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Document Title

**Tier 2 Summary of the Metabolism and Residues Data for
Flupyradifurone (BYI 02960)**

Data Requirements

Regulation (EC) No 1107/2009

**Regulatory Directive 2003-01/Canada/PMRA
OPPTS guidelines/US/EPA**

**Annex II A
Section 4, Point 6
Document M**

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

Date

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Author(s)

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Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

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**IIA 6 Metabolism and Residues Data****IIA 6.1 Stability of residues****IIA 6.1.1 Stability of residues during storage of samples**

As residue samples in trials with BYI 02960 on crops and animal were routinely stored frozen for longer periods of time prior to their analysis, the effects of frozen storage on the residue levels were investigated.

General remark:

In this summary section (KIIA 6.1.1), the name DFEAF will be used for the metabolite BYI 02960-difluoroethyl-amino-furanone, which is relevant to the tested residue definition:

Name	Metab. No.	Standard "dossier name"
DFEAF	M34	BYI 02960-difluoroethyl-amino-furanone

► PLANT MATRICES

The longest periods of frozen storage of samples from plant residue studies (field residue, processing, or rotational crop trials) are shown in table 6.1.1-1 below:

Table 6.1.1-1: Periods of frozen storage (approx. -18°C) of plant-based samples (between sampling and analysis) for matrices relevant to this dossier

Crop	Sample material Matrix	Longest storage duration (d)	Study	
			Report no.	Annex point KIIA...
lettuce	head	300	10-2503	6.6.3/01
	outer leaves			
	inner head parts	224	10-3223	6.5.4/01
	inner leaves			
	washed inner leaves			
hops	washing water	225	10-3223	6.5.4/01
	green cone	219	10-3407	6.5.4/02
	dried cone	223	10-2225	6.3.2/01
	beer			
	hops draf	220	10-3407	6.5.4/02
barley	brewer's yeast			
	grain			
	straw	480	10-2503	6.6.3/01
carrot	green material	540	10-2503	6.6.3/01
	root	257	10-2503	6.6.3/01
turnip	root (body)	180	10-2503	6.6.3/01
	leaf	177	10-2503	6.6.3/01

Based on this information and on the fact that the use of BYI 02960 will be supported on a wide array of additional crops, storage stability data was required for the relevant residues of BYI 02960 in plant matrices.



Report:	KIIA 6.1.1/01, Timberlake, B.C.; [REDACTED], A.M. 2012
Title:	Storage stability of BYI 02960, difluoroacetic acid, and difluoroethyl-amino-furanone in plant matrices
Report No. & Document No.:	RARVP046, dated April 3, 2012 M-428412-01-1
Guidelines:	– EPA Ref. OPPTS 860.1380 Storage Stability Data – OECD Guideline for the Testing of Chemicals No. 506, Stability of Pesticide Residues in Stored Commodities – PMRA Residue Chemistry Guidelines, Reg. Dir. 98-02, Section 5, Storage Stability Data
GLP:	yes (certified laboratory)

I. Materials and Methods

To determine the freezer storage stability of the relevant residues of BYI 02960 in plant materials, individual 5-g control samples of orange fruit (high acid content), spinach leaves and tomato fruit (high water content), wheat grain (high starch content), bean seed (high protein content), coffee bean and soybean seed (high oil content), and sugar cane were separately spiked with 5.0 µg of either BYI 02960 parent compound, DFA, or DFEAF. Except for the day-0 analysis, samples were stored in glass containers in a freezer at an average temperature of -23°C (maximum -12°C with a single exception of a very brief interval at 6.5°C, too short for the samples to thaw) for later use. For day-0 analysis, three treated samples of each material were chosen, as well as one control sample of each. Samples were then also analysed after nominal intervals of 1, 2½, 5-6, and 12 months. (The intended final storage period to be covered in this study is 2 years.) At each of these intervals, two treated samples of each material were removed from storage and analyzed, as well as a control sample and two samples for concurrent recovery. Samples used for concurrent recoveries were prepared at the same time and stored in the same fashion as the control samples, and spiked on the day of analysis.

BYI 02960 and its metabolites were analytically determined using analytical method 01304 (cf. report RARVP013, Li & Schoening, 2012; KIIA 4.3/03), which was validated prior to and parallel to the residue analysis of the samples. The LOQ was 0.01 mg/kg for parent and DFEAF and 0.02 mg/kg for DFA, expressed in BYI 02960 equivalents.

II. Findings

The mean values of the concurrent recovery rates per compound, sample material, and spiking level were in the range of 82-106%, with relative standard deviations in the range of 5-14%. Details of recovery data are shown in table 6.1.1-5.

At day 0, average residue recoveries of BYI 02960 ranged from 91-111% of nominal; of DFEAF ranged from 101-110% of nominal; and of DFA ranged from 79-101% of nominal. In samples analysed after approximately 1 year of frozen storage (362-372 days), storage stability recoveries, corrected to day 0, ranged from 84-114% for BYI 02960, 79-90% for DFEAF, and 74-104% for DFA. At all sampling dates and in all sample materials, the relevant components of the residue of BYI 02960



were above 70%. Even in the case of the lower values in the given ranges, there was no evidence of any continued degradation of any of the analytes in any of the sample materials. Thus, all analytes can be considered stable in all relevant plant matrix types for a period of at least one year.

All storage stability results are summarised below in tables 6.1.1-2 to 6.1.1-4.

III. Conclusions

During a storage period of 12 months under deep-freezer conditions, the components of the relevant residues of BYI 02960 (including parent compound, DFEAF, and DFA) were stable in orange fruit, spinach leaves and tomato fruit, wheat grain, bean seed, coffee bean and soybean seed, and sugar cane, representing a wide array of plant-based sample materials. These results validate the residue values reported in supervised field trials and processing studies with respect to storage stability of samples frozen prior to analysis.

(Some samples in the field rotational crop studies were stored for longer periods of up to 16-18 months; the affected samples were green material, straw, and grain from cereal crops. The storage stability study as reported and summarized in this section will be continued until a storage period of 2 years has been covered. Another interim report will be submitted at approx. 18 months of storage.)

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.1.1-2: Summary of stability data for deep-frozen samples fortified with **BYI 02960**
 – samples fortified at 1 mg/kg

Matrix	Storage period (d)	Concurrent recovery (%)	Recovery in stored samples (%)		
			apparent	normalized to day 0	corrected for recovery
sugar cane	0	(100)	100	100	100
	29	101	92	92	91
	77	91	85	85	93
	149	98	94	94	96
	372	87	93	93	107
coffee bean (green)	0	(100)	94	100	100
	33	97	93	99	96
	81	81	81	85	99
	152	85	104	110	122
	370	93	83	88	89
orange fruit	0	(100)	96	100	100
	28	89	84	88	95
	77	86	95	98	111
	148	104	84	87	81
	365	102	80	83	79
soybean seed	0	(100)	93	100	100
	28	93	93	101	100
	75	93	96	104	104
	148	91	97	105	106
	371	96	98	106	103
navy bean	0	(100)	111	100	100
	26	91	107	96	117
	75	94	110	99	117
	148	100	123	111	123
	364	113	127	114	112
tomato fruit	0	(100)	95	100	100
	28	90	92	97	103
	76	86	89	94	102
	148	107	105	111	98
	370	89	95	101	107
spinach	0	(100)	91	100	100
	26	96	97	106	101
	75	90	85	93	94
	147	94	104	114	110
	364	119	97	106	81
wheat grain	0	(100)	94	100	100
	27	93	90	95	97
	76	89	82	87	93
	186	97	109	116	113
	362	92	77	81	83

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.1.1-3: Summary of stability data for deep-frozen samples fortified with DFA
 – samples fortified at 1 mg/kg

Matrix	Storage period (d)	Concurrent recovery (%)	Recovery in stored samples (%)		
			apparent	normalized to day 0	corrected for recovery
sugar cane	0	(100)	98	100	100
	29	97	96	99	100
	77	97	92	94	94
	149	86	100	102	116
	372	97	99	101	102
coffee bean (green)	0	(100)	90	100	100
	33	90	89	98	99
	81	77	76	84	99
	152	80	94	104	118
	370	73	79	87	108
orange fruit	0	(100)	95	100	100
	28	98	100	106	102
	77	89	99	105	112
	148	87	93	98	107
	365	94	98	103	104
soybean seed	0	(100)	79	100	100
	28	89	71	89	79
	75	93	82	103	88
	148	75	98	124	131
	371	73	79	99	107
navy bean	0	(100)	96	100	100
	26	96	99	104	104
	75	98	108	113	110
	148	79	100	104	127
	364	80	78	82	98
tomato fruit	0	(100)	101	100	100
	28	99	96	95	97
	76	94	94	93	100
	148	84	98	96	117
	370	98	105	104	108
spinach	0	(100)	100	100	100
	26	98	97	97	99
	75	90	87	87	97
	147	85	100	99	117
	364	70	74	74	106
wheat grain	0	(100)	92	100	100
	27	97	98	107	102
	76	88	94	102	107
	186	78	98	107	126
	362	72	71	78	100



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.1.1-4: Summary of stability data for deep-frozen samples fortified with **BYI 02960-difluoroethylaminofuranone** – samples fortified at 1 mg/kg

Matrix	Storage period (d)	Concurrent recovery (%)	Recovery in stored samples (%)		
			apparent	normalized to day 0	corrected for recovery
sugar cane	0	(100)	110	100	100
	29	89	81	74	91
	77	93	88	80	95
	149	117	112	102	96
	372	87	93	85	108
coffee bean (green)	0	(100)	101	100	100
	33	100	94	93	94
	81	89	88	87	99
	152	118	100	99	85
	370	90	86	85	95
orange fruit	0	(100)	104	100	100
	28	100	109	105	109
	77	98	94	90	96
	148	119	110	105	92
	365	97	92	88	95
soybean seed	0	(100)	107	100	100
	28	99	106	99	107
	75	103	94	87	91
	148	117	117	108	100
	371	84	85	79	101
navy bean	0	(100)	102	100	100
	26	109	102	100	93
	75	107	93	91	87
	148	116	103	101	89
	364	93	88	86	94
tomato fruit	0	(100)	110	100	100
	28	96	116	105	121
	76	103	100	91	98
	148	118	101	92	86
	370	97	84	76	87
spinach	0	(100)	106	100	100
	26	107	109	103	102
	75	101	96	91	96
	147	114	109	102	95
	364	105	95	90	91
wheat grain	0	(100)	103	100	100
	27	101	106	103	105
	76	96	91	88	94
	186	104	99	96	95
	362	92	90	88	98



Table 6.1.1-5: Recovery data for the relevant residues of BYI 02960 in various plant matrices

Study No. GLP Year	Crop, Matrix	a.s./metabolite	n	Spike level (mg/kg)	Recovery (%)				
					Individual recoveries	Min	Max	Mean	RSD
RARVP046 GLP: yes 2011	spinach leaf	BYI 02960	11	1.0	84, 90, 99, 93, 99, 92, 89, 95, 95, 115, 124	84	124	98	12
		difluoroacetic acid	11	1.0	101, 99, 101, 97, 99, 90, 89, 87, 84, 69, 70	69	101	90	13
		BYI 02960-difluoroethyl-aminofuranone	11	1.0	102, 112, 104, 106, 108, 99, 102, 118, 110, 112, 98	98	112	106	6
	orange fruit	BYI 02960	11	1.0	101, 93, 95, 88, 89, 104, 67, 111, 97, 107, 97	67	111	95	12
		difluoroacetic acid	11	1.0	94, 93, 96, 98, 99, 102, 76, 87, 86, 94, 94	76	102	93	8
		BYI 02960-difluoroethyl-aminofuranone	11	1.0	104, 103, 105, 102, 98, 115, 82, 120, 119, 101, 92	82	120	104	11
	soybean seed	BYI 02960	11	1.0	97, 90, 91, 95, 92, 92, 94, 88, 94, 96, 95	88	97	93	3
		difluoroacetic acid	11	1.0	66, 95, 76, 88, 90, 90, 96, 75, 75, 74, 73	66	96	82	13
		BYI 02960-difluoroethyl-aminofuranone	11	1.0	112, 109, 101, 92, 106, 104, 102, 116, 118, 84, 85	84	118	103	11
	navy bean	BYI 02960	11	1.0	105, 104, 125, 93, 90, 96, 93, 104, 97, 106, 121	90	125	103	11
		difluoroacetic acid	11	1.0	96, 91, 100, 95, 96, 100, 96, 79, 78, 82, 78	78	100	90	10
		BYI 02960-difluoroethyl-aminofuranone	11	1.0	109, 94, 103, 121, 98, 103, 111, 118, 115, 98, 88	94	121	105	10
	sugarcane	BYI 02960	11	1.0	97, 105, 99, 107, 96, 92, 89, 98, 99, 83, 90	83	107	96	7
		difluoroacetic acid	11	1.0	100, 97, 97, 95, 98, 98, 97, 85, 86, 96, 97	85	100	95	5
		BYI 02960-difluoroethyl-aminofuranone	11	1.0	116, 114, 100, 88, 90, 101, 84, 116, 117, 87, 86	84	117	100	14

Continued on next page...



