Document Title

Document N - Tier 3 Summary of the Active Substance for

BYI 02960 (Flupyradifurone)
and its formulation
Sivanto (Flupyradifurone, BYI 02960) SL 200

Data Requirements

Regulation (EC) No 1107/2009
Regulatory Directive 2003-01/Canada/PMRA
OPPTS guidelines/US/EPA

Document N

According to OECD format guidance for industry data submissions on plant protection products and their active substances

Date

2012-05-02

Author(s)

Kwiatkowski, Pat; Rauen, Hans Werner; Spiegel, Kathrin; Garside, Christina M.

Bayer CropScience

M-429792-01-1

OWNERSHIP STATEMENT

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Chapter 1 The active substance, its properties, uses, proposed classification and labelling

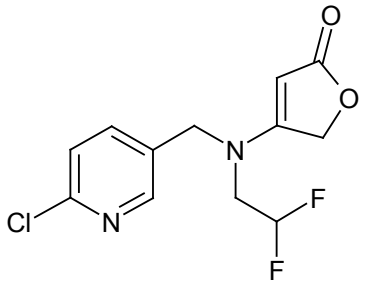
1.1 Identity of the active substance and preparations containing it

All relevant information and data concerning the identity of BYI 02960 (flupyradifurone) and the formulated product Sivanto® SL 200 have been provided in the relevant Tier II summaries except the confidential information which is included in Document J.

Active substance:

Company code: BYI 02960

Structural formula:



ISO common name: flupyradifurone

Chemical name:
IUPAC: 4-[[(6-chloropyridinyl)methyl](2,2-difluoroethyl)amino]furan-2(5H)-one
CAS: 2(5H)-furanone, 4-[[(6-chloro-3-pyridinyl)methyl](2,2-difluoroethyl)amino]-

CAS #:	951659-40-8
Empirical formula:	C12 H11 Cl F2 N2 O2
Molecular weight:	288.68 g/mol

1.2 Physical and chemical properties

Pure BYI 02960 is a white powder with no characteristic odour and with a melting point at atmospheric pressure of 69.0 °C, while the technical grade a.s. is a pale beige powder with a solvent odour. BYI 02960 has no boiling point; pure a.s decomposes at a temperature of 270 °C and technical grade a.s. at a temperature of 245 °C; for technical grade, exothermal decomposition occurs in the temperature range of 245 – 400 °C with a maximum overall energy of 836-938 J/g. The vapour pressure of purified BYI 02960 was determined to be 9.1 x 10^{-7} Pa at 20 °C. Henry's law constant at 20 °C was calculated to be 8.2 x 10^{-8} Pa x m^3/mol at pH 4, 7 and 9. The water solubility of pure active substance at 20.0 °C was found to be 3.2 g/L at pH 4 and 7 and 3.0 g/L at pH 9. The pH values for 1%

aqueous suspensions of pure a.s. and technical grade a.s. in water were 6.6 and 6.2, respectively. No dissociation in water was observed in a pH range of 1 to 12.

The solubility of BYI 02960 in organic solvents at 20 °C was found to be 0.0005 g/L in heptane, 3.7 g/L in toluene and >250 g/L in dichloromethane, acetone, ethyl acetate and dimethyl sulfoxide.

The logP value at 25 °C was 1.2 for BYI 02960, independent of pH. With a log Pow well below 3, concentration in environmental compartments is not expected. BYI 02960 is hydrolytically stable at ambient temperature from pH 4 to 9. Even after 5 days at 50 °C, less than 5% degradation was observed. Under aqueous photolysis conditions, BYI 02960 degrades quickly with a calculated half-life of 1.75 days at latitude 33.3º N. Based on the Atkinson calculation, the half-life in air is expected to be short; there is little risk of long term transport.

BYI 02960 is not flammable, autoflammable or explosive. It is not surface active and has no oxidizing or reducing properties. Technical grade material is stable in the presence of metal ions; it was also shown to be stable in polypropylene and polyethylene containers after 24 months at ambient temperature. Overall, risk during transportation is minimal.

The plant protection product Sivanto® SL 200 appears as a clear brown liquid and has weak odour. The preparation has a flash point greater than 100 °C and a self ignition temperature of 420 °C. Sivanto® SL 200 is not explosive and has no oxidizing properties. The pH value of 5.4 is within the range that naturally occurs in soil. Sivanto® SL 200 was proven stable in accelerated storage stability testing at 54°C; storage stability under practical and commercial conditions will be acceptable. Overall, the technical properties of Sivanto® SL 200 indicate that no problems are anticipated when used according to label directions. Risks during transport of product are minimal.

1.3 Details of uses and further information

BYI 02960 is a new xylem mobile insecticide that is very effective against aphids, leafhoppers, whiteflies, scales and psyllids. The BYI 02960 formulation Sivanto® SL 200 is intended to be used in agriculture on a wide range of crops such as coffee, cocoa, cotton, citrus, pome and stone fruits, grapes, fruiting and leafy vegetables (indoor and outdoor), and hops. Seed treatment for soybean and cereals is also being developed, as are uses on various ornamentals plants.

In the EU the representative uses for Annex-1-inclusion consist of lettuce as a low and hops as a high crop.

1.4 Classification and labelling

1.4.1 Classification and labelling a.i

European Union
Based on the toxicological and eco-toxicological data presented in the dossier and summarized in the Material Safety Data Sheet for the active substance, the following risk phrases and hazard symbols are proposed for the classification and labelling of the active substance BYI 02960 in the EU:

According to EC Directive 1999/45/EC:

Hazard symbol(s):	Xn, N
Indications of danger	Harmful, dangerous for the environment

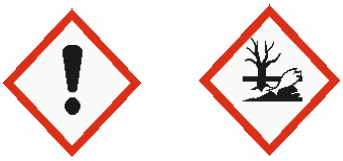
Risk phrases:	22	Harmful if swallowed
	50/53	Very toxic to aquatic organisms may cause long-term adverse effects in the aquatic environment

Safety phrases:	60	This material and its container must be disposed of as hazardous waste
	61	Avoid release to the environment. Refer to special instructions (safety data sheets)

According to Regulation (EC) No. 1272/2008 on classification, labelling and packaging of substances and mixtures, as amended:

Classified as hazardous for supply/use

Risk symbols:



Signal Word: Warning

Hazard Statements:	H302	Harmful if swallowed
	H400	Very toxic to aquatic life
	H410	Very toxic to aquatic life with long lasting effects
Precautionary Statements:	P273	Avoid release to the environment
	P301+ P312	IF SAWLLOWED: call a poison centre or doctor/physician if you feel unwell
	P501	Dispose of contents/container in accordance with local regulation

1.4.2 Classification and labelling PPP

European Union

Based on the toxicological and eco-toxicological data presented in the dossier and summarized in the Material Safety Data Sheet for the formulated product, the following risk phrases and hazard symbols are proposed for the classification and labelling of Sivanto® SL 200 in the EU:

According to EC Directive 1999/45/EC:

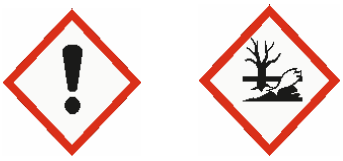
Hazard symbol(s):	Xn, N
Indications of danger	Harmful, dangerous for the environment

Risk phrases:	20	Harmful by inhalation
	43	May cause sensitization by skin contact
	50/53	Very toxic to aquatic organisms may cause long-term adverse effects in the aquatic environment
Safety phrases:	2	Keep out of reach of children
	13	Keep away from food, drink and animal feeding stuffs
	20/21	When using do not eat, drink or smoke
	24	Avoid contact with skin and eyes
	35	This material and its container must be disposed in a safe way
	37	Wear suitable gloves
	57	Use appropriate container to avoid environmental contamination

According to Regulation (EC) No. 1272/2008 on classification, labelling and packaging of substances and mixtures, as amended:

Classified as hazardous for supply/use

Risk symbols:



Signal Word: Warning

Hazard Statements:

H332	harmful if inhaled
H317	may cause an allergic skin reaction
H410	Very toxic to aquatic life with long lasting effects
EUH410	To avoid risk to human health and the environment, comply with the instructions for use

Precautionary Statements:

P280	Wear protective gloves/protective clothing/eye protection/ face protection
P304 + P340	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing
P309 + P311	If exposed or if you feel unwell: Call a POISON CENTER or a physician
P501	Dispose of contents/container in accordance with local regulation

Chapter 2 Methods of analysis

2.1 Methods for analysis of the active substance as manufactured

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. The limit of quantification is 0.5 g/kg (0.05%) for each impurity except for water (LOQ=1.0 g/kg)

2.2 Methods for formulation analysis

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.

2.3 Methods for residue analysis

2.3.1 Multi-residue methods for residue analysis

The residue definition for enforcement is proposed as parent plus difluoroacetic acid (DFA) in plant and animal matrices. However, only parent can be determined by the European multi-residue methods DFG S19 or QuEChERS or by the US FDA multi-residue method. None of the extraction processes described in either of the methods would allow appropriate extraction of DFA. Thus, a specific method was developed for enforcement purposes.

2.3.2 Methods for residue analysis of plants and plant products

The LC-MS/MS method 01330 was developed as an enforcement method for the determination of BYI 02960 and difluoroacetic acid (DFA) residues in/on plant substrates. Residues are extracted from plant matrix twice using a mixture of acetonitrile/water with formic acid (2.2 mL/L). After dilution, an aliquot of the raw extract is filtered and analysed by reversed HPLC and electrospray MS/MS; residues are quantified against standards in matrix. The method was successfully validated for specificity, linearity, recovery rates/accuracy, precision/repeatability and interferences/matrix effects. The limit of quantitation (LOQ) for BYI 02960, defined as the lowest validated fortification level, was 0.01 mg/kg in all matrices tested, except hop cones, in which it was 0.05 mg/kg. For DFA, the LOQ was 0.02 mg/kg in all matrices, except hop cones, where it was 0.10 mg/kg. The calculated limit of detection (LOD) was estimated to be at least 3 times lower than the respective LOQ, based on the linearity response data and matrix interference observed in control samples.

The method was also successfully validated in an independent laboratory for specificity, linearity, recovery rates/accuracy, precision/repeatability and interferences/matrix effects. The method meets all guideline criteria to determine the residues of BYI 02960 and metabolite DFA in/on plant matrices at 0.01 and 0.02 mg/kg parent equivalents, respectively.

Summary of validated matrices and corresponding LOQs

Analyte	Matrix	LOQ [mg/kg]
Enforcement method 01330		
BYI 02960	lettuce (head) rape (seed) orange (fruit) wheat (grain) hop (cone)	0.01 0.01 0.01 0.01 0.05
difluoroacetic acid	lettuce (head) rape (seed) orange (fruit) wheat (grain) hop (cone)	0.02 0.02 0.02 0.02 0.10
ILV of method 01330		
BYI 02960	lettuce (head) orange (fruit) wheat (grain)	0.01 0.01 0.01
difluoroacetic acid	lettuce (head) orange (fruit) wheat (grain)	0.02 0.02 0.02

2.3.3 Methods for residue analysis of food of animal origin

Method 01214 was developed as an enforcement method for animal matrices. It determines BYI 02960 and its metabolite difluoroacetic acid (DFA) by HPLC-MS/MS. Residues are extracted from animal matrix twice using a mixture of acetonitrile/water. For fat and milk, n-heptane is used for an additional extraction. After addition of formic acid and dilution, an aliquot of the raw extract is filtered and quantification performed by reversed HPLC and electrospray MS/MS against standards in matrix. The method was successfully validated for specificity, linearity, recovery rates/accuracy, precision/repeatability and interferences/matrix effects. The LOQs established for BYI 02960 and DFA in all matrices are 0.01 and 0.02 mg/kg parent equivalents, respectively. LODs were estimated to be 0.003 and 0.006 mg/kg parent equivalents for BYI 02960 and metabolite DFA, respectively.

The method was also successfully validated in an independent laboratory for specificity, linearity, recovery rates/accuracy, precision/repeatability and interferences/matrix effects. The method meets all guideline criteria to determine the residues of BYI 02960 and metabolite DFA in/on animal matrices at 0.01 and 0.02 mg/kg parent equivalents, respectively.

2.3.4 Methods for residue analysis of soil

Modification M001 to the analytical method 01074 was developed for the determination of BYI 02960 and its metabolites DFA and 6-CNA in soil. The method was validated using three different soils. The limit of quantification (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.

Residues are extracted from soil samples of ca. 20 g 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 BYI 02960-DFA are eliminated by using an internal standard solution of isotopically labelled reference items which is added to the sample solutions after extraction. A subsample is filtered to

remove fine particles of the soil, and identification and quantification of parent compound and metabolite DFA is accomplished 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. Recoveries for each fortification level were in an acceptable range (70 - 110%).

This method was successfully validated according to the European requirements (96/46/EC of 16 July 1996 amending Directive 91/414/EEC), the Guidance Document on Residue Analytical Methods (SANCO/825/00 rev.7 of March 17, 2004) and BBA Guideline for Residue Analytical Methods of July 21, 1998 and is therefore recommended for monitoring of BYI 02960 residues in soil.

2.3.5 Methods for residue analysis of water

The method MR-12/022 (M-428019-01-1) was developed for determination of BYI 02960 in surface and drinking water with an estimated limit of quantification of 0.05 µg/L.

2.3.6 Methods for residue analysis of air

A method was developed for determination of BYI 02960 in air with a limit of quantification of $\leq 7 \mu g/m^3$.

Principle of the method: The air to be analysed is passed through XAD adsorption tubes at a flow rate of about 0.83 L/min for 6 hours (total volume of ~0.3 m^3). Following sampling, the adsorptive material in the tubes is extracted with acetonitrile, the extract diluted into water with 0.1% formic acid and by liquid chromatography with tandem mass spectrometric detection (LC-MS/MS), monitoring two daughter ion transitions. The method was successfully validated at the LOQ and 10 fold the LOQ (7 and 70 $\mu g/m^3$, respectively).

Chapter 3 Impact on human and animal health

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

3.1.1 Absorption, distribution, metabolism and excretion

The absorption, distribution, metabolism, and excretion (ADME) of BYI 02960 have been studied in rats following a single oral or i.v. administration of [^{14}C]BYI 02960 labelled in the furanone ring, the pyridinylmethyl bridge or the difluoroethyl side chain. In total, eight ADME experiments have been performed:

Experiment	Label	Dose, Route	Sex	Duration (hours)	Comment
1	[Pyridinylmethyl-^{14}C]	2 mg a.s./kg bw, Oral	Male	72	Low Dose, Male
2	[Pyridinylmethyl-^{14}C]	2 mg a.s./kg bw, Oral	Female	72	Low Dose, Male
3	[Pyridinylmethyl-^{14}C]	200 mg a.s./kg bw, Oral	Male	72	High Dose, Male
4	[Pyridinylmethyl-^{14}C]	200 mg a.s./kg bw, Oral	Female	72	High Dose, Male
5	[Pyridinylmethyl-^{14}C]	2 mg a.s./kg bw, Intravenous	Male	72	Low Dose, Male
6	[Furanone-4-^{14}C]	2 mg a.s./kg bw, Oral	Male	168	Low Dose, Male
7	[Furanone-4-^{14}C]	2 mg a.s./kg bw, Oral	Female	168	Low Dose, Male
8	[Ethyl-1-^{14}C]	2 mg a.s./kg bw, Oral	Male	72	Low Dose, Male

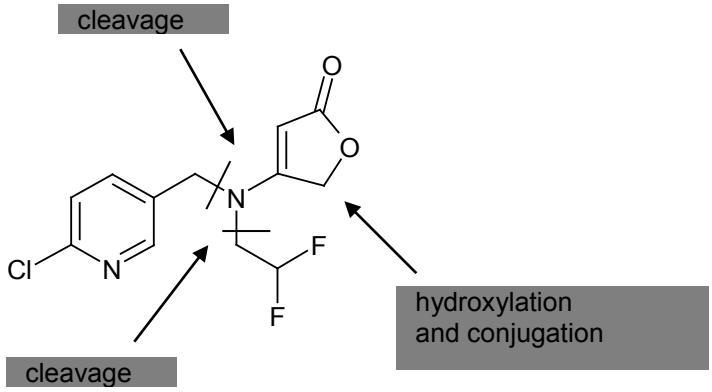
Absorption of [^{14}C]BYI 02960 following oral administration was rapid, reaching peak plasma concentrations (C_{max}) between 1 and 4 hours after oral administration; in total, >80% of the orally administered dose was absorbed independent of the label position. Plasma level decline in male rats receiving [^{14}C]BYI 02960 by i.v. injection was essentially identical from 8 to 72 hours after administration to that of males that were dosed orally.

Excretion of radioactivity was rapid, being nearly complete within 24-48 hours after administration. Only after administration of [furanone-4-^{14}C]BYI 02960 between 1 and 3% of the administered radioactivity was exhaled. This demonstrated that for a small portion of the dose the furanone ring of the molecule obviously was opened and underwent biotransformation to C1 fragments. The main route of excretion was renal. Female rats had slightly higher renal excretion rate than male rats. Comparison of the excretion patterns in male rats from oral vs. i.v. experiments shows equivalent urinary excretion levels and rates; as expected, faecal excretion was somewhat higher in rats receiving [^{14}C]BYI 02960 orally.

In all experiments, only 0.1 to 4% of the administered dose remained in the tissues and gastrointestinal tract (GIT) at 72 or 168 hours after oral administration. As expected for a compound with a log P_{OW} of 1.2, overall retention in tissues was low. The radioactivity remaining in the tissues from the low dose experiments following oral dosing were negligible (≤0.016 mg/kg). In the high dose experiments, the highest radioactive residues were found in the red blood cells, GIT, liver and kidney after oral administration of [pyridinylmethyl-^{14}C]BYI 02960 at a dose level of 200 mg/kg bw.

In urine, the parent compound was the predominant component, representing 36 – 74% of the administered dose. Metabolites identified in urine were BYI 02960-OH and its conjugates, BYI 02960-OH-gluA and BYI 02960-OH-SA and the cleavage products BYI 02960-hippuric acid, BYI 02960-des-difluoroethyl, 6-CNA, DFA, BYI 02960-difluoroethyl-amino-furanone. Additionally BYI 02960-iso-OH was identified at minor amounts. BYI 02960-OH and parent compound were the major components found in faeces.

The following figure shows the sites of the molecule involved in the major metabolic reactions:

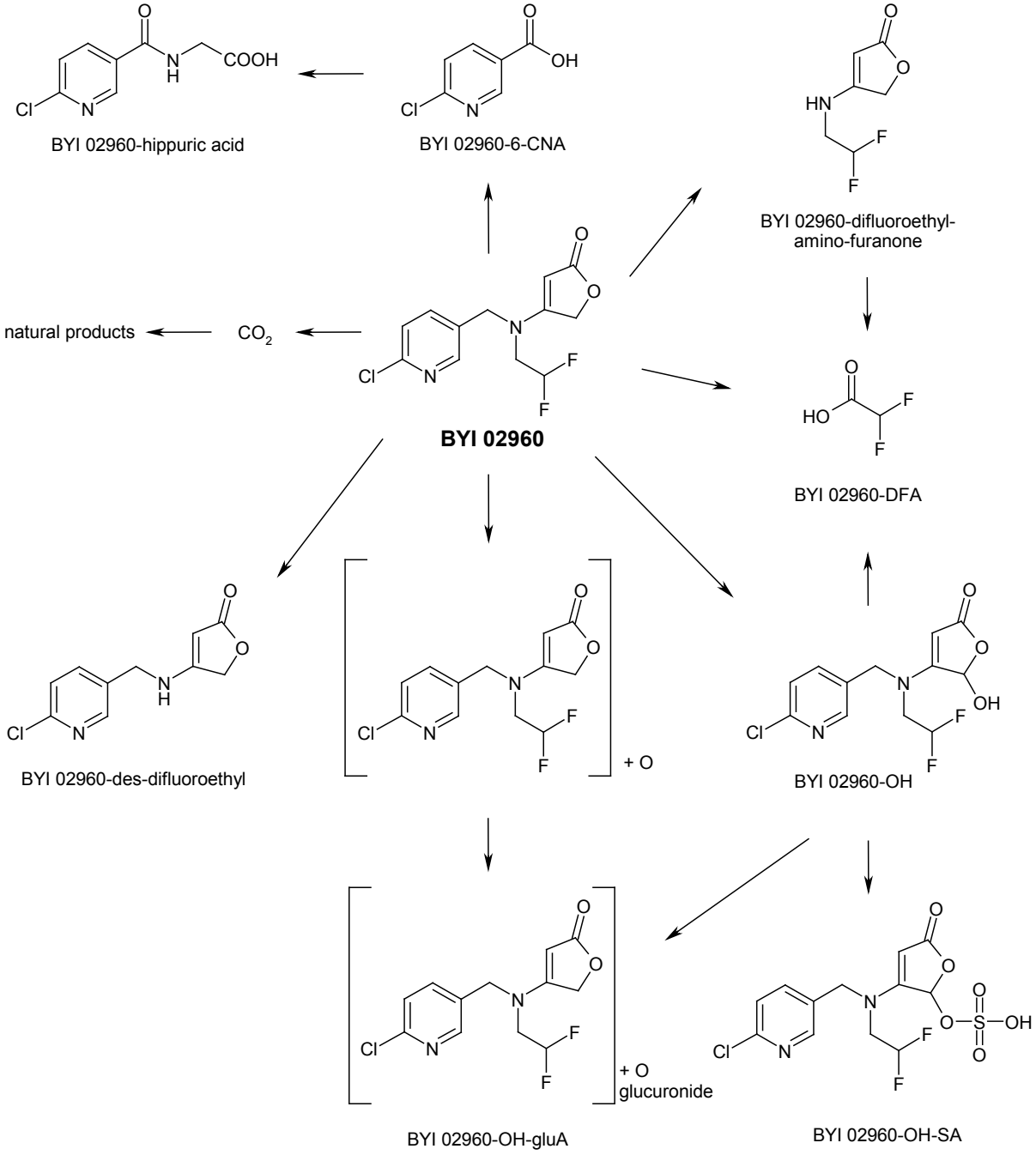


In summary, the principal metabolic reactions of BYI 02960 in rats were:

- hydroxylation followed by conjugation with glucuronic acid or sulfate,
- cleavage of the difluoroethyl group forming BYI 02960-des-difluoroethyl and difluoroacetic acid (DFA),
- cleavage of the molecule at the pyridinylmethylene bridge forming BYI 02960-difluoroethyl-amino-furanone and BYI 02960-6-CNA, which was further conjugated with glycine to BYI 02960-hippuric acid

The metabolic pathway of BYI 02960 in the rat is shown below.

Proposed metabolic pathway of BYI 02960 in the rat



In addition, quantitative whole body autoradiography (QWBA) experiments were performed by administering a single oral dose of [furanone-4-^{14}C]- or [pyridinylmethyl-^{14}C]BYI 02960 to male and female rats at a dose rate of 5 mg a.s./kg body weight. Excretion rates and patterns were essentially the same as observed in the ADME studies. Any accumulation or significant retention of [^{14}C]BYI 02960 residues in male and female rats can be excluded, except incorporated C1- or C2 fragments resulting from metabolic degradation of the furanone moiety.

Organ metabolism studies were also conducted. In a first organ metabolism study, male and female rats were orally administered [furanone-4-^{14}C]BYI 02960. Total radioactivity was determined in urine

for the time period 0 – 6 h as well as in plasma, liver, kidney, muscle (leg) and fat (perirenal) at sacrifice. The metabolism was investigated in urine and plasma, as well as in extracts of liver, kidney, muscle, and fat.
Over the 0 – 6 h collection period, the renal excretion in female rats was slightly higher than in male rats; parent compound was the largest radioactive component (approx. 22% of the dose in males and 38% in females). In tissues, 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 expected for 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 muscle. In all samples of plasma, organs and tissues, BYI 02960 was by far the largest component, accounting for more than 72% of the total radioactivity. No metabolite accounted for more than 12% of the total radioactivity. Metabolism was qualitatively similar in male and female rats with some quantitative differences resulting from higher degradation in males.

In the second organ metabolism study, male and female rats were orally administered a single dose of 3 mg/kg [ethyl-1-^{14}C]BYI 02960. Animals were sacrificed 1 h, 6 h, and 24 h after dosing. The total radioactivity was determined at different time points in urine; determinations in plasma, liver, kidney, muscle and fat were made only at sacrifice. Metabolism was investigated in urine, plasma, and in extracts of liver, kidney, muscle, and fat.

Parent compound was the main constituent observed in urine at all time points. In organs, plasma and tissues taken 24 hours after administration, difluoroacetic acid (DFA) was by far the dominant metabolite, accounting for more than 50% of the radioactivity.

3.1.2 Acute toxicity

The acute toxicity of active substance BYI 02960 was low for all routes evaluated (oral, dermal and inhalation). The oral LD_{50} for rats was equal to 2000 mg/kg body weight (bw). The rat acute dermal LD_{50} was > 2000 mg/kg bw. The rat acute inhalation LC_{50} (4-hour) was > 4671 mg/m^3, the highest achievable concentration.

BYI 02960 was not irritating to rabbit skin and caused only slight eye irritation (redness of the conjunctivae) which reversed within 48 hours. No evidence of skin sensitization (delayed contact hypersensitivity) was seen in a modified LLNA in NMRI mice.

Results and proposed classification according to OECD criteria are shown below.

Type of study	Species	Results	OECD Classification
Oral LD_{50}	Rat	4/6 mortalities observed at 2000 mg/kg; none at 300 mg/kg	Category 4 (LD_{50} cut off = 2000 mg/kg)
Dermal LD_{50}	Rat	LD_{50} > 2 000 mg/kg,	Category 5 / Unclassified
Inhalation LC_{50}	Rat	4 hour LC_{50} > 4671 mg/m^3	Category 5 / Unclassified
Primary skin irritation	Rabbit	Non irritating	Category 5 / Unclassified
Eye irritation	Rabbit	Slight redness of conjunctivae, reversed within 48 hours	Category 5 / Unclassified
Skin sensitization	Mouse	Non sensitising	Category 5 / Unclassified

The acute toxicity for Sivanto® SL 200 is also low for all routes tested with rat acute oral and dermal LD_{50} values of >2000 mg/kg and an inhalation LC_{50} of >3496 mg/ m^3. There were no signs of skin or eye irritation in rabbits. Sivanto® SL 200concentrated was found to be very slightly sensitising in the modified LLNA in NMRI mice.

