	no	yes

Thus, these studies confirm the results of the semi-field studies.

In the two field studies on oil seed rape, no adverse effects on honey bees were seen during the exposure period nor during the observation period which included overwintering. The only treatment-related effects were a short-term (two days) reduction on flight intensity in both studies and some behavioural anomalies in a small fraction of bees for one day in one of the two studies. These effects apparently did not lead to direct or delayed effects on survival or wellbeing of the colonies as compared to the control colonies. Exposure in the field studies was confirmed with residue measurements in nectar and pollen both from forager bees and from hive matrices.

Also, a long-term feeding study was submitted in which colonies were forced to feed on up to 10 mg/kg diet for seven weeks. This is clearly a worst-case exposure for the GAPs of the semi-field and field studies (2x 200 g a.s./ha foliar spray and in some cases also background soil concentration included). In these trials, the initial concentration in pollen and nectar taken from forager bees could be above 10 mg/kg on the first days, but this quickly decreased to below 10 mg/kg. In the hives, the concentration in pollen or nectar never exceeded a maximum value of 10 mg/kg and also decreased to much below that level soon. This study had some shortcomings but it can be concluded that a 7-week exposure to 10mg flupyradifurone/kg diet does not lead to collapse of a colony, including overwintering.

The overall data package shows that the spraying of 2x 200 g a.s./ha on a bee-attractive crop 1x before and 1x during full flowering and active foraging of honey bees does not result in adverse acute or long-term (including overwintering) effects on honey bee colonies.

This data package covers the proposed uses in the non-attractive crops lettuce and hops with application rates of 1x125 and 1x150 g a.s./ha, respectively. For these crops, the main concern is via flowering weeds especially in the hop plantations. The above studies in highly attractive crops indicate that a low risk is expected from these uses via nectar and pollen of flowering weeds.

Expert discussion in Pesticide Peer Review meeting 124 (December 2014):

The RMS concluded that the data sets indicate that overall no adverse acute or long-term effects (including over-wintering evaluation) were indicated for a spray of 2 x 200 g a.s./ha on bee attractive crops during full flowering and active foraging (1 application prior to flowering and 1 application during flowering). The experts at the meeting agreed with the conclusion of the RMS noting that some slight transient treatment related effects may have been observed.

B.9.4.4.6 Risk assessment for other exposure routes

B.9.4.4.6.1 Exposure via honeydew

Aphids are expected to be more sensitive to flupyradifurone than bees because they are target pest species. If they are indeed more sensitive, buildup of honeydew at a level that can harm honeybees is not expected. However, this needs to be confirmed with data. The notifier has submitted dose-response studies with aphids (KIIIA1 6.1.1) . However, actual LD50 values were not found in the dose-response studies and the doses are expressed in g a.s./ha; therefore they are not useful for a comparison with the toxicity to honeybees. The notifier also submitted an LD50-study with the green peach aphid (all these data are summarised under B.9.9.2). The raw data of the LD50-study with the green peach aphid need to be submitted before conclusions can be drawn about the usefulness of the study. However, based on the conclusion above that the risk via direct overspray and via nectar and pollen of highly attractive flowering crops is acceptable, a high risk via honeydew (directly oversprayed or produced (much) later by aphids) is not expected either.

Expert discussion in Pesticide Peer Review meeting 124 (December 2014):

The RMS included a consideration of the risk via insect honey dew in the DAR. The RMS considers that the higher tier studies were sufficient to cover the risk to honey bees via this route of exposure. The experts agreed with the conclusions of the RMS that this route of exposure was addressed.

B.9.4.4.6.2 Exposure via guttation and other water sources

The relevance of exposure via guttation droplets and other water sources for honey bee risk assessment is not yet clear. The risk via guttation droplets is generally estimated to be highest from droplets on young seedlings grown from treated seeds. The applicant submitted two studies in which the residue level in young oil seed rape seedlings, sown in soil with a background concentration covering the PECplateau of the proposed uses, was measured. A highest level of 21 mg/L was found (initial level, quickly declining). This is comparable to the NOEC-value from the chronic honey bee laboratory study with technical flupyradifurone (10-d feeding of 26 mg/kg in highly bee attractive sugar solution). Therefore, a high risk via guttation is not indicated. Based on the conclusion above (see section higher tier toxicity data and risk assessment) that the risk via direct overspray and via nectar and pollen of highly attractive flowering crops is acceptable, a high risk via other water sources (e.g. puddles, surface water) is not expected either.

B.9.4.4.6.3 Exposure via succeeding crops

In order to account for potential side-effects on honey bees from a potential carry-over of soil-borne flupyradifurone residues into potentially bee-attractive, succeeding flowering cops, a worst-case soil-plateau concentration was established in two out of four semi-field studies in Phacelia tanacetifolia as well as in both long term honey bee health field studies in W-OSR. In all of these four studies which evaluated potential side-effects on honey bees, nominally 300 g flupyradifurone a.s./ha was applied onto the soil at the day of crop planting, to be immediately incorporated down to depth of 20 cm to establish a worst-case, non-aged flupyradifurone soil plateau concentration within the root zone of the planted, bee-attractive flowering crop. The analytically determined soil concentration in these four studies was in the range of 36 - 76 µg flupyradifurone a.s./kg soil.

These analytically determined soil concentrations can be compared to the soil plateau concentration that has been calculated in the fate section. The PECplateau for lettuce of 0.016 mg/kg (over 20 cm) is clearly covered. For hops, the reported PECplateau is 0.080 mg/kg, but this is calculated over 5 cm for perennial crops without tillage. In case of succeeding crops other than hops, tillage will take place and it can be assumed that the soil concentration will be spread over 20 cm, leading to a soil concentration of 0.020 mg/kg. Thus, risk via succeeding crops of the use in hops is also covered with the effect semi-field and field studies.

European Commission

Flupyradifurone

VOLUME 3

ANNEX B

B.9 Ecotoxicology

Rapporteur Member State: The Netherlands

January 2014 November 2014 December 2014

Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible approval of flupyradifurone under Regulation (EC)1107/2009

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NB for the Pesticide Peer Review meeting of December 2014, an addendum was made as a discussion document. This addendum is integrated in this revised DAR of December 2014. Thus, all relevant information can be found in the document in hand.

B.9.5 Effects on non-target terrestrial arthropods (IIA 8.8, IIIA 10.5)

B.9.5.1 Laboratory toxicity studies (IIA 8.8.1, IIIA 10.5.1, IIIA 10.5.2)

STUDY IIA, 8.8.1.1/01

Characteristics

reference	:	Jans D. (2010a)	species	:	*Aphidius rhopalosiphi*
type of study	:	Laboratory toxicity study	exposure duration	:	2 days
year of execution	:	2009	nominal dose	:	0.5, 1.1, 2.2, 4.7, 10, 20, 40, 80 and 160 g a.s./ha
GLP statement	:	Yes	dosing method	:	Dry residues on glass plates
guideline	:	Mead-Briggs *et al*. (2000), Candolfi *et al* (2001)	acceptability	:	Acceptable
test substance	:	BYI 02960 SL 200 G (200 g/L SL formulation), batch 2009-001253	LR50	:	<0.5 g a.s./ha
a.s. content	:	17.0 % w/w (199.8 g/L) BYI 02960			

Methods

The effect of fresh residues of BYI 02960 SL 200 G (200 g/L SL formulation, 17.0 % w/w (199.8 g/L) BYI 02960) on survival of the parasitic wasp *Aphidius rhopalosiphi*, confined under laboratory conditions over laboratory-treated glass plates, was determined.

Two trials 1 and 2 were performed. Treatment rates in trial 1 were 10, 20, 40, 80 and 160 g a.s./ha. Trial 2 was performed at lower treatment rates since in trial 1 100% mortality was observed at all treated rates within 24 hours. Treatment rates in trial 2 were 0.5, 1.1, 2.2, 4.7 and 10 g a.s./ha. Treatments with BYI 02960 SL 200 G and a positive control (dimethoate, 0.04 g a.s./ha) were tested in 200 L spray solution/ha against water-treated controls. Laboratory treated glass plates were allowed to dry for 1 hour and incorporated into test units (4/treatment), forming floor and ceiling respectively. Fifteen adult wasps (<48 hours old) were added to each test unit. The test units were kept in a controlled environment room until study end. Mortality was assessed during 48 hours.

Results

Control mortality was 1.7% after 24 hours in trial 1 and 0% after 48 hours in trial 2. Mortality was 100% at all treated rates of BYI 02960 SL 200 G in trial 1 and 2, except for the 0.5 g a.s./ha treatment in trial 2 (85% mortality and corrected mortality). The LR50 is <0.5 g a.s./ha. The reference substance gave the expected response in both tests (100% mortality within 24 or 48 hours).

Conclusion

LR50 <0.5 g a.s./ha.

Guidelines & Limitations

The study is acceptable.

STUDY IIA, 8.8.1.1/02

Characteristics

reference	:	Jans D. (2010b)	species	:	*Typhlodromus pyri*
type of study	:	Laboratory toxicity study	exposure duration	:	7 days
year of execution	:	2009	nominal dose	:	2, 4, 9, 19 and 40 g a.s./ha
GLP statement	:	Yes	dosing method	:	Dry residues on glass plates
guideline	:	Blümel *et al*. (2000) , Candolfi *et al* (2001)	acceptability	:	Acceptable
test substance	:	BYI 02960 SL 200 G (200 g/L SL formulation), batch 2009-001253	LR50	:	17 g a.s./ha
a.s. content	:	17.0 % w/w (199.8 g/L) BYI 02960			

Methods

The effect of fresh residues of BYI 02960 SL 200 G (200 g/L SL formulation, 17.0 % w/w (199.8 g/L) BYI 02960) on survival of the predatory mite *Typhlodromus pyri*, confined under laboratory conditions over laboratory-treated glass plates, was determined.

Treatments with BYI 02960 SL 200 G at 2, 4, 9, 19 and 40 g a.s./ha and a positive control (dimethoate, 4 g a.s./ha) were tested in 200 L spray solution/ha against water-treated controls. Laboratory treated glass plates were allowed to dry for 1 hour and incorporated into test units (5 per treatment, 5 for the control) to form the floor. A layer of sticky material provided an exposure area of each test unit into which twenty protonymphs were added. The test units were kept in a controlled environment room until study end. Survival was assessed over a 7-day period.

Results

Results are summarised in Table B.9.5.1-01. The LR50 (Probit analysis) was 17 g a.s./ha (95% CI 13-21 g a.s./ha). The reference substance gave the expected response (80% mortality within 7 days).

Table B.9.5.1-02 Effects of BYI 02960 SL 200 G on *Typhlodromus pyri* under laboratory conditions

Treatment	Dose (g a.s./ha)	Mortality (%) after 7 days	Corrected mortality (%)[(A)]
Water control	-	4	-
BYI 02960 SL 200 G	2	10	6.3
BYI 02960 SL 200 G	4	10	6.3
BYI 02960 SL 200 G	9	28*	25.0
BYI 02960 SL 200 G	19	52*	50.0
BYI 02960 SL 200 G	40	90*	89.6

* statistically significant difference from control at 5% level.
(A) According to Abbott formula

Conclusion

LR50 17 g a.s./ha.

Guidelines & Limitations

The study is acceptable.

STUDY IIIA, 10.5.2/01

Characteristics

reference	:	Jans D. (2010e)	species	:	*Aphidius rhopalosiphi*
type of study	:	Extended laboratory toxicity study	exposure duration	:	2 days
year of execution	:	2010	nominal dose	:	0.5, 0.89, 1.58, 2.81 and 5.0 g a.s./ha
GLP statement	:	Yes	dosing method	:	Dry residues on barley seedlings
guideline	:	Mead-Briggs *et al*. (2000), Mead-Briggs *et al*. (draft 2006), Candolfi *et al* (2001)	acceptability	:	Acceptable
test substance	:	BYI 02960 SL 200 (200 g/L SL formulation), batch 2009-001253	LR50	:	2.02 g a.s./ha
a.s. content	:	17.0 % w/w (199.8 g/L) BYI 02960	ER50	:	>0.89 g a.s./ha; <1.58 g a.s./ha

Methods

The effect of fresh residues of BYI 02960 SL 200 (200 g/L SL formulation, 17.0 % w/w (199.8 g/L) BYI 02960) on survival and reproduction of the parasitic wasp *Aphidius rhopalosiphi*, confined under laboratory conditions over laboratory-sprayed barley seedlings, was determined.

Treatment rates were 0.5, 0.89, 1.58, 2.81 and 5.0 g a.s./ha. Treatments with BYI 02960 SL 200 and a positive control (dimethoate, 3.0 g a.s./ha) were tested in 400 L spray solution/ha against water-treated controls. Potted 5-day old barley plants (6 replicate pots per treatment), that had been sprayed with 10% fructose solution one hour earlier, were treated with the test substance using a calibrated laboratory sprayer. The residue was allowed to dry for about 1 hour, the plants were enclosed within acrylic cylinders and five female <48 h old wasps were added to each replicate. The test units were kept in a controlled environment room until study end. Assessments of repellence were made during the first 3 hours and wasp mortality was assessed during 48 hours. Thereafter, 15 surviving healthy females of the control and the three lowest treatment rates were removed and individually confined over untreated aphid-infested barley plants for 24 hours. The wasps were then removed and the plants were left for a further 11 days before the number of aphid mummies was recorded.

Results

No repellent effect of the test item was observed. Control mortality was 3.3% after 48 hours. At 0.5, 0.89, 1.58, 2.81 and 5.0 g a.s./ha, respectively, mortality after 48 hours was 0%, 13.3%, 40.0%, 70.0% and 90.0%. The LR50 (Probit analysis) was 2.02 (95% CI 1.61-2.55) g a.s./ha. At 0.5, 0.89 and 1.58 g a.s./ha, respectively, reduction of reproduction was -6.4%, -2.9% and 57.7%, respectively (reproduction not tested at higher concentrations). The effect on reproduction at 1.58 g a.s./ha was statistically significant at the 5% uncertainty level. The ER50 was not reported but is between 0.89 and 1.58 g a.s./ha. The reference substance gave the expected response (100% mortality within 48 hours).

Conclusion

LR50 2.02 g a.s./ha, ER50 >0.89 g a.s./ha.

Guidelines & Limitations

The study is acceptable.

STUDY IIIA, 10.5.2/02

Characteristics

reference	:	Jans D. (2010f)	species	:	*Typhlodromus pyri*
type of study	:	Extended laboratory toxicity study	exposure duration	:	14 days
year of execution	:	2010	nominal dose	:	15, 32, 67, 142 and 300 g a.s./ha
GLP statement	:	Yes	dosing method	:	Dry residues on bean leaf discs
guideline	:	Blümel *et al*. (2000), Candolfi *et al* (2001)	acceptability	:	Acceptable
test substance	:	BYI 02960 SL 200 (200 g/L SL formulation), batch 2009-001253	LR50		177 g a.s./ha
a.s. content	:	17.0 % w/w (199.8 g/L) BYI 02960	ER50		>142 g a.s./ha

Methods

The effect of fresh residues of BYI 02960 SL 200 (200 g/L SL formulation, 17.0 % w/w (199.8 g/L) BYI 02960) on survival and reproduction of the predatory mite *Typhlodromus pyri*, confined under laboratory conditions over laboratory-treated bean leaf discs, was determined.

Treatments with BYI 02960 SL 200 at 15, 32, 67, 142 and 300 g a.s./ha and a positive control (Dimethoate EC 400 g/L, 400 g/L dimethoate, 40 g a.s./ha) were tested in 200 L spray solution/ha against water-treated controls. Bean leaf discs were treated with the test substance using a calibrated laboratory sprayer and allowed to dry for about 1 hour prior to incorporation into test units (5 per treatment and for the control) to form the floor. A thin layer of surrounding insect trap glue provided an exposure area within each test unit into which twenty protonymphs were added. The test units were kept in a controlled environment room until study end. Survival was assessed over a 7-day period. Fecundity assessments were carried out in the control and all treatments except the highest during a second week in the same treated test units.

Results

Results are summarised in Table B.9.5.1-02. The LR50 (Probit analysis) was 177 g a.s./ha (95% CI 151-205 g a.s./ha). The ER50 is >142 g a.s./ha. The reference substance gave the expected response (100% mortality within 7 days).

Table B.9.5.1-02 Effects of BYI 02960 SL 200 on *Typhlodromus pyri* under extended laboratory conditions

Treatment	Dose (g a.s./ha)	Mortality (%) after 7 days	Corrected mortality (%)[(A)]	Eggs/ female	Reduction of reproduction (%)
Water control	-	13.0	-	4.5	-
BYI 02960 SL 200	15	12.0	-1.1	4.3	6.0
BYI 02960 SL 200	32	9.0	-4.6	4.5	0.6
BYI 02960 SL 200	67	19.0	6.9	4.3	5.1
BYI 02960 SL 200	142	46.0*	37.9	4.8	-6.8[(C)]
BYI 02960 SL 200	300	79.0*	75.9	n.t.[(B)]	-

* statistically significant difference from control at 5% level.
(A) According to Abbott formula
(B) n.t. = not tested.
(C) Negative value represents increase relative to control, hence no adverse effect.

Conclusion

LR50 177 g a.s./ha, ER50 >142 g a.s./ha.

Guidelines & Limitations

The study is acceptable.

STUDY IIIA, 10.5.2/03

Characteristics

Reference	:	Jans D. (2010g)	Species	:	*Coccinella septempunctata*
Type of study	:	Extended laboratory toxicity study	Exposure duration	:	13-17 days
Year of execution	:	2009-2010	Nominal dose		Trial 1: 8, 17, 35, 72 and 150 g a.s./ha; Trial 2: 100, 160, 250, 380 and 600 g a.s./ha
GLP statement	:	Yes	Dosing method	:	Dry residues on detached bean leaves
Guideline	:	Schmuck *et al.* (2000)	Acceptability	:	Acceptable
Test substance	:	BYI 02960 SL 200 (200 g/L SL formulation), batch 2009-001253	LR50	:	273.9 g a.s./ha
a.s. content	:	17.0 % w/w (199.8 g/L) BYI 02960	Reproduction		No effects up to 250 g a.s./ha

Methods

The effect of freshly dried residues of BYI 02960 SL 200 (200 g/L SL formulation, 17.0 % w/w (199.8 g/L) BYI 02960) on survival and reproduction of the ladybird *Coccinella septempunctata*, confined under laboratory conditions over laboratory-treated bean leaf discs, was determined.

Two trials 1 and 2 were performed, since in the first trial no effects were found up to the highest test dose of 150 g a.s./ha. Treatments with BYI 02960 SL 200 at 8, 17, 35, 72 and 150 g a.s./ha (trial 1) or 100, 160, 250, 380 and 600 g a.s./ha (trial 2) and a positive control (Dimethoate EC 400 g/L, a.s. dimethoate, 12 g a.s./ha) were tested in 200 L water/ha against water-treated controls. Detached bean leaves (*Phaseolus vulgaris*) were treated with the test substance using a calibrated laboratory sprayer and allowed to dry for about 1 hour. After application a disc was cut out of the bean leaf which was incorporated into a test unit (40 units per treatment and for the control) to form the floor (treated side up). In each unit one 4-day old larva was individually confined. The test units were kept in a controlled environment room until study end. Assessments of mortality were made up to adulthood (duration of exposure phase 13 days for trial 1 and 17 days for trial 2). Surviving ladybirds were transferred to untreated reproduction containers and egg counts were initiated one week after the first eggs in the control could be observed. Eggs were counted during two weeks, and hatching success was determined.

Results

Mortality and reproduction data are summarised in the Tables below. The toxic reference gave the expected response. The LR50 (Probit analysis) was 273.9 (95% CI 185.9-330.8) g a.s./ha. The IOBC guideline by Candolfi *et al* (2000) states that the assessment of reproduction is of a qualitative nature,

and that there is no effect on reproduction if the number of fertile eggs per viable female per days exceeds 2.0, which was the case for all tested BYI 02960 SL 200 treatments up to 250 g a.s./ha.

Table B.9.5.1-03 Effects of BYI 02960 SL 200 on *Coccinella septempunctata* under extended laboratory conditions (trial 1)

Test item		BYI 02960 SL 200 (g/L)				
Test organism		*Coccinella septempunctata*				
Exposure on:		Detached bean leaves				
		Mortality [%]			Reproduction	
Treatment	g as/ha	Uncorr.	Corr.	P-Value(*)	Fertile eggs per female and day	Fertility [hatching rate in %]
Control	0	22.5			10.4	92.0
Test item	8	32.5	12.9	0.906 n.sign.	15.5	91.4
Test item	17	25.6	4.1	1.000 n.sign.	17.6	95.7
Test item	35	15.0	-9.7	1.000 n.sign.	9.5	87.3
Test item	72	25.0	3.2	1.000 n.sign.	15.5	93.5
Test item	150	47.5	32.3	0.085 n.sign.	21.6	95.4
Reference item	12	92.5	90.3		n.a.	n.a.

* Fisher's Exact test (one-sided), p-values are adjusted according to Bonferroni-Holm
n.a. not assessed
n.sign. not significant

Table B.9.5.1-04 Effects of BYI 02960 SL 200 on *Coccinella septempunctata* under extended laboratory conditions (trial 2)

Test item		BYI 02960 SL 200 (g/L)				
Test organism		*Coccinella septempunctata*				
Exposure on:		Detached bean leaves				
		Mortality [%]			Reproduction	
Treatment	g as/ha	Uncorr.	Corr.	P-Value(*)	Fertile eggs per female and day	Fertility [hatching rate in %]
Control	0	22.5			13.9	91.9
Test item	100	30.0	9.7	0.306 n.sign.	16.1	92.0
Test item	160	37.5	19.4	0.222 n.sign.	21.0	92.3
Test item	250	57.5	45.2	0.004 sign.	25.6	91.3
Test item	380	77.5	71.0	<0.001 sign.	n.a.	n.a.
Test item	600	97.5	96.8	<0.001 sign.	n.a.	n.a.
Reference item	12	100.0	100.0		n.a.	n.a.

LR_{50}: 273.9 g as/ha; 95 % Confidence Interval: (185.9 - 330.8) (calculated with Probit analysis)

* Fisher's Exact test (one-sided), p-values are adjusted according to Bonferroni-Holm
n.a. not assessed
n.sign. not significant
sign. significant

Conclusion

LR50 273.9 g a.s./ha; no effect on reproduction up to 250 g a.s./ha.

Guidelines and limitations

The study is acceptable.

STUDY IIIA, 10.5.2/04

Characteristics

reference	:	Jans D. (2010h)	species	:	*Aleochara bilineata*
type of study	:	Extended laboratory study	exposure duration	:	28 days
year of execution	:	2009-2010	nominal dose	:	Trial 1: 10, 21, 45, 95 and 200 g a.s./ha; Trial 2: 300 g a.s./ha
GLP statement	:	Yes	exposure method	:	Solution of test substance in water was sprayed on soil
Guideline	:	Grimm *et al.* (2000), Candolfi *et al* (2001)	acceptability	:	Acceptable
test substance	:	BYI 02960 SL 200 (200 g/L SL formulation), batch 2009-001253	ER50	:	>300 g a.s./ha
a.s content	:	17.0 % w/w (199.8 g/L) BYI 02960			

Methods

The effect of BYI 02960 SL 200 (200 g/L SL formulation, 17.0 % w/w (199.8 g/L) BYI 02960) on the reproductive capacity of the rove beetle *Aleochara bilineata*, exposed under extended laboratory conditions to fresh residues on natural soil, was determined.

Two trials 1 and 2 were performed, since in the first trial no effects were found up to the highest test rate of 200 g a.s./ha. Test units were plastic vessels (24 x 24 x 10.5 cm), which were covered with gauze, and contained LUFA 2.1 soil (4-5.5 cm high) moistened to 35% of WHC (0.74-0.81% oc, pH 5.1, 86.3-86.6% particles >0.063 mm, characterised (DIN) as sand or silty sand). The test substance was dissolved in water and sprayed on the soil using a calibrated laboratory sprayer in 400 L water/ha. The treatment rates for BYI 02960 SL 200 were 10, 21, 45, 95 and 200 g a.s./ha (trial 1) and 300 g a.s./ha (trial 2). The toxic standard was Dimethoate EC 400 g/L, sprayed onto the soil at a rate of 788.8 g a.s./ha in 400 L water/ha. The control was left untreated. Twenty adult beetles (10 females and 10 males) were introduced into each treated test unit immediately after application (4 replicate units per treatment). Yellow mealworm larvae were placed on the soil surface as a food source about 1 hour after application and then every 2-3 days afterwards. Seven, 14 and 21 days after application, 500 *Delia antiqua* pupae were placed into each test unit for parasitisation. The test units were kept in a controlled environment room until study end. Soil water losses were replaced every 2-3 days. Four weeks after application, the beetles were removed from the exposure units and discarded. Five weeks after application, the pupae were sieved out of the soil and transferred into untreated hatching cages, which were maintained to assess hatching rate until the hatching rate in the controls had fallen below 2 beetles per replicate per day.

Results

The reported results on reproduction are summarised in the tables below. The number of emerged beetles in the water treated control satisfied the criterion (≥400 per replicate) in Grimm *et al* (IOBC, 2000). The reference substance gave the expected response. As there were no statistically significant effects on reproduction at any test dose, the ER50 is >300 g a.s./ha.

Table B.9.5.1-05 Effects of BYI 02960 SL 200 on *reproduction of A. bilineata* under extended laboratory conditions (trial 1)

Test item		BYI 02960 SL 200 (g/L)		
Test organism		*Aleochara bilineata*		
Exposure on:		Spray deposits on sandy soil (LUFA 2.1)		
		Reproductive capacity		
Treatment	g as/ha	Hatched beetles per introduced female (number)	Red. rel. to control [%]	P-Value(#)
Control	0	76.4		
Test item	10	70.6	7.6	0.219 n.sign.
Test item	21	62.5	18.2	0.261 n.sign.
Test item	45	76.7	-0.4	0.279 n.sign.
Test item	95	66.3	13.2	0.289 n.sign.
Test item	200	63.7	16.5	0.296 n.sign.
Reference item	788.8	27.1	64.5	
# one-way ANOVA, Williams test (one-sided) n.sign. not significant				

Table B.9.5.1-06 Effects of BYI 02960 SL 200 on *reproduction of A. bilineata* under extended laboratory conditions (trial 2)

Test item		BYI 02960 SL 200 (g/L)		
Test organism		*Aleochara bilineata*		
Exposure on:		Spray deposits on sandy soil (LUFA 2.1)		
		Reproductive capacity		
Treatment	g as/ha	Hatched beetles per introduced female (number)	Red. rel. to control [%]	P-Value(#)
Control	0	71.2		
Test item	300	62.0	13.0	0.322 n.sign.
Reference item	788.8	21.5	69.8	
ER_{50} > 300 g as/ha # one-way ANOVA, Williams test (one-sided) n.sign. not significant				

Conclusion

ER50 >300 g a.s./ha (natural soil with 0.74-0.81% organic carbon).

Guidelines and limitations

The study is acceptable. The age of the beetles at test initiation was not reported, but the reproductive performance of the control was acceptable and the response of the reference product confirmed the sensitivity of the test organisms.

STUDY IIIA, 10.5.3/01

Characteristics

reference	:	Jans D. (2010i)	species	:	*Aphidius rhopalosiphi*
type of study	:	Extended laboratory toxicity study (aged residues)	exposure duration	:	2 days
year of execution	:	2010	nominal dose	:	2 x 250 g a.s./ha (10 days interval)
GLP statement	:	Yes	dosing method	:	Semi-field aged residues on maize seedlings
guideline	:	Mead-Briggs *et al*. (2000), Mead-Briggs *et al*. (2009), Candolfi *et al* (2001)	acceptability	:	Acceptable
test substance	:	BYI 02960 SL 200 G (200 g/L SL formulation), batch 2009-001253	Endpoint	:	No adverse effects on survival and reproduction following exposure to 56-day aged residues
a.s. content	:	17.0 % w/w (199.8 g/L) BYI 02960			

Methods

Maize seedlings were treated with BYI 02960 SL 200 G (200 g/L SL formulation, 17.0 % w/w (199.8 g/L) BYI 02960) and aged up to 56 days under semi-field conditions. The effect of residues of BYI 02960 SL 200 G on survival and reproduction of the parasitic wasp *Aphidius rhopalosiphi*, confined under laboratory conditions over leaves cut from the treated maize seedlings, was determined.

The study was conducted in Höchst, Frankfurt am Main, Germany. The treatment regime for BYI 02960 SL 200 G was two times 250 g a.s./ha in 400 L water/ha (T1 and T2), with a treatment interval of 10 days (treatment dates 07 and 17 May, 2010). The study included a positive control (Dimethoate EC 400 g/L, 3.0 g a.s./ha) in 400 L water/ha, and water-treated controls. Treatments with BYI 02960 SL 200 G were applied under semi-field conditions to potted maize plants using a plot sprayer. Aging of the spray residues on the potted maize plants took place under natural semi-field conditions with rain protection (Plexi-glass, UV permeable). The temperature ranged from 5.2 to 39.3°C and the relative humidity from 20.8 to 99.9% during the aging time of the maize plants. Bioassays were performed with pieces of maize leaf (about 25 cm long) taken from treated plants at 0, 14, 28, 42, 49 and 56 days after the second treatment. One piece of leaf was transplanted in a pot. A wet piece of paper was wrapped around the leaf part which was put in the pot bottom, and the pot was filled with quartz sand up to 0.5 - 2 cm below the upper pot rim, so that the leaf stood in an upright position. Finally the leaf was sprayed with a 10% fructose solution and left to dry. Then the pot was filled with quartz sand completely to the pot rim. The pots were placed on a water absorbent mat to allow the pots to soak up water. Each pot with one treated maize leaf was enclosed within a clear polyacrylic cylinder, into which five female <48 h old wasps were introduced. The top of the cylinder was closed with a fine mesh gauze. Each bioassay was performed with 6 replicate pots. The test units were kept in a controlled environment room until study end. Assessments of repellence were made during the first 3 hours and wasp mortality was assessed during 48 hours. During the 4th, 5th and 6th bioassay, 15 surviving females of the control and the BYI 02960 SL 200 G treatment were removed and individually confined over untreated aphid-infested barley plants for 24 hours. The wasps were then removed and the plants were left for a further 11 days before the number of aphid mummies was recorded.

The reference item was applied under semi-field conditions only at the second application day of the test item. In the second to the sixth bioassay the reference item was freshly applied in the laboratory using a calibrated laboratory sprayer on detached leaves taken from untreated maize plants, which had been stored till then under outdoor conditions.

Results

The results are summarised in the Table below. The reference substance gave the expected response in all bioassays. Validity criteria of Mead-Briggs *et al.* (2009) were satisfied: <10% control mortality, ≥5 mummies per control female, no more than 2 control wasps producing zero reproduction.

A repellent effect of the test item was observed up to 28 days after the second treatment. A statistically significant effect on survival was noted up to 49 days after the 2nd treatment, and a statistically significant effect on reproduction was recorded at 42 days after treatment. No more statistically significant or biologically relevant effect on survival and reproduction) occurred by day 56 after the 2nd treatment.

Table B.9.5.1-07 Effects of fresh and semi-field aged residues of BYI 02960 SL 200 on semi-field treated maize plants on survival and reproduction of *A. rhopalosiphi*

Start bioassay:	0 DAA[a]	14 DAA[a]	28 DAA[a]	42 DAA[a]	49 DAA[a]	56 DAA[a]
	Mortality (%) after 48 h					
Control:	6.7	0.0	0.0	10.0	0.0	0.0
Test item:	100.0	90.0	76.7	36.7	20.0	6.7
Reference item:	100.0	100.0	100.0	100.0	100.0	100.0
	Corrected Mortality (%)					
Test item:	**100.0** (p-value < 0.001, significant[b])	**90.0** (p-value < 0.001, significant[b])	**76.7** (p-value < 0.001, significant[b])	**29.6** (p-value 0.015, significant[b])	**20.0** (p-value 0.012 significant[b])	**6.7** (p-value 0.246, not significant[b])
Reference item:	100.0	100.0	100.0	100.0	100.0	100.0
	Repellency (mean values)					
	% Wasps on plant					
Control:	58.2	48.2	62.0	50.0	46.0	44.5
Test item:	18.6	25.3	43.0	31.3	54.7	47.2
Reference item:	56.7	37.0	55.5	45.0	40.8	45.8
	Rel. to control (%)					
Test item	**68.1** (p-value 0.007, significant[d])	**47.5** (p-value 0.006, significant[d])	**30.6** (p-value 0.012, significant[d])	**37.3** (p-value 0.062, not significant[d])	**-18.8** (p-value 0.051, not significant[c])	**-6.0** (p-value 0.416, not significant[d])
Reference item:	2.6	23.2	10.5	10.0	11.2	-3.0
	Reproduction					
	Number of mummies per female					
Control:	n.a.	n.a.	n.a.	**15.4**	**18.0**	**24.1**
Test item:	n.a.	n.a.	n.a.	**1.6**	**11.2**	**24.0**
	Reduction rel. to control (%)					
Test item:	n.a.	n.a.	n.a.	**89.9** (p-value < 0.001, significant[g])	**37.6** (p-value 0.181, not significant[c])	**0.6** (p-value 0.499, not significant[h])

n.a.: not assessed; [a] Days after 2nd application; [b] Fisher's Exact test (one-sided); [c] one-way ANOVA, Williams test (one-sided); [d] one-way ANOVA, Dunnett test (one-sided); [e] Welch test (one-sided); [f] Dunnett test (two-sided); [g] Wilcoxon test (one-sided)

Conclusion

Two treatments T1 and T2 of maize plants in spring (7 and 17 May) in Germany at 250 g a.s./ha with 10 days interval: statistically significant effects on survival of *A. rhopalosphi* up to 49 days after T2, and on reproduction at 42 days after T2; no effects by day 56 after T2.

Guidelines & Limitations

The study is acceptable.

STUDY IIIA, 10.5.3/02

Characteristics

reference	:	Schwarz A. (2010)	species	:	*Orius laevigatus*
type of study	:	Extended laboratory toxicity study (aged residues)	exposure duration	:	8-15 days
year of execution	:	2010	nominal dose	:	2 x 250 g a.s./ha (10 days interval)

GLP statement	:	Yes	dosing method	:	Semi-field aged residues on apple trees
guideline	:	Bakker et al. (2000)	acceptability	:	Acceptable
test substance	:	BYI 02960 SL 200 G (200 g/L SL formulation), batch 2009-001067	Endpoint	:	No adverse effects on survival and reproduction following exposure to 42-day aged residues
a.s. content	:	201.0 g/L BYI 02960			

Methods

Apple trees were treated with BYI 02960 SL 200 G (200 g/L SL formulation, 201.0 g/L BYI 02960) and aged up to 42 days under semi-field and field conditions. The effect of residues of BYI 02960 SL 200 G on survival and reproduction of the predatory bug *Orius laevigatus*, confined under laboratory conditions over leaves cut from treated apple trees, was determined.

The study was conducted in Rossdorf, Germany. The treatment regime for BYI 02960 SL 200 G was two times 250 g a.s./ha in 400 L water/ha (T1 and T2), with a treatment interval of 10 days (treatment dates 31 May and 10 June, 2010). The study included a positive control (Perfekthion, 414.8 g/L dimethoate, 37 g a.s./ha in 400 L water/ha for treatment of apple trees in the field, 10 g a.s./ha in 200 L water/ha for treatment of detached apple leaves in the laboratory), and water-treated controls. Treatments with BYI 02960 SL 200 G were applied under semi-field conditions to apple trees using a plot sprayer (BBCH 17-27 and 17-31 at 1st and 2nd application, respectively). Aging of the spray residues on the apple trees took place under natural semi-field conditions with rain protection (transparent, light permeable roof) up to 19 days after T2. Thereafter, the roof was removed, but on two more occasions (2 and 1 day, respectively), the plants were protected against rain due to forecast of heavy rain. The temperature ranged from 7 to 37°C and the relative humidity from 34 to 100% during the aging time of the apple trees. Bioassays were performed with detached leaves taken from treated trees at 0, 14, 28 and 42 days after the second treatment.

Leaf discs were cut from detached leaves from treated trees and incorporated into test units, forming the floor (treated side upwards). One 2nd instar nymph (4-5 days old) was added to each replicate unit. There were 50 replicates for each treatment, the control and the reference product. Food (*Ephestia* eggs) was added to the exposure units at least twice weekly. The test units were kept in a controlled environment room until study end. Assessments of mortality were made up to adulthood (during 8-15 days). During the 28DAT and 42DAT bioassay, when at least 80% of the control test organisms had developed into adults, all surviving males and females of a treatment were transferred to an untreated mating vessel. After mating, mated females were placed individually in untreated oviposition units consisting of a cylinder placed on a leaf disc from a leaf from an untreated bean plant, and the eggs were counted after 2 days and incubated to determine hatching success. The females were then transferred to new untreated oviposition units and after 2 days the eggs were again counted and incubated to determine hatching success.

The reference item was applied under semi-field conditions only at the second application day of the test item. In the second to the fourth bioassay, the reference item was freshly applied in the laboratory using a calibrated laboratory sprayer on detached leaves taken from untreated apple trees.

Results

The results are summarised in the Table below. The reference substance gave the expected response in all bioassays. Validity criteria of Bakker *et al.* (2000) were satisfied in all bioassays: <25% control mortality, ≥2 eggs per control female per day and <5 bugs producing zero values, >70% hatching rate.

A statistically significant effect on survival was noted up to 28 days after the 2nd treatment, and no statistically significant effects on reproduction were recorded at 28 and 42 days after treatment. No more statistically significant or biologically relevant effect on survival and reproduction) occurred by day 42 after the 2nd treatment.

Table B.9.5.1-08 Effects of fresh and semi-field aged residues of BYI 02960 SL 200 G on semi-field treated apple trees on survival and reproduction of *O. laevigatus*

bioassay	treatment	rate (g a.s./ha)	morta-lity (%)	corr. morta-lity (%)[(a)]	fecun-dity (eggs/ female/ day)	effect on fecun-dity (%)	fertility (% nym-phal hat-ching rate)	effect on fertility (%)
1 (day of T2)	control	-	14	-	n.t.	n.t.	n.t.	n.t.
	BYI 02960 SL 200 G	2 x 250	100*	100	n.t.	n.t.	n.t.	n.t.
	Perfekthion	37	100*	100	n.t.	n.t.	n.t.	n.t.
2 (14DAT2)	control	-	10	-	n.t.	n.t.	n.t.	n.t.
	BYI 02960 SL 200 G	2 x 250	78*	76	n.t.	n.t.	n.t.	n.t.
	Perfekthion	10	100*	100	n.t.	n.t.	n.t.	n.t.
3 (28DAT2)	control	-	2	-	7.2	-	91	-
	BYI 02960 SL 200 G	2 x 250	26*	25	5.5	23	97	-6.5[(B)]
	Perfekthion	10	100*	100	n.t.	n.t.	n.t.	n.t.
4 (42DAT2)	control	-	18	-	4.8	-	88	-
	BYI 02960 SL 200 G	2 x 250	26	9.8	3.1	35	77	13
	Perfekthion	10	100*	100	n.t.	n.t.	n.t.	n.t.

* statistically significant difference from control at 5% uncertainty level.
n.t. = not tested
(A) According to Abbot.
(B) Note: negative value, hence no adverse effect.

Conclusion

Two treatments T1 and T2 of apple trees in spring (31 May and 10 June) in Germany at 250 g a.s./ha with 10 days interval: statistically significant effects on survival of *O. laevigatus* up to 28 days after T2, no effects on reproduction at 28 and 42 days after T2; no adverse effects by day 42 after T2.

Guidelines & Limitations

The study is acceptable.

B.9.5.2 Semi-field and field studies (IIA 8.8.2, IIIA 10.5.3 and IIIA 10.5.4)

IIIA 10.5.4/01

Data requirement 5.2

Applicant to submit more comprehensive study summary of the two studies Aldershof S. & Bakker F. (2012). Furthermore the present table B.9.5.2-02 should be presented in a readable version. More detailed information will be necessary to understand the visualised results and especially the data standing behind it (e.g. validity criterion).

See also expert consultation 5(60).

See reporting table 5(58)

During the EU evaluation of Flupyradifurone SL 200 (BYI 02960 SL 200) EFSA set a data requirement for the applicant to provide a better readable extended version of the study summaries that were provided in the DAR with an improved quality of the included graphs and tables (see above). The applicant provided these. The sections of the applicant's summary that provide additional information and/or graphs/figures of improved graphical quality are added to the RMS' summary below.

Characteristics

reference	:	Aldershof S. & Bakker F. (2012a)	test organism	:	Field populations of arthropods
type of study	:	Non-target arthropod field study (off-crop)	exposure duration	:	8 weeks
year of execution	:	2010-2011	nominal dose	:	1 application at 0.51, 1.7, 5.1 and 21 g a.s./ha
GLP statement	:	Yes	dosing method	:	Sprayed on grassland
guideline	:	IOBC (Hassan, 1992), Brown (1998), Candolfi *et al.* (2000, 2001), De Jong *et al.* (2010)	acceptability	:	Acceptable
test substance	:	BYI 02960 SL 200, batch 2010-001067	conclusion	:	NOAER population, effect class 2: 21 g a.s./ha NOER community: 21 g a.s./ha NOER population: 5.1 g a.s./ha
a.s. content	:	201.0 g a.s./L (17.1% w/w)			

Objective

This field study was designed to assess the potential adverse effects on Non-Target Arthropods (NTA) in off-crop habitats that might occur at various distances from a treated area for current and future use pattern of the test item. The study was set up to enable an assessment of community- and population level ecotoxicological standards, in particular the NOER (No Observed Effect Rate), the NOEAER and the LOEAER (No and Lowest Observed Ecologically Adverse Effect Rate, respectively).