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Table 6.1.1-5 (cont'd): Recovery data for the relevant residues of BYI 02960 in various plant matrices

Study No. GLP Year	Crop, Matrix	a.s./metabolite	n	Spike level (mg/kg)	Recovery (%)				
					Individual recoveries	Min	Max	Mean	RSD
RARVP046 GLP: yes 2011	coffee bean, green	BYI 02960	11	1.0	92, 97, 95, 100, 94, 83, 80, 88, 83, 100, 86	80	100	91	8
		difluoroacetic acid	11	1.0	89, 89, 94, 92, 87, 77, 77, 80, 79, 72, 75	72	94	83	9
		BYI 02960-difluoroethyl-aminofuranone	11	1.0	103, 103, 97, 108, 92, 92, 86, 120, 117, 92, 89	89	120	100	11
	tomato fruit	BYI 02960	11	1.0	95, 108, 81, 88, 91, 86, 87, 105, 109, 97, 81	81	109	93	11
		difluoroacetic acid	11	1.0	99, 105, 100, 99, 99, 96, 91, 82, 85, 101, 94	82	105	96	7
		BYI 02960-difluoroethyl-aminofuranone	10	1.0	112, 108, 98, 94, 103, 102, 120, 116, 94, 99	94	120	105	9
	wheat grain	BYI 02960	11	1.0	97, 87, 99, 96, 90, 96, 82, 90, 104, 103, 82	82	104	93	8
		difluoroacetic acid	11	1.0	85, 98, 92, 95, 99, 97, 79, 81, 75, 70, 73	70	99	86	13
		BYI 02960-difluoroethyl-aminofuranone	11	1.0	97, 104, 107, 103, 99, 109, 84, 104, 104, 92, 92	84	109	100	8

► **ANIMAL MATRICES**

The longest storage periods for samples from animal residue studies (feeding studies) are shown in table 6.1.1-6 below:

Table 6.1.1-6: Periods of frozen storage (approx. -15 to -20°C) of animal-based samples (between sampling and analysis) for matrices relevant to this dossier

Animal	Sample material Matrix	Analyte group*	Longest storage duration (d)	Study Report no.	Annex point KIIA...
ruminant	milk	A	16	RARVP050	6.4.2/01
		B	25		
	cream	A & B	11		
	whey	A	5		
		B	7		
	fat	A	14		
		B	40		
	kidney	A	22		
		B	41		
	liver	A	21		
		B	37		
	muscle	A	19		
		B	41		
	urine	A & B	33		
poultry	eggs	A & B	12	RARVP041	6.4.1/01
	fat	A & B	11		
	liver	A & B	11		
	muscle	A & B	11		
	excreta	A & B	18		

* analyte group A comprises BYI 02960, BYI 02960-acetyl-AMCP, and BYI 02960-OH
analyte group B comprises DFA

Based on the information presented here, no storage stability data was required for the relevant residues of BYI 02960 in most animal matrices. Only samples with DFA in bovine fat, kidney, liver, muscle, and urine; as well as with BYI 02960, BYI 02960-acetyl-AMCP, and BYI 02960-OH in bovine urine were stored for periods of >1 month.



Report:	KIIA 6.1.1/02, [REDACTED], S.M., & [REDACTED], A.M.; 2012
Title:	BYI 02960 – Magnitude of the Residue in Dairy Cows
Report No. & Edition No.	RARVP050 M-428416-01-1
Guidelines:	– OPPTS 860.1480 – Meat/milk/poultry/eggs – OECD Guideline 505 – APVMA Residue Guideline No. 23 – DACO 7.5 – Meat/milk/poultry/eggs
GLP:	yes (certified laboratory)

I. Materials and Methods

To determine the freezer storage stability of the relevant residues of BYI 02960 in animal materials, individual control samples of bovine fat, kidney, liver, and muscle were separately spiked with DFA, at a nominal concentration of 0.20 mg/kg. Samples were stored in a freezer at a temperature of -15°C. For day-0 analysis, three treated samples of each material were chosen, as well as one control sample of each. Samples were then analysed after an interval of 43 days, in order to cover the longest period of storage for these matrices in the GLP feeding study. At this interval, two treated samples of each material were removed from storage and analyzed, as well as a control sample and two samples for concurrent recovery. Samples used for concurrent recoveries were prepared at the same time and stored in the same fashion as the control samples, and spiked on the day of analysis.

(In addition to the samples and matrices mentioned previously, urine samples were stored for 33 days between sampling and analysis. Although this is longer than one month, it is only very marginally so – an exceedance of only 10%. As the primary purpose of the urine residue values in the feeding study was to elucidate transfer factors for the two main components of the residue [BYI 02960 and DFA], the similarity of this very water-rich matrix with some of those included in the plant storage stability study [KIIA 6.1.1/01] indicates that the major residue components are, indeed, stable in frozen storage in urine.)

DFA was analytically determined using analytical method 01304 (cf. report RARVP013, Li & Schoening, 2012; KIIA 4.3/03), which was validated prior to and parallel to the residue analysis of the samples. The LOQ of the method was 0.02 mg/kg for DFA, expressed in BYI 02960 equivalents.

II. Findings

The mean values of the concurrent recovery rates per compound, sample material, and spiking level were in the range of 60-88%, with standard deviations < 10%. Details of recovery data are shown in table 6.1.1-8.

At day 0, average residue recoveries of DFA ranged from 60-86% of nominal. In samples analysed after 43 days of frozen storage, storage stability recoveries, normalized to day 0, ranged from 100-109%. When corrected for concurrent recovery, they ranged from 97-100%. There was no evidence of any continued degradation of DFA in any of the sample materials. Thus, it can be considered stable in all relevant animal matrix types over the tested period of 43 days.

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All storage stability results are summarised below in table 6.1.1-7.

III. Conclusions

During a storage period of 43 days under deep-freezer conditions, DFA was stable in bovine fat, kidney, liver, and muscle. These results validate the residue values reported in the cattle feeding study with respect to storage stability of samples frozen prior to analysis. In addition, data from the plant storage stability study (KIIA 6.1.1/01) in watery matrices indicate that the crucial components of the residue in urine, BYI 02960 parent compound and DFA, are stable in storage, thus validating the residue values reported in the cattle feeding study.

Table 6.1.1-7: Summary of stability data for deep-frozen samples fortified with **DFA**
– samples fortified at 0.20 mg/kg

Matrix	Storage period (d)	Concurrent recovery (%)	Recovery in stored samples (%)		
			apparent	normalized to day 0	corrected for recovery
bovine fat	0	-	85.8	100	100
	43	87.8	86.0	100.2	98.0
bovine kidney	0	-	68.7	100	100
	43	72.5	72.5	105.5	100
bovine muscle	0	-	65.3	100	100
	43	69.0	69.0	105.6	100
bovine liver	0	-	60.3	100	100
	43	68.0	65.8	109.1	96.7

Table 6.1.1-8: Concurrent recovery data for DFA in various animal matrices

Study No. GLP Year	Matrix	a.s./metabolite	n	Spike level (mg/kg)	Recovery (%)				
					Individual recoveries	Min	Max	Mean	RSD
RARVP050 GLP: yes 2011	bovine fat	difluoroacetic acid	5	0.20	80, 87, 91, 88, 88	80	91	87	4.7
	bovine kidney	difluoroacetic acid	5	0.20	70, 72, 65, 73, 72	65	73	70	4.6
	bovine muscle	difluoroacetic acid	5	0.20	63, 66, 68, 68, 71	63	71	67	4.4
	bovine liver	difluoroacetic acid	5	0.20	58, 61, 63, 71, 65	58	71	64	7.7

Additional recoveries for these matrices are presented with the feeding study, cf. KIIA 6.4.2/01

IIA 6.1.2 Stability of residues in sample extracts

The storage stability of residues of BYI 02960 in extracts was tested during development of the analytical methods.

Since the validity of the methods depends on factors such as reproducibility and the possibility of interruption during the work process, it must be ensured that the stability during possible storage of samples in extracts is always guaranteed. Additionally, when conducting residue analysis on regular samples, the entire analytical procedure is routinely monitored by performing concurrent recoveries with each sample set.

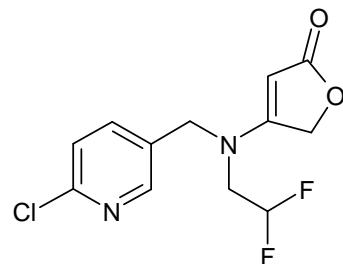
During the course of the method validations, stability was shown in all tested matrix extracts for at least 4-8 days, when stored in the dark in a refrigerator at $4^{\circ}\text{C} \pm 3^{\circ}\text{C}$.



IIA 6.2 Metabolism, distribution and expression of residues

BYI 02960 (common name: flupyradifurone) is a new insecticide being developed by Bayer CropScience. The chemical structure and nomenclature for the active substance are provided below.

Chemical structure:



Common name:

Flupyradifurone

Company code:

BYI 02960

IUPAC name:

4-[(6-chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino]furan-2(5H)-one

CAS name:

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

CAS #:

951659-40-8

Empirical formula:

C₁₂H₁₁ClF₂N₂O₂

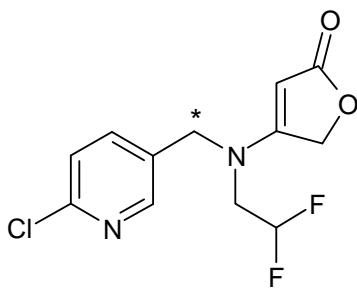
Molecular weight:

288.68 g/mol

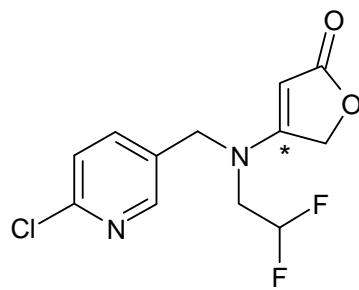
The metabolism of BYI 02960 has been investigated in target plants (apple, cotton, rice, tomato, potato) using different application techniques (foliar application, soil application, tuber treatment and granular application) and in confined rotational crops. The representative rotational crops were wheat, Swiss chard and turnips which were studied at three plant back intervals.

The metabolic fate of BYI 02960 has also been investigated in livestock (lactating goats and laying hens) in addition to the rat.

As BYI 02960 contains separate ring systems, two different radiolabels were used in all plant and animal studies. These label positions are shown below:



* denotes position of radiolabel, labelled in the methyl group of the pyridinylmethyl moiety



* denotes position of radiolabel, labelled in the 4-position of the furanone ring

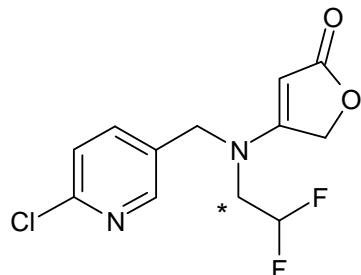
[pyridinylmethyl-¹⁴C]BYI 02960

[furanone-4-¹⁴C]BYI 02960

A third radiolabel 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. The fate of this radiolabel was investigated in a tomato study as a crop being representative for soil (drench) application. Two rat metabolism studies (one on absorption, distribution, excretion, and

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

metabolism in male rats and one on metabolism in organs and tissues of male and female rats) were also conducted with [ethyl-1-¹⁴C]BYI 02960.



* denotes position of radiolabel, labelled in the ethyl group of the difluoroethyl amino moiety

[ethyl-1-¹⁴C]BYI 02960

Additionally, residue levels of difluoroacetic acid were estimated by LC-MS/MS according to the conditions of residue analytical method 01304 in selected samples collected in the plant and confined rotational crop metabolism studies conducted with either [pyridinylmethyl-¹⁴C]BYI 02960 or [furanone-4-¹⁴C]BYI 02960. Thus, DFA levels have been determined in all crops included in the BYI02960 metabolism program. For livestock tissues, difluoroacetic acid levels were estimated on basis of the rat data. Additionally, high resolution LC-MS analyses (non-GLP) were performed for selected samples and confirmed the estimations.