Results and proposed classification according to OECD criteria are shown below. Sivanto® SL 200 requires the following classification for toxicity according to GHS criteria.

Type of study	Species	Results	OECD Classification
Oral LD_{50}	Rat	LD_{50} > 2000 mg/kg	Category 5/ Unclassified
Dermal LD_{50}	Rat	LD_{50} > 2 000 mg/kg,	Category 5 / Unclassified
Inhalation LC_{50}	Rat	4 h LC_{50} females = 3496 mg/m^3 males >4483 mg/m^3	Xn, R20, Category 4
Primary skin irritation	Rabbit	Non irritating	Category 5 / Unclassified
Eye irritation	Rabbit	Non irritating	Category 5 / Unclassified
Skin sensitization	Mouse	Very slightly sensitising	Xi, R43, Category 2, H317

3.1.3 Genotoxicity

Technical grade BYI 02960 was tested in a standard battery of *in vitro* and *in vivo* genotoxicity studies and mutagenicity tests carried out according to the current OECD and European guidelines in compliance with GLP requirements. There was no indication of gene mutation in either the presence or absence of metabolic activation in either the bacterial reverse mutation or mammalian gene mutation tests. The *in vitro* chromosome aberration test and the *in vivo* mouse micronucleus tests were also both negative. These studies demonstrate that BYI 02960 has no genotoxic potential.

The results of genotoxicity studies on parent compound are summarized below.

Mutagenicity tests with BYI 02960	Metabolic Activation	Results
A. *In vitro* tests		
Ames Test (M-354173-01-1)	+/-	Negative
Ames Test (M-420539-02-1)	+/-	Negative
Chromosome aberrations (V79 cells) (M-359746-01-1)	+/-	Negative
HPRT Test (V79 cells) (M-359743-01-1)	+/-	Negative
B. *In vivo* tests	Dose levels	
Micronucleus Test in male mice – oral administration (M-353785-01-1)	10, 20 and 40 mg/kg	Negative
Micronucleus Test in female mice – oral administration (M-420536-01-1)	12.5, 25 and 50 mg/kg	Negative

Genotoxicity studies on select plant and environmental metabolites have also been performed. None of the compounds has been shown to have any genotoxic potential.

3.1.4 Sub-chronic and chronic toxicity

Overview:
BYI 02960 belongs to the butenolide chemical class and acts as a nicotinic acetylcholine receptor agonist. As such, the insecticidal mode of action is reflected in animal studies where, at sufficiently high doses, signs of effects on the central nervous system are observed.

Short term and sub-chronic toxicity studies have been conducted with BYI 02960. Range finding (28 day) studies were performed in rat, mouse and dog to establish appropriate dose levels in the 90 day studies. Results across species indicate that liver is a common target organ in all species, kidney is affected in the mouse and the dog as well as thyroid in rat and skeletal muscle in dog (observed in both 90 day and 1 year dog studies at dietary dose levels of 1200 and 1000 ppm, respectively, indicating no increase in severity occurs with longer term exposure) The lowest subchronic NOAEL is 12 mg/kg/day.

Summary of BYI 02960 short-term toxicity

Studies (Dose levels in feed)	NOEL/NOAEL		LOAEL		Adverse effects at high dose levels
	ppm	mg/kg/d	ppm	mg/kg/d	
28-day rat study 0, 75, 200 & 350 mg/kg/day	-	75	-	200	Liver: centrilobular hepatocellular hypertrophy, both sexes Thyroid: Minimal diffuse follicular cell hypertrophy in males only at 200 mg/kg/day
28-day rat study 0, 500 & 5000 ppm	500	33.6	5000	385	Liver: slight to moderate diffuse centrilobular hepatocellular hypertrophy Thyroid: Minimal to slight diffuse follicular cell hypertrophy Decreased T4, increased TSH, BROD and UDPGT inductions
90-day rat study 0, 100, 500 & 2500 ppm	500	30/38	2500	156/186	Liver: centrilobular hepatocellular hypertrophy in both sexes Thyroid: follicular cell hypertrophy in males only
28-day mouse study 0, 300, 600 & 1200 ppm	960 to 1080	166 to 186	>960 to 1080	>166 to 186	Only slight body weight decrease
90-day mouse study 0, 100, 500 & 2500 ppm	500	80.6/98.1	2500	407/473	Liver: increased diffuse hepatocellular vacuolations Kidney: decreased multifocal/diffuse Corticoepithelial vacuolation
28-day dog study 0, 500, 2000 & 4000 ppm	2000	62/77	4000	118/131	Liver: centrilobular glycogen accumulation decreased in incidence and/or severity

Table continued on next page…

Studies (Dose levels in feed)	NOEL/NOAEL		LOAEL		Adverse effects at high dose levels
	ppm	mg/kg/d	ppm	mg/kg/d	
90-day dog study 0, 400, 1200 & 3600/2400 ppm	400	12/12	1200	33/41	Liver: increased absolute and relative weight in both sexes; brown pigment in Kupffer cells in females (high dose) Kidney: increased relative weights in both sexes Skeletal muscle: myofiber atrophy/degeneration in both sexes

Chronic toxicity and oncogenicity have been investigated in rats (2 year study) mice (18 month oncogenicity) and dog (1 year toxicity). Target organs were consistent with those observed in the 90 day studies. No relevant treatment-related neoplastic changes were observed at any dose level tested. were observed in either the rat or mouse oncogenicity studies. Results of the dog, mouse and rat studies are summarized below.

Summary of BYI 02960 chronic toxicity and oncogenicity

Studies (Dose levels in ppm)	NOEL/NOAEL		LOAEL		Target organ(s) and treatment-related effects
	ppm	mg/kg/day	ppm	mg/kg/day	
1 year dog study (0, 150, 300 & 1000)	300	7.8/7.8 (M/F)	1000	28.1/28.2 (M/F)	Minimal to slight degeneration of skeletal muscle (gastrocnemius and biceps femoris) in both sexes
2 year rat carcinogenicity (0, 80, 400 & 2000)	400	15.8/22.5 (M/F)	2000	80.8/120 (M/F)	Target organs: liver & thyroid in either sex; lung in females No tumours
Mouse oncogenicity (0, 70, 300 & 1500)	300	43/53 (M/F)	1500	224/263 (M/F)	Target organs: liver either sex; kidney in males No tumours

Short term as well as acute rat studies have also been conducted on selected plant metabolites. Findings indicate these metabolites of BYI 02960 have similar systemic effect and are of similar or lesser toxicity when compared to parent. Overall results are presented below.

Study	Species	Results
DFA		
Rat Acute Oral Study	Sprague Dawley Rat	300 mg/kg bw $< LD_{50} \leq$ 2000 mg/kg bw
14-day range-finding study	Wistar rat	NOAEL= 500 ppm (equating to 51 mg/kg/day) based on clinical chemistry changes
90-day dietary study	Wistar rat	NOAEL = 200 ppm (12.7/15.6 mg/kg bw in M/F, equal to 38/ 47 mg/kg/day in parent equivalents) 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-DFEAF		
Rat Acute Oral Study	Sprague Dawley Rat	LD_{50} cut off $\geq$ 2000 mg/kg bw
Range-finding dietary rat study	Wistar Rat	Lower blood glucose concentration from 1280 ppm (equating to 135 mg/kg/day) in females
28-day dietary study	Wistar Rat	NOAEL = 3000 ppm (243 and 273 mg/kg/day in males and females, respectively) based on body weight effects.
BYI 02960-CHMP		
Rat Acute Oral Study	Sprague Dawley Rat	LD_{50} in males = 1842 mg/kg bw LD_{50} in females = 1483 mg/kg bw
90-day dietary study	Sprague Dawley Rat	NOEL= 800 ppm (48.9 mg/kg/day) in males and NOEL= 4000 ppm (275.9 mg/kg/day) in females BWG and FC decreases, increase in alkaline phosphatase activity, eosinophilic intranuclear inclusions in proximal tubular epithelium of kidney
6-CNA		
Rat Acute Oral Study	Sprague Dawley Rat	LD_{50} >5000 mg/kg bw

3.1.4.1 Sub-chronic and chronic toxicity in the mouse

In the 90-day mouse study, BYI 02960 was administered to C57BL/6J mice at 100, 500 and 2500 ppm (equating to 16, 81 and 407 mg/kg/day in males and 19, 98 and 473 mg/kg/day in females). Effects were limited to 2500 ppm except that lower mean body weight gain was observed in males during the first week of the study at 500 ppm. A lower body weight was observed in both sexes throughout the study. A slight reduction in mean food consumption was observed in females between study days 1 and 22. A lower mean total cholesterol concentration, higher mean urea concentrations and slightly lower total protein concentrations were observed in both sexes, whilst higher mean alkaline phosphatase activity was noted in males and mean alanine and aspartate aminotransferase activities were higher in females. In females, mean albumin concentrations were slightly lower. The target organs were the liver (with higher mean absolute and relative weights in females, pale liver in the females and slight increase in severity of diffuse hepatocellular vacuolation in both sexes) and the kidney (with lower mean absolute and relative weights to brain weight ratio in males and loss of the normal multifocal/diffuse cortical epithelial vacuolation in males also). The No Observed Adverse Effect Level was 500 ppm (equating to 80.6 mg/kg body weight/day) in males and the NOAEL in females (equating to 98.1 mg/kg body weight/day).

In the mouse carcinogenicity study, Wistar rats were administered BYI 02960 through the diet at dietary levels of 70, 300 or 1500 ppm. At 1500 ppm, mean body weight was progressively decreased in both sexes throughout the study compared to control means. No relevant treatment-related neoplastic changes were observed at any dose level tested. The target organs were the liver and the kidney. The changes observed in the liver were higher liver weights and a higher incidence and severity of diffuse hepatocellular vacuolation (mainly centrilobular) noted in males, whilst a decreased incidence of diffuse hepatocellular macrovacuolation (mainly periportal) was noted in females. In the kidney, lower weight, decreased incidence and severity of bilateral basophilic tubules, focal cortical

mineralization and corticoepithelial vacuolation were noted in males. At 300 ppm, the only histopathological changes were noted in males and consisted of a higher incidence and severity of diffuse hepatocellular vacuolation (mainly centrilobular) in the liver together with a decreased severity of corticoepithelial vacuolation in the kidney. Both changes were considered to be treatment-related but not adverse. A dose level of 300 ppm (equivalent to 43 mg/kg/day in males and 53 mg/kg/day in females) was considered to be a No Observed Adverse Effect Level (NOAEL) in both sexes over an 18-month period of dietary administration.

3.1.4.2 Sub-chronic and chronic toxicity in the rat

Wistar rats were administered BYI 02960 in the diet at 100, 500 and 2500 ppm (equating to 6.0, 30.2 and 156 mg/kg/day in males and 7.6, 38.3 and 186 mg/kg/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. Significant findings were limited to the group treated at 2500 ppm, except for a reduced mean body weight gain observed in females during the first and the last weeks of the study. At 2500 ppm, a lower body weight was observed in both sexes throughout the study. Throughout the recovery phase of the study the mean body weight of males and females remained lower than the control group. A slight reduction in mean food consumption was observed for males during the first four days of the study and thereafter on several occasions and for the females from the first week of the study until Study Week 7. A higher mean platelet count was observed in females. Mean total bilirubin and glucose concentrations were slightly lower in both sexes and mean total cholesterol and triglyceride concentrations were slightly higher when compared to the controls. The change observed for total bilirubin was considered to be partially reversible in females. The other treatment-related changes were considered to be reversible. The target organs were also the liver (with higher relative weights to body weight ratio and centrilobular hepatocellular hypertrophy in both sexes) and the thyroid gland (dark aspect at necropsy and minimal follicular cell hypertrophy in some males). These findings were totally reversible. The NOAEL in this study was 500 ppm equating to 30.2 and 38.3 mg/kg/day in males and females, respectively.

In the rat combined chronic toxicity and carcinogenicity study, animals were administered BYI 02960 through the diet at 80, 400 or 2000 ppm. Lower body weight and body weight gain were observed in females at 2000 ppm throughout the study and slightly lower cumulative body weight gain was observed in males during the first year. Higher mean leukocyte counts associated with higher mean absolute lymphocyte and neutrophil counts were observed in males from the end of the first year. Slightly higher cholesterol concentrations were seen in the females throughout the study. No relevant treatment-related neoplastic changes were observed at any dose level tested. The target organs were the liver and the thyroid in both sexes and the lung in females. The effects seen in the liver in the 2000 ppm treated male and female groups were higher mean liver to body weight ratios associated with centrilobular hypertrophy, centrilobular hepatocellular macrovacuolation, lower incidences of periportal hepatocellular vacuolation and eosinophilic, mixed and tigroid foci of altered hepatocytes. In addition, higher incidences of brown pigments in Kupffer cells, interstitial mononuclear cell infiltrate and periportal hepatocellular macrovacuolation were observed in females. Changes were also observed in the thyroid gland including higher incidences of follicular cell hypertrophy and of follicular cell pigment in both sexes at the final sacrifice and increased incidences of colloid alteration in males and females at the interim sacrifice and in males only at final sacrifice. In the lung, higher

incidences of foamy macrophages and chronic interstitial and perivascular inflammation were observed in females at final sacrifice. At 400 ppm, the findings were limited to centrilobular hypertrophy (minimal) in the liver and of colloid alteration in the thyroid gland observed in males. However these changes were considered not to be adverse since they were minimal and/or not associated with other relevant changes.

The No Observed Adverse Effect Level over a 12-month period of dietary administration with BYI 02960 to the Wistar rat was 400 ppm in both sexes (equivalent to 18.5 mg/kg body weight/day in males and 25.3 mg/kg body weight/day in females). The No Observed Adverse Effect Level over a 24-month period of dietary administration with BYI 02960 to the Wistar rat was 400 ppm in both sexes (equivalent to 15.8mg/kg/day weight/day in males and 22.5 mg/kg body weight/day in females).

3.1.4.3 Sub-chronic toxicity in the dog

BYI 02960 was administered via the diet to beagle dogs (4/sex/dose) of both sexes at nominal concentrations of 400, 1200 or 3600/2400 ppm for at least 90 days (equating approximately to 12, 33 or 102/85 mg/kg body weight/day in males and 12, 41 or 107/78 mg/kg body weight/day in 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.
In the high dose group, compound-related clinical findings were unsteady and stiff back legs and lower back on study days 44, 53, and 54 in one male and on study day 44 for one female. Lower body weight was observed in males and females, during the first week of the study at 3600/2400 ppm and in males only at 1200 ppm. Food consumption was also reduced at the beginning of the study in both sexes at 3600/2400 ppm and in males only at 1200 ppm. Higher creatine phosphokinase, aspartate aminotransferase, and alanine aminotransferase activities were observed at the 2-month test interval in both sexes at 3600/2400 and 1200 ppm. Lower red blood cell count, hemoglobin concentration, and hematocrit were observed at 3600/2400 ppm at 1, 2, and 3 months in both sexes. The target organs were the liver at 3600./2400 ppm (with higher absolute and relative weights in both males and females and minimal brown pigments in Kupffer cells in females), the kidney (with higher relative weights in both sexes at 3600/2400 ppm and males only at 1200 ppm) and skeletal muscle (with minimal to slight myofiber atrophy/degeneration in both sexes at 3600/2400 ppm and 1200 ppm). The NOAEL in this study was 400 ppm for males and females equating to 12 mg/kg/day.

In a one year toxicity study, male and female Beagle dogs (4/sex/dietary level) were fed control feed or feed containing BYI 02960 at dietary concentrations of 150, 300, or 1000 ppm (approximately equal to 4.6/4.1, 7.8/7.8, 28.1/28.2 mg/kg body weight/day in males/females, respectively). Test substance-related effects were limited to degeneration noted in skeletal muscle (gastrocnemius, biceps femoris) of males and females at 1000 ppm only. Minimal to slight, focal to multifocal areas of degeneration of skeletal muscles were noted in males (gastrocnemius – incidence 2/4; biceps femoris – incidence 3/4) and females (gastrocnemius – incidence 2/4; biceps femoris – incidence 3/4). Degeneration of the myofiber comprised one or more of the following changes: atrophy, necrosis, and/or presence of inflammatory cells around the affected myofiber. Skeletal muscles evaluated from 150 ppm and 300 ppm males and females were comparable to controls. Based on the micropathology findings, the Lowest Observed Adverse Effect Level (LOAEL) in this study was 1000 ppm, which was equivalent to 28.1 and 28.2 mg/kg body weight/day for male and female dogs, respectively. Based

on the lack of adverse compound-related effects, a dietary level of 300 ppm (equivalent to 7.8 mg/kg body weight/day for both sexes) was considered to be a No Observed Adverse Effect Level (NOAEL).

3.1.4.4 Reproductive and developmental toxicity

The reproductive toxic potential of BYI 02960 was tested in a multigeneration study in rats and in developmental toxicity studies in rats and rabbits. The developmental toxicity study in rats (main study) was supplemented by a second study (supplementary study) to further precise the maternal effect dose.

In a pilot one generation rat reproduction study, BYI 02960 was administered in the feed to Wistar rats (10 animals/dose/sex) at nominal dietary concentrations of 0, 200, 700, and 2000 ppm. Males exhibited a very slight decline in body weight gain over 15 weeks of treatment with the test substance at 2000 ppm. Females showed declines in absolute body weight and body weight gain as well as declines in food consumption throughout the premating period at 2000 ppm and decline in body weight gain at 700 ppm. Statistically significant body weight declines were also observed throughout gestation and lactation at 2000 ppm; at 700 ppm declines in body weight during lactation with significance observed by lactation Day 14 were observed. Females treated at 2000 ppm also exhibited test substance-related decreases in absolute and relative spleen weight. At 2000 and 700 ppm, declines in absolute male and female pup weight were observed beginning PND 14 and continuing to PND 21 with significance only observed for the females. Body weight gain for the males and females was also declined, relative to controls. No test substance-related findings were observed on reproductive parameters.

In the definitive 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. In the P-generation and F_1-generation, females from the 1800 ppm treated group exhibited declines in body weight during premating, gestation and laction. In the P-generation males treated at 1800 ppm, increased absolute and relative liver weights were observed as well as increased absolute thyroid weights. Minimal centrilobular hypertrophy of the liver was observed in the males and correlated with the increased liver weights. Declines in body weight were also observed in the females treated at 500 ppm from the P-generation during the premating period and the females from the F_1-generation during premating, gestation and lactation periods.

F_1-offspring from the 1800 ppm parental group showed a significant decline in body weight at birth and during lactation. No decline in body weight was observed at birth for the F_2-offspring, but a significant decline was observed during lactation. In the F_1-offspring a significant delay in preputial separation and a slight nonstatistical delay in vaginal patency were observed in parallel with the decreased body weight. However, no effect on anogenital distance was observed in the F_2-generation pups. In both generations, variations in brain, thymus and spleen weights in males and/or females were observed and are considered to be due to the decreased body weights observed at this same dietary level and not a direct effect of the test substance. At 500 ppm a decline in body weight was observed in F_2-generation pups. Variations in brain, thymus and spleen weights in males and/or females were also observed in the F_2-offspring and are considered to be due to the decreased body weights.
A slight decrease in litter size was noted in the F_2-generation pups at 1800 ppm. The decline in litter size (9.2) is just outside of this laboratory's historical control range (9.8–11.8), and is attributable to

declines in total weight gain during gestation for the F_1-adults occurring concomitantly with a decline in the total number of implantation sites in the F_1-adults. There was no test substance-related effects observed on the viability of the pups after delivery at any dietary level tested.

The parental systemic NOAEL was 500/100 ppm in males and females, respectively (32.3/7.8 mg BYI 02960/kg bw/day) based upon liver and thyroid effects in P-generation males and body weight effects in females. The reproductive NOAEL was 500 ppm (32.3/39.2 mg BYI 02960/kg bw/day in males and females, respectively) based upon decreased cycle number, litter size and number of implants in F1 generation. The offspring NOAEL was 100 ppm (7.8 mg BYI 02960/kg bw/day) based upon body weight effects in F_2 pups.

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. At 150 mg/kg/day, there was a mean maternal body weight loss of 5.7 g between GD 6-8, compared to a weight gain of 5.9 g in the concurrent controls. In addition, between GD 8-10, the mean body weight gain was reduced by 24% when compared to the control group. Mean food consumption was reduced by between 9 and 27% on all intervals between GD 6 and 12. At 50 mg/kg/day, the mean maternal body weight gain was reduced by 49% and mean food consumption was reduced by 8% between GD 6-8, when compared to the concurrent controls. At 150 mg/kg/day, the mean absolute liver weight was 13% higher than controls. At cesarean section, mean fetal body weights for combined sexes and females were marginally reduced compared to the controls (by 2 to 3%, not statistically significant). At the fetal skeletal examination, the incidences of two variations ("parietal (uni/bi): incomplete ossification" and "hyoid centrum: incomplete ossification") were higher than in the control group and were indicative of a slightly delayed fetal development. The NOEL for maternal toxicity was 15 mg/kg/day and the NOEL for developmental toxicity was 50 mg/kg/day.

In a complementary study, 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 and the NOEL for developmental toxicity was 50 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. A dose level of 40 mg/kg/day BYI 02960 resulted in maternal toxicity as evidenced by body weight loss, significantly reduced body weight gain and food consumption between GD 6 and 10, and lower mean maternal corrected body weight change compared to control animals. Fetal development was unaffected by treatment at any dose level tested. A dose level of 15 mg/kg/day was considered to be a No Observed Adverse Effect Level (NOAEL) for maternal toxicity, while a dose level of 40 mg/kg/day was considered to be a NOAEL for developmental toxicity.

A summary of reproduction and developmental toxicity study results can be found below.

Type of study (Dose levels)	NOAEL (mg/kg/d)	LOAEL (mg/kg/d)	Adverse effects at LOAEL / target organs	
Reproductive toxicity studies				
One-generation rat 0, 200, 700, 2000 ppm	50.1/17.5 (M/F)	147.5/60 (M/F)	Parent	*Males:* Slight declines in BWG *Females:*Decreased BW and /or BWG (premating, gestation, and lactation)
	147.5/168.9 (M/F)	>147.5/168.9 (M/F)	Repro-duction	No effects
	17.5	60. 9	Offspring	Decreased BW and BWG
Two-generation rat 0, 100, 500, 1800 ppm	32.3/7.8 (M/F) [500/100 ppm]	119.8/39.2 (M/F) [1800/500 ppm]	Parents	*Males:* Increased liver weights (P) Increased thyroid weights (P) Increased incidence of centrilobular hypertrophy (minimal - P) *Females:* Decreased BW (premating, gestation, and lactation; F_1) Decreased BWG (premating; P and F_1) Decreased terminal body weights (P & F_1)
	32.3/39.2 (M/F) [500/500 ppm]	119.8/140.2 (M/F) [1800/1800 ppm]	Repro-duction	Decreased cycle number (F_1), litter size (F_1), and number of implants (F_1)
	7.8 (M/F) [100 ppm]	39.8 (M/F) [500 ppm]	Offspring	Decreased BW and BWG (F_2); with Secondary to BW decreases: organ weight changes in brain, thymus, and spleen
Developmental toxicity studies				
Rat 0, 15, 50, 150 mg/kg/d	15 (Maternal)	50	Dams	Decreased mean BWG and food consumption (FC)
	50 (Develop.)	150	Fetuses	Decreased fetal BW; Reduced ossification of a few skull bones
Complementary rat toxicity 0, 20, 30 mg/kg/d	30	>30	Dams	No maternal toxicity
Rabbit, 0, 7.5, 15, 40 mg/kg/d	15 (Maternal)	40	Dams	Decreased BW, BWG, corrected BWG, and FC (GD6-10)
	40 (Develop.)	>40	Fetuses	No treatment-related effects

3.1.5 Neurotoxicity (acute, delayed and sub-chronic)

Acute and subchronic neurotoxicity studies have been conducted with BYI 02960. There have been no signs of delayed neurotoxic effects in any standard toxicity or neurotoxicity study conducted with BYI 02960. The active substance is not an organophosphate insecticide, and therefore delayed neurotoxicity studies are not required.

In an acute neurotoxicity study, technical grade BYI 02960 was administered by gavage in a single dose to rats at 0, 50, 200 and 800 mg/kg. Compound related effects were observed at all dose

concentrations in both sexes. Findings associated with neurotoxicity were observed at the time-of-peak effect including piloerection, lower muscle tone, rapidrespiration, 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. The only treatment-relatedeffects at 50 mg/kg were limited to higher incidences of piloerection in both sexes and dilated pupils in females only. A follow-up single dose study was performed in order to establish a clear NOAEL. In this follow-up study, females only were used as they were considered equally or more sensitive than males at higher dose levels. Females were administered doses of 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 and 173 mg BYI 02960/kg body wt/day for males and females, respectively).

Neurotoxicity study results are summarized below.

Type of study **Dose levels**	**NO(A)EL (mg/kg/d)**	**LOAEL (mg/kg/d)**	**Adverse effects / target organs**
Acute neurotoxicity in the rat 0, 20, 35, 50, 200 and 800 mg/kg bw	35 (M/F)	50 (M/F)	Piloerection and dilated pupils - At high dose levels: lower muscle tone, rapid respiration, gait incoordination, tremors, reduced motor activity, impaired righting reflex, impaired flexor and tail pinch responses
90-day neurotoxicity in the rat 0, 100, 500, 2500 ppm	143/173 (M/F)	> 143/173 (M/F)	None

3.2 Toxicological end point for assessment of risk following long-term dietary exposure (ADI)

The potential risk for consumers is linked to chronic exposure of potential residues of BYI 02960 in the food. Therefore the ADI should be based on the results of long term studies: In accordance with internationally accepted procedures the ADI (or Reference Dose RfD) is estimated on the basis of the No-Observed-Adverse-Effect Level (NOAEL) obtained in a chronic toxicity study in the most sensitive species. The long term study results for BYI 02960 are presented below.