Methods

The effect of BYI 02960 SL 200 (201 g/L BYI 02960) on non-target arthropod populations was investigated in Renkum (The Netherlands) under field conditions after 1 spray application on grassland at dose levels of 0.51, 1.7, 5.1 and 21 g a.s./ha on 30 June 2010, with water control and reference product (Karate Zeon, a.s. lambda cyhalothrin, 40 g a.s./ha), using a design to determine the NOER, NOEAER and LOEAER.

Test material

Test item:	BYI 02960 SL 200
Specification No.:	102000021884-01
Type:	Formulated product (soluble (liquid) concentrate)
Chemical state and description:	Clear brown liquid
Batch No.:	2010-001067
Sample description:	TOX 08907-00
Nominal content of active ingredient:	BYI 02960: 200 g/L
Analytical content of active ingredient:	BYI 02960: 17.1% w/w, 201.0 g/L according to certificate of analysis
Density:	1.175 g/mL at 20°C
Stability of test compound:	Approved until 14.06.2012, when stored at 25 ± 5°C
Reference item:	Karate Zeon (lambda-cyhalothrin)
Batch No.:	BSN902273
MITOX ID	20100625FB01
Nominal content of active ingredient:	Lambda-cyhalothrin: 100 g/L (9.5%)
Stability of test compound:	Approved until June 2012
Controls:	Water
In-life dates	June 27 to August 30, 2010
Location:	3 km north of the village Renkum, 10 km west of the village Arnhem in the Netherlands.
Field description:	The meadow was located in an agricultural landscape (meadows, low crops, some bushes). The field was delimited on the western side by a road, on the north- and eastern side by a hedgerow and meadows, on the south side by a dirt road and meadows.

Trial lay out and treatment

Prior to trial lay out, the test site had been uncultivated grassland with low agricultural input (occasional mowing for hay production). The site was surrounded by agricultural fields. For approximately 20 years prior to the test no pesticides or herbicides were used on the test field, and during three years before the start of the study no mowing was performed. The soil had pH 4.5 and 7.1% organic matter (reported particle size distribution was incorrect, soil class was not reported).

On the test site, 24 plots (24 m x 24 m each) were established in a checkerboard design, such that treatment plots alternated with untreated plots, and no treatment plots were directly adjacent to one another. The BYI 02960 SL 200 treatments, the water control and the reference product were each assigned to four replicate plots, arranged in a randomised block design.

Figure NL-01: Plot layout

Applications were made in 200 L water/ha on 30 June 2010, using a calibrated lift-mounted sprayer with 2 booms (total length 12 m). Spray deposit distribution during application was checked with water-sensitive papers at 2 locations in the test field and found to be adequate. In addition, water-sensitive papers were placed next to a treated plot on 2 locations, to monitor possible spray drift, these papers

did not show any contact with spray solution. The actual rate was confirmed to be within 7.3% of target by measuring the remaining spray liquid after applications of each plot.

Arthropod sampling

The soil-surface and plant-dwelling arthropod communities were sampled just before application (0-1 days before treatment), and 1, 2, 4 and 8 weeks after application (last sampling on 25 August 2010). On each sampling occasion, soil and surface dwelling arthropods were sampled using pitfall traps (10 per plot, 3-5 day sampling period), small, low and highly mobile plant inhabiting arthopods were collected using suction sampling, and mites and other low mobile small plant inhabiting arthropods were collected using weed sampling (weeds were cut off just above the soil surface from two 0.25 m^2 subplots of each plot) followed by Berlese-Tullgren extraction. Sampling was confined to the centre of each plot; pitfalls were placed in a central area of approximately 2 x 2 m around the plot centre and suction and weed sampling was done outside this central area. Arthropods were identified to the lowest possible taxonomic level.

Environmental conditions / irrigation

Climatic data were recorded on a weather station 8 km away from the test site. On the application day all treatments were applied during dry and calm weather conditions. No rainfall was recorded up to 10 days after treatment, when 29.3 mm fell. Total rainfall during the experimental period (2 months) was 267 mm.

Calculations and statistical analysis

<u>Evaluation at the community level (multivariate statistics)</u>

Data evaluation was done separately for the different sample types, and additionally with a dataset based on data from all sample types pooled. Final conclusions were based on results obtained from analyses of the pooled dataset.

Community level effects on non-target arthropods were examined using multivariate analysis techniques. The ordination technique used was Principal Response Curve method (PRC) developed by Van den Brink *et al.* (1996) and van den Brink and ter Braak (1999). The arthropod community data gathered in this study were analyzed using redundancy analysis. Data were log transformed (2*natural log of densities +1) prior to analysis. To assess the significance of differences between treatment groups and the control on each sampling moment separately, Monte Carlo permutation tests were performed per sampling date.

<u>Evaluation of separate taxa (univariate statistics)</u>

Only taxa that occurred at densities above 10 (total number of individuals in the sum of all water control treatments per sampling date) were considered. If taxonomically relevant, taxa were analyzed pooled if densities of individual taxa were too low for evaluation. Abbott's formula was used to express treatment effects relative to the control. The statistical significance of among treatment differences in

population density was determined separately for all time points using the nonparametric Mann-Whitney U test.

To reduce noise, taxa with Coefficient of Variation (CV) exceeding 1.4 (i.e. 140%) on more than three sampling moments were not used for effect evaluations. In addition, taxa with very unequal starting populations among treatments were excluded from evaluations. Taxa excluded from univariate evaluations were also excluded from the multivariate datasets. Taxa with CV exceeding 1.2 or 1.4 on several sampling moments were removed from the datasets used for multivariate analyses. As far as could be assessed, data did not indicate that these taxa that were excluded from analyses were adversely affected by the test item treatments.

Results of statistical analyses obtained from multivariate and univariate evaluations were in principle considered at an alpha level of 5%. However, for the evaluation of treatment related trends, statistical significances at an alpha level of 10% were considered in the context of graphical analysis of the results and if appropriate included by the reviewer in effect classification decisions.

Effects were classified according to De Jong *et al.* (2010). Different effect classes are listed below. For this study with a total post-application sampling period of two months only effect classes 1, 2, 3 and 8 are applicable. According to De Jong *et al.* (2010), recovery should be demonstrated on two consecutive sampling moments. Due to the 4 week interval between the last two sampling moments this would result in a very conservative effect classification. Effect class 3 was therefore subdivided in class 3a (no longer statistically significant on the last two sampling dates) and class 3b (no longer statistically significant on the last sampling date).

Table B.9.5.2-01 / Table NL-01 Effect classes according to De Jong *et al.* (2010)

Effect class	Description	Criteria
1	Effects could not be demonstrated (NOER)	• No (statistically significant) effects observed as a result of the treatment • Observed differences between treatment and controls show no clear causal relationship
2	Slight and transient effects	• Quantitatively restricted response of one or a few taxa and only observed on one sampling occasion
3	Pronounced short term effects; recovery within two months after first application	• Clear response of taxa, but full recovery within two months after the first application • Effects observed at two or more sampling instances
4	Pronounced effects; recovery within four months after first application	• Clear response of taxa, effects last longer than two months but full recovery within four months after the first application • Effects observed at two or more sampling instances
5	Pronounced effects; recovery within eight months after first application	• Clear response of taxa, effects last longer than four months but full recovery within eight months after the first application • Effects observed at two or more sampling instances
6	Pronounced effects; full recovery one year after first application	• Clear response of taxa, effects last longer than eight months but full recovery within one year after first application • Effects observed at two or more sampling instances
7	Pronounced effects; full recovery more than one year after first application	• Clear response of taxa, effects last longer than twelve months after the first application but full recovery found within the study period • Effects observed at two or more sampling instances
8	Pronounced effects; no recovery within the study period	• Clear response of taxa, no recovery within the duration of the study • Effects observed at two or more sampling instances

Note: For this study (see text), effect class 3 was subdivided in class 3a (no longer statistically significant on the last two sampling dates) and class 3b (no longer statistically significant on the last sampling date).

Results

Power analysis

For a rough estimation of the overall power of the univariate tests, Abbott values and P-values obtained from comparisons with the control and the toxic reference (Mann Whitney-U test) were presented in scatter plots. The minimum detection level for alpha= 0.05 was approximately 50% in the dataset including all sample types (used for final evaluations).

Arthropod species composition and abundance

Arthropod taxa with sufficiently high numbers for univariate analysis and acceptable CV (see before) were:

- *Acari*: 34 taxa, 8 of which were analysed (*Tydeoidea*; *Phytoseiid* female mites (mainly of the species *Graminaseius graminis* and *Typhlodromus kerkirae)*; other *Gamasida*; *Cunaxidae*; phytophagous *Tetranychidae*; *Tarsonemidae*; *Oribatida*).
- *Araneae*: *Lycosidae* (predominantly adult *Pardosa*); crab spider *Xysticus*; *Gnaphosidae*; *Phrurolithus* (*Clubionidae*); two taxa of the family *Linyphiidae*; juvenile *Araneidae*; *Phalangida*.
- *Coleoptera*: mainly *Carabidae* (*Amara*, *Harpalus*, *Poecilus*, *Pterostichus* and *Trechus*, other adult *Carabidae*) and *Staphylinidae* (*Aleocharinae*, *Staphilininae*, *Tachyporinae*, *Xantholininae*, other

Staphilinidae), and a few plant feeding taxa (*Elateridae*, *Alticinae* (*Chrysomelidae*), *Apion* (*Curculionidae*), other *Curculionidae*).

- *Hymenoptera*: *Formicidae*, *Braconidae* (*Alysiinae*, other *Braconidae*), *Chalcidoidea* (*Encyrtidae*, *Eulophidae*, *Mymaridae*, *Pteromalidae*), *Scelionidae*, other *Proctotrupoidea*.
- *Homoptera*: *Aphidoidae*, *Cicadellidae* (juvenile and adult stages).
- *Heteroptera*: *Miridae* (juvenile and adult stages), other *Heteroptera* (juvenile and adult stages).
- Other arthropods: *Collembola* (*Entomobryidae*, *Symphypleona*, *Isotomidae*); *Thysanoptera (juvenile and adult stages)*; *Diptera* (*Cecidomyiidae*, *Sciaridae*, *Phoridae*, *Agromyzidae*, *Chloropidae*, other *Acalyptrata*); *Isopoda (*suborder *Oniscidea*); *Chilopoda*

This list was compared by the reviewer to Table 4 of De Jong et al. (2010), which gives an overview of taxa which should be present in off-field studies in sufficient numbers to allow univariate analyses with a sufficient power to allow for a comparison with the relevant regulatory threshold. The guidance states: “This overview is considered generally applicable to field studies in Europe. When certain taxa are lacking, this does not mean that the study is unreliable per se, but the evaluator should be triggered to ask questions about the reasons for the lack of certain taxa, and it should be argued in the study report why these taxa were not sampled, or why they are lacking. The other way around, under local conditions for some taxa a more precise level than indicated in Table 4 would be of importance. When this is known, these taxa could be included in the study.”

This list was compared by the reviewer to Table 4 of De Jong et al. (2010), which gives an overview of taxa which should be present in off-field studies in sufficient numbers to allow univariate analyses with a sufficient power to allow for a comparison with the relevant regulatory threshold. The guidance states: “This overview is considered generally applicable to field studies in Europe. When certain taxa are lacking, this does not mean that the study is unreliable per se, but the evaluator should be triggered to ask questions about the reasons for the lack of certain taxa, and it should be argued in the study report why these taxa were not sampled, or why they are lacking. The other way around, under local conditions for some taxa a more precise level than indicated in Table 4 would be of importance. When this is known, these taxa could be included in the study.”

The Coleopteran taxa Coccinellidae and Lathridiidae, Lepidoptera, Orthoptera, and the Aranean taxa Thomisidae were not present in this study. However, all of these except for Coccinellidae were present in the other field study (see below). Both field studies are taken into account in the risk assessment. Furthermore, several other Coleopteran taxa are present in the current study and Coccinellidae are represented in a first tier (laboratory) study, in which they were not very sensitive. Therefore, the study is considered sufficiently representative for the off-field environment.

Altogether, a total of 72 taxa were sufficiently abundant to be subjected to population level evaluations. Multivariate analyses showed that before application, arthropod species composition and abundance was rather similar in all plots. This was deduced from the fact that all sample scores were

plotted relatively close together. No clustering per block or per treatment could be detected. The test field was considered suitable for the study.

Reference product

Multivariate analysis showed that the reference treatment induced immediate and persistent (at least 4 weeks) adverse effects on the arthropod fauna sampled in grasslands. On three consecutive post-application sampling moments the response was statistically significant (see Figure B.9.5.2-01 / Figure NL-03). Univariate analysis showed that many taxa were adversely and statistically significantly affected by the reference item treatment (see Table B.9.5.2-02 / Table NL-03). The validity criterion for the reference treatment was clearly met (at least 50% effect on at least one sample date for at least 10% of the taxa evaluated; De Jong *et al.*, 2010). No recovery was seen for several taxa in the positive reference treatment, indicating that for the experimental period chosen, the plot size was adequate to demonstrate persistent treatment related effects. The response of the reference treatment confirmed the validity of the study.

Figure NL-02: Proportion of Abbott values above 50% in the reference treatment. X-axis indicates sampling moments (weeks after application)

Community responses

The results for community response (pooled data from all sampling methods) are summarised in Figure B.9.5.2-01 / Figure NL-03 (first ordination axis). The second ordination axis was not statistically significant for any of the test item rates and the percentage of variation due to treatment and captured by the first axis (23.3%) was high compared to the percentage captured by the second axis (9.9%), hence the overall community response is well described by the PRC from the first ordination axis. Based on pooled data, treatment with the insecticide BYI 02960 SL 200 in an off-field grassland habitat did not lead to statistically significant effects on prevailing arthropod communities at any of the rates tested up to 21 g a.s./ha. Examination of community responses obtained from separate datasets

did not reveal any treatment related adverse effect on arthropod communities either~~(data not shown in this summary)~~.

Figure B.9.5.2-01 / Figure NL-03 Principal Response Curve (first ordination axis)
Test- and reference items were analyzed separately but for comparison plotted in one graph. PRC analyses comprised data from weed- (W), pitfall- (P) and suction (S) samples. Encircled data points are statistically significant (Monte-Carlo Permutation test, alpha = 0.05). Test item dose rates are in g a.s./ha.

Table NL-02: Statistical evaluation of the PRC

	Statistically significant at alpha = 0.1
	Statistically significant at alpha = 0.05

treatments included in analysis	% Variance accounted for by		% Variance explained by treatment regime captured by			
	time	treatment	ax1	ax2	P-value ax1	P-value ax2
Test item rates	28.5	12.2	12.3	10.6	0.995	0.913
Reference	26.7	17	61.6	13.5	0.030	0.921
P-values at individual sampling moments (Monte Carlo Permutation Test)						
All data	sample	0.51 g	1.7 g	5.1 g	21 g	Reference
29-Jun-10	1	0.894	0.932	0.815	0.987	1.000
07-Jul-10	2	0.810	0.664	0.334	0.827	0.032
14-Jul-10	3	0.874	0.811	0.375	0.932	0.030
28-Jul-10	4	0.780	0.668	0.813	0.875	0.028
25-Aug-10	5	0.917	0.913	0.825	0.962	0.344
Weed samples	sample	0.51 g	1.7 g	5.1 g	21 g	Reference
29-Jun-10	1	0.912	0.974	0.598	0.406	0.819
07-Jul-10	2	0.801	0.940	0.326	0.655	0.160
14-Jul-10	3	0.896	0.818	0.432	0.660	0.088
28-Jul-10	4	0.944	0.767	0.481	0.818	0.026
25-Aug-10	5	0.689	0.886	0.962	0.592	0.550
Pitfall samples	sample	0.51 g	1.7 g	5.1 g	21 g	Reference
30-Jun-10	1	0.968	0.667	0.629	1.000	0.976
07-Jul-10	2	0.796	0.449	0.126	0.883	0.217
14-Jul-10	3	0.824	0.567	0.330	0.784	0.570
28-Jul-10	4	0.669	0.732	0.826	0.649	0.033
25-Aug-10	5	0.894	0.921	0.965	0.929	0.242
Suction samples	sample	0.51 g	1.7 g	5.1 g	21 g	Reference
29-Jun-10	1	0.975	0.964	0.969	0.769	0.894
07-Jul-10	2	0.934	0.605	0.537	0.162	0.027
14-Jul-10	3	0.527	0.859	0.548	0.462	0.023
28-Jul-10	4	1.000	1.000	0.679	0.954	0.099
25-Aug-10	5	0.826	0.703	0.527	0.579	0.028

Population responses

The results for arthropod abundance (univariate analysis) in the BYI 02960 SL 200 treatments and the reference treatment at pre-test and after application are summarised in the Table below.

Table B.9.5.2-02 / Table NL-03 Comparison of arthropod abundance in control and BYI 02960 SL 200 treated plots one day before treatment (-1) and 1, 2, 4 and 8 weeks after last treatment.

		sample (weeks after application)	-1	1	2	4	8	-1	1	2	4	8	-1	1	2	4	8	-1	1	2	4	8	-1	1	2	4	8
	order	taxon / treatment	0.51 g a.s./ha					1.7 g a.s./ha					5.1 g a.s./ha					21 g a.s./ha					reference				
W	ACARI	Phytoseiidae (female)														↓								↓		↓↓	
W		Gamasida other (female)																						↓		↓	
W		Gamasida Nymph and male																						↓		↓	
W		Cunaxidae																		↓↓				↓↓		↓↓	
W		Tarsonemidae																									
W		Tetranychidae																↓									
W		Tydeoidea																						↓↓		↓	
W		Oribatida (total)												↓↓	↑												
P	COLLEMBOLA	Symphypleona																								↑↑	↑↑
P		Isotomidae																			↑					↑	
P		Entomobryidae							↑																	↑	
P	THYSANOPTERA	Thysanoptera		↑																							
P	HOMOPTERA	Aphidoidea												↓	↓↓	↓											
P		Cicadellidae (juveniles)						↓↓	↓																		↓↓
P	HETREOPTERA	Heteroptera juveniles							↓↓									↑									
P		Heteroptera adults																					↑				
P	HYMENOPTERA	Scelionidae	↑↑											↑													
P	COLEOPTERA	*Amara*	↑						↑		↑↑			↑	↑												
P		*Harpalus*																									
P		*Poecilus*																									
P		*Pterostichus*									↓↓						↓↓										
P		*Trechus*												↑↑													
P		Carabidae (other adults)															↓↓										↓
P		Alticinae								↑																	
P		Elateridae												↑↑													
P		*Apion*																									
P		Curculionidae other					↓↓				↓↓						↓										
P		Aleocharinae																									
P		Staphylininae	↓↓				↑	↓↓					↓					↓↓									
P		Tachyporinae						↓																↓↓	↓↓		
P		Xantholininae									↓				↓↓			↓		↓↓							↓
P		Staphylinidae (other adults)	↑					↑										↑					↑↑				
P	ISOPODA	Isopoda (all)																									
P	CHILOPODA	Chilopoda (all)												↓↓			↑		↓	↓↓							
P	ACARI	Oribatida																									
P	ARANEAE	Phalangida (juveniles)																									
P		Gnaphosidae		↓																							
P		*Phrurolithus* (adult)					↑↑								↑										↓		
P		*Alopecosa* (juveniles)																									
P		*Alopecosa* (adult)								↑↑																	
P		*Pardosa* (adult)												↑	↑↑									↓	↓↓		
P		*Trochosa*				↓																		↑			
P		*Pachygnatha*																						↓↓			
P		Erigoninae																									
P		Linyphiidae other		↓↓				↓												↓							
S	THYSANOPTERA	Thysanoptera (juveniles)																						↓↓		↓↓	
S		Thysanoptera (adults)																						↓↓	↓↓		
S	HOMOPTERA	Aphidoidea					↓↓																	↓↓	↓↓		
S		Cicadellidae (juveniles)																								↓↓	
S		Cicadellidae (adults)		↓↓													↑							↓			
S	HETEROPTERA	Miridae (adults)				↓↓					↓													↓↓		↓↓	
S		Miridae (juveniles)															↓↓		↓↓					↓↓			
S		Heteroptera other																									↑↑
S	DIPTERA	Cecidomyiidae																									
S		Sciaridae											↓↓														
S		Phoridae																									
S		Agromyzidae			↓↓										↓↓									↓↓	↓↓	↓	
S		Chloropidae																						↓↓	↓		
S		Acalyptrata (others)																						↓↓			
S	HYMENOPTERA	Formicidae																						↓↓	↓↓		
S		Alysiinae																	↓↓					↓↓			
S		Braconidae (other)				↓													↓↓					↓↓			
S		Encyrtidae							↑↑	↑				↑↑													
S		Eulophidae		↓↓	↓↓		↓↓																				
S		Mymaridae																									
S		Pteromalidae						↑↑					↑↑										↑				
S		Scelionidae																									
S		Proctotrupoidea other																									
S	COLEOPTERA	Alticinae															↓					↓		↓	↓↓	↓↓	↓↓
S	ARANEAE	*Xysticus* (juvenile)																						↓↓	↓		
S		*Pachygnatha* (juvenile)																						↓↓			
S		Araneidae (juvenile)																						↓↓	↓↓	↓↓	↓↓

↓↓ Numbers reduced compared to the control, statistically significant at alpha=0.05
↓ Numbers reduced compared to the control, statistically significant at alpha=0.1
W=weed; P=pitfall, S=suction
↑↑ Numbers increased compared to the control, statistically significant at alpha=0.05
↑ Numbers increased compared to the control, statistically significant at alpha=0.1

Reported evaluation of population responses

At rates up to and including 5.1 g a.s./ha, on single sampling moments statistically significant differences compared to the control were observed for several taxa, but no consistent trend in time or relation to the dose rate was found, and these observations are therefore considered to be unrelated

to treatment. For few taxa a statistically significant reduction compared to the control was seen one or two weeks after application in the highest BYI 02960 SL 200 rate of 21 g a.s./ha.

At 21 g a.s./ha, the statistically significant reductions of the mite taxon Cunaxidae 2 weeks after treatment and of the Hymenoptera taxa Alysiinae and other Braconidae one week after treatment were considered to be treatment related adverse effects, followed however by fast recovery. The statistically significant reductions of juvenile Miridae (Heteroptera taxon) one week after treatment were considered to be unrelated to the test item treatment since at the time the reduction occurred densities were low and declining, and relative differences in densities between treatment plots were not consistent in time. The statistically significant reductions of Xantholininae (Coleoptera) 2 weeks after treatment were not likely to be related to the treatment since there was no clear dose related response on any of the sampling moments, and densities in the highest test item rate were lowest before application took place. The author of the report did not comment on the statistically significant reduction of Chilopoda 2 weeks after treatment.

The author of the report drew the following conclusions:

- BYI 02960 SL 200 applied at a rate of 21 g a.s./ha is the community NOER (No Observed Effect Rate), effect class 1.
- The predatory mite Cunaxidae and the hymenopteran parasitoid Braconidae were adversely affected on one sampling moment only shortly after application in the highest test rate. Both populations recovered within the next sampling moment one or two weeks later. BYI 02960 SL 200 applied at 21 g a.s./ha (effect class 2), is therefore classified as the population NOEAER (no observed ecologically adverse effect rate).

Evaluation by reviewer

The reported conclusions for Cunaxidae, Alysiinae and other Braconidae are acceptable. Figures B.9.5.2-02 to -04 / NL-04 to -06 show that recovery indeed occurs quickly. The Y-axis shows the number of arthropods per treatment (total of all plots per treatment), on a log-scale.

Figure B.9.5.2-02 / Figure NL-04Effects on Cunaxidae (Prostigmata, Acari) [Weed samples]

Figure B.9.5.2-03 / Figure NL-05 Effects on Alysiinae (Braconidae, Hymenoptera) [Suction samples]

Figure B.9.5.2-04 / Figure NL-06 Effects on other Braconidae (Hymenoptera) [Suction samples]

The effects on Xantholininae, juvenile Miridae and Chilopoda are graphically displayed in Figure B.9.5.2-05 to –07 / Figure NL-07 to NL-09.

After 2 weeks, statistically significant reductions of 62% of Xantholininae were recorded at 5.1 and 21 g a.s./ha, respectively, and a weak dose-effect relationship is present. However, before and after the 2-week sampling there is no dose-effect relationship at all (therefore the statistically significant reduction after 4 weeks at 1.7 g a.s./ha is not considered treatment related), and in addition there were reductions of 56% (p=0.24) and 81% (p=0.091) at 5.1 and 21 g a.s./ha, respectively, at the pre-test sampling. The conclusion of the author (effect on Xantholininae after 2 weeks not likely to be related to the treatment) is supported.

Figure B.9.5.2-05 / Figure NL-07 Effects on Xantholininae (Staphylinidae, Coleoptera) [Pitfall samples]

Because of the strongly declining populations in all treatments (including the control), it is not unlikely that the statistically significant reduction of juvenile Myridae of 80% relative to the control at 21 g a.s./ha one week after treatment is unrelated to the test item treatment. This is supported by the fact that at the 2-week and 4-week sampling the populations of juvenile Myridae at 21 g a.s./ha were highest of all treatment and the control, and that there is no dose-response relation after one week; in fact, the second highest dose rate of 5.1 g a.s./ha has comparable individuals to the control while the lowest dose rate of 0.51 g a.s./ha has the second lowest number of individuals). The conclusion of the author (effect on juvenile Myridae unrelated to the treatment) is supported.

Figure B.9.5.2-06 / Figure NL-08 Effects on juvenile Miridae (Heteroptera) [Suction samples]

Chilopoda were collected in relatively low numbers from pitfall traps. A statistically significant reduction of 84% relative to the control ($p < 0.05$) was recorded one week after treatment at 5.1 g a.s./ha, and of 78% ($p < 0.1$) after 1 week and of 67% ($p < 0.05$) after 2 weeks at 21 g a.s./ha. However, during the pre-test sampling the numbers of Chilopoda were already lower than those of the control at 5.1 and 21 g a.s./ha (88% and 50%, respectively). Inspection of Figure B.9.5.2-04 / Figure NL-09 shows that at 21 g a.s./ha, the development of the population of Chilopoda is comparable to that of the control throughout the test period (the curves are essentially parallel), and that the pre-test difference was maintained throughout the study. The statistically significant reduction of Chilopoda after 2 weeks at 21 g a.s./ha is not likely to be a treatment related effect.

Figure B.9.5.2-07 / Figure NL-09: Effects on Chilopoda [Pitfall samples]

Hence, the reported evaluation of the study results is considered to be acceptable.

Conclusion

BYI 02960 SL 200, single application to grassland on 30 June 2010 in The Netherlands: community NOER 21 g a.s./ha, effect class 1; population NOEAER 21 g a.s./ha (effect class 2).
Some taxa showed a statistically significant increase. These could potentially result from indirect effects.

Guidelines & Limitations

Comments were incorporated in the above summary. The study is acceptable. The actual concentration of BYI 02960 in the spray solution was not confirmed by chemical analysis. However, the test material was a certified product, and since it was a Soluble Concentrate (SL), inhomogeneity in the spray tank is not likely to occur.

IIIA 10.5.4/02

Data requirement 5.2 Applicant to submit more comprehensive study summary of the two studies Aldershof S. &

Bakker F. (2012). Furthermore the present table B.9.5.2-02 should be presented in a readable version. More detailed information will be necessary to understand the visualised results and especially the data standing behind it (e.g. validity criterion).

See also expert consultation 5(60).

See reporting table 5(58)

During the EU evaluation of Flupyradifurone SL 200 (BYI 02960 SL 200) EFSA set a data requirement for the applicant to provide a better readable extended version of the study summaries that were provided in the DAR with an improved quality of the included graphs and tables (see above). The applicant provided these. The sections of the applicant's summary that provide additional information and/or graphs/figures of improved graphical quality are added to the RMS' summary below.

Characteristics

reference	:	Aldershof S. & Bakker F. (2012b)	test organism	:	Field populations of arthropods
type of study	:	Non-target arthropod field study (off-crop)	exposure duration	:	8 weeks
year of execution	:	2010	nominal dose	:	1 application at 0.51, 1.7, 5.1 and 21 g a.s./ha
GLP statement	:	Yes	dosing method	:	Sprayed on grassland
guideline	:	IOBC (Hassan, 1992), Brown (1998), Candolfi *et al.* (2000, 2001), De Jong *et al.* (2010)	acceptability	:	Acceptable
test substance	:	BYI 02960 SL 200, batch 2010-001067	conclusion	:	NOAER population, effect class 3a: 21 g a.s./ha, NOAER population, effect class 2: 5.1 g a.s./ha; NOER community: 21 g a.s./ha NOER population: 1.7 g a.s./ha
a.s. content	:	201.0 g a.s./L (17.1% w/w)			

Objective

This field study was designed to assess the potential adverse effects on Non-Target Arthropods (NTA) in off-crop habitats that might occur at various distances from a treated area for current and future use patterns of the test item. By analogy to regulatory studies in e.g. aquatic environments the study was set up to enable an assessment of community- and population level ecotoxicological standards, in particular the NOER (No Observed Effect Rate), the NOEAER and the LOEAER (No and Lowest Observed Ecologically Adverse Effect Rate, respectively).

Methods

The effect of BYI 02960 SL 200 (201 g/L BYI 02960) on non-target arthropod populations was investigated in Sainte Maure de Peyriac, Lot et Garonne, South-France, under field conditions after 1

spray application on grassland at dose levels of 0.51, 1.7, 5.1 and 21 g a.s./ha on 20 July 2010, with water control and reference product (Karate Zeon, a.s. lambda cyhalothrin, 40 g a.s./ha), using a design to determine the NOER, NOEAER and LOEAER.

Test material

Test item:	BYI 02960 SL 200
Specification No.:	102000021884-01
Type:	Formulated product (soluble (liquid) concentrate)
Chemical state and description:	Clear brown liquid
Batch No.:	2010-001067
Sample description:	TOX 08907-00
Nominal content of active ingredient:	BYI 02960: 200 g/L
Analytical content of active ingredient:	BYI 02960: 17.1% w/w, 201.0 g/L according to certificate of analysis
Density:	1.175 g/mL at 20°C
Stability of test compound:	Approved until 14.06.2012, when stored at 25 ± 5°C
Reference item:	Karate Zeon (lambda-cyhalothrin)
Batch No.:	BSN9H2472
MITOX ID	20100505JR01
Nominal content of active ingredient:	Lambda-cyhalothrin: 100 g/L (9.5%)
Stability of test compound:	Approved until May 2011
Controls:	Water
In-life dates	13 July until 14 September 2010
Location:	The field was situated 1.5 km east of the village Sainte Maure de Peyriac, Lot et Garonne (47), in the South-West of France.
Field description:	The meadow was delimited on the northeastern side by a road, on the northwestern side by a house and gardens, at the southeast and southwest side by vinyards. It was gently sloping down towards the southwest.

Trial lay out and treatment

Prior to trial lay out, the test site had been uncultivated grassland with little agricultural input (occasional mowing for hay production). The site was surrounded by agricultural fields. For >5 years prior to the test no pesticides or herbicides were used on the test field. In January 2010, a low dose of fertiliser was applied on the field. Three months before the start of the study the field was mown (no mowing was performed during the study period). The soil had pH 6.1 and 4.1% organic matter (reported particle size distribution was incorrect, soil class was not reported). *Festuca pratensis* (meadow fescue) and *Trifolium pratense* (red clover) were the dominant plant species.

On the test site, plots (22 m x 22 m each) were established in a checkerboard design, such that treatment plots alternated with untreated plots, and no treatment plots were directly adjacent to one another. The BYI 02960 SL 200 treatments, the water control and the reference product were each assigned to four replicate plots, arranged in a randomised block design.

Figure SWF-01: Plot layout

Applications were made in 200 L water/ha on 20 July 2010, using a calibrated tractor-mounted sprayer with 2 booms (total length 7.5 m). Spray deposit distribution during application was checked with water-sensitive papers at 1 location in the test field and found to be adequate. In addition, water-sensitive papers were placed next to a treated plot to monitor possible spray drift, these papers did not

show any contact with spray solution. The actual rate was confirmed to be within 5.4% of target by measuring the remaining spray liquid after applications of each plot.

Arthropod sampling

The soil-surface and plant-dwelling arthropod communities were sampled just before application (3-6 days before treatment), and 1, 2, 4 and 8 weeks after application (last sampling on 14 September 2010). On each sampling occasion, soil and surface dwelling arthropods were sampled using pitfall traps (10 per plot, 4-7 day sampling period), small, low and highly mobile plant inhabiting arthropods were collected using suction sampling, and mites and other low mobile small plant inhabiting arthropods were collected using weed sampling (weeds were cut off just above the soil surface from two 0.25 m^2 subplots of each plot) followed by Berlese-Tullgren extraction. Sampling was confined to the centre of each plot; pitfalls were placed in a central area of approximately 2 x 2 m around the plot centre and suction and weed sampling was done outside this central area. Arthropods were identified to the lowest possible taxonomic level.

Environmental conditions / irrigation

Climatic data were recorded on a weather station <4 km away from the test site. On the application day all treatments were applied during dry and calm weather conditions. No rainfall was recorded on the day of application (1.2 and 16.4 mm on 1 and 2 days after application, respectively, no more rain up to day 12 after application).

Calculations and statistical analysis

<u>Evaluation at the community level (multivariate statistics)</u>

Data evaluation was done separately for the different sample types, and additionally with a dataset based on data from all sample types pooled. Final conclusions were based on results obtained from analyses of the pooled dataset.

Community level effects on non-target arthropods were examined using multivariate analysis techniques. The ordination technique used was Principal Response Curve method (PRC) developed by van den Brink *et al.* (1996) and van den Brink and ter Braak (1999). The arthropod community data gathered in this study were analyzed using redundancy analysis. Data were log transformed (2*natural log of densities +1) prior to analysis. To assess the significance of differences between treatment groups and the control on each sampling moment separately, Monte Carlo permutation tests were performed per sampling date.

<u>Evaluation of separate taxa (univariate statistics)</u>

Only taxa that occurred at densities above 10 (total number of individuals in the sum of all water control treatments per sampling date) were considered. If taxonomically relevant, taxa were analyzed pooled if densities of individual taxa were too low for evaluation. Abbott's formula was used to express treatment effects relative to the control. The statistical significance of among treatment differences in

population density was determined separately for all time points using the nonparametric Mann-Whitney U test.

To reduce noise, taxa with Coefficient of Variation (CV) exceeding 1.2 (i.e. 120%) on more than three sampling moments were not used for effect evaluations. Taxa excluded from univariate evaluations were also excluded from the multivariate datasets. The author of the report stated that, as far as could be assessed, data did not indicate that these taxa that were excluded from analyses were adversely affected by the test item treatments.

Results of statistical analyses obtained from multivariate and univariate evaluations were in principle considered at an alpha level of 5%. However, for the evaluation of treatment related trends, statistical significances at an alpha level of 10% were considered in the context of graphical analysis of the results and if appropriate included in effect classification decisions.

Effects were classified according to De Jong *et al.* (2010). Different effect classes are listed below. For this study with a total post-application sampling period of two months only effect classes 1, 2, 3 and 8 are applicable. According to De Jong *et al.* (2010), recovery should to be demonstrated on two consecutive sampling moments. Due to the 4 week interval between the last two sampling moments this would result in a very conservative effect classification. Effect class 3 was therefore subdivided in class 3a (no longer statistically significant on the last two sampling dates) and class 3b (no longer statistically significant on the last sampling date).

Table B.9.5.2-01 / Table SWF-01 Effect classes according to De Jong et al. (2010)

Note: For this study (see text), effect class 3 was subdivided in class 3a (no longer statistically significant on the last two sampling dates) and class 3b (no longer statistically significant on the last sampling date).

Effect class	Description	Criteria
1	Effects could not be demonstrated (NOER)	• No (statistically significant) effects observed as a result of the treatment • Observed differences between treatment and controls show no clear causal relationship
2	Slight and transient effects	• Quantitatively restricted response of one or a few taxa and only observed on one sampling occasion
3	Pronounced short term effects; recovery within two months after first application	• Clear response of taxa, but full recovery within two months after the first application • Effects observed at two or more sampling instances
4	Pronounced effects; recovery within four months after first application	• Clear response of taxa, effects last longer than two months but full recovery within four months after the first application • Effects observed at two or more sampling instances
5	Pronounced effects; recovery within eight months after first application	• Clear response of taxa, effects last longer than four months but full recovery within eight months after the first application • Effects observed at two or more sampling instances
6	Pronounced effects; full recovery one year after first application	• Clear response of taxa, effects last longer than eight months but full recovery within one year after first application • Effects observed at two or more sampling instances
7	Pronounced effects; full recovery more than one year after first application	• Clear response of taxa, effects last longer than twelve months after the first application but full recovery found within the study period • Effects observed at two or more sampling instances
8	Pronounced effects; no recovery within the study period	• Clear response of taxa, no recovery within the duration of the study • Effects observed at two or more sampling instances

Note: For this study (see text), effect class 3 was subdivided in class 3a (no longer statistically significant on the last two sampling dates) and class 3b (no longer statistically significant on the last sampling date).

Results

Power analysis

For a rough estimation of the overall power of the univariate tests, Abbott values and P-values obtained from comparisons with the control and the toxic reference (Mann Whitney-U test) were presented in scatter plots. The minimum detection level for alpha= 0.05 was approximately 50% in the dataset including all sample types (used for final evaluations).

Arthropod species composition and abundance

Arthropod taxa with sufficiently high numbers for univariate analysis and acceptable CV (see before) were:

- *Acari*: 22 taxa, 9 of which were analysed (*Phytoseiid* female mites (mainly *Graminaseius graminis* and *Neoseiulus alpinus*); other *Gamasida* (adults and *juveniles* , including *Phytoseiidae*); *Tetranychidae*; *Tarsonemidae*; *Tydeoidea*; *Oribatida*).

- *Araneae*: *Linyphiidae (Meioneta, Erigoninae,* other *Linyphiidae*); *Lycosidae* (predominantly *Pardosa*, juveniles and adults); lynx spider *Oxyopes; Gnaphosidae*; several genera of *Thomisidae*; *Philodromidae*; *Thomisoidea*; *Theridiidae*; *Araneidae*.
- *Coleoptera*: mainly *Carabidae* (most abundant was *Harpalus*) and *Staphylinidae* (*Aleocharinae*, *Staphilininae*, *Xantholininae)* and a few plant feeding taxa (*Alticinae (Chrysomelidae)*, *Apion (Curculionidae), Latridiidae,* juvenile Coleoptera (including *Dermestidae and Carabidae)*).
- *Hymenoptera*: *Formicidae*, *Braconidae*, *Chalcidoidea* (*Aphilinidae*, *Pteromalidae*, *Trichogrammatidae, Mymaridae*, *Encyrtidae* and *Eulophidae*), *Proctotrupoidea* (*Scelionidae*, *Ceraphronidae* and other *Proctotrupoidea*), *Cynipoidea* (*Figitidae*).
- *Homoptera*: *Aphidoidae*, *Cicadellidae* (juvenile and adult stages).
- *Heteroptera*: *Lygeidae*, *Miridae*, *Tingidae*, *Cydnidae*.
- Other arthropods: *Collembola* (*Entomobryidae*, *Symphypleona*, *Isotomidae, Poduromorpha*); *Orthoptera* (*Acrididae*); *Thysanoptera*; *Lepidoptera*; *Diptera* (*Nematocera* (*Cecidomyiidae)*, *Orthorrrhapha* (*Dolichopodidae*, *Hybotidae*), *Phoridae*, *Chloropidae*, *Acalyptrata* (*Agromyzidae*), juvenile *Diptera*); *Isopoda (Oniscidae).*

This list was compared by the reviewer to Table 4 of De Jong et al. (2010), which gives an overview of taxa which should be present in off-field studies in sufficient numbers to allow univariate analyses with a sufficient power to allow for a comparison with the relevant regulatory threshold. The guidance states: “This overview is considered generally applicable to field studies in Europe. When certain taxa are lacking, this does not mean that the study is unreliable per se, but the evaluator should be triggered to ask questions about the reasons for the lack of certain taxa, and it should be argued in the study report why these taxa were not sampled, or why they are lacking. The other way around, under local conditions for some taxa a more precise level than indicated in Table 4 would be of importance. When this is known, these taxa could be included in the study.”

Only the Coleopteran taxa Coccinellidae was not present in this study. This was also not present in the other field study (see above). However, several other Coleopteran taxa are present in the current study and Coccinellidae are represented in a first tier (laboratory) study, in which they were not very sensitive. Therefore, the study is considered sufficiently representative for the off-field environment.

Altogether, a total of 79 taxa were sufficiently abundant to be subjected to population level evaluations. Multivariate analyses showed that before application, arthropod species composition and abundance was rather similar in all plots. This was deduced from the fact that all sample scores were plotted relatively close together. No clustering per block or per treatment could be detected. The test field was considered suitable for the study.

Reference product

Multivariate analysis showed that the reference treatment induced immediate and persistent (>8 weeks) adverse effects on the arthropod fauna sampled in grasslands. On all post-application sampling moments the response was statistically significant (see Figure B.9.5.2-05 Figure SWF-3). Univariate analysis showed that nearly all taxa were adversely and statistically significantly affected by the reference item treatment (see Table B.9.5.2-03 Table SWF-03). One week after the application 62.2% of the analysed taxa showed effects >50%. During the following sampling period the proportion of taxa with effects >50% decreased slowly from 59.5% after 2 weeks down to 33.8% after 8 weeks. The validity criterion for the reference treatment was clearly met (at least 50% effect on at least one sample date for at least 10% of the taxa evaluated; De Jong *et al.*, 2010). No recovery was seen for many taxa in the positive reference treatment, indicating that for the experimental period chosen, the plot size was adequate to demonstrate persistent treatment related effects. The response of the reference treatment confirmed the validity of the study.