Numerous metabolites were identified in the metabolism studies. The chemical structures and report names used in the summaries are given at the very end of this summary and in the List of Metabolites presented in Document N.

All residue values given in mg/kg refer to parent compound equivalents if not indicated otherwise.

The overall summary of the metabolism in plants and livestock is given in KIIA 6.11.1.

IIA 6.2.1 In plants, at least three crops from three different crop categories

Metabolism studies were conducted in crops representing four different crop categories (fruits, root vegetables, oilseeds and cereals). The studies reflected the intended use patterns of the active substance for foliar and soil treatment (including tuber treatment, granular application and soil drench) as summarized in the table below.

Table 6.2.1-1: Crops and application techniques used in the metabolism studies

Crop group	Crop	Application technique
fruits	apple	foliar application
fruits	tomato	soil drench
root crops	potato	tuber treatment / in-furrow application
oilseeds	cotton	foliar application
cereals	rice	foliar application / granular application

In Europe, soil drench applications will not be developed; however, soil applications are important in other regions which will apply for import tolerances in future, e.g. NAFTA (USA, Canada) and South America (Brazil). Therefore, the metabolism studies covering soil drench applications will be presented in this dossier, as well. These studies also provide information on uptake of soil metabolites, which is also of interest for crops receiving early foliar applications where a portion of the spray solution will hit the ground.

Metabolism, distribution and expression of residues in tomato (soil drench)

Metabolism studies in tomatoes were conducted with [furanone-4-¹⁴C]-, [pyridinylmethyl-¹⁴C]- and [ethyl-1-¹⁴C]BYI 02960:

Report:	KIIA 6.2.1/01, Justus, K.; 2011
Title:	Metabolism of [furanone-4- ¹⁴ C]BYI 02960 in tomatoes
Report No & Edition No	MEF-11/016 M-411352-01-2
Guidelines:	OECD 501 Metabolism in Crops US EPA Residue Chemistry Test Guideline OPPTS 860.1300: Nature of the Residue – Plants, Livestock PMRA Regulatory Directive Dir98-02: Residue Chemistry Guidelines Section 2: Nature of the Residue – Plants, Livestock Japanese MAFF, 12 Nousan 8147 European Parliament and Council Regulation (EC) No 1107/2009
GLP	yes

Executive Summary

The metabolism of [furanone-4-¹⁴C]BYI 02960 in tomatoes was investigated according to the maximum envisaged use pattern globally. Four tomato plants were treated by soil drench application with [furanone-4-¹⁴C]BYI 02960 formulated as an SL 200. The first application was performed at BBCH 15 (5th leaf on main shoot unfolded) and a second application at BBCH 51 (1st inflorescence visible and first bud erect). The single application rate corresponded to 300 g a.s./ha; the total application rate was 600 g a.s./ha.



At 6 to 36 days after the last application the flowers were sampled from one tomato plant, and at 69 to 92 days after the last application the fruits were harvested from the remaining three plants. The TRR values are shown in the following table:

Table 6.2.1-2: TRR values in tomato fruits and flowers after drench application of [furanone-4-¹⁴C]BYI 02960

Matrix	Timing and Application	PHI (days)*	ppm (mg a.s. equiv./kg)
tomato fruits	two drench applications, at BBCH 15 and 14 days later; 2 x 300 g a.s./ha	69	0.096
tomato flowers		n.a.	0.721

* PHI: preharvest interval (corresponds to days after last treatment (DAT) at the start of harvest/sampling)
n.a. not applicable, flowers are not a RAC

The fruits and flowers were conventionally extracted four times with acetonitrile/water mixtures, and 84.8% and 93.6% of the TRR were released, respectively.

Parent compound and metabolites in the extracts of tomato flowers and fruits were analysed by HPLC. Identification was performed by HPLC and/or TLC co-chromatography with reference compounds as well as by comparison of HPLC profiles. Besides parent compound, all major metabolites (>10% of the TRR) and one minor metabolite were identified.

Parent compound was the main component in both matrices and represented 35.9% of the TRR in tomato fruits and 77.9% in tomato flowers. Two major metabolites were detected in tomato fruits: The natural compound glucose (or isomeric carbohydrates) represented 27.5% of the TRR and BYI 02960-difluoroethyl-amino-furanone represented 10.3% of the TRR. In tomato flowers, no radioactive glucose (or isomeric carbohydrates) was detected. BYI 02960-difluoroethyl-amino-furanone was detected as a minor metabolite and represented 9.2% of the TRR. The metabolite BYI 02960-OH-glyc was detected as minor metabolite in tomato fruits and flowers and represented 5.5% and 6.6% of the TRR, respectively.

[Furanone-4-¹⁴C]BYI 02960 was moderately metabolised in tomatoes. The following metabolic routes were observed:

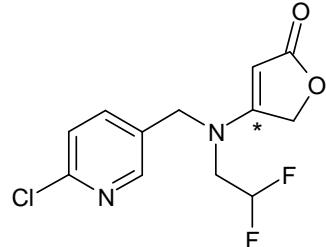
- complete degradation of the furanone moiety and incorporation of carbon atoms into the natural compound pool, i.e. into glucose (or isomeric carbohydrates),
- cleavage of the pyridinylmethylamine bond and formation of BYI 02960-difluoroethylamino-furanone and
- hydroxylation of the methylene group of the furanone moiety followed by conjugation with glucose

On the basis of these results, a metabolic pathway of [furanone-4-¹⁴C]BYI 02960 in tomatoes can be proposed.

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure		* position of the radiolabel
Radiolabelled test material	[furanone-4- ¹⁴ C]BYI 02960	
Specific radioactivity (before radiodilution) (after radiodilution)	3.94 MBq/mg (106.46 µCi/mg) 1.31 MBq/mg (35.50 µCi/mg)	
Chemical Purity	> 99% (HPLC)	
Radiochemical purity	> 99% (HPLC and TLC)	

The supplied radiolabelled test compound [furanone-4-¹⁴C]BYI 02960 was dissolved in acetonitrile. Radiodilution and formulation of the test compound was performed prior to the first and the second application: Therefore, an adequate portion of the non-radiolabelled test compound was dissolved in an adequate volume of the stock solution of the radiolabelled test compound. The solution was evaporated to dryness. The remainder was dissolved in an adequate amount of SL 200 blank formulation using a magnetic stirrer. Preparation of each application solution was done by dissolving the formulation in water. The specific activity of each application solution was 1.31 MBq/mg (35.50 µCi/mg).

2. Soil: "Einheitserde T", pH (CaCl₂) = 5.8, 85% white moor peat and 15% clay, enriched with water soluble nutrient salt mixture (ENS)

3. Plant Tomato, variety "Philona", representative for fruiting crops

B. Study Design

Experimental conditions:

Four tomato plants (variety: Philona) were cultivated, each in a 30 L planting bucket with a surface diameter of 38 cm filled with "Einheitserde T". The plants were cultivated in the greenhouse of the test facility (controlled temperature, humidity and light conditions).

The tomato plants were treated with SL 200 formulated [furanone-4-¹⁴C]BYI 02960 by drench application. Two applications were performed, each at an application rate of 300 g a.s./ha. The first application was conducted at a growth stage of BBCH 15 (5th leaf on main shoot unfolded) and the second at a growth stage of BBCH 51 (first inflorescence visible and first bud erect). The interval between the two applications was 14 days. The aqueous application solution was poured into a circular furrow prepared in the soil around each tomato plant. At each application, a volume of 200 mL was applied in total, corresponding to 132.64 MBq or to 100.0 mg a.s. (= 25.0 mg a.s./plant). Based on a



planting density of 12,000 tomato plants/ha in agricultural practice, the application rate corresponds to 300 g a.s./ha, which was anticipated to be the maximum single drench application rate in agricultural practice.

Sampling:

When the plants reached the growth stage BBCH 61 (first flower of the first inflorescence open), the open flowers were sampled from one of the four tomato plants in order to determine the nature of the residues in flowers. The flowers were collected with their receptacles and were stored in a freezer ($\leq -18^{\circ}\text{C}$) on the same day. Sampling of newly opened flowers was continued 2 - 3 times a week over a total period of approx. four weeks until end of flowering (at BBCH 69: 9 or more inflorescences with open flowers). The tomato plant from which the flowers were cut was sampled, cut in pieces and stored in a freezer ($\leq -18^{\circ}\text{C}$) for optional metabolite investigations. The frozen flowers were homogenized with a high speed blender in liquid nitrogen (Polytron). The homogenised flower sample was stored in a freezer ($\leq -18^{\circ}\text{C}$) until extraction.

On the day when the three remaining tomato plants reached the growth stage BBCH 81 (10% of fruits show typical fully ripe colour), the ripe tomato fruits were harvested and were stored in a freezer ($\leq -18^{\circ}\text{C}$). Harvesting of newly ripe fruits was continued 2 - 3 times a week over a total period of approx. three weeks until the plants produced no new fruits (at BBCH 89: fully ripe). The frozen fruits were cut in pieces and were homogenised with a high speed blender (Polytron). The homogenised tomato fruits samples was stored in portions in a freezer ($\leq -18^{\circ}\text{C}$) until extraction.

C. Analytical Procedures

Extraction:

The homogenised tomato flowers were extracted three times with a mixture of acetonitrile/water (8:2, v/v) and one time with a mixture of acetonitrile/water (1:1, v/v), and a portion of the homogenised tomato fruits was extracted four times with a mixture of acetonitrile/water (8:2, v/v) using a high speed blender (Polytron). After each extraction step, extracts and solids were separated by centrifugation. The radioactivity in the extracts was determined by LSC and in the solids by combustion followed by LSC. The actual TRR value of the RAC was determined by summing up the radioactivity measured in the extracts and in the remaining solids.

All tomato fruit extracts were combined and the first two tomato flower extracts were combined. The combined extracts were subjected to a clean-up step using a pre-conditioned SPE RP 18 cartridge (Phenomenex, Strata C18-E). The flow-through fraction (percolate) was collected and the cartridge was rinsed with acetonitrile/water (8:2, v/v) and methanol/dichloromethane (1:1, v/v). The percolate and the acetonitrile/water fraction were combined and concentrated by rotary evaporation in vacuo. The concentrated extracts of fruits and flowers were analysed by HPLC.

Quantification:

Parent compound and metabolites in the extracts were analysed by reversed phase HPLC coupled to a radioactivity detector with a glass scintillator cell. The HPLC chromatograms (= metabolite profiles) were integrated for quantification of compounds.

**Identification and characterisation:**

In tomato fruits (RAC), the major components (parent compound and one major metabolite) were identified by co-chromatography with radiolabelled reference compounds using two independent chromatographic systems (reversed phase HPLC and normal phase TLC). Minor metabolites (residue levels ≤ 0.01 mg/kg) were identified by HPLC co-chromatography, only.

In tomato flowers (additional plant matrix), parent compound was also identified by HPLC and TLC co-chromatography (two independent chromatographic systems) with an authentic reference compound. The minor metabolites (<10% of the TRR) were identified by HPLC comparison (comparison of HPLC profiles of tomato fruits and tomato flowers) and by HPLC co-chromatography. The conjugate BYI 02960-OH-glyc was additionally cleaved by alkaline hydrolysis and its corresponding aglycon was identified by HPLC co-chromatography.

Storage stability:

Detailed evidence was provided in the report to show that the quantified pattern of parent compound and metabolites adequately reflected the residue components at harvest. The extraction experiments and the first HPLC analyses of tomato fruits and flowers were performed not later than two months after harvest. HPLC chromatograms recorded at different times during the study showed that the profiles of the extracts did not change significantly during the analytical work up (including identification and characterization of metabolites) which did not exceed a period of 6 months in total. In accordance with the OECD Guidance for the Testing of Chemicals 501 (2007), it was therefore concluded, that the residues in the extracts were sufficiently stable during the experimental period of the study and that the chromatograms represented the metabolic pattern in the samples at harvest.

II. Results and Discussion

The metabolism of [furanone-4- ^{14}C]BYI 02960 was investigated in tomato fruits and flowers following two drench applications. The total radioactive residue (TRR) in fruits and flowers accounted for 0.096 mg/kg and 0.721 mg/kg, respectively. The major portion of radioactivity was extracted conventionally by acetonitrile/water mixtures (84.8% to 93.6% of the TRR) as shown in Table 6.2.1-3. Parent compound was the main residue in both extracts and was identified by HPLC and TLC co-chromatography (i.e. with two independent chromatographic systems) with an authentic reference compound.