Type of study Dose levels		NOEL/NOAEL		LOEL		Target organ(s) and treatment-related effects
		ppm	mg/kg/day	ppm	mg/kg/day	
1 year dog study		300	7.8/7.8 (M/F)	1000	28.1/28.2 (M/F)	Minimal to slight degeneration of skeletal muscle (gastrocnemius and biceps femoris) in both sexes
2 year rat carcinogenicity		400	15.8/22.5 (M/F)	2000	80.8/120 (M/F)	Target organs: liver & thyroid in either sex; lung in females No tumours
Mouse oncogenicity		300	43/53 (M/F)	1500	224/263 (M/F)	Target organs: liver either sex; kidney in males No tumours
Rat Multigeneration	Parent	500/ 100 (M/F)	32.3/7.8 (M/F)	1800/ 500 (M/F)	119.8/39.2 (M/F)	*Males:* ↑ liver weights (P) ↑ thyroid weights (P) ↑ incidence of centrilobular hypertrophy (minimal - P) *Females:* ↓ BW (premating, gestation, and lactation; F_1) ↓ BWG (premating; P and F_1) ↓ terminal body weights (P & F_1)
	Reprod	500	32.3/39.2 (M/F)	1800	119.8/140.2 (M/F)	↓ cycle number (F_1), litter size (F_1), and number of implants (F_1)
	Pups	100	7.8 (M/F)	500	39.8 (M/F)	↓ BW and BWG (F_2); with Secondary to BW ↓, organ weight changes in brain, thymus, and spleen

In accordance with internationally accepted procedures, the ADI (or Reference Dose RfD) is derived from the lowest No-Observed-Adverse-Effect Level (NOAEL) obtained in a chronic/long term toxicity study in the most sensitive species with a safety factor of 100 (10 X for inter- and 10X for intra-species sensitivity) applied if no additional severe toxic effects have been observed.

The rat and dog appear equally sensitive based upon the fact the NOAELs from the 1 year dog and 2-generation reproduction studies are almost identical (7.9 and 7.8 mg/kg bw/day, respectively), and the mouse appears less sensitive to long term exposure to BYI 02960. For BYI 02960, there were no indications of mutagenic or oncogenic toxicity, nor were any effects or concern noted in reproductive or developmental toxicity studies. Therefore, taking into account the toxicological profile of BYI 02960, a margin of safety (MOS) of 100 is considered to be appropriate.

The lowest NOAEL of 7.8 mg/kg/day was observed in the rat 2-generation reproduction study based on body weight effects in parental females. Based on these considerations, the following ADI value (= chronic Reference Dose cRfD) is proposed for BYI 02960:

- **ADI = 7.8 mg/kg/day / 100 = 0.078 mg/kg/day**

3.3 Toxicological end point for assessment of risk following acute dietary exposure - ARfD (Acute reference dose)

The potential acute risk for consumers is mainly linked to single exposure to possible residue of BYI 02960 in the food. Studies conducted with BYI 02960 which define effects that can be attributed to a single exposure are:

Type of study		NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ(s) and treatment-related effects
Acute neurotoxicity in the rat		35 (M/F)	50 (M/F)	Piloerection and dilated pupils at 50 mg/kg More severe effects at 200 mg/kg and higher
Rat developmental toxicity	Dams	15	50	Decreased mean BWG and FC
	Fetuses	50	150	Decreased fetal BW, reduced ossification of a few skull bones
Rat complementary toxicity	Dams	30	>30	No maternal toxicity
Rabbit developmental toxicity	Dams	15	40	Decreased BW, BWG and FC (GD6-10)
	Fetuses	40	>40	No treatment-related effects

Assessing the relative severity of effects across studies, only slight body weight gain effects were observed at the top dose in the developmental toxicity studies in both rats and rabbits. More severe toxic effects were seen in the acute neurotoxicity study, where typical signs of nicotinergic insecticides have been observed. Therefore, it seems appropriate to set the ARfD based on the NOAEL of the acute neurotoxicity study. Taking into account the toxicological profile of BYI 02960, a margin of safety **(MOS) of 100** is considered to be appropriate. Based on these considerations, the following ARfD value is proposed for BYI 02960:

- **ARfD = 35 mg/kg / 100 = 0.35 mg/kg**

3.4 Toxicological end points for assessment of occupational and bystander risks - AOEL / MOE

BYI 02960 has low acute toxicity to mammals irrespective of the route of exposure (oral, percutaneous or inhalation). It is not a skin sensitizer; it is not irritating to skin or eyes. Therefore there are no acute risk factors of special concern.

Exposure risks to typical operators are generally related to short term rather than to chronic exposures; therefore, the NOAELs derived from short term and developmental toxicity studies should be taken into account for the purposes of establishing an AOEL for the EU.

Subchronic studies on rodents and dogs indicate that liver and kidney are common target organs as well as thyroid in rat and skeletal muscle in dog. In the rat 2-generation reproduction study, slight decreases in cycle number, litter size and number of implants are observed in the second generation at the top dose where significant body weight effects are observed in the dams. Limited body weight effects are also observed at the intermediate dose in the dams with 20.5% decrease in body weight gain compared to controls in the first generation during premating and 5.9% decrease in body weight or 16.3% decrease in body weight gain in the second generation during premating. Body weight effects during gestation and lactation are seen only in the second generation.

The relevant NOAELs to be considered for calculation of the operator exposures are summarised in the following table.

Studies		**NOEL/NOAEL**		**LOEL**		**Target organ(s) and treatment-related effects**
		ppm	**mg/kg/day**	**ppm**	**mg/kg/day**	
90-day dog study		400	12/12 (M/F)	1200	**33**/41 (M/F)	Liver: increased weight in both sexes; brown pigment in Kupffer cells in females (high dose) Kidney: increased relative weights in both sexes Skeletal muscle: myofiber atrophy/ degeneration in both sexes
90-day rat study		500	30/38 (M/F)	2500	156/186 (M/F)	Liver: centrilobular hepatocellular hypertrophy in both sexes Thyroid: follicular cell hypertrophy in males only
90-day mouse study		500	80.6/98.1 (M/F)	2500 (M/F)	407/473 (M/F)	Liver: increased diffuse hepatocellular vacuolations Kidney: decreased multifocal/diffuse Corticoepithelial vacuolation
Rat teratology	Dams	-	30	-	50	Decreased mean BWG and food consumption (FC)
	Foetus	-	50	-	150	Decreased fetal BW; Reduced ossification of a few skull bones
Rabbit teratology	Dams	-	15	-	40	Decreased BW, BWG, corrected BWG, and FC (GD6-10)
	Foetus	-	40	-	>40	No treatment-related effects

From this table, comparing Lowest Observed Adverse Effect Levels (LOAELs), the most sensitive species is the dog with a subchonic LOAEL of 33 mg/kg/day, based on myofiber atrophy seen in skeletal muscle. Thus the 90-day dog study was chosen to set the AOEL.

Available studies indicate that BYI 02960 is well absorbed by rats following oral administration by gavage. Therefore, adjustment for oral absorption is not necessary when calculating the systemic AOEL for the EU.

Based on BYI 02960's overall toxicological profile, the current conventional (EU) Uncertainty Factor (UF) of **100** is considered to be appropriate for setting AOEL. For this reason the following systemic AOEL is proposed

- **AOEL $_{systemic}$ = 12 mg/kg bw/day / 100 = 0.12 mg/kg bw/day**

3.5 Drinking water limit

According to the criteria set by the WHO, exposure through drinking water should not account for more than 10% of the ADI and calculation of the acceptable drinking water concentration should be based on an average water consumption of 2 litres per person per day and a body weight of 60 kg. The individual calculation steps are shown as follow:

$ADI_{10\%}$: = 0.0078 mg/kg bw/day
$ADI_{10\%}$: = 0.0078 x 60 = 0.468 mg/person/day

- **MAC = 0.468 mg / 2 = 0.234 mg/L**

Based on these considerations a limit of 0.234 mg/L is proposed as the maximum acceptable concentration in drinking water per day.

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

There are no impurities of concern in technical grade BYI 02960. All impacts on human and animal health are based upon consideration of active substance only.

3.6.1 Operators – estimates relevant for Europe

Sivanto® SL 200 is a water soluble concentrate containing 200 g of insecticide BYI 02960/L. The proposed safe uses are foliar sprays onto hops and lettuce. Applications of Sivanto® SL 200 will be achieved via field crop sprayers, broadcast air assisted sprayers and by hand-held devices in greenhouses. Water will be the diluent/carrier in all cases. Usage information pertinent to operator exposure is summarized below.

Application parameters for 'BYI 02960 SL 200

Crop	Application technique	Max no of application	Spray volume (L/ha)	Max dose rate (g BYI 02960 / ha)
Lettuce (field)	FCS	1	500 – 1000	125
Hops	BAA	1	2000 – 3000	150
Lettuce (greenhouse)	HH-GH	2	500 – 1000	125

FCS = Field crop sprayer, BAA = Broadcast air assisted sprayer, HH-GH = Hand-held application in greenhouses

Consideration of the AOEL

The proposed AOEL for BYI 02960 is based on the NOAEL from the 90-day dog study (NOAEL: 12 mg/kg bw/day) with no adjustment for oral absorption necessary. Applying the standard safety factor of 100, the AOEL is 0.12 mg/kg bw/day.

Consideration of dermal absorption

Dermal absorption data are available for BYI 02960 from *in vitro* studies with human/rat skin and from an *in vivo* study with rats. Based upon the results of these studies it is proposed to use 22% and 15% dermal absorption to calculate systemic exposure of BYI 02960 from the concentrate and the spray dilution, respectively.

Consideration for estimation of operator exposure estimates

Operator exposures to Sivanto® SL 200 during the intended tractor mounted ground boom spray application in the field as well as during broadcast air assisted application to hops will be estimated using the EU wide accepted German model[1] as well as the UK-POEM[2]. In addition, the exposure scenario for the greenhouse application is estimated with the 'Greenhouse Model'.

The results of the exposure calculations are summarized below for the German, UK and greenhouse models. Even assuming no PPE, all scenarios clearly result in acceptable exposure levels to operators.

[1] Lundehn, J.-R.; Westphal, D.; Kieczka, H.; Krebs, B.; Löcher-Bolz, S.; Maasfeld, W.; Pick, E.-D. (1992): Uniform Principles for Safeguarding the Health of Applicators of Plant Protection Products (Uniform Principles for Operator Protections); Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem, n° 277, 1992

[2] Scientific Subcommittee on Pesticides and British Agrochemicals Joint Medical Panel., Estimation of Exposure and Absorption of Pesticides by Spray Operators (UK MAFF) 1986 and the Predictive Operator Exposure Model (POEM) – A User's Guide (UK MAFF) 1992, revised model 2007

Predicted systemic exposure as a proportion of the AOEL

Crop	Model	PPE	Total systemic exposure (mg/kg bw/day)	% of AOEL[#]
Lettuce (field)	German model	No PPE[1)]	0.0217	18
		With PPE[2)]	0.00102	<1
	UK-POEM	No PPE[1)]	0.0776	65
		With PPE[2)]	0.00685	6
Hop	German model	No PPE[1)]	0.0155	13
		With PPE[2)]	0.00475	4
	UK-POEM	No PPE[1)]	0.0451	38
		With PPE[2)]	0.0175	15
Lettuce (greenhouse)	Low crops (standard scenario)	No PPE[1)]	0.00321	3
		With PPE[2)]	0.00090	<1
	Low crops (intensive scenario)	No PPE[1)]	0.0929	77
		With PPE[3)]	0.00316	3

[#] BYI 02960: AOEL = 0.12 mg/kg bw/day

1) One layer of typical work wear (e.g. trousers and a long sleeved shirt) as well as sturdy foot wear
2) In addition to typical work wear (see 1) protective gloves are worn during mixing and loading as well as when handling contaminated surfaces.
3) Instead of typical work wear spray tight trousers as protective clothing have to be worn. In addition protective gloves are worn during mixing/loading and application.

Based on these results there is no unacceptable risk anticipated for the operator with the intended uses of Sivanto® SL 200 even when only typical work clothing, i.e. one layer of work clothing (e.g. a coverall) is worn.

3.6.2 Operators – estimates relevant for North America

Europe and North America are not participating in a joint review for BYI 02960. The submission of the BYI 2960 dossier in NAFTA will occur later than in Europe, and therefore NAFTA assessments are not available at this time.

3.6.3 Bystanders

Plant protection products can be applied to crops in areas that may be accessible to the public. Individuals might therefore be exposed who are not actively involved in the application of these products. The individual may be temporarily located in the vicinity of the application (the so-called 'bystander') or working or living in the vicinity of the application (the so-called 'resident'). Exposure scenarios associated with the product application are evaluated for bystanders and for residents (including children) for both outdoor scenarios. Since during spraying operations in greenhouses no bystanders will be present in greenhouses, no assessment is required for this scenario.

Calculations have been performed according to the German guideline published in 2008 (Martin et al., 2008)[3]. Exposure estimates and percent of the proposed systemic AOEL assuming an AOEL of 12 mg/kg bw/day are summarised in the following table for a 60 kg adult and a 16.15 kg child.

[3] S. Martin , D. Westphal , M. Erdtmann-Vourliotis , F. Dechet , C. Schulze-Rosario, F. Stauber, H. Wicke and G. Chester (2008): Guidance for Exposure and Risk Evaluation for Bystanders and Residents exposed to Plant Protection Products during and after Application, J. Verbr. Lebensm. 3, 272 - 281.

Scenario	Crop	Person	Total systemic exposure* (mg/kg bw/day)	% of AOEL#
Bystander	Lettuce	Adult	0.00013	<1
		Child	0.00011	<1
Resident		Adult	0.00001	<1
		Child	0.00002	<1
Bystander	Hops	Adult	0.00318	3
		Child	0.00249	2
Resident		Adult	0.00023	<1
		Child	0.00044	<1

*: Assumes a 60 kg adult and a 16.15 kg child.

There should be no concern for potential adverse effects on bystanders resulting from agricultural uses of Sivanto® SL 200.

3.6.4 Workers

Sivanto® SL 200 is proposed for application to hops and to lettuce in the field and in greenhouses. These crops require re-entry activities such as harvesting. Re-entry exposure is evaluated according to the re-entry model published by Hoernicke et al. (1998)[4] together with transfer coefficients relating to the appropriate tasks.

Considerations on DFR values:
Dislodgeable foliar residues were experimentally determined under actual use conditions for lettuce. With a conservative approach the highest DFR_M values observed in the course of the experiments are presented below.

Crop	DFR_M [µg/cm²]	Observed on
Lettuce (field, northern Europe)	0.291	Day 0 after 1st application (DAFT 0)
Lettuce (field, southern Europe)	0.264	Day 0 after 2nd application (DAFT 10)
Lettuce (Greenhouse)	0.316	Day 0 after 2nd application (DAFT 10)

DAFT= Days after first treatment

For hops no measured dislodgeable foliar residues are available. As default figures proposals from EUROPOEM II (3µg a.s./cm² per 1 kg a.s./ha) as well as from the German guidance (1µg a.s./cm² per 1 kg a.s./ha) are available. Since data from the dislodgeable foliar residue trials with lettuce have shown that measured values are somewhat in between these two default figures (and corresponding more to the US-EPA default of 2 µg/cm² per kg a.s./ha), the default value from EUROPOEM II has been chosen for hops for a conservative assessment.

[4] Hoernicke, E.; Nolting, H.G.; Westphal, D.: Label instructions for the protection of workers re-entering crop growing areas after application of plant protection products; Nachrichtenbl. Deut. Pflanzenschutzd.50 (10), 267 - 269, 1998 (document no.: M-107544-01-1)

A summary of the exposure calculations and risk assessment is presented below. The estimated systemic worker exposure to BYI 02960 is well below the proposed AOEL in both crops.

Predicted systemic exposures as a proportion of the AOEL

Scenario	Crop	Substance	Total systemic exposure (mg/kg bw/day)	% of AOEL#
Worker	Lettuce	BYI 02960	0.0232	19
	Hops	BYI 02960	0.0660	55

BYI 02960: AOEL = 0.12 mg/kg bw/day

As this scenario – re-entry immediately after the sprays have dried – is considered to represent the worst case scenario, there is no unacceptable risk anticipated for the worker when performing re-entry activities in lettuce/hops treated with Sivanto® SL 200.

3.6.5 Consumers

Dietary risk assessment was performed according to the EFSA model (PRIMo revision 2). For the evaluation of the **chronic exposure** the model uses 5 WHO diets relevant to the EU and 22 national diets from 13 different EU Member States. For the evaluation of the acute exposure 19 national diets from 11 different EU Member States are used.

The assessment of the chronic uptake of BYI 02960 residues with food is made based on the proposed MRLs and the Acceptable Daily Intake (ADI) of 0.078 mg/kg bw/day, which was established based on the lowest NOAEL obtained in a chronic toxicity study in the most sensitive species (rat 2-generation reproduction study and 1-year dog study). The TMDIs of parent BYI 02960 calculated according to the model amounts to between 0.8% (PL general population) and 29.4% (WHO Cluster Diet B) of the ADI. The "top ten" most critical values ranged from 15.3% to 29.4% of the ADI. All ADI usage values in these evaluations are well below 100%; thus, a further, more refined risk assessment is not required for these models.

For the **acute risk assessment** the ARfD considered was 0.35 mg/kg bw (established based on the NOAEL determined in the acute neurotoxicity study in the rat). The intake estimation was calculated using the EFSA model (PRIMo revision 2) including diets from various countries in Europe. In a first, very conservative approach, the MRL values derived from supervised residues trials were used in the calculations for the edible part of plant commodities (as opposed to the standard approach using HR values). For animal matrices, MRL values were also used. The results of the calculations are summarized in the table below.

BYI 02960 – IESTI calculation (EFSA/PRIMo rev. 2) Calculation based on MRL values

IESTI 1		IESTI 2	
% of the ARfD	**Commodity**	**% of the ARfD**	**Commodity**
CHILDREN			
53.8	lettuce	32.3	lettuce
7.5	broad-leaf endive (scarole)	7.5	broad-leaf endive (scarole)
6.2	wheat	6.2	wheat
6.2	rocket (rucola)	6.2	rocket (rucola)
5.6	lamb's lettuce	5.6	lamb's lettuce
ADULTS	*(GEN. POPULATION)*		
22.0	lettuce	13.2	lettuce
3.8	lamb's lettuce	3.8	lamb's lettuce
3.4	wheat	3.4	wheat
3.2	rice	3.2	rice
3.1	barley	3.1	barley

The results summarised indicate that the maximum contribution to the ARfD is approx. 54% for lettuce (children, IESTI 1 calculation), and thus far below 100%. (It should be noted that the use in lettuce does not include broad-leafed endive; use in "scarole" will not be registered in the EU and thus will not appear on any label. Its appearance in the list above is due to the proposed MRL for rotational leafy vegetables.) Despite using the most conservative approach for the assessment of the acute risk (based on MRLs instead of HRs), it is evident that no acute risk for consumers will arise from the uses of BYI 02960 as presented in this dossier.

Chapter 4 Residues

4.1 Definition of the residues relevant to MRLs

The metabolism of BYI 02960 was investigated in plants (primary crops and confined rotational crops) and livestock (laying hen and lactating goat). Two different radiolabels were used in the plant and livestock studies: [pyridylmethyl-^{14}C]BYI 02960 and [furanone-4-^{14}C]BYI 02960. A third radiolabel ([ethyl-1-^{14}C]BYI 02960) was used for one additional plant study after soil degradation studies indicated the formation of significant amounts of difluoroacetic acid (DFA) after application of BYI 02960 to soil. This study showed that DFA was formed to a major extent in edible and non-edible matrices. Therefore all plant samples obtained in the metabolism studies and selected livestock samples were analysed subsequently for non-radiolabelled difluoroacetic acid.

Based on the overall primary crop and confined rotational crop metabolism results (including the additional analysis for difluoroacetic acid), the residue definition for **enforcement** of BYI 02960 residues in plants is proposed as parent **BYI 02960** plus **DFA**.

Based on the livestock metabolism results (including the additional analysis for difluoroacetic acid), the residue definition for **enforcement** of BYI 02960 residues in livestock matrices is proposed as parent **BYI 02960** plus **DFA**.

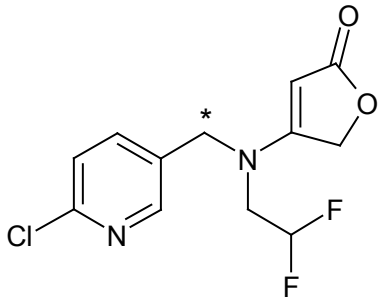
4.1.1 Definition of the residues in plants relevant to MRLs

Primary Crop Metabolism:

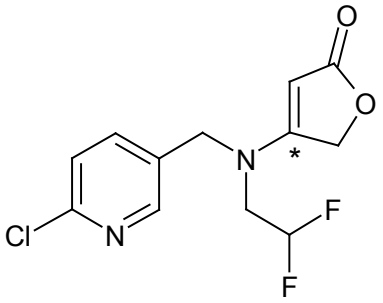
Target crop metabolism studies have been performed on apple, tomato, potato, cotton, and rice (representing five diverse crops) using foliar, soil, or seed treatment application with [^{14}C]BYI 02960 (labelled in the furanone ring or pyridinylmethyl position) formulated as 200 SL (for foliar and soil applications) and 480 FS (for seed treatment applications). The metabolism studies were conducted at rates covering the anticipated worst case GAPs.

	Fruiting Crops	**Root/Tuber Crops**	**Pulses and Oilseeds**	**Cereals (Rice)**
Foliar Treatment	Apple 1) Application at BBCH 69 2) Applications at BBCH 69 and at 14 days PHI		Cotton 1) Application at BBCH 15-18 2) Applications at BBCH 15-18 and at 14 days PHI	Paddy Rice 1) Applications at BBCH 13-15 (1 day after transplanting) 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 application into the planting hole at transplanting

The positions for the [^{14}C] labels are shown below:

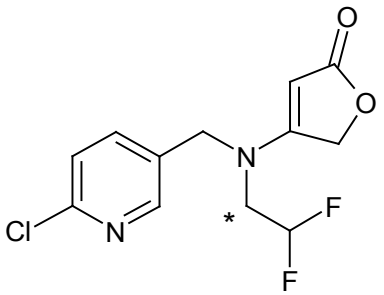


[pyridinylmethyl-^{14}C]BYI 02960



[furanone-4-^{14}C]BYI 02960

During the investigation of the environmental fate of BYI 02960, 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]BYI 02960, all sample materials from the ongoing studies were analysed for DFA by LC-MS/MS according to residue analytical method 01304 (data collection method RARVP013) to get an estimate of the residue levels. In addition, BCS conducted a target crop metabolism study with [ethyl-^{14}C]BYI 02960 applied as a soil drench to tomato to address possible metabolites specific for this radiolabel and generate sample material with incurred residues for radiovalidation of extraction efficiency.



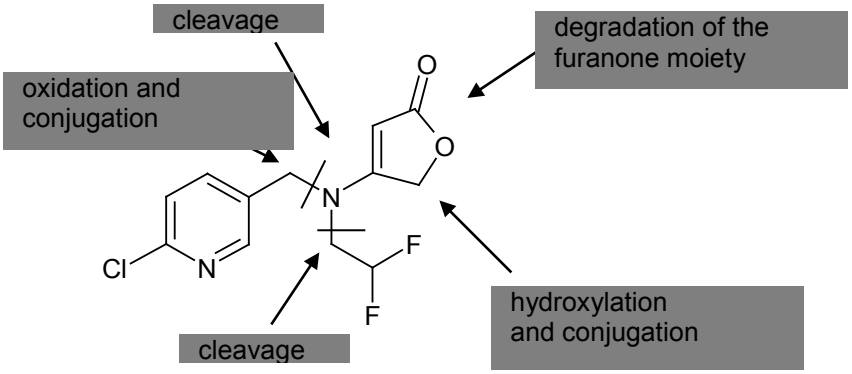
[ethyl-1-^{14}C]BYI 02960

The BYI 02960 plant metabolism studies have shown a reasonably consistent metabolic profile across both foliar and soil application. The available metabolism data (including the analysis of the non-radioactive DFA) from the five diverse crops are adequate to define the nature of the BYI 02960 residue in target crops. On basis of the metabolites identified, biotransformation of BYI 02960 in target crops 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 or 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 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),

- 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, and
- hydroxylation of the furanone moiety followed by conjugation.

The positions involved in the metabolic degradation are summarised in the following figure.



The only BYI 02960 residues that were consistently observed at significant levels across all primary crops and matrices were the parent compound and difluoroacetic acid (DFA), both of which will be specific to BYI 02960 crop use. All other major metabolites were detected in individual primary crop RACs only and generally at low concentrations. Only the natural compound glucose/carbohydrates which was formed from the metabolism and incorporation of ^{14}C from the furanone moiety into the natural plant constituents showed higher mg/kg levels.

Tomato metabolism (soil drench):

The metabolism of the insecticide BYI 02960 was investigated in tomato fruits and tomato flowers in three studies following two soil drench applications of: (1) [furanone-4-^{14}C]BYI 02960, (2) [pyridinylmetyl-^{14}C]BYI 02960 or (3) [ethyl-1-^{14}C]BYI 02960. Total radioactive residue was measured in fruits and flowers, and metabolites were identified or characterised dependent on levels found.

Based upon the results of all three tomato metabolism studies, it can be concluded that BYI 02960 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 summarised below.

TRR values and distribution of parent compound and metabolites in tomato fruits after drench application of BYI 02960

	Tomato fruits					
Radiolabel	[furanone-4-^{14}C]		[pyridinylmethyl-^{14}C]		[ethyl-1-^{14}C]	
TRR [mg/kg] =	0.096		0.130		0.201	
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
BYI 02960	35.9	0.034	24.2	0.031	10.0	0.020
DFA					*86.6*	*0.174*
glucose/carbohydrates	*27.5*	*0.026*				
6-CNA			*13.2*	*0.017*		
CHMP-di-glyc			*37.1*	*0.048*		
CHMP-glyc			*5.1*	*0.007*		
CHMP			*3.3*	*0.004*		
difluoroethyl-amino-furanone	*10.3*	*0.010*			*2.2*	*0.004*
OH-glyc	5.5	0.005	3.4	0.004	0.6	0.001
Total identified	**79.2**	**0.076**	**86.3**	**0.112**	**99.5**	**0.200**
Total characterised	**4.3**	**0.004**	**12.2**	**0.016**	**---**	**---**
Analysed extract(s)	83.5	0.080	98.5	0.128	99.5	0.200
Extract(s) not analysed	1.3	0.001	---	---	---	---
Total extracted	**84.8**	**0.081**	**98.5**	**0.128**	**99.5**	**0.200**
Unextractable (PES*)	15.2	0.015	1.5	0.002	0.5	0.001
Accountability	**100.0**	**0.096**	**100.0**	**0.130**	**100.0**	**0.201**

* post extraction solids
Label specific metabolites are printed in italics.