Figure SWF-02:Proportion of Abbott values above 50% in the reference treatment. X-axis indicates sampling moments (weeks after application)

Community responses

The results for community response (pooled data from all sampling methods) are summarised in Figure B.9.5.2-08 Figure SWF-3 (first ordination axis). As the second ordination axis was not statistically significant for any of the test item rates and the percentage of variation due to treatment was low in all test item rates (12.2%), the second axis was not considered in the evaluation. The PRC graph showed differences between the highest two test item rates and the control, which were of small magnitude and short duration, and which were not statistically significant at any sampling moment. When examining community responses obtained from separate datasets, it appeared that the mite community (weed samples) and the arthropod community sampled from the soil surface (pitfall samples) did not show adverse responses to any of the BYI 02960 SL 200 treatments. It was only the

arthropod community collected with suction sampling that showed signs of transient adverse treatment related effects, but no statistically significant effects were found on any sampling moment in any of the datasets analysed.

Figure B.9.5.2-08 / Figure SWF-3: Principal Response Curve (first ordination axis)

Test- and reference items were analyzed separately but for comparison plotted in one graph. PRC analyses comprised data from weed (W)-, pitfall (P)- and suction (S) samples. Encircled data points are statistically significant (Monte-Carlo Permutation test, alpha = 0.05). The arrow indicates the application day. Test item dose rates are in g a.s./ha

Table SWF-02: Statistical evaluation of the PRC

	Statistically significant at alpha = 0.1
	Statistically significant at alpha = 0.05

treatments included in analysis	% Variance accounted for by		% Variance explained by treatment regime captured by			
	time	treatment	ax1	ax2	P-value ax1	P-value ax2
Test item rates	28.5	12.2	12.3	10.6	0.995	0.913
Reference	26.7	17	61.6	13.5	0.030	0.921
P-values at individual sampling moments (Monte Carlo Permutation Test)						
All data	sample	0.51 g	1.7 g	5.1 g	21 g	Reference
17-Jul-10	1	0.953	0.923	0.823	0.421	0.928
27-Jul-10	2	1.000	0.772	0.925	0.405	0.030
03-Aug-10	3	0.783	1.000	0.878	0.385	0.031
17-Aug-10	4	1.000	0.789	0.921	0.902	0.034
14-Sep-10	5	0.923	0.756	0.725	0.666	0.030
Weed samples	sample	0.51 g	1.7 g	5.1 g	21 g	Reference
14-Jul-10	1	0.932	0.650	0.169	0.187	0.722
27-Jul-10	2	0.704	0.072	0.844	0.311	0.031
03-Aug-10	3	0.652	1.000	0.953	0.676	0.417
17-Aug-10	4	0.631	0.646	0.860	0.521	0.494
14-Sep-10	5	0.383	0.720	0.549	0.326	0.703
Pitfall samples	sample	0.51 g	1.7 g	5.1 g	21 g	Reference
17-Jul-10	1	0.853	0.823	0.783	0.073	0.670
27-Jul-10	2	0.943	0.724	0.650	0.558	0.034
03-Aug-10	3	0.827	0.827	0.389	0.211	0.245
17-Aug-10	4	0.724	0.182	0.650	0.884	0.048
14-Sep-10	5	0.992	0.885	0.895	0.870	0.253
Suction samples	sample	0.51 g	1.7 g	5.1 g	21 g	Reference
14-Jul-10	1	0.975	0.964	0.969	0.769	0.894
27-Jul-10	2	0.934	0.605	0.537	0.162	0.027
03-Aug-10	3	0.527	0.859	0.548	0.462	0.023
17-Aug-10	4	1.000	1.000	0.679	0.954	0.099
14-Sep-10	5	0.826	0.703	0.527	0.579	0.028

Population responses

The results for arthropod abundance (univariate analysis) in the BYI 02960 SL 200 treatments and the reference treatment at pre-test and after application are summarised in the Table below.

Table B.9.5.2-03 Comparison of arthropod abundance in control and BYI 02960 SL 200 treated plots before treatment (-1) and 1, 2, 4 and 8 weeks after last treatment.

Table B.9.5.2-03 / Table SWF-03: Comparison of arthropod abundance in control and BYI 02960 SL 200 treated plots before treatment (-1) and 1, 2, 4 and 8 weeks after last treatment.

Order		sample (weeks after application) / Treatment	0.51 g a.s./ha					1.7 g a.s./ha					5.1 g a.s./ha					21 g a.s./ha					reference				
			-1	1	2	4	8	-1	1	2	4	8	-1	1	2	4	8	-1	1	2	4	8	-1	1	2	4	8
ACARI	WEED	Phytoseiidae (female)																	↑					↓			
		Total other Gamasida							↓										↑					↓			
		Tarsonemidae			↑		↑	↓												↓		↑		↑			↑
		Tetranychidae																						↓			
		Tydeoidea			↓				↓					↓						↓					↓		
		Oribatida													↑									↓	↑	↓	
COLLEMBOLA		Symphypleona					↓																				
		Poduromorpha																					↑				
		Isotomidae									↑																
		Entomobryidae																		↑							↑
ORTHOPTERA		Orthoptera																						↓	↓	↓	↓
THYSANOPTERA		Thysanoptera																									
HOMOPTERA		Aphidoidea							↑							↓											
		Cicadellidae (juveniles)																							↓	↓	↓
HETREOPTERA		Heteroptera juveniles																									
		Heteroptera adults																		↓							
DIPTERA		Phoridae																								↑	
		Chloropidae																		↑	↑						↓
HYMENOPTERA		Chalcidoidea																									
		Proctotrupoidea (6)																						↓	↓		
COLEOPTERA		Harpalus spp.								↑					↑												
		Carabidae (other adults)												↑										↑			
	PITFALL	Lathridiidae																								↓	
		Staphylinidae																									
		Coleoptera (juveniles)																							↓		
		Isopoda (all)																		↑		↑		↑			
		Chilopoda (all)								↓											↑						
ACARI		Oribatida																				↑				↑	
ARANEAE		Gnaphosidae		↑		↓	↓																↑				↓
		Thomisoidea							↓							↑					↓	↓		↓	↓		↓
		Pardosa (juveniles)																								↓	↓
		Pardosa (adult)												↑					↑					↓	↓		
		Lycosidae others																					↑				↓
		Erigoninae																						↓			↓
		Linyphiidae other																	↑								
COLLEMBOLA		Symphypleona																		↓							
		Entomobryidae																								↑	
THYSANOPTERA		Thysanoptera					↓															↓		↓	↓		↓
HOMOPTERA		Aphidoidea																		↓	↓	↓		↓	↓	↓	
		Coccoidea (juveniles)																			↓		↓				↓
		Coccoidea (adults)														↓											
		Cicadellidae (juveniles)												↓						↓	↓	↓		↓	↓	↓	↓
		Cicadellidae (adults)													↑	↑							↑	↓	↓		
		Fulgoromorpha (juveniles)																		↑					↓		
		Fulgoromorpha (adults)																									↑
		Fulgoromorpha																							↓		
HETEROPTERA		Heteroptera juv																							↓		
		Heteroptera adult					↑					↑										↑					
LEPIDOPTERA		Lepidoptera (adults)										↑															
DIPTERA		Cecidomyiidae																			↑	↑		↓	↓	↓	↓
		Orthorrhapha																									
		Phoridae					↑							↓							↓				↑		
		Agromyzidae								↓					↓									↓	↓		↓
		Chloropidae																						↓			
		Diptera juveniles																									↓
HYMENOPTERA		Formicidae																						↓			
		Braconidae																						↓		↓	
		Aphelinidae																									
		Encyrtidae					↓									↓						↓					
	SUCTION	Eulophidae												↓										↓			
		Mymaridae																	↑								
		Pteromalidae																			↑			↓	↓		
		Trichogrammatidae																						↓			↓
		Scelionidae																			↓				↓		
		Ceraphronidae																								↓	
		other Proctotrupoidea																									
		Cynipoidea																									
COLEOPTERA		Alticinae							↓					↓						↓	↓			↓			
		Apion spp.																						↓			
		Lathridiidae																									
		Coleoptera juveniles																								↓	
ACARI		Oribatida																									
ARANEAE		Runcinia (juv.)							↓					↓	↓					↓	↓			↓	↓	↓	
		Xysticus (juv.)																						↓	↓	↓	
		Philodromidae juveniles																						↓			
		Oxyopes (juv.)																						↓	↓	↓	
		Theridiidae																									
		Araneidae (juv.)										↓														↓	↓
		Erigoninae			↓					↓														↓	↓	↓	↓
		Meioneta																						↓		↓	
		Linyphiidae other																				↑		↓	↓		

↓ Numbers reduced compared to the control, statistically significant at alpha=0.05

↓ Numbers reduced compared to the control, statistically significant at alpha=0.1

↑ Numbers increased compared to the control, statistically significant at alpha=0.05

↑ Numbers increased compared to the control, statistically significant at alpha=0.1

Summary of reported evaluation of population responses (minor modifications/additions by reviewer)

Three taxa (all collected with suction sampling) showed statistically significant adverse response patterns that were considered related to the test item treatment.

Aphidoidea were reduced compared to the control on three consecutive sampling moments at 21 g a.s./ha (effects 93%, 79% and 69% after 1, 2 and 4 weeks, respectively; statistically significant at α <0.05 only after 4 weeks). At the last sampling densities were similar to control densities. In all density graphs below, the Y-axis shows the number of arthropods per treatment (total of all plots per treatment), on a log-scale.

Figure B.9.5.2-09 / Figure SWF-04: Effects on Aphidoidea [Suction samples]

Juvenile *Cicadellidae* collected with suction sampling were statistically significantly reduced compared to the control 1 and 2 weeks after application at 21 g a.s./ha (effects 89% and 77%, respectively). On the last two sampling moments (4 and 8 weeks after application) densities were still reduced compared to the control (52% and 59%, respectively), but differences were no longer statistically significant (α 0.083 and 0.15, respectively). Densities in the 5.1 g a.s./ha rate were statistically significantly reduced by 70% on the sampling moment immediately after treatment. During the remainder of the sampling period densities were similar to the control in this test item treatment. These effects were not observed with juvenile *Cicadellidae* collected with pitfall sampling. *Cicadellidae* species in arable habitats on perennial host plants as in grasslands mostly have two or more generations per year. Adults at later sampling events are the grown-up juveniles of the earlier sampling events and would also occur in a lower abundance if treatment related reductions of

abundance of juveniles occur. Nevertheless, no response was observed in adult Cicadellids 2 and 6 weeks after the reduction of juveniles. There were even higher numbers of adults relative to the control not only in test plots of 21 g as/ha but also in all other plots treated with BYI 02960 SL 200. This supports the assumption that the observed effect at the test rate of 21 g a.s./ha was only of short term. The winged adults are more mobile than the larvae and could quickly stabilize the population by migration and reproduction.

Figure B.9.5.2-10 / Figure SWF-05: Effects on juvenile Cicadellidae [Suction samples]

Figure B.9.5.2-10 / Figure SWF-06: Effects on juvenile Cicadellidae [Pitfall samples]

Plant eating *Alticinae* were statistically significantly reduced in the 5.1 g and 21 g a.s./ha rate one or two weeks after application. Reductions in these two test item treatments varied between approximately 70% and 90%. Differences compared to the control were not statistically significant on the last two sampling moments. Flea beetles can also walk normally and fly, indicating a large potential for recovery. (Effect figure given in next section)

For the following taxa (all collected with suction sampling) reductions compared to the control occurred incidentally, but no consistent trend in time or relation to the dose rate was found.

Juvenile *Runcinia* (Thomisidae) were moderately but statistically significantly reduced one week after application, both in the 21 g a.s./ha rate and in the 5.1 g a.s./g ha rate (see Figure B.9.5.2-11), but pre-application densities were very low. Another taxon of the family Thomisidae, Xysticus, was present at higher numbers during application, and showed no adverse response to the test item treatment.

Addition by reviewer: in addition to the statistically significant reductions of 40% and 60% after 1 week at 5.1 and 21 g a.s./ha, respectively, a statistically significant reduction of 50% after 1 week was recorded at 1.7 g a.s./ha. For the reasons given above, this reduction at 1.7 g a.s./ha is also not likely to be a treatment related effect.

Figure B.9.5.2-11 / Figure SWF-07: Effects on juvenile Runcinia (Thomisidae) [Suction samples]

Phoridae (Diptera) were statistically significantly reduced 1 week after application (5.1 g a.s./ha) or 2 weeks after application (21 g a.s./ha). Otherwise dynamics were similar to the control. Phoridae populations collected with pitfalls were not reduced compared to the control, nor were Phoridae populations in reference treatment plots (suction and pitfall samples).

Addition by reviewer: the reduction of 61% after 1 week at 5.1 g a.s./ha is likely to be an artifact since no treatment related effect was observed after 1 week at 21 g a.s./ha.

Figure B.9.5.2-12 / Figure SWF-08: Effects on Phoridae (Diptera) [Suction samples]

Figure B.9.5.2-12 / Figure SWF-09: Effects on Phoridae (Diptera) [Pitfall samples]

The author of the report drew the following conclusions:

- BYI 02960 SL 200 applied at a rate of 21 g a.s./ha is the community NOER (No Observed Effect Rate), effect class 1.
- Only three taxa were adversely affected. At the test rate of 5.1 g a.s./ha these effects were only on single sampling occasions significant (effect class 2) and at the rate of 21 g a.s./ha they all recovered within the ecologically acceptable time frame of two months (effect class 3a and 3b). The highest rate tested in this study, BYI 02960 SL 200 applied at 21 g a.s./ha, is therefore classified as the population NOEAER (no observed ecologically adverse effect rate).

Evaluation by reviewer

The reported description and interpretation of the effects on *Aphidoidea* and juvenile *Cicadellidae* as summarised above are acceptable. The position of the author that reductions of *Runcinia* and *Phoridae* are incidental events with an uncertain relationship to treatment is supported.

Effects on *Alticinae* are displayed in Figure B.9.5.2-13 Figure SWF-10 and summarised in Table B.9.5.2-04 Table SWF-04. After 1 week, reductions were 70%, 84% and 84% at 1.7, 5.1 and 21 g a.s./ha, respectively (statistically significant at α 0.083, <0.05 and 0.059, respectively). After 2 weeks, a statistically significant reduction of 91% was recorded at 21 g a.s./ha, and no other statistically significant reductions (α <0.1) were recorded at lower treatment rates after 2 weeks and at all treatment rates after 4 and 8 weeks. However, after 4 and 8 weeks, at 21 g a.s./ha the numbers of *Alticinae* were still reduced by 89-91% relative to the control, and although these differences are no longer statistically significant, they could indicate that recovery had not occurred. However, Figure B.9.5.2-13 Figure SWF-10 shows that at 21 g a.s./ha a reduction of the population of *Alticinae* relative to the control occurred during the first 2 weeks after treatment, but, although the numbers of *Alticinae* did not return to control values during the remainder of the study, the development *rate* of the control and 21 g a.s./ha populations was very similar (as indicated by the essentiall parallel population-time graphs). The lower numbers at the end of the study would then be the result of the lower numbers at the start of the period of similar development (after 4 weeks). Therefore it may be concluded that at 21 g a.s./ha *Alticinae*, although initially adversely affected, recovered within 4 weeks. Hence, the reported evaluation of the study results and the reported conclusions are considered to be acceptable.

Figure B.9.5.2-13 / Figure SWF-10: Effects on Alticinae (Chrysomelidae) [Suction samples]

Table B.9.5.2-04 / Table SWF-04: Effects on Alticinae (reductions with α <0.1 marked in bold)

Rate g a.s./ha	Week after application									
	-1		1		2		4		8	
	% redn	α	% redn	α	% redn	α	% redn	α	% redn	α
0.51	4	0.88	60	0.47	25	0.56	53	0.47	40	0.77
1.7	-11	0.77	**70**	0.08	91	0.19	84	0.38	73	0.38
5.1	-7	0.66	**84**	0.03	62	0.38	76	0.56	75	0.31
21	-11	0.47	**84**	0.06	**91**	0.05	89	0.22	91	0.15

Conclusion

BYI 02960 SL 200, single application to grassland on 20 July 2010 in South-France: community NOER 21 g a.s./ha, effect class 1; population NOEAER 5.1 g a.s./ha (effect class 2); population NOEAER 21 g a.s./ha (effect class 3b because of effects on *Aphidoidea*).

Guidelines & Limitations

Comments were incorporated in the above summary. The study is acceptable. The actual concentration of BYI 02960 in the spray solution was not confirmed by chemical analysis. However, the test material was a certified product, and since it was a Soluble Concentrate (SL), inhomogeneity in the spray tank is not likely to occur.
Several taxa showed a statistically significant increase at certain sampling points. Three taxa showed this increase at the highest dose rate at the last sampling point. These observations may result from indirect effects and therefore be relevant, but they were not explained in the report. No further information is available on this issue. Note that it would not change the outcome of the risk assessment, since this is based on a lower dose rate.

B.9.5.3 Summary and risk assessment

Procedures for risk assessment were in agreement with the recommendations in the Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (Working Document Sanco/10329/2002 rev 2 final, 17 October 2002, referring to ESCORT 2). The recommendations of ESCORT 3, a follow-up workshop, are taken into account for this DAR for issues where guidance is lacking.

In glasshouse crops for which Integrated Pest Management systems are used, introduced natural enemies may be exposed to flupyradifurone and, considering the results of in-field risk calculations for the field uses (see below), significantly affected This issue is not assessed in the framework of the DAR but can be taken account at Member State level.

Toxicity tests on non-target arthropods have been performed with BYI 02960 SL 200 G on the species *Typhlodromus pyri*, *Aphidius rhopalosiphi*, *Coccinella septempunctata*, *Aleochara bilineata* and *Orius laevigatus*. Furthermore, two full-fauna off-crop field studies were conducted. Table B.9.5.3-01 below summarises the results of laboratory testing with non-target arthropod species.

Table B.9.5.3- 01 Ecotoxicological endpoints for arthropods other than bees (BYI 02960 SL 200)

Test species, Dossier-File-No. Reference	**Tested Formulation, Study type, Exposure**	**Ecotoxicological Endpoint**

Test species, Dossier-File-No. Reference	Tested Formulation, Study type, Exposure	Ecotoxicological Endpoint
Aphidius rhopalosiphi Jans, 2010 KIIA 8.8.1.1/01 KIIIA1 10.5.1/01	BYI 02960 SL 200 (g/L) Laboratory, glass plates 0.5 g a.i./ha 1.1 g a.i./ha 2.2 g a.i./ha 4.7 g a.i./ha 10 g a.i./ha 10 g a.i./ha 20 g a.i./ha 40 g a.i./ha 80 g a.i./ha 160 g a.i./ha	LR_{50} < 0.5 g a.i./ha Corr. Mortality [%] 85 100 100 100 100 100 100 100 100 100
Typhlodromus pyri Jans, 2010 KIIA 8.8.1.2/01 KIIIA 10.5.1/02	BYI 02960 SL 200 (g/L) Laboratory, glass plates 2 g a.i./ha 4 g a.i./ha 9 g a.i./ha 19 g a.i./ha 40 g a.i./ha	LR_{50} 17 g a.i./ha Corr. Mortality [%] 6.3 6.3 25.0 50.0 89.6
Aphidius rhopalosiphi Jans, 2010 KIIIA1 10.5.2/01	BYI 02960 SL 200 (g/L) Extended lab., exposure on potted barley seedlings 0.5 g a.i./ha 0.89 g a.i./ha 1.58 g a.i./ha 2.81 g a.i./ha 5.0 g a.i./ha	LR_{50} 2.02 g a.i./ha, ER_{50} >0.89 <1.58 g a.i./ha Corr. Mortality [%] / Effect on Reproduction [%] / Repellency rel. to control [%] -3.4 [A] / -6.4 [B] / -42.0 [C] sign. 10.3 / -2.9 [B] / -45.8 [C] n.sign. 37.9 / 57.7 / -26.3 [C] n.sign. 69.0 / n.a. / -67.5 [C] sign. 89.7 / n.a. / -43.7 [C] sign.
Typhlodromus pyri Jans, 2010 KIIIA1 10.5.2/02	BYI 02960 SL 200 (g/L) Extended lab., exposure on detached broad bean leaf discs 15 g a.i./ha 32 g a.i./ha 67 g a.i./ha 142 g a.i./ha 300 g a.i./ha	LR_{50} 177 g a.i./ha, ER_{50} >142 g a.i./ha Corr. Mortality [%] / Effect on Reproduction [%] -1.1 [A] / 6.0 -4.6 [A] / 0.6 6.9 / 5.1 37.9 / -6.8 [B] 75.9 / n.a.
Coccinella septempunctata Jans, 2010 KIIIA1 10.5.2/03	BYI 02960 SL 200 (g/L) Extended lab., exposure on detached broad bean leaves Control 8 g a.i./ha 17 g a.i./ha 35 g a.i./ha 72 g a.i./ha 150 g a.i./ha Control 100 g a.i./ha 160 g a.i./ha 250 g a.i./ha 380 g a.i./ha 600 g a.i./ha	LR_{50} 273.9 g a.i./ha Corr. Mortality [%] / Eggs/Female/Day / Hatching [%] - / 10.4 / 92.0 12.9 / 15.5 / 91.4 4.1 / 17.6 / 95.7 -9.7 [A] / 9.5 / 87.3 3.2 / 15.5 / 93.5 32.3 / 21.6 / 95.4 - / 13.9 / 91.9 9.7 / 16.1 / 92.0 19.4 / 21.0 / 92.3 45.2 / 25.6 / 91.3 71.0 / n.a. / n.a. 96.8 / n.a. / n.a.

Test species, Dossier-File-No. Reference	Tested Formulation, Study type, Exposure	Ecotoxicological Endpoint		
Aleochara bilineata Jans, 2010 KIIIA1 10.5.2/04	BYI 02960 SL 200 (g/L) Extended lab., spray deposits on soil (LUFA 2.1) 10 g a.i./ha 21 g a.i./ha 45 g a.i./ha 95 g a.i./ha 200 g a.i./ha 300 g a.i./ha	ER_{50} > 300 g a.i./ha Effect on Reproduction [%] 7.6 18.2 -0.4 [B] 13.2 16.5 13.0		
Aphidius rhopalosiphi Jans, 2010 KIIIA1 10.5.3/01	BYI 02960 SL 200 (g/L) Aged residue spray deposits on maize plants, 2 appl. of 250 g a.i./ha (spray interval of 10 d)	Corr. Mortality [%]	Effect on Reproduction [%]	Repellency rel. to control [%]
	Residues aged for 0 d:	100	n.a.	68.1 sign.
	Residues aged for 14 d:	90.0	n.a.	47.5 sign.
	Residues aged for 28 d:	76.7	n.a.	30.6 sign.
	Residues aged for 42 d:	29.6	89.9	37.3 n.sign.
	Residues aged for 49 d:	20.0	37.6	-18.8 [C] n.sign.
	Residues aged for 56 d:	6.7	0.6	-6.0 [C] n.sign.
Orius laevigatus Schwarz, 2010 KIIIA1 10.5.3/02	BYI 02960 SL 200 (g/L) Aged residue spray deposits on apple trees, 2 appl. of 250 g a.i./ha (spray interval of 10 d)	Corr. Mortality [%]	Effect on Fecundity [%]	Effect on Fertility [%]
	Residues aged for 0 d:	100	n.a.	n.a.
	Residues aged for 14 d:	75.6	n.a.	n.a.
	Residues aged for 28 d:	24.5	23.0	-6.5 [D]
	Residues aged for 42 d:	9.8	34.7	12.9

A: A negative value indicates a lower mortality in the treatment than in the control.
B: A negative value indicates a higher reproduction rate in the treatment than in the control.
C: A negative value indicates a higher percentage of wasps found on plants in the treatment than in the control.
D: A negative value indicates a higher nymphal hatching rate in the treatment than in the control.
n.a.: Not assessed.
sign.: statistically significant at 5%-level (only indicated for repellency).
n.sign.: not statistically significant (only indicated for repellency).

The tier 1 glass plate studies indicate that insects, as represented by *Aphidius rhopalosiphi* (LR50 < 0.5 g a.i./ha), are more sensitive to the exposure of BYI 02960 SL 200 G than mites like *Typhlodromus pyri* (LR50 17.3 g a.i./ha). This has been confirmed by extended laboratory studies for the same two species. A comparison of the extended laboratory results for *Aphidius rhopalosiphi* (LR50 2.02 g a.i./ha, ER50 >0.89 and <1.58 g a.i./ha) with the results from the additionally tested species *Coccinella septempunctata* (LR50 273.9 g a.i./ha and no effects on reproduction below the LR50) and *Aleochara bilineata* (ER50 >300 g a.i./ha) gives clear evidence, that *Aphidius rhopalosiphi* is by far the most sensitive species.

Hence, aged residue studies were conducted with *Aphidius rhopalosiphi* and - in addition - with the predatory bug *Orius laevigatus*. The results showed that *Orius laevigatus* is also susceptible to the exposure of BYI 02960 SL 200 G (effects <50% after 28 days of aging) but clearly less sensitive than

Aphidius rhopalosiphi (effects <50% after 49 days of aging). For this reason, the risk assessment of *Orius laevigatus* is considered to be covered by the risk assessment for *Aphidius rhopalosiphi*.

Data have been submitted on *Typhlodromus pyri* and *Aphidius rhopalosiphi* (i.e. two indicator species) as well as three crop-relevant species (*Coccinella septempunctata, Aleochara bilineata* and *Orius laevigatus*). From these studies a risk assessment is performed according to the recommendations of ESCORT 2.

Tier 1 risk assessment

In the 1st tier, the LR50 values of laboratory glassplate tests with the two indicator species are taken into account. The hazard quotient (HQ) is calculated as follows:

In field-HQ = max. single application rate * MAF / LR_{50}

Off-field HQ = max. single application rate * MAF * (drift factor/VDF)*correction factor / LR_{50}

- Max. single application rate = 125 g a.i./ha (lettuce), 150 g a.i./ha (hops)
- MAF (multiple application factor) = 1 (only 1 application)
- Drift factor = 19.33% (hop, 3 m distance, 1 application; ESCORT2) 2.77% (lettuce (field crops), 1m distance, 1 application; ESCORT2)
- VDF (vegetation distribution factor) = 10 (default value as recommended by the Terrestrial Guidance Document, to take into account the 3-dimensional structure of the off-field vegetation)
- Correction factor = 10 (uncertainty factor for the extrapolation from indicator species to all off-field non-target arthropods; default value for tier 1 risk assessment according to the Terrestrial Guidance Document)

The risk is considered acceptable if the calculated HQ is < 2. See the Tables below.

Table B.9.5.3-02 HQ for terrestrial non-target arthropods for the in-field scenario

Crop (field uses)	**Species**	**Appl. rate [g a.i./ha]**	**MAF**	**LR_{50} [g a.i./ha]**	**HQ**	**Trigger**
Hops	*A. rhopalosiphi*	150	1	<0.5	**>300**	2
Hops	*T. pyri*	150	1	17	**8.8**	2
Lettuce	*A. rhopalosiphi*	125	1	<0.5	**>250**	2
Lettuce	*T. pyri*	125	1	17	**7.4**	2

Conclusion: For both standard species, the in-field HQ values are above the trigger of concern, indicating a need for refinement.

Table B.9.5.3-03 HQ for terrestrial non-target arthropods for the off-field scenario

Crop	Species	Appl. rate [g a.i./ha]	MAF	Drift [%]	VDF	Corr. factor	LR_{50} [g a.i./ha]	HQ	Trigger
Hops	*A. rhopalosiphi*	150	1	19.33	10	10	<0.5	**>58**	2
Hops	*T. pyri*	150	1	19.33	10	10	17	1.7	2
Lettuce	*A. rhopalosiphi*	125	1	2.77	10	10	<0.5	**>6.9**	2
Lettuce	*T. pyri*	125	1	2.77	10	10	17	0.2	2

Conclusion: The HQ values for *Typhlodromus pyri* are below the trigger of concern for both uses. However, both HQ values for *Aphidius* are above the trigger of 2, indicating a need for refinement.

Tier 2 risk assessment

In the 2nd tier, the results of extended laboratory tests are taken into account (LR50, ER50 and/or effect percentages). These are available for four species.

The exposure is calculated as:

In-field: Application rate * MAF

In-field (high crops) Application rate * MAF * 0.5

Off-field: Application rate * MAF * (drift factor / VDF) *correction factor

- Max. single application rate = 125 g a.i./ha (lettuce), 150 g a.i./ha (hops)
- MAF (multiple application factor) = 1 (only 1 application)
- Factor 0.5 for high crops: The correction factor of 0.5 for the in-field exposure assessment in three-dimensional crops according to ESCORT2 (see footnote “a” in legend on page 19 of the ESCORT2 document) can be applied according to the notifier. However, application in hops can start from a stage in which the plants are still small, BBCH 31. The BBCH scale describes the hop plants between BBCH 31 and 38 as follows:

  Principal growth stage 3: Elongation of bines
  31 Bines have reached 10% of top wire height
  32 Bines have reached 20% of top wire height
  33 Bines have reached 30% of top wire height
  3 . Stages continuous till . . .
  38 Plants have reached the top wire

  At the beginning of the application period (BBCH 31-37), the plants are still thin and small (ca. 40cm based on a top wire size of 4m) and it is not justified to use the factor of 0.5. Furthermore, it is unclear whether the correction factor can really also be used for hops. Since no justification/validation for the use of the factor of 0.5 for other crops than orchards and vineyards, the crops mentioned in ESCORT 2, is available, the risk is calculated without correction factor.
- Drift factor = 19.33% (hop, 3 m distance, 1 application; ESCORT2; considered relevant for all grwoth stages since the same application machinery will be used throughout the season); 2.77% (lettuce (field crops), 1m distance, 1 application; ESCORT2)

- VDF (vegetation distribution factor) = 10 (default value as recommended by the Terrestrial Guidance Document, to take into account the 3-dimensional structure of the off-field vegetation; it can only be applied in the context of 2D test systems)
- Correction factor = 5 (uncertainty factor for the extrapolation from indicator species to all off-field non-target arthropods; default value for tier 2 risk assessment)

Table B.9.5.3-04 Exposure calculation for in-field assessment

Crop / no. of applications	Appl. rate [g a.i./ha]	MAF	factor to account for high crops	in-field PEC_{max}. [g/ha]
Hops BBCH 31-38 / 1	150	1	n.a.	150
Lettuce / 1	125	1	n.a.	125

Table B.9.5.3-05 Exposure calculation for off-field risk assessment

Appl. rate [g a.i./ha]	MAF	Drift [%]	Veg. distr. factor	Correction factor	off-field $PEC_{max.}$ [g/ha]	Remark
150	1	19.33	-	5	145	in case of 3-D study design
150	1	19.33	10	5	14.5	in case of 2-D study design
125	1	2.77	-	5	17.3	in case of 3-D study design
125	1	2.77	10	5	1.73	in case of 2-D study design

Tier 2 in-field risk assessment

The in-field exposure PEC values are compared to the results of the extended laboratory studies.

Table B.9.5.3-06 In-field risk assessment based on study results from extended laboratory studies

Test Species	in-field $PEC_{max.}$, [g a.i./ha]	LR_{50}/ ER_{50} [g a.i./ha]	Trigger	Refined assessment required?
Use in hops, 1 x 150 g a.i./ha				
Typhlodromus pyri	150	177 / >142	Effects are < 50%	no*
Aphidius rhopalosiphi	150	2.02 / >0.89	Effects are < 50%	**yes**
Coccinella septempunctata	150	273.9 / no effects up to 250	Effects are < 50%	no
Aleochara bilineata	150	>300	Effects are < 50%	no
Use in lettuce, 1 x 125 g a.i./ha				
Typhlodromus pyri	125	177 / >142	Effects are < 50%	no
Aphidius rhopalosiphi	125	2.02 / >0.89	Effects are < 50%	**yes**
Coccinella septempunctata	125	273.9 / no effects up to 250	Effects are < 50%	no
Aleochara bilineata	125	>300	Effects are < 50%	no

* No adverse effects on reproduction were seen up to 142 g a.s./ha. The LR50 is 177 g a.s./ha. It is likely that at 150 g a.s./ha no adverse effects on *Typhlodromus* will occur.

The higher tier in-field risk assessment for *Typhlodromus, Coccinella* and *Aleochara* indicates that no unacceptable adverse effects are to be expected in the in-field area for arthropod species with a similar sensitivity as these species. However, the in-field risk assessment for *Aphidius rhopalosiphi* indicates that initial effects in the in-field area cannot be excluded. Therefore, further refinement is needed.

Refined in-field risk assessment

The results of the tier 2 risk assessment indicate that initial effects on species with a similar sensitivity as *Aphidius rhopalosiphi* cannot be excluded. As a consequence, an aged residue study was performed to demonstrate the potential for recovery for *Aphidius rhopalosiphi*, the most sensitive tested species, and in addition for the predatory bug *Orius laevigatus*.

An extended laboratory aged residue study has been performed on *Aphidius rhopalosiphi* (Jans, 2010; KIIIA1 10.5.3/01). In this study, BYI 02960 SL 200 was applied 2 times at a rate of 250 g a.i./ha with a 10 days interval on potted maize plants. Spray residues were aged under semi-field conditions. Bioassays with freshly dried residues, residues aged for 14 days and for 28 days, respectively, resulted in a corrected morality of 100, 90 and 76.7%, respectively. After an ageing time of 42, 49 and 56 days, corrected mortalities of 29.6, 20.0 and 6.7% were recorded, respectively. Exposure to residues aged for 42 days resulted in a 89.9% reduction in reproduction relative to the control. No effects on reproduction >50% were observed after an ageing time of 49 and 56 days, respectively. Statistically significant repellency was recorded in the first, second and third bioassay (aged 0, 14 and 28 days), while in the fourth, fifth and sixth bioassay (aged 42, 49 and 56 days) no repellent effects were observed.

A second extended laboratory aged residue study has been performed on *Orius laevigatus* (Schwarz, 2010; KIIIA1 10.5.3/02). BYI 02960 SL 200 was applied 2 times at a rate of 250 g a.i./ha with an application interval of 10 days onto potted apple plants. *Orius laevigatus* showed a corrected mortality of 100 and 75.6%, when exposed to fresh residues of the test item and residues aged for 14 days, respectively. After an ageing time of 28 and 42 days, a corrected mortality of 24.5 and 9.8% was observed, respectively. No adverse effects on reproduction > 50% were observed after an ageing time of 28 and 42 days, respectively. The results indicated that *Orius laevigatus* is as well susceptible to the exposure of BYI 02960 SL 200 G (effects <50% after 28 days of ageing) but clearly less sensitive as *Aphidius rhopalosiphi* (effects <50% after 49 days of ageing).

These aged residue studies indicate that the potential for recovery is given even after 2 applications at a rate of 250 g a.i./ha within 7 weeks for the most sensitive species, *Aphidius rhopalosiphi*. For *Orius laevigatus*, residues aged for only 4 weeks already had no adverse effect on mortality and reproduction. Since the intended use pattern includes only single applications at a rate of up to

150 g a.i./ha in the field it can be concluded that the potential for recovery is given within a few weeks after the application and no unacceptable in-field risk for non-target arthropods has to be expected from the use of BYI 02960 SL 200 according to the proposed use pattern.
It is noted that the ageing in the studies was done under semi-field conditions and on plants which could potentially cause more growth dilution than other plant species, and therefore the aged residue studies do not represent all possible field conditions. Thus, a generic 'potential for recovery' period cannot be quantified. However, RMS considers that a potential for recovery within one year (the required period for in-field risk assessment according to ESCORT 2) has been shown.

Tier 2 off-field risk assessment

The off-field exposure PEC values are compared to the results of the extended laboratory studies.

Table B.9.5.3-07 Off-field risk assessment based on study results from extended laboratory studies

Test Species	**off-field $PEC_{max.}$, [g a.i./ha]**	**LR_{50}/ ER_{50} [g a.i./ha]**	**Trigger**	**Refined assessment required?**
Use in hops, 1 x 150 g a.i./ha				
Typhlodromus pyri	14.5	177 / >142	Effects are < 50%	no
Aphidius rhopalosiphi	145	2.02 / >0.89	Effects are < 50%	**yes**
Coccinella septempunctata	14.5	273.9 / no effects up to 250	Effects are < 50%	no
Aleochara bilineata	14.5	>300	Effects are < 50%	no
Use in lettuce, 1 x 125 g a.i./ha				
Typhlodromus pyri	1.73	177 / >142	Effects are < 50%	no
Aphidius rhopalosiphi	17.3	2.02 / >0.89	Effects are < 50%	**yes**
Coccinella septempunctata	1.73	273.9 / no effects up to 250	Effects are < 50%	no
Aleochara bilineata	1.73	>300	Effects are < 50%	no

The maximum PEC off-field for the use in hop (worst-case) is calculated to be 14.5 g/ha for 2D-test systems and 144.9 g/ha for 3D-test systems. For *Typhlodromus*, *Coccinella* and *Aleochara* no effects > 50% neither on mortality nor on reproduction were observed in extended laboratory studies on natural substrate at a rate of 142 (*Typhlodromus*), 250 (*Coccinella*, 2nd trial) and 300 g a.i./ha (*Aleochara*), respectively (see Table B.9.5.3-07). However, for *Aphidius*, initial effects cannot be excluded, indicating a need for further refinement.

Refined off-field risk assessment

To assess off-field effects of BYI 02960 SL 200 on naturally occurring arthropod communities under more realistic conditions, two full-fauna field studies were conducted on grassland as surrogate for off-field habitats in the Netherlands and in Southwestern France.

BYI 02960 SL 200 was applied in a dose-response design at drift rates (0.51, 1.7, 5.1 and 21 g a.i./ha) to grassland habitats with little agricultural input in the Netherlands and Southwestern France. These sites held a diverse and representative off-crop non-target arthropod community. Four replicate plots of 24 x 24 m or 22 x 22 m each were used per treatment (4 application rates, control, reference treatment = 24 plots in total). Arthropods were sampled comprehensively using three different sampling methods (pitfall, suction and weed/Berlese sampling) shortly before the application and 1, 2, 4 and 8 weeks after the application. Overall community changes relative to the control were analyzed using multivariate statistics and depicted by Principal Response Curves (PRC).
In addition, effects on individual arthropod populations were analyzed with univariate statistics for taxa that were sufficiently abundant. In both studies, the toxic reference item treatment caused clear responses both at the arthropod community level and at the population level, demonstrating that the test system was sufficiently sensitive to detect toxic effects.
Both field tests contained sufficient numbers for analysis of effects on the Hymenopteran family Braconidae, to which the most sensitive first tier species *Aphidius rhopalosiphi* belongs.

<u>Results of the trial in the Netherlands:</u>
At community level, no statistically significant effects on arthropod communities were found at any of the rates tested up to and including 21 g a.i./ha. Therefore, 21 g a.i./ha is the community NOER (No Observed Effect Rate) of BYI 02960 SL 200. In addition, 72 taxa were sufficiently abundant for a univariate statistical evaluation at population level. In the three lowest test rates, none of the taxa showed a consistent treatment-related response. Predatory mites of the family *Cunaxidae* and hymenopteran parasitoids of the family *Braconidae* showed transient adverse effects only at one sampling moment shortly after application of the highest test rate (21 g a.i./ha). Both taxa recovered within one week. In conclusion, the population NOEAER (No Observed Ecologically Adverse Effect Rate) of BYI 02960 SL 200 is 21 g a.i./ha and the population NOER (No Observed Effect Rate) is 5.1 g a.i./ha.

<u>Results of the trial in France:</u>
At community level, no statistically significant effects on arthropod communities were found at any of the rates tested up to and including 21 g a.i./ha. Therefore, 21 g a.i./ha is the community NOER (No Observed Effect Rate) of BYI 02960 SL 200.
At the population level, 79 taxa were sufficiently abundant for population level evaluations. Only three phytophagous taxa were adversely affected.
At the test rate of 5.1 g a.i./ha, significant adverse effects on the chrysomelid beetles *Alticinae* and on juvenile leafhoppers (*Cicadellidae*) occurred at a single sampling occasion. At the rate of 21 g a.i./ha,

Alticinae and *Cicadellidae* showed statistically significant reductions at two sampling moments after application, but recovered already 4 weeks after the application. Aphids (*Aphidoidea*) were reduced at several sampling moments after the application of 21 g a.i./ha; however, the reduction was only statistically significant at the penultimate sampling moment. The observed effects on *Aphidoidea* were to be expected since aphids are a target pest species for the proposed uses of BYI 02960 SL 200 in lettuce and hop. However, aphids can be expected to recover quickly from population declines due to their high asexual reproductive potential. No significant reduction in *Aphidoidea* abundance was observed at the last sampling moment two months after application of the test item. The highest rate tested in this study - BYI 02960 SL 200 applied at 21 g a.i./ha - is therefore classified as the population NOEAER (No Observed Ecologically Adverse Effect Rate) with effect class 3a; the second highest dose rate – 5.1 g a.i./ha is the NOEAER with effect class 2.

In the Terrestrial ecotoxicology guidance document and in ESCORT 2, there is no clear guidance on what endpoints should be used from field studies for risk assessment. The report from the ESCORT 3 workshop contains some relevant recommendations: (chapter 1.8.1.2, p.9) ‘ Class 3 effects may be appropiate for the off-crop in-field margin, and Class 1 and 2 are appropiate for off-field area’; (chapter 5.3, p.40) ‘ Considering field studies for off-crop risk assessment, no effect or only transient effects (e.g. de Jong et al. 2010) are considered acceptable’; and (chapter 7.1, p.49): ‘At the level of field studies, the NOER community and the NOEAER population should be used for the off-field risk assessment.’.

The drift values used to calculate the off-field exposure were determined at distances from the *crop*. In the current risk assessment, differentiation between ‘ off-crop in-field’ and ‘ off-field’ is not done. Depending on MS, crop type, farmer, etc., there may or may not be an in-field off-crop buffer zone. To ensure that the off-field is adequately protected, in this risk assessment the situation without off-crop in-field zone is assumed.