In tomato fruits, two additional major metabolites (>10% of TRR and >0.01 mg/kg) were detected: a polar fraction which was assigned to glucose (or isomeric carbohydrates) and BYI 02960-difluoroethyl-amino-furanone. The identity of the carbohydrate was elucidated by HPLC and TLC co-chromatography before and after derivatisation with benzoyl chloride in pyridine. While correspondence with the reference compounds D-glucose and pentabenzoyl-D-glucose was clearly shown, the chromatographic methods were not considered selective enough to discriminate the configuration of the sugar moiety. Therefore, the isolated polar fraction was assigned more generally as glucose/carbohydrates. The minor metabolites BYI 02960-difluoroethyl-amino-furanone and BYI 02960-OH-glyc were identified by HPLC co-chromatography only, since their residue levels were rather low (≤ 0.01 mg/kg). The configuration of the hexose in BYI 02960-OH-glyc was identified

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as D-glucose in the corresponding apple metabolism study due to the specific enzymatic treatment with β -glucosidase.

In tomato flowers, the minor metabolites BYI 02960-difluoroethyl-amino-furanone and BYI 02960-OH-glyc were identified by HPLC comparison (comparison of HPLC profiles of tomato fruits and tomato flowers). For BYI 02960-difluoroethyl-amino-furanone, the assignment was additionally confirmed by HPLC co-chromatography. The conjugate BYI 02960-OH-glyc was cleaved by alkaline hydrolysis and the resulting aglycon BYI 02960-OH was additionally identified by HPLC co-chromatography.

The distribution of the parent compound and metabolites is shown in Table 6.2.1-4. In total, 79.2% and 93.6% of the TRR were identified in tomato fruits and flowers, respectively.

Table 6.2.1-3: Distribution of radioactivity in the extracts of the tomato matrices fruits and flowers

	tomato fruits		tomato flowers	
TRR [mg/kg] =	0.096			0.721
	% of TRR	mg/kg	% of TRR	mg/kg
Conventionally extracted	84.8	0.081	93.6	0.675
Extract for analysis	83.5	0.080	93.6	0.675
Losses (not analysed)	1.3	0.001	---	---
Total extracted	84.8	0.081	93.6	0.675
Unextractable (PES*)	15.2	0.015	6.4	0.046
Accountability	100.0	0.096	100.0	0.721

* post extraction solids

Table 6.2.1-4: TRR values and distribution of parent compound and metabolites in tomatoes (fruit and flowers) after drench application of [furanone-4-¹⁴C]BYI 02960

	tomato fruits		tomato flowers	
TRR [mg/kg] =	0.096			0.721
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg
<i>Conventional extraction</i>				
BYI 02960 (parent compound)	35.9	0.034	77.9	0.561
glucose/carbohydrates	27.5	0.026	---	---
difluoroethyl-amino-furanone	10.3	0.010	9.2	0.066
OH-glyc	5.5	0.005	6.6	0.048
Total identified	79.2	0.076	93.6	0.675
unknown 1	4.3	0.004	---	---
Total characterised	4.3	0.004	<0.1	<0.001
Analysed extract(s)	83.5	0.080	93.6	0.675
Extract(s) not analysed	1.3	0.001	---	---
Total extracted	84.8	0.081	93.6	0.675
Unextractable (PES*)	15.2	0.015	6.4	0.046
Accountability	100.0	0.096	100.0	0.721

* post extraction solids

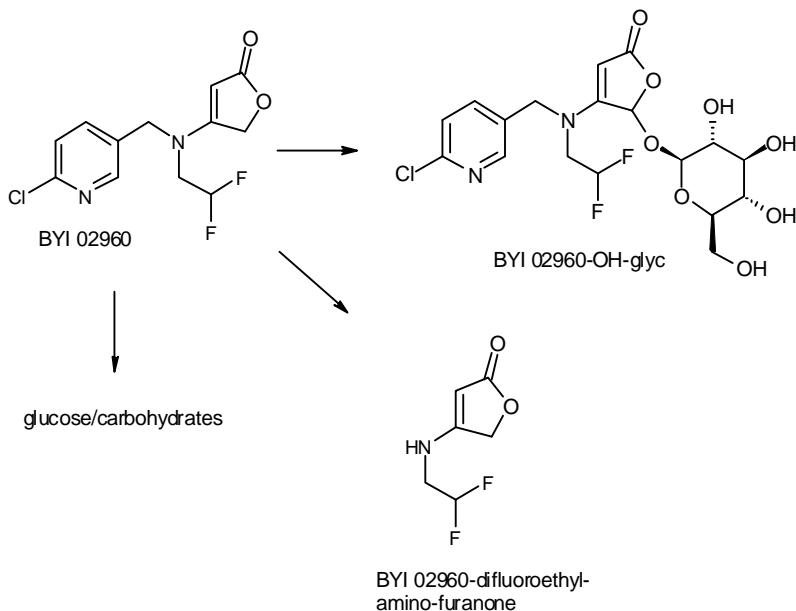
III. Conclusions

Based on the metabolites identified the following metabolic routes were deduced:

- 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
- hydroxylation of the methylene group of the furanone moiety followed by conjugation with glucose.

Thus, [furanone-4-¹⁴C]BYI 02960 was metabolised moderately in tomatoes. On the basis of the results of this study it is concluded that the metabolism of [furanone-4-¹⁴C]BYI 02960 in tomatoes is well understood and the following metabolic pathway is proposed.

Figure 6.2.1-1: Proposed metabolic pathway of [furanone-4-¹⁴C]BYI 02960 in tomatoes





Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Report:	KIIA 6.2.1/02, Justus, K.; 2011
Title:	Metabolism of [pyridinylmethyl- ¹⁴ C]BYI 02960 in tomatoes
Report No & Edition No	MEF-11/182 M-411500-01-2
Guidelines:	OECD 501 Metabolism in Crops US EPA Residue Chemistry Test Guideline OPPTS 860.1300: Nature of the Residue – Plants, Livestock PMRA Regulatory Directive Dir98-02: Residue Chemistry Guidelines Section 2: Nature of the Residue – Plants, Livestock Japanese MAFF, 12 Nousan 8147 European Parliament and Council Regulation (EC) No 1107/2009
GLP	yes

Executive Summary

The metabolism of [pyridinylmethyl-¹⁴C]BYI 02960 in tomatoes was investigated according to the maximum envisaged use pattern globally. Four tomato plants were treated by soil drench application with [pyridinylmethyl-¹⁴C]BYI 02960 formulated as an SL 200. The first application was performed at BBCH 15 (5th leaf on main shoot unfolded) and a second application at BBCH 51 (first inflorescence visible and first bud erect). The single application rate corresponded to 300 g a.s./ha; the total application rate was 600 g a.s./ha.

At 3 to 36 days after the last application the flowers were sampled from one tomato plant and at 73 to 92 days after the last application the fruits were harvested from the remaining three tomato plants. The TRR values are shown in the following table:

Table 6.2.1-5: TRR values in tomato fruits and flowers after drench application of [pyridinylmethyl-¹⁴C]BYI 02960

Matrix	Timing and Application	PHI (days)*	ppm (mg a.s. equiv./kg)
tomato fruits	two drench applications, at BBCH 15 and 14 days later; 2 x 300 g a.s./ha	73	0.130
tomato flowers		n.a.	1.254

* PHI: preharvest interval (corresponds to days after last treatment (DAT) at the start of harvest/sampling)
n.a. not applicable, flowers are not a RAC

The fruits and flowers were conventionally extracted four times with acetonitrile/water mixtures and 98.5% and 96.5% of the TRR were released, respectively.

Parent compound and metabolites in the extracts of tomato flowers and fruits were analysed by HPLC. Identification was performed by HPLC co-chromatography with reference compounds, by comparison of HPLC profiles or by HPLC-MS/MS analysis. Besides parent compound, two major metabolites and three minor metabolites were identified.

Parent compound was a major component in both matrices and represented 24.2% of the TRR in tomato fruits and 66.2% in tomato flowers. In tomato fruits, the label-specific metabolite BYI 02960-CHMP-di-glyc was the main constituent (37.1% of the TRR), followed by parent compound and the label-specific metabolite 6-CNA (13.2% of the TRR). Additionally, three minor metabolites



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BYI 02960-CHMP-glyc (5.1% of the TRR), BYI 02960-OH-glyc (3.4% of the TRR) and BYI 02960-CHMP (3.3% of the TRR) were identified. In tomato flowers, parent compound represented the predominant portion besides four minor metabolites. The minor metabolites were identical with the metabolites identified in the tomato fruits, except for BYI 02960-CHMP, which was not present in flowers.

[Pyridinylmethyl-¹⁴C]BYI 02960 was moderately metabolised in tomatoes. The following metabolic routes were observed:

- cleavage of the pyridinylmethylamine bond followed by conjugation with carbohydrates or by oxidation of the methylene group to a carboxylic group, which were the major metabolic routes, and
- hydroxylation of the methylene group of the furanone moiety followed by conjugation with glucose, which was the minor metabolic route.

On the basis of these results, a metabolic pathway of [pyridinylmethyl-¹⁴C]BYI 02960 in tomatoes can be proposed.

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	 * position of the radiolabel
Radiolabelled test material	[pyridinylmethyl- ¹⁴ C]BYI 02960
Specific radioactivity (before radiodilution) (after radiodilution)	4.37 MBq/mg (118.08 µCi/mg) 1.46 MBq/mg (39.37 µCi/mg)
Chemical Purity	> 99% (HPLC)
Radiochemical purity	> 98% (HPLC and TLC)

The supplied radiolabelled test compound [pyridinylmethyl-¹⁴C]BYI 02960 was dissolved in acetonitrile. Radiodilution and formulation of the test compound was performed prior to the first and the second application: Therefore, an adequate portion of the non-radiolabelled test compound was dissolved in an adequate volume of the stock solution of the radiolabelled test compound. The solution was evaporated to dryness. The remainder was dissolved in an adequate amount of SL 200 blank formulation using a magnetic stirrer. Preparation of each application solution was done by dissolving the formulation in water. The specific activity of each application solution was 1.46 MBq/mg (39.37 µCi/mg).

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

2. Soil: "Einheitserde T", pH (CaCl₂) = 5.8, 85% white moor peat and 15% clay, enriched with water soluble nutrient salt mixture (ENS)

3. Plant Tomato, variety "Philona", representative for fruiting crops

B. Study Design

Experimental conditions:

Four tomato plants (variety: Philona) were cultivated, each in a 30 L planting bucket with a surface diameter of 38 cm filled with "Einheitserde T". The plants were cultivated in the greenhouse of the test facility (controlled temperature, humidity and light conditions). The tomato plants were treated with SL 200 formulated [pyridinylmethyl-¹⁴C]BYI 02960 by drench application. Two applications were performed, each at an application rate of 300 g a.s./ha. The first application was conducted at a growth stage of BBCH 15 (5th leaf on main shoot unfolded) and the second at a growth stage of BBCH 51 (first inflorescence visible and first bud erect). The interval between the two applications was 14 days. The aqueous application solution was poured into a circular furrow prepared in the soil around each tomato plant. At each application, a total volume of 200 mL was applied, corresponding to 145.67 MBq or to 100.0 mg a.s. (= 25 mg a.s./plant). Based on a planting density of 12,000 tomato plants/ha in agricultural practice, the application rate corresponds to 300 g a.s./ha, which was anticipated to be the maximum single drench application rate in agricultural practice.

Sampling:

When the plants reached the growth stage BBCH 61 (first flower of the first inflorescence open), the open flowers were sampled from one of the four tomato plants in order to determine the nature of the residues in flowers. The flowers were collected with their receptacles using scissors and were stored in a freezer ($\leq -18^{\circ}\text{C}$) on the same day. Sampling of newly opened flowers was continued 2 - 3 times a week over a total period of approx. five weeks until the end of flowering (at BBCH 69: 9 or more inflorescences with open flowers). The tomato plant from which the flowers were cut was sampled, cut in pieces and stored in a freezer ($\leq -18^{\circ}\text{C}$) for optional metabolite investigations. The frozen flowers were homogenized with a high speed blender in liquid nitrogen (Polytron). The homogenised flower sample was stored in a freezer ($\leq -18^{\circ}\text{C}$) until extraction.