Potato metabolism (tuber treatment and in-furrow application)

The metabolism of the insecticide BYI 02960 was investigated in potatoes in two studies following tuber or soil application with (1) [furanone-4-^{14}C]BYI 02960 or (2) [pyridinylmethyl-^{14}C]BYI 02960. In both studies, the total radioactive residues (TRR) in the potato tubers, representing the edible raw agricultural commodity (RAC), were low after tuber treatment and after in-furrow application. The metabolite profiles of all tuber extracts were very similar, and at harvest, the predominant portion was always parent compound BYI 02960. However, subsequent analysis of the extracts on the non-radiolabelled metabolite difluoroacetic acid – which was not detectable with the radiolabels used - revealed even higher concentrations of this metabolite compared to the parent compound (0.39 mg/kg after tuber treatment and 0.54 mg/kg after in-furrow application).

When considering the results from both metabolism studies conducted on potato, it can be concluded that BYI 02960 is moderately metabolised in this crop. A total of 2 major and 10 minor metabolites were found. The distribution of parent compound and metabolites in the edible commodity potato tuber is summarized below.

TRR values and distribution of parent compound and metabolites in potato tuber after tuber treatment and in-furrow application of BYI 02960

	Potato tuber							
Radiolabel	[furanone-4-^{14}C]				[pyridinylmethyl-^{14}C]			
	tuber treatment		in-furrow appl.		tuber treatment		in-furrow appl.	
TRR [mg/kg] =	0.078		0.171		0.076		0.115	
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
BYI 02960	40.0	0.031	56.9	0.097	40.2	0.031	44.1	0.051
6-CNA					*21.5*	*0.016*	*18.4*	*0.021*
6-CNA-glycerol-gluA					---	---	*2.3*	*0.003*
CHMP-di-glyc					*4.4*	*0.003*	*5.3*	*0.006*
CHMP-glyc					*3.7*	*0.003*	*2.4*	*0.003*
CHMP					*3.9*	*0.003*	*3.9*	*0.004*
difluoroethyl-amino-furanone	*4.2*	*0.003*	*2.9*	*0.005*				
OH-glyc	6.6	0.005	4.4	0.007	6.7	0.005	4.7	0.005
Total identified	**50.8**	**0.039**	**64.2**	**0.110**	**80.5**	**0.061**	**80.9**	**0.093**
Total characterised	**12.4**	**0.010**	**7.4**	**0.013**	**9.3**	**0.007**	**5.8**	**0.007**
Analysed extract(s)	63.1	0.049	71.5	0.122	89.8	0.068	86.7	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	0.052	75.3	0.129	93.4	0.071	90.4	0.104
Unextractable (PES*)	33.0	0.026	24.7	0.042	6.6	0.005	9.6	0.011
Accountability	**100.0**	**0.078**	**100.0**	**0.171**	**100.0**	**0.076**	**100.0**	**0.115**

* post extraction solids
Label specific metabolites are printed in italics.

Apple metabolism (foliar application):

The metabolism of the insecticide BYI 02960 was investigated in apple fruits in two studies following foliar application with (1) [furanone-4-^{14}C]BYI 02960 or (2) [pyridinylmethyl-^{14}C]BYI 02960. In order to gain information on the fate of the difluoroethane moiety of BYI 02960, 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. In both metabolism studies, single and double application experiments were done. In the single application experiment, apple trees were treated once at the end of flowering (BBCH 69), whereas in the double application experiment, the trees were additionally treated with the same rate at 14 days before harvest. As expected, in both studies the residues in the double application experiment were dominated by parent compound.

When considering the results from both metabolism studies conducted on apple, it can be concluded that BYI02960 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 in fruits) were found. The distribution of parent compound and metabolites in the edible commodity apple fruit is summarized below.

TRR values and distribution of parent compound and metabolites in apple fruit after foliar application of BYI 02960

	Apple fruits							
Radiolabel	[furanone-4-^{14}C]				[pyridinylmethyl-^{14}C]			
	single appl.		double appl. w/o surface wash		single appl.		double appl. w/o surface wash	
TRR [mg/kg] =	0.280		1.286		0.079		0.545	
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
BYI 02960	7.4	0.021	73.6	0.946	43.1	0.034	85.6	0.467
glucose/carbohydrates	*71.7*	*0.201*	*14.2*	*0.182*				
6-CNA					*5.0*	*0.004*	*1.5*	*0.008*
CHMP-glyc					*4.7*	*0.004*	*0.9*	*0.005*
CHMP					*4.0*	*0.003*	*0.8*	*0.004*
difluoroethyl-amino-furanone	*3.2*	*0.009*	*0.2*	*0.003*				
acetic acid-glyc	0.3	0.001	0.5	0.007	3.5	0.003	0.8	0.004
OH-glyc	0.4	0.001	1.1	0.014	4.9	0.004	1.7	0.009
acetic acid	0.2	0.001	0.7	0.009	3.0	0.002	1.1	0.006
AMCP-difluoroethanamine					*8.4*	*0.007*	*4.1*	*0.023*
difluoroethyl-OH-glyc					1.4	0.001	---	---
OH	---	---	0.8	0.01	0.8	0.001	1.0	0.005
Total identified	**83.4**	**0.234**	**91.1**	**1.171**	**78.9**	**0.062**	**97.6**	**0.532**
Total characterised	**2.0**	**0.005**	**0.1**	**0.002**	**15.3**	**0.012**	**0.5**	**0.002**
Analysed extract(s)	85.3	0.239	91.2	1.173	94.2	0.074	98.0	0.534
Extract(s) not analysed	1.2	0.003	0.7	0.009	---	---	0.6	0.004
Total extracted	86.5	0.242	91.9	1.182	94.2	0.074	98.7	0.538
Unextractable (PES*)	13.5	0.038	8.1	0.104	5.8	0.005	1.3	0.007
Accountability	**100.0**	**0.280**	**100.0**	**1.286**	**100.0**	**0.079**	**100.0**	**0.545**

* 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 0.69 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 0.12 mg a.s. equiv./kg.

Cotton metabolism (foliar application):

The metabolism of the insecticide BYI 02960 was investigated in the raw agricultural commodities cotton seeds and gin trash and as well in lint following two different spray application scenarios of (1) [furanone-4-^{14}C]BYI 02960 or (2) [pyridinylmethyl-^{14}C]BYI 02960. In the single spray application experiment, cotton plants were treated once at an early growth stage (BBCH 16). In the double spray application experiment, one plant was treated at the early growth stage (BBCH 15) and additionally at 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 of BYI 02960, the extracts obtained in the cotton 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.

When considering the results from both cotton metabolism studies, it can be concluded that BYI02960 is rather extensively metabolised in this crop. A total of 4 major and approx. 15 minor metabolites were found. The edible commodity cotton seed showed 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 the table below followed by results for the feed commodity gin trash.

TRR values and distribution of parent compound and metabolites in cotton seeds after foliar application of BYI 02960

	Cotton seed			
Radiolabel	[pyridinylmethyl-^{14}C]			
	single appl.		double appl.	
TRR [mg/kg] =	0.045		0.068	
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg
BYI 02960	---	---	23.4	0.016
6-CNA	*16.2*	*0.007*	*5.0*	*0.003*
OH-glyc/ acetic acid	---	---	4.9	0.003
Total identified	**16.2**	**0.007**	**33.3**	**0.023**
Total characterised	**5.7**	**0.003**	**5.2**	**0.003**
Analysed extract(s)	21.9	0.010	38.5	0.026
Extract(s) not analysed	6.5	0.003	27.6	0.019
Total extracted	28.3	0.013	66.1	0.045
Unextractable (PES*)	71.7	0.032	33.9	0.023
Accountability	**100.0**	**0.045**	**100.0**	**0.068**

* post extraction solids
Label specific metabolites are printed in italics

TRR values and distribution of parent compound and metabolites in gin trash after foliar application of BYI 02960

	Gin trash							
Radiolabel	[furanone-4-^{14}C]				[pyridinylmethyl-^{14}C]			
	single appl.		double appl.		single appl.		double appl.	
TRR [mg/kg] =	0.191		2.767		0.310		2.344	
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
BYI 02960	40.0	0.076	54.4	1.505	26.3	0.082	53.2	1.247
6-CNA					20.2	0.063	2.2	0.053
glyoxylic acid	0.9	0.002	1.6	0.044	2.1	0.007	1.5	0.035
acetic acid-glyc	---	---	2.3	0.063	---	---	3.7	0.087
OH-glyc/ acetic acid	15.7	0.030	20.8	0.577	13.7	0.043	22.4	0.526
OH	13.1	0.025	0.6	0.016	14.5	0.045	1.3	0.030
chloro/ bromo	0.6	0.001	2.3	0.063	---	---	2.1	0.049
Total identified	**70.3**	**0.134**	**81.9**	**2.268**	**76.8**	**0.238**	**86.5**	**2.028**
Total characterised	**7.4**	**0.014**	**11.5**	**0.319**	**14.2**	**0.044**	**6.9**	**0.162**
Analysed extract(s)	77.7	0.148	93.4	2.586	91.0	0.282	93.4	2.190
Extract(s) not analysed	2.6	0.005	2.3	0.065	0.9	0.003	3.8	0.089
Total extracted	80.3	0.153	95.8	2.651	92.0	0.285	97.2	2.279
Unextractable (PES*)	19.7	0.038	4.2	0.116	8.0	0.025	2.8	0.065
Accountability	**100.0**	**0.191**	**100.0**	**2.767**	**100.0**	**0.310**	**100.0**	**2.344**

* post extraction solids
Label specific metabolites are printed in italic.

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.

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 and 0.06 mg a.s. equiv./kg 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.

Rice metabolism (granular and foliar applications):

The metabolism of the insecticide BYI 02960 was investigated in rice kernels, straw and husks following two different application scenarios of (1) [furanone-4-^{14}C]BYI 02960 or (2) [pyridinylmethyl-^{14}C]BYI 02960. 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 could not 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.

When considering the results from both metabolism studies conducted on rice, it can be concluded that BYI 02960 is rather moderately metabolised in this crop. A total of 3 major and approx. 25 minor metabolites were found. 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 below.

TRR values and distribution of parent compound and metabolites in rice kernels after granular and foliar application of BYI 02960

	Rice kernels							
Radiolabel	[furanone-4-^{14}C]				[pyridinylmethyl-^{14}C]			
	granule application		foliar application		granule application		foliar application	
TRR [mg/kg] =	0.140		0.659		0.050		0.620	
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
BYI 02960	23.1	0.032	56.6	0.373	69.6	0.035	75.2	0.467
glucose/carbohydrates	*26.9*	*0.038*	*3.6*	*0.023*				
6-CNA					*4.7*	*0.002*	*3.1*	*0.019*
glyoxylic acid	---	---	---	---	---	---	0.4	0.003
acetic acid-glyc	---	---	0.4	0.003	---	---	0.6	0.003
acetic acid	---	---	6.1	0.04	---	---	7.8	0.048
OH	---	---	---	---	---	---	0.4	0.002
chloro/ bromo	---	---	1.7	0.011	---	---	1.5	0.009
Total identified	**50.1**	**0.070**	**68.4**	**0.450**	**74.3**	**0.037**	**88.9**	**0.552**
Total characterised	**4.2**	**0.006**	**17.2**	**0.113**	**---**	**---**	**7.4**	**0.045**
Analysed extract(s)	54.3	0.076	85.6	0.564	74.3	0.037	96.4	0.597
Extract(s) not analysed	14.4	0.020	1.6	0.010	---	---	0.9	0.007
Total extracted	68.7	0.096	87.2	0.574	74.3	0.037	97.3	0.604
Unextractable (PES*)	31.3	0.044	12.8	0.085	25.7	0.013	2.7	0.017
Accountability	**100.0**	**0.140**	**100.0**	**0.659**	**100.0**	**0.050**	**100.0**	**0.620**

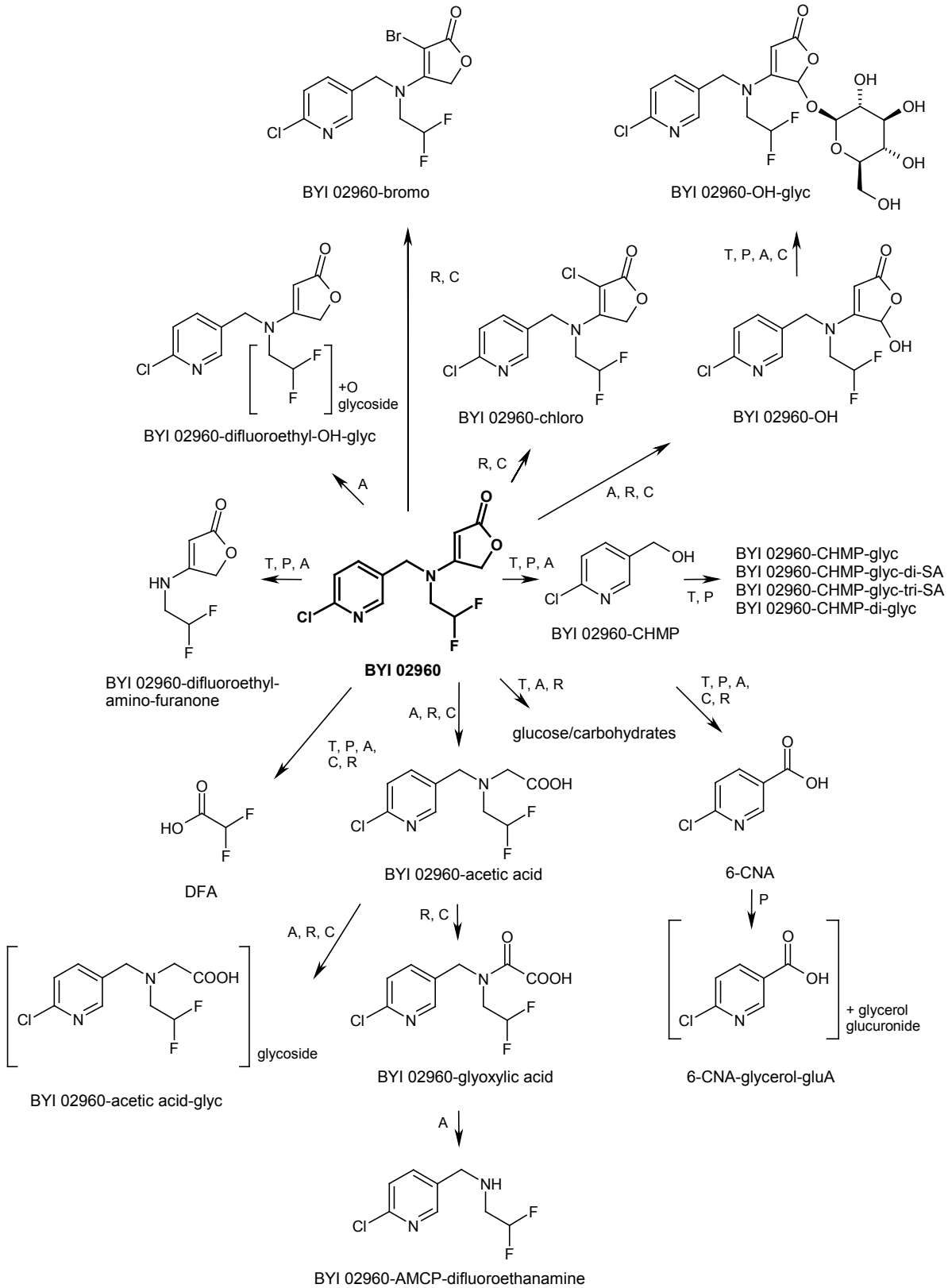
* post extraction solids
Label specific metabolites are printed in italic.

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. Difluoroacetic acid accounted for 0.06 mg a.s. equiv./kg 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.

BYI 02960 parent compound and difluoroacetic acid (DFA) were consistently observed at significant levels across all primary crop matrices. Thus, the **residue definition for enforcement** of BYI 02960

residues in primary crops is proposed as parent **BYI 02960** and **DFA**. An overall metabolic pathway for primary crops is shown below.

Proposed metabolic pathway of BYI 02960 in primary crops



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 [furanone-4-^{14}C]BYI 02960 or [pyridinylmethyl-^{14}C]BYI 02960. In each study, 400 g a.s./ha 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 grain, Swiss chard and turnip leaves and roots.

The total radioactive residues (TRR) of the raw agricultural commodities (RACs) of the rotational crops in the three subsequent rotations are summarized below. The TRR values in all RACs declined significantly from the first to the third rotation.

TRR values in the different RACs of the three rotations after soil application of BYI 02960

TRR	wheat				Swiss chard		turnips	
[mg/kg]	[furanone-4-^{14}C]BYI 02960							
	forage	hay	straw	grain	imm.	mature	leaves	roots
1st rotation	0.783	2.003	6.290	0.478	0.848	0.871	0.679	0.074
2nd rotation	0.193	1.081	1.519	0.103	0.311	0.263	0.158	0.014
3rd rotation	0.111	0.254	0.462	0.047	0.180	0.152	0.090	0.008
	[pyridinylmethyl-^{14}C]BYI 02960							
1st rotation	1.407	2.409	9.015	0.177	1.358	1.483	0.815	0.072
2nd rotation	0.308	1.009	2.148	0.057	0.332	0.438	0.230	0.022
3rd rotation	0.117	0.321	0.491	0.017	0.135	0.130	0.083	0.008

Similar to the target crop metabolism studies, parent BYI 02960 was by far the major radioactive residue found in the rotational crop matrices, representing <1 - 64% of the TRR in wheat forage, hay, straw, and grain, 25 - 57% of the TRR in Swiss chard, and 31 - 72% of the TRR in turnip roots and tops.

Nine other metabolites were found at >10% TRR in at least one RAC at one rotational interval.

- 6-CNA-glycerol-gluA, 3 isomers (11 - 33% of TRR, specific to wheat RACs, found in all rotations),
- BYI 02960-bromo-amino-furanone (up to 11% of TRR, only >10% TRR in wheat hay in 3rd rotation),
- BYI 02960-difluoroethyl-amino-furanone (up to 17% of TRR, major in all Swiss chard rotations),
- BYI 02960-glyoxylic acid (up to 16% of TRR, only > 10% in 1st rotation wheat forage, hay, straw and turnip root),
- BYI 02960-OH-glyc (up to 28% of TRR, major in all Swiss chard and turnip leaves rotations),
- BYI 02960-OH (10% of TRR in wheat grain in 1st rotation only, corresponding to a level of 0.02 mg/kg), and
- Glucose/carbohydrates (up to 70% of TRR, in wheat grain and turnip roots).

Additional LC-MS/MS analysis of all crop matrices of all rotations on difluoroacetic acid (DFA) according to residue analytical method 01304 (data collection method RARVP013) revealed that DFA was the main metabolite in wheat grains (all rotations) and in turnip roots (1st rotation) and a major metabolite in all other RACs.

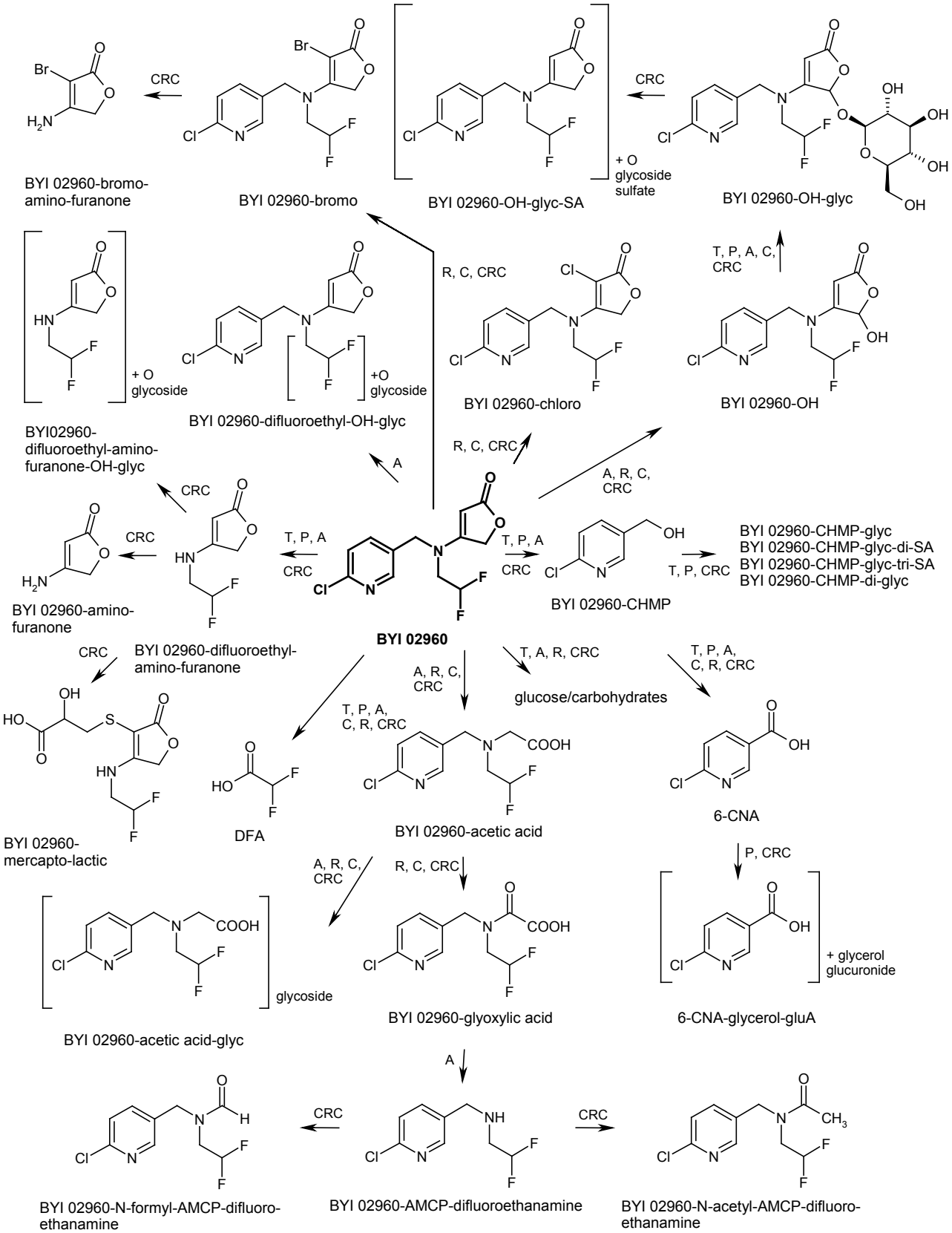
On basis of these results and the available confined rotational crop data from the studies with pyridinylmethyl- and furanone-ring-labelled BYI 02960, the nature of the BYI 02960 residue in rotational crops has been derived. BYI02960 was rather extensively metabolised in rotational crops. The proposed metabolic pathway is very similar to the one of the target plants. No additional main metabolic routes were detected and thus the main reactions involved were:

- 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 counterpart BYI 02960-CHMP followed by several conjugation reactions,
- cleavage or complete degradation 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, and
- halogenation 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.

A consolidated pathway for confined rotational crops and primary crops is shown below:

Proposed metabolic pathway of BYI 02960 in primary and confined rotational crops



T = tomato P = potato A = apple C = cotton R = rice

CRC = confined rotational crop

BYI 02960 parent compound and/or difluoroacetic acid (DFA) were consistently observed at significant levels across all rotational crop matrices. In addition, BYI 02960-difluoroethyl-amino-furanone was detected as a significant metabolite in the human consumable commodity Swiss chard.

Based on the overall confined rotational crop metabolism results, samples of field rotational crops were analysed for parent BYI 02960, DFA and BYI 02960-difluoroethyl-amino-furanone. None of the field samples showed BYI 02960-difluoroethyl-amino-furanone residues above the LOQ (0.01 mg/kg). Therefore, the residue definition for **enforcement** of BYI 02960 residues in rotational crops is proposed as parent **BYI 02960** plus **DFA** in accordance with the proposal for primary crops.

4.1.2 Definition of the residues in food of animal origin relevant to MRLs

Livestock Metabolism:

The metabolism of BYI 02960 was investigated in laying hens as a model for poultry and lactating goats as a model for ruminants following oral administration of [pyridinylmethyl-^{14}C]BYI 02960 and [furanone-4-^{14}C]BYI 02960 for both species.

Six hens were orally dosed once daily in the morning for 14 consecutive days with an aqueous 0.5% Tragacanth® suspension of 1.0 mg/kg body weight which corresponded to approximately 16.7 mg a.s./kg dry feed/day. 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.

One goat 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 which corresponded to approximately 26.6 mg a.s./kg dry feed/day. 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 parent compound and metabolites.

In case of laying hens the overall recovery of radioactivity was high (96.1% of the total dose) after administration of [pyridinylmethyl-^{14}C]BYI 02960. However, following the dosage of [furanone-4-^{14}C]BYI 02960 the recovery was lower (82.2% of the total dose). This is probably due to the partial instability of the labelling position and the formation of $^{14}CO_2$, an observation which was also made in rat studies with the [furanone-4-^{14}C]-labelled test compound. The total radioactive residues in the eggs and edible tissues as well as the concentrations of the identified metabolites are summarised in the following table.