In both field studies, the community NOER is 21 g a.s./ha.
As NOEAER, three values were set:

Study in NL: 21 g a.s./ha is the population level NOEAER with effect class 2

Study in SW-FR: 5.1 g a.s./ha is the population level NOEAER with effect class 2; 21 g a.s./ha is the population level NOEAER with effect class 3a

Based on the recommendations of ESCORT 3, the community NOER and the community NOEAER values with effect class 2 are considered and the lowest of these is taken. Thus, the relevant endpoint for the risk assessment assessment would be 5.1 g a.s./ha based on the population NOEAER effect class 2 from the study in France. Because two field studies are available, the RMS does not consider an additional safety factor on this value necessary.

The relevant endpoint for the risk assessment from these studies was discussed in Pesticide Peer Review meeting 124 of December 2014. The studies had a checkerboard design, meaning that there were areas of low dose and control which could serve as source of recolonisation for the populations. The experts questioned whether there was more possibility for recolonisation in these studies than there would be in a normal agricultural off-field situation. It was therefore decided in the meeting that the NOER values could be used from these studies as these do not (less likely) account for recovery/ recolonisation.

In both field studies, the community NOER is 21 g a.s./ha.
The NOER population is:

Study in NL: 5.1 g a.s./ha
Study in SW-FR: 1.7 g a.s./ha

The lowest from these values can be used in the risk assessment, i.e. 1.7 g a.s./ha.

It was extensively discussed in the pesticide peer review meeting whether a safety factor should be applied to this value to account from uncertainties in extrapolating the results of field studies to the realistic worst case field situation. Overall, the majority of the experts agreed that no additional safety factor is required in this case given that the endpoint is a NOER and that two studies (accounting for the representativeness of the taxa) are available.

Refined potential exposure:
In the original DAR, the calculation of the off-field PEC was confusing, as reference was made to the term ‘correction factor’ while this is only appropiate for extended laboratory studies and not for field studies. This term has therefore been removed from the table below. The possible need for a safety factor, accounting for uncertainty when using field studies in the risk assessment, has already been discussed above. The vegetation distribution factor is not applicable because of the 3-D study design. The refined off-field exposure is calculated in the table below. For the use in hops, a risk is identified using the default drift value; therefore the exposure at increasing distances from the crop (and therefore decreasing drift values) is also shown.

Table B.9.5.3-08 Refined exposure calculation for off-field risk assessment

Crop / no. of applications	Appl. rate [g a.i./ha]	MAF	Distance from crop	Drift [%]	off-field PECmax. [g/ha]	Endpoint based two full-fauna field studies [g/ha]
Hops / 1	150	1	3*	19.33	29	1.7
	150	1	5	11.57	17	1.7
	150	1	10	5.77	8.7	1.7
	150	1	15	3.84	5.8	1.7
	150	1	20	1.79	2.7	1.7
	150	1	30	0.56	0.84	1.7
Lettuce / 1	125	1	1*	2.77	3.5	1.7
	125	1	5	0.57	0.71	1.7

*Default

Table B.9.5.3-08 E.13b shows that ~~the off-crop exposure is below the relevant off-field endpoint of 5.1 g a.s./ha for the use in lettuce. Therefore, the off-field risk from the use in lettuce is acceptable.~~

~~t~~he expected exposure (29 g a.s./ha) is above the relevant endpoint (1.7 ~~5.1~~ g a.s./ha) for the use in hops at the default distance from the crop. The table shows that a buffer zone of at least 30~~20~~ m would be required for the exposure value to fall below the required level. This means that acceptable use can be achieved based on the European guidance. The off-field risk from the use in hops is acceptable provided that a buffer zone of 30~~20~~ m is used.

For lettuce, the expected exposure (3.5 g a.s./ha) is above the relevant endpoint (1.7 ~~5.1~~ g a.s./ha) at the default distance from the crop. The table shows that a buffer zone of at least 5 m would be required for the exposure value to fall below the required level.

Note that other options for risk mitigation may be possible (e.g. drift reducing nozzles or the presence of off-crop in-field buffer zones which would give the option to use a higher endpoint). Risk mitigation measures are set at Member State level.

B.9.6 Effects on earthworms (IIA 8.9, IIIA 10.6)

B.9.6.1 Acute toxicity to earthworms (IIA 8.9.1)

STUDY IIA, 8.9.1/01

Characteristics

reference	:	Leicher T. (2010a)	purity	:	96.2%
type of study	:	Acute toxicity	species	:	*Eisenia foetida*
year of execution	:	2009-2010	exposure duration	:	14 days
GLP statement	:	Yes	nominal conc.	:	5.6, 10, 18, 32, 56, 100, 178, 562 and 1000 mg/kg soil d.w.
Guideline	:	OECD 207 (1984)	dosing method	:	Soil was treated with mixture of test substance and quartz sand
test substance	:	BYI 02960 (tech.), batch 2009-000239	LC50	:	185.6 mg a.s./kg soil d.w. (10% organic matter)

Methods

In an acute toxicity study, earthworms (*Eisenia fetida*, adults, mean weight 350 mg) were exposed to BYI 02960 technical for 14 days in artificial soil (OECD 207 substrate, with 10% organic matter). The test was conducted in 1.5 L glass jars covered with glass lids, containing 595 g d.w. artificial soil.

Two trials 1 and 2 were performed. Treatment rates in trial 1 were 5.6, 10, 18, 32, 56 and 100 mg/kg soil d.w.. Trial 2 was performed at higher treatment rates since in trial 1 only 3% mortality was observed at the highest treatment rate after 14 days. Treatment rates in trial 2 were 178, 562 and 1000 mg/kg soil d.w..

Each treatment and the controls were tested in 4 replicates of 10 worms each. Test soils were prepared by treating the artificial soil with a mixture of the test substance and quartz sand. The control was treated with quartz sand only. Jars were maintained at 20±2°C under continuous light (400-800 lux). Soil moisture was in the range 53-58% of WMHC at the start and the end of the test. The soil pH was in the range 5.6-5.7 at the start and end of the test. A reference product (2-chloroacetamide) was tested in a separate test about 1 year earlier and gave an acceptable result (14-day LC50 13.2 mg/kg soil d.w. in artificial soil with 10% organic matter).

Results

Test results are summarised in the table below. The results of the control group satisfied the guideline requirements (OECD 207, 1984): ≤10% mortality. The 14-day LC50 (Probit analysis) was 192.9 mg/kg soil d.w. (95% CI 143.3-276.9 mg/kg soil d.w.), equivalent to 185.6 mg a.s./kg soil d.w..

Table B.9.6.1-01 Acute toxicity of BYI 02960 technical to *Eisenia fetida*

Nominal concentration (mg/kg soil d.w.)	Mortality at 14 days (%)	Mean weight change survivors over 14 days (%)
control	0	-3
5.6	0	-12*
10	0	-13*
18	0	-17*
32	0	-20*
56	0	-23*
100	3	-25*

178	23	-25*
316	95	-38*
562	100	-
1000	100	-

* statistically significant difference from control at 5% level.

Conclusion

14-day LC50 185.6 mg a.s./kg soil d.w. (soil with 10% organic matter).

Guidelines and limitations

The study was performed according to OECD 207 (1984) and is acceptable. Test rates were expressed as mg t.i./kg soil d.w. (mg test item/kg soil d.w.). Test rates were not corrected for active ingredient content of the technical active substance (96.2%). The correction of the LC50 from 192.9 mg/kg soil d.w. to 185.6 mg a.s./kg soil d.w. was performed by the reviewer.

STUDY IIIA, 10.6.2/01

Characteristics

reference	:	Leicher T. (2010e)	a.s. content	:	17.1% w/w (201.0 g/L) BYI 02960
type of study	:	Acute toxicity	species	:	*Eisenia foetida*
year of execution	:	2010	exposure duration	:	14 days
GLP statement	:	Yes	nominal conc.	:	100, 178, 316, 562, 1000 and 1780 mg test item/kg soil d.w.
Guideline	:	OECD 207 (1984), OPPTS 850.6200	dosing method	:	Soil was treated with solution of test substance in water
test substance	:	BYI 02960 SL 200 G (200 g/L SL formulation), batch 2009-001067	LC50	:	121 mg a.s./kg soil d.w. (5% organic matter)

Methods

In an acute toxicity study, earthworms (*Eisenia fetida*, adults, mean weight 310 mg) were exposed to BYI 02960 SL 200 G (200 g/L SL formulation, 17.1% w/w (201.0 g/L) BYI 02960) for 14 days in artificial soil (OECD 207 substrate, with 5% peat). The test was conducted in 1.5 L glass jars covered with glass lids, containing 595 g d.w. artificial soil. Treatment rates were 100, 178, 316, 562, 1000 and 1780 mg test item/kg soil d.w.. Each treatment and the controls were tested in 4 replicates of 10 worms each. Test soils were prepared by treating the artificial soil with a solution of the test substance in water. The control was treated with water only. Jars were maintained at 20±2°C under continuous light (400-800 lux). Soil moisture was in the range 57-58% of WMHC at the start and the end of the test. The soil pH was in the range 6.1-6.2 at the start and the end of the test. A reference product (2-chloroacetamide) was tested in a separate test about 7 months earlier and gave an acceptable result (14-day LC50 26.1 mg test item/kg soil d.w. in artificial soil with presumably 10% organic matter).

Results

Test results are summarised in the table below. The results of the control group satisfied the guideline requirements (OECD 207, 1984): ≤10% mortality. The 14-day LC50 (Probit analysis) was 709 mg test item/kg soil d.w. (95% CI 245-2187 mg test item/kg soil d.w.), equivalent to 121 mg a.s./kg soil d.w..

Table B.9.6.1-03 Acute toxicity of BYI 02960 SL 200 G to *Eisenia fetida*

Nominal concentration (mg test item/kg soil d.w.)	Mortality at 14 days (%)	Mean weight change survivors over 14 days (%)
control	0	+4
100	0	-16*
178	3	-22*
316	0	-28*
562	28	-36*
1000	78	-48*
1780	100	-

* statistically significant difference from control at 5% level.

Conclusion

14-day LC50 121 mg a.s./kg soil d.w. (soil with 5% organic matter).

Guidelines and limitations

The study was performed according to OECD 207 (1984) and is acceptable.

STUDY IIA, 8.9.1/02

Characteristics

reference	:	Leicher T. (2010b)	purity	:	95.8%
type of study	:	Acute toxicity	species	:	*Eisenia foetida*
year of execution	:	2010	exposure duration	:	14 days
GLP statement	:	Yes	nominal conc.	:	31.3, 62.5, 125, 250, 500 and 1000 mg/kg soil d.w.
Guideline	:	OECD 207 (1984)	dosing method	:	Soil was treated with mixture of test substance and quartz sand
test substance	:	BYI 02960-difluoroacetic acid, batch number BCOO 5984-1	LC50	:	>958 mg pure metabolite/kg soil d.w. (10% organic matter)

Methods

In an acute toxicity study, earthworms (*Eisenia fetida*, adults, mean weight 320 mg) were exposed to difluoroacetic acid (metabolite of BYI 02960) for 14 days in artificial soil (OECD 207 substrate, with 10% organic matter). The test was conducted in 1.5 L glass jars covered with glass lids, containing 595 g d.w. artificial soil. Treatment rates were 31.3, 62.5, 125, 250, 500 and 1000 mg/kg soil d.w.. Each treatment and the controls were tested in 4 replicates of 10 worms each. Test soils were prepared by treating the artificial soil with a mixture of the test substance and quartz sand. The control was treated with quartz sand only. Jars were maintained at 20±2°C under continuous light (400-800 lux). Soil moisture was in the range 51-54% of WMHC at the start and the end of the test. The soil pH was 6.2-6.3 at the start of the test and 6.0 at the end. A reference product (2-chloroacetamide) was tested in a separate test about 1 year and 4 months earlier and gave an acceptable result (14-day LC50 13.2 mg/kg soil d.w. in artificial soil with 10% organic matter).

Results

Test results are summarised in the table below. The results of the control group satisfied the guideline requirements (OECD 207, 1984): ≤10% mortality. The 14-day LC50 was >1000 mg/kg soil d.w. equivalent to >958 mg pure metabolite/kg soil d.w..

Table B.9.6.1-02 Acute toxicity of difluoroacetic acid to *Eisenia fetida*

Nominal concentration (mg/kg soil d.w.)	Mortality at 14 days (%)	Mean weight change survivors over 14 days (%)
control	0	+6
31.3	0	+5
62.5	0	-2*
125	0	-4*
250	2.5	-6*
500	0	-8*
1000	0	-11*

* statistically significant difference from control at 5% level.

Conclusion

14-day LC50 >958 mg pure metabolite/kg soil d.w. (soil with 10% organic matter).

Guidelines and limitations

The study was performed according to OECD 207 (1984) and is acceptable. Test rates were expressed as mg t.i./kg soil d.w. (mg test item/kg soil d.w.). Test rates were not corrected for active ingredient content of the test item (95.8%). The correction of the LC50 from >1000 mg/kg soil d.w. to >958 mg pure metabolite/kg soil d.w. was performed by the reviewer.

STUDY IIA, 8.9.1/03

Characteristics

reference	:	Wetton P.M. (1999)	purity	:	99.7%
type of study	:	Acute toxicity	species	:	*Eisenia foetida*
year of execution	:	1999	exposure duration	:	14 days
GLP statement	:	Yes	nominal conc.	:	1000 mg/kg soil d.w.
Guideline	:	OECD 207 (1984)	dosing method	:	Test substance was added directly to soil.
test substance	:	IC-0 = 6-chloronicotinic acid (6-CNA); batch MF-4267	LC50	:	>1000 mg/kg soil d.w. (10% organic matter)

Methods

In an acute toxicity limit study, earthworms (*Eisenia fetida*, adults, mean weight about 400 mg) were exposed to IC-0 (6-chloronicotinic acid, metabolite of BYI 02960) for 14 days in artificial soil (OECD 207 substrate, with 10% organic matter). The test was conducted in 1 L glass jars covered with perforated plastic film, containing 500 g d.w. artificial soil. The test substance (3.5 g) was added directly to 3.5 kg of the test substrate. The treatment rate was 1000 mg/kg soil d.w.. The control was untreated. The treatment and the control were tested in 6 replicates of 10 worms each. Jars were maintained at 21°C under continuous light (598-700 lux). Soil moisture was 24-25% of soil d.w. at the start and 20-21% and the end of the test. This level is considered to be adequate (MWHC of OECD 207 substrate with 10% peat is about 50-55%). The soil pH was 6.1-6.3 at the start of the test and 6.2-6.4 at the end. A reference product (2-chloroacetamide) was tested once every 6 months in a separate test and gave an acceptable result (14-day LC50 23 mg/kg soil d.w. in artificial soil with 10% organic matter).

Results

Mortality in the control and the IC-0 treatment was 5% and 2%, respectively, after 14 days. Earthworm weights were unaffected by the treatment with IC-0. The 14-day LC50 was >1000 mg/kg soil d.w..

Conclusion

14-day LC50 >1000 mg/kg soil d.w. (soil with 10% organic matter).

Guidelines and limitations

The study was performed according to OECD 207 (1984) and is acceptable.

B.9.6.2 Sublethal effects on earthworms (IIA 8.9.2)

STUDY IIA, 8.9.2/01 & IIIA, 10.6.3/01

Characteristics

reference	:	Leicher T. (2010c)	species	:	*Eisenia fetida andrei*
type of study	:	Sublethal toxicity study	exposure duration	:	8 weeks
year of execution	:	2010	nominal conc.	:	8.9, 15.8, 28.1, 50 and 89 mg test item/kg soil d.w.
GLP statement	:	Yes	dosing method	:	Soil was treated with a dispersion of test substance in water
guideline	:	OECD 222 & ISO 11268-2	acceptability	:	Acceptable
test substance	:	BYI 02960 SL 200 G (200 g/L SL formulation), batch 2009-001253	NOEC	:	8.9 mg test item/kg soil d.w. (1.5 mg a.s./kg soil d.w.) (10% organic matter)
a.s. content	:	17.0 % w/w (199.8 g/L) BYI 02960			

Methods

In a chronic toxicity study, earthworms (*Eisenia fetida andrei*, clitellate adults, 250-450 mg at the start) were exposed to BYI 02960 SL 200 G (200 g/L SL formulation, 17.0 % w/w (199.8 g/L) BYI 02960) for 4 weeks in artificial soil (OECD 222 substrate with 10% peat). After this period, the adults were removed and cocoons remained in the test vessels for 4 more weeks. The test was conducted in 16.5 x 12 x 6 cm plastic boxes covered with perforated transparent lids containing 500 g of soil (based on d.w.). To prepare the highest test concentration, the test substance was dispersed in deionised water and mixed with the test soil. Lower concentrations were prepared by serial dilution. Nominal soil concentrations of 8.9, 15.8, 28.1, 50 and 89 mg test item/kg soil d.w. were tested in 4 replicates of 10 worms each, with an untreated control tested in 8 replicates. Vessels were maintained at 18-22°C under a 16 hours light (400-800 lux) and 8 hours dark photoperiod. Cattle manure (5 g per container per event) was added as food on day 1 after application and weekly thereafter for the first 4 weeks. Soil moisture was maintained at 49-52% of MWHC during the test. The soil pH was 6.13 at the start of the test and 6.4-6.5 at the end. After four weeks, survival and growth rate of adults were determined, and after eight weeks the number of offspring. In a separate study conducted about 1 year earlier, a positive control (carbendazim) was tested.

Results

The positive control gave the expected response (NOEC 1.25 mg a.s./kg soil d.w., effects on reproduction from 2.5 mg a.s./kg soil d.w.). Test results are summarised in the Table below. The results of the control group satisfied the guideline requirements (OECD 222, 2004): ≤10% adult mortality, ≥30 offspring per replicate, CV for reproduction ≤30%.

None of the differences in weight change between the BYI 02960 SL 200 G treatments and the control were statistically significant at the 5% level. Based on statistically significant reductions of the number of offspring at 15.8 mg test item/kg soil.d.w. and above, the NOEC is 8.9 mg test item/kg soil d.w., equivalent to 1.5 mg a.s./kg soil d.w..

Table B.9.6.2-01 Chronic toxicity of BYI 02960 SL 200 G to *Eisenia fetida*

Concentration (mg test item/kg soil d.w.)	Adult mortality after 28 days (%)	Mean weight change over 28 days (%)	No. of juveniles per replicate
Control	0	+44.6	220.0 (CV 10.3%)
8.9	0	+48.1	205.3
15.8	2.5	+52.0	190.0*
28.1	5	+52.9	188.8*
50	0	+43.8	162.8*
89	0	+45.4	130.0*

* statistically significant difference from control at 5% level.

Conclusion

NOEC 8.9 mg test item/kg soil d.w., equivalent to 1.5 mg a.s./kg soil d.w. (soil with 10% organic matter).

Guidelines and limitations

The study was performed according to OECD 222 (2004) and is acceptable.

STUDY IIA, 8.9.2/02

Characteristics

reference	:	Leicher T. (2010d)	species	:	*Eisenia fetida andrei*
type of study	:	Sublethal toxicity study	exposure duration	:	8 weeks
year of execution	:	2010	nominal conc.	:	11, 20, 35, 62 and 110 mg test item/kg soil d.w.
GLP statement	:	Yes	dosing method	:	Test item was added to soil mixed with quartz sand
Guideline	:	OECD 222 & ISO 11268-2	acceptability	:	Acceptable
test substance	:	BYI 02960-difluoroacetic acid; batch code BCS-AA56716-01-01, batch number BCOO 5984-1-1	NOEC	:	59 mg pure metabolite/kg soil d.w. (10% organic matter)
Purity	:	95.8%			

Methods

In a chronic toxicity study, earthworms (*Eisenia fetida andrei*, clitellate adults, 250-450 mg at the start) were exposed to BYI 02960-difluoroacetic acid (metabolite of BYI 02960) for 4 weeks in artificial soil (OECD 222 substrate with 10% peat). After this period, the adults were removed and cocoons

remained in the test vessels for 4 more weeks. The test was conducted in 16.5 x 12 x 6 cm plastic boxes covered with perforated transparent lids containing 500 g of soil (based on d.w.). To prepare the test substrates, the test substance was mixed with quartz sand and the mixture was added to the artificial soil substrate. Nominal soil concentrations of 11, 20, 35, 62 and 110 mg test item/kg soil d.w. were tested in 4 replicates of 10 worms each, with a control treated with quartz sand only tested in 8 replicates. Vessels were maintained at 18-22°C under a 16 hours light (400-800 lux) and 8 hours dark photoperiod. Cattle manure (5 g per container per event) was added as food on day 1 after application and weekly thereafter for the first 4 weeks. Soil moisture was maintained at 55-56% of MWHC during the test. The soil pH was 6.01 at the start of the test and 6.4-6.5 at the end. After four weeks, survival and growth rate of adults were determined, and after eight weeks the number of offspring. In a separate study conducted about 1 year earlier, a positive control (carbendazim) was tested.

Results

The positive control gave the expected response (NOEC 1.25 mg a.s./kg soil d.w., effects on reproduction from 2.5 mg a.s./kg soil d.w.). Test results are summarised in the Table below. The results of the control group satisfied the guideline requirements (OECD 222, 2004): ≤10% adult mortality, ≥30 offspring per replicate, CV for reproduction ≤30%.

None of the differences in weight change between the difluoroacetic acid treatments and the control were statistically significant at the 5% level. Based on a statistically significant reduction of the number of offspring at 110 mg test item/kg soil.d.w., the NOEC is 62 mg test item/kg soil d.w., equivalent to 59 mg pure metabolite/kg soil d.w..

Table B.9.6.2-02 Chronic toxicity of BYI 02960-difluoroacetic acid to *Eisenia fetida*

Concentration (mg test item/kg soil d.w.)	Adult mortality after 28 days (%)	Mean weight change over 28 days (%)	No. of juveniles per replicate
Control	0	+66.6	264.8 (CV 11.6%)
11	0	+61.8	259.8
20	0	+63.3	373.5
35	0	+60.4	282.3
62	0	+61.5	230.3
110	0	+60.2	190.0*

* statistically significant difference from control at 5% level.

Conclusion

NOEC 59 mg pure metabolite/kg soil d.w. (soil with 10% organic matter).

Guidelines and limitations

The study was performed according to OECD 222 (2004) and is acceptable. Test rates were not corrected for active ingredient content of the test substance (95.8%). The correction of the NOEC from 62 mg test item/kg soil d.w. to 59 mg pure metabolite/kg soil d.w. based on test item purity of 95.8% was performed by the reviewer.

STUDY IIA, 8.9.2/03

Characteristics

reference	:	Leicher T. (2011)	species	:	*Eisenia fetida andrei*
type of study	:	Sublethal toxicity study	exposure duration	:	8 weeks
year of execution	:	2010-2011	nominal conc.	:	9.5, 16.8, 30.0, 53.4, 95.0 and 100 mg test item/kg soil d.w.
GLP statement	:	Yes	dosing method	:	Test item was added to soil mixed with quartz sand
guideline	:	OECD 222 & ISO 11268-2	acceptability	:	Acceptable
test substance	:	6-chloronicotinic acid (6-CNA = IC-0); batch AE F161089 00 1B99 0001	NOEC	:	95 mg/kg soil d.w. (soil with 5% organic matter)
purity	:	98.8%			

Methods

In a chronic toxicity study, earthworms (*Eisenia fetida andrei*, clitellate adults, 250-450 mg at the start) were exposed to 6-chloronicotinic acid (6-CNA = IC-0, metabolite of BYI 02960) for 4 weeks in artificial soil (OECD 222 substrate with 5% peat). After this period, the adults were removed and cocoons remained in the test vessels for 4 more weeks.

Two trials 1 and 2 were performed. The treatment rate in trial 1 was 100 mg test item/kg soil d.w. (limit test). Trial 2 was performed at lower treatment rates since in trial 1 a statistically significant reduction of body weight gain and rate of reproduction was observed. Treatment rates in trial 2 were 9.5, 16.8, 30.0, 53.4 and 95.0 mg test item/kg soil d.w..

The test was conducted in 16.5 x 12 x 6 cm plastic boxes covered with perforated transparent lids containing 500 g of soil (based on d.w.). To prepare the test substrates, the test substance was mixed with quartz sand and the mixture was added to the artificial soil substrate. In the limit test (trial 1), the nominal soil concentration of 100 mg test item/kg soil d.w. was tested in 8 replicates of 10 worms, with a control treated with quartz sand also tested in 8 replicates. In the dose-response test (trial 2), the nominal soil concentrations of 9.5, 16.8, 30.0, 53.4 and 95.0 mg test item/kg soil d.w. were tested in 4 replicates of 10 worms each, with a control treated with quartz sand tested in 8 replicates. Vessels were maintained at 18-22°C under a 16 hours light (400-800 lux) and 8 hours dark photoperiod. Cattle manure (5 g per container per event) was added as food on day 1 after application and weekly thereafter for the first 4 weeks. Soil moisture was maintained at 56-65% of MWHC during the test. The soil pH in trial 1 was 6.3 at the start of the test and 6.7-7.0 at the end. The soil pH in trial 2 was 5.8 at the start of the test and 6.6 at the end. After four weeks, survival and growth rate of adults were determined, and after eight weeks the number of offspring. In a separate study conducted about 1 year earlier, a positive control (carbendazim) was tested.

Results

The positive control gave the expected response (NOEC 1.25 mg a.s./kg soil d.w., effects on reproduction from 2.5 mg a.s./kg soil d.w.). Test results are summarised in the Table below. The results of the control group satisfied the guideline requirements in both tests (OECD 222, 2004): ≤10% adult mortality, ≥30 offspring per replicate, CV for reproduction ≤30%.

In the dose-response test, weight change and reproduction rate of the 6-chloronicotinic acid treatment was statistically significantly lower (5% level) than in the control. In the dose-response test, none of

the differences in weight change and reproduction rate between the 6-chloronicotinic acid treatments and the control were statistically significant at the 5% level. Based on a statistically significant reduction of the number of offspring at 100 mg test item/kg soil.d.w., the NOEC is 95 mg test item/kg soil d.w (no correction required considering the high test item purity).

Table B.9.6.2-03 Chronic toxicity of 6-chloronicotinic acid to *Eisenia fetida*

Concentration (mg test item/kg soil d.w.)	Adult mortality after 28 days (%)	Mean weight change over 28 days (%)	No. of juveniles per replicate
Limit test			
Control	0	+72.8	211.9 (CV 14.2%)
100	0	+56.7*	107.4*
Dose-response test			
Control	0	+59.2	215.1(CV 13.6%)
9.5	0	+55.8	208.0
16.8	0	+56.0	199.0
30.0	0	+49.9	222.3
53.4	0	+49.5	180.0
95.0	0	+53.3	186.0

* statistically significant difference from control at 5% level.

Conclusion

NOEC 95 mg/kg soil d.w..(soil with 5% organic matter).

Guidelines and limitations

The study was performed according to OECD 222 (2004) and is acceptable. An explanation for the somewhat conflicting results of the limit test and the findings at 95 mg/kg in the dose-response test was not provided by the author of the report. Further questions are considered to be redundant considering the order of magnitude of the NOEC.

B.9.6.4 Earthworms field studies (IIA 10.6.4)

IIIA 10.6.4/01

Characteristics

reference	:	Menke U. (2012)	test organism	:	Field populations of earthworms
type of study	:	Earthworm field study	exposure duration	:	11 months
year of execution	:	2010-2011	nominal dose	:	One application at 300, 600 or 1500 g a.s./ha
GLP statement	:	Yes	dosing method	:	Sprayed on grassland
guideline	:	BBA VI, 2-3 (1994), ISO 11268-3 Part 3 (1999)	acceptability	:	~~Acceptable~~ **Not acceptable**
test substance	:	BYI 02960 SL 200 G (200 g/L SL formulation), batch 2010-001067	conclusion	:	~~No lasting effect after a spring application on grassland at 1500 g a.s./ha (soil with 1.98% OC)~~
a.s. content	:	17.1% w/w (201 g/L) BYI 02960			

Methods

The effect of BYI 02960 SL 200 G (200 g/L SL formulation, 17.1% w/w (201 g/L) BYI 02960) on earthworm populations was investigated in Germany near the Höfchen Experimental Station of the

Bayer CropScience AG under field conditions after one spray application on grassland at dose levels of 300, 600 and 1500 g a.s./ha on 25 May 2010, with untreated control and reference product (Carbendazim SC 500, 10000 g a.s./ha).

Trial lay out and treatment

Prior to trial lay out, the test site had been cultivated as grassland for many years. In the three years preceding the test onset, the field had not been treated with pesticides. During the study period, agricultural practice was conducted: plots were mown 12 days before application and the mulch was removed 5 days before application. Mulching was performed 6 times during the remaining study period (21 June, 20 August, 17 September, 8 October and 26 October 2010 and 31 March 2011), the mulch was left on the surface. A pre-sampling of earthworms was performed 8 days before application from 16 spots across the study site and revealed sufficiently high and diverse earthworm populations (4.8/m^2 *L. terrestris* (anecic), 72/m^2 *L. rubellis* + *L. castaneus* (epigeic), 46/m^2 *A. caliginosa* (endogeic), 5.0/m^2 *A. chlorotica* (endogeic), 128/m^2 total earthworms)

The soil characteristics are shown in Table B.9.6.3-01 (determined from soil sampled on April 04, 2011). To establish the test site, 10 x 10 m plots were established. The BYI 02960 SL 200 G treatment, the untreated control and the reference product were each assigned to four replicate plots. Applications were made in 400 L water/ha on 25 May 2010, according to normal agricultural practice (no further information).

Table B.9.6.3-01 Properties of study soil

Parameter	value parameter
% sand	9.4
% silt	72.5
% clay	18.1
Texture[(A)]	Silt loam
pH	6.41
% total carbon	2.05
% anorganic carbon	0.07
WHC (%)	46.1

(A) DIN 4220

Earthworm sampling

From each plot earthworms were sampled (4 areas of 0.25 m^2 within the inner 6 x 6 m area of each plot) by formalin extraction. Samplings were conducted on 22-23 June 2010 (4 weeks after treatment), 27-28 October and 05 November 2010 (5 months after treatment) and on 5-6 April 2011 (11 months after treatment). Adult earthworms and juveniles were identified to species level and their numbers and weights recorded. Before each sampling (including the pre-test sampling) the efficiency of the formalin extraction method was checked and the sampling efficiency was 69%, 61%, 73% and 74% at pre-test and after 4 weeks, 5 months and 11 months, respectively (hence above the criterion of at least 60% specified in ISO 11268-3). In addition to the above, surface monitoring for direct mortality effects on earthworms was performed the first few days after application.

Irrigation

No irrigation was applied as 15.3 mm of rain fell on the day after application, with a total of 59.4 mm during the first 9 days after application.

Environmental conditions

Climatic data were recorded at the nearby experimental farm Laacher Hof of the Bayer CropScience AG.

Chemical soil analysis

Soil cores (10 cm deep, 50 mm diameter, 20 cores per plot) were collected within 24 hours after treatment and analysed for BYI 02960 using method 01074/M0001 (extraction followed by LC-MS/MS, LOQ 5 µg/kg, concurrent validation by fortified samples at 5, 300, 600 and 1500 µg/kg (n=1 at each level), recoveries 68% (5 µg/kg) and 93-98% (300-1500 µg/kg). Method 01074 was validated in soil according to SANCO/3029/99 rev.4 of 11/07/00, but method 01074/M0001 differed from method 01074 in that it used an internal standard whereas method 01074 employed matrix matched standards. Although method 01074/M0001 is therefore not considered to be fully validated, further information is not considered necessary, since (1) sampling of soil is not mandatory according to ISO 11268-3 and according to De Jong *et al.* (2006) (De Jong *et al.*, Guidance for Summarising Earthworm Field Studies, RIVM Report 601506006/2006); (2) three concurrent recoveries were determined for

fortification levels representative for those of the study samples, and found to be excellent (93-98%), indicating that the use of an internal standard leads to accurate results in this range.

Soil samples were stored frozen for about 10 months prior to analysis. Storage stability in soil was not demonstrated. Further information is not considered necessary, since (see results) the measured concentrations in soil (means per treatment rate in range 118-136% of expected concentrations) were within the acceptable range of 50-150% specified in Kula *et al.* (2006) (Kula C., Heimbach F., Riepert F. and Rombke J. 2006. technical recommendations for the update of the ISO earthworm field test guideline (ISO 11268-3). Journal of Soil and Sediments 6, 182-186).

Statistical analysis

Total and species abundance and biomass data were statistically evaluated by the Wilcoxon, Mann and Whitney U-test (α=0.05, two-sided). Shannon-Weaver diversity indices were calculated for each sampling point for adult, juvenile and sum of adult and juvenile earthworms separately.

Results

Environmental conditions

The environmental conditions are shown in the Table below.

Table B.9.6.4-02 Monthly climatic conditions (mean values)

Month	temperature		cumulated precipitation		sunshine	
	°C	compared to long-term*)	mm	compared to long-term*)	hours	compared to long-term *) and **)
May, 2010 *)	11.23	13.9	96.5	61.6	138.94	187.3
June, 2010 *)	18.00	16.6	12.3	77.5	259.28	171.2
July, 2010 *)	21.15	18.7	88.0	71.3	249.26	185.6
August, 2010 *)	17.05	18.4	203	70.0	132.31	184.3
September, 2010 *)	13.57	14.9	109	61.6	131.56	134.1
October, 2010 **)	10.01	10.8	60.6	61.4	115.21	99.4
November, 2010 **)	6.2	6.3	129	62.1	29.28	54.6
December, 2010 **)	- 1.63	3.6	67.5	69.4	18.29	36.9
January, 2011 **)	2.87	2.8	128.2	60.8	46.31	46.0
February, 2011 **)	3.83	3.4	58.3	47.3	69.58	70.2
March, 2011 **)	7.27	6.1	17.3	58.0	217.47	103.6
April, 2011 **)	13.72	9.3	42.2	55.3	233.08	151.8

*) mean of 1966 – 2006
**) mean of 1966 – 2005

Soil analysis

Samples taken from the control plots contained low residues of BYI 02960 (5.28 µg/kg; at least 45 times lower than the residues in any of the plots of the lowest BYI 02960 treatment). After application,

the mean measured concentration (mean of 4 plots) of BYI 02960 at nominal treatment rates of 300, 600 and 1500 g a.s./ha, respectively, was 271, 472 and 1234 µg/kg soil, corresponding to 136%, 118% and 123% of the expected PECsoil of 200, 400 and 1000 µg/kg soil, calculated for a soil depth of 10 cm and an assumed soil density of 1.5 kg/dm^3. The measured soil concentrations were within the range of 50-150% indicated by Kula *et al* (2006) as the acceptable range.

Earthworm surface searches

Some earthworms (at the most 2 on any day) could be detected on the soil surface of treated and control plots. The data did not indicate any difference between treated and control plots.

Earthworm abundance and biomass

The results for earthworm abundance and biomass are summarised in the tables below. At the pre-test count, mean earthworms abundance was 128 individuals per square meter, which is sufficient to provide valid data (the guideline requires a minimum of 60). Mean earthworm density in the control plots was 68, 402 and 264 individuals per square meter at 4 weeks, 5 months and 11 months after treatment. The low density in the control after 4 weeks can be explained by the dry and warm weather conditions at that time in combination with the sampling type (under these weather conditions especially endogeic species may enter diapause and they would then be missed with formalin extraction, which requires earthworm activity; hand sorting would have probably resulted in higher numbers). It may hamper statistical evaluation, but is still higher than the required number of 60, and since the main endpoints from this study are derived from samplings at later stages and high densities were achieved at those stages, it does not invalidate the study.

The reference product carbendazim gave the expected response, with a statistically significant reduction of total earthworm abundance and biomass after 4 weeks (effect >50%) and 5 months (effect >50% on biomass), confirming the validity of the study.

The collected adult earthworms with sufficient abundance belonged to 2 endogeic taxa, 2 epigeic taxa and 1 anecic taxon. During the pre-test sampling, the most abundant endogeic species was *Aporrectodea caliginosa* (36% of total earthworms) followed by *Allolobophora chlorotica* (4% of total earthworms); the most abundant anecic earthworm species was *Lumbricus terrestris* (4% of total earthworms). The most abundant epigeic species was *Lumbricus rubellis*/*Lumbricus castaneus* (57% of total earthworms). Because of similar external taxonomic characters of the immature (juvenile) epigeic worms, a classification to the individual species level was sometimes not possible. Therefore *L. rubellus* and *L. castaneus* were combined and are presented as "total epigeic earthworms" or "juvenile epigeic earthworms " in Table B.9.6.3-02 to B.9.6.3-04. The class "epigeic species" may also

contain a small percentage of juvenile *L. terrestris*, which, based on their size, could not be classified as *L. terrestris*.

At 4 weeks after treatment, at 300 g a.s./ha no statistically significant or biologically relevant adverse effects on abundance and biomass were recorded. At 600 g a.s./ha, statistically significant reductions of biomass of total earthwoms (-33%) and of total adult earthworms (-41%) were recorded. At 1500 g a.s./ha, statistically significant reductions were recorded of abundance of total earthwoms (-33%) and total adult earthworm (-44%), and of abundance (-45%) and biomass (-47%) of adult epigeic earthworms, the latter associated with statistically significant reduced biomass (-46%) of adult *L. rubellis*. Non-statistically significant reductions of abundance of total *L. terrrestris* at 600 and 1500 g a.s./ha (-60% and -57%, respectively) and associated reductions of total anecic earthworms (-60% and -57%, respectively) are considered to be of doubtful biological relevance considering the very low number of adults and juvenile *L. terrrestris* in the control (2.5/m^2 and 7.6/m^2, respectively).

At 5 months after treatment, at 300 g a.s./ha no statistically significant or biologically relevant adverse effects on abundance and biomass were recorded. At 5 months after treatment, statistically significant biologically relevant and treatment related effects were reductions of abundance of adult *L. caliginosa* (-38%, 1500 g a.s./ha) and associated adult endogeic earthworms (-37%, 1500 g a.s./ha) and biomass of adult *L. caliginosa* (-32% and -45% at 600 and 1500 g a.s./ha, respectively), and associated reductions of biomass of adult and total endogeic earthworms (-45% and -42%, respectively, 1500 g a.s./ha) and of total *A. caliginosa* (-30% and -42%, 600 and 1500 g a.s./ha, respectively).

At 600 and 1500 g a.s./ha, statistically significant reductions of abundance of juvenile anecic earthwoms were recorded (-55% and -50%, respectively), corresponding with identical statistically significant reductions of juvenile *L. terrestris*. The abundance and biomass of total *L. terrestris*, and the associated abundance and biomass of total anecic earthworms, were statistically significantly reduced at 600 g a.s./ha (-57%, -61%, -57% and -61%, respectively). The biomass of adult *L. terrestris* and the associated biomass of adult anecic earthworms was statisticaly significantly reduced at 600 g a.s./ha (-63% and -63%, respectively). These findings are considered to be of doubtful biological relevance in view of (1) the lack of a dose-response relationship in the range 300-1500 g a.s./ha for the effects on abundance of juvenile anecic earthworms and juvenile *L. terrestris*; (2) the inverse dose-response relationship for the effects on abundance and biomass of total *L. terrestris* and total anecic earthworms; (3) the inverse dose-response relationship for the effects on biomass of adult *L. terrestris* and adult anecic earthworms; (4) the relatively low numbers of juvenile *L. terrestris* in the control (10.4 individuals/m^2).

At 11 months after treatment, at 300 g a.s./ha no statistically significant or biologically relevant adverse effects on abundance and biomass were recorded. At 600 and 1500 g a.s./ha, no statistically significant and/or biologically relevant reductions of abundance and biomass of any taxon were determined, although statistically significant reductions of abundance and biomass of juvenile anecic earthworms (range -50% to 65%) were recorded, corresponding with identical statistically significant reductions of juvenile *L. terrestris*. These findings however are considered to be of doubtful biological relevance in view of the low numbers of juvenile *L. terrestris* in the control (8.4 individuals/m^2) and the very low numbers of adult *L. terrestris* in the control (3.2 individuals/m^2). Several other effects of >50% relative to the control were recorded at this sampling, but these effects were considered to be due to biological variation and unrelated to the treatment as they were not statistically significant, mostly based on very low numbers of individuals in the control, and in most cases represented increases relative to the control, hence not adverse effects. It is concluded that under the conditions tested, the use of BYI 02960 SL 200 G will not present a sustained unacceptable effect on biomass and abundance of earthworm communities.

Tables B.9.6.3-03 to -05 show the results. Bold values represent statistically significant reductions; these cells are marked yellow for the test item treatments.