On the day when the three remaining tomato plants reached the growth stage BBCH 81 (10% of fruits show typical fully ripe colour), the ripe tomato fruits were harvested and were stored in a freezer ($\leq -18^{\circ}\text{C}$). Harvesting of newly ripe fruits was continued 2 - 3 times a week over a total period of approx. three weeks until the plants produced no new fruits (at BBCH 89: fully ripe). The frozen fruits were cut in pieces and were homogenised with a high speed blender (Polytron). The homogenised tomato fruit samples were stored in portions in a freezer ($\leq -18^{\circ}\text{C}$) until extraction.

C. Analytical Procedures

Extraction:

The homogenised tomato flowers were extracted three times with a mixture of acetonitrile/water (8:2, v/v) and one time with a mixture of acetonitrile/water (1:1, v/v) and a portion of the homogenised tomato fruits was extracted four times with a mixture of acetonitrile/water (8:2, v/v) using a high speed blender (Polytron). After each extraction step, extracts and solids were separated by centrifugation. The radioactivity in the extracts was determined by LSC, in the solids by combustion



followed by LSC. The actual TRR value of the RAC was determined by summing up the radioactivity measured in the extracts and in the remaining solids.

All tomato fruit extracts were combined, and the first two tomato flower extracts were combined. The combined extracts were subjected to a clean-up step using a pre-conditioned SPE RP 18 cartridge (Phenomenex, Strata C18-E). The flow-through fraction (percolate) was collected and the cartridge was rinsed with 100 mL of acetonitrile/water (8:2, v/v) and methanol/dichloromethane (1:1, v/v). The percolate and the acetonitrile/water fraction were combined and concentrated by rotary evaporation in vacuo. The concentrated extracts of fruits and flowers were analysed by HPLC.

Quantification:

Parent compound and metabolites in the extracts were analysed by reversed phase HPLC coupled to a radioactivity detector with a glass scintillator cell. The HPLC chromatograms (= metabolite profiles) were integrated for quantification of compounds.

Identification and characterisation:

Parent compound was identified in tomato fruits and flowers by reversed phase HPLC co-chromatography using a radiolabelled reference compound. Confirmation of the assignment by a second chromatographic method was shown in the tomato study performed with [furanone-4-¹⁴C]BYI 02960. The main metabolite in tomato fruits was identified by LC-MS/MS analysis after isolation of the compound by semi-preparative HPLC. An additional major metabolite was identified by HPLC co-chromatography with an authentic reference compound. Confirmation of the assignment by a second chromatographic method was not needed due to its low residue level. All minor metabolites detected in fruits or flowers were identified either by reversed phase HPLC co-chromatography using authentic reference compounds or by HPLC comparison.

Storage stability:

The extraction experiments and the HPLC analyses of tomato fruits and flowers for quantitative evaluation were performed not later than 1.5 months after harvest. Extract stability was demonstrated by comparing the HPLC chromatograms recorded at different time points during the study. The profiles did not change significantly during the whole course of the study. It was therefore concluded, that the residues in the extracts were sufficiently stable during the experimental period of the study (which did not exceed a period of approx. 6 to 7 months in total) and that the chromatograms represented the metabolic pattern in the samples at harvest. Thus, no additional storage stability data have to be provided according to OECD Guidance for the Testing of Chemicals 501 (2007).

II. Results and Discussion

The metabolism of [pyridinylmethyl-¹⁴C]BYI 02960 was investigated in tomato fruits and flowers following two drench applications. The total radioactive residue (TRR) in fruits, which represent the edible RAC, accounted for 0.130 mg/kg. The TRR was considerably higher in flowers and accounted for 1.254 mg/kg. A major portion of radioactivity was extracted conventionally by acetonitrile/water mixtures (96.5% to 98.5% of the TRR) as shown in Table 6.2.1-6. Parent compound was the main residue in flowers and the second major residue in fruits.

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

In tomato fruits, two major metabolites (>10% of TRR and >0.01 mg/kg) were detected: BYI 02960-CHMP-di-glyc (main compound in fruits) and 6-CNA. BYI 02960-CHMP-di-glyc was identified by LC-MS/MS analysis after isolation by semi-preparative HPLC. The metabolite 6-CNA was identified by HPLC co-chromatography using an authentic reference compound, as were the minor metabolite BYI 02960-CHMP and its conjugate BYI 02960-CHMP-glyc. The configuration of the carbohydrate in the conjugate was assigned to D-glucose in accordance to the reference compound used. The minor metabolite BYI 02960-OH-glyc was identified after isolation with semi-preparative HPLC and chromatographic comparison with radiolabelled reference compounds. For chromatographic comparison an acidic reversed phase HPLC method was used to ensure separation and differentiation from BYI 02960-acetic acid, a metabolite which was identified e.g. in the apple metabolism and confined rotational crops studies and which co-eluted with BYI 02960-OH-glyc using the neutral profiling method.

In tomato flowers, the same metabolites were detected as in the fruits, with the exception of BYI 02960-CHMP, which was not detected. All metabolites represented less than 10% of the TRR and were identified by HPLC co-chromatography with authentic reference compounds or by HPLC comparison.

The distribution of the parent compound and metabolites is shown in Table 6.2.1-7. In total, 86.3% and 96.5% of the TRR were identified in tomato fruits and flowers, respectively.

Table 6.2.1-6: Distribution of radioactivity in the extracts of the tomato matrices fruits and flowers after drench application of [pyridinylmethyl-¹⁴C]BYI 02960

TRR [mg/kg] =	tomato fruits		tomato flowers	
	% of TRR	mg/kg	% of TRR	mg/kg
Conventionally extracted	98.5	0.128	96.5	1.209
Extract for analysis	98.5	0.128	96.5	1.209
Losses (not analysed)	---	---	---	---
Total extracted	98.5	0.128	96.5	1.209
Unextractable (PES*)	1.5	0.002	3.5	0.044
Accountability	100.0	0.130	100.0	1.254

* post extraction solids

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.2.1-7: TRR values and distribution of parent compound and metabolites in tomatoes (fruit and flowers) after drench application of [pyridinylmethyl-¹⁴C]BYI 02960

	tomato fruits		tomato flowers	
TRR [mg/kg] =	0.130		1.254	
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg
<i>Conventional extraction</i>				
BYI 02960 (parent compound)	24.2	0.031	66.2	0.829
6-CNA	13.2	0.017	7.0	0.087
CHMP-di-glyc	37.1	0.048	8.0	0.100
CHMP-glyc	5.1	0.007	9.5	0.119
CHMP	3.3	0.004	---	---
OH-glyc	3.4	0.004	5.9	0.073
Total identified	86.3	0.112	96.5	1.209
unknown 1	5.5	0.007	---	---
unknown 2	3.8	0.005	---	---
unknown 3	3.0	0.004	---	---
Total characterised	12.2	0.016	<0.1	<0.001
Analysed extract(s)	98.5	0.128	96.5	1.209
Extract(s) not analysed	---	---	---	---
Total extracted	98.5	0.128	96.5	1.209
Unextractable (PES*)	1.5	0.002	3.5	0.044
Accountability	100.0	0.130	100.0	1.254

* post extraction solids

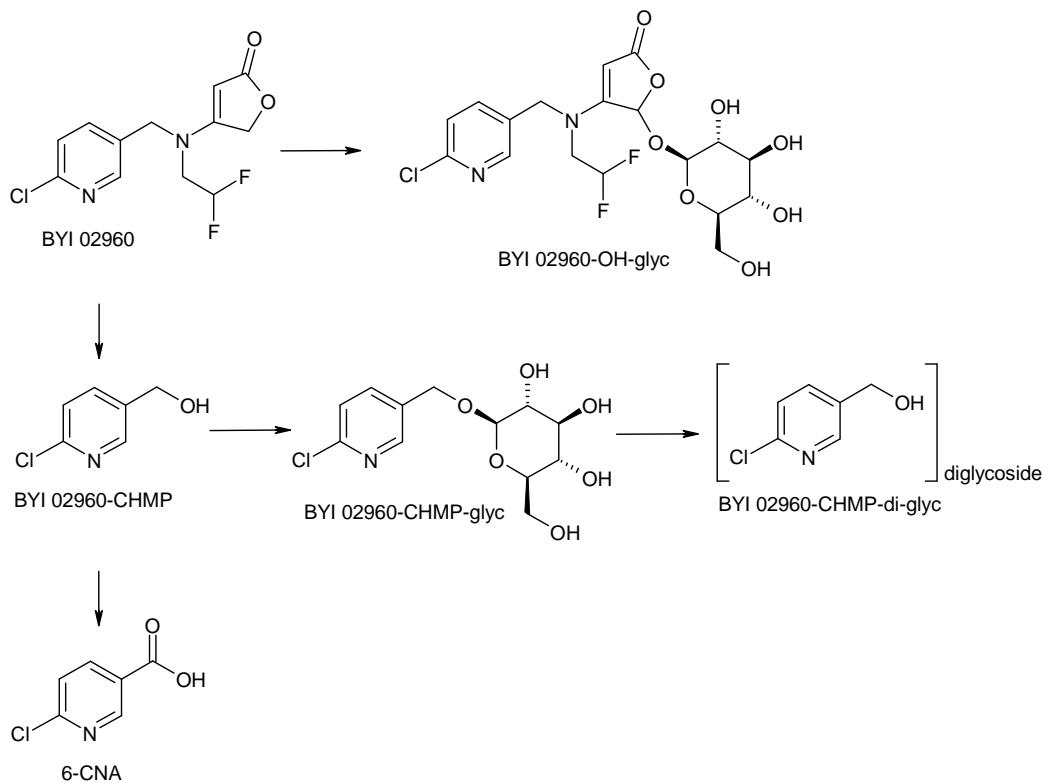
III. Conclusions

Based on the metabolites identified the following metabolic routes were deduced:

- cleavage of the pyridinylmethylamine bond followed by conjugation with carbohydrates or followed by oxidation of the methylene group to a carboxylic group, which were the major metabolic routes, and
- hydroxylation of the methylene group of the furanone moiety followed by conjugation with glucose, which was the minor metabolic route.

Thus, [pyridinylmethyl-¹⁴C]BYI 02960 was metabolised moderately in tomatoes. On the basis of the results of this study it is concluded that the metabolism of [pyridinylmethyl-¹⁴C]BYI 02960 in tomatoes is well understood and the following metabolic pathway is proposed.

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

 Figure 6.2.1-2: Proposed metabolic pathway of [pyridinylmethyl-¹⁴C]BYI 02960 in tomatoes




Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Report:	KIIA 6.2.1/03, Unold, M., Justus, K.; 2011
Title:	Metabolism of [ethyl-1- ¹⁴ C]BYI 02960 in tomatoes
Report No & Edition No	MEF-11/498 M-413996-01-2
Guidelines:	OECD 501 Metabolism in Crops US EPA Residue Chemistry Test Guideline OPPTS 860.1300: Nature of the Residue – Plants, Livestock PMRA Regulatory Directive Dir98-02: Residue Chemistry Guidelines Section 2: Nature of the Residue – Plants, Livestock Japanese MAFF, 12 Nousan 8147 European Parliament and Council Regulation (EC) No 1107/2009
GLP	yes

Executive Summary

The metabolism of [ethyl-1-¹⁴C]BYI 02960 in tomatoes was investigated according to the maximum envisaged use pattern. Three tomato plants were treated by soil drench application with [ethyl-1-¹⁴C]BYI 02960 formulated as an SL 200. The first application was performed at BBCH 14-15 (4th to 5th leaf on main shoot unfolded) and a second application at BBCH 51-59 (first to 9th inflorescence visible and first bud erect). The single application rate corresponded to 300 g a.s./ha; the total application rate was 600 g a.s./ha.

The flowers were sampled from one tomato plant at 1 to 36 days after the last application and the fruits were harvested from the remaining two tomato plants at 56 to 86 days after the last application. The TRR values are shown in the following table:

Table 6.2.1-8: TRR values in tomato fruits and flowers after drench application of [ethyl-1-¹⁴C]BYI 02960

Matrix	Timing and Application	PHI (days)*	ppm (mg a.s. equiv./kg)
tomato fruits	two drench applications, at BBCH 14-15 and 14 days later; 2 x 300 g a.s./ha	56	0.201
tomato flowers		n.a.	2.230

* PHI: preharvest interval (corresponds to days after last treatment (DAT) at the start of harvest/sampling)
n.a. not applicable, flowers are not a RAC

The fruits and flowers were extracted conventionally. Three extraction steps with acetonitrile/water mixtures released 99.5% and 98.3% of the TRR from fruits and flowers, respectively.