Radioactive residues of parent compound and metabolites in eggs and edible organs and tissues of laying hens following oral administration of 14 daily doses of [pyridinylmethyl-14C]- or [furanone-4-^{14}C] BYI02960 at a dose rate of 1.0 mg/kg

	Pyridinylmethyl-^{14}C								**Furanone-4-^{14}C**							
	Eggs (day 3-13)		Muscle		Fat		Liver		Eggs (day 2-7)		Muscle		Fat		Liver	
Total radioactivity (TRR) (mg/kg)	0.084		0.070		0.021		0.435		0.540		0.183		0.427		2.178	
BYI 02960-	%TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Endogenous fatty acids									52.0	0.281	8.1	0.015	95.9	0.410	51.5	1.121
lactato-mercaptyl-nicotinic acid	4.0	0.003	3.6	0.002			15.5	0.068								
acetyl-cysteinyl-nicotinic acid							0.3	0.001								
6-CNA	7.2	0.006	8.8	0.006	1.8	<0.001	6.4	0.028								
des-difluoroethyl-OH-SA			2.1	0.001	5.6	0.001	3.1	0.014	0.1	0.001	0.5	0.001			0.2	0.004
acetyl-AMCP	23.1	0.019	40.2	0.028	28.5	0.006	6.3	0.027								
des-difluoroethyl	8.9	0.007	9.9	0.007	5.0	0.001	1.8	0.008	1.2	0.006	2.6	0.005			0.8	0.017
AMCP-difluoroethanamine-SA							0.3	0.001								
OH-SA	5.1	0.004	1.8	0.001	16.2	0.003	22.5	0.098	0.6	0.003					5.1	0.112
OH	18.0	0.015	8.1	0.006	5.5	0.001	1.5	0.007	2.3	0.013	2.4	0.004			0.8	0.018
Parent compound	19.8	0.017	9.8	0.007	15.3	0.003	0.9	0.004	2.3	0.013	2.9	0.005			0.5	0.010
Total identified	86.2	0.072	84.2	0.059	77.9	0.016	58.6	0.255	58.5	0.316	16.5	0.030	95.9	0.410	58.9	1.282

For lactating goats the recovery of radioactivity was also higher for the pyridinylmethyl-label (88.8% of the total dose) as compared to the furanone-label (78.9% of the total dose). The same explanation as for the hen studies would be applicable here. The total radioactive residues in the milk and edible tissues as well as the concentrations of the identified metabolites are summarised in the following table.

Radioactive residues of parent compound and metabolites in milk and edible organs and tissues of lactating goats following oral administration of 5 daily doses of [pyridinylmethyl-^{14}C]- or [furanone-4-^{14}C] BYI02960 at a dose rate of 1.0 mg/kg

	Pyridinylmethyl-^{14}C									
	Milk		Muscle		Fat		Kidney		Liver	
TRR (mg/kg)	0.186		0.356		0.106		1.869		1.215	
BYI 02960-	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Cysteinyl-nicotinic acid							6.1	0.114	4.8	0.058
Hippuric acid	9.1	0.017					9.5	0.178	0.8	0.010
Methylthio-glyoxylic acid	1.5	0.003	1.3	0.005						
OH-gluA (isomer 1)							6.0	0.112		
OH-gluA (isomer 2)							9.3	0.175	1.4	0.016
OH-gluA (isomer 3)							8.4	0.158		
OH-gluA (isomer 4)							7.5	0.141		
AMCP-difluoroethanamine							1.1	0.020	1.2	0.015
Des-difluoroethyl										
OH							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
	Furanone-4-^{14}C									
	Milk		Muscle		Fat		Kidney		Liver	
TRR (mg/kg)	1.046		0.539		0.265		1.472		1.746	
BYI 02960-	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Lactose	66.8	0.698								
OH-gluA (isomer 1)							2.2	0.032		
OH-gluA (isomer 2)							2.2	0.032		
OH-gluA (isomer 3)							4.7	0.069		
OH-gluA (isomer 4)							3.5	0.052		
Des-difluoroethyl							1.3	0.019		
OH			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

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

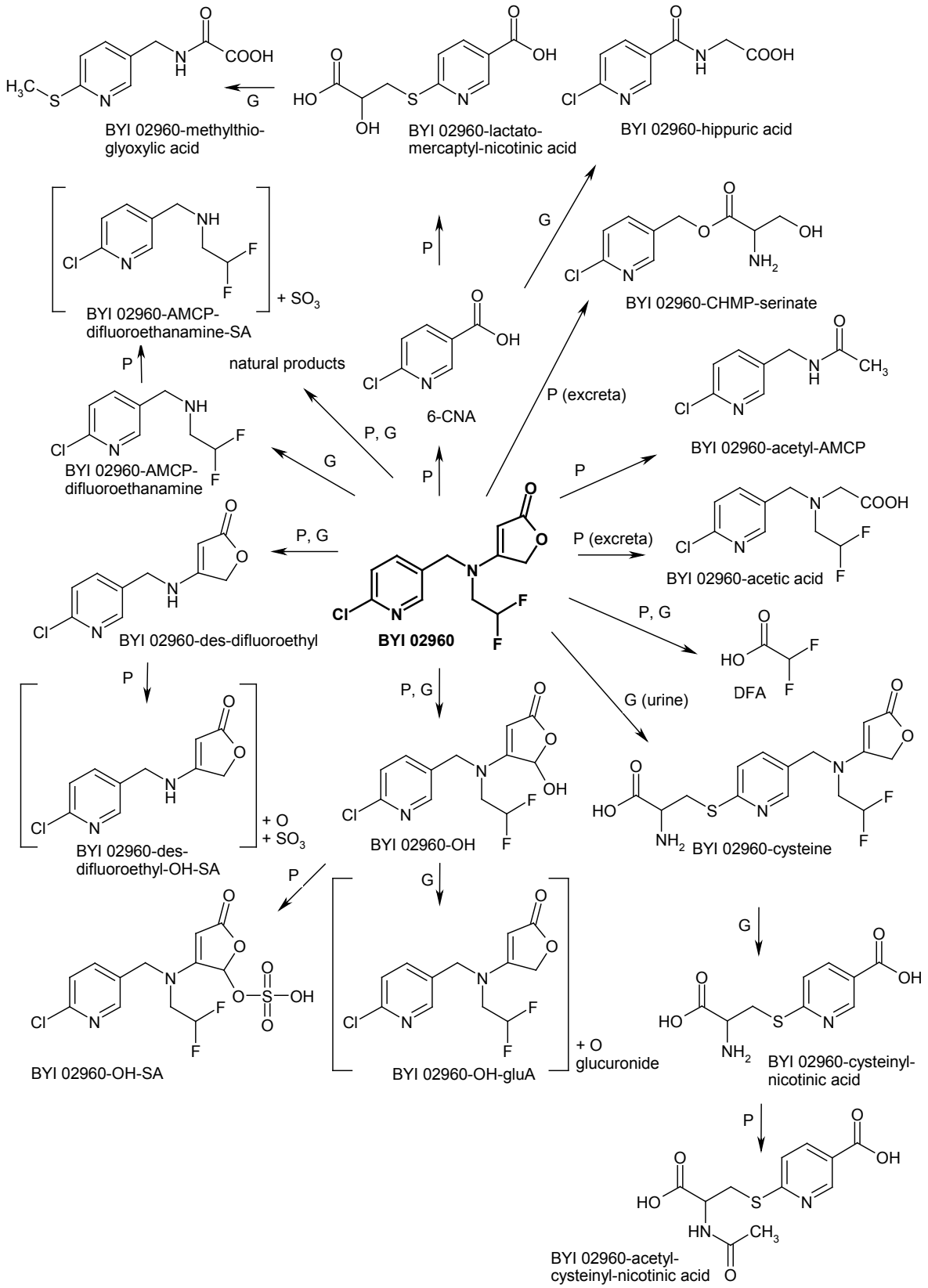
radioactivity originating from this label is incorporated into the natural compound pool. i.e. in fatty acids in all poultry tissues and lactose in the milk of goats.
Notwithstanding these observations, the unchanged parent compound is a significant, if not the dominating constituent of the residue in 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 proposed metabolic pathway of BYI 02960 in edible tissues of livestock, milk and eggs is shown on the next page. 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, and
- cleavage of the difluoroethyl group forming BYI 02960-des-difluoroethyl and difluoroacetic acid.

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-^{14}C]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.

Proposed metabolic pathway of BYI 02960 in lactating goats and laying hens



G = goat P = poultry

These main metabolic reactions detected in livestock were also observed in the rat studies on absorption, distribution, metabolism and elimination (ADME) of BYI 02960 (cf. 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.
In the ADME studies, parent compound was highly bioavailable and rather moderately metabolised. Generally, the metabolic profiles in urines and faeces were very similar for both sexes and the dose rates tested, but male rats exhibited a higher rate of metabolite formation compared to female animals. In all low dosed tests with male and female rats the unchanged active substance was found between 40.9% and 77.7% of the given dose. The hydroxylated parent compound and its conjugates are major rat and livestock metabolites. The same is true for 6-CNA and its glycine conjugate hippuric acid. Also BYI 02960-des-difluoroethyl was found in rats. In livestock, some further conjugates of 6-CNA occur, i.e. BYI 02960-lactato-mercaptyl nicotinic acid, BYI 02960-cysteinyl-nicotinic acid and BYI 02960-acetyl-cysteinyl-nicotinic acid. The formation of these conjugates is considered a typical detoxification reaction so that these metabolites would also be covered by 6-CNA. It should also be noted that these metabolites occur only at very low concentrations in the livestock metabolism studies which have been conducted at exaggerated dose levels. Finally, there is a group of livestock metabolites which do no longer contain the furanone ring (BYI 02960-AMCP-difluoroethanamine, BYI 02960-AMCP-difluoroethanamine-SA and BYI 02960-acetyl-AMCP). There is no equivalent metabolite in the rat. These metabolites occur at maximum concentrations of 0.028 mg/kg (muscle of poultry) so that one would expect residues below 0.01 mg/kg in the poultry feeding study at the 1X-dose level (cf. KIIA 5.10). BYI 02960-acetyl-AMCP as the main metabolite of this group was included in the analytical method for data collection used in the poultry feeding study (cf. KIIA 6.4.1). With the exception of one muscle sample in which a concentration of 0.003 mg/kg was determined, the residue levels of BYI 02960-acetyl-AMCP were in all cases below the LOD of 0.003 mg/kg thus confirming the assumption made on the basis of the results of the metabolism study.

Considering the results of the livestock metabolism studies and as well the results of the feeding studies, the sum of parent compound **BYI 02960** and metabolite **DFA,** expressed as BYI 02960 equivalents, is proposed as **residue definition for enforcement**.

4.2 Residues relevant to consumer safety

Primary crops:

Numerous residue trials have been conducted to support the use of BYI 02960 in/on various crops. In the Annex II dossier, only the so-called "safe uses" have been described (lettuce and hops), data on other crops will be submitted in a separate document later in 2012.

The residue trials in lettuce and hops were conducted in Europe to support the use of BYI 02960 as foliar spray application.

Supervised residue trials:

For **lettuce**, 18 field trials and 9 greenhouse trials were conducted during the 2010 and 2011 growing seasons. The residue trials were conducted according to the critical GAPs being representative for field and greenhouse use.

In order to support the field use in the EU of BYI 02960 in lettuce, 9 valid trials were conducted in the northern European residue region and 9 trials were conducted in the southern European residue region in the years 2010-2011. BYI 02960 was applied twice as an SL 200 formulation at an active substance rate of 125 g/ha per treatment. The application intervals were 9-11 days and the envisaged pre-harvest interval (PHI) was 3 days. The GAP reflects the intended use of a retail-sale formulation for private home and garden uses and is the most critical envisaged use in respect to MRL setting. The total residue of BYI 02960 (comprising parent BYI 02960 and its metabolites DFA and BYI 02960-difluoroethyl-amino-furanone), observed at day 3 (= PHI) ranged from 0.14 to 3.0 mg/kg in the northern European trials and from 0.39 to 3.2 mg/kg in the southern European trials in lettuce head. The respective median values were 0.71 mg/kg and 1.2 mg/kg.

In the greenhouse trials, BYI 02960 was also applied twice as an SL formulation (BYI 02960 SL 200), at 10-day intervals, with an envisaged PHI of 3 days. The total residue of BYI 02960 observed at day 3 (= PHI) ranged from 0.80 to 6.0 mg/kg in lettuce head, with a median of 2.2 mg/kg.

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 somewhat higher total residues in lettuce than did the field uses. Thus the residue results obtained from the greenhouse trials will be representative for the MRL calculation.

In order to support the use in the EU of BYI 02960 in **hops**, 8 valid trials were conducted in the northern European residue region in the years 2010-2011. BYI 02960 was applied once as an SL 200 formulation at an active substance rate of 120 g/ha. Samples of both green and dried hop cones were taken at several intervals after the final application, including the envisaged PHI of 21 days. The total residue of BYI 02960 observed at day 21 (= PHI) ranged from <0.04 to 60.87 mg/kg in green hop cones. The median value was 0.47 mg/kg. When evaluating the highest residues in dried cones, residues ranged from 0.61-2.4 mg/kg at relevant sampling intervals (either day 21 or, in three trials, day 28), with a median of 1.2 mg/kg.

Livestock feeding studies:

Livestock feeding studies were conducted with BYI 02960 on poultry and cattle in order to elucidate the levels of relevant residues in poultry and cattle tissues and in eggs and milk. The studies were designed to cover the regulatory needs of various regions in the world in which BYI 02960 is to be registered, including the EU, NAFTA, and Australia.

The test substance used in the feeding study should be representative of the residue in the feedstuffs. In the case of the new BCS insecticide BYI 02960, by far the major part of the residue in plants is formed by parent compound BYI 02960 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 could be applied. After discussion with the EU Rapporteur (Ctgb, NL), it was decided to feed parent BYI 02960 and derive separate transfer factors for the total residue of BYI 02960 (parent compound BYI 02960 plus DFA), as well as separately for DFA.

While the nature of the calculations/evaluations for the total residue is relatively straightforward, using the study data to evaluate the metabolite DFA required careful consideration. As agreed with the Rapporteur Member State, 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 BYI 02960 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.

Poultry:

BYI 02960 was administered orally (via capsule) to laying hens for 29 consecutive days at average dose rates of 1.5 mg/kg feed (1X EU dose), 6.5 mg/kg feed (4.3X, which approximated a 1X NAFTA dose), 19.4 mg/kg feed (13X), and 65.1 mg/kg feed (43X). 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 plus DFA** in poultry tissues at sacrifice in the EU 1X 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 on days 24 and 28. In the EU 1X dose group, the residues (BYI 02960 plus DFA) amounted to 0.061 and 0.057 mg/kg, respectively. The residues of **DFA** in poultry tissues at sacrifice in the EU 1X dosing group were 0.083 mg/kg in muscle, 0.029 mg/kg in fat, and 0.10 mg/kg in liver, expressed in parent compound equivalents. Prior to sacrifice, residues in eggs were measured on days 24 and 28. In the EU 1X dose group, the DFA residues amounted to 0.051 and 0.047 mg/kg, respectively.

Residue levels reached a plateau in eggs. Though residues were highest at day 28, residue data from the 43X 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 (pyridinyl-methyl label) or day 9 (furanone label).

Depuration occurred quickly. Total BYI 02960 residues in eggs, fat, liver, and muscle from the 43X dose level hens declined from 1.722 ppm, 1.230 ppm, 3.480 ppm, and 2.410 ppm, 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.

Cattle:

BYI 02960 was administered orally (via capsule) to dairy cows for 29 consecutive days at average dose rates of 4.8 mg/kg feed (1.3X EU dose), 27 mg/kg feed (6.3X, which approximated a 0.9X NAFTA dose), 55 mg/kg feed (13X), and 147 mg/kg feed (34X). 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 EU 1.3X 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 in the three lower dose groups on day 28. In the EU 1.3X dose group, the residues (BYI 02960 + DFA) amounted to 0.043 mg/kg. The **DFA** residues in bovine tissues at sacrifice in the EU 1.3X dosing group were always <0.02 mg/kg (ranging between 0.004 mg/kg in milk to 0.018 mg/kg in kidney).

Residue levels reached a plateau in milk. Highest residue was determined at day 17 in the 34X 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 34X 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.

Processing:

Field trials were conducted in order to determine "processing" factors for total residues of BYI 02960 from lettuce heads to parts thereof and from hops (dried cones) to beer.

For **lettuce heads**, the mean value of total residue "processing" factors for the outer leaves was 1.8, and 0.73 to the inner head parts. Further washing is of little consequence, with mean "processing" factors for unwashed or washed inner leaves of 0.76 and 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.

For **hops** (dried cones), 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.

Succeeding crops:

The field rotational crop data described in the Annex II dossier reflect only the "main" study, i.e. covering multiple rotations and 3 rotational crop groups (root, leafy, and cereal crops). Further data for many other crop groups will be submitted in a separate document later in 2012.

Supervised residue trials:

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 vegetables, cereals) were planted on the plots at 3 intervals thereafter.

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 BYI 02960-difluoroethyl-amino-furanone. 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 0.06-0.14 mg/kg (median 0.08 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 0.08 mg/kg (n=4).
- 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).

4.3 Residues relevant to worker safety

See chapter 3.6.4.

4.4 Proposed MRLs and compliance with existing MRLs

4.4.1 Compliance with existing MRLs

BYI 02960 and its formulation are submitted for the first time to the EU Rapporteur (Ctgb, NL). The uses of the Global Joint Review partner countries Australia, Brazil, Canada and USA will be submitted in a separate document later in 2012 since the submission of the BYI 02960 dossier in these countries will occur later than in Europe. Therefore, no MRLs or Import Tolerances in any other country in the world have been set, yet.

4.4.2 Proposed MRLs

Plant matrices:

The proposed residue definition for enforcement is slightly different than the one for risk assessment, as it does include only parent compound BYI 02960 and DFA, and not BYI 02960-difluoroethyl-amino-furanone. However, BYI 02960-difluoroethyl-amino-furanone did not play a major role in any of the trials and thus its effect on the MRL calculation is negligible. The total residue values determined for risk assessment are therefore also valid for MRL calculations.

For **primary crops**, the MRL proposals are based on the results of the greenhouse studies on lettuce and the field trials on hops. These uses reflect only the "safe uses" - further uses will be submitted later in 2012 to cover numerous other crops.

For **rotational crops**, the MRL proposals are based on the results of 4 "main" field rotational crop trials, which cover each 3 rotations and 3 rotational crop groups (root, leafy, and cereal crops). Further data for many other crop groups will be submitted later in 2012.

Animal matrices

The proposed residue definition for enforcement in animal matrices is identical to the one made for risk assessment. It consists of the sum of parent compound BYI 02960 and its metabolite DFA, calculated as parent equivalents.

For **animal matrices**, the MRL proposals are based on the results of the livestock feeding studies in poultry and cattle. They are presented for animal tissues (muscle, fat, liver, kidney) and products (eggs and milk), resulting from exposure of livestock to feed crops treated with BYI 02960.

MRL calculations were made according to the statistical methods described in EU guideline 7039/VI/95 and the German BBA-Guideline, Part IV, 3-6 (1990), using methods I (including elimination of outliers) and II; and to the OECD calculator.

The following MRLs for the relevant residue of BYI 02960 have been proposed in the Annex II dossier based on uses described:

Proposed MRLs based on envisaged uses of BYI 02960

Commodity	MRL proposals* (mg/kg)
lettuce and similar plants**	7.0
hops (dried cone)	4.0
rotational root crops	0.30
rotational leafy veg. crops	0.30
rotational cereal crops	1.5

Commodity	MRL proposals* (mg/kg)
eggs	0.15
poultry meat (muscle)	0.20
poultry fat	0.07
poultry liver/offal	0.30
milk	0.07
bovine meat (muscle)	0.20
bovine fat	0.15
bovine liver	0.30
bovine kidney	0.40
other bovine offal	0.40

* MRLs reflect the sum of BYI 02960 and DFA, expressed in parent equivalents
** no registrations are sought in endive ("scarole") and similar crops

4.5 Proposed import tolerances

Numerous residue trials have been conducted to support the use of BYI 02960 in/on various crops and in various regions. In the presented Annex II dossier, only the so-called "safe uses" have been described (lettuce and hops). The uses of the Global Joint Review partner countries Australia, Brazil, Canada and USA will be submitted in a separate document later in 2012 since the submission of the BYI 02960 dossier in these countries will occur later than in Europe. Harmonized MRLs considering import tolerances will be presented in the separate document submitted later in 2012.

4.6 Basis for differences, if any, in conclusions reached having regard to established or proposed CAC MRLs

Please refer to chapter 4.5.

Chapter 5 Fate and behaviour in the environment

5.1 Definition of the residue relevant to the environment

5.1 Definition of the residue relevant to the environment

In Europe the definition of the residue relevant for risk assessment is:

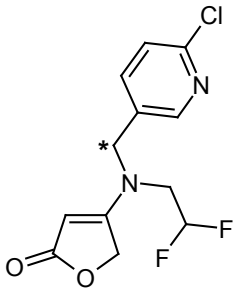
Soil:	BYI 02960, DFA, 6-CNA
Surface water:	BYI 02960, DFA, 6-CNA, BYI 02960-succinamide, BYI 02960-azabicyclosuccinmaide
Groundwater:	BYI 02960, DFA and 6-CNA
Air:	BYI 02960

The definition of the residue relevant for monitoring

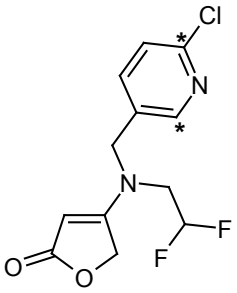
Soil:	BYI 02960
Groundwater:	BYI 02960
Surface water:	BYI 02960
Air:	BYI 02960

5.2 Fate and behaviour in soil

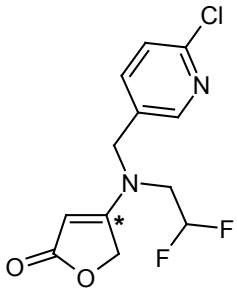
The laboratory studies concerning the fate and behaviour of BYI 02960 in soil were conducted using different radiolabelled forms; the structure of BYI 02960 and the positions of the different radiolabels are as follows (* indicates position of radiolabel):



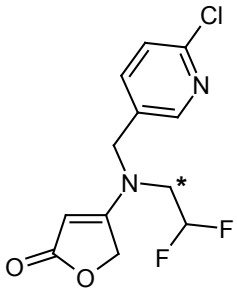
[Pyridinyl-methyl-14C] BYI 02960 (PYM-^{14}C)



[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)

The biotransformation of [pyridinylmethyl-14C] BYI 02960 was studied in four European soils, [furanone-4-14C] BYI 02960 was studied in four European soils and two US soils. [Ethyl-1-14C]BYI 02960 was studied in three European soils and [pyridine 2, 6-14C] BYI 02960 was studied in one European and two US soils. All studies were conducted for a maximum period of 120 days under aerobic conditions in the dark at approx. 20 °C and 55% WHCmax (max. water holding capacity, European soil) or pF 2 to 2.5 (US soils). Additionally for the [furanone-4-14C] BYI 02960 and [pyridine 2,6-14C]BYI 02960 gamma irradiated soil samples were investigated in the US. BYI 02960 was applied at nominal application rates equivalent to 200 to 410 g/ha field rate.

In all studies mineralization to $^{14}CO_2$was high, ranging from 12.0 to 58.6 % maximum levels depending on soil type and position of label. Volatile organic compounds were negligible (≤ 0.1% AR).
With [pyridinylmethyl-^{14}C]BYI 02960 and [furanone-4-^{14}C]BYI 02960 no major metabolites were detected except for $^{14}CO_2$ formed up to 39% for the FUR label and up to 59% for the PYR label. Non-extractable residues (NER) where up to 17% with the PYR label and 34% with the FUR label. The investigations with gamma irradiated soils indicated a biological component to the formation of NER.
With [ethyl-1-^{14}C]BYI 02960 one major metabolite difluoroacetic acid (max. 33.9% of AR and declining during the study) was formed. Again, significant amounts of 14CO2 (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.
In the studies with [pyridine 2,6-14C]BYI 02960, the mineralization to ^{14}CO2 was significant (up to 57%AR) with the formation of minor metabolites in the European soil and one US soil. In the second US soil, one major metabolite, which was identified as 6-chloronicotinic acid, was formed at maximum of 17.1%, this was also identified as a minor metabolite in the European soil. NER formation was in the range of max. 11.3 to 25.5% of AR in the three soils. The formation of NER was shown to have a biological component.

An aerobic soil degradation study on three European soils has been performed with 6-CNA in support of a previously approved plant protection product. Degradation was rapid.

The anaerobic degradation of BYI 02960 was investigated in a single European silt loam soil with ^{14}C PYR, FUR and ETH labelled BYI 02960 and two US soils with ^{14}C PYR label, only. Results from both studies show that BYI 02960 and metabolites DFA and 6-CNA are expected to be stable under anaerobic/flooded soil conditions.

The phototransformation of [pyridinylmethyl-^{14}C]- and [furanone-4-^{14}C]BYI 02960 was studied on a US loam soil at a concentration corresponding to a field rate of ca. 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. In addition, photolysis was studied on air dried samples. In the irradiated test systems, BYI 02960 slightly decreased over 5 days (<10%). No volatiles or major soil transformation products were detected. Phototransformation on soil is of minor importance for the degradation of BYI 02960 under outdoor conditions.