Table B.9.6.3-03 Earthworm abundance in control and reductions of earthworm abundance (mean of 4 0.25 m^2 plots per treatment) in field soil **4 weeks after treatment** with BYI 02960 SL 200 G (200 g/L BYI 02960) at 300, 600 and 1500 g a.s./ha or with Carbendazim SC 500 (10000 g a.s./ha). page 478

	abundance (number per 0.25 m^2)									biomass (gram per 0.25 m^2)								
	control	300 g a.s./ha		600 g a.s./ha		1500 g a.s./ha		carbendazim		control	300 g a.s./ha		600 g a.s./ha		1500 g a.s./ha		carbendazim	
taxon	no.	no.	% effect	no.	% effect	no.	% effect	no.	% effect	g	g	% effect	g	% effect	g	% effect	g	% effect
total L. terrestris	2.5	2.3	-7	1.0	-60	1.1	-57	**0.19***	**-92**	3.0	4.4	45	1.6	-46	2.0	-35	**0.20***	**-93**
total L. rubellis	classification to individual species level not possible, *L. rubellus* and *L. castaneus* were combined, see "total epigeic earthworms"																	
total L. castaneus																		
total A. caliginosa	0.75	0	-	0	-	0	-	0		0.35	0	-	0	-	0	-	0	-
total A. chlorotica																		
total anecic earthworms	2.5	2.3	-7	1.0	-60	1.1	-57	**0.19***	**-92**	3.0	4.4	45	1.6	-46	2.0	-35	**0.20***	**-93**
total endogeic earthworms	0.75	0	-	0	-	0	-	0		0.35	0	-	0	-	0	-	0	-
total epigeic earthworms	14	18	27	12	-10	10	-25	**0.31***	**-98**	6.9	7.8	14	5.2	-24	4.6	-32	**0.13***	**-98**
total earthworms	17	20	17	13	-21	**11***	**-33**	**0.5**	**-97**	10	12	19	**6.9***	**-33**	6.6	-35	**0.33***	**-97**
adult *L. terrestris*	0.63	0.88	40#	0.50	-20#	0.44	-30#	0	-100#	1.6	2.9	85#	0.94	-39#	1.4	-10#	0	-100#
adult *L. rubellis*	7.1	7.4	4	4.6	-35	4.2	-41	**0.13***	**-98**	5.7	6.0	6	3.5	-38	**3.1***	**-46**	**0.12***	**-98**
adult *L. castaneus*	0.63	0.25	-60#	0.19	-70#	0.06	-90#	0	-100#	0.11	0.03	-74#	0.04	-69#	0.01	-90#	0	-100#
adult *A. caliginosa*	0.13	0	-	0	-	0	-	0	-	0.27	0	-	0	-	0	-	0	-
adult *A. chlorotica*																		
adult anecic earthworms	0.63	0.88	40#	0.50	-20#	0.44	-30#	0	-100#	1.6	2.9	85#	0.94	-39#	1.4	-10#	0	-100#
adult endogeic earthworms	0.13	0	-	0	-	0	-	0	-	0.27	0	-	0	-	0	-	0	-
adult epigeic earthworms	7.7	7.6	-1	4.8	-38	**4.3***	**-45**	**0.13***	**-98**	5.8	6.1	5	3.6	-38	**3.1***	**-47**	**0.12***	**-98**
total adult earthworms	8.4	8.5	1	5.3	-38	**4.7***	**-44**	**0.13***	**-99**	7.6	8.9	17	**4.5***	**-41**	4.5	-41	**0.12***	**-98**
juvenile *L. terrestris*	1.9	1.4	-23#	0.50	-73#	0.63	-67#	0.19	-90#	1.5	1.5	4	0.69	-53	0.58	-61	0.2	-86
juvenile *L. rubellis*	classification to individual species level not possible, *L. rubellus* and *L. castaneus* were combined, see "juvenile epigeic earthworms"																	
juvenile *L. castaneus*																		
juvenile *A. caliginosa*	0.63	0	-	0	-	0	-	0	-	0.08	0	-	0	-	0	-	0	-
juvenile *A. chlorotica*																		
juvenile anecic earthworms	1.9	1.4	-23#	0.5	-73#	0.63	-67#	0.19	-90#	1.5	1.5	4	0.69	-53	0.58	-61	0.2	-86
juvenile endogeic earthworms	0.63	0	-	0.0	-	0	-	0	-	0.08	0	-	0	-	0	-	0	-
juvenile epigeic earthworms	6.1	9.9	63	7.6	26	6.1	1	**0.19***	**-97**	1.1	1.8	63	1.7	56	1.5	43	**0.01***	**-99**
total juvenile earthworms	8.6	11	32	8.1	-5	6.8	-21	**0.38***	**-96**	2.6	3.3	25	2.4	-10	2.1	-20	**0.22***	**-92**

Empty cell: not recorded at that sampling.

Negative and postive % effect values, respectively, indicate a reduced and increased abundance or biomass compared to the control; positive % effect values do not represent an adverse effect.

Not statistically analysed because of too low abundance

- Not analysed because of too low abundance

* Statistically significant difference compared to control at the 5% level

Table B.9.6.3-04 Earthworm abundance in control and reductions of earthworm abundance (mean of 4 0.25 m^2 plots per treatment) in field soil **5 months after treatment** with BYI 02960 SL 200 G (200 g/L BYI 02960) at 300, 600 and 1500 g a.s./ha or with Carbendazim SC 500 (10000 g a.s./ha).

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	abundance (number per 0.25 m^2)									biomass (gram per 0.25 m^2)								
	control	300 g a.s./ha		600 g a.s./ha		1500 g a.s./ha		carbendazim		control	300 g a.s./ha		600 g a.s./ha		1500 g a.s./ha		carbendazim	
taxon	no.	no.	% effect	no.	% effect	no.	% effect	no.	% effect	g	g	% effect	g	% effect	g	% effect	g	% effect
total L. terrestris	5.4	4.4	-17	**2.3***	**-57**	3.9	-27	**0.63***	**-88**	11	12	7	**4.3***	**-61**	11	-3	**0.53***	**-95**
total L. rubellis	classification to individual species level not possible, *L. rubellus* and *L. castaneus* were combined, see "total epigeic earthworms"																	
total L. castaneus																		
total A. caliginosa	28	32	16	23	-16	17	-37	16	-42	6.9	7.3	6	**4.8***	**-30**	**4.0***	**-42**	4.5	-35
total A. chlorotica	2.6	2.4	-10	5.1	95	1.8	-31	0.63	-76	0.31	0.45	44#	0.67	115#	0.20	-35#	0.09	-72#
total anecic earthworms	5.4	4.4	-17	**2.3***	**-57**	3.9	-27	**0.63***	**-88**	11	12	7	**4.3***	**-61**	11	-3	**0.53***	**-95**
total endogeic earthworms	30	34	14	28	-6	19	-37	17	-45	7.2	7.7	7	5.5	-23	**4.2***	**-42**	4.5	-37
total epigeic earthworms	65	54	-17	77	19	64	-1	**35***	**-46**	28	23	-16	31	13	27	-1	**17***	**-39**
total earthworms	100	93	-8	108	7	87	-13	**52***	**-48**	46	43	-7	41	-10	42	-8	**22***	**-52**
adult *L. terrestris*	2.8	3	9	1.1	-59	2.6	-5	**0.06***	**-98**	9.4	11	12	**3.5***	**-63**	9.1	-3	**0.15***	**-98**
adult *L. rubellis*	11	9.3	-13	11	6	9.4	-12	7.8	-27	11	9.8	-12	12	6	10	-9	**6.5***	**-42**
adult *L. castaneus*	6.9	8.9	29	10	48	7.1	3	3.3	-52	1.2	1.6	34	1.7	48	1.2	4	0.61	-48
adult *A. caliginosa*	13	15	18	9.9	-23	**7.9***	**-38**	8.2	-36	5.4	5.4	0	**3.7***	**-32**	**3.0***	**-45**	3.5	-35
adult *A. chlorotica*	1.1	1.6	39#	2.6	128#	0.81	-28#	0.38	-67#	0.2	0.28	39#	0.49	142#	0.14	-30#	0.07	-65#
adult anecic earthworms	2.8	3.0	9	1.1	-59	2.6	-5	**0.06***	**-98**	9.4	11	12	**3.5***	**-63**	9.1	-3	**0.15***	**-98**
adult endogeic earthworms	14	17	19	12	-11	**8.8***	**-37**	8.6	-39	5.6	5.7	1	4.1	-26	**3.1***	**-45**	3.6	-36
adult epigeic earthworms	18	18	3	22	23	17	-6	11	-37	13	11	-9	14	8	11	-10	**7.1***	**-44**
total adult earthworms	34	38	10	35	3	28	-19	**20***	**-43**	28	28	0	21	-23	24	-14	**11***	**-61**
juvenile *L. terrestris*	2.6	1.4	-45	**1.2***	**-55**	**1.3***	**-50**	**0.56***	**-79**	1.4	1.1	-26	0.73	-48	1.4	-2	**0.38***	**-73**
juvenile *L. rubellis*	classification to individual species level not possible, *L. rubellus* and *L. castaneus* were combined, see "juvenile epigeic earthworms"																	
juvenile *L. castaneus*																		
juvenile *A. caliginosa*	15	17	14	13	-10	9.4	-37	**7.9***	**-47**	1.5	1.7	14	1.2	-20	1.1	-29	0.94	-37
juvenile *A. chlorotica*	1.5	0.81	-46#	2.6	71#	1.0	-33#	0.25	-83#	0.11	0.06	-42#	0.19	66#	0.06	-44#	0.02	-85#
juvenile anecic earthworms	2.6	1.4	-45	**1.2***	**-55**	**1.3***	**-50**	**0.56***	**-79**	1.4	1.1	-26	0.73	-48	1.4	-2	**0.38***	**-73**
juvenile endogeic earthworms	16	18	9	16	-3	10	-36	**8.1***	**-50**	1.6	2.1	29	1.4	-14	1.1	-30	0.96	-40
juvenile epigeic earthworms	47	36	-24	55	17	47	0	**24***	**-49**	15	12	-21	18	17	16	7	**9.9***	**-35**
total juvenile earthworms	66	55	-17	72	9	59	-11	**33***	**-51**	18	15	-17	20	9	19	3	**11***	**-38**

Empty cell: not recorded at that sampling.

Negative and postive % effect values, respectively, indicate a reduced and increased abundance or biomass compared to the control; positive % effect values do not represent an adverse effect.

Not statistically analysed because of too low abundance

- Not analysed because of too low abundance

* Statistically significant difference compared to control at the 5% level

Table B.9.6.3-05 Earthworm abundance in control and reductions of earthworm abundance (mean of 4 0.25 m^2 plots per treatment) in field soil **11 months after treatment** with BYI 02960 SL 200 G (200 g/L BYI 02960) at 300, 600 and 1500 g a.s./ha or with Carbendazim SC 500 (10000 g a.s./ha). page 482

	abundance (number per 0.25 m^2)									biomass (gram per 0.25 m^2)								
	control	300 g a.s./ha		600 g a.s./ha		1500 g a.s./ha		carbendazim		control	300 g a.s./ha		600 g a.s./ha		1500 g a.s./ha		carbendazim	
taxon	no.	no.	% effect	no.	% effect	no.	% effect	no.	% effect	g	g	% effect	g	% effect	g	% effect	g	% effect
total L. terrestris	2.9	3.4	15	2.3	-21	2.4	-17	**0.69***	**-77**	5.0	9.7	95	5.4	9	7.3	46	**1.2***	**-75**
total L. rubellis	classification to individual species level not possible, *L. rubellus* and *L. castaneus* were combined, see "total epigeic earthworms"																	
total L. castaneus																		
total A. caliginosa	15	19	27	19	29	16	9	12	-17	3.1	3.9	24	3.8	21	3.2	2	3.1	-1
total A. chlorotica	1.3	1.2	-5#	3.1	150#	0.88	-30#	1.4	10#	0.14	0.16	13#	0.37	162#	0.12	-12#	0.23	63#
total anecic earthworms	2.9	3.4	15	2.3	-21	2.4	-17	**0.69***	**-77**	5.0	9.7	95	5.4	9	7.3	46	**1.2***	**-75**
total endogeic earthworms	16	20	25	22	38	17	6	13	-15	3.3	4.1	24	4.2	27	3.34	2	3.4	2
total epigeic earthworms	47	43	-8	51	8	50	5	38	-19	27	22	-19	29	8	26	-3	27	0
total earthworms	66	66	1	75	14	69	4	53	-21	35	35	1	38	10	36	4	31	-10
adult *L. terrestris*	0.81	2.3	185#	1.3	54#	1.7	108#	0.19	-77#	3.1	8.6	175#	4.5	44#	6.6	110#	0.83	-73#
adult *L. rubellis*	12	9.9	-18	12	3	11	-11	17	44	11	9.2	-18	11	-1	11	-6	18	58
adult *L. castaneus*	3.1	5.4	76	5.4	78	4.3	39	4.3	39	1.2	0.91	-26	0.93	-25	0.75	-40	0.78	-37
adult *A. caliginosa*	5.3	6.8	28	6.8	28	5.8	9	5.6	5	1.9	2.3	23	2.3	26	1.9	3	2.1	15
adult *A. chlorotica*	0.75	0.63	-17#	1.8	133#	0.75	0#	1.4	83#	0.1	0.09	-3#	0.23	138#	0.11	15#	0.23	135#
adult anecic earthworms	0.81	2.3	185#	1.3	-54#	1.7	108#	0.19	-77#	3.1	8.6	175#	4.5	44#	6.6	110#	0.83	-73#
adult endogeic earthworms	6.1	7.4	23	8.6	41	6.6	8	6.9	14	2.0	2.4	21	2.6	31	2.0	3	2.4	21
adult epigeic earthworms	15	15	1	18	18	15	-1	22	43	12	10	-17	12	3	11	-7	18	53
total adult earthworms	22	25	14	28	26	23	6	29	30	17	21	23	19	14	20	16	22	26
juvenile *L. terrestris*	2.1	1.1	-50	**1.1***	**-50**	**0.75***	**-65**	**0.5***	**-76**	1.9	1.1	-40	0.91*	-51	0.72*	-61	0.41*	-78
juvenile *L. rubellis*	classification to individual species level not possible, *L. rubellus* and *L. castaneus* were combined, see "juvenile epigeic earthworms"																	
juvenile *L. castaneus*																		
juvenile *A. caliginosa*	9.3	12	27	12	29	10	9	6.5	-30	1.3	1.3	2	1.5	15	1.3	2	0.99	-23
juvenile *A. chlorotica*	0.5	0.56	13#	1.4	175#	0.13	-75#	0	-100#	0.04	0.04	-3#	0.14	216#	0.01	-74#	0	-100#
juvenile anecic earthworms	2.1	1.1	-50	**1.1***	**-50**	**0.75***	**-65**	**0.50***	**-76**	1.9	1.1	-40	**0.91***	**-51**	**0.72***	**-61**	**0.41***	**-78**
juvenile endogeic earthworms	9.8	12	26	13	37	10	5	6.5	-33	1.3	1.7	27	1.6	21	1.3	-1	0.99	-25
juvenile epigeic earthworms	32	28	-13	33	3	35	8	17	-48	14	11	-21	16	12	14	0	8.2	-44
total juvenile earthworms	44	41	-6	48	8	46	4	24	-46	18	14	-19	19	6	16	-7	9.6	-46

Empty cell: not recorded at that sampling.

Negative and postive % effect values, respectively, indicate a reduced and increased abundance or biomass compared to the control; positive % effect values do not represent an adverse effect.

Not statistically analysed because of too low abundance

- Not analysed because of too low abundance

* Statistically significant difference compared to control at the 5% level

Shannon-Weaver diversity index (Hs)

The results are summarised in the Table below. The species diversity in all BYI 02960 SL 200 G treatments tended to be reduced relative to the control at 4 weeks after application, although a dose-relationship was not apparent. At 5 and 11 months after application, diversity indices in treated plots were comparable to those in control plots.

Table B.9.6.4-06 Shannon-Weaver diversity index (Hs)

treatment	diversity (H_S) 4 weeks after the application	diversity (H_S) 5 months after the application	diversity (H_S) 11 months after the application
sum of adult and juvenile earthworms			
control	0.59	0.89	0.79
BYI 300	0.36	0.92	0.86
BYI 600	0.27	0.80	0.85
BYI 1500	0.31	0.77	0.75
Carbendazim	0.66	0.74	0.72
adult earthworms			
control	0.60	1.37	1.18
BYI 300	0.46	1.38	1.36
BYI 600	0.47	1.38	1.34
BYI 1500	0.38	1.40	1.32
Carbendazim	0	1.12	1.08
juvenile earthworms			
control	0.77	0.79	0.75
BYI 300	0.38	0.80	0.77
BYI 600	0.23	0.70	0.79
BYI 1500	0.31	0.62	0.63
Carbendazim	0.69	0.68	0.68

Conclusion

BYI 02960 SL 200 G did not induce lasting adverse effects on the earthworm community following a spring application in grassland up to 1500 g a.s./ha (1.98% organic carbon), corresponding to a measured concentration of 1234 µg/kg soil.

Remarks

The study was performed according to ISO 11268-3 and is acceptable. The abundance of endogeic and epigeic earthworms during the study was high, but that of anecic earthworms (*L. terrestris*) was in general too low in the control plots to allow reliable conclusions for this category. The RMS considered

that the species distribution, including the low numbers of anecic earthworms (*L. terrestris*) is considered to be a feature of the test plots, and does not invalidate the study. However, in the pesticide peer review meeting 124 of December 2014, it was noted that the effects of the test substance on the number and the biomass of juveniles of *Lumbricus terrestris* looked significant and constant over the test period of 11 months showing a concentration dependent trend without recovery. It was also noted that if the effects on *L. terrestris* are disregarded as proposed by the RMS, the effects on anecic (burrowing) earthworms cannot be estimated since *L. terrestris* was the only representative of this group.

In the peer review meeting it was furthermore noted that the abundance of other species besides *L. terrestris* was quite low. The results of sampling collected after 4 weeks were questioned due to too hot and dry conditions. However, it was noted that the abundance in general was sufficient at the beginning of the test. After 4 weeks, the abundance in the control (although individual groups had a low number which does not match with the recommendations of the guideline) was higher than the treated plots.

Low residues of the a.s. were found in the control plots. Contamination may have occurred via spray drift. Since the residues are at least 45 times lower than those in the treatment plots, RMS considers that ~~the results of the study can still be used~~ this does not invalidate the study.

The residue concentration in soil was measured over 10 cm. Usually, for spray applications it is assumed that the initial residues are distributed over a 5 cm layer, therefore it the approach used may be worst case (expressed over 5 cm the endpoint could be doubled). It is however unknown whether the residues in the 10 cm soil cores were evenly distributed or indeed only present in the top 5 cm layer. ~~Therefore, the risk assessment should be based on the endpoint mentioned under study conclusion.~~

Overall, the experts in the pesticide peer review meeting considered the study as not reliable for risk assessment.

B.9.6.4 Summary and risk assessment

Procedures for risk assessment were in agreement with the recommendations in the Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC (Working Document Sanco/10329/2002 rev 2 final, 17 October 2002). Two soil metabolites require further consideration according to the fate section: DFA and 6-CNA.

B.9.6.4.1 Acute risk to earthworms

The table below presents a summary of the acute toxicity studies on earthworms.

Table B.9.6.4.1-01 Acute effects of BYI 02960 and relevant metabolites on earthworms

Test species	Test design	Ecotoxicological endpoint			Reference
BYI 02960					
Eisenia fetida	acute, 14 d (10% peat in test soil)	LC_{50}	185.6	mg a.i./kg dws	Leicher (2010) KIIA 8.9.1/01
BYI 02960 SL 200 G					
Eisenia fetida	acute, 14 d (5% peat in test soil)	LC_{50}	121	mg a.i./kg dws	Leicher (2010) KIIIA 10.6.2/01
DFA					
Eisenia fetida	acute, 14 d (10% peat in test soil)	LC_{50}	> 958	mg p.m./kg dws	Leicher (2007) KIIA 8.9.1/02
6-CAN					
Eisenia fetida	acute, 14 d (10% peat in test soil)	LC_{50}	> 1000	mg p.m./kg dws	Wetton (1999) KIIA 8.9.1/03

dws = dry weight soil
a.i. = active ingredient; p.m. = pure metabolite

For lipophilic substances (log POW > 2) the Terrestrial Guidance Document recommends to apply an additional assessment factor of 2 for the ecotoxicological endpoints (LC50, NOEC), if the study was conducted in artificial soil with a high content of organic matter (i.e. 10 % peat), to consider the possible sorption of these compounds to the organic matter. However, BYI 02960 and its metabolites have a log POW < 2 and no additional assessment factor has to be applied.

The toxicity values are compared with the PECsoil values from section B.8.3 (maximum concentration after multiple year use: $PEC_{initial}$ after one application + $PEC_{accumulation}$). The $PEC_{initial}$ is expressed over 5 cm. For hops, the $PEC_{accumulation}$ is expressed over 5 cm (perennial crop without tillage). For lettuce, the $PEC_{accumulation}$ is expressed over 20 cm (annual crop with tillage). The acute TER values are presented in Table B.9.6.3.1-02.

Table B.9.6.4.1-02 TER_{acute} calculations for earthworms

Compound test design	End point	[mg/kg soil]	$PEC_{max,accu}$ [mg/kg soil]	TER_{acute}	Trigger	Refined risk assessment?
Hops						
BYI 02960	LC_{50}	185.6	0.160	2.3E+3	10	
BYI 02960 SL 200	LC_{50}	121	0.160	7.6E+2	10	No
DFA	LC_{50}	>958	0.009	> 11E+4	10	
6-CNA	LC_{50}	>1000	0.007	> 14E+4	10	
Lettuce biennial						
BYI 02960	LC50	185.6	0.141	1.3E+3	10	
BYI 02960 SL 200	LC50	121	0.141	8.6E+2	10	No
DFA	LC50	>958	0.014	> 68E+3	10	
6-CNA	LC50	>1000	0.012	≥ 83E+3	10	
Lettuce annual						
BYI 02960	LC50	185.6	0.063	2.9E+3	10	
BYI 02960 SL 200	LC50	121	0.063	1.9E+3	10	No
DFA	LC50	>958	0.006	> 16E+4	10	
6-CNA	LC50	>1000	0.005	> 20E+4	10	

The TER values in Table B.9.6.4.1-02 are far above the Annex VI 91/414 EEC trigger of 10. Hence the acute risk to earthworms is considered to be acceptable.

B.9.6.4.2 Chronic risk to earthworms

The table below presents a summary of the chronic toxicity studies on earthworms.

Table B.9.6.4.1-03 Chronic effects of BYI 02960 and relevant metabolites on earthworms

Test species	Test design	Ecotoxicological endpoint	Reference
BYI 02960 SL 200 G			
Eisenia fetida	reproduction, 56 d (10% peat in test soil)	NOEC 1.5 mg a.i./kg dws	Leicher (2010) KIIIA1 10.6.3/01
DFA			
Eisenia fetida	reproduction, 56 d (10% peat in test soil) mixing	NOEC 59 mg p.m./kg dws	Leicher (2010) KIIA 8.9.2/02
6-CNA			
Eisenia fetida	reproduction, 56 d (10% peat in test soil) mixing	NOEC 95 mg p.m./kg dws	Leicher (2011) KIIA 8.9.2/03

dws = dry weight soil
a.i. = active ingredient; p.m. = pure metabolite

The toxicity values are compared with the PECsoil values from section B.8.3 (maximum concentration after multiple year use: $PEC_{initial}$ after one application + $PEC_{accumulation}$). The $PEC_{initial}$ is expressed over

5 cm. For hops, the $PEC_{accumulation}$ is expressed over 5 cm (perennial crop without tillage). For lettuce, the $PEC_{accumulation}$ is expressed over 20 cm (annual crop with tillage). The long-term TER values are presented in Table B.9.6.3.1-02.

Table B.9.6.4.1-04 $TER_{long-term}$ calculations for earthworms

Compound test design	End point	[mg/kg soil]	$PEC_{max,accu}$ [mg/kg soil]	TER_{lt}	Trigger	Refined risk assessment?
Hops						
BYI 02960 SL 200	NOEC	1.5	0.160	9.4	5	No
DFA	NOEC	59	0.009	6.6E+3	5	
6-CNA	NOEC	95	0.007	14E+3	5	
Lettuce biennial						
BYI 02960 SL 200	NOEC	1.5	0.141	11	5	No
DFA	NOEC	59	0.014	4.2E+3	5	
6-CNA	NOEC	95	0.012	7.9E+3	5	
Lettuce annual						
BYI 02960 SL 200	NOEC	1.5	0.063	24	5	No
DFA	NOEC	59	0.006	9.8E+3	5	
6-CNA	NOEC	95	0.005	19E+3	5	

The TER values in Table B.9.6.4.1-04 are above the Annex VI 91/414 EEC trigger of 5. Hence the chronic risk to earthworms is considered to be acceptable.

B.9.6.4.3 Field studies with earthworms

The above risk assessments indicate that no acute or long-term risk is to be expected for earthworms from the proposed uses of BYI 02960 in hops and lettuce.

A field study with earthworms was performed nevertheless. In this one year earthworm field study, BYI 02960 SL 200 G was sprayed on grassland at 300, 600 and 1500 g a.i./ha corresponding to measured concentrations of 271, 472 and 1234 µg/kg soil (mean of four plots). Reduction of abundance (-33%) and biomass (-38%) at 1500 g a.i./ha and biomass at 600 g a.i./ha (-36%) after 1 month were seen, but there was full recovery of earthworm population after 11 months. The measured concentrations in soil at the start of the study (up to 1.234 mg/kg soil measured over 10 cm, see remarks to the field study above) cover the expected concentrations at the proposed uses (up to 0.160 mg/kg soil). ~~The study confirms the expected acceptable risk to earthworms.~~ However, the study was considered invalid by the experts of the pesticide peer review meeting of December 2014. Therefore,

this study cannot be used for the risk assessment. This has no influence on the outcome of the risk assessment, since this was already acceptable based on laboratory studies.

B.9.7 Effects on soil non-target macro-organisms (IIIA 10.6.6)

B.9.7.1 Toxicity studies with soil non-target macro-organisms other than earthworms

STUDY IIA, 8.14/01

Characteristics

reference	:	Frommholz U. (2009b)	species	:	*Folsomia candida*
type of study	:	Laboratory reproduction toxicity study	exposure duration	:	28 days
year of execution	:	2009	nom. conc.	:	Run 1: 8.8, 13.2, 19.9, 29.8 and 44.6 mg test item/kg soil d.w.; Run 2: 5.88, 7.06 and 8.47 mg test item/kg soil d.w.
GLP statement	:	Yes	dosing method	:	Test substance was dissolved in water and mixed into the soil
guideline	:	ISO 11267 (1999)	acceptability	:	Acceptable
test substance	:	BYI 02960 SL 200 G (200 g/L SL formulation), batch 2009-001253	NOEC	:	8.47 mg test item/kg (1.44 mg a.s./kg), soil with 5% organic matter
a.s. content	:	17.0% w/w (199.8 g/L) BYI 02960			

Methods

In a 28-day reproduction study, springtails (*Folsomia candida*) were exposed to BYI 02960 SL 200 G (200 g/L SL formulation, 17.0% w/w (199.8 g/L) BYI 02960) for 28 days in artificial soil prepared according to OECD 207, but with 5% peat. The test was performed in two runs, since in the first run statistically significant effects on reproduction were noted at the lowest test concentration. Test concentrations were 8.8, 13.2, 19.9, 29.8 and 44.6 mg test item/kg soil d.w. in run 1, and 5.88, 7.06 and 8.47 mg test item/kg soil d.w. in run 2. The test substance was dissolved in water and mixed into the soil. The control was treated with water only. A positive control (Betosip, 157 g/L phenmedipham, 50-200 mg product/kg soil d.w.) was tested less than one year earlier. The test was conducted in 100 mL glass jars (5 for each treatment and the control) covered with glass lids containing 30 g of moist soil and 10 springtails (10-12 days old) at test initiation. Containers were maintained at 18-22°C under a 16 h light regime (400-800 lux). Soil moisture was maintained at 41-49% of MWHC. The pH was 5.6-6.1 at the start and 5.3-5.7 at 28 days, when adult survival and the number of juveniles were assessed.

Results

The positive control gave the expected response (NOEC <50 mg product/kg soil d.w.; test conducted in soil with 5% peat, therefore effects on reproduction were higher than recommended by ISO 11267: effects at 100-200 mg product/kg soil d.w.). Other results are summarised in the table below. The results of the control group satisfied the guideline requirements: ≤20% adult mortality, ≥100 instar juveniles per test vessel, CV for reproduction ≤30%. The most sensitive test endpoint, reproduction, as measured by the number of offspring, was significantly reduced at 8.8 mg test item/kg and above. The NOEC is 8.47 mg test item/kg soil d.w., equivalent to 1.44 mg a.s./kg soil d.w..

Table B.9.7-01 Effects of BYI 02960 SL 200 G on *Folsomia candida*

Test item Test object Exposure		BYI 02960 SL 200 G *Folsomia candida* Artificial Soil		
Nominal concentration mg/kg artificial soil dry weight test item	a.s.	Adult mortality (%)	Mean number of juveniles±SD	Reproduction (% of control)
First test run				
Control		2	1340 ± 60	-
8.8	1.5	2	1111 ± 120	83*
13.2	2.3	6	934 ± 144	70*
19.9	3.4	12	680 ± 130	51*
29.8	5.1	14	427 ± 169	32*
44.6	7.6	44	196 ± 61	15*
Second test run				
Control		4	1202 ± 101	-
5.88	1.00	14	1289 ± 104	107
7.06	1.20	14	1308 ± 97	109
8.47	1.44	12	1166 ± 121	97
$NOEC_{reproduction}$ (mg test item/kg soil dry weight)				8.47
$LOEC_{reproduction}$ (mg test item/kg soil dry weight)				8.8

The calculations were performed with unrounded values
* = statistically significant (William's T-test one-sided-smaller, α = 0.05)

Conclusion

NOEC 8.47 mg test item/kg soil d.w. equivalent to 1.44 mg a.s./kg soil d.w. (soil with 5% organic matter).

Guidelines and limitations

The study was conducted in agreement with ISO 11267 (1999) and is acceptable.

STUDY IIA, 8.14/02

Characteristics

reference	:	Frommholz U. (2010a)	species	:	*Folsomia candida*
type of study	:	Laboratory reproduction toxicity study	exposure duration	:	28 days
year of execution	:	2010	nom. conc.	:	100 mg test item/kg soil d.w.
GLP statement	:	Yes	dosing method	:	Test item was added to soil mixed with quartz sand
guideline	:	OECD 232	acceptability	:	Acceptable
test substance	:	BYI 02960-difluoroacetic acid; batch BCOO 5984-1-1	NOEC	:	95.8 mg pure metabolite/kg soil d.w. (soil with 5% organic matter)
a.s. content	:	95.8%			

Methods

In a 28-day reproduction study, springtails (*Folsomia candida*) were exposed to BYI 02960-difluoroacetic acid (metabolite of BYI 02960) for 28 days in artificial soil prepared according to OECD 207, but with 5% peat. The test concentration was 100 mg test item/kg soil d.w.. To prepare the test

substrate, the test substance was mixed with quartz sand and the mixture was added to the artificial soil substrate. The control was treated with quartz sand only. A positive control (boric acid, 44-225 mg/kg soil d.w.) was tested less than one year earlier. The test was conducted in 100 mL glass jars (8 for each treatment and the control) covered with glass lids containing 30 g of moist soil and 10 springtails (10-12 days old) at test initiation. Containers were maintained at 18-22°C under a 16 h light regime (400-800 lux). Soil moisture was maintained at 42-49% of MWHC. The pH was 5.6 at the start and 5.6-5.7 at 28 days, when adult survival and the number of juveniles were assessed.

Results

The positive control gave the expected response (EC50 for reproduction 96 mg/kg soil d.w.). Other results are summarised in the table below. The results of the control group satisfied the guideline requirements: ≤20% adult mortality, ≥100 instar juveniles per test vessel, CV for reproduction ≤30%. The NOEC is 100 mg test item/kg soil d.w., equivalent to 95.8 mg pure metabolite/kg soil d.w..

Table B.9.7-02 Effects of BYI 02960-difluoroacetic acid on *Folsomia candida*

Test item Test object Exposure	Metabolite BYI 02960-difluoroacetic acid *Folsomia candida* Artificial soil		
mg test item/kg soil dry weight nominal concentration	Adult mortality (%)	Mean number of juveniles±SD	Reproduction (% of control)
Control	2.5	1337 ± 175	-
100	1.3	1268 ± 146	95 n.s.
$NOEC_{reproduction}$ (mg test item/kg soil dry weight) $LOEC_{reproduction}$ (mg test item/kg soil dry weight)			≥100 >100

The calculations were performed with un-rounded values
n.s. = statistically not significant (Student-t test one-sided-smaller, α = 0.05)

Conclusion

NOEC 95.8 mg pure metabolite/kg soil d.w. (soil with 5% organic matter).

Guidelines and limitations

The study was performed according to OECD 232 and is acceptable. Test rates were not corrected for active ingredient content of the test substance (95.8%). The correction of the NOEC from 100 mg test item/kg soil d.w. to 95.8 mg pure metabolite/kg soil d.w. based on test item purity of 95.8% was performed by the reviewer.

STUDY IIA, 8.14/03

Characteristics

reference	:	Frommholz U. (2011b)	species	:	*Folsomia candida*
type of study	:	Laboratory reproduction toxicity study	exposure duration	:	28 days

year of execution	:	2011	nom. conc.	:	Run 1: 100 mg test item/kg soil d.w.; Run 2: 10, 17, 30, 52 and 90 mg test item/kg soil d.w.
GLP statement	:	Yes	dosing method	:	Test item was added to soil mixed with quartz sand
guideline	:	OECD 232	acceptability	:	Acceptable
test substance	:	6-chloronicotinic acid (6-CNA = IC-0); batch M12653	NOEC	:	90 mg/kg soil d.w. (soil with 5% organic matter)
a.s. content	:	98.8%			

Methods

In a 28-day reproduction study, springtails (*Folsomia candida*) were exposed to 6-chloronicotinic acid (6-CNA = IC-0, metabolite of BYI 02960) for 28 days in artificial soil prepared according to OECD 207, but with 5% peat. The test was performed in two runs, since in the first run a statistically significant effect on reproduction were noted at the only tested concentration. Test concentrations were 100 mg test item/kg soil d.w. in run 1, and 10, 17, 30, 52 and 90 mg test item/kg soil d.w. in run 2. To prepare the test substrate, the test substance was mixed with quartz sand and the mixture was added to the artificial soil substrate. The control was treated with quartz sand only. A positive control (boric acid, 44-225 mg/kg soil d.w.) was tested less than one year earlier. The test was conducted in 100 mL glass jars (8 for each treatment and the control in run 1, 8 for the control and 4 for the treatments in run 2), covered with glass lids, containing 30 g of moist soil and 10 springtails (10-12 days old) at test initiation. Containers were maintained at 18-22°C under a 16 h light regime (400-800 lux). Soil moisture was maintained at 43-48% of MWHC. The pH was 5.6-5.9 at the start and 5.5-5.8 at 28 days, when adult survival and the number of juveniles were assessed.

Results

The positive control gave the expected response (EC50 for reproduction 91 mg/kg soil d.w.). Other results are summarised in the table below. The results of the control group satisfied the guideline requirements: ≤20% adult mortality, ≥100 instar juveniles per test vessel, CV for reproduction ≤30%.
The NOEC is 90 mg test item/kg soil d.w. (no correction required considering the high test item purity).

Table B.9.7-03 Effects of 6-chloronicotinic acid on *Folsomia candida*

Test item Test object Exposure	6-chloronicotinic acid *Folsomia candida* Artificial soil		
mg test item/kg soil dry weight	Adult mortality (%)	Mean number of juveniles±SD	Reproduction (% of control)
1st test run			
Control	13.8	1541.4 ± 165.6	-
100	20.0	1258.9 ± 132.5	81.7*
2nd test run			
control	2.5	1566.9 ± 166.0	-
90	5.0	1485.8 ± 124.8	94.8 n.s.
52	5.0	1434.3 ± 155.0	91.5 n.s.
30	7.5	1573.5 ± 95.4	100.4 n.s.
17	5.0	1641.3 ± 51.4	104.7 n.s.
10	5.0	1583.0 ± 93.6	101.0 n.s.
$NOEC_{reproduction}$ (mg test item/kg soil dry weight) $LOEC_{reproduction}$ (mg test item/kg soil dry weight)			**90** **100**

The calculations were performed with un-rounded values
*= statistically significant (Student's-t test one-sided-smaller, α = 0.05)
n.s. = statistically not significant (William's-t test one-sided-smaller, α = 0.05)

Conclusion

NOEC 90 mg/kg soil d.w. (soil with 5% organic matter).

Guidelines and limitations

The study was performed according to OECD 232 and is acceptable.

STUDY IIA, 8.14/04

Characteristics

reference	:	Kratz M.-A. (2009a)	species	:	*Hypoaspis aculeifer*
type of study	:	Laboratory reproduction toxicity study	exposure duration	:	14 days
year of execution	:	2009	nom. conc.	:	100, 178, 316, 562 and 1000 mg test item/kg soil d.w.
GLP statement	:	Yes	dosing method	:	Test substance was dissolved in water and mixed into the soil
guideline	:	OECD 226	acceptability	:	Acceptable
test substance	:	BYI 02960 SL 200 G (200 g/L SL formulation), batch 2009-001253	NOEC	:	1000 mg test item/kg soil d.w. (170 mg a.s./kg soil d.w.), soil with 5% organic matter
a.s. content	:	17.0% w/w (199.8 g/L) BYI 02960			

Methods

In a 14-day reproduction study, soil mites (*Hypoaspis aculeifer*) were exposed to BYI 02960 SL 200 G (200 g/L SL formulation, 17.0% w/w (199.8 g/L) BYI 02960) at 100, 178, 316, 562 and 1000 mg test item/kg dry soil for 14 days in artificial soil prepared according to OECD 226 (5% organic matter). The test substance was dissolved in water and mixed into the soil. The control was treated with water only. A positive control (dimethoate, 0.98-8.92 mg/kg soil d.w.) was tested less than one year earlier. The

test was conducted in glass containers (four for each treatment and eight for the control), covered with glass lids and containing 20 g d.w. test substrate and 10 mites (adult, fertilised, 28-day old females) at test initiation. Containers were maintained at 18-22°C under a 16 h light regime (400-800 lux). Soil moisture was maintained at 47-50% of MWHC. The pH was 5.6-5.8 at the start and 5.5-5.8 at 14 days, when adult survival and the number of juveniles were assessed using an extraction method.

Results

The positive control gave the expected response (EC50 for reproduction 5.45 mg a.s./kg dw). Other results are summarised in the table below. The results of the control group satisfied the guideline requirements (OECD 226, 2008): ≤20% adult mortality, ≥50 juveniles per test vessel, CV for reproduction ≤30%. None of the differences between the test item treatments and the control were statistically significant at the 5% level. The NOEC is 1000 mg test item/kg soil d.w., equivalent to 170 mg a.s./kg soil d.w..

Table B.9.7-04 Effects of BYI 02960 SL 200 G on *Hypoaspis aculeifer*

Test item Test object Exposure	BYI 2960 SL 200 G *Hypoaspis aculeifer* Artificial Soil		
mg test item/kg dry weight artificial soil	% mortality (Adults)	Mean number of juveniles per test vessel ± standard dev.	Reproduction (% of control)
Control	1.3	361.9 ± 23.4	---
100	2.5	334.5 ± 44.8	92.4
178	5.0	377.3 ± 22.8	104.2
316	0.0	387.5 ± 23.1	107.1
562	0.0	384.3 ± 19.3	106.2
1000	0.0	382.0 ± 43.4	105.6
			Reproduction
NOEC (mg test item/kg dry weight artificial soil) LOEC (mg test item/kg dry weight artificial soil)			≥ 1000 mg t.i./kg > 1000 mg t.i./kg

* statistical significance (Williams test one-sided smaller, α = 0.05)

Conclusion

NOEC 1000 mg test item/kg soil d.w., equivalent to 170 mg a.s./kg soil d.w. (soil with 5% organic matter).

Guidelines and limitations

The study was conducted in agreement with OECD 226 (2008) and is acceptable.

STUDY IIA, 8.14/05

Characteristics

reference	:	Kratz M.-A. (2010)	species	:	*Hypoaspis aculeifer*
type of study	:	Laboratory reproduction toxicity study	exposure duration	:	14 days
year of execution	:	2010	nom. conc.	:	Run 1: 63, 125, 250, 500 and 1000 mg test item/kg soil d.w.; Run 2: 1000 mg test item/kg soil d.w.

GLP statement	:	Yes	dosing method	:	Test item was added to soil mixed with quartz sand
guideline	:	OECD 226	acceptability	:	Acceptable
test substance	:	BYI 02960-difluoroacetic acid; batch BCOO 5984-1-1	NOEC	:	958 mg pure metabolite/kg soil d.w. (soil with 5% organic matter)
a.s. content	:	95.8%			

Methods

In a 14-day reproduction study, soil mites (*Hypoaspis aculeifer*) were exposed to BYI 02960-difluoroacetic acid (metabolite of BYI 02960) for 14 days in artificial soil prepared according to OECD 226 (5% organic matter). The test was performed in two runs, since in the first run in at least one replicate of each treatment and the control a few juveniles were inadvertantly introduced at the start of the test. Test concentrations were 63, 125, 250, 500 and 1000 mg test item/kg soil d.w. in run 1, and 1000 mg test item/kg soil d.w. in run 2. To prepare the test substrate, the test substance was mixed with quartz sand and the mixture was added to the artificial soil substrate. The control was treated with quartz sand only. A positive control (dimethoate, 1.0-10 mg/kg soil d.w.) was tested less than one year earlier. The test was conducted in glass containers (four for each treatment and eight for the control in run 1; eight for the treatment and the control in run 2), covered with glass lids and containing 20 g d.w. test substrate and 10 mites (adult, fertilised, 35-day old (run 1) or 28-day old (run 2) females) at test initiation. Containers were maintained at 18-22°C under a 16 h light regime (400-800 lux). Soil moisture was maintained at 43-53% of MWHC. The pH was 5.6-6.3 (run 1) or 5.5 (run 2) at the start and 5.4-6.1 (run 1) or 4.9-5.4 (run 2) at 14 days, when adult survival and the number of juveniles were assessed using an extraction method.

Results

The positive control gave the expected response (EC50 for reproduction 5.7 mg a.s./kg dw). Other results are summarised in the table below. The results of the control group satisfied the guideline requirements (OECD 226, 2008): ≤20% adult mortality, ≥50 juveniles per test vessel, CV for reproduction ≤30%. Negative % mortality values were obtained in the first test run because together with the adult females inadvertently juveniles were transferred to the test vessels at the start of the test and became adult during the test run. The numbers of juveniles of individual replicates obtained during the 1st test run suggested that inadvertently inserted juveniles didn't reproduce during the test run. Therefore all counted juveniles were considered as reproduction of the initially inserted adults by the author of the report. However, the results of the first run may be ignored, since the results of the properly performed second run clearly demonstrated that the NOEC is 1000 mg test item/kg soil d.w., equivalent to 958 mg pure metabolite/kg soil d.w..