Parent compound and metabolites in the extracts of tomato flowers and fruits were analysed by HPLC. Identification was performed by HPLC and/or TLC co-chromatography with reference compounds, as well as by comparison of HPLC profiles. Besides parent compound, one major metabolite and two minor metabolites were identified.

The label-specific metabolite difluoroacetic acid (DFA) was the main component detected in both matrices and represented 86.6% of the TRR in tomato fruits and 59.8% in tomato flowers. Since difluoroacetic acid was also detected as a major soil metabolite in the aerobic soil degradation studies, it can be expected that at least a part of the residues detected in tomato fruits and flowers originated



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

from the uptake of the soil metabolite. Parent compound was the second major component in tomato fruits and flowers and represented 10.0% and 33.0% of the TRR, respectively. BYI 02960-OH-glyc and BYI 02960-difluoroethyl-amino-furanone were minor metabolites (<5% of the TRR) detected in fruits and flowers.

[Ethyl-1-¹⁴C]BYI 02960 was metabolised to a significant extent in tomatoes. The following metabolic routes were observed:

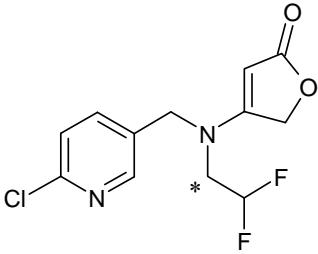
- oxidative cleavage of the difluoroethylamine bond and formation of difluoroacetic acid (this degradation process occurs also in the soil and thus uptake of difluoroacetic acid from the soil is supposed as well)
- cleavage of the pyridinylmethylamine bond and formation of BYI 02960-difluoroethylamino-furanone and
- hydroxylation of the methylene group of the furanone moiety followed by conjugation with glucose

On the basis of these results, a metabolic pathway of [ethyl-1-¹⁴C]BYI 02960 in tomatoes can be proposed.

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure		* position of the radiolabel
Radiolabelled test material	[ethyl-1- ¹⁴ C]BYI 02960	
Specific radioactivity (before radiodilution) (after radiodilution)	3.93 MBq/mg (106.28 µCi/mg) 1.31 MBq/mg (35.46 µCi/mg)	
Chemical Purity	> 99% (HPLC)	
Radiochemical purity	> 99% (HPLC and TLC)	

The supplied radiolabelled test compound [ethyl-1-¹⁴C]BYI 02960 was dissolved in acetonitrile. Radiodilution and formulation of the test compound was performed prior to the first and the second application: Therefore, an adequate portion of the non-radiolabelled test compound was dissolved in an adequate volume of the stock solution of the radiolabelled test compound. The solution was evaporated to dryness. The remainder was dissolved in an adequate amount of SL 200 blank formulation using a magnetic stirrer. Preparation of each application solution was done by dissolving the formulation in water. The specific activity of each application solution was 1.31 MBq/mg (35.46 µCi/mg).



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

2. Soil: "Einheitserde T", pH (CaCl₂) = 5.8, 85% white moor peat and 15% clay, enriched with

water soluble nutrient salt mixture (ENS)

3. Plant Tomato, variety "Philona", representative for fruiting crops

B. Study Design

Experimental conditions:

Three tomato plants (variety: Philona) were cultivated each in a 30 L planting bucket with a surface diameter of 38 cm. The planting buckets have been filled with "Einheitserde T". The plants were cultivated in the greenhouse of the test facility (controlled temperature, humidity and light conditions).

The tomato plants were treated with SL 200 formulated [ethyl-1-¹⁴C]BYI 02960 by drench application. Two applications were performed, each at an application rate of 300 g a.s./ha. The first application was conducted at a growth stage of BBCH 14-15 (4th to 5th leaf on main shoot unfolded) and the second at a growth stage of BBCH 51-59 (1st to 9th inflorescence visible and first bud erect). The interval between the two applications was 14 days. The aqueous application solution was poured into a circular furrow prepared in the soil around the tomato plant. At each application, a total volume of 150 mL was applied, corresponding to 98.50 MBq or to 75.1 mg a.s. (= 25 g a.s./plant). Based on a planting density of 12,000 tomato plants/ha in agricultural practice, the application rate was 300 g a.s./ha. The total rate corresponds to the anticipated maximum application rate in agricultural practice.

Sampling:

When the plants reached the growth stage BBCH 61 (first flower of the first inflorescence open), the open flowers were sampled from one of the three tomato plants in order to determine the nature of the residues in flowers. The flowers were collected with their receptacles using scissors and were stored in a freezer ($\leq -18^{\circ}\text{C}$) on the same day. Sampling of newly opened flowers was continued 2 - 3 times a week over a total period of approx. four weeks until end of flowering (at BBCH 69: 9 or more inflorescences with open flowers). The tomato plant from which the flowers were cut was sampled, cut in pieces and stored in a freezer ($\leq -18^{\circ}\text{C}$) for optional metabolite investigations. The frozen flowers were homogenized with a high speed blender in liquid nitrogen (Polytron). The homogenised flower sample was stored in a freezer ($\leq -18^{\circ}\text{C}$) until extraction.

On the day when the two remaining tomato plants reached the growth stage BBCH 81 (10% of fruits show typical fully ripe colour), the ripe tomato fruits were harvested and were stored in a freezer ($\leq -18^{\circ}\text{C}$) on the same day. Harvesting of newly ripe fruits was continued 2 - 3 times a week over a total period of approx. four weeks until the plants produced no new fruits (at BBCH 89: fully ripe). The frozen fruits were crushed and homogenised with a high speed blender (Polytron). The homogenised tomato fruits samples was stored in portions in a freezer ($\leq -18^{\circ}\text{C}$) until extraction.

C. Analytical Procedures

Extraction:

The homogenised tomato flowers and tomato fruits were extracted three times with a mixture of acetonitrile/water (8:2, v/v) using a high speed blender (Polytron). After each extraction step, extracts and solids were separated by centrifugation. The radioactivity in the extracts was determined by LSC, in the solids by combustion followed by LSC. The actual TRR value of the RAC was determined by summing up the radioactivity measured in the extracts and in the remaining solids.



The first two tomato fruit extracts were combined and as well the first two tomato flower extracts. The combined extracts were subjected to a clean-up step using a pre-conditioned SPE RP 18 cartridge (Phenomenex, Strata C18-E). The flow-through fraction (percolate) was collected and the cartridge was rinsed with 100 mL of acetonitrile/water (8:2, v/v) and methanol/dichloromethane (1:1, v/v). The percolate and the acetonitrile/water fraction were combined and concentrated by rotary evaporation in vacuo. The concentrated extract was analysed by HPLC.

Quantification:

Parent compound and metabolites in the extracts were analysed by reversed phase HPLC coupled to a radioactivity detector with a glass scintillator cell. The HPLC chromatograms (= metabolite profiles) were integrated for quantification of compounds.

Identification and characterisation:

Parent compound and metabolites were identified by co-chromatography with reference compounds or by comparison of chromatographic profiles. For identification of the label-specific main metabolite, three different stationary phases were used for TLC co-chromatography. Thus, dissimilar TLC systems confirmed the assignment unambiguously.

Storage stability:

The extraction experiments and the first HPLC analysis of the tomato fruits were performed within approx. 1.5 months after harvest. The time period between harvest and analysis was approx. 3 months for tomato flowers. Thus, no additional storage stability data have to be provided according to OECD Guidance for the Testing of Chemicals 501 (2007).

All extracts were analysed on the next day after the start of extraction. Extract stability was demonstrated by comparing the HPLC chromatograms recorded at different times during the study. The profiles did not change significantly during the analytical work. In the corresponding tomato metabolism studies performed with [pyridinylmethyl-¹⁴C]BYI 02960 and [furanone-4-¹⁴C]BYI 02960, it was shown that the profiles of tomato fruits and flowers extracts did not significantly change during a period of at least six months. It was therefore concluded, that the residues in the extracts were sufficiently stable during the experimental period of the study and that the chromatograms represented the metabolic pattern in the samples at harvest.

II. Results and Discussion

The metabolism of [ethyl-1-¹⁴C]BYI 02960 was investigated in tomato fruits and flowers following two drench applications. The total radioactive residue (TRR) in fruits, which represent the edible RAC, accounted for 0.201 mg/kg. The TRR was considerably higher in flowers and accounted for 2.230 mg/kg. By far the main portion of radioactivity was extracted conventionally by acetonitrile/water mixtures (99.5% to 98.3% of the TRR) as shown in Table 6.2.1-9. The label-specific metabolite difluoroacetic acid (DFA) was the main residue in both extracts, followed by parent compound. Two additional minor metabolites (<5% of TRR) were identified in tomato fruits and flowers: BYI 02960-difluoroethyl-amino-furanone and BYI 02960-OH-glyc.

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Parent compound was identified in tomato fruits and flowers by HPLC co-chromatography using a non-radiolabelled reference compound and by comparison of the metabolite profiles of all tomato studies conducted with BYI 02960 labelled in the different moieties of the molecule. In the tomato metabolism study with [furanone-4-¹⁴C]BYI 02960, parent compound was additionally identified in tomato fruits and flowers by normal phase TLC co-chromatography as different chromatographic technique. The main metabolite difluoroacetic acid was identified after semi-preparative isolation by TLC co-chromatography with an authentic reference compound. Two dissimilar systems (normal phase and reversed phase TLC) and one modified normal phase system were applied. TLC co-chromatography with three different stationary phases showed chromatographic correspondence of the radioactivity in the isolated fraction with the radiolabelled reference compound difluoroacetic acid. In tomato flowers this metabolite was identified by comparison of the HPLC profiles of fruits and flowers.

The minor metabolite BYI 02960-difluoroethyl-amino-furanone was identified in tomato fruits by reversed phase HPLC co-chromatography with a non-radiolabelled reference compound. This metabolite was identified in tomato flowers by comparison of the two profiles. The minor metabolite BYI 02960-OH-glyc was identified in tomato fruits and flowers by comparison of the HPLC-profile with a corresponding profile obtained in the tomato metabolism study performed with [furanone-4-¹⁴C]BYI 02960. In the latter study, the metabolite BYI 02960-OH-glyc had been identified in tomato fruits by reversed phase HPLC co-chromatography with a radiolabelled reference compound and additionally in tomato flowers by co-chromatography of the aglycon after alkaline hydrolysis with a non-radiolabelled reference compound.

The TRR and the distribution of parent and metabolites in the extracts is shown in Table 6.2.1-10. In total, 99.5% and 98.3% of the TRR were identified in the tomato fruits and flowers, respectively.

Table 6.2.1-9: Distribution of radioactivity in the extracts of the tomato matrices fruits and flowers after drench application of [ethyl-1-¹⁴C]BYI 02960

	tomato fruits		tomato flowers	
	% of TRR	mg/kg	% of TRR	mg/kg
TRR [mg/kg] =	0.201		2.230	
Conventionally extracted	99.5	0.200	98.3	2.192
Extract for analysis	99.5	0.200	98.3	2.192
Losses (not analysed)	n.q.	n.q.	n.q.	n.q.
Total extracted	99.5	0.200	98.3	2.192
Unextractable (PES*)	0.5	0.001	1.7	0.037
Accountability	100.0	0.201	100.0	2.230

* post extraction solids

n.q. not quantified



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.2.1-10: TRR values and distribution of parent compound and metabolites in tomatoes (fruit and flowers) after drench application of [ethyl-1-¹⁴C]BYI 02960

	tomato fruits		tomato flowers	
TRR [mg/kg] =	0.201		2.230	
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg
<i>Conventional extraction</i>				
BYI 02960 (parent compound)	10.0	0.020	33.0	0.736
difluoroacetic acid	86.6	0.174	59.8	1.334
difluoroethyl-amino-furanone	2.2	0.004	3.1	0.068
OH-glyc	0.6	0.001	2.4	0.054
Total identified	99.5	0.200	98.3	2.192
Total characterized	---	---	---	---
Analysed extract(s)	99.5	0.200	98.3	2.192
Extract(s) not analysed	---	---	---	---
Total extracted	99.5	0.200	98.3	2.192
Unextractable (PES*)	0.5	0.001	1.7	0.037
Accountability	100.0	0.201	100.0	2.230

* post extraction solids

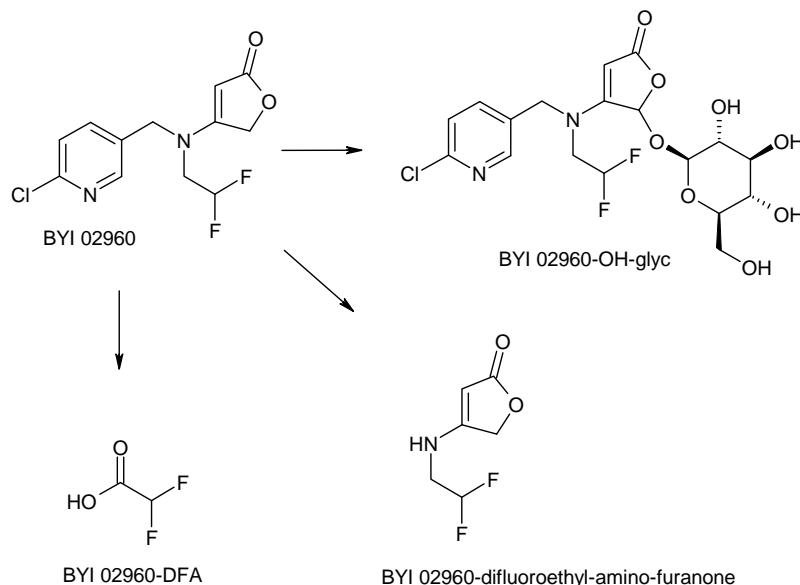
III. Conclusions

Based on the metabolites identified the following metabolic routes were deduced:

- oxidative cleavage of the difluoroethylamine bond and formation of difluoroacetic acid,
- cleavage of the pyridinylamine bond, and formation of BYI 02960-difluoroethyl amino-furanone, and
- hydroxylation of the methylene group of the furanone moiety followed by conjugation with glucose.