Soil dissipation of BYI 02960 under field conditions was investigated after application of BYI 02960 SL 200 at a rate of 250 g a.i./ha onto bare soil plots at six European sites representative for both Northern and Southern Europe. Soil samples were taken from day 0 to 540 days post-application to a maximum depth of 100 cm. Soil samples were analysed for BYI 02960 and metabolite BYI 02960-DFA (difluoroacetic acid) using LC-MS/MS; the limit of quantification (LOQ) was 5.0 µg/kg, and the limit of detection (LOD) was 1.5 µg/kg for both analytes. Residues essentially remained in the upper 0-20 cm soil layer with only low amounts below the LOQ detected to a maximum depth of 30 cm. At study completion, the remaining BYI 02960 residues in soil corresponded to 2.9 to 29.8% of the applied amount. BYI 02960 showed biphasic degradation behaviour. The calculated DT_{50} of BYI 02960 ranged between 8.3 and 251 days. In general the field dissipation behaviour observed for

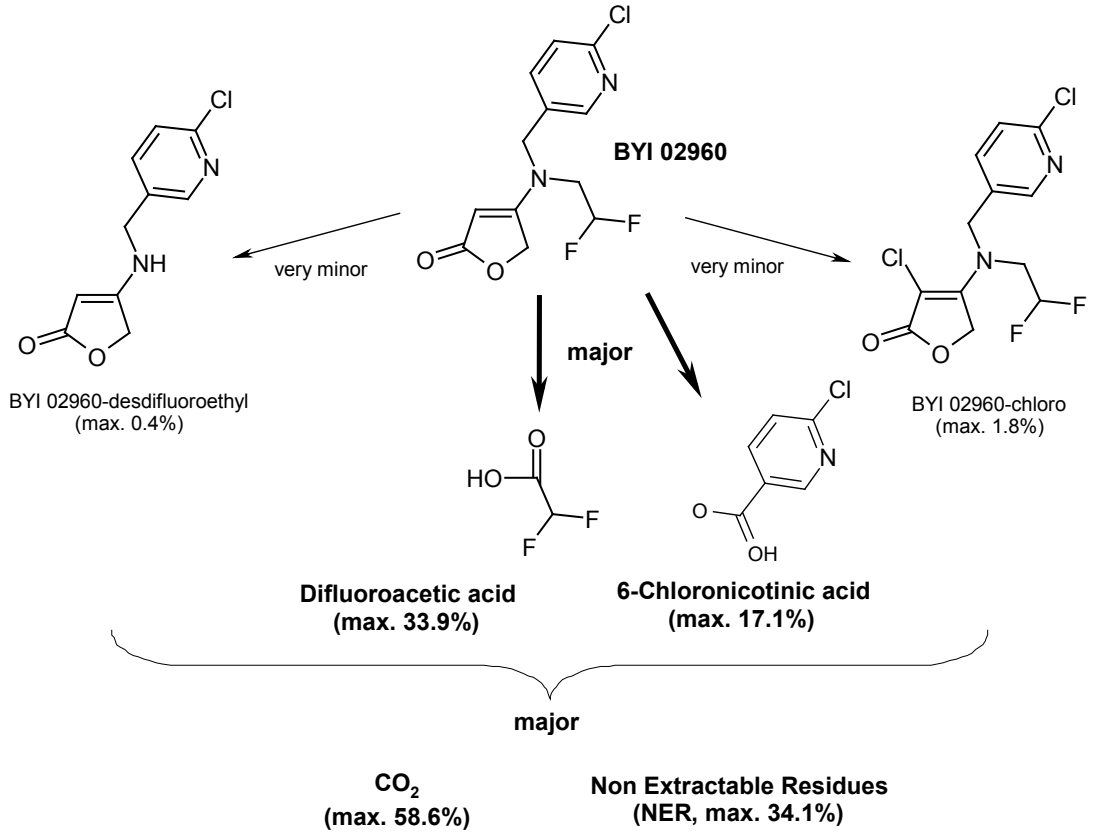
BYI 02960 and soil metabolite BYI 02960-DFA was comparable to that found in the standardized laboratory studies

In summary, the degradation of BYI 02960 in and on soil has been extensively investigated in the laboratory with appropriately labelled compound. Under aerobic conditions, BYI 02960 degrades at a moderate rate in soil and is extensively mineralized. Two metabolites have been observed at >10% AR in at least 1 soil: difluoroacetic acid (DFA) and 6-chloronicotinic acid (6-CNA). Non-extractable residues have been shown to be strongly bound to the solid (humin) fractions of soil. Degradation under anaerobic or photolytic conditions is not expected to significantly contribute to the overall environmental dissipation of BYI 02960. Results of field dissipation studies support the laboratory findings.

Considering the results from laboratory soil metabolism studies and terrestrial field dissipation studies the major route(s) of dissipation for BYI 02960 are:

- cleavage of the difluoroethyl group producing difluoroacetic acid (BYI 02960-DFA),
- cleavage of the molecule at the pyridinylmethylene bridge with subsequent oxidation to form 6-CNA
- mineralization to CO_2.

The soil degradation pathway for BYI 02960 is shown below:



Note: The degradates to be observed as well as the given maximum values are highly dependent on radiolabel and kind of soil used

Kinetic evaluations of the overall soil degradation results have been performed for parent compound and metabolites. In order to determine the best-fit kinetic model for the degradation of BYI 02960 in soil over time, SFO, DFOP and FMOC 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. Across all laboratory studies conducted with BYI 02960, the trigger value geometric mean DT_{50} for BYI 02960 is 73 days. DT_{50} input values for EU modelling were determined as follows:

For PEC_{soil} the calculation considered the DFOP kinetics from the field dissipation studies, non-normalised, (fast phase 0.2 days, slow phase 462 days)

For groundwater a tiered approach was taken using the DT_{50} values calculated as follows

For Tier 1 modelling, the 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 value of 94.8 days.

For Tier 2a (DFOP) according to FOCUS (2009), DFOP degradation kinetics are considered in leaching modelling based on the procedure described in FOCUS (2006).
To obtain common DFOP parameters across all soils, 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.
A fast compartment DT_{50fast} of 33 days and a slow compartment DT_{50slow} of 95 days were derived.

Tier 2a (TDS): Time-dependent sorption (TDS) data of BYI 02960 on four soils were derived via curve fitting. These parameters are required to address TDS processes in regulatory exposure modelling.[5]. Experimental soil data were re-calculated to fit the TDS model, resulting in a geomean DT_{50} of 58 days, a K_{OM} of 46.5 L/kg and a Freundlich exponent of 0.860.

For 6-CNA, the trigger value DT_{50} is 3.1 days and the DT_{50}value for modelling is 3.0 days.
For DFA the trigger value DT_{50} is 62 days and the DT_{50} for modelling is 44.7 days.

Mobility
Freundlich adsorption and desorption constants KF and KOC of BYI 02960 have been determined in batch equilibrium experiments conducted in the dark at 20 ± 1 °C with 4 European and 2 US soils using radiolabelled test substance ([PYM-14C]BYI 02960). The adsorption phase of the study was carried out using pre-equilibrated air-dried soils in 0.01 M aqueous CaCl2 solution with soil/solution ratios ranging from 1:1 to 1:4. BYI 02960 was applied at concentrations of nominal 1.0, 0.3, 0.1, 0.03, and 0.01 mg/L. The desorption phase was performed by supplying pre-adsorbed soil samples with fresh 0.01 M aqueous CaCl2 solution for one desorption cycle for all soils. Additionally, for the highest concentration on US soils two additional desorption cycles were performed with 24 hours

[5] Beulke, S., van Beinum, W., Boesten, J., ter Horst, M. (2010): Proposed guidance on how aged sorption studies for pesticides should be conducted, analysed and used in regulatory assessments. The Food and Environment research Agency, York, UK and Alterra, Wageningen, The Netherlands

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 92.9 - 94.7%.
The calculated adsorption constants Kf (ads) of the Freundlich isotherms for the four European test soils ranged from 2.08 to 3.82 mL/g, and the mean KOC(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. For the two US soils, the calculated adsorption constants Kf (ads) of the Freundlich isotherms for the two test soils were 0.597 and 2.512 mL/g, and the mean KOC(ads) was 107.8 mL/g. The Freundlich exponent 1/n was 0.8505 and 0.9021 (mean 0.8763). The desorption KF(des) and the normalized KOC(des) values were significantly higher (i.e. 2 to 3.6 times higher) than those obtained for the adsorption phase, indicating that the test item once adsorbed to soil is not readily desorbed.

The biotransformation and time dependent sorption of [pyridinylmethyl-14C]BYI 02960 was studied in four European soils for a maximum period of 120 days under aerobic conditions in the dark at ca. 20 °C and 55% WHCmax (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 CaCl2-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 analysed and quantified by TLC with HPLC as the confirmatory method.

At the beginning of the study (DAT-0; equivalent to approximately two hours) the distribution coefficient values RTDS were 1.46, 1.91, 3.35 and 4.66 mL/g. Depending on the aging time, these values increased with time; coefficient values RTDS at the end of the study were 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 RTDS values increased by a factor of 4.4, 2.9, 3.2 and 2.6 (mean of all four soils = 3.3).

In summary, in standard batch equilibrium studies on 6 soils the adsorption Koc for for BYI 02960 ranged from 74.9 to 132.2 mL/g (arithmetic mean Koc98.4, mean 1/n 0.866) indicating medium mobility in soil; desorption Kdoc values were higher indicating significantly stronger sorption. In time dependent sorption studies the sorption of BYI 02960 was shown to increase over time with an ageing factor of 2.4 to 4.4. The time-dependent sorption data generated 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.

The equilibrium sorption of soil metabolite 6-chloronicotinic was studied in experiments using five soils and an aquatic pond sediment. The arithmetic mean 1/n value for soil was 0.949, adsorption Koc values in soils ranged from 70 to 258, averaging 88.0 L/kg (excluding the sediment and 1 soil with very low carbon content). Similar Kf and Kdes values for all soils and the pond sediment indicate a reversible equilibration between adsorption and first desorption phases. 6-CNA can be classified as medium to high mobility for adsorption and desorption.
The equilibrium sorption of difluoroacetic acid 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. DFA can be classified as very mobile for adsorption with low mobility for desorption.

Volatility

For compounds with a vapor pressure < 10^{-5} Pa at 20 °C, volatilization from soil surfaces or from plant surfaces is not considered relevant. Based on BYI 02960's vapor pressure value of 9.1 x 10^{-9} Pa , volatilization is not expected.

Estimation of concentration of BYI 02960 and its metabolites in soil

Calculations were based on a simple first tier approach 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). As hops and lettuce have not been defined in the FOCUS groundwater guidance paper, vines and cabbage were chosen as surrogate crops.

PEC_{soil} calculations were based on the DT_{50} of 0.2 days for the fast and 462 days for the slow degrading compartments (DFOP, worst case of field dissipation studies, non-normalised). The maximum PEC_{soil} values of BYI 02960 are summarised below for hops and lettuce.

Maximum PEC_{soil} (of BYI 02960 in hops in the upper 5 cm, DFOP decay

	Time [days]	BYI 02960 Hops, 1 x 150 g/ha	BYI 02960 Lettuce, 1 x 125 g/ha (field use)
		PEC_{soil} [mg/kg]	TWA_{soil} [mg/kg]
Initial	0	0.080	0.125-

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 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.

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

	Residues distributed over …	Seasonal $PEC_{s, max}$, max. soil residue in 1st year	Long-term plateau / background conc. C_{min}	Long-term maximum conc. C_{max}	Background C_{min} + max. of 1 year in 5 cm
	[cm]	[mg/kg]	[mg/kg]	[mg/kg]	[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 x 125 g/ha	5	0.125	0.125	0.250	
	10	0.063	0.063	0.125	0.188
	20	0.031	0.031	0.063	**0.156**

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).

Predicted environmental concentrations in soil were also calculated for the major soil metabolites DFA and 6-CNA using the approach, scenarios and application rates described for the calculations for the parent compound with compound specific parameters are summarised below.

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 the following table.

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 x 125 g/ha of parent	0.014	0.012

5.3 Fate and behaviour in water

The fate and behaviour of BYI 02960 in aquatic systems was investigated under standardized laboratory conditions using radiolabeled test substance. Additionally, degradation was investigated in pond water and sediment in outdoor microcosms as an aquatic model ecosystem.

In a laboratory study, BYI 02960 was stable to hydrolysis in sterile buffer solutions at pH 4, 7 and 9, indicating the active substance will be hydrolytically stable under environmental conditions. In two aqueous photolysis studies, BYI 02960 degraded very rapidly in sterile buffer and natural water. The major degradates were identified as BYI 02960-succinamide (found at max. 39.6% of applied) and BYI 02960-azabicyclosuccinamide (found at max. 25.9% of applied). 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). BYI 02960 should rapidly degrade in the aqueous environment if exposed to sunlight.

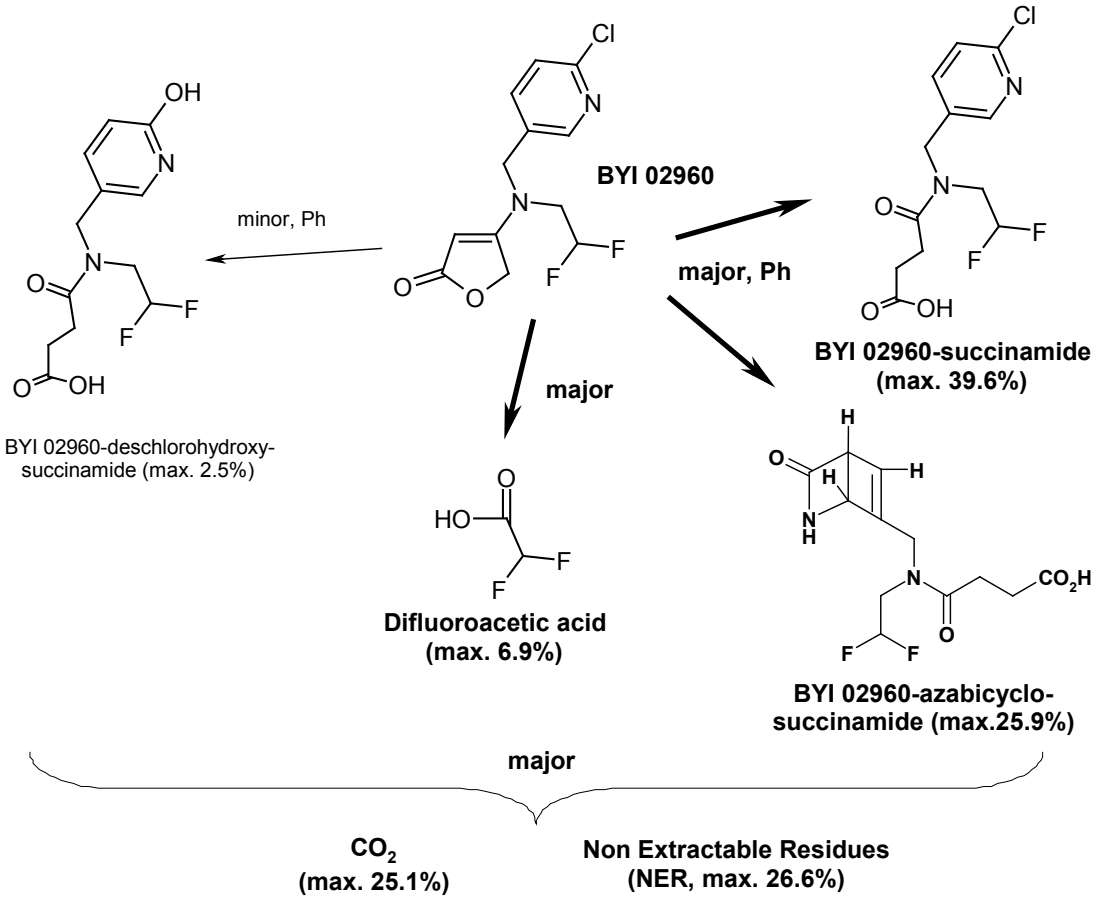
The degradation of BYI 02960 under aerobic aquatic conditions was studied in two water-sediment systems using [furanone-4-^{14}C]-, [ethyl-1-^{14}C]-, and [pyridine-2,6-^{14}C]-labelled BYI 02960. Dissipation of BYI 02960 from the water phase was mainly characterized by rapid partitioning into the sediment where it is slowly degraded and mineralized. DFA was observed as a degradation product of [ethyl-1-^{14}C]BYI 02960 in both water/sediment systems tested. In the water phases DFA accounted for up to 6.0% of applied radioactivity and in the sediment extracts for a maximum of 0.9% of the applied radioactivity. No further significant degradation products were observed in any of the studies except for mineralization to carbon dioxide (max. 8.5% of applied) and formation of non-extractable residue (NER, max. 26.6% of applied). The DT_{50} value for BYI 02960 in the entire water/sediment systems ranged from 193 to 285 days.

In a separate aerobic water-sediment study the degradation behaviour of DFA applied as the test item was investigated. Mineralization to carbon dioxide (max. 25.1% of applied) and formation of NER (max. 15.8% of applied) was observed. The total system degradation half-life for DFA was 249 days.

In a supportive study the fate of BYI 02960 (tech.) was investigated in pond water and sediment in outdoor microcosms as an aquatic model ecosystem for lentic aquatic freshwater systems with different trophic levels. The dissipation of BYI 02960 from the supernatant water phase occurred by translocation into the sediment and by degradation; the mean DT50 for parent compound was 81 days. The overall degradation (mean of 95 days) was faster under the prevailing outdoor conditions compared to the standardized laboratory water sediment studies. The more rapid degradation may in part be due to the enhanced photodegradation due to outdoor test conditions as well as more persistent microbial activity.

The anaerobic aquatic degradation of [pyridine-2,6-^{14}C]BYI 02960 was studied in two pond water/sediment systems. No major degradates were formed in the test systems. $^{14}CO_2$ levels were≤0.1% and no organic volatiles were observed. BYI 02960 is stable under anaerobic aquatic conditions.

The overall aquatic degradation pathway for BYI 02960 is shown below.



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

Estimation of concentration of BYI 02960 and its metabolites in surface water

Predicted environmental concentrations in surface water and sediment (PEC_{sw} and PEC_{sed}) of BYI 02960 and its metabolites have been calculated for the use in hop and lettuce in Europe employing the tiered FOCUS Surface Water approach. FOCUS STEP 1 and 2 calculations were made for all compounds. For BYI 02960 Step 3 calculations and Step 4 calculations considering potential mitigation measures (spray drift buffers, run-off buffers and drift reducing nozzles) were performed., The calculations consider 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.

The maximum PEC values for Steps 1 and 2 are given below for parent and metabolites.

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

Crop Appl. 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 x 125 g/ha (F)	2 (N-EU)	6.410	6.249
	2 (S-EU)	11.78	11.51

Maximum PEC_{sw} and PEC_{sed} values for metabolites of BYI 02960 at Step 1 and 2

Crop	FOCUS	difluoroacetic acid		6-chloronicotinic acid		-succinamide		-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	Step 1	5.810	0.380	4.177	3.675	4.065	<0.001	2.499	<0.001
	Step 2 N-EU	0.743	0.050	0.232	0.204	4.065	<0.001	2.499	<0.001
	Step 2 S-EU	1.268	0.086	0.463	0.408	4.065	<0.001	2.499	<0.001
Lettuce 1 x 125 g/ha (F)	Step 2 N-EU	0.682	0.046	0.289	0.255	0.484	<0.001	0.297	<0.001
	Step 2 S-EU	1.339	0.091	0.579	0.509	0.484	<0.001	0.297	<0.001

F = Field use,

Maximum PEC_{sw} and PEC_{sed} for BYI 02960 at Step 3

	BYI 02960			
Step 3	Hop (F)		Lettuce (F)	
Scenario	Entry route *	$PEC_{sw, max}$ [µg/L]	Entry route *	$PEC_{sw, max}$ [µg/L]
D3 (ditch, 1st)	-[1]	-	S	0.830
D3 (ditch, 2nd)	-	-	S	0.840
D4 (pond, 1st)	-	-	D	1.035
D4 (stream, 1st)	-	-	S	0.794
D6 (ditch, 1st)	-	-	D	1.268
R1 (pond, 1st)	S	0.394	R	0.060
R1 (stream, 1st)	S	5.531	R	0.858
R1 (pond, 2nd)	-	-	R	0.097
R1 (stream, 2nd)	-	-	R	1.186
R2 (stream, 1st)	-	-	R	1.586
R2 (stream, 2nd)	-	-	R	0.940
R3 (stream, 1st)	-	-	R	2.226
R3 (stream, 2nd)	-	-	R	3.570
R4 (stream, 1st)	-	-	S	0.522
R4 (stream, 2nd)	-	-	R	4.808

* S= spray drift, D=drainflow. R= runoff
[1] not a defined crop for this scenarion

Maximum PEC_{sw} and PEC_{sed} for BYI 02960 at Step 4 considering different mitigation measures:

	BYI 02960		
Step 4	**Hop (F)**		
Scenario	**Buffer Zone (no spray zone)**	**Drift reduction**	**$PEC_{sw, max}$ [µg/L]**
R1 (stream)	0m	90%	0.553
R1 (stream)	5m	90%	0.452
R1 (stream)	10 m	75%	0.589
R1 (stream)	20 m	50%	0.354

Buffer Width & Type	**FOCUS Scenario**	**PECsw [µg/L] Drift Reduction**			
		25%	**50%**	**75%**	**90%**
0m (drift)	D3 (ditch, 1st)	0.632	0.434	0.235	0.117
	D3 (ditch, 2nd)	0.643	0.446	0.249	0.130
	D4 (pond, 1st)	1.034	1.034	1.033	1.033
	D4 (stream, 1st)	0.721	0.721	0.721	0.721
	D6 (ditch, 1st)	1.268	1.268	1.268	1.268
	R1 (stream, 1st)	0.858	0.858	0.858	0.858
	R1 (stream, 2nd)	1.186	1.186	1.186	1.186
	R2 (stream, 1st)	1.586	1.586	1.586	1.586
	R2 (stream, 2nd)	0.940	0.940	0.940	0.940
	R3 (stream, 1st)	2.226	2.226	2.226	2.226
	R3 (stream, 2nd)	3.570	3.570	3.570	3.570
	R4 (stream, 2nd)	4.808	4.808	4.808	4.808
5m (drift)	D3 (ditch, 1st)	0.252	0.145	0.091	0.059
	D3 (ditch, 2nd)	0.265	0.158	0.105	0.073
	D4 (pond, 1st)	1.035	1.034	1.033	1.033
	D4 (stream, 1st)	0.721	0.721	0.721	0.721
	D6 (ditch, 1st)	1.268	1.268	1.268	1.268
	R1 (stream, 1st)	0.858	0.858	0.858	0.858
	R1 (stream, 2nd)	1.186	1.186	1.186	1.186
	R2 (stream, 1st)	1.586	1.586	1.586	1.586
	R2 (stream, 2nd)	0.940	0.940	0.940	0.940
	R3 (stream, 1st)	2.226	2.226	2.226	2.226
	R3 (stream, 2nd)	3.570	3.570	3.570	3.570
	R4 (stream, 2nd)	4.808	**4.808**	4.808	4.808
10 m (drift and run-off)	D4 (pond, 1st)	1.034	1.033	1.033	1.033
	D4 (stream, 1st)	0.721	0.721	0.721	0.721
	D6 (ditch, 1st)	1.268	1.268	1.268	1.268
	R2 (stream, 1st)	0.716	0.716	0.716	0.716
	R3 (stream, 1st)	1.009	1.009	1.009	1.009
	R3 (stream, 2nd)	1.630	1.630	1.630	1.630
	R4 (stream, 2nd)	2.184	2.184	2.184	2.184
20m (drift and run-off)	D4 (pond, 1st)	1.033	1.033	1.033	1.033
	D4 (stream, 1st)	0.721	0.721	0.721	0.721
	D6 (ditch, 1st)	1.268	1.268	1.268	1.268
	R2 (stream, 2nd)	0.220	0.220	0.220	0.220
	R3 (stream, 1st)	0.528	0.528	0.528	0.528
	R3 (stream, 2nd)	0.856	0.856	0.856	0.856
	R4 (stream, 2nd)	1.144	1.144	1.144	1.144

Estimation of concentration of BYI 02960 and its metabolites in groundwater

The predicted environmental concentrations in groundwater (PEC_{gw}) for the active substance were calculated in a stepwise approach following the tiered approach proposed by the FOCUS (2009) , Tier 1 standard calculations, Tier 2a using DFOP and Tier 2a using time-dependent sorption (TDS), based on the simulation models PEARL and PELMO following the recommendations of the FOCUS working group on groundwater scenarios.

Tier 1: standard calculations following the recommendations of FOCUS (2009) with the DT_{50} values derived from standard laboratory soils moisture standardised to DT_{50} values at 100% field capacity (FC)/pF 2. The 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

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 2a (TDS): following the model of Boesten et al. (1989) implemented in PEARL and PELMO FOCUS (2009), time-dependent sorption (TDS) was evaluated using the TDS parameters determined according to Beulke et al. (2010). Time-dependent sorption (TDS) data of BYI 02960 on four soils were derived via curve fitting. These parameters are required as input for regulatory exposure modelling.[6].

Groundwater concentrations, considering the different tiered approach and annual/biannual applications are summarized below:

[6] Beulke, S., van Beinum, W., Boesten, J., ter Horst, M. (2010): Proposed guidance on how aged sorption studies for pesticides should be conducted, analysed and used in regulatory assessments. The Food and Environment research Agency, York, UK and Alterra, Wageningen, The Netherlands

Use in Hops

Scenario	BYI 02960 Application in hops, every year					
	Tier 1		Tier 2a		Tier 2a	
			DFOP approach		TDS approach	
	PEARL	PELMO	PEARL	PELMO	PEARL	PELMO
	PEC_{gw} [µg/L]	PEC_{gw} [µg/L]	PEC_{gw} [µg/L]	PEC_{gw} [µg/L]	PEC_{gw} [µg/L]	PEC_{gw} [µg/L]
Châteaudun	0.453	0.415	0.272	0.251	0.116	0.100
Hamburg	0.579	0.634	0.346	0.380	0.175	0.175
Kremsmuenster	0.430	0.481	0.257	0.287	0.135	0.157
Piacenza	0.359	0.442	0.213	0.263	0.118	0.157
Porto	0.220	0.286	0.133	0.171	0.056	0.087
Sevilla	0.223	0.063	0.137	0.039	0.042	0.007
Thiva	0.183	0.155	0.111	0.095	0.030	0.023

Scenario	BYI 02960 Application in hops, every 2nd year					
	Tier 1		Tier 2a		Tier 2a	
			DFOP approach		TDS approach	
	PEARL	PELMO	PEARL	PELMO	PEARL	PELMO
	PEC_{gw} [µg/L]	PEC_{gw} [µg/L]	PEC_{gw} [µg/L]	PEC_{gw} [µg/L]	PEC_{gw} [µg/L]	PEC_{gw} [µg/L]
Châteaudun	0.193	0.168	0.116	0.102	0.044	0.035
Hamburg	0.283	0.249	0.172	0.151	0.069	0.065
Kremsmuenster	0.172	0.196	0.103	0.118	0.049	0.056
Piacenza	0.154	0.211	0.093	0.127	0.041	0.065
Porto	0.093	0.122	0.056	0.073	0.020	0.030
Sevilla	0.091	0.022	0.056	0.015	0.014	0.002
Thiva	0.073	0.059	0.045	0.037	0.010	0.006

Scenario		BYI 02960 Application in lettuce, every year (Field use)					
		Tier 1		Tier 2a		Tier 2a	
				DFOP approach		TDS approach	
		PEARL	PELMO	PEARL	PELMO	PEARL	PELMO
		PEC_{gw} [µg/L]	PEC_{gw} [µg/L]	PEC_{gw} [µg/L]	PEC_{gw} [µg/L]	PEC_{gw} [µg/L]	PEC_{gw} [µg/L]
Châteaudun	(1st)	0.413	0.298	0.269	0.194	0.085	0.053
	(2nd)	0.556	0.396	0.355	0.257	0.111	0.070
Hamburg	(1st)	0.809	0.724	0.630	0.462	0.263	0.194
	(2nd)	1.081	0.983	0.751	0.708	0.329	0.272
Jokioinen		0.325	0.269	0.213	0.175	0.055	0.046
Kremsmuenster	(1st)	0.595	0.517	0.382	0.347	0.181	0.157
	(2nd)	0.698	0.637	0.451	0.417	0.216	0.198
Porto	(1st)	0.327	0.413	0.216	0.255	0.090	0.133
	(2nd)	0.643	0.713	0.431	0.461	0.176	0.247
Sevilla	(1st)	0.018	0.005	0.016	0.005	0.001	<0.001
	(2nd)	0.025	0.006	0.025	0.006	0.001	<0.001
Thiva		0.313	0.221	0.224	0.149	0.047	0.036
1st = first/early season crop, 2nd = second/late season crop							

Scenario		BYI 02960 Application in lettuce, every 2nd year (Field use)					
		Tier 1		Tier 2a		Tier 2a	
				DFOP approach		TDS approach	
		PEARL	PELMO	PEARL	PELMO	PEARL	PELMO
		PEC_{gw} [µg/L]	PEC_{gw} [µg/L]	PEC_{gw} [µg/L]	PEC_{gw} [µg/L]	PEC_{gw} [µg/L]	PEC_{gw} [µg/L]
Châteaudun	(1st)	0.154	0.111	0.095	0.068	0.025	0.015
	(2nd)	0.207	0.142	0.126	0.087	0.034	0.020
Hamburg	(1st)	0.379	0.298	0.230	0.179	0.099	0.075
	(2nd)	0.443	0.401	0.270	0.244	0.132	0.107
Jokioinen		0.109	0.088	0.068	0.055	0.014	0.011
Kremsmuenster	(1st)	0.242	0.210	0.146	0.128	0.064	0.052
	(2nd)	0.291	0.255	0.177	0.155	0.075	0.067
Porto	(1st)	0.137	0.177	0.082	0.105	0.032	0.052
	(2nd)	0.253	0.305	0.153	0.182	0.061	0.086
Sevilla	(1st)	0.006	0.002	0.004	0.001	<0.001	<0.001
	(2nd)	0.009	0.002	0.006	0.002	<0.001	<0.001
Thiva		0.109	0.069	0.067	0.043	0.014	0.009
1st = first/early season crop, 2nd = second/late season crop							

The results indicate that even considering the most conservative tier 1 calculations and annual applications there are scenarios with PECgw concentrations below the trigger for the outdoor use in both crops. Considering the Tier 2 a calculations there are more scenarios below the trigger and at the highest tier (TDS with application every 2nd year) in hop all scenarios are below the trigger while in lettuce all except the late use in the scenario Hamburg are below the trigger of 0.1 µg/L.