Table B.9.7-05 Effects of BYI 02960-difluoroacetic acid on *Hypoaspis aculeifer*

1st test run

Test item Test object Exposure	BYI 2960-DFA (BCS-AA56716) *Hypoaspis aculeifer* Artificial Soil		
	% mortality (Adults)	Mean number of juveniles per test vessel ± standard dev.	Reproduction (% of control)
Control	5.0	369.9 ± 44.3	---
63	-7.5	393.0 ± 50.1	106.3
125	2.5	356.5 ± 39.4	96.4
250	-10.0	436.0 ± 43.6	117.9
500	-7.5	377.3 ± 18.3	102.0
1000	5.0	393.3 ± 48.1	106.3

No statistical significance (Williams test one-sided smaller, α = 0.05)

2nd test run

Test item Test object Exposure	BYI 2960-DFA (BCS-AA56716) *Hypoaspis aculeifer* Artificial Soil		
mg test item/kg dry weight artificial soil	% mortality (Adults)	Mean number of juveniles per test vessel ± standard dev.	Reproduction (% of control)
Control	1.3	380.6 ± 37.7	---
1000	0.0	391.9 ± 29.9	103.0

Conclusion

NOEC 958 mg pure metabolite/kg soil d.w. (soil with 5% organic matter).

Guidelines and limitations

The study was conducted in agreement with OECD 226 (2008) and is acceptable.

STUDY IIA, 8.14/06

Characteristics

reference	:	Kratz M.-A. (2011)	species	:	*Hypoaspis aculeifer*
type of study	:	Laboratory reproduction toxicity study	exposure duration	:	14 days
year of execution	:	2011	nom. conc.	:	100 mg test item/kg soil d.w.
GLP statement	:	Yes	dosing method	:	Test item was added to soil mixed with quartz sand
guideline	:	OECD 226	acceptability	:	Acceptable
test substance	:	6-chloronicotinic acid (6-CNA = IC-0); batch AE F161089 00 1B99 0001	NOEC	:	100 mg/kg soil d.w. (soil with 5% organic matter)
a.s. content	:	98.8%			

Methods

In a 14-day reproduction study, soil mites (*Hypoaspis aculeifer*) were exposed to 6-chloronicotinic acid (6-CNA = IC-0, metabolite of BYI 02960) for 14 days in artificial soil prepared according to OECD 226 (5% organic matter). To prepare the test substrate, the test substance was mixed with quartz sand and the mixture was added to the artificial soil substrate. The control was treated with quartz sand only. The test concentration was 100 mg test item/kg soil d.w.. A positive control (dimethoate, 1.0-10 mg/kg soil d.w.) was tested less than one year earlier. The test was conducted in glass containers (eight for each treatment and the control), covered with glass lids and containing 20 g d.w. test

substrate and 10 mites (adult, fertilised, 28-day old females) at test initiation. Containers were maintained at 18-22°C under a 16 h light regime (400-800 lux). Soil moisture was maintained at 44-48% of MWHC. The pH was 5.6 at the start and 6.7-6.8 at 14 days, when adult survival and the number of juveniles were assessed using an extraction method.

Results

The positive control gave the expected response (EC50 for reproduction 5.7 mg a.s./kg dw). Other results are summarised in the table below. The results of the control group satisfied the guideline requirements (OECD 226, 2008): ≤20% adult mortality, ≥50 juveniles per test vessel, CV for reproduction ≤30%. The NOEC is 100 mg test item/kg soil d.w. (no correction required considering the high test item purity).

Table B.9.7-06 Effects of 6-chloronicotinic acid on *Hypoaspis aculeifer*

Test item Test object Exposure	6-chloronicotinic acid (AE F161089) *Hypoaspis aculeifer* Artificial Soil		
mg test item/kg dry weight artificial soil	% mortality (Adults)	Mean number of juveniles per test vessel ± standard dev.	Reproduction (% of control)
Control	16.3	264.0 ± 54.5	---
100	21.3	252.0 ± 130.2	95.5
			Reproduction
NOEC (mg test item/kg dry weight artificial soil) LOEC (mg test item/kg dry weight artificial soil)			≥ 100 > 100

No statistical significance (Welch-t test for inhomogeneous variances one-sided smaller, α = 0.05)

Conclusion

NOEC 100 mg/kg soil d.w. (soil with 5% organic matter).

Guidelines and limitations

The study was conducted in agreement with OECD 226 (2008) and is acceptable.

B.9.7.2 Summary and risk assessment for soil non-target macro-organisms other than earthworms

The Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (Sanco 10329/2002, rev 2 final) states that a 'higher degree of scrutiny' for the soil organism risk assessment is required if the field DT90 values in soil are >100 days. The field DT90 values for BYI 02960 and the laboratory DT90 (no field studies available) for metabolites DFA and 6-CNA are >100 days. Therefore, studies with *Folsomia candida* and *Hypoaspis aculeifer* were performed with the formulated product and the two metabolites. See Table B.9.7-07.

Table B.9.7-07 Effects on other soil non-target macro-organisms

Test species	Test design	Ecotoxicological endpoint			Reference
BYI 02960 SL 200 G					
Folsomia candida	chronic, 28 d (5% peat in test soil)	NOEC	1.44	mg a.s./kg dws	Frommholz (2009) KIIA 8.14/01, KIIIA1 10.6.6/01
Hypoaspis aculeifer	chronic, 14 d (5% peat in test soil)	NOEC	≥ 170	mg a.s./kg dws	Kratz (2010) KIIA 8.14/04, KIIIA1 10.6.6/02
DFA					
Folsomia candida	chronic, 28 d (5% peat in test soil)	NOEC	≥ 95.8	mg p.m./kg dws	Frommholz (2010) KIIA 8.14/02
Hypoaspis aculeifer	chronic, 14 d (5% peat in test soil)	NOEC	≥ 958	mg p.m./kg dws	Kratz (2010) KIIA 8.14/05
6-CNA					
Folsomia candida	chronic, 28 d (5% peat in test soil)	NOEC	90	mg p.m./kg dws	Frommholz (2010) KIIA 8.14/03
Hypoaspis aculeifer	chronic, 14 d (5% peat in test soil)	NOEC	≥ 100	mg p.m./kg dws	Kratz, A. (2011) KIIA 8.14/06

dws = dry weight soil
p.m. = pure metabolite

The toxicity values are compared with the PECsoil values from section B.8.3 (maximum concentration after multiple year use: $PEC_{initial}$ after one application + $PEC_{accumulation}$). The $PEC_{initial}$ is expressed over 5 cm. For hops, the $PEC_{accumulation}$ is expressed over 5 cm (perennial crop without tillage). For lettuce, the $PEC_{accumulation}$ is expressed over 20 cm (annual crop with tillage). The long-term TER values are presented in Table B.9.6.3.1-08 and -09.

Table B.9.6.4.1-08 $TER_{long\text{-}term}$ calculations for *Folsomia candida*

Compound test design	End point	[mg/kg soil]	$PEC_{max,accu}$ [mg/kg soil]	TER_{lt}	Trigger	Refined risk assessment?
Hops						
BYI 02960 SL 200	NOEC	1.44	0.160	9.0	5	No
DFA	NOEC	≥ 95.8	0.009	11E+3	5	
6-CNA	NOEC	90	0.007	13E+3	5	
Lettuce biennial						
BYI 02960 SL 200	NOEC	1.44	0.141	10	5	No
DFA	NOEC	≥ 95.8	0.014	6.8E+3	5	
6-CNA	NOEC	90	0.012	7.5E+3	5	
Lettuce annual						
BYI 02960 SL 200	NOEC	1.44	0.063	23	5	No
DFA	NOEC	≥ 95.8	0.006	16E+3	5	
6-CNA	NOEC	90	0.005	18E+3	5	

Table B.9.6.4.1-09 $TER_{long\text{-}term}$ calculations for *Hypoaspis aculeifer*

Compound test design	End point	[mg/kg soil]	$PEC_{max,accu}$ [mg/kg soil]	TER_{lt}	Trigger	Refined risk assessment?
Hops						
BYI 02960 SL 200	NOEC	≥ 170	0.160	≥ 1.1E+3	5	No
DFA	NOEC	≥ 958	0.009	≥ 11E+4	5	
6-CNA	NOEC	≥ 100	0.007	≥ 14E+3	5	
Lettuce biennial						
BYI 02960 SL 200	NOEC	≥ 170	0.141	≥ 1.2E+3	5	No
DFA	NOEC	≥ 958	0.014	≥ 68E+3	5	
6-CNA	NOEC	≥ 100	0.012	≥ 8.3E+3	5	
Lettuce annual						
BYI 02960 SL 200	NOEC	≥ 170	0.063	≥ 2.7E+3	5	No
DFA	NOEC	≥ 958	0.006	≥ 16E+4	5	
6-CNA	NOEC	≥ 100	0.005	≥ 20E+3	5	

The TER values in Table B.9.6.4.1-08 and -09 are above the Annex VI 91/414 EEC trigger of 5. Hence the chronic risk to soil macro-organisms other than earthworms is considered to be acceptable.

B.9.7.3 Effects on organic matter breakdown - Litterbag studies

STUDY IIIA, 10.6.2/01 (8.16.2/01)

Characteristics

reference	:	Leicher, T. (2011)	species	:	Litterbag study in loamy silt soil, 1.9% OM
type of study	:	Litterbag study	exposure duration	:	
year of execution	:	2010-2011	nom. conc.	:	450 g a.s./ha (total application consisting of first and second application of nominal 150 and 300 g as/ha)
GLP statement	:	Yes	measured conc.		237 µg as/kg
guideline	:	OECD 56 (2006)	dosing method	:	plateau concentration incorporated followed by annual concentration oversprayed
test substance	:	BYI 02960 SL 200 G, Batch no: 2010-001067, density 1.175 g/mL	acceptability	:	Acceptable
a.s. content	:	201 g/L analysed, 17.1% w/w	NOEC	:	237 µg as/kg d.w. (soil with 1.9% organic matter)

Description

Litterbag study was performed at the Bayer Experimental farm Höfchen, Burscheid, Germany.

Site characteristics

Silty loam, pH 6.47, 1.09% organic carbon (equivalent to 1.9 o.m.), microbial C 477 mg/kg dry soil. Cation exchange capacity 14.5 meq/100 g. Since 2007 the following plant protection products were applied: Basagran DP (bentazone, herbicide), Stomp SC (pendimethalin, herbicide) and Tristar (acetamiprid, insecticide)

Application, concentrations, replicates

Study was conducted in twelve plots, six treatment plots and six untreated plots with a size of 9 m x 9 m = 81 m^2 each.

On June 14, 2010, the test item was applied at a rate of 150 g as/ha (corresponding to 750 mL test item/ha) in 300 L water/ha, and incorporated into the upper 10 cm soil layer. Assuming a soil bulk density of 1500 L/kg, this procedure leads to a corresponding concentration of 0.1 mg as/kg soil in a depth of 0-10 cm, which represents the calculated plateau concentration . Details about calculation of the plateau concentration are not presented in the litterbag report, plateau concentration was provided by the sponsor. On June 16, untreated summer wheat seed sort ‘Chamsin’ was sown at a seeding rate of 230 kg seed/ha. Then, litterbags were buried after sowing. Litterbags (12x22 cm, mesh size 8mm) were filled with 4 g dry wheat straw. In the centre of each plot, 40 litterbags were buried horizontally at a depth of 5 cm with a distance of about 15 cm between each other in each direction. On the same day the second application was performed, at the annual rate of 300 g as/ha (corresponding to 1490 mL test item/ha) in 300 L water/ha.

Sampling of spray solutions.

Samples of spray solutions were taken before application of the plateau concentration. Spray solution samples were not analysed, however, because no need was present, given the clear results of the soil samples.

Soil sampling and analysis.

On July 14, 2007, directly after incorporation of the first application and on June 21, 2010, (5 days after burying of the litterbags and application of annual rate), samples were taken from the soil (each sample consisted of two soil cores per plot). Soil samples of 10 cm depth were randomly taken outside the litterbag area and avoiding a zone of about 1 m from the edge of each plot. Samples were stored frozen until analysis. Soil samples were analysed according to Analytical method 01074/M001. This method is suitable for determination of the active substance (BYI 02960) and the metabolites 6-chloronicotinic acid and difluoroacetic acid using HPLC-MS/MS. Only the active substance will be analysed. LOQ of the method is 5.0 µg/kg for the active substance. Soil samples were kept in the freezer until analysis in March-May 2011.

Removal and processing of litterbags

On three occasions (29, 92 and 217 days after burying (July 15, September 16 and January 19), eight litterbags per plot and sampling time were selected in a randomised procedure. Of the 40 litterbags per plot, eight litterbags were used per sampling time. Litterbags were air-dried to interrupt microbial degradation. Contents were ground and sieved in order to remove any soil for the degraded straw. The remaining mixture of straw and residual mineral soil was dried at a temperature of 35º C for a minimum of 2 hours, before the loss by ignition was determined (30 minutes at 600 ºC).

Calculations and statistical evaluation

Weight difference of the straw between the start of the experiment and the actual sampling time was calculated and used to calculate the degree of degradation. Because separation of litter and soil organic matter is not complete, in accordance with the guidance a correction was applied to correct for possible contribution of soil organic matter to the ash-free dry weight.

Cochran's test was conducted to test for homogeneity of variances and Kolmogorov-Smirnov Test was conducted to test for normal distribution. A Student-t test (two sided, α = 0.05) was performed to determine statistically significant differences between control and treatment. If conditions for homogeneity and/or and normality were not met, data transformation was applied and otherwise the non-parametric Mann-Whitney Pairwise U-test was chosen.

Results

Weather conditions

As no precipitation occurred at June 16 and 17, after the application of the annual rate, plots were irrigated with 6 mm water on June 18. Sum of precipitation at June 20 of 9.5 mm was considered enough for exposure of the soil organisms to the product. Monthly precipitation during test period was 12.2, 88.0, 203.0, 109.0, 60.6, 129.0, 67.5 and 113.9 mm in the months June to January, respectively.

Measured soil concentrations.

Mean concentrations of flupyradifurone at June 14 (directly after application and incorporation of the plateau concentration) were 89 µg as/kg dry soil in the treated plots, which is 89% of the nominal amount of 100 µg as/kg dry soil (= calculated plateau concentration) (see Table 9.9.1-1 .). At June 21 (5 days after application of the annual rate), mean measured concentration in the treated plots was 237 µg as/kg dry soil , which is 79% of the theoretical amount 5 days after application of the annual

rate and including the plateau rate. These data were corrected for analytical recovery rate of 104%. All calculations assume a soil depth of 10 cm and a soil density of 1.5 g/mL. Concentrations in the samples of the control plots were all <LOQ.

Table 9.9.1-1 . Nominal and analytically verified amount of flupyradifurone, means from 6 plots (copied from report Table 4)

	Nominal application rate	
	[µg a.s./kg dry soil]	[g a.s./ha]
Plateau concentration	100	150
Cumulative annual application	200	300
∑	300	450
	Analysed concentrations	
	[µg a.s./kg dry soil]	% of nominal amount
Plateau concentration [soil samples taken after incorporation]	89	89
Plateau concentration + Cumulative annual application [soil samples taken six days after application]	237	79

Degradation of straw

The degree of degradation was determined by calculating the weight difference of the straw between the start of the experiment and the actual sampling time.

Table 9.9.1-2 .Ddegradation of 4 g straw (means of 6 plots. Table copied from report summary.

Means of 4 plots	Control	BYI 02960 SL 200 G	% of Control[1)]	% Effect[2)]
0 – 29 d *			92	8
g straw degraded	0.93	0.86		
% straw degraded	23.37	21.56		
0 – 92 d *			118	- 18
g straw degraded	2.12	2.51 **		
% straw degraded	52.95	62.73		
0 – 217 d *			97	3
g straw degraded	3.06	2.96		
% straw degraded	76.38	74.06		

* day 0 was set on June 16, 2010, when the litterbags had been buried
** statistically significant difference to the control (student-t test, α = 0.05, two sided)
1) corresponding to degraded straw in g, formula: (mass loss treatment*100)/mass loss control
2) corresponding to degraded straw in g, formula: ((mass loss control-mass loss treatment)/mass loss control)*100

Coefficients of variation for average straw degradation in the control were 20.72% for 0-29 d; 8.25% for 0-92 d; 7.24% for 0-217 d. Coefficients of variation for average straw degradation in the treatment were 16.37% for 0-29 d; 8.25% for 0-92 d; 3.73% for 0-217 d.

The authors of the study report conclude that the data in Table 9.9.1-2 show that soil residues of flupyradifurone SL 200 G anticipated after long-term use (plateau concentration plus an annual application rate) have no influence on organic matter breakdown after 1, 3 and 7 months.

Remarks

The test meets the validity criteria. Degradation in the control is >60%.

Analysis of the soil samples was performed 8-11 months after sampling. As samples were kept in the freezer this will not have affected the outcome. Measured concentrations were in the range of nominal concentrations.

The residue concentration in soil was measured over 10 cm. This is relevant for the plateau part of the concentration, which was incorporated over 10 cm, but may be worst case for the spray part of the concentration. Usually, for spray applications it is assumed that the initial residues are distributed over a 5 cm layer, therefore it the approach used may be worst case (expressed over 5 cm the endpoint could be doubled). It is however unknown whether the residues in the 10 cm soil cores were evenly distributed or mainly present in the top 5 cm layer, and for the incorporated part of the concentration, 10 cm is relevant. Therefore, the risk assessment should be based on the endpoint mentioned under study conclusion.

Weather conditions were relatively dry in June. This might have influenced soil litter degradation in the first period. However at least 60% soil litter degradation was found at the end of the study in the control plots. Coefficients of variation meet the recommended validity criterion of ≤ 40% for straw degradation in the control plots for data within the first 6 months.
It should be noted that at the second sampling moment a statistically significant difference (18% higher degradation in treatment) was found between control and treatment. This is not considered to be an adverse effect of the treatment. It can be concluded that at a measured initial concentration of 237 µg as/kg dry soil no statistically significant effects were found on soil litter degradation 217 d after treatment. A NOEC ≥ 237 µg as/kg dry soil can be used for risk assessment.

STUDY IIIA, 10.6.2/02 (8.16.2/02)

Characteristics

reference	:	Leicher, T. (2011)	species	:	Litterbag study in loamy silt soil, 1.9% OM
type of study	:	Litterbag study	exposure duration	:	
year of execution	:	2010-2011	nom. conc.	:	415 g a.s./ha (total application consisting of first and second application of nominal 150 and 265 g as/ha)
GLP statement	:	Yes	measured conc.		243 µg as/kg
guideline	:	OECD 56 (2006)	dosing method	:	plateau concentration incorporated followed by sowing of treated seed
test substance	:	spray treatment: BYI 02960 SL 200 G, Batch no: 2010-001067, density 1.175 g/mL seed treatment: BYI 02960 FS 480 G, Batch no 2010-001101, density 1.191 g/mL	acceptability	:	Acceptable
a.s. content	:	spray treatment: 201 g/L analysed, 17.1% w/w seed treatment: 481.4 g/L analysed, 40.4% w/w	NOEC	:	243 µg as/kg d.w. (soil with 1.9% organic matter)

Description

Litterbag study was performed at the Bayer Experimental farm Höfchen, Burscheid, Germany.

Site characteristics

Silty loam, pH 6.47, 1.09% organic carbon (equivalent to 1.9 o.m.), microbial C 477 mg/kg dry soil. Cation exchange capacity 14.5 meq/100 g. Since 2007 the following plant protection products were applied: Basagran DP (bentazon, herbicide), Stomp SC (pendimethalin, herbicide) and Tristar (acetamiprid, insecticide)

Application, concentrations, replicates

Study was conducted in twelve plots, six treatment plots and six untreated plots with a size of 9 m x 9 m = 81 m^2 each. On June 14, 2010, the SL 200 G product was applied on June 14, 2010, at a rate of 150 g as/ha (corresponding to 750 mL test item/ha) in 300 L water/ha and the test item was incorporated into the upper 10 cm soil layer. Assuming a soil bulk density of 1500 L/kg, this procedure leads to a corresponding concentration of 0.1 mg as/kg soil in a depth of 0-10 cm which represents the calculated plateau concentration . Details about calculation of the plateau concentration are not

presented in the litterbag report, plateau concentration was provided by the sponsor. A second application was performed two days later at June 16, 2010, in form of treated summer wheat seed sort 'Chamsin' treated with the FS 480 G product at a rate of 115.37 g as/100 kg seed. The seeding rate of 230 kg seed/ha led to 265 g as/ha. Untreated seed was sown in the control plots. Litterbags (12x22 cm, mesh size 8mm) were filled with 4 g dry wheat straw. In the centre of each plot, 40 litterbags were buried horizontally at a depth of 5 cm with a distance of about 15 cm between each other in each direction. Litterbags were buried on June 16, 2010 after sowing.

Sampling of spray solutions.

Samples of spray solutions were taken before application of the plateau concentration. Samples were not analysed, however, because no need was present, given the clear results of the soil samples.

Soil sampling and analysis.

On July 14, 2007, directly after incorporation of the first application and on June 21, 2010, (5 days after application of annual rate and burying of the litterbags), samples were taken from the soil (each sample consisted of two soil cores per plot). Soil samples of 10 cm depth were randomly taken outside the litterbag area and avoiding a zone of about 1 m from the edge of each plot. Samples were stored frozen until analysis. Soil samples were analysed according to Analytical method 01074/M001 for determination of the active substance. This method is suitable for determination of the active substance (BYI 02960) and the metabolites 6-chloronicotinic acid and difluoroacetic acid using HPLC-MS/MS. Only the active substance will be analysed. LOQ of the method is 5.0 µg/kg for the active substance. Soil samples were kept in the freezer until analysis in March-May 2011.

Removal and processing of litterbags

On three occasions (29, 92 and 217 days after burying (July 15, September 16 and January 19), eight litterbags per plot and sampling time were selected in a randomised procedure. Of the 40 litterbags per plot, eight litterbags were used per sampling time. Litterbags were air-dried to interrupt microbial degradation. Contents were ground and sieved in order to remove any soil for the degraded straw. The remaining mixture of straw and residual mineral soil was dried at a temperature of 35º C for a minimum of 2 hours, before the loss by ignition was determined (30 minutes at 600 ºC).

Calculations and statistical evaluation

Weight difference of the straw between the start of the experiment and the actual sampling time was calculated and used to calculate the degree of degradation. Because separation of litter and soil organic matter is not complete, in accordance with the guidance a correction was applied to correct for possible contribution of soil organic matter to the ash-free dry weight.

Cochran's test was conducted to test for homogeneity of variances and Kolmogorov-Smirnov Test was conducted to test for normal distribution. A Student-t test (two sided, α = 0.05) was performed to determine statistically significant differences between control and treatment. If conditions for homogeneity and/or and normality were not met, data transformation was applied and otherwise the non-parametric Mann-Whitney Pairwise U-test was chosen.

Results

Weather conditions

As no precipitation occurred at June 16 and 17, after the application of the annual rate, plots were irrigated with 6 mm water on June 18. Sum of precipiation at June 20 of 9.5 mm was considered enough for exposure of the soil organisms to the product. Monthly precipitation during test period was 12.2, 88.0, 203.0, 109.0, 60.6, 129.0, 67.5 and 113.9 mm in the months June to January, respectively.

Measured soil concentrations.

Mean measured concentration of flupyradifurone at June 14 (directly after application and incorporation of the plateau concentration) was 94 µg as/kg dry soil in the treated plots, which is 94% of the nominal amount of 100 µg as/kg dry soil (= calculated plateau concentration) (see Table 9.9.1-3). At June 21 (5 days after application of the annual rate) mean measured concentration in the treated plots was 243 µg as/kg dry soil , which is 88% of the theoretical amount 5 days after application of the annual rate and including the plateau rate. These data were corrected for analytical recovery rate of 104%. All calculations assume a soil depth of 10 cm and a soil density of 1.5 g/mL. Concentrations in the samples of the control plots were all <LOQ.

Table 9.9.1-3 . Nominal Degradation of 4 g straw (means of 6 plots) Nominal and analytically verified amount of flupyradifurone, means from 6 plots (copied from report).

	Nominal application rate	
	[µg a.s./kg dry soil]	[g a.s./ha]
Plateau concentration	100	150
Cumulative annual application	177	265
∑	277	415
	Analysed concentrations	
	[µg a.s./kg dry soil]	% of nominal amount
Plateau concentration [soil samples taken after incorporation]	94	94
Plateau concentration + Cumulative annual application [soil samples taken six days after application]	243	88

The degree of degradation was determined by calculating the weight difference of the straw between the start of the experiment and the actual sampling time.

Table 9.9.1-4. Degradation of 4 g straw (means of 6 plots), Table copied from summary of report.

Degradation of 4 g straw:

Means of 4 plots	Control	BYI 02960	% of Control[1)]	% Effect[2)]
0 – 29 d *			100	0
g straw degraded	0.93	0.93		
% straw degraded	23.37	23.37		
0 – 92 d *			99	1
g straw degraded	2.12	2.09		
% straw degraded	52.95	52.35		
0 – 217 d *			105	-5
g straw degraded	3.06	3.20		
% straw degraded	76.38	80.08		

* day 0 was set on June 16, 2010, when the litterbags had been buried
1) corresponding to degraded straw in g, formula: (mass loss treatment*100)/mass loss control
2) corresponding to degraded straw in g, formula: ((mass loss control-mass loss treatment)/mass loss control)*100

Coefficients of variation for average straw degradation in the control was 20.72% for 0-29 d; 8.25% for 0-92 d; 7.24% for 0-217 d. Coefficients of variation for average straw degradation in the treatment 31.42% for 0-29 d; 4.49% for 0-92 d; 6.81% for 0-217 d.
The authors of the study report conclude that the data in Table 9,9,1-3 show that soil residues of BYI 02960 SL 200 G (flupyradifurone) anticipated after long-term use (plateau concentration) plus treated seed application with BYI 02960 FS 480 G (an annual application rate) have no influence on organic matter breakdown after 1, 3 and 7 months.

Remarks

The test meets the validity criteria. Degradation in the control is >60%. Apparently, the same control was used for this study and the previous one (IIIA 10.6.2/01). Analysis of the soil samples was performed 8-11 months after sampling. As samples were kept in the freezer this will not have affected the outcome. Measured concentrations were in the range of nominal concentrations.
The residue concentration in soil was measured over 10 cm. This is relevant for the plateau part of the concentration, which was incorporated over 10 cm, but may be worst case for the spray part of the concentration. Usually, for spray applications it is assumed that the initial residues are distributed over a 5 cm layer, therefore it the approach used may be worst case (expressed over 5 cm the endpoint could be doubled). It is however unknown whether the residues in the 10 cm soil cores were evenly distributed or mainly present in the top 5 cm layer, and for the incorporated part of the concentration, 10 cm is relevant. Therefore, the risk assessment should be based on the endpoint mentioned under study conclusion.
Weather conditions were relatively dry in June. This might have influenced soil litter degradation in the first period. However at least 60% soil litter degradation was found at the end of the study in the

control plots. Coefficients of variation meet the recommended validity criterion of ≤ 40% for straw degradation in the control plots for data within the first 6 months At a measured initial concentration of 243 µg as/kg dry soil no statistically significant effects were found on litter degradation. A NOEC ≥ 243 µg/kg dry soil can be used for risk assessment.

Summary and risk assessment

Two litterbag studies, one similating spray treatment and one seed treatment, both performed in Germany, show no adverse effects on organic matter breakdown after exposure to ca. 0.24 mg BYI 02960/kg soil dw. These tested concentrations are higher than the calculated maximum concentrations of at most 0.160 mg/kg a.s. (measured over 10 cm) for the proposed uses in hops and lettuce ($PEC_{initial}$ expressed over 5 cm + $PEC_{accumulation}$ expressed over 5 (hops) or 20 (lettuce) cm). Therefore the risk to organic matter breakdown is considered to be low.

B.9.8 Effects on soil microbial activity (IIA 8.10, IIIA 10.7)

B.9.8.1 Nitrogen transformation (IIA 8.10.1) and Carbon mineralization (IIA 8.10.2)

STUDY IIA, 8.10.1/01

Characteristics

reference	:	Frommholz U. (2009a)	species	:	Soil microflora
type of study	:	Inhibition of nitrification in soil	exposure duration	:	28 days
year of execution	:	2009	nominal conc.	:	0.4 and 4.0 mg test item/kg soil d.w.
GLP statement	:	Yes	dosing method	:	Soil was treated with test substance mixed with quartz sand
guideline	:	OECD 216 (2000)	acceptability	:	Acceptable
test substance	:	BYI 02960 a.s., batch BYI02960-01-03 (original batch 2009-000239)	NOEC	:	3.8 mg a.s./kg soil d.w.
a.s. content	:	96.2%			

Methods

The effect of BYI 02960 technical on nitrification in a loamy sand soil was determined under laboratory conditions.

The test soil (properties see Table below, sieved to 2 mm) was taken from an agricultural field that was untreated with agrochemicals and fertilisers for the previous 9 years. Some of the soil properties were not determined on the batch of test soil but on another batch of soil collected within one year from the same plot where the test soil was taken (see footnote (A) under the Table below). This is considered to be acceptable, since texture, CEC and organic carbon content are not considered to change significantly within one year on a plot that is left undisturbed. The soil was used in the test within 1 month from sampling in the field and stored at 4°C prior to use in the test. A batch of soil of 900 g d.w. was treated with the test substance mixed with quartz sand. Lucerne meal was added to a final concentration of 0.5%. Treatment rates were 0.4 and 4.0 mg test item/kg soil d.w.. After mixing, aliquots (300 g d.w.) were dispensed into 500 mL brown glass bottles closed with perforated plastic lids (3 replicate flasks per concentration and for the control). Control soil was treated with quartz sand only. Treated soils were incubated under aerobic conditions at 18-22°C in the dark. Soil moisture was maintained at 40-45% MWHC. On day 0, and after 7, 14 and 28 days, aliquots of 10 g d.w. were removed from each replicate and extracted with 1M KCl. Nitrate, nitrite and ammonia were measured photometrically using an autoanalyser. The soil pH was measured at the start and end of the test and ranged between 6.3 and 6.4.

Table B.9.8.1-01 Properties of the study soil

Parameter	Value
Texture	loamy sand (DIN)[(A)]
% particles >0.063/0.002-0.063/<0.002 mm	10.4/17.4/72.2[(A)]
Maximum water holding capacity (% w/w)	42.65
pH (KCl)	6.3
% organic carbon	1.57[(A)]
Microbial biomass (mg/kg soil)	902 (5.7% of organic carbon)
CEC (meq/100 g soil d.w.)	6.63[(A)]

(A) This property was determined annually on soil from the same plot where the test soil was taken. The value or data shown do not pertain to the batch of test soil but to another batch of soil collected from the same plot where the test soil was taken.

Results

Nitrite was not detected in any sample. Ammonium-N was in the range 11-14 mg/kg on day 0 and 7 and then decreased to 3 and 2 mg/kg after 14 and 28 days, respectively. Nitrate levels and nitrate formation rates are summarised in the Table below. The variation (CV) between nitrate levels of control replicates was ≤10% at any sampling, satisfying the validity criterion of OECD 216 (≤15%). After 28 days, nitrate formation rates differed from untreated soil by 1-2%. The NOEC is 4.0 mg test item/kg soil d.w., equivalent to 3.8 mg a.s./kg soil d.w..

Table B.9.8.1-02 Effect of BYI 02960 technical on nitrification

	nitrate (mg NO_3-N/kg soil d.w.)		
day	control	0.4 mg test item/kg soil d.w.	4.0 mg test item/kg soil d.w.
0	4.69	4.78	4.52
7	7.85	9.16	8.44
14	32.46	33.69	36.49
28	56.91	57.67	61.19
	nitrate formation during interval (mg NO_3-N/kg soil d.w./time interval)		
0-7	3.16	4.38	3.92
7-14	24.62	24.54	28.05
14-28	24.45	23.97	24.71
	% deviation (nitrate formation rate) from control:		
0-7	-	39	24
7-14	-	0	14*
14-28	-	2	1

* statistically significantly different from control at 5% level.

Conclusion

NOEC 3.8 mg a.s./kg soil d.w..

Guidelines and limitations

The study was conducted according to OECD 216 (2000) and is acceptable. The soil was not adapted to the test conditions prior to treatment, but the adequate increase of nitrate levels in the control over a 28-day period demonstrated that the soil was viable during the test.

STUDY IIA, 8.10.1/02

Characteristics

reference	:	Frommholz U. (2011a)	species	:	Soil microflora
type of study	:	Inhibition of nitrification in soil	exposure duration	:	28 days
year of execution	:	2011	nominal conc.	:	1.33 mg/kg soil d.w.
GLP statement	:	Yes	dosing method	:	Soil was treated with test substance mixed with quartz sand
guideline	:	OECD 216 (2000)	acceptability	:	Acceptable
test substance	:	6-chloronicotinic acid (6-CNA = IC-0); origin batch no. M12653	NOEC	:	1.33 mg/kg soil d.w.
a.s. content	:	98.8%			

Methods

The effect of 6-chloronicotinic acid (6-CNA = IC-0, metabolite of BYI 02960) on nitrification in a silty sand soil was determined under laboratory conditions.

The test soil (properties see Table below, sieved to 2 mm) was taken from an agricultural field that was untreated with agrochemicals and fertilisers for the previous 5 years. The soil was used in the test within about 2 months from sampling in the field and stored at 4°C prior to use in the test. A batch of soil of 900 g d.w. was treated with the test substance mixed with quartz sand. Lucerne meal was added to a final concentration of 0.5%. The treatment rate was 1.33 mg/kg soil d.w.. After mixing, aliquots (300 g d.w.) were dispensed into 500 mL brown glass bottles closed with perforated plastic lids (3 replicate flasks per concentration and for the control). Control soil was treated with quartz sand only. Treated soils were incubated under aerobic conditions at 18-22°C in the dark. Soil moisture was maintained at 42-45% MWHC. On day 0, and after 7, 14 and 28 days, aliquots of 10 g d.w. were removed from each replicate and extracted with 1M KCl. Nitrate, nitrite and ammonia were measured photometrically using an autoanalyser. The soil pH was measured at the start and end of the test and was 7.1 at all samplings.

Table B.9.8.1-03 Properties of the study soil

Parameter	Value
Texture	Silty sand (DIN)
% particles >0.063/0.002-0.063/<0.002 mm	8.0/29.7/62.3
Maximum water holding capacity (% w/w)	39.37
pH (KCl)	7.08
% organic carbon	0.83
Microbial biomass (mg/kg soil)	231 (2.78% of organic carbon)
CEC (meq/100 g soil d.w.)	12.4

Results

Nitrite was not detected in any sample. Ammonium-N was 4, 2, 0.8-1.0 and 0.3-0.4 mg/kg on day 0, 7, 14 and 28, respectively. Nitrate levels and nitrate formation rates are summarised in the Table below. The variation (CV) between nitrate levels of control replicates was ≤7% at any sampling, except on day 7, when it was 32%. The value of 32% does not comply with the validity criterion of OECD 216 (≤15%), but this high value on a single sampling is associated with low nitrate levels on that day (denitrification), and does not invalidate the study. After 28 days, nitrate formation rates differed from untreated soil by 1%. The NOEC is 1.33 mg/kg soil d.w..

Table B.9.8.1-04 Effect of 6-chloronicotinic acid (6-CNA = IC-0, metabolite of BYI 02960) on nitrification

	nitrate (mg NO_3-N/kg soil d.w.)	
day	control	1.33 mg/kg soil d.w.
0	14.74	14.62
7	2.47	1.49
14	16.68	14.85

28	29.02	27.29
	nitrate formation during interval (mg NO_3-N/kg soil d.w./time interval)	
0-7 7-14 14-28	-12.27 14.21 12.34	-13.13 13.36 12.44
	% deviation (nitrate formation rate) from control:	
0-7 7-14 14-28	- - -	7 6 1

It was reported that in the most recent test with the reference item sodium chloride, 16 g sodium chloride/kg soil d.w. had a distinct and long-term (>28 days) influence on microbial mineralization of nitrogen.

Conclusion

NOEC 1.33 mg/kg soil d.w..

Guidelines and limitations

The study was conducted according to OECD 216 (2000) and is acceptable. The soil was not adapted to the test conditions prior to treatment, but the adequate increase of nitrate levels in the control over a 28-day period demonstrated that the soil was viable during the test.

STUDY IIA, 8.10.2/03

Characteristics

reference	:	Schulz L. (2011)	species	:	Soil microflora
type of study	:	Inhibition of respiration in soil	exposure duration	:	28 days
year of execution	:	2011	nominal conc.	:	0.4 and 4.0 mg test item/kg soil d.w.
GLP statement	:	Yes	dosing method	:	Soil was treated with test substance mixed with quartz sand
guideline	:	OECD 217 (2000)	acceptability	:	Acceptable
test substance	:	BYI 02960 a.s., batch BYI02960-01-03 (original batch 2009-000239)	NOEC	:	3.8 mg a.s./kg soil d.w.
purity	:	96.2%			

Methods

The effect of BYI 02960 technical on respiration in a sandy loam soil was determined under laboratory conditions.

The test soil (properties see Table below, sieved to 2 mm) was taken from an agricultural field that was untreated with agrochemicals for the previous 21 years and with organic and inorganic fertiliser for the previous 8 years. It was used in the test within 6 weeks from sampling in the field and acclimated to the test conditions for 7 days prior to treatment. Aliquots (1000 g d.w.) were treated with a mixture of the test substance and quartz sand, mixed and dispensed into 4 L steel flasks. Treatment rates were 0.4 and 4.0 mg test item/kg soil d.w.. Control soil was treated with quartz sand only. Treated soil (3 replicate flasks per concentration) was incubated under aerobic conditions at 20-22°C

in the dark. Soil moisture was maintained at 45-50% MWHC. On day 0, and after 7, 14 and 28 days, aliquots of 100 g d.w. were removed from each replicate, amended with glucose (4 g/kg soil, which exerted a maximum response in a pretest), and oxygen consumption was measured for 12 hours. Evaluation was based on the cumulative oxygen consumption after 12 hours. The soil pH was measured at the start and end of the test and ranged between 6.2 and 6.3. A positive control (Dinoterb, 6.8, 16 and 27 mg/kg soil d.w.) was tested in a field soil about 6 months earlier, and gave the expected response (effects on respiration up to 48%).

Table B.9.8.1-05 Properties of the study soil

Parameter	Value
Texture	sandy loam (USDA)
% particles >0.05/0.002-0.05/<0.002 mm	53/37/10
Maximum water holding capacity (% w/w)	36.67
pH (water)	6.4
% organic carbon	1.39
Microbial biomass (mg/100 g soil)	33.04 (2.38% of organic carbon)
CEC (meq/100 g soil d.w.)	10.0

Results

The results are summarised in the Table below. The variation (CV) between oxygen consumption levels of control replicates was ≤2.2% at any sampling, satisfying the validity criterion of OECD 217 (≤15%). After 28 days, the cumulative oxygen consumption values differed from untreated soil by 0.1-0.9%. The NOEC is 4.0 mg test item/kg soil d.w., equivalent to 3.8 mg a.s./kg soil d.w..

Table B.9.8.1-06 Effect of BYI 02960 technical on soil respiration

Days after application	Control	0.40 mg test item/kg soil dry weight equivalent to 0.3 kg test item/ha		4.00 mg test item/kg soil dry weight equivalent to 3 kg test item/ha	
	O_2 consumption [mg/kg soil d.w./h]	O_2 consumption [mg/kg soil d.w./h]	Deviation from control [%][1]	O_2 consumption [mg/kg soil d.w./h]	Deviation from control [%][1]
0	12.54	12.51	-0.2	12.26*[2]	-2.2
7	11.27	11.28	+0.1	11.27	±0.0
14	10.61	10.95	+3.2	10.34	-2.5
28	9.64	9.66	+0.1	9.56	-0.9

The calculations were performed with unrounded values.
[1] based on O_2 consumption; - = inhibition; + = stimulation
*[2] statistically significantly different to control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

Conclusion

NOEC 3.8 mg a.s./kg soil d.w..

Guidelines and limitations0

The study was conducted according to OECD 217 (2000) and is acceptable.

STUDY IIIA, 10.7.1/01

Characteristics

reference	:	Frommholz U. (2010b)	species	:	Soil microflora
type of study	:	Inhibition of respiration in soil	exposure duration	:	28 days
year of execution	:	2010	nominal conc.	:	1.66 and 16.59 µL test item/kg soil d.w.
GLP statement	:	Yes	dosing method	:	Soil was treated with test substance mixed with quartz sand
guideline	:	OECD 217 (2000)	acceptability	:	Acceptable
test substance	:	BYI 02960 SL 200 G (200 g/L SL formulation), batch 2010-001067	NOEC	:	16.59 µL test item/kg soil d.w. (3.335 mg a.s./kg soil d.w.)
a.s. content	:	201.0 g/L BYI 02960			

Methods

The effect of BYI 02960 SL 200 G (200 g/L SL formulation, 201.0 g/L BYI 02960) on respiration in a loamy sand soil was determined under laboratory conditions.