[Ethyl-1-¹⁴C]BYI 02960 was metabolised rather extensively in the present study. Since it is known that parent degrades to difluoroacetic acid in soil, the difluoroacetic acid residue is probably the result of both uptake of this metabolite from the soil and degradation of the parent compound BYI 02960 in the plant. On the basis of the results of this study it is concluded that the metabolism of [ethyl-1-¹⁴C]BYI 02960 in tomatoes is well understood and the following metabolic pathway is proposed.

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Figure 6.2.1-3: Proposed metabolic pathway of [ethyl-1-¹⁴C]BYI 02960 in tomatoes**Overall Conclusions Tomato (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-4-¹⁴C]BYI 02960, (2) [pyridinylmethyl-¹⁴C]BYI 02960 or (3) [ethyl-1-¹⁴C]BYI 02960. Total radioactive residue was measured in fruits and flowers and metabolites identified or characterized dependent on levels found.

(1) Label 1: [furanone-4-¹⁴C]BYI 02960

At harvest, the total radioactive residue (TRR) in the tomato fruits was low (0.096 mg/kg). Parent compound was the main component detected. Besides parent compound, two major label-specific metabolites were present: The natural compound glucose (or isomeric carbohydrates) and BYI 02960-difluoroethyl-amino-furanone. Furthermore, two minor metabolites were detected. One of them was identified as the non-label specific metabolite BYI 02960-OH-glyc. In tomato flowers, the same metabolites were identified as in the fruits, with the exception of glucose which was only present in fruits. Although the same metabolites were detected, the proportions differed significantly. Most significant was that parent compound was by far the main constituent in flowers and represented nearly 80% of the TRR.

On basis of the metabolites identified, the metabolic pathway was deduced. One major metabolic route was the complete degradation of the furanone moiety and incorporation of the carbon atoms into the natural compound pool, i.e. most probably into glucose. Cleavage of the pyridinylmethylamine bond and formation of BYI 02960-difluoroethylamino-furanone was also an important route. The cleavage of the molecule was also detected in the tomato studies conducted with the other two radiolabels. In the study with [ethyl-1-¹⁴C]BYI 02960, BYI 02960-difluoroethylamino-furanone was detected in nearly identical concentrations and in the study with [pyridinylmethyl-¹⁴C]BYI 02960, BYI 02960-



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

CHMP and 6-CNA were identified as corresponding counterparts. A minor metabolic route was the hydroxylation of the methylene group of the furanone moiety followed by conjugation with glucose. This metabolic route resulted in the non-label specific metabolite BYI 02960-OH-glyc, which was identified in corresponding concentrations in the tomato study performed with [ethyl-1-¹⁴C]- and [pyridinylmethyl-¹⁴C]BYI 02960. Thus, the results of the present metabolism study in tomatoes are in very good conformity with the results of the corresponding studies performed with [ethyl-1-¹⁴C]- and [pyridinylmethyl-¹⁴C]BYI 02960.

(2) Label 2: [pyridinylmethyl-¹⁴C]BYI 02960

At harvest, the total radioactive residue (TRR) in the tomato fruits, representing the edible raw agricultural commodity (RAC), was low (0.130 mg/kg). Three major components were detected in the fruits: Metabolite BYI 02960-CHMP-di-glyc, parent compound BYI 02960 and 6-CNA. Besides the major components, six minor metabolites were detected, three of them were identified: BYI 02960-CHMP and BYI 02960-CHMP-glyc, the label-specific precursor metabolites of BYI 02960-CHMP-di-glyc, and BYI 02960-OH-glyc, a metabolite common to all three radiolabels tested. In tomato flowers, the same metabolites were identified as in the fruits, with the exception of BYI 02960-CHMP. However, the proportions of the compounds differed significantly. Most significant was that parent compound was by far the main constituent and represented more than 60% of the TRR.

The major metabolic route was cleavage of the pyridinylmethylamine bond followed by conjugation with carbohydrates or by oxidation of the methylene group to a carboxylic group. Cleavage of the molecule was also confirmed in the tomato study performed with [furanone-4-¹⁴C]- and [ethyl-1-¹⁴C]BYI 02960. A minor metabolic route was hydroxylation of the methylene group of the furanone moiety followed by conjugation with glucose. This metabolic route resulted in the non-label specific metabolite BYI 02960-OH-glyc, which was identified in corresponding concentrations in the tomato studies performed with [furanone-4-¹⁴C]- and [ethyl-1-¹⁴C]BYI 02960. Thus, the results of the present metabolism study in tomatoes are in good conformity with the results of the corresponding studies performed with the other radiolabels.

(3) Label 3: [ethyl-1-¹⁴C]BYI 02960

At harvest, the total radioactive residue (TRR) in the tomato fruits, representing the edible raw agricultural commodity (RAC), was 0.201 mg/kg. The TRR in flowers accounted for 2.230 mg/kg. These TRR values were significantly higher compared to those obtained in the tomato metabolism studies conducted with [pyridinylmethyl-¹⁴C]BYI 02960 and [furanone-4-¹⁴C]BYI 02960. Since it is known that parent degrades to difluoroacetic acid in soil, the difluoroacetic acid residue is probably the result of both uptake of this metabolite from the soil and degradation of the parent compound BYI 02960 in the plant. Difluoroacetic acid was by far the main compound detected in fruits and flowers, followed by parent compound. Additionally, two minor metabolites were detected. They were identified as BYI 02960-difluoroethyl-amino-furanone and BYI 02960-OH-glyc.

Parent compound and all metabolites not specific to the [ethyl-1-¹⁴C]-label were detected in comparable amounts as in the tomato metabolism studies conducted with the other radiolabels. The major metabolic route in the present study observed was oxidative cleavage of the difluoroethylamine bond and formation of difluoroacetic acid. Cleavage of the pyridinylamine bond was also observed and led to the formation of BYI 02960-difluoroethyl amino-furanone. The latter molecular cleavage

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

was also confirmed in the tomato study with [furanone-4-¹⁴C]BYI 02960 and as well in the tomato study with [pyridinylmethyl-¹⁴C]BYI 02960, in which BYI 02960-CHMP and 6-CNA were detected as corresponding counterparts. A minor metabolic route was hydroxylation of the methylene group of the furanone moiety followed by conjugation with glucose. This metabolic route resulted in the non-label specific metabolite BYI 02960-OH-glyc, which was identified in comparable concentrations in the other two tomato studies performed with [furanone-4-¹⁴C]- and [pyridinylmethyl-¹⁴C]BYI 02960. Thus, the results of the present metabolism study in tomatoes are in good conformity with the results of the corresponding studies performed with the other radiolabels.

When considering the results from all metabolism studies conducted on tomato, it can be concluded that BYI02960 is rather extensively metabolised in this crop. A total of 6 major and 6 minor metabolites were found, and all major and 3 minor have been identified. The distribution of parent compound and metabolites in the edible commodity tomato fruits is summarized in Table 6.2.1-11.

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.2.1-11: TRR values and distribution of parent compound and metabolites in tomato fruits after drench application of radiolabelled 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
<i>difluoroacetic acid</i>					86.6	0.174
<i>glucose/carbohydrates</i>	27.5	0.026				
<i>6-CNA</i>			13.2	0.017		
<i>CHMP-di-glyc</i>			37.1	0.048		
<i>CHMP-glyc</i>			5.1	0.007		
<i>CHMP</i>			3.3	0.004		
<i>difluoroethyl-amino-furanone</i>	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.

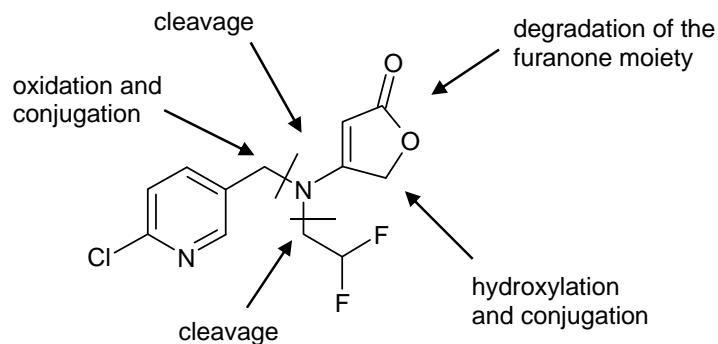
On basis of the metabolites identified, biotransformation of BYI 02960 in tomato proceeds by the following pathways:

- oxidative cleavage of the difluoroethylamine bond and formation of difluoroacetic acid
- complete degradation of the furanone moiety and incorporation of carbon atoms into the natural compound pool, e.g. into glucose/carbohydrates
- cleavage of the pyridinylmethylamine bond and formation of BYI 02960-difluoroethylamino-furanone and its corresponding counterpart BYI 02960-CHMP, which was either conjugated with carbohydrates or oxidised to 6-chloronicotinic acid (6-CNA)
- hydroxylation of the furanone moiety followed by conjugation with glucose

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

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

Figure 6.2.1-4: Positions involved in metabolic degradation of BYI 02960 in tomato fruits and flowers





Metabolism, distribution and expression of residues in potato (tuber treatment and in-furrow application)

Metabolism studies in potatoes were conducted with [furanone-4-¹⁴C]- and [pyridinylmethyl-¹⁴C]BYI 02960:

Report:	KIIA 6.2.1/04, Justus, K.; 2011
Title:	Metabolism of [furanone-4- ¹⁴ C]BYI 02960 in potatoes
Report No & Edition No	MEF-10/769 M-415234-01-2
Guidelines:	OECD 501 Metabolism in Crops US EPA Residue Chemistry Test Guideline OPPTS 860.1300: Nature of the Residue – Plants, Livestock PMRA Regulatory Directive Dir98-02: Residue Chemistry Guidelines Section 2: Nature of the Residue – Plants, Livestock Japanese MAFF, 12 Nousan 8147 European Parliament and Council Regulation (EC) No 1107/2009
GLP	yes

Executive Summary

The metabolism of [furanone-4-¹⁴C]BYI 02960 in potatoes was investigated according to the maximum envisaged use pattern using two different methods of application. In one experiment, the seed potato tubers were treated with [furanone-4-¹⁴C]BYI 02960 formulated as an FS 480 at an application rate of 10.0 g a.s./dt (= 254 g a.s./ha, seed density 25 dt/ha). In the other experiment, [furanone-4-¹⁴C]BYI 02960, formulated as an SL 200, was sprayed in the furrow onto the soil at an application rate of 626 g a.s./ha (seed density 22 dt/ha) prior to planting of the seed potatoes. The potato tubers were harvested at maturity in both experiments. Concurrently, the leaves and roots as well as the remainders of the seed potatoes were sampled. The TRR values determined are shown in the following table:

Table 6.2.1-12: TRR values in potato tubers and the remaining part of the plant after tuber treatment or in-furrow application of [furanone-4-¹⁴C]BYI 02960

Matrix	Timing and Application	PHI (days)*	ppm (mg a.s. equiv./kg)
potato tubers	tuber treatment at planting (BBCH 03), 10.0 g a.s./dt	97	0.078
potato leaves and roots		97	6.97
remainders of the seed potatoes		97	36.21
potato tubers	in-furrow application at planting (BBCH 03), 626 g a.s./ha	97	0.171
potato leaves and roots		97	7.01
remainders of the seed potatoes		97	3.43

* PHI: preharvest interval (corresponds to days after last treatment (DAT) at the start of harvest/sampling)

The potato tubers (edible raw agricultural commodity) were extracted conventionally. Four extraction steps with acetonitrile/water mixtures released 67.0% of the TRR in the tuber treatment experiment and 75.3% of the TRR in the in-furrow treatment experiment.