PEC_{gw} of Metabolites

The predicted concentrations in groundwater for the metabolites are summarised below considering tier 1 and/or tier 2 simulations

	Application in hops, Tier 1							
	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	
Scenario	PEARL	PELMO	PEARL	PELMO	PEARL	PELMO	PEARL	PELMO
Châteaudun	1.074	0.939	0.553	0.489	0.010	0.009	0.004	0.004
Hamburg	1.423	1.395	0.678	0.669	0.011	0.013	0.006	0.006
Kremsmuenster	0.941	0.953	0.467	0.467	0.009	0.010	0.004	0.004
Piacenza	0.753	0.709	0.393	0.360	0.007	0.009	0.003	0.005
Porto	0.597	0.581	0.290	0.288	0.006	0.007	0.003	0.003
Sevilla	0.635	0.477	0.334	0.266	0.005	0.002	0.002	0.001
Thiva	0.596	0.602	0.300	0.271	0.004	0.004	0.002	0.002

	Application in hops, every year, Tier 2							
	Difluoroacetic acid (DFA) PEC_{gw} [µg/L]				Difluoroacetic acid (DFA) PEC_{gw} [µg/L]			
	Tier 2a DFOP				Tier 2a TDS			
	every year		every 2nd year		every year		every 2nd year	
Scenario	**PEARL**	**PELMO**	**PEARL**	**PELMO**	**PEARL**	**PELMO**	**PEARL**	**PELMO**
Châteaudun	1.008	0.875	0.528	0.464	1.007	0.895	0.516	0.450
Hamburg	1.386	1.335	0.667	0.656	1.388	1.335	0.656	0.647
Kremsmuenster	0.903	0.931	0.458	0.473	0.912	0.933	0.439	0.448
Piacenza	0.667	0.655	0.360	0.329	0.701	0.671	0.364	0.343
Porto	0.513	0.500	0.248	0.248	0.541	0.538	0.261	0.263
Sevilla	0.565	0.407	0.302	0.241	0.572	0.420	0.296	0.234
Thiva	0.523	0.504	0.272	0.235	0.514	0.525	0.254	0.230

	Application in lettuce, Tier 1							
	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	
Scenario	**PEARL**	**PELMO**	**PEARL**	**PELMO**	**PEARL**	**PELMO**	**PEARL**	**PELMO**
Châteaudun (1st)	1.476	1.085	0.687	0.515	0.009	0.006	0.004	0.003
(2nd)	1.701	1.325	0.793	0.607	0.012	0.008	0.005	0.003
Hamburg (1st)	2.382	1.815	1.110	0.901	0.015	0.015	0.008	0.006
(2nd)	2.632	2.322	1.254	1.120	0.020	0.019	0.009	0.008
Jokioinen	2.373	2.013	1.106	0.954	0.008	0.007	0.003	0.003
Kremsmuenster (1st)	1.461	1.261	0.712	0.611	0.012	0.011	0.005	0.005
(2nd)	1.583	1.445	0.750	0.713	0.014	0.013	0.006	0.006
Porto (1st)	0.760	0.715	0.361	0.315	0.008	0.010	0.004	0.005
(2nd)	1.155	1.105	0.589	0.556	0.015	0.016	0.006	0.007
Sevilla (1st)	0.383	0.289	0.193	0.134	<0.001	<0.001	<0.001	<0.001
(2nd)	0.577	0.410	0.273	0.187	<0.001	<0.001	<0.001	<0.001
Thiva	1.056	0.875	0.519	0.412	0.007	0.005	0.003	0.002
1st = first/early season crop, 2nd = second/late season crop								

	Application in hops, every year, Tier 2							
	Difluoroacetic acid (DFA) PEC_{gw} [µg/L]				Difluoroacetic acid (DFA) PEC_{gw} [µg/L]			
	Tier 2a DFOP (parent)				Tier 2a TDS (parent)			
	every year		every 2nd year		every year		every 2nd year	
Scenario	**PEARL**	**PELMO**	**PEARL**	**PELMO**	**PEARL**	**PELMO**	**PEARL**	**PELMO**
Châteaudun (1st)	1.377	1.025	0.633	0.473	1.363	0.994	0.628	0.469
(2nd)	1.761	1.369	0.807	0.616	1.603	1.236	0.723	0.561
Hamburg (1st)	2.305	1.769	1.075	0.828	2.289	1.744	1.067	0.865
(2nd)	2.728	2.473	1.282	1.127	2.278	1.930	1.049	0.926
Jokioinen	2.448	2.014	1.098	0.935	2.572	2.202	1.221	1.072
Kremsmuenster (1st)	1.493	1.245	0.687	0.581	1.377	1.208	0.675	0.574
(2nd)	1.658	1.573	0.761	0.724	1.542	1.417	0.721	0.676
Porto (1st)	0.620	0.590	0.296	0.276	0.698	0.668	0.330	0.306
(2nd)	1.298	1.212	0.598	0.564	1.083	1.038	0.544	0.515
Sevilla (1st)	0.336	0.240	0.150	0.108	0.351	0.257	0.171	0.119
(2nd)	0.567	0.373	0.241	0.164	0.517	0.353	0.246	0.164
Thiva	1.219	0.955	0.537	0.414	0.957	0.808	0.449	0.347
1st = first/early season crop, 2nd = second/late season crop								

The results indicate that for 6-CNA there are no concerns for groundwater, all calculated value are < the trigger.

For DFA the concentration may exceed 0.1 µg/L, and may also exceed 0.75 µg/L. Therefore for DFA the relevance in groundwater needs to be assessed in accordance with Sanco/221/2000 –rev.10 (2003): Guidance document on the assessment of the relevance of metabolites in groundwater. In accordance with the guidance document DFA has been shown to be non-relevant (not biologically active, not genotoxic and no concerns for toxicity). DFA is included in the dietary risk assessment as it is a constituent of the residue definition in plants.

5.4 Fate and behaviour in air

Based on the estimation method developed by Roger Atkinson and co-workers the chemical lifetime of the BYI 02960 in the air was assessed by the program AOPWINTM, version 1.92a (U.S. EPA, 2008). The half-life time (t1/2) 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. As a consequence of the short half-life in air, no long-range transport of BYI 02960 in the atmosphere is likely to occur. From the low vapour pressure of the substance it is concluded that very little BYI02960 will enter the atmosphere.

Chapter 6 Effects on non-target species

The available toxicity data and the relevant exposure estimates as combined in the respective risk assessments for terrestrial vertebrates, aquatic organisms, honeybees, non-target arthropods, earthworms & soil macro-organisms, soil micro-organisms and non-target terrestrial plants indicate that no adverse short-term or long-term effects on these species are to be expected from the use of Sivanto 200SL on hops or lettuce when applied according to the use directions.

Environmentally relevant metabolites of BYI 02960 (found in plant metabolism and environmental fate) of the applied parent compound) 6-CNA (soil only), DFA (soil and water) and BYI 02960-succinamide and BYI 02960- azabicyclosuccinamide (both water only) are also addressed as appropriate in the risk assessment for honey bees, soil and aquatic organisms.

6.1 Effects on terrestrial vertebrates

Summary of the study and risk assessment results on birds

In acute oral toxicity studies, BYI 02960 was moderately toxic to bobwhite quail and canaries. Parent compound was not toxic to quail or mallard ducks in 5 day dietary studies. The lowest reproductive NOEC of 302 mg/kg a.s./kg food was observed in bobwhite quail.The following overview table summarises the results of the studies on birds conducted with the active substance BYI 02960.

Test organisms	Duration	Test substance	Ecotoxicological endpoint	
Northern Bobwhite quail	acute	tech.	LD_{50}	232 mg a.i./kg b.w.
Northern Bobwhite quail	Acute	BYI 02960 200SL	LD_{50}	431 mg a.i/kg bw
Canary	acute	tech.	LD_{50}	330 mg a.i./kg b.w.
Chicken (hen)	acute	tech.	LD_{50}	>2000 mg a.i./kg b.w.
Chicken (hen)	acute	BYI 02960 200SL	LD_{50}	>2000 mg product /kg b.w.
Northern Bobwhite quail	5-day-dietary	tech.	LC_{50}	>4876 mg a.i./kg food (*> 470 mg a.i./kg bw/d*)
Mallard duck	5-day-dietary	tech.	LC_{50}	> 4741 mg a.i./kg food *(> 825 mg a.i./kg bw/d*)
Northern Bobwhite quail	Reproduction	tech.	NOAEL	302 mg a.i/kg food (40 mg a.i/kg bw/d)
Mallard duck	Reproduction	tech.	NOAEL	845 mg a.i/kg bw/food *81 mg a.i/kg bw/d*)

EU Risk assessment for birds:

The risk assessment procedure follows the EFSA Guidance Document on Risk Assessment for Birds & Mammals (2009). For 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.
The calculation of acute and long-term Toxicity to Exposure Ratio (TER) is defined as follows:

Acute risk: $TER_A = LD_{50}$ [mg as/kg bw] / DDD_{AC}

Long-term risk: $TER_{LT} = LD_{50}/10$ [mg as/kg bw] / DDD_{mean}

Where

DDD Daily dietary dose

Summary of calculated TER values for birds

According to the conservative Tier 1 risk assessment based on the lowest LD_{50}, the risk to birds from the proposed use of Sivanto® SL 200 on hops and lettuce is acceptable.

Summary of acute TER calculations

Crop (BBCH)	Generic focal species	Active substance	TER_A	Assessment step
Hops (≥ 20)	Small insectivorous bird "finch" (Chaffinch)	BYI 02960	61	Tier 1
Hops (20-39)	Small granivorous bird "finch" (Goldfinch)		126	Tier 1
Lettuce (10-19)	Medium herbivorous/granivorous bird "pigeon" (Wood pigeon)	BYI 02960	20	Tier 1
Lettuce (10-49)	Small granivorous bird "finch" (Serin)		68	Tier 1
Lettuce (10-49)	Small omnivorous bird “lark” (Woodlark)		77	Tier 1
Lettuce (10-19)	Small insectivorous bird “wagtail” (Yellow wagtail)		69	Tier 1

Summary of long-term TER calculations

Crop (BBCH)	Generic focal species	Active substance	TER_{LT}	Assessment step
Hops (≥ 20)	Small insectivorous bird "finch" (Chaffinch)	BYI 02960	28	Tier 1
Hops (20-39)	Small granivorous bird "finch" (Goldfinch)		51	Tier 1
Lettuce (10-19)	Medium herbivorous/granivorous bird "pigeon" (Wood pigeon)	BYI 02960	9	Tier 1
Lettuce (10-49)	Small granivorous bird "finch" (Serin)		28	Tier 1
Lettuce (10-49)	Small omnivorous bird “lark” (Woodlark)		32	Tier 1
Lettuce (10-19)	Small insectivorous bird “wagtail” (Yellow wagtail)		31	Tier 1

The TER values for drinking water exposure also exceed the a-priori acceptability triggers of 10 and 5 in the worst case risk assessments. No unacceptable risk is to be expected from the use of the product according to the intended use pattern.

Summary of the study and risk assessment results on mammals

A summary of the results of the ecotoxicologically relevant studies on mammals conducted with BYI 02960 is shown in the following table.

Test organisms	Duration	Test substance	Ecotoxicological endpoint
Rat	acute	tech.	LD_{50} ca. 2000 mg a.i./kg bw
Rat	acute	SL200	LD_{50} > 2000 mg product/kg bw (equivalent to > 400 g a.i./kg bw*)
Rat	chronic	tech.	$LOAEL_{female}$ 500 ppm (equivalent to doses of 39.2 mg/kg bw/d**)

*assuming an analytical content of 200 g/L

** the slight decrease in maternal body weight observed in the 2-generation reproduction study at 500 ppm was not considered ecologically relevant

EU Risk assessment for small mammals:

The risk assessment procedure for wild mammals follows the same principles as described in detail under point 10.1 for birds, i.e. EFSA Guidance Document on Risk Assessment for Birds & Mammals (2009). The acute risk to mammals from the proposed uses in hops and lettuce is acceptable based upon the Tier 1 assessment.

Summary of TER_{AC} values

Crop (BBCH)	Generic focal species	Active substance	TER_A	Assessment step
Hops (≥20)	Small insectivorous mammal “shrew” Common shrew	BYI 02960	1984	Tier 1
Hops (≥ 40)	Small herbivorous mammal "vole" Common vole		262	Tier 1
Hops (20 – 39)	Small omnivorous mammal “mouse” Wood mouse		1246	Tier 1
Lettuce (≥20)	Small insectivorous mammal “shrew” Common shrew	BYI 02960	2381	Tier 1
Lettuce (40-49)	Small herbivorous mammal "vole" Common vole		94	Tier 1
Lettuce (All season)	Large herbivorous mammal “lagomorph” Rabbit		366	Tier 1
Lettuce (10-49)	Small omnivorous mammal “mouse” Wood mouse		747	Tier 1

The TER_{LT} values for small herbivorous mammals were below the trigger of 5 in the reproductive/long-term risk assessment for both uses, indicating a need for refinement. The refined risk assessment utilized the reproduction LOAEL of 39.2 mg/kg bw/day in combination with measured residue data that allows refinement of the time-weighted average residue concentrations (21-d f_{TWA}) of BYI 02960 on foliage as potential diet of small herbivorous mammals and 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. The long term risk is judged acceptable based on this higher tier assessment.

Summary of TER_{LT} values

Crop (BBCH)	Generic focal species	Active substance	TER_{LT}	Assessment step
Hops (≥20)	Small insectivorous mammal "shrew" Common shrew	BYI 02960	52	Tier 1
Hops (≥ 40)	Small herbivorous mammal "vole" Common vole		4.5	Tier 1
			12.6-63.4	refined
Hops (20 – 39)	Small omnivorous mammal "mouse" Wood mouse		25	Tier 1
Lettuce (≥20)	Small insectivorous mammal "shrew" Common shrew	BYI 02960	62	Tier 1
Lettuce (40-49)	Small herbivorous mammal "vole" Common vole		1.6	Tier 1
			4.5-22.8	refined
Lettuce (All season)	Large herbivorous mammal "lagomorph" Rabbit		8	Tier 1
Lettuce (10-49)	Small omnivorous mammal "mouse" Wood mouse		15	Tier 1

The TER values for drinking water exposure also indicate safe uses on both hops and lettuce.

6.2 Effects on aquatic species

The following overview tables summarise the results of the studies on aquatic species conducted with the BYI 02960 and metabolites.

Toxicity of BYI 02960 to aquatic organisms

Test species	Test system	Test duration	Endpoint [mg a.i. /L]	
BYI 02960 tech. – freshwater fish				
Oncorhynchus mykiss (rainbow trout)	static acute	96 h	LC_{50} NOEC	> 74.2 (mm)[1] 74.2 (mm)
Pimephales promelas (fathead minnow)	static acute	96 h	LC_{50} NOEC	**> 70.5 (mm)** 70.5 (mm)
Cyprinus carpio (carp)	static acute	96 h	LC_{50} NOEC	> 100 (mm) 100 (mm)
Pimephales promelas (fathead minnow)	early life stage (ELS), flow-through	35 d	NOEC LOEC	**4.41 (mm)** 8.41 (mm)
Sivanto 200SL – freshwater fish				
Oncorhynchus mykiss (rainbow trout)	static acute	96 h	LC_{50} NOEC	>105 (mm) <105(mm)
Cyprinus carpio (common carp)	static acute	96 h	LC_{50} NOEC	108 (mm) 108 (mm)
BYI 02960 tech. – freshwater invertebrates				
Daphnia magna (water flea)	static acute	48 h	EC_{50} NOEC	**> 77.6 (mm)** 77.6 (mm)
Daphnia magna (water flea)	chronic, static renewal	21 d	NOEC LOEC	**3.2 (nom)**[2] 6.4 (nom)
Chironomus riparius (chironomid)	static acute	48 h	EC_{50} **NOEC**	**0.062 (nom)** 0.025 (nom)
Chironomus riparius (chironomid)	Static chronic, spiked water	28 d	NOEC EC_{50}	0.0105 (mi) 0.0353 (mi)

Sivanto 200SL – freshwater invertebrates				
Daphnia magna (water flea)	static acute	48 h	EC_{50} NOEC	684 mg form./L (equivalent to 117.0 (mm)) 125 mg form./L (equivalent to 21.4 (mm))
Chironomus riparius (chironomid)	chronic test – spiked water	28 d	**NOEC** LOEC $EC_{15\ emergence}$ $EC_{15\ developmental}$	**0.012 (mi)** [3] 0.024 (mi) 0.0132 (mi) 0.0233 (mi)
BYI 02960 tech. - algae and plants				
Pseudokirchneriella subcapitata (green alga)	growth inhibition static	96 h	E_rC_{50} NOE_rC	**> 80 (nom)** 80 (nom)
Lemna gibba (duck weed)	growth inhibition static renewal	7 d	$E_bC_{50\ (frond\ no.)}$ $E_rC_{50(frond\ no)}$	> 67.7 (mm) 67.7(mm)
Sivanto SL200 - algae				
Pseudokirchneriella subcapitata (green alga)	growth inhibition test	72 h	E_rC_{50} NOEC	> 250 mg form./L (equivalent to 42.5 (mm)) 250 mg form./L (equivalent to 42.5 (mm))
BYI 02960 tech. - marine organisms				
Cyprinodon variegatus (sheepshead minnow)	static acute	96 h	LC_{50} NOEC	> 83.9 (mm) 83.9 (mm)
Crassostrea virginica (eastern oyster)	acute, flow-through	96 h	EC_{50} NOEC	> 29 (mm) 29 (mm)
Americamysis bahia (saltwater mysid)	flow-through	96 h	EC_{50} NOEC	0.26 (mm) 0.12 (mm)
Americamysis bahia (saltwater mysid)	Life cycle, flow-through	28d	NOEC LOEC	0.0132 (mm) 0.0236 (mm)
BYI 02960 tech. - amphibians				
Xenopus laevis (African clawed frog)	Static acute	48 h	LC_{50} NOEC	> 73.8 (mm) 73.8 (mm)

[1] mm = mean measured concentration, [2] nom = nominal concentration, [3] mi = initially measured concentration

Bold value: Endpoint considered relevant for risk assessment

Toxicity of BYI 02960 metabolites to aquatic organisms

Test species	Test system	Test duration	Endpoint [mg p.m.[1]/L]	
BYI 02960 – succinamide				
Oncorhynchus mykiss (rainbow trout)	static acute	96 h	**LC_{50}** NOEC	**> 100 (nom)** [2] 100 (nom)
Daphnia magna (water flea)	chronic, static renewal	21 d	**NOEC** LOEC	**43.3 (nom)** 100 (nom)
Chironomus riparius (chironomid)	static acute	48 h	**EC_{50}** NOEC	**> 100 (mi)** [4] 71 (mi)
Pseudokirchneriella subcapitata (green alga)	growth inhibition test	72 h	**E_rC_{50}** NOE_rC	**> 10 (nom)** 10 (nom)
BYI 02960 – azabicyclosuccinamide				
Chironomus riparius (chironomid)	static acute	48 h	**EC_{50}** NOEC	**> 100 (mi)** [4] 71 (mi)

DFA (tested as Sodium difluoro acetate)			
Oncorhynchus mykiss (rainbow trout)	static acute	96 h	**LC_{50} > 10 (nom)[2]** NOEC 10 (nom)
Daphnia magna (water flea)	static acute	48 h	**EC_{50} > 10 (nom)** NOEC 10 (nom)
Chironomus riparius (chironomid)	static chronic, spiked water	28 d	LOEC > 100 (nom) **NOEC 100 (nom)**
Pseudokirchneriella subcapitata (green algae)	growth inhibition static	72 h	**E_rC_{50} > 10 (nom)** NOE_rC 10 (nom)
6-Chloronicotinic acid			
Daphnia magna	acute, static renewal	48 h	**EC_{50} > 95.1 (mm)** NOEC 95.1 (mm)[3]
Chironomus tenants (chironomid)	static acute	96 h	**LC_{50} 1 (mi)**[4] NOEC 1 (mi)
Chironomus riparius (chironomid)	static chronic, spiked water	28 d	LOEC > 100 (nom) **NOEC 100 (nom)**
Pseudokirchneriella subcapitata (green algae)	growth inhibition test	72 h	**E_rC_{50} > 100 [A] (nom)** NOEC 100 (nom)

[1] p.m. = pure metabolite in case of studies on metabolites, [2] nom = nominal concentration
[3] mm = mean measured concentration, [4] mi = initially measured concentration
[A] E_rC_{50} at a test concentration of 100 mg pure metabolite/L, pH adjusted (pH 7.5 - 8.2)

The results indicate that BYI 02960 exhibits minimal toxicity to fish, algae, amphibians and aquatic plants. When considering aquatic invertebrates BYI 02960 is of low toxicity to daphnids but, as expected for an insecticide, exhibits a selective toxicity to the aquatic insect *Chironomus riparius*. The toxicity of BYI 02960's metabolites to aquatic organism was examined with a range species, including *C. riparius*, the most sensitive aquatic species with parent. The results indicate that all the metabolites are exhibit only limited toxicity to aquatic organisms and are orders of magnitude less toxic than parent and therefore are not of ecotoxicological concern.

Risk assessment
The risk assessment is considered acceptable when the TER_A is > 100 and the TER_{LT} is > 10.
In accordance with the tiered European risk assessment scheme, initially Step 2 calculations are assessed, followed by Step 3 which consider more realistic exposure scenarios and Step 4 which consider potential mitigation measures.

For fish, algae, daphnids and aquatic plants the TER trigger was clearly exceeded considering Step 2 calculations.

Compound	**Species**	**Timescale**	**FOCUS step**	**TER**	
				Hops	Lettuce
BYI 02960	Fish	Acute	**2**	> 4061	> 5985
	Fish	Long-term	**2**	254	374
	Daphnia	Acute	**2**	> 4470	> 6587
	Daphnia	Long-term	**2**	184	272
	Algae	Long-term	**2**	> 4608	> 6791
	Aquatic plants	Long-term	**2**	> 3900	> 5747

For aquatic insects, as represented by chironomids, the trigger was not met at Step 2 for either the acute or long-term risk assessment, therefore the risk assessment at Step 3 and Step 4.

Summary of aquatic risk assessment for acute risk, use on hops

Compound	Species	Scenario	Focus Step	Mitigation	TER Acute
					Hops
BYI 02960	Chironomids	R1 pond	3	Not required	157
		R1 stream	4	No buffer, 90% drift reduction	112

Summary of aquatic risk assessment for chronic risk, use on hops

Compound	Species	Scenario	Focus Step	Mitigation	TER Acute
					Hops
BYI 02960	Chironomids	R1 pond	3	Not required	30
		R1 stream	4	No buffer, 90% drift reduction	22

Summary of aquatic risk assessment for acute risk, use on lettuce (outdoors)

Compound	***Species***	**Scenario**	**Focus Step**	**Mitigation required**	**TER Acute**
					Lettuce
BYI 02960	*Chironomids*	D3 (ditch, 1st)	4	No buffer, 50% drift reducing nozzles	173
		D3 (ditch, 2nd)	4	No buffer, 50% drift reducing nozzles	139
		D4 (pond, 1st)	#	#	#
		D4 (stream, 1st)	#	#	#
		D6 (ditch, 1st)	#	#	#
		R1 (pond, 1st)	3	Not required	1033
		R1 (pond, 2nd)	3	Not required	639
		R1 (stream, 1st)	4	10m run-off buffer	159
		R1 (stream, 2nd)	4	10m run-off buffer	115
		R2 (stream, 1st)	4	20 m run-off buffer	165
		R2 (stream, 2nd)	4	10m run-off buffer	147
		R3 (stream, 1st)	4	20 m runoff buffer	117
		R3 (stream, 2nd)	#	#	#
		R4 (stream, 1st)	3	Not required	119
		R4 (stream, 2nd)	#	#	#
# trigger not exceeded with maximum standard mitigation					

Summary of aquatic risk assessment for chronic risk, use on lettuce (outdoors)

Compound	*Species*	Scenario	Focus Step	Mitigation required	TER Acute
					Lettuce
BYI 02960	*Chironomids*	D3 (ditch, 1st)	3	Not required	14
		D3 (ditch, 2nd)	3	Not required	14
		D4 (pond, 1st)	3	**Not required**	12
		D4 (stream, 1st)	3	**Not required**	15
		D6 (ditch, 1st)	#	#	#
		R1 (pond, 1st)	3	**Not required**	200
		R1 (pond, 2nd)	3	**Not required**	124
		R1 (stream, 1st)	3	**Not required**	14
		R1 (stream, 2nd)	3	Not required	10
		R2 (stream, 1st)	4	10 m run-off buffer	
		R2 (stream, 2nd)	3	Not required	
		R3 (stream, 1st)	4	10 m run-off buffer	
		R3 (stream, 2nd)	#	20m run-off buffer	
		R4 (stream, 1st)	3	Not required	
		R4 (stream, 2nd)	#	20m run-off buffer	
# trigger not exceeded with maximum standard mitigation					

Conclusions – aquatic risk assessment

The acute risk assessment resulted in the more critical risk assessment than the long-term risk assessment, therefore the following the required mitigation is summarized for the acute risk.