The test soil (properties see Table below, sieved to 2 mm) was taken from an agricultural field that was untreated with agrochemicals and fertiliser for the previous 4.5 years. The soil was stored at 4°C for 3 months prior to use in the test. Batches of soil were then treated with a mixture of the test substance and quartz sand and mixed. Treatment rates were 1.66 and 16.59 µL test item/kg soil d.w.. Control soil was treated with quartz sand only. Aliquots of treated soil (350 g d.w.) were dispensed into 500 mL brown flasks (3 replicate flasks per concentration), that were closed with parafilm and incubated at 18-22°C in the dark. Soil moisture was maintained at 42-49% MWHC. On day 0, and after 7, 14 and 28 days, aliquots of 25 g d.w. were removed from each replicate, amended with glucose (2 g/kg soil, which exerted a maximum response in a pretest), and carbon dioxide production was measured for 12 hours. Evaluation was based on the cumulative carbon dioxide production after 12 hours. The soil pH was measured at the start and end of the test and was 7.0 at all times. The most recent test with a positive control (sodium chloride, 16 g/kg soil d.w.), conducted in an agricultural soil, gave the expected response (long-term (>28 days) effects on carbon transformation).

Table B.9.8.1-07 Properties of the study soil

Parameter	Value
Texture	Loamy sand (DIN)
% particles >0.063/0.002-0.063/<0.002 mm	61/28/10
Maximum water holding capacity (% w/w)	37.55
pH (water)	7.27
% organic carbon	1.46
Microbial biomass (mg/100 g soil)	20.3 (1.39% of organic carbon)
CEC (meq/100 g soil d.w.)	12.9

Results

The results are summarised in the Table below. The variation (CV) between oxygen consumption levels of control replicates was ≤4.3% at any sampling, satisfying the validity criterion of OECD 217 (≤15%). After 28 days, the cumulative oxygen consumption values differed from untreated soil by 5-6%. The NOEC is 16.59 µL test item/kg soil d.w., equivalent to 3.335 mg a.s./kg soil d.w..

Table B.9.8.1-08 Effect of BYI 02960 SL 200 G on soil respiration

Days after treatment	Application rates µL BYI 02960 SL 200 G/kg dry wt. soil				
	control	1.66		16.59	
	mg CO_2 /h/kg dry wt soil	mg CO_2 /h/kg dry wt soil	% of control	mg CO_2 /h/kg dry wt soil	% of control
0	147.2±4.0	136.8±0.8	93 *w	140.0±0.9	95 *w
7	197.3±8.5	162.7±1.7	82 *)	159.6±4.5	81 *)
14	163.8±6.3	154.8±3.3	94 n.s.	161.1±4.9	98 n.s.
28	142.5±2.7	133.9±0.5	94 *)	135.4±2.7	95 *)

*) Statistically significant difference to the control (Student t-test, α= 0.05, two-sided)
n.s. No statistically significant difference to the control (t-test, α= 0.05, two-sided)
*w = Statistically significant difference to the control (Welch-t Test for non-homogeneous variances, two-sided, α = 0.05).

Conclusion

NOEC 16.59 µL test item/kg soil d.w. equivalent to 3.335 mg a.s./kg soil d.w..

Guidelines and limitations

The study was conducted according to OECD 217 (2000) and is acceptable. Test flasks were closed with parafilm during incubation, which may hamper air exchange. However, respiration rates of the control on day 7-28 were not remarkably different from those on day 0, hence incubation conditions should have been adequate.

STUDY IIIA, 10.7.1/02

Characteristics

reference	:	Frommholz U. (2010c)	species	:	Soil microflora
type of study	:	Inhibition of nitrification in soil	exposure duration	:	28 days
year of execution	:	2010	nominal conc.	:	1.66 and 16.59 µL test item/kg soil d.w.
GLP statement	:	Yes	dosing method	:	Soil was treated with test substance mixed with quartz sand
guideline	:	OECD 216 (2000)	acceptability	:	Acceptable
test substance	:	BYI 02960 SL 200 G (200 g/L SL formulation), batch 2010-001067	NOEC	:	16.59 µL test item/kg soil d.w. (3.335 mg a.s./kg soil d.w.)
a.s. content	:	201.0 g/L BYI 02960			

Methods

The effect of BYI 02960 SL 200 G (200 g/L SL formulation, 201.0 g/L BYI 02960) on nitrification in a loamy sand soil was determined under laboratory conditions.

The test soil (properties see Table below, sieved to 2 mm) was taken from an agricultural field that was untreated with agrochemicals and fertiliser for the previous 4.5 years. The soil was stored at 4°C for 3 months prior to use in the test. A batch of soil of 900 g d.w. was treated with the test substance mixed with quartz sand. Lucerne meal was added to a final concentration of 0.5%. Treatment rates

were 1.66 and 16.59 µL test item/kg soil d.w.. Control soil was treated with quartz sand only. After mixing, aliquots (300 g d.w.) were dispensed into 500 mL brown glass bottles closed with perforated plastic lids (3 replicate flasks per concentration and for the control). Treated soils were incubated under aerobic conditions at 18-22°C in the dark. Soil moisture was maintained at 42-50% MWHC. On day 0, and after 7, 14 and 28 days, aliquots of 10 g d.w. were removed from each replicate and extracted with 1M KCl. Nitrate, nitrite and ammonia were measured photometrically using an autoanalyser. The soil pH was measured at the start and end of the test and ranged between 7.0 and 7.3.

Table B.9.8.1-09 Properties of the study soil

Parameter	Value
Texture	Loamy sand (DIN)
% particles >0.063/0.002-0.063/<0.002 mm	61/28/10
Maximum water holding capacity (% w/w)	37.55
pH (water)	7.27
% organic carbon	1.46
Microbial biomass (mg/100 g soil)	20.3 (1.39% of organic carbon)
CEC (meq/100 g soil d.w.)	12.9

Results

Nitrite was not detected in any sample. Ammonium-N was in the range 4.5-5.1 mg/kg on day 0 and decreased to 0.64-0.77 mg/kg after 28 days. Nitrate levels in control soil were 12, 4.1, 14 and 27 mg/kg on day 0, 7, 14 and 28, respectively. The variation (CV) between nitrate levels of control replicates was ≤8% at any sampling, satisfying the validity criterion of OECD 216 (≤15%). Nitrate formation rates are summarised in the Table below. After 28 days, nitrate formation rates differed from untreated soil by 5-11%. The NOEC is 16.59 µL test item/kg soil d.w., equivalent to 3.335 mg a.s./kg soil d.w..

Table B.9.8.1-10 Effect of BYI 02960 SL 200 G on nitrification

Time Interval (days)	Application rates											
	BYI 02960 SL 200 G											
	control			1.66 µL/kg dry weight soil				16.59 µL/kg dry weight soil				
	Nitrate-N[1)]			Nitrate-N[1)]			% difference to control	Nitrate-N[1)]			% difference to control
0-7	-1.13	±	0.04	-1.25	±	0.03	**11** n.s.	-1.01	±	0.09	**10** n.s.
7-14	1.35	±	0.05	1.28	±	0.26	**5** n.s.w	1.50	±	0.08	**11** n.s.w
14-28	0.99	±	0.09	1.04	±	0.10	**5** n.s.	1.09	±	0.04	**11** n.s.

1) Rate: Nitrate-N in mg/kg dry weight soil/time interval/day, mean of 3 replicates and standard deviation

n.s. = No statistically significant difference to the control (Student-t Test, two-sided, α = 0.05).

n.s.w = No statistically significant difference to the control (Welch-t Test for non-homogeneous variances, two-sided, α = 0.05).

Conclusion

NOEC 16.59 µL test item/kg soil d.w. equivalent to 3.335 mg a.s./kg soil d.w..

Guidelines and limitations

The study was conducted according to OECD 216 (2000) and is acceptable. The soil was not adapted to the test conditions prior to treatment, but the adequate increase of nitrate levels in the control over a 28-day period demonstrated that the soil was viable during the test.

B.9.8.2 Risk assessment

B.9.8.2.1 Risk assessment of the active substance and formulation

Laboratory studies on microbial turnover are available for the active substance BYI 02960, the formulation BYI 02960 SL 200 G, and the metabolite 6-CNA (N-turnover only).
The results indicate that BYI 02960 and BYI 02960 SL 200 G have no adverse impact on microbial C- and N-turnover in soil. NOECs were clearly above the expected exposure in soil.
Overall, it can be concluded that the functioning of soil micro-organisms is not at risk if BYI 02960 SL 200 G is applied according to the recommended use pattern.

The toxicity of BYI 02960 SL 200 G on soil non-target micro-organisms is summarised in the table below.

Table B.9.8.2-01 Effects on soil non-target micro-organisms

Test species	Test design	Ecotoxicological endpoint		Reference
BYI 02960 SL 200 G				
C-cycle	28 d	no influence	16.59 µL prod/kg dws (≡3.335 mg a.s./kg dws)	Frommholz (2010) KIIIA1 10.7.1/01
N-cycle	28 d	no influence	16.59 µL prod/kg dws (≡3.335 mg a.s./kg dws)	Frommholz (2010) KIIIA1 10.7.1/02
BYI 02960				
N-cycle	28 d	no influence	3.8 mg a.i./kg dws	Frommholz (2009) KIIA 8.10.1/01
C-cycle	28 d	no influence	3.8 mg a.i./kg dws	Schulz (2011) KIIA 8.10.2/01
6-CNA				
N-cycle	28 d	no influence	1.33 mg p.m./kg dws	Frommholz (2011) KIIA 8.10.1/02

Risk assessment

According to current regulatory requirements the risk is acceptable, if the effect of the recommended application rate of a compound / product on nitrogen or carbon mineralisation is < 25% after 100 days. In the studies, deviations from the control did not exceed 25% after 28 days, at tested concentrations which are higher than the calculated maximum concentrations of at most 0.160 mg/kg a.s. and 0.012 mg 6-CNA for the proposed uses.

The metabolite 6-CNA was shown to be of general low toxicity for soil organisms (earthworm LC50 >1000 mg/kg, NOEC 95 mg/kg; Hypoaspis NOEC 100 mg/kg; Folsomia NOEC 90 mg/kg). The NOEC of the metabolite 6-CNA for microbial N-turnover was determined to be 111- fold higher than the worst case PEC in soil for 6-CNA. The high margin of safety indicates that microbial C-turnover is not at risk due to formation of 6-chloronicotinic acid in soil.

Similar to 6-chloronicotinic acid the metabolite DFA acid was shown to be of general low toxicity for soil organisms (earthworm LC50 >958 mg/kg, NOEC 59 mg/kg). Assuming a 10-fold higher toxicity of DFA in comparison with to 6-CNA with regard to microbial C- and N-turnover, the risk could still be considered as acceptable.

Furthermore, the metabolites are likely to have been formed in the litterbag studies, in which no risk was identified to organic matter breakdown.

Based on the above, no further studies are required. The risk to soil micro-organisms is considered to be low.

B.9.9 Effects on other non-target organisms (flora and fauna) believed to be at risk (IIA 8.12 and IIA 8.14; IIIA 10.8 and IIIA 10.9)

B.9.9.1 Effects on non-target terrestrial plants (IIA 8.12; IIIA 10.8)

STUDY IIA, 8.12/01

Characteristics

reference	:	Gosch H. (2010a)	species	:	Cucumber (*Cucumis sativa*), buckwheat (*Fagopyrum esculentum*), oilseed rape (*Brassica napus*), sugar beet (*Beta vulgaris*), soybean (*Glycine max*), lettuce (*Lactuca sativa*), tomato (*Lycopersicon esculentum*), onion (*Allium cepa*), oat (*Avena sativa*), ryegrass (*Lolium perenne*), corn (*Zea mays*)
type of study	:	Vegetative vigour	exposure duration	:	21 days
year of execution	:	2010	nominal conc	:	410 g a.s./ha
GLP statement	:	Yes	treatment method	:	Test substance was suspended in water and sprayed on plants
guideline	:	OPPTS 850.4150, OECD 227 (2006)	acceptability	:	Acceptable
test substance	:	BYI 02960 SL 200 g/L (200 g/L SL formulation), batch 2009-001253	ER_{50}	:	>410 g a.s./ha
a.s. content	:	17.0% w/w (199.8 g/L) BYI 02960			

Methods

In a greenhouse screening study (limit test), BYI 02960 SL 200 g/L (SL formulation, 17.0% w/w (199.8 g/L) BYI 02960) was applied to 4 monocot and 7 dicot plant species at a dose rate of 410 g a.s./ha, with a negative (water) control. Applications were made in 200 L water/ha post-emergence of target plants (applications to the plant canopy at the 2-4 true leaf stage (except onion, 1-2 leaf stage) using a calibrated laboratory sprayer. The concentration of BYI 02960 was determined by HPLC/UV in the stock solution and found to be 99% of target. Each treatment was tested in 8 replicate pots containing 4 seedlings per pot. The pot soil was a silt loam soil, pH ($CaCl_2$) 7.31, 1.30% organic carbon. Test conditions were: temperature 10-31°C, daily mean relative humidity 40-100%, at least 16-hour light period. Watering was performed when necessary by bottom irrigation of the pots. Liquid NPK fertiliser was added 7 days after application in the saucers. Assessments of phytotoxicity were made weekly by visual observations using a rating scale of 0 to 100% (comparison to controls) where 0 represented no injury and 100% complete damage. Survival, was recorded weekly and plant height and plant dry weight were recorded 21 days after treatment.

Results

The results are summarised in the Table below. The results of the control group satisfied the guideline requirements (OECD 227, 2006) for all tested species: ≥90% plant survival, 0% phytotoxic damage, (seedling emergence rate was not reported). Visual toxicity was at the most rated as slight (20% effect relative to control), and effects observed included chlorosis, necrosis and stunting. In the absence of any effect on any parameter of >50%, for all parameters and test species the ER50 is >410 g a.s./ha. The NOER is <410 g a.s./ha for oilseed rape and buckwheat, based on a statistically significant

reduction of shoot dry weight, although the effects were small (7-12%). For all other species, the NOER is 410 g a.s./ha.

Table B.9.9.1-01 Effect of BYI 02960 SL 200 g/L on vegetative vigour after 21 days

Species	Survival (% inhibition)	Phytotoxicity	Shoot Length (% reduction)	Shoot Dry Weight (% inhibition)
Dicotyledoneae				
Beta vulgaris	0	0	-2.3	6.5
Brassica napus	0	0 - 10%	-1.0	**7.0**
Cucumis sativus	0	10 - 20%	-2.1	4.5
Fagopyrum esculentum	0	10%	3.1	**12.0**
Glycine max	0	0	0.6	-11.1
Lactuca sativa	0	0	-4.0	0.7
Lycopersicon esculentum	0	10 - 20%	3.1	10.9
Monootyledoneae				
Allium cepa	0	0	-2.8	-9.3
Avena sativa	0	0	2.1	-1.9
Lolium perenne	0	0	4.2	-3.0
Zea mays	0	0 - 10%	0.3	4.1

Bold figures for shoot dry weight are statistically significant (Pairwise Mann-Whitney-U-test, one sided smaller; $p \leq 0.05$)

Conclusion

For all parameters and test species, ER50 >410 g a.s./ha.

Guidelines and limitations

The study was performed according to OECD 227 (2006) and is acceptable.

STUDY IIA, 8.12/02

Characteristics

reference	:	Gosch H. (2010b)	species	:	Cucumber (*Cucumis sativa*), buckwheat (*Fagopyrum esculentum*), oilseed rape (*Brassica napus*), sugar beet (*Beta vulgaris*), soybean (*Glycine max*), lettuce (*Lactuca sativa*), tomato (*Lycopersicon esculentum*), onion (*Allium cepa*), oat (*Avena sativa*), ryegrass (*Lolium perenne*), corn (*Zea mays*)
type of study	:	Seedling emergence	exposure duration	:	21 days
year of execution	:	2010	nominal conc	:	410 g a.s./ha
GLP statement	:	Yes	treatment method	:	Test substance was suspended in water and sprayed on soil
guideline	:	OPPTS 850.4100, OECD 208	acceptability	:	Acceptable
test substance	:	BYI 02960 SL 200 g/L (200 g/L SL formulation), batch 2009-001253	ER_{50}	:	>410 g a.s./ha
a.s. content	:	17.0% w/w (199.8 g/L) BYI 02960			

Methods

In a greenhouse screening study (limit test), BYI 02960 SL 200 g/L (SL formulation, 17.0% w/w (199.8 g/L) BYI 02960) was applied to 4 monocot and 7 dicot plant species at a dose rate of 410 g a.s./ha, with a negative (water) control. Applications were made in 200 L water/ha to the soil containing the seeds, using a calibrated laboratory sprayer. The concentration of BYI 02960 was determined by HPLC/UV in the stock solution and found to be 99-100% of target. Each treatment was tested in 8 replicate pots containing 5 seeds per pot. The pot soil was a silt loam soil mixed with 30% sand, pH ($CaCl_2$) 7.24, 0.63% organic carbon. Test conditions were: temperature 10-31°C, daily mean relative humidity 40-100%, at least 16-hour light period. Watering was performed when necessary by bottom irrigation of the pots. Liquid fertiliser was added to the soil prior to sowing (none thereafter). Assessments of phytotoxicity were made weekly by visual observations using a rating scale of 0 to 100% (comparison to controls) where 0 represented no injury and 100% complete damage. Emergence and survival were recorded weekly and plant height and plant dry weight were recorded 21 days after treatment.

Results

The results are summarised in the Table below. The results of the control group satisfied the guideline requirements (OECD 208) for all tested species: ≥70% emergence, ≥90% plant survival, 0% phytotoxic damage. Visual toxicity was at the most rated as moderate (40% effect relative to control), and effects observed included necrosis, leaf deformation and stunting. In the absence of any effect on any parameter of >50%, for all parameters and test species the ER50 is >410 g a.s./ha. The NOER is <410 g a.s./ha for buckwheat, based on a statistically significant reduction of shoot height and shoot dry weight. For all other species, the NOER is 410 g a.s./ha.

Table B.9.9.1-02 Effect of BYI 02960 SL 200 g/L on seedling emergence after 21 days

Plant Species	Emergence (% inhibition)	Survival (% inhibition)	Phytotoxicity	Shoot Length (% reduction)	Shoot Dry Weight (% inhibition)
Dicotyledoneae					
Beta vulgaris	15.4	6.1	0	1.8	-7.4
Brassica napus	2.8	0	0	-1.3	-10.5
Cucumis sativus	-2.9	0	0	-5.3	-8.6
Fagopyrum esculentum	2.6	0	0 - 40%	**13.8**	**19.7**
Glycine max	8.3	0	0 - 30%	7.0	10.7
Lactuca sativa	-2.6	0	0	2.9	1.1
Lycopersicon esculentum	7.9	0	0 - 20%	-2.0	-16.3
Monocotyledoneae					
Allium cepa	-3.3	-3.4	0	3.6	6.5
Avena sativa	7.5	0	0 - 20%	0.1	-1.0
Lolium perenne	-12.5	0	0	4.6	7.4
Zea mays	7.5	0	0 - 20%	4.2	13.1

Bold figures for shoot dry weight are statistically significant (Pairwise Mann-Whitney-U-test, one sided smaller; p ≤ 0.05)

Conclusion

For all parameters and test species, ER50 >410 g a.s./ha.

Guidelines and limitations

The study was performed according to OECD 208 and is acceptable.

B.9.9.2 Effects on other non-target species (flora and fauna) from available preliminary data (IIA 8.14; IIIA 10.9)

STUDY IIA, 8.14.1/01

Characteristics

reference	:	Malsam O. and Voertse A. (2010)	species	:	*Phaedon cochleariae* (mustard beetle); *Myzus persicae* (green peach aphid); *Nilaparvata lugens* (brown plant hopper); *Aphis gossypii* (cotton aphid); *Bemisia tabaci* (silver leaf whitefly).
type of study	:	Insecticidal efficacy	exposure duration	:	6-10 days
year of execution	:	Not reported	nom. dose	:	4-500 g/ha or 0.0064-0.8 mg/L (microtiter plate testing) or 0.032-20 mg/L (whole plant test)
GLP statement	:	No	dosing method	:	Test item was dissolved in water and sprayed on plant (substrate) or poured on soil.
guideline	:	Not applicable	acceptability	:	Acceptable
test substance	:	BYI02960, batch 2009-000239; difluoroacetic acid; batch BCOO5984-1	Conclusion	:	difluoroacetic acid has no effficacy compared to parent BYI02960
a.s. content	:	BYI02960: 96.2%; difluoroacetic acid: 95.8%			

Methods

The insecticidal activity of BYI02960 and its metabolite difluoroacetic acid were compared in leaf disc (microtiter plate) and in whole plant screening systems.

Microtiter plate testing

Microtiter plate testing employed the following species: *Phaedon cochleariae* (mustard beetle), *Myzus persicae* (green peach aphid) and *Nilaparvata lugens* (brown plant hopper). For Myzus and Phaedon, leaf discs were placed in the cavity of microtiter plates and the microtiter plate cavities were then sprayed with BYI02960 or difluoroacetic acid at rate 4, 20, 100 and 200 g/ha. In case of Myzus, the leaf discs were already infected with Myzus instars at the time of treatment. In case of Phaedon, 2 larvae were placed on the leaf discs after spraying. After 7 days (Phaedon) or 6 days (Myzus), survival was determined. For testing of Nilaparvata, small rice plants were grown in perlite in 10-cm high tubes plugged into each cavity of the microtiter plate. Test compound solutions of BYI02960 or difluoroacetic acid were sprayed onto the plants using a spray concentration of 0.0064, 0.032, 0.16 and 0.8 mg/L, and the plants were infested with plant hoppers afterwards.

Greenhouse test

Greenhouse testing employed the following species: *Myzus persicae* (green peach aphid), *Aphis gossypii* (cotton aphid) and *Bemisia tabaci* (silver leaf whitefly). Testing involved either whole plant spraying (all 3 species) or a soil drench (Myzus only). During whole plant spraying, the test plants (see table below) were sprayed with BYI02960 or difluoroacetic acid using a spray concentration of 0.032, 0.16, 0.8, 4 and (*Bemisia tabaci* only) 20 mg/L. The plants were already infested at the time of treatment. During the drench test (performed on Myzus only), a solution of BYI02960 or difluoroacetic acid in water was poured on the soil of a pot in which a cabbage plant was growing and which was already infested at the time of treatment.

Results

The results are summarised in the tables below. Whereas at the highest test dose or test concentration 90-100% efficacy of BYI02960 was recorded in the 5 insect species, no insecticidal efficacy at all was observed for difluoroacetic acid up to the highest test dose or test concentration. The metabolite difluoroacetic acid does not exhibit any insecticidal efficacy compared to parent BYI02960.

Table B.9.9.1-03 Insecticidal efficacy of BYI 02960 and difluoracetic acid in microtiter plate test system.

Microtiter plate test	Test object	MYZUPE	PHAEDON	Test object	NILALU
	Test plant	Chinese cabbage	Chinese cabbage	Test plant	Rice
	Application	Spray	Spray	Application	Spray
	Evaluation	6 d	7 d	Evaluation	7 d
	Conc. (g/ha)	Insecticidal efficacy (%)		Conc. (g/ha)	Efficacy (%)
BYI02960 (BCS-BZ89914)	500	**100**	**100**	0,8	**90**
	100	**100**	**100**	0,16	**50**
	20	**90**	**0**	0,032	**30**
	4	**70**	**0**	0,0064	**0**
Difluoroacetic acid (BCS-AA56716)	500	**0**	**0**	0,8	**0**
	100	**0**	**0**	0,16	**0**
	20	**0**	**0**	0,032	**0**
	4	**0**	**0**	0,0064	**0**

Table B.9.9.1-04 Insecticidal efficacy of BYI 02960 and difluoracetic acid in greenhouse tests on plants.

	Test object	MYZUPE	APHIGO	BEMITA	Test object	MYZUPE
Whole plant spray test	Test plant	Bell pepper	Cotton	Cotton	Test plant	Cabbage
	Application	Spray	Spray	Spray	Application	Drench
	Evaluation	6 d	6 d	8 d	Evaluation	6 d
	Conc. (ppm)	Insecticidal efficacy (%)			Conc. (ppm)	
BYI02960 (BCS-BZ89914)	20			98	20	
	4	100	100	98	4	100
	0,8	75	95	0	0,8	75
	0.16	0	0	0	0.16	0
	0.032	0	0		0.032	0
Difluoroacetic acid (BCS-AA56716)	20			0	20	
	4	0	0	0	4	0
	0,8	0	0	0	0,8	0
	0.16	0	0	0	0.16	0
	0.032	0	0		0.032	0

Conclusion

Difluoroacetic acid does not exhibit any insecticidal efficacy compared to parent BYI02960.

Guidelines and limitations

The study is acceptable.

Study IIA 8.16.1/08

Reference	:	Nauen 2013	Test species	:	*Myzus persicae*
Type of study	:	aphid, oral toxicity (2 tests)	Exposure duration	:	24 h
Year of execution	:	2013	Dosing method	:	oral
GLP statement	:	no	Acceptability	:	Not acceptable; may be used as supplemental information provided that the raw data are submitted.
Guideline	:	no specific guideline available	LC50 flupyradifurone	:	0.036 mg as/kg diet / 0.081 pg as/aphid
Test substance and purity	:	Flupyradifurone , purity > 99 % Imidacloprid, purity > 99 %	LC50 imidacloprid	:	0.17 mg as/kg diet / 0.38 pg as/aphid

Description

The effect of imidacloprid and flupyradifurone on the aphid, *Myzus persicae* Sulzer, was assessed in a 24 h continuous feeding test in the laboratory. The test was carried out with an insecticide susceptible strain of the green peach aphid, *Myzus persicae* Sulzer (Homoptera: Aphididae). The strain was maintained on cabbage at 22°C, 50% relative humidity and a photoperiod of 16h (light):8h (dark).

Oral activity of the insecticides was tested with a feeding bioassay using artificial (Parafilm®) double membranes, so-called sachets, as `synthetic leaves´ (Elbert et al., 1991; Nauen et al., 1996). In the described bioassay, the oral LD50-value of imidacloprid against *Myzus persicae* was determined by feeding on an artificial diet and based on the ingestion of a radiolabelled sugar, N-acetyl-D-[14C] glucosamine (Elbert et al., 1991). The uptake of the radiolabelled sugar was measured by liquid

scintillation counting of tissue-solubilized aphids allowed the calculation of the amount of simultaneously ingested imidacloprid based on its concentration in µg/mL (ppm) diet.

The LD50-value obtained earlier for imidacloprid was 0.54 pg/aphid based on a measured LC50-value of 0.24 ppm. The objectives of the current study were (1) to repeat the recently published oral LC50-value of imidacloprid against *M. persicae*, (2) to determine the oral LC50-value of flupyradifurone against *M. persicae* and (3) to calculate its LD50-value based on the repeated imidacloprid data. Each sachet (diameter 2.8 cm) contained 0.15 mL of an aqueous solution of 15% (w/v) sucrose with different concentrations of insecticide covering a potential mortality range from 0 to 100%. Concentrations used were: 0, 0.00256, 0.0128, 0.064, 0.32, 1.6, 8 and 40 mg as/L. A toxic standard was not included in this test. Bioassays were performed twice using three replicates per concentration with seven concentrations per test. Prior to the bioassays the aphids were transferred to small petri dishes for a 4-5 h starvation period. Subsequently 10 to 15 apterous adults (approx. weight 200 µg per aphid) were transferred to one small petri dish (feeding cage) for each concentration and the sachet was stretched over the petri dish. A piece of yellow cellophane was placed over the sachet to enhance the probing activity of aphids. The number of dead aphids was recorded after 2 h. Percentage mortality (dead aphids and those not able to walk in a coordinated manner) was scored after 24 h. Test conditions: 23°C (± 1°C). RH and light conditions were not reported.

Calculations

LC50-values for both compounds were calculated from probit regressions using GraphPad Prism v5.0 software (GraphPad Software, San Diego, California, USA).

The uptake of the radiolabelled sugar measured by liquid scintillation counting of tissue-solubilized aphids allowed the calculation of the amount of ingested imidacloprid based on its concentration in µg/mL diet.

Results

Results are reported in the following tables and figure (copied from the report)

Table 2. Mean aphid mortality (given in %) at different insecticide concentrations tested (24 h).

Compound	Percentage mortality [%] at test concentrations						
	40	8	1.6	0.32	0.064	0.0128	0.00256
Flupyridafurone	100	100	100	99	73	17	3
Imidacloprid	100	100	99	70	27	10	0

Table 3. Log-dose probit-mortality data (24 h) for flupyradifurone and imidacloprid against apterous adults of *M. persicae* in an oral feeding bioassay based on artificial double membranes. Study year 1991 refers to data published by Elbert et al. (1991).

Insecticide	study year	LC-50 value (µg/mL)	95% CL
Flupyradifurone	2013	0.036	0.033 – 0.039
Imidacloprid	2013	0.17	0.12 – 0.23
	1991	0.24	0.078 – 0.72

*Based on data published by Elbert *et al.* (1991) PF-NACHR. Bayer 44, 113 - 136

Figure 1. Dose-response curves obtained for flupyradifurone and imidacloprid against *M. persicae* in an oral feeding bioassay (24 h).

Oral LD50-value for flupyradifurone

Based on the LC50-value of 0.24 µg/mL obtained for imidacloprid in feeding bioassays including radiolabelled N-acetyl-D-[14C] glucosamine, Elbert et al. (1991) calculated an oral LD50-value of 0.54 pg/aphid for imidacloprid. The value is based on the amount of radiolabel taken up at individual imidacloprid concentrations, i.e. the higher the concentration of insecticide the lower the ingested amount of radiolabel due to the onset of symptoms of poisoning preventing aphids from further feeding. Considering the fact that an oral LC50-value of 0.24 µg/mL for imidacloprid corresponded to the uptake of 0.54 pg/aphid and that the oral LC50-value for imidacloprid as reported by Elbert et al. (1991) was confirmed, the author concludes that the LD50-value of flupyradifurone against M. persicae is significantly lower based on its LC50-value of 0.036 µg/mL

Remarks by RMS

A toxic reference is not included in the test. Not all test conditions are reported, RH is missing, light conditions are missing. Raw data are not presented, no information is available on the variability between replicates within the treatments. The number of aphids per treatment is reported to be between 10 to 15. Since no exact numbers are reported, recalculation of the results is impossible. Furthermore it is not clear if the results from this study are realistic and comparable to results deriving from aphids exposed to treated leaves. Thus, there is a number of shortcomings in this report. However, it is acknowledged that there are no harmonised test guidelines for aphids yet. The study indicates that the toxicity of flupyradifurone to aphids is higher than that of imidacloprid. This information could be used in the risk assessment of flupyradifurone, provided that the raw data are submitted so that the results can be recalculated by RMS.

B.9.9.3 Summary and risk assessment

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev2 final, 2002). It is restricted to off-field situations, as non-target plants are non-crop plants located outside the treated area. Spray drift from the treated areas may lead to residues of a product in off-crop areas. Exposure is not relevant for glasshouse uses.

In the case of a non-herbicide, screening results and/or Tier 1 studies give first information about the likelihood for terrestrial plant effects. The risk can be considered acceptable if there are no data indicating more than 50% phytotoxic effect at the maximum application rate.

Seedling emergence (Gosch (2010), KIIIA1 10.8.1.3/01) and vegetative vigour studies Gosch (2010), KIIIA1 10.8.1.2/01) have been conducted with BYI 02960 SL 200 G following OECD testing guidelines 208 and 227, respectively (see Annex Points IIIA1 10.8.1.3 and 10.8.1.2). Each study included 11 species which were tested at the maximum application rate of 410 g a.i./ha.
In the case of BYI 02960 SL 200 G neither the tier 1 seedling emergence nor the vegetative vigour studies showed phytotoxic effects >50% at the maximum application rate of 410 g a.i./ha. Therefore, the ER50 values are set at >410 g a.s./ha.

For the proposed field uses in hops and lettuce, the following scenario is used to describe the exposure to non-target plants:

off-field exposure= Application rate * MAF * drift factor

For the MAF and drift factor, similar assumptions are made as for the off-field risk assessment for non-target arthropods.
As the proposed uses of BYI 02960 SL 200 formulation involves one application only, the multiple application factor (MAF) is 1. Drift values are 19.33% for hops (3 m distance) and 2.77% for lettuce (field crops, 1m distance).

The calculated exposure for the respective uses and the TER values are given in Table B.9.9.3-01.

Table B.9.9.2-01 Exposure values and TERs for non-target plants

Crop	Max. single dose [g a.s./ha]	number of applications	MAF	Drift [%]	Exposure [g a.s./ha]	ER_{50} [g a.s./ha]	TER	Trigger
hops	150	1	1	19.33	30	>410	>14	5
lettuce	125	1	1	2.77	3.5	>410	>118	5

1: drift in vegetables, > 50 cm.

For both uses, the TERs are above the trigger of 5. A low risk for non-target plants is expected.

B.9.10 Effects on biological methods for sewage treatment (IIA 8.15)

B.9.10.1 Effects of the active substance

STUDY IIA, 8.15/01

Characteristics

reference	:	Caspers C. (2010)	water solubility	:	3000-3200 mg/L at pH 4-9 & 20°C
type of study	:	Activated sludge respiration inhibition test	exposure duration	:	3 hours
year of execution	:	2010	nominal conc.	:	100, 180, 320, 560 and 1000 mg test item/L
GLP statement	:	Yes	treatment method	:	Test substance was stirred in water for 15 hours and added to test flasks
guideline	:	OECD 209, EC C.11	acceptability	:	Acceptable
test substance	:	BYI 02960; technical, batch 2009-000239	3-hour EC50	:	>962 mg a.s./L
purity	:	96.2%			

Methods

The effect of BYI 02960 technical on the rate of respiration of bacterial populations from activated sludge was determined after a contact time of 3 hours. The sludge was obtained from the municipal sewage treatment plant of Cologne-Stammheim, Germany (final concentration in test 0.8 g solids/L). BYI 02960 technical was added to the test vessels with 130 mL of water and the mixtures were stirred for 15 hours at ambient temperature. Thereafter synthetic nutrient medium and activated sludge suspension (pH 7.5) were added, and water was added to a total volume of 250 mL (test concentrations 100, 180, 320, 560 and 1000 mg test item/L). Each treatment was tested in one flask, the untreated control was tested in 2 flasks. The test solutions were aerated for 3 hours at 20°C. At the end of the 3 hour contact time, the oxygen consumption was measured.

Results

The concurrently determined EC50 of the reference product 3,5-dichlorophenol (8.7 mg/L) was in the acceptable range of 2 to 25 mg/L (OECD 209 (2010)). The respiratory rates in the two replicate control flasks were 24.9 and 26.0 mg O_2/L/hour, hence satisfying the criteria of OECD 209 (2010): CV <30% and >20 mg O_2/L/hour.

At 100, 180, 320, 560 and 1000 mg test item/L, respectively, the inhibition percentage of the respiratory rate was 0, 0, 0, 8.6 and 23.3%. The 3-hour EC50 is >1000 mg test item/L, equivalent to >962 mg a.s./L.

Conclusion

3-hour EC50 >962 mg a.s./L.

Guidelines and Limitations

The study was performed according to OECD 209 and is acceptable.

B.9.10.2 Risk assessment

Emission of flupyradifurone to sewage treatment plants may occur following the proposed use of Sivanto in glasshouses. The expected concentration in the STP is not calculated because EU harmonised guidance is lacking. However, based on the results of the activated sludge respiration test, which obtained an EC_{50} > 962 mg a.s./L, no effect on biological methods of sewage treatment are expected.

B.9.11 POP/PBT/vPvB assessment

Assessment of the properties with regard to the POP criteria of the Stockholm Convention or the vPvB and PBT criteria				
Active substance flupyradifurone				
		POP/vPvB	PBT	
Persistence	DT_{50} water	> 2 months (60 d)	> 120 d	yes water/sed: DT50w max 123.8
	DT_{50} soil	> 6 months (180 d)	> 120 d	yes lab: DT50 max 235 field: DT50 max 251
	DT_{50} sediment	> 6 months (180 d)	> 120 d	yes water/sed: DegT50system max 285
	Conclusion: P criterion fulfilled?			yes → P
Potential for extensive trans-port into the environment	$DT_{50\ air}$ (direct and indir-ect phototrans-formation)	> 2 d / --	--	no
Bioaccumulation	BCF	> 5000	> 2000	no
Toxicity	NOEC aquatic organisms	--	< 0.01 mg/L	no NOEC *C. riparius* 0.010 mg/L

The active substance flupyradifurone does not fulfil all the POP/vPvB criteria. The active substance does not fulfil all the P and B criteria completely (B not applicable) and should not be classified as a potential PBT active substance.

B.9.12 References relied on

Scientific peer reviewed open literature.

The following data requirements and open points were identified in the evaluation table for ecotoxicology:

Open point 5.18 RMS to present an evaluation of the literature review completed by the applicant in an addendum. See reporting table 5(79)
Data requirement 5.5 Applicant to provide a search of the scientific peer-reviewed open literature relevant to the scope of the application, dealing with side-effects on health, the environment and non-target species and published within the last 10 years before the date of submission of dossier, being conducted and reported in accordance with the Guidance of EFSA on the submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009 (EFSA Journal 2011;9(2):2092) for the aqueous photolysis metabolites BYI 02960-succinamide and BYI 02960-azabicyclosuccinamide that do nor appear to have been included in the available literature review. See reporting table 5(79)

The applicant has provided a search of the scientific peer-reviewed open literature for flupyradifurone and metabolites (including the aqueous photolysis metabolites BYI 02960-succinamide and BYI 02960-azabicyclosuccinamide). They provide a list of bibliographic databases searched, the search input parameters and the search results. They also provide the following summary of their literature review:

"*A literature review was conducted for flupyradifurone and its metabolites according to the requirements of Regulation EC 1107/2009.*
The total number of literature findings within the last ten years before submission were 342, all referring to two metabolites. A large number of keywords, grouped by the categories "health", "environment" and "non target species" has been used as a text mining filter. From the total of 342 references, 136 publications were identified which gave a matching overlap with one or more of the keywords used. All 342 references were reviewed by scientific experts and categorized.
The filter was merely tested, and improved, as a tool to be applied in future literature reviews for other compounds. From those references which could not be allocated to one of the categories "health", "environment" or "non target species", none was identified by the scientific experts to provide relevant information for regulatory purposes, i.e. no false negative findings were identified.
None of the studies was considered relevant as defined by the EFSA Guidance document on 'submission of scientific peer-reviewed open literature for the approval of pesticide active substances

under Regulation (EC) no. 1107/2009', i.e. as "dealing with side-effects on health, the environment and non-target species".
In the area of toxicology, only one publication was evaluated as "relevant". Fortunately this publication was already known and the result was therefore already assessed and fully integrated in the supplementary dossier Therefore, no reference had to be discussed separately in the dossier, as all risk assessments remained fully valid after consideration of all references.
In conclusion, no information was identified which was considered to have an impact on an endpoint or would result in the need to adapt a risk assessment in the flupyradifurone dossier."

Remarks RMS

A literature search and review was provided by the applicant. The search has been conducted in line with the EFSA guidance. Of all identified papers (342) the relevant ones (136) have been selected by the applicant in a transparent way using a text mining filter. The applicant provided a general justification for evaluating the relevant papers. As no study-by-study justifications were given the RMS can not properly verify whether the 136 relevant papers were correctly evaluated in terms of reliability/validity/acceptability for regulatory use. The one study that was considered relevant by the applicant is evaluated in the DAR (B.6). RMS notes that flupyradifurone is a new active substance worldwide. Therefore, public literature is not expected to be an important new information source for the active substance itself.

References for the active substance

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. Claimed Y/N	Owner
KIIA 8.1.1 /01	[redacted].	2010	Toxicity of BYI 02960 technical during an acute oral LD50 with the northern bobwhite quail (Colinus virginianus) [redacted] Bayer CropScience, Report No.: EBRVP022, Edition Number: M-386036-01-1 Date: 2010-07-14 GLP/GEP: yes, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. Claimed Y/N	Owner
KIIA 8.1.1 /02	[redacted]	2011	Toxicity of BYI 02960 technical during an acute oral LD50 with the canary (Serinus canaria) [redacted] Bayer CropScience, Report No.: EBRVP036, Edition Number: M-408514-01-1 Date: 2011-05-25 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.1.2 /01	[redacted]	2010	Toxicity of BYI 02960 technical during an acute dietary LC50 with the mallard duck (Anas platyrhynchos) [redacted] Bayer CropScience, Report No.: EBRVP020, Edition Number: M-388718-01-1 Date: 2010-08-26 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.1.2 /02	[redacted]	2010	Toxicity of BYI 02960 technical during an acute dietary LC50 with the northern bobwhite quail (Colinus virginianus [redacted] Bayer CropScience, Report No.: EBRVP021, Edition Number: M-394535-01-1 Date: 2010-11-10 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.1.4 /01	[redacted]	2011	Toxicity of BYI 02960 technical on reproduction to the mallard duck (Anas platyrhynchos) [redacted] Bayer CropScience, Report No.: EBRVP018-1, Edition Number: M-412917-02-1 Date: 2011-08-25 ...Amended: 2012-03-19 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.1.4/01a	Anon.	2013	Mallard repro, BYI 2960, revised data [redacted] Bayer CropScience Edition no. M-450547-01-1 (supplement to edition M-412917-02-1) Date: 2013-03-22 GLP/GEP: yes , unpublished	Yes	BCS
KIIA 8.1.4/01b	[redacted]	2013	SynTech response for the mallard reproduction study exposed to BYI 02960 [redacted] Bayer CropScience Edition Number: M-450549-01-1 Date: 2013-03-26 GLP/GEP: no , unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. Claimed Y/N	Owner
KIIA 8.1.4 /02	[redacted]	2012	Toxicity of BYI 02960 technical on reproduction to the northern bobwhite quail (Colinus virginianus) [redacted] Bayer CropScience, Report No.: EBRVP019, Edition Number: M-424704-01-1 Date: 2012-02-09 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.1.4 /02a	[redacted]	2013	SynTech response for the quail reproduction study exposed to BYI 02960 [redacted] Bayer CropScience Edition Number: M-451967-01-1 Date: 2013-04-18 GLP/GEP: no , unpublished	Yes	BCS
KIIA 8.2.1.1 /01	[redacted]	2010	Acute toxicity of BYI 02960 technical to the rainbow trout (Oncorhynchus mykiss) under static conditions [redacted] Bayer CropScience, Report No.: EBRVP041, Edition Number: M-390611-01-1 Date: 2010-09-27 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.2.1.1 /02	[redacted]	2011	Acute toxicity of BYI 02960 to Xenopus laevis under flow-through conditions [redacted] Bayer CropScience, Report No.: EBRVP187, Edition Number: M-417822-01-1 Date: 2011-11-18 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.2.1.2 /01	[redacted]	2010	Acute toxicity of BYI 02960 technical to the fathead minnow (Pimephales promelas) under static conditions [redacted] Bayer CropScience, Report No.: EBRVP035, Edition Number: M-392560-01-1 Date: 2010-10-21 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.2.1.2 /02	[redacted]	2011	Acute toxicity of BYI 02960 (tech.) to fish (Cyprinus carpio) under static conditions (limit test) [redacted] Report No.: EBRVP186, Edition Number: M-420407-01-2 Date: 2011-12-19 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.2.1.3 /01	[redacted]	2011	Acute toxicity of BYI 02960 succinamide (tech.) to fish (Oncorhynchus mykiss) under static conditions (limit test) [redacted] Report No.: EBRVP203, Edition Number: M-414293-01-2 Date: 2011-09-21 GLP/GEP: yes, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. Claimed Y/N	Owner
KIIA 8.2.1.3 /02	[redacted]	2011	Acute toxicity of sodium difluoro acetate (BCS AB60481, tech.) to fish (Oncorhynchus mykiss) under static conditions (limit test) [redacted], Report No.: EBRVP080, Edition Number: M-413889-01-2 Date: 2011-09-12 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.2.4 /01	[redacted]	2011	Early life stage toxicity of BYI 02960 technical to the Fathead minnow (Pimephales promelas) under flow-through conditions [redacted] Bayer CropScience, Report No.: EBRVP033, Edition Number: M-409339-01-1 Date: 2011-06-14 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.3.1.1 /01	Banman, C. S.; Lam, C. V.	2009	Acute toxicity of BYI 02960 to Daphnia magna under static conditions Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: EBRVP032, Edition Number: M-357476-01-1 Date: 2009-10-14 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.3.1.1 /02	Bruns, E.	2011	Acute toxicity of BCS-AB60481 to the waterflea Daphnia magna in a static laboratory test system - limit test- Bayer CropScience, Report No.: EBRVP079, Edition Number: M-409326-01-2 Date: 2011-06-10 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.3.1.1 /03	McElligott, A.	1997	Acute toxicity (48 hours) to Daphnids (Daphnia magna) under semi-static conditions IC-0 Rhone-Poulenc Agro, Sophia Antipolis, France Bayer CropScience, Report No.: C007748, Edition Number: M-196569-01-1 Date: 1997-05-20 GLP/GEP: yes, unpublished	No	NISSO
KIIA 8.3.1.2 /01	Bruns, E.	2011	Acute toxicity of BYI 02960 (tech.) to larvae of Chironomus riparius in a 48 h static laboratory test system Bayer CropScience, Report No.: EBRVP026, Edition Number: M-414739-01-2 Date: 2011-09-26 GLP/GEP: yes, unpublished ...also filed: KIIA 8.5.1 /01	Yes	BCS

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. Claimed Y/N	Owner
KIIA 8.3.1.2 /02	Bruns, E.	2011	Acute toxicity of BYI 02960-succinamide to larvae of Chironomus riparius in a 48 h static laboratory test system Bayer CropScience, Report No.: EBRVP202, Edition Number: M-417386-01-2 Date: 2011-11-17 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.3.1.2 /03	Bruns, E.	2012	Acute toxicity of BYI 02960-azabicyclosuccinamide (BCS-CS64875) to larvae of Chironomus riparius in a 48 h static laboratory test system Bayer CropScience, Report No.: EBRVP207, Edition Number: M-424404-01-1 Date: 2012-02-02 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.3.1.2 /04	Bowers, L. M.; Lam, C. V.	1998	Acute toxicity of 6-chloronicotinic acid (a metabolite of imidacloprid) to Chironomus tentans under static renewal conditions Bayer Corporation, Stilwell, KS, USA Bayer CropScience, Report No.: 108127, Edition Number: M-048448-01-1 Date: 1998-04-23 GLP/GEP: yes, unpublished	No	NISSO
KIIA 8.3.2.1 /01	Riebschlaeger, T.	2011	Effects of BYI 02960 (techn.) on development and reproductive output of the waterflea Daphnia magna in a static-renewal laboratory test system Bayer CropScience, Report No.: EBRVP209, Edition Number: M-414066-01-2 Date: 2011-09-15 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.3.2.1 /02	Riebschlaeger, T.	2012	Influence of BYI02960-succinamide (tech.) on development and reproductive output of the waterflea Daphnia magna in a static-renewal laboratory test system Bayer CropScience, Report No.: EBRVP185, Edition Number: M-424700-01-2 Date: 2012-02-10 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.3.2.2 /01	Bruns, E.	2011	Chironomus riparius 28-day chronic toxicity test with BYI 02960 (tech.) in a water-sediment system using spiked water Bayer CropScience, Report No.: EBRVP025, Edition Number: M-401792-01-2 Date: 2011-02-14 GLP/GEP: yes, unpublished ...also filed: KIIA 8.5.2 /01	Yes	BCS

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. Claimed Y/N	Owner
KIIA 8.3.2.2 /02	Bruns, E.	2011	Chironomus riparius 28-day chronic toxicity test with Sodium difluoroacetate in a water-sediment system using spiked water - limit test Bayer CropScience, Report No.: EBRVP181, Edition Number: M-415913-01-2 Date: 2011-10-06 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.3.2.2 /03	Bruns, E.	2011	Chironomus riparius 28-day chronic toxicity test with 6-Chloronicotinic acid in a water-sediment system using spiked water - limit test Bayer CropScience, Report No.: EBRVP183, Edition Number: M-416604-02-2 Date: 2011-10-18 ...Amended: 2011-12-20 GLP/GEP: yes, unpublished	Yes	BCS
Q 14 /01 (relevant for KIIA 8.3.2.2)	Hager, J.	2014	Flupyradifurone (BYI 02960): BCS opinion on the need for additional data on mayflies (Ephemeroptera) Bayer CropScience, Report No.: M-496218-01-1, Edition Number: M-496218-01-1 Date: 2014-08-29 GLP/GEP: n.a., unpublished	Yes	BCS
KIIA 8.4 /01	Banman, C. S.; Lam, C. V.	2010	Toxicity of BYI 02960 technical to the green alga Pseudokirchneriella subcapitata Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: EBRVP030, Edition Number: M-397552-01-1 Date: 2010-12-10 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.4 /02	Bruns, E.	2011	Pseudokirchneriella subcapitata growth inhibition test with BCS-AB60481 - limit test Bayer CropScience, Report No.: EBRVP077, Edition Number: M-409118-01-2 Date: 2011-06-10 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.4 /03	Sobczyk, H.	2011	Pseudokirchneriella subcapitata growth inhibition test with BYI 02960 - succinamide - limit test Bayer CropScience, Report No.: EBRVP184, Edition Number: M-414090-01-2 Date: 2011-09-23 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.4 /04	Bruns, E.	2012	Pseudokirchneriella subcapitata growth inhibition test with 6 - chloronicotinic acid Bayer CropScience, Report No.: EBRVP242, Edition Number: M-424145-01-2 Date: 2012-02-01 GLP/GEP: yes, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. Claimed Y/N	Owner
KIIA 8.5.1 /01	Bruns, E.	2011	Acute toxicity of BYI 02960 (tech.) to larvae of Chironomus riparius in a 48 h static laboratory test system Bayer CropScience, Report No.: EBRVP026, Edition Number: M-414739-01-2 Date: 2011-09-26 GLP/GEP: yes, unpublished ...also filed: KIIA 8.3.1.2 /01	Yes	BCS
KIIA 8.5.2 /01	Bruns, E.	2011	Chironomus riparius 28-day chronic toxicity test with BYI 02960 (tech.) in a water-sediment system using spiked water Bayer CropScience, Report No.: EBRVP025, Edition Number: M-401792-01-2 Date: 2011-02-14 GLP/GEP: yes, unpublished ...also filed: KIIA 8.3.2.2 /01	Yes	BCS
KIIA 8.6 /01	Banman, C. S.; Alexander, T. M.; Lam, C. V.	2010	Toxicity of BYI 02960 technical to duckweed (Lemna gibba G3) under static-renewal conditions Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: EBRVP043, Edition Number: M-398376-01-1 Date: 2010-12-21 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.7.1 /01	Schmitzer, S.	2012	Revised final report no.: 1 - Effects of BYI 02960 (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 41121035, Edition Number: M-308904-02-1 Date: 2012-01-14 ...Amended: 2012-03-22 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.7.1 /02	Schmitzer, S.	2010	Effects of BYI02960 - difluoroethyl - amino - furanone (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 60291035, Edition Number: M-398557-01-2 Date: 2010-12-21 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.7.1 /03	Schmitzer, S.	2011	Effects of BYI 02960 - hydroxy (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 63901035, Edition Number: M-409606-01-2 Date: 2011-06-15 GLP/GEP: yes, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. Claimed Y/N	Owner
KIIA 8.7.1 /04	Schmitzer, S.	2010	Effects of difluoroacetic acid (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 56331035, Edition Number: M-367915-01-2 Date: 2010-04-29 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.7.1 /05	Schmitzer, S.	2010	Effects of 6-chloronicotinic acid (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 60281035, Edition Number: M-395279-01-2 Date: 2010-11-19 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.7.1 /06	Schmitzer, S.	2010	Effects of 6-chloro-picolylalcohol (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 50911035, Edition Number: M-361234-01-2 Date: 2010-01-11 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.7.1 /07	Vergé, E	2012	Flupyradifurone SL 200 G: Acute contact toxicity to the bumblebee Bombus terrestris L.(Hymenoptera, Apidae) under laboratory conditions (multi doses test) Source: Eurofins-GAB GmbH, Niefern-Oeschelbronn, Germany Edition Number: M-443696-01-1 Study Completion Date: 2012-12-13 GLP: yes, unpublished	Yes	BCS
KIIA 8.8.1.1 /01	Jans, D.	2010	Toxicity to the parasitoid wasp Aphidius rhopalosiphi (DESTEPHANI-PEREZ) (Hymenoptera: Braconidae) using a laboratory test; BYI 02960 SL 200 g/L Bayer CropScience, Report No.: CW09/079, Edition Number: M-366965-01-2 Date: 2010-04-15 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.8.1.2 /01	Jans, D.	2010	Toxicity to the predatory mite Typhlodromus pyri SCHEUTEN (Acari, Phytoseiidae) using a laboratory test; BYI 02960 SL 200 g/L Bayer CropScience, Report No.: CW09/073, Edition Number: M-366957-01-2 Date: 2010-04-15 GLP/GEP: yes, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. Claimed Y/N	Owner
KIIA 8.9.1 /01	Leicher, T.	2010	BYI 02960 (tech.): Acute toxicity to earthworms (Eisenia fetida) tested in artificial soil Bayer CropScience, Report No.: LRT/RG-A-131/09, Edition Number: M-363742-01-2 Date: 2010-02-18 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.9.1 /02	Leicher, T.	2010	BYI 02960-difluoroacetic acid: acute toxicity to earthworms (Eisenia fetida) tested in artificial soil Bayer CropScience, Report No.: LRT/RG-A-135/10, Edition Number: M-368835-01-2 Date: 2010-04-12 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.9.1 /03	Wetton, P. M.	1999	Acute toxicity to earthworms (Eisenia foetida) IC-0 Safepharm Lab. Ltd., Derby, United Kingdom Bayer CropScience, Report No.: C007758, Edition Number: M-196591-01-1 Date: 1999-08-25 GLP/GEP: yes, unpublished	No	NISSO
KIIA 8.9.2 /01	Leicher, T.	2010	BYI 02960 SL 200 G: Effects on survival, growth and reproduction on the earthworm Eisenia fetida tested in artificial soil Bayer CropScience, Report No.: LRT-RG-R-76/09, Edition Number: M-392964-01-2 Date: 2010-10-21 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.9.2 /02	Leicher, T.	2010	BYI 02960 Difluoroacetic acid: Effects onsurvival, growth and reproduction on the earthworm Eisenia fetida tested in artifical soil with 10 % peat Bayer CropScience, Report No.: LRT-RG-R-81/10, Edition Number: M-398061-01-2 Date: 2010-12-16 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.9.2 /03	Leicher, T.	2011	6-chloronicotinic acid (AE F161089): Effects on survival, growth and reproduction on the earthworm Eisenia fetida tested in artificial soil with 5 % peat Bayer CropScience, Report No.: LRT-RG-R-101/11, Edition Number: M-413562-02-2 Date: 2011-09-05 ...Amended: 2012-02-23 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.10.1 /01	Frommholz, U.	2009	BYI 02960 a.s.: Determination of effects on nitrogen transformation in soil Bayer CropScience, Report No.: FRM-N-130/09, Edition Number: M-359803-01-2 Date: 2009-12-03 GLP/GEP: yes, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. Claimed Y/N	Owner
KIIA 8.10.1 /02	Frommholz, U.	2011	6-chloronicotinic acid (AE F161089): Determination of effects on nitrogen transformation in soil Bayer CropScience, Report No.: FRM-N-156/11, Edition Number: M-408028-01-2 Date: 2011-05-20 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.10.2 /01	Schulz, L.	2011	BYI 02960 a.s.: Effects on the activity of soil microflora (carbon transformation test) BioChem Agrar GmbH, Gerichshain, Germany Bayer CropScience, Report No.: 11 10 48 058 C, Edition Number: M-417194-01-2 Date: 2011-11-11 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.11.1 /01	Banman, C. S.; Lam, C. V.	2009	Acute toxicity of BYI 02960 technical to the sheepshead minnow (Cyprinodon variegatus) under static conditions Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: EBRVP034, Edition Number: M-357479-01-1 Date: 2009-10-14 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.11.1 /01a	Bruns, E.; Hoffmann, K.; Hager, J.	2013	Flupyradifurone (BYI 02960) - BCS opinion on the reproduction endpoint of the chronic study with Americamysis bahia Bayer CropScience AG, Monheim, Germany Edition Number: M-452374-01-1 Edition Date: 2013-04-26 GLP/GEP: No	Yes	BCS
KIIA 8.11.1 /01b	Springer, T	2013	Effect size, variation, and dependability of conclusions from NOEC and ECx calculations for for project 149A-242B, BYI 02960: A flow-through life-cycle toxicity test Wildlife International Edition Number: M-452382-01-1 Edition Date: 2013-04-25 GLP/GEP: No	Yes	BCS
KIIA 8.11.1 /02	Gallagher, S. P.; Kendall, T. Z.; Krueger, H. O.	2009	BYI 02960: A 96-hour shell deposition test with the eastern oyster (Crassostrea virginica) Wildlife International Ltd., Easton, MD, USA Bayer CropScience, Report No.: EBRVP023, Edition Number: M-361668-01-1 Date: 2009-12-01 GLP/GEP: yes, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. Claimed Y/N	Owner
KIIA 8.11.1 /03	Gallagher, S. P.; Kendall, T. Z.; Krueger, H.O.	2009	BYI 02960: A 96-hour static acute toxicity test with the saltwater mysid (Americamysis bahia) Wildlife International Ltd., Easton, MD, USA Bayer CropScience, Report No.: 149A-236, Edition Number: M-364620-01-1 Date: 2009-12-08 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.11.1 /04	Claude, M. B.; Kendall, T. Z.; Krueger, H. O.	2011	BYI 02960: A flow-through life-cycle toxicity test with the saltwater mysid (Americanysis bahia) Wildlife International Ltd., Easton, MD, USA Bayer CropScience, Report No.: EBRVP038, Edition Number: M-420783-01-1 Date: 2011-09-08 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.12 /01	Gosch, H.	2010	BYI 02960 SL 200 g/L - Effects on the vegetative vigour of eleven species of non-target terrestrial plants (Tier 1) Bayer CropScience, Report No.: VV 10/002, Edition Number: M-397734-01-2 Date: 2010-12-14 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.12 /02	Gosch, H.	2010	BYI 02960 SL 200 g/L - Effects on the seedling emergence and growth of eleven species of non-target terrestrial plants (Tier 1) Bayer CropScience, Report No.: SE10/001, Edition Number: M-397727-01-2 Date: 2010-12-14 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.14 /01	Frommholz, U.	2009	BYI 02960 SL 200 G: Influence on the reproduction of the collembola species Folsomia candida tested in artificial soil with 5 % peat Bayer CropScience, Report No.: FRM-COLL-75/09, Edition Number: M-359728-01-2 Date: 2009-12-02 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.14 /02	Frommholz, U.	2010	Metabolite BYI 02960-difluoroacetic acid: Influence on the reproduction of the collembolan species Folsomia candida tested in artificial soil Bayer CropScience, Report No.: FRM-COLL-85/10, Edition Number: M-368675-01-2 Date: 2010-05-12 GLP/GEP: yes, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. Claimed Y/N	Owner
KIIA 8.14 /03	Frommholz, U.	2011	6-chloronicotinic acid (AE F161089): Influence on the reproduction of the collembolan species Folsomia candida tested in artificial soil Bayer CropScience, Report No.: FRM-COLL-111/11, Edition Number: M-407861-01-2 Date: 2011-05-18 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.14 /04	Kratz, M.-A.	2009	BYI 02960 SL 200 G: Influence on mortality and reproduction on the soil mite species Hypoaspis aculeifer tested in artificial soil with 5 % peat Bayer CropScience, Report No.: KRA-HR-19/09, Edition Number: M-358752-01-2 Date: 2009-11-10 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.14 /05	Kratz, M.-A.	2010	BYI 02960-DFA (BCS-AA56716): Influence on mortality and reproduction on the soil mite species Hypoaspis aculeifer tested in artificial soil with 5 % peat Bayer CropScience, Report No.: KRA-HR-27/10, Edition Number: M-390091-01-2 Date: 2010-09-15 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.14 /06	Kratz, M.-A.	2011	6-chloronicotinic acid (AE F161089): Influence on mortality and reproduction on the soil mite species Hypoaspis aculeifer tested in artificial soil Bayer CropScience, Report No.: KRA-HR-44/11, Edition Number: M-404434-01-2 Date: 2011-03-24 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.14.1 /01	Voerste, A.; Malsam, O.	2010	Determination of the insecticidal efficacy of the metabolite difluoroacetic acid (BCS-AA56716) compared to the parent compound BYI02960 (BCS-BZ89914) Bayer CropScience, Report No.: VAR 2010-01, Edition Number: M-386333-01-1 Date: 2010-06-10 GLP/GEP: no, unpublished	Yes	BCS
KIIA 8.15 /01	Caspers, N.	2010	Activated sludge, respiration inhibition test with BYI 02960 (tech.) Currenta GmbH & Co. OHG, Leverkusen, Germany Bayer CropScience, Report No.: 2010/0089/01, Edition Number: M-377311-01-1 Date: 2010-06-21 GLP/GEP: yes, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. Claimed Y/N	Owner
KIIA 8.16.1 /02	Kling, A.	2012	BYI 02960-difluoroethyl-amino-furanone (BYI 02960-DFEAF) - Assessment of chronic effects to the honey bee, Apis mellifera L., in a 10 days continuous laboratory feeding limit test Eurofins Agroscience Services EcoChem GmbH, Niefern-Öschelbronn, Germany Bayer CropScience, Report No.: S11-01959, Report includes Trial Nos.: S11-01959-L1_BLCF Edition Number: M-425174-01-2 Date: 2012-02-14 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.16.1 /03	Kling, A.	2012	BYI 02960-hydroxy - Assessment of chronic effects to the honey bee, Apis mellifera L., in a 10 days continuous laboratory feeding limit test Eurofins Agroscience Services EcoChem GmbH, Niefern-Öschelbronn, Germany Bayer CropScience, Report No.: S11-01960, Report includes Trial Nos.: S11-01960-L1_BLCF Edition Number: M-425212-01-2 Date: 2012-02-15 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.16.1 /04	Kling, A.	2012	Difluoroacetic acid - Assessment of chronic effects to the honey bee, Apis mellifera L., in a 10 days continuous laboratory feeding limit test Eurofins Agroscience Services EcoChem GmbH, Niefern-Öschelbronn, Germany Bayer CropScience, Report No.: S11-01939, Report includes Trial Nos.: S11-01939-L1_BLCF Edition Number: M-425105-01-1 Date: 2012-02-13 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.16.1 /05	Kling, A.	2012	6-chloronicotinic acid - Assessment of chronic effects to the honey bee, Apis mellifera L., in a 10 days continuous laboratory feeding limit test Eurofins Agroscience Services EcoChem GmbH, Niefern-Öschelbronn, Germany Bayer CropScience, Report No.: S11-01957, Report includes Trial Nos.: S11-01957-L1_BLCF Edition Number: M-425155-01-2 Date: 2012-02-13 GLP/GEP: yes, unpublished	Yes	BCS

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KIIA 8.16.1 /06	Kling, A.	2012	6-chloropicolyl alcohol - Assessment of chronic effects to the honey bee, Apis mellifera L., in a 10 days continuous laboratory feeding limit test Eurofins Agroscience Services EcoChem GmbH, Niefern-Öschelbronn, Germany Bayer CropScience, Report No.: S11-01958, Report includes Trial Nos.: S11-01958-L1_BLCF Edition Number: M-425159-01-2 Date: 2012-02-13 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.16.1 /07	Nikolakis, A.; Theis, M.; Przygoda, D.	2011	BYI 02960 tech.: Effects of exposure to spiked diet on honeybee larvae (Apis mellifera carnica) in an in vitro laboratory testing design Bayer CropScience, Report No.: E 318 3897-9, Edition Number: M-406645-01-2 Date: 2011-05-02 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.16.1 /08	Nauen, R.	2013	Determination of the oral LD50-value for flupyradifurone against the green peach aphis, Myzus persicae Sulzer (Homoptera: Aphididae); Bayer CropScience AG, Monheim, Germany; Report No.: M-461449-01-1; Document No.: M-461449-01-1; August 01, 2013; Pages: 6	Yes	BCS
KIIA 8.16.1 /09	Gladbach, D.; Theis, M.; Przygoda, D.; Nikolakis, A.	2013	Assessment of chronic effects of BYI 02960 tech. to the honey bee, Apis mellifera L., in a 10 days continuous laboratory feeding test; Bayer CropScience AG, Monheim, Germany; Report No.: E 318 4561-8; Document No.: M-462475-01-1; August 26, 2013; Pages: 96	Yes	BCS
KIIA 8.16.2 /01	Leicher, T.	2011	BYI 02960: Effects on soil litter degradation after spray application Bayer CropScience, Report No.: LRT-SLD-45/11, Edition Number: M-413408-01-2 Date: 2011-09-06 GLP/GEP: yes, unpublished	Yes	BCS
KIIA 8.16.2 /02	Leicher, T.	2011	BYI 02960: Effects on soil litter degradation if applied as seed treatment Bayer CropScience, Report No.: LRT-SLD-46/11, Edition Number: M-413416-01-2 Date: 2011-09-06 GLP/GEP: yes, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. Claimed Y/N	Owner
KIIA 8.16.2/03	Nikolakis, A.; Krieg, V.; Aumeier, P.; Gladbach, D.	2012	Honey bee colony feeding study, evaluating the effects of BYI 02960-fortified sugar- and pollen diet on the development of honey bee colonies under confined semi-field conditions, followed by a post-exposure field observation period Bayer CropScience, Report No.: E 319 4111-0, Edition Number: M-438748-01-1 Date: 2012-09-24 GLP/GEP: yes, unpublished		
KIIA 8.16.2/04	Gould, T. J.; Lawrence, J.; Harbin, A. M.	2012	Determination of residues of BYI 02960 in blossoms, nectar, and pollen when applied via soil drench and Foliar Spray to melon under semi-field Conditions in North Carolina; Eurofins Agroscience Services, Mebane, NC, USA; Report No.: RARVP019; Document No.: M-435037-01-1; July 24, 2012; Pages: 209	Yes	BCS
KIIA 8.16.2/05	Bocksch, S.	2012a	Determination of residues of BYI 02960 applied via drench application in watermelon in the semi-field; Eurofins Agroscience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany; Report No.: S09-01391; Document No.: M-424666-01-2; February 09, 2012; Pages: 173	Yes	BCS
KIIA 8.16.2/06	Bocksch, S.	2012b	Determination of residues of BYI 02960 applied via drench application in tomato in the semi-field; Eurofins Agroscience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany; Report No.: S10-03818; Document No.: M-424683-01-2; February 10, 2012; Pages: 108	Yes	BCS
KIIA 8.16.2/07	Bocksch, S.	2012c	Determination of residues of BYI 02960 applied via drench application in watermelon in the semi-field; Eurofins Agroscience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany; Report No.: S10-03825; Document No.: M-424675-01-2; February 08, 2012; Pages: 117	Yes	BCS
KIIA 8.16.2/08	Rexer, H. U.	2013	Determination of residues of BYI 02960 after application of BYI 02960 SL 200 G once before and once during flowering in a semi-field honeybee (Apis mellifera L.) study in Phacelia tanacetifolia in 2012; Eurofins-GAB GmbH, Niefern-Oeschelbronn, Germany; Report No.: S12-00038; Document No.: M-457246-01-1; May 31, 2013; Pages: 192	Yes	BCS
KIIA 8.16.2/09	Rexer, H. U.	2012c	A field study to determine residues of BYI 02960 in guttation liquid from winter oil-seed rape (OSR) plants in Northern Germany in 2010/2011; Eurofins Agroscience Services GmbH, Niefern-Oeschelbronn, Germany; Report No.: S10-03312; Document No.: M-438826-01-1; September 27, 2012; Pages: 107	Yes	BCS

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KIIA 8.16.2/10	Rexer, H. U.	2012d	A field study to determine residues of BYI 02960 in guttation liquid from winter oil-seed rape (OSR) plants in France in 2010/2011; Eurofins Agroscience Services GmbH, Niefern-Oeschelbronn, Germany; Report No.: S10-03313; Document No.: M-438829-01-1; September 27, 2012; Pages: 106	Yes	BCS

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Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. claimed	Owner
KIIIA1 10.1.6 /01	[redacted]	2012	Toxicity of BYI 02960 SL 200 during an acute oral LD50 with the Northern bobwhite quail (Colinus virginianus) [redacted] Bayer CropScience, Report No.: EBRVP093, Edition Number: M-424312-01-1 Date: 2012-02-03 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA1 10.1.6 /02	[redacted]	2012	Acute oral toxicity of chicken (Gallus gallus domesticus) with BYI 02960 SL 200, according to OECD 223 - limit test- [redacted] Report No.: BAR/LD 142, Edition Number: M-423043-01-2 Date: 2012-01-06 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA1 10.2.2.1 /01	[redacted]	2010	Acute toxicity of BYI 02960 SL 200 G to fish (Oncorhynchus mykiss) under static conditions-limit test [redacted] Report No.: EBRVP098, Edition Number: M-398721-02-2 Date: 2010-12-20 ...Amended: 2012-01-19 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA1 10.2.2.1 /02	[redacted].	2011	Acute toxicity of BYI 02960 SL 200 G to fish (Cyprinus carpio) under static conditions (limit test) [redacted], Report No.: EBRVP199, Edition Number: M-420485-01-2 Date: 2011-12-19 GLP/GEP: yes, unpublished	Yes	BCS

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KIIIA1 10.2.2.2 /01	Riebschlaeger, T.	2010	Acute toxicity of BYI 02960 SL 200 G to the waterflea Daphnia magna in a static laboratory test system Bayer CropScience, Report No.: EBRVP097, Edition Number: M-393738-01-2 Date: 2010-10-28 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA1 10.2.2.3 /01	Bruns, E.	2010	Pseudokirchneriella subcapitata growth inhibition test with BYI 02960 SL 200 G Bayer CropScience, Report No.: EBRVP095, Edition Number: M-397244-01-2 Date: 2010-12-20 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA1 10.2.6.2 /01	Silke, G.	2011	Chironomus riparius 28-day chronic toxicity test with BYI 02960 SL 200 G in a water-sediment system using spiked water Bayer CropScience, Report No.: EBRVP182, Edition Number: M-416145-01-2 Date: 2011-10-17 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA1 10.3.3 /01	Wolf, C.	2004	Generic field monitoring of birds and mammals in hop-cultivation in Southern Germany Bayer CropScience, Report No.: WFC/FS 009, Edition Number: M-123479-01-1 Date: 2004-11-12 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA1 10.3.3 /02	Uceda, L.	2012	Determination of the residues of BYI 02960 in/on barley and wheat after spray application of BYI 02960 SL 200 in the field in Germany, Southern France and Italy Bayer S.A.S., Bayer CropScience, Lyon, France Bayer CropScience, Report No.: 11-2958, Report includes Trial Nos.: 11-2958-01 11-2958-02 11-2958-03 11-2958-04 Edition Number: M-427494-01-1 Date: 2012-03-22 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA1 10.3.3 /03	Scherr, F.; Ellerich, C.	2013	Scherr, F.; Ellerich, C.; Statement on residue dissipation of flupyradifurone in treated foliage of lettuce: kinetic evaluation; Bayer CropScience AG, Monheim, Germany; Report No.: EnSa-12-0154; Document No.: M-428040-02-1; August 09, 2013; Pages: 127	Yes	BCS

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KIIIA1 10.3.3 /04	Sur, R.; Ellerich, C.	2012	Statement on residue dissipation of flupyradifurone in treated foliage of monocotyledonous plants: kinetic evaluation Bayer CropScience, Report No.: EnSa-12-0155, Edition Number: M-428041-01-1 Date: 2012-03-23 GLP/GEP: no, unpublished	Yes	BCS
KIIIA1 10.4.2.1 /01	Schmitzer, S.	2009	Revised final report no.: 1 - Effects of BYI 02960 SL 200 G (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 52331035, Edition Number: M-359920-02-1 Date: 2009-12-03 ...Amended: 2012-03-22 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA1 10.4.3 /01	Porch, J. R.; Krueger, H. O.	2011	BYI 02960 200 SL: A foliage residue toxicity study with the honeybee Wildlife International Ltd., Easton, MD, USA Bayer CropScience, Report No.: EBRVP044, Report includes Trial Nos.: 149-220 Edition Number: M-413084-01-1 Date: 2011-07-13 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA 10.4.5/01	Rexer, H. U.	2012a	Assessment of side effects on the honeybee (Apis mellifera L.), exposed to winter oil-seed rape, grown from seeds treated with BYI 02960 FS 480 G and sequentially sprayed with BYI 02960 SL 200 G during immediate pre- and full flowering in a long-term field study in Northern Germany Source: Eurofins Agroscience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Edition Number: M-438818-01-1 Date: 2012-09-27 GLP: yes, unpublished	Yes	BCS
KIIIA 10.4.5/02	Rexer, H. U.	2012b	Assessment of side effects on the honeybee (Apis mellifera L.), exposed to winter oil-seed rape, grown from seeds treated with BYI 02960 FS 480 G and sequentially sprayed with BYI 02960 SL 200 G during immediate pre- and full flowering in a long-term field study in France Source: Eurofins Agroscience Services GmbH, Niefern-Oeschelbronn, Germany Edition Number: M-438819-01-1 Date: 2012-07-24 GLP: yes, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. claimed	Owner
KIIIA1 10.4.6.1 /01	Nikolakis, A.; Theis, M.; Przygoda, D.	2011	BYI 02960 tech.: Effects of exposure to spiked diet on honeybee larvae (Apis mellifera carnica) in an in vitro laboratory testing design Bayer CropScience, Report No.: E 318 3897-9, Edition Number: M-406645-01-2 Date: 2011-05-02 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA1 10.4.7 /01	Schnorbach, H. J.	2012a	Evaluation of the effects of BYI 02960 SL 100 on honey bees (Apis mellifera) in a semifield tunnel test in full-flowering Phacelia tanacetifolia Bayer CropScience, Report No.: IA08DVG048G001, Edition Number: M-427040-01-1 Date: 2012-03-15 GLP/GEP: no, unpublished	Yes	BCS
KIIIA1 10.4.7 /02	Schnorbach, H. J.	2012b	Evaluation of the effects of BYI 02960 SL 200 on honey bees (Apis mellifera) in a semifield tunnel test in full-flowering Phacelia tanacetifolia Bayer CropScience, Report No.: IA09DVG051K619, Edition Number: M-427046-01-1 Date: 2012-03-15 GLP/GEP: no, unpublished	Yes	BCS
KIIIA1 10.4.7 /03	Rentschler, S.	2012a	BYI 02960 SL 200 G: A semi-field study in Germany 2009 to evaluate effects of spray applications in Phacelia tanacetifolia on the honeybee Apis mellifera L. (Hymenoptera, Apidea) Eurofins Agroscience Services EcoChem GmbH, Niefern-Öschelbronn, Germany Bayer CropScience, Report No.: S09-00854, Report includes Trial Nos.: S09-00854-01_BZEU Edition Number: M-425576-01-2 Date: 2012-02-17 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA1 10.4.7 /04	Proebsting, A.	2012a	BYI 02960 SL 200 G: A semi-field study in Denmark 2010 to evaluate effects of spray applications in Phacelia tanacetifolia on the honeybee Apis mellifera L. (Hymenoptera, Apidae) Eurofins Agroscience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Bayer CropScience, Report No.: S10-01954, Report includes Trial Nos.: S10-01954-01_BZEU Edition Number: M-423156-01-2 Date: 2012-01-19 GLP/GEP: yes, unpublished	Yes	BCS

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KIIIA1 10.4.7 /05	Proebsting, A.	2012b	BYI 02960 SL 200 G: A semi-field study in Italy 2011 to evaluate effects of spray applications in Phacelia tanacetifolia on the honeybee Apis mellifera L. (Hymenoptera, Apidae) Eurofins Agroscience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Bayer CropScience, Report No.: S10-01955, Report includes Trial Nos.: S10-01955-01-BZEU Edition Number: M-423172-01-2 Date: 2012-01-18 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA1 10.4.7 /06	Rentschler, S.	2012b	Determination of side-effects of BYI 02960 SL 200 G on honey bee (Apis mellifera L.) brood under semi-field conditions Eurofins Agroscience Services, EcoChem GmbH, Niefern-Oeschelbronn, Germany Bayer CropScience, Report No.: S10-03819, Report includes Trial Nos.: S10-03819-01 Edition Number: M-427438-01-1 Date: 2012-03-19 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA1 10.5.1 /01	Jans, D.	2010	Toxicity to the parasitoid wasp Aphidius rhopalosiphi (DESTEPHANI-PEREZ) (Hymenoptera: Braconidae) using a laboratory test; BYI 02960 SL 200 g/L Bayer CropScience, Report No.: CW09/079, Edition Number: M-366965-01-2 Date: 2010-04-15 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA1 10.5.1 /02	Jans, D.	2010	Toxicity to the predatory mite Typhlodromus pyri SCHEUTEN (Acari, Phytoseiidae) using a laboratory test; BYI 02960 SL 200 g/L Bayer CropScience, Report No.: CW09/073, Edition Number: M-366957-01-2 Date: 2010-04-15 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA1 10.5.2 /01	Jans, D.	2010	Toxicity to the parasitoid wasp Aphidius rhopalosiphi (DESTEPHANI-PEREZ) (Hymenoptera: Braconidae) using an extended laboratory test on barley BYI 02960 SL 200 (g/L) Bayer CropScience, Report No.: CW09/083, Edition Number: M-366970-01-2 Date: 2010-04-13 GLP/GEP: yes, unpublished	Yes	BCS

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KIIIA1 10.5.2 /02	Jans, D.	2010	Toxicity to the predatory mite Typhlodromus pyri SCHEUTEN (Acari, Phytoseiidae) using an extended laboratory test on Phaseolus vulgaris BYI 02960 SL 200 (g/L) Bayer CropScience, Report No.: CW09/076, Edition Number: M-366968-01-2 Date: 2010-04-13 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA1 10.5.2 /03	Jans, D.	2010	Toxicity to the ladybird beetle Coccinella septempunctata L. (Coleoptera, Coccinellidae) using an extended laboratory test on Phaseolus vulgaris; BYI 02960 SL 200 (g/L) Bayer CropScience, Report No.: CW09/074, Edition Number: M-384754-01-2 Date: 2010-06-30 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA1 10.5.2 /04	Jans, D.	2010	Chronic toxicity (ER50) of BYI 02960 SL 200 (g/L) to the rove beetle Aleochara bilineata Gyll. (Coleoptera: Staphylinidae) under extended laboratory conditions Bayer CropScience, Report No.: CW09/072, Edition Number: M-384433-01-2 Date: 2010-06-30 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA1 10.5.3 /01	Jans, D.	2010	Toxicity to the parasitoid wasp Aphidius rhopalosiphi (DESTEPHANI-PEREZ) (Hymenoptera: Braconidae) in an extended laboratory test (under semi-field conditions aged residues on Zea mays) BYI 02960 SL 200 (g/L) Bayer CropScience, Report No.: CW10/018, Edition Number: M-396372-01-2 Date: 2010-11-30 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA1 10.5.3 /02	Schwarz, A.	2010	Effects of BYI 02960 SL 200 (g/L) on the predatory bug Orius laevigatus extended laboratory study - aged residue test IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 58691053, Edition Number: M-394033-01-2 Date: 2010-10-28 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA1 10.5.4 /01	Aldershof, S.; Bakker, F.	2012	A field study to assess the effects of BYI 02960 SL 200 g/L on the non-target, surface- and plantdwelling, arthropod fauna of a grassland habitat (off-crop) in The Netherlands during summer MITOX Consultants, Amsterdam, The Netherlands Bayer CropScience, Report No.: B154FFN, Edition Number: M-425092-01-2 Date: 2012-02-17 GLP/GEP: yes, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. claimed	Owner
KIIIA1 10.5.4 /02	Aldershof, S.; Bakker, F.	2012	A field study to assess the effects of BYI 02960 (SL 200 g/L) on the non-target, surface- and plant-dwelling arthropod fauna of a grassland habitat (off-crop) in SW France during summer MITOX Consultants, Amsterdam, The Netherlands Bayer CropScience, Report No.: B153FFN, Edition Number: M-425080-01-2 Date: 2012-02-17 GLP/GEP: yes, unpublished	Yes	BCS
Q 15 /01 (relevant for KIIIA 10.5.4)	Neumann, P.	2014	Extended summaries of the non-target arthropod off-crop field studies conducted for flupyradifurone SL 200 (including the evaluation of the EU RMS) Bayer CropScience, Report No.: M-496127-01-1, Edition Number: M-496127-01-1 Date: 2014-09-09 GLP/GEP: n.a., unpublished	Yes	BCS
KIIIA1 10.6.2 /01	Leicher, T.	2010	BYI 02960 SL 200 G: acute toxicity to earthworms (Eisenia fetida) tested in artificial soil with 5 percent peat Bayer CropScience, Report No.: LRT/RG-A-143/10, Edition Number: M-397720-01-2 Date: 2010-12-14 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA1 10.6.3 /01	Leicher, T.	2010	BYI 02960 SL 200 G: Effects on survival, growth and reproduction on the earthworm Eisenia fetida tested in artificial soil Bayer CropScience, Report No.: LRT-RG-R-76/09, Edition Number: M-392964-01-2 Date: 2010-10-21 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA1 10.6.4 /01	Menke, U.	2012	BYI 02960 SL 200 G: Effects on the earthworm fauna of a grassland area within one year Bayer CropScience, Report No.: MNU/RG-F-8/12, Edition Number: M-426607-01-1 Date: 2012-03-07 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA1 10.6.6 /01	Frommholz, U.	2009	BYI 02960 SL 200 G: Influence on the reproduction of the collembola species Folsomia candida tested in artificial soil with 5 % peat Bayer CropScience, Report No.: FRM-COLL-75/09, Edition Number: M-359728-01-2 Date: 2009-12-02 GLP/GEP: yes, unpublished	Yes	BCS

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP/GEP status (where relevant), published or not	Data protect. claimed	Owner
KIIIA1 10.6.6 /02	Kratz, M.-A.	2009	BYI 02960 SL 200 G: Influence on mortality and reproduction on the soil mite species Hypoaspis aculeifer tested in artificial soil with 5 % peat Bayer CropScience, Report No.: KRA-HR-19/09, Edition Number: M-358752-01-2 Date: 2009-11-10 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA1 10.7.1 /01	Frommholz, U.	2010	BYI 02960 SL 200 G : Determination of effects on carbon transformation in soil Bayer CropScience, Report No.: FRM-C-116/10, Edition Number: M-395469-01-2 Date: 2010-11-23 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA1 10.7.1 /02	Frommholz, U.	2010	BYI 02960 SL 200 G: Determination of effects on nitrogen transformation in soil Bayer CropScience, Report No.: FRM-N-148/10, Edition Number: M-396112-01-2 Date: 2010-11-29 GLP/GEP: yes, unpublished	Yes	BCS
KIIIA1 10.8.1.2 /01	Gosch, H.	2010	BYI 02960 SL 200 g/L - Effects on the vegetative vigour of eleven species of non-target terrestrial plants (Tier 1) Bayer CropScience, Report No.: VV 10/002, Edition Number: M-397734-01-2 Date: 2010-12-14 GLP/GEP: yes, unpublished	Yes	BCS
KIII A, 10.8.1.3 /01	Gosch, H.	2010	BYI 02960 SL 200 g/L - Effects on the seedling emergence and growth of eleven species of non-target terrestrial plants (Tier 1) Bayer CropScience, Report No.: SE10/001, Edition Number: M-397727-01-2 Date: 2010-12-14 GLP/GEP: yes, unpublished	Yes	BCS

European Commission

Flupyradifurone

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ANNEX C

Rapporteur Member State: The Netherlands

November 2014

~~January 2014~~

Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible approval of flupyradifurone under Regulation (EC) 1107/2009

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