In hops a safe use for aquatic organisms can be shown in scenario R1 pond with no mitigation measures and in R1 stream with no buffer zone and 90% drift reducing nozzles,

In lettuce a safe use for aquatic organisms can be shown with no mitigation measures for scenarios R1 (pond) and R4 stream (early seasons use) and for most scenarios considering mitigation measures of 10 to 20m run-off buffers. For some scenarios(D4, D6 and the late season use in R3 and R4) standard mitigation was not sufficient and specific options may need to be developed for those regions where these scenarios apply.

6.3 Effects on bees and other arthropod species

BYI 02960 has been extensively tested to establish the safety profile for the anticipated use of Sivanto® SL 200 on flowering crops. The intrinsic acute and chronic toxicity to honey bees has been investigated in laboratory studies for parent compound and metabolites identified in flowers in a tomato metabolism study. BYI 02960 larval toxicity has also been investigated. In addition, an extensive semi-field testing program has been conducted.

Intrinsic toxicity of BYI 02960 and metabolites to honey bees:

BYI 02960 and Sivanto® SL 200 toxicity in laboratory acute oral and contact honey bee toxicity studies of 1.2/3.2 µg a.i./bee and 122.8/15.7 µg a.i./bee, respectively. All tested metabolites were non-toxic.
In chronic feeding tests, no effects were seen for any compound at levels of 10,000 µg a.i./L in sugar solution. In addition, parent BYI 02960 was tested for toxicity to honey bee larvae. There were no adverse effect noted in the chronic *in-vitro* laboratory study on honey bee larvae and their development at concentrations of up to and including 10,000 µg a.i./kg larval diet, establishing that honey bee larvae/brood are not more sensitive to BYI 02960 as compared to adult bees.

Acute toxicity of BYI 02960 and metabolites to honey bees in the laboratory

Test substance	Ecotoxicological Endpoint	
BYI 02960	LD_{50} - oral 48 h LD_{50} - contact 96 h	**1.2 µg a.i./bee** 122.8 µg a.i./bee
Sivanto® SL 200	LD_{50} - oral 48 h LD_{50} - contact 72 h	**3.2 µg a.i./bee** 15.7 µg a.i./bee
BYI 02960-DFEAF	LD_{50} - oral 48 h LD_{50} - contact 48 h	>81.5 µg a.i./bee >100 µg a.i./bee
BYI 02960-OH	LD_{50} - oral 48 h LD_{50} - contact 48 h	>105.3 µg a.i./bee >100 µg a.i./bee
DFA	LD_{50} - oral 48 h LD_{50} - contact 48 h	>107.9 µg a.i./bee >100 µg a.i./bee
6-CNA	LD_{50} - oral 48 h LD_{50} - contact 48 h	>107.1 µg a.i./bee >100 µg a.i./bee
BYI 02960 -CHMP	LD_{50} - oral 48 h LD_{50} - contact 48 h	>106.7 µg a.i./bee >100 µg a.i./bee

Bold values: Endpoints considered relevant for risk assessment

Chronic toxicity of BYI 02960 and metabolites to honey bees in the laboratory

Test substance	Ecotoxicological Endpoint: NOEC / NOED	
BYI 02960	NOEC (nominal) NOED (nominal)	10000 µg a.i./L 0.464 µg a.i./bee/day
BYI 02960-DFEAF	NOEC (nominal) NOED (nominal)	10000 µg a.i./L 0.435 µg a.i./bee/day
BYI 02960-OH	NOEC (nominal) NOED (nominal)	10000 µg a.i./L 0.420 µg a.i./bee/day
DFA	NOEC (nominal) NOED (nominal)	10000 µg a.i./L 0.379 µg a.i./bee/day
6-CNA	NOEC (nominal) NOED (nominal)	10000 µg a.i./L 0.418 µg a.i./bee/day
BYI 02960-CHMP	NOEC (nominal) NOED (nominal)	10000 µg a.i./L 0.413 µg a.i./bee/day

EPPO risk assessment for bees
An indication of hazard (Hazard Quotient or Q_H) can be derived by calculating the ratio between the application rate (expressed in g or mL/ha) and the lowest laboratory contact and oral LD_{50} (expressed in µg/bee).

Q_{HO} and Q_{HC} resp. = Application rate [g or mL/ha] / LD_{50} oral or LD_{50} contact [µg/bee]

Q_H values are calculated using data from the studies performed with technical material and the corresponding formulated product. Q_H values higher than 50 indicate the need of higher tiered tests to clarify the actual risk to honey bees.

The hazard quotients for contact exposure for both proposed safe uses are well below 50 (<10), and therefore no unacceptable risk to bees is to be expected via the contact route of exposure. The QHO value for formulated products is acceptable for both uses, but for BYI 02960 technical, the empirical trigger value of 50 is exceeded, thereby requiring a refined risk assessment.

Hazard quotients for bees – oral exposure

Crop	Exposure route	LD_{50} [µg a.i./bee]	Application rate [g/ha]	Hazard quotient Q_{HO}	Trigger	Refined risk assessment
BYI 02960 SL 200						
Hops	oral	3.2	150	47	50	No
Lettuce	oral	3.2	125	39	50	No
BYI 02960 (technical)						
Hops	oral	1.2	150	125	50	Yes
Lettuce	oral	1.2	125	104	50	Yes

Refined risk assessment for honeybees

The use in hops and lettuce will result in only very limited exposure of honey bees, in commercial cultivation only female hops are grown and these do not provide nectar or honey to bees. Lettuce are harvested before flowering and therefore do not provide a crop to which honey bees are attracted.

In addition there are a total of six semi-field studies in which, 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). In two research, pilot studies, BYI 02960 was applied during bee flight at a rate of 75 and 150 g a.i./ha, respectively, and in a 2nd pilot research semi-field study, BYI 02960 was applied during bee flight at a rate of 150 g a.i./ha.
Additionally four GLP-compliant honey bee semi-field tunnel studies have been conducted with multiple applications of BYI 02960 to the highly bee attractive surrogate crop *Phacelia tanacetifolia*. In all four studies, the Phacelia-crop received a foliar application just before onset of flowering in addition to a full-flowering treatment, with bees present, both at rates corresponding to 200 g a.i./ha. In two of the studies, there was in addition a soil treatment at a rate corresponding to 300 g a.i./ha on the day Phacelia-seeds were sown. Observations made in all studies included mortality, foraging activity, behaviour, brood, food, and population development as well as on colony vitality throughout the entire study. In one study, a detailed quantitative digital brood assessment of individually marked cells was conducted.

The results of all semi-field (tunnel) studies under forced worst-case exposure conditions were consistent; in all studies, there were no adverse effects on mortality, foraging activity, behaviour, brood-, food- and population development as well as on overall colony vitality. The findings in the tunnel studies under forced exposure conditions are in line with the low toxicity observed in the *in-vitro* oral acute and chronic laboratory studies with BYI 02960 and BYI 02960 metabolites.

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. The laboratory findings have been consistently confirmed in multiple 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 while honey bees are foraging, without adverse effects on honey bees.

Non-target arthropods other than bees

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.

The tier 1 glass plate studies indicate that insects, as represented by *Aphidius rhopalosiphi* (LR_{50} < 0.5 g a.i./ha), are more sensitive than mites like *Typhlodromus pyri* (LR_{50} 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* (LR_{50} 2.02 g a.i./ha, ER_{50} >0.89 g a.i./ha) with the results from the additionally tested species *Coccinella septempunctata* (LR_{50} 273.9 g a.i./ha and no effects on reproduction below the LR_{50}) and *Aleochara bilineata* (ER_{50} >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).

Risk assessment

The tier 1 and 2 risk assessment for in-field exposure and off-crop exposure demonstrated that a refine risk assessment was required for species such as *Aphidius rhopalosiphi.*

To address the in-field risk 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.* In these studies BYI 02960 SL200 G was applied twice at 250g a.i./ha with a 10 day interval, spray residues were aged under semi-field conditions. For the *Aphidius rhopalosiphi study* 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 aging time of 49 and 56 days.

In the study with *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 aging 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 aging time of 28 and 42 days, respectively.

The aged residue studies indicate that the potential for recovery 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 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.

To address the off-field risk of BYI 02960 SL 200 to 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 South-western France. In these studies BYI 02960 SL 200 was applied in a dose-response design at drift rates 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. Arthropods were sampled shortly before the application and 1, 2, 4 and 8 weeks after the application. The results of the two field studies demonstrate that an exposure to BYI 02960 SL 200 at 21 g a.i./ha does not adversely affect arthropod communities in off-field habitats (community NOER in both studies 21 g a.i./ha). The taxa which were statistically significantly reduced at the highest tested rate of 21 g a.i./ha all recovered within 4 to 8 weeks after the application (population NOEAER in both studies 21 g a.i./ha). As the maximum off-field PEC lies below 21 g a.i./ha for the proposed use patterns in hops and lettuce, no unacceptable adverse effects on non-target arthropods are to be expected in the off-field area

Therefore it can be concluded that no adverse effects can be expected either in-field or off-field following the use of BYI 02960 SL200G according to the use pattern.

6.4 Effects on earthworms and other soil macro-organisms

A summary of the toxicity of BYI 02960 soil metabolites as well as Sivanto® SL 200 to earthworms and other soil macro-organisms is provided in the tables below.

Effects of BYI 02960 and metabolites on earthworms and other soil non-target macro-organisms

Test species	Test design	Ecotoxicological endpoint[1,2]		
BYI 02960				
Eisenia fetida	acute, 14 d (10% peat in test soil)	LC_{50}	192.9	mg a.i./kg dws
DFA				
Eisenia fetida	acute, 14 d (10% peat in test soil)	LC_{50}	> 1000	mg p.m./kg dws
Eisenia fetida	reproduction, 56 d (10% peat in test soil) mixing	NOEC	62.0	mg p.m./kg dws
Folsomia candida	chronic, 28 d (5% peat in test soil)	NOEC	100	mg p.m./kg dws
Hypoaspis aculeifer	chronic, 14 d (5% peat in test soil)	NOEC	1000	mg p.m./kg dws
6-CNA				
Eisenia fetida	acute, 14 d (10% peat in test soil)	LC_{50}	> 1000	mg p.m./kg dws
Eisenia fetida	reproduction, 56 d (10% peat in test soil) mixing	NOEC	95	mg p.m./kg dws
Folsomia candida	chronic, 28 d (5% peat in test soil)	NOEC	90	mg p.m./kg dws
Hypoaspis aculeifer	chronic, 14 d (5% peat in test soil)	NOEC	100	mg p.m./kg dws

[1] dws = dry weight soil, [2]p.m. = pure metabolite

Effects of Sivanto® SL 200 on earthworms and other soil non-target macro-organisms

Test species	Test item	Test design	Ecotoxicological endpoint
Eisenia fetida	Sivanto® SL 200	acute, 14 d (5% peat in test soil)	LC_{50} 709 mg prod./kg dws
Eisenia fetida	Sivanto® SL 200	reproduction, 56 d (10% peat in test soil)	NOEC 8.9 mg prod./kg dws
Earthworm fauna	Sivanto® SL 200	Field study on grassland one year 300, 600 and 1500 g a.i./ha	Significant 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; full recovery of earthworm population after 11 months
Folsomia candida	Sivanto® SL 200	chronic, 28 d (5% peat in test soil)	NOEC 8.47 mg prod./kg dws
Hypoaspis aculeifer	Sivanto® SL 200	chronic, 14 d (5% peat in test soil)	NOEC $\geq$ 1000 mg prod./kg dws

The risk assessment procedure follows current regulatory requirements and the Guidance Document on Terrestrial Ecotoxicology. TER calculations for earthworms indicate acceptable risk.

TER calculations for earthworms

Compound test design	End point	[mg/kg soil]	$PEC_{max,accu}$ [mg/kg soil]	TER_A / TER_{LT}	Trigger	Refined risk assessment?
Hops						
Sivanto® SL 200 acute	LC_{50}	709	0.470	1509	10	
Sivanto® SL 200 chronic	NOEC	8.9	0.470	19	5	
BYI 02960 acute	LC_{50}	192.9	0.160	1206	10	
DFA acute	LC_{50}	> 1000	0.009	> 111 111	10	No
DFA chronic	NOEC	62.0	0.009	6889	5	
6-CNA acute	LC_{50}	> 1000	0.007	> 142 857	10	
6-CNA chronic	NOEC	95	0.007	13 571	5	
Lettuce						
Sivanto® SL 200 acute	LC50	709	0.685	1035	10	
Sivanto® SL 200 chronic	NOEC	8.9	0.685	13	5	
BYI 02960 acute	LC50	192.9	0.156	1237	10	
DFA acute	LC50	> 1000	0.014	> 71 429	10	No
DFA chronic	NOEC	62.0	0.014	4429	5	
6-CNA acute	LC50	> 1000	0.012	> 83 333	10	
6-CNA chronic	NOEC	95	0.012	7917	5	

The collembolan species *F. candida* was determined to be the most sensitive species to BYI 02960 SL 200 in soil with a NOEC (reproduction) of 8.47 mg BYI 02960 SL 200/kg, slightly lower than the 8.9 mg/kg on earthworm. The calculated TER value for lettuce is 12 for *F. candida,* indicating that collembolan populations are not at risk if BYI 02960 SL 200 is applied at rates of 0.625 L/ha in lettuce (which also covers the use in hops).

For 6-CNA, *F. candida* is also slightly more sensitive than earthworm, but as the TER for earthworm is >10,000 the difference has no real impact on the risk assessment. As all TER values are above the trigger of concern, there is no unacceptable risk for earthworms and soil non-target macro-organisms.

6.5 Effects on soil micro-organisms

Laboratory studies on microbial turnover are available for the active substance BYI 02960, the formulation Sivanto® SL 200, and the metabolite 6-CNA (N-turnover only) at rates far exceeding the anticipated single application or seasonal use rate. 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. Results of treated soil compared to the control showed little change in turn-over after 28 days, indicating low risk to soil micro-organisms. Overall, it can be concluded that the functioning of soil micro-organisms is not at risk if Sivanto® SL 200 is applied according to the recommended use pattern.

The toxicity of Sivanto® SL 200 on soil non-target micro-organisms is summarised below.

Effects on soil non-target micro-organisms

Test species	Test design	Ecotoxicological endpoint	
Sivanto® SL 200			
C-cycle	28 d	no influence	12.44 L prod./ha (≡16.59 µL prod./kg dws)
N-cycle	28 d	no influence	12.44 L prod./ha (≡16.59 µL prod./kg dws)
BYI 02960			
N-cycle	28 d	no influence	3 kg a.i./ha (≡4.0 mg a.i./kg dws)
C-cycle	28 d	no influence	3 kg a.i./ha (≡4.0 mg a.i./kg dws)
6-CNA			
N-cycle	28 d	no influence	1.0 kg p.m./ha (≡1.33 mg p.m./kg dws)

6.6 Effects on other non-target organisms (flora and fauna)

The effect of Sivanto® SL 200 on seedling emergence and vegetative vigour of terrestrial non-target plants has been tested in course of two Tier-1 limit tests with a single rate of 410 g a.i./ha. This rate covers multiple applications of lower rates multiplied with a MAF (multiple application factor).
At this rate no inhibitory effect above 20% was observed in any of the ten species tested. In the off-crop area non-target plants are exposed to spray-drift only. It can be concluded that terrestrial non-target plants are not at risk when BYI 02960 SL200 is applied at rates recommended according to good agricultural practice

6.7 Effects on biological methods of sewage treatment

In the test on the inhibition of the respiration rate of activated sludge with BYI 02960 an EC_{50} of greater than 10000 mg a.i./L had been established. Based on this study result no adverse effects on sewage treatment processes by BYI 02960 are to be expected.

6.8 Environmental risk mitigation

The risk assessment indicates that risk mitigation may be required for exposure to aquatic water bodies to protect aquatic insects. The required mitigation depends on the considered scenario ranging from

no mitigation to 20m run-off buffers (VFS). Specific mitigation measures may be required for some regions where the standard mitigation was not sufficient to enable the trigger to be exceeded.

Chapter 7 Efficacy data and information

7.1 Effectiveness

Sivanto SL200 (Flupyradifurone, developed by BCS under the product code BYI 02960 Specification number: 1020000021884) is proposed as an insecticide in agriculture and in ornamentals to control mainly sucking insects after foliar spray application. The 2 representative uses chosen for Flupyradifurone SL200 in the EU are: Control of Nasonovia ribisnigri in lettuce (field and greenhouse) and Phorodon humuli in hops.

7.1.1 Intended use

Sivanto is a systemic insecticide for foliar spray use and intended mainly for controlling sucking pest such as aphids, hoppers and whiteflies. Sivanto can control important CNI resistant pest populations such as Bemisia tabaci, Phorodon humuli and Empoasca sp. Spectrum extension to mealybugs, leafminer, weevils and (flea) beetles is under examination.
Sivanto can be applied curative and preventive but is most effective when applied at threshold level to the vegetation, and with spray volumes ensuring a good coverage of the targeted plant parts, based on the size and density of the treated crop.

7.1.2 Mode of action

Flupyradifurone is the active ingredient (active substance) of the new systemic foliar insecticide SivantoTM. It belongs to the chemical class of butenolides, and acts as a nAChR antagonist:
The a.i. interacts with insect nicotinic acethylcholine receptors, a class of neurotransmitter-gated cation channels which are involved in excitatory neurotransmission. Like the naturally occurring neurotransmitter acetylcholine, Flupyradifurone acts as an agonist, i.e., the binding of Flupyradifurone to the receptor protein induces a depolarising ion current and causing excitation of the nerve cell which can be measured by electrophysiological methods. In contrast to acetylcholine, Flupyradifurone cannot be inactivated by the acetylcholinesterase. The lasting effect of the product results in a disorder of the nervous system of the insect and subsequently death.

7.1.3 Crops

Sivanto SL 200 is intended to be used as an insecticide in agriculture on a range of crops such as vegetables, fruits, grapes, hops, cotton, tobacco and coffee, cocoa plantations as well as ornamentals

7.1.4 Effectiveness against the lettuce aphid (*Nasonovia ribisnigri*)

Sivanto (Flupyradifurone SL 200) at 75-125 g a.s/ha provided a good control, similar to that given by commonly used product Calypso, and better than those of Movento or Plenum, which are slower acting products. 125 g a.s/ha could provide a better lasting efficacy than the lower application rate, 75 g a.s/ha, at the same level as Calypso, and better than Plenum.
In the Mediterranean Zone, although not statistically different, a dose response from 75 g a.s/ha to 150 g a.s/ha could be observed when considering the initial efficacy. The lasting efficacy of

Flupyradifurone SL200 g/L at 125-150 g a.s/ha was higher than at 75 g a.s/ha. At 125 g a.i/ha Flupyradifurone performed better than Confidor at 100 g a.s/ha, and much better than Plenum at 200 g a.s/ha.
According to the presented results, the dose of 125 g a.s/ha provided the optimum overall control (initial and lasting efficacy) and should be considered as effective against Nasonovia ribisnigri in lettuce (field and protected) for which activity of Flupyradifurone SL200 is claimed.

7.1.5 Effectiveness against the damson hop aphid (*Phorodon humuli*)

A dose rate of 0.75 L/ha Flupyradifurone SL200 (150 g a.s./ha) provided the optimum overall control and should be considered as effective against damson hop aphid (Phorodon humuli) in hop, for which activity of Flupyradifurone SL200 is claimed. This dose rate proved similar or higher efficacy than the current standard product Teppeki. Lower tested dose rates of 0.5 and 0.6 L/ha proved similar or lower efficacy level than imidacloprid-based products (Confidor 70 WG and Confidor 200 SL). A slower initial effect of the lower rates was identified. Since 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 is considered as the minimum effective dose rate. The results are supported by data from Germany, Czech Republic (Maritime zone) and Poland (North-East zone).

7.2 Information on the occurrence or possible occurrence of the development of resistance

Flupyradifurone interacts with insect nicotinic acetylcholine receptors (nAChRs), a class of neurotransmitter-gated cation channels which are involved in excitatory neurotransmission, a target also known for neonicotinoid insecticides, nicotine, sulfoxaflor and spinosyns. However flupyradifurone is a butenolide insecticide and chemically different from neonicotinoids, but it binds as an agonist to the same receptor site. Therefore flupyradifurone can be assigned to IRAC (Insecticide Resistance Action Committee) mode of action Group 4, which includes all insecticidal agonists of the nAChR.

7.3 Effects on the yield of treated plants or plant products in terms of quantity and/or quality

7.3.1 Effects on the quality of plants or plant products

No adverse effects of parent and DFA have been observed on neither survival nor emergence, survival, growth, shoot length and shoot weight of 11 plant species tested. Beyond, the bioefficacy studies did not reveal any negative impact on the quality of plants or plant products.

7.3.2 Effects on transformation processes

There is no evidence that Sivanto SL 200 has any effects on transformation processes.

7.3.3 Effects on yield of treated plants or plant products

No adverse effects of parent and DFA have been observed on neither survival nor emergence, survival, growth, shoot length and shoot weight of 11 plant species tested. Beyond, the bioefficacy studies did not reveal any negative impact on the yield of plants or plant products

7.4 Phytotoxicity to target plants (including different cultivars), or to target plant products

Sivanto SL 200 has been tested in field development trials in the European Union, which demonstrated high efficacy against targeted pests and appropriate crop safety. No phytotoxic effect was observed in the Minimum Effective Dose trials in various lettuce types and varieties as well as in varieties of hop. The zonal biological dossier will provide all the relevant information in detail as required in the zone to which the application is made.

7.5 Observations on undesirable or unintended side effects e.g. on beneficial and other non-target organisms, on succeeding crops, other plants or parts of treated plants used for propagating purposes (e.g. seed, cuttings, runners)

7.5.1 Impact on 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.

7.5.2 Impact on adjacent crops

No adverse effects of parent and DFA have been observed on neither survival nor emergence, survival, growth, shoot length and shoot weight of 11 plant species tested. Therefore, negative effects on adjacent crops are very unlikely to happen.

7.5.3 Impact on seed viability

No adverse effects of parent and DFA have been observed on neither survival nor emergence, survival, growth, shoot length and shoot weight of 11 plant seeds tested.

7.5.4 Impact on beneficial and other non-target organisms

Selectivity towards beneficial insects and predatory mites is a requirement for a modern IPM-compatible product. Side effects of Sivanto on beneficial arthropods have been tested in various semi-field and field trials. So far, Sivanto can be considered safe to most beneficial insects and specifically to pollinators.

7.6 Conclusions

Flupyradifurone (BYI 02960), trade name Sivanto, is intended to be used as an insecticide in agriculture on a range of crops such as vegetables, fruits, coffee and cocoa plantations as foliar spray or soil drench and as seed treatment product in soybean and, eventually, cereals. Sivanto is a systemic insecticide, flexible in application (foliar, soil and ST) and mainly intended for sucking pest control such as aphids, hoppers and whiteflies. Sivanto can control important CNI resistant pest populations such as whitefly and certain hoppers. Spectrum extension to mealybugs, leafminer, weevils and (flea) beetles is under examination.

Flupyradifurone is a new systemic foliar insecticide belonging to the butenolid group acts as a nAChR antagonist.

Sivanto is most effective when applied as a preventive or threshold treatment. Studies have shown that the product can often be applied at reduced spray volumes in comparison to standard commercial applications due to its highly systemic nature. However, good coverage of the target plant parts, based on the size and density of the treated crop, is recommended to facilitate maximum uptake by leaf surfaces.

Sivanto does not exhibit cross-resistance to insecticides from other chemical classes. Sivanto can control important CNI resistant pest populations such as Brown Plant Hopper (BPH) and whitefly.

Selectivity towards beneficial insects and predatory mites is a requirement for a modern IPM-compatible product. Side effects of Sivanto on beneficial arthropods are tested in various semi-field and field trials. So far, Sivanto can be considered safe to beneficial insects and specifically to pollinators.

Chapter 8 Overall Conclusions

Proposed decision

Bayer CropScience proposes to approve BYI 02960 (Flupyradifurone) for use as active substance in the territory of the European Union.

Further information to be submitted

IIIA 2.7.5 Shelf life test following storage for 2 years at ambient temperature

The studies mentioned below are still under preparation for the Global Joint Review and are of no relevance for the submission in the EU:

Toxicology:

- Developmental neurotoxicity study (required by USA and Canada)
- Acute oral toxicity test and Ames test of BYI02960-bromo (required by Japan)
- Acute oral toxicity test for the photo-metabolites BYI 02960 succinamide and BYI 02960 azabicyclosuccinamide (presumably required by USA and Canada)
- Salmonella typhimurium reverse mutation assay and Micronucleus assay in the mouse with the formulation Sivanto SL 200 (required by Brazil)

Ecotox:

- Further bee studies

Environmental fate:

- US field dissipation studies
- Rate of degradation in aerobic soils with Brazilian soils (required for Brazil)
- Adsorption/desorption study with Brazilian soils (required by Brazil)
- Terrestrial model ecosystem (may be required at MS level in Europe)