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Parent compound and metabolites in the extracts of potato tubers were analysed by HPLC.

Identification was performed by HPLC co-chromatography with reference compounds and by comparison of HPLC profiles. Parent compound represented 40.0% and 56.9% of the TRR in potato tubers. Besides parent compound, which was the predominant compound in the extracts, two minor metabolites representing less than 10% of the TRR and accounting for less than 0.01 mg/kg were identified: BYI 02960-OH-glyc and BYI 02960-difluoroethyl-amino furanone.

[Furanone-4-¹⁴C]BYI 02960 was moderately metabolised in potatoes. The following metabolic routes were observed:

- hydroxylation of the methylene group of the furanone moiety followed by conjugation with glucose, and
- cleavage of the pyridinylmethylamine bond.

On the basis of these results, a metabolic pathway of [furanone-4-¹⁴C]BYI 02960 in potatoes can be proposed.

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure		* position of the radiolabel
Radiolabelled test material	[furanone-4- ¹⁴ C]BYI 02960	
Specific radioactivity	3.94 MBq/mg (106.46 µCi/mg)	
Chemical Purity	> 99% (HPLC)	
Radiochemical purity	> 99% (HPLC and TLC)	

The supplied radiolabelled test compound [furanone-4-¹⁴C]BYI 02960 was dissolved in acetonitrile. Formulation of the test compound was performed prior to the application. For both formulations (FS 480 and SL 200), adequate parts of this stock solution were transferred into special glass vials and evaporated to dryness. For the preparation of the FS 480 formulation, the blank formulation was added to the test item and was homogenized using a ball mill. Afterwards the mixture was suspended in water by stirring or swirling. For the preparation of the SL 200 formulation, the liquid blank formulation was added to the test item using a magnetic stirrer. The mixture was diluted with water to get the aqueous application solution.

2. Soil: “Monheim 4” (sandy loam soil from Germany), pH (CaCl₂) = 6.8, 69% sand, 18% silt and 13% clay, 1.2% organic carbon, cation exchange capacity (CEC) of 8.3 meq/100 g

3. Plant Potato, variety “Cilena”, representative for root crops



B. Study Design

Experimental conditions:

Two experiments were performed representing the intended application types (tuber treatment and in-furrow application). Each experiment was conducted with three seed potatoes (variety: Cilena) in a planting container with a surface area of 0.5 m². The plants were cultivated in the glass-roofed vegetation area of the test facility. The plants were grown similar to natural temperature and light conditions, but protected from rainfall. The plants were watered by pouring onto the soil in the planting containers.

Tuber treatment experiment:

The formulated test compound was applied to the seed potatoes at a target rate of 10 g a.s./dt. For this purpose, three seed potatoes were treated with a defined volume of the aqueous application suspension. The potatoes were placed in the furrows before application to prevent any loss of the application suspension. To ensure a regular treatment, the seed potatoes were treated on the upper side, allowed to dry, then turned and treated on the other side. The furrow was closed when the application suspension was dried on the seed potatoes. A total volume of 763.2 µL was applied, corresponding to 50.2 MBq or to 12.7 mg a.s.. The actual seed treatment rate was 10.0 g a.s./dt, corresponding to 254 g a.s./ha. The seed density was 25 dt/ha.

In-furrow treatment experiment:

For the in-furrow treatment experiment, a defined volume of the aqueous application solution was filled into the bottle of a hand pump sprayer. The application solution was sprayed onto the soil in the furrow. The empty pump sprayer was rinsed with 3 mL water, 0.5 mL of the rinse was sampled for radioactivity measurement and the rest of the rinse was sprayed onto the soil in the furrow. Three seed potatoes were placed into the furrow and the furrow was closed. The empty pump sprayer was again rinsed with 3 mL water. The remaining radioactivity in the rinsing solution was determined by LSC. The losses were subtracted from the initial amount of radioactivity. An amount of 123.3 MBq or 31.3 mg a.s. was applied to the three potatoes, corresponding to an actual application rate of 626 g a.s./ha. The seed density was 22 dt/ha.

Sampling:

Potato tubers were sampled as raw agricultural commodity (RAC) from the tuber treatment experiment and from the in-furrow treatment experiment at maturity of the potato plants (BBCH 97). The leaves and roots, as well as the remainders of the seed potatoes were sampled at the same time to determine the TRR by combustion analysis.

The potato tubers were allowed to dry at room temperature for one night. Adhering soil was removed. The potato tuber samples from the two experiments were each washed with 1 L of water and the radioactivity in the wash water was determined. The potato tubers were cut into small cubes. The cubes were randomized by shuffling and stored in a freezer ($\leq -18^{\circ}\text{C}$) in aliquots.

The leaves and roots as well as the remainders of the seed potatoes were cut in pieces, liquid nitrogen was added and the mixtures were homogenised using a high speed blender (Polytron PT6000). The powdered samples were stored in a freezer ($\leq -18^{\circ}\text{C}$).



C. Analytical Procedures

Extraction:

The cut potato tubers of the tuber treatment and the in-furrow treatment experiment were extracted three times with a mixture of acetonitrile/water (8:2, v/v) and one time with a mixture of acetonitrile/water (1:1, v/v) using a high speed blender (Polytron PT6000). The radioactivity in the extracts was determined by LSC and in the solids by combustion followed by LSC. The actual TRR value of the RAC was determined by summing up the radioactivity measured in the extracts and in the remaining solids. The first two extracts of each experiment were combined and subjected to a clean-up step using an SPE RP 18 cartridge (Phenomenex, Strata C18-E, 20 g), which was conditioned with acetonitrile beforehand. The flow-through fraction (percolate) was collected and the cartridge was rinsed with 100 mL of acetonitrile/water (8:2, v/v) and methanol/dichloromethane (1:1, v/v). The percolate and the acetonitrile/water fraction were combined and concentrated by rotary evaporation in vacuo for HPLC analysis.

The remaining solids after conventional extraction of the potato tubers in the in-furrow treatment experiment were subjected two times to exhaustive extraction with acetonitrile/water (1:1, v/v) under microwave assistance (120°C for 20 min.). After each extraction step, extracts and solids were separated by centrifugation. The radioactivity in the extracts was determined by LSC, in the solids by combustion followed by LSC. The two extracts were combined and concentrated by rotary evaporation in vacuo for HPLC analysis. In the tuber treatment experiment, the solids remaining after conventional extraction amounted to 0.026 mg/kg, only. Moreover, since the distribution of the radioactivity in the extracts and solids after conventional extraction was nearly identical within the in-furrow and the tuber treatment experiments and the conventional extracts showed a very similar metabolite pattern, exhaustive extraction of the solids of the tuber treatment experiment was not considered necessary.

Quantification:

Parent compound and metabolites in the extracts were analysed by reversed phase HPLC coupled to a radioactivity detector with a glass scintillator cell. The HPLC chromatograms (= metabolite profiles) were integrated for quantification of compounds.

Identification and characterisation:

Parent compound and metabolites were identified by co-chromatography with reference compounds and/or by comparison with the HPLC profiles obtained in the potato metabolism study with [pyridinylmethyl-¹⁴C]BYI 02960.

Storage stability:

The conventional extractions and the first HPLC analyses of the potato tuber extracts were performed not later than three months after harvest. The extracts were analysed after two and four days following the start of extraction. The solids after conventional extraction were extracted with microwave assistance and were analysed within five and a half months after harvest.

According to OECD Guidance for the Testing of Chemicals 501 (2007), storage stability data are not necessary for samples analysed within 6 months of collection. Therefore it was concluded that the data



in hand provide adequate evidence to show the stability of the compounds and no additional investigations deemed necessary.

In addition, the extract stability was demonstrated by comparing the HPLC chromatograms recorded at different time points during the study. The profiles did not change significantly during the whole course of the study. The stability of extracts was demonstrated for parent and metabolites for at least two months which covers the time period of all analytical investigations including identification and characterisation of metabolites.

II. Results and Discussion

The metabolism of [furanone-4-¹⁴C]BYI 02960 was investigated in potato tubers following two different soil application methods. In one experiment, tubers of seed potatoes were treated with [furanone-4-¹⁴C]BYI 02960 formulated as an FS 480 at an application rate of 10.0 g a.s./dt (= 254 g a.s./ha). In the other experiment [furanone-4-¹⁴C]BYI 02960, formulated as an SL 200, was sprayed in the furrow onto the soil prior to planting of the seed potatoes at an application rate of 626 g a.s./ha..

At harvest, the total radioactive residues (TRR) in potato tubers were low after tuber treatment and after in-furrow application and accounted for 0.078 mg/kg and 0.171 mg/kg, respectively. The TRR values in the remaining plant parts (leaves, roots and remainders of the seed potatoes) were considerably higher. These plant parts were only sampled for optional analysis to support identification of metabolites, if needed.

A major portion of radioactivity in the potato tubers was extracted conventionally by acetonitrile/water mixtures (67.0% to 69.0% of the TRR) as shown in Table 6.2.1-13. Additionally, 6.3% of the TRR was released after exhaustive extraction of the remaining solids with microwave assistance as shown for the in-furrow experiment.

Parent compound was the main residue in both extracts. Two additional minor metabolites (<10% of TRR and <0.01 mg/kg) were detected: BYI 02960-difluoroethyl-amino-furanone and BYI 02960-OH-glyc. The assignment of parent compound and metabolite BYI 02960-OH-glyc was based on the identification achieved in the potato study with [pyridinylmethyl-¹⁴C]BYI 02960. The HPLC profiles were compared and corresponding peaks were assigned. In the potato study with [pyridinylmethyl-¹⁴C]BYI 02960, all major metabolites were identified by co-chromatography with authentic reference compounds using two different chromatographic systems (HPLC and TLC), and minor metabolites were identified by HPLC co-chromatography. In the present study, the identification of parent compound (main constituent in the profile) was confirmed additionally by HPLC co-chromatography with an authentic reference compound. Co-chromatographic investigations were performed with the extracts obtained after tuber treatment and after in-furrow application.

The minor, label-specific metabolite BYI 02960-difluoroethyl-amino-furanone was identified by HPLC co-chromatography with an authentic reference compound in the tuber extract obtained after in-furrow application. In the profile of the tuber treatment experiment, the metabolite was assigned by comparison of the two profiles.

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

The TRR values and the distribution of the parent and metabolites is shown in Table 6.2.1-14. In total, 50.8% and 64.2% of the TRR were identified in potato tubers, respectively.

Table 6.2.1-13: Distribution of radioactivity in the extracts of the potato tubers after tuber treatment or in-furrow application of [furanone-4-¹⁴C]BYI 02960

	tuber treatment		in-furrow application	
	potato tubers		potato tubers	
TRR [mg/kg] =	0.078		0.171	
	% of TRR	mg/kg	% of TRR	mg/kg
Conventionally extracted	67.0	0.052	69.0	0.118
Extract for analysis	63.1	0.049	65.3	0.111
Losses (not analysed)	3.9	0.003	3.8	0.006
Microwave extraction	---	---	6.3	0.011
Extract for analysis	---	---	6.2	0.011
Losses (not analysed)	---	---	<0.1	<0.001
Total extracted	67.0	0.052	75.3	0.129
Unextractable (PES*)	33.0	0.026	24.7	0.042
Accountability	100.0	0.078	100.0	0.171

* post extraction solids

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.2.1-14: TRR values and distribution of parent compound and metabolites in potato tubers after tuber treatment and in-furrow application of [furanone-4-¹⁴C]BYI 02960

	tuber treatment		in-furrow application	
	potato tubers		potato tubers	
TRR [mg/kg] =	0.078		0.171	
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg
<i>Conventional extraction</i>				
BYI 02960 (parent compound)	40.0	0.031	50.6	0.086
difluoroethyl-amino-furanone	4.2	0.003	2.9	0.005
OH-glyc	6.6	0.005	4.4	0.007
Subtotal identified	50.8	0.039	57.9	0.099
unknown 1	7.2	0.006	5.5	0.009
unknown 2	1.7	0.001	---	---
unknown 3	2.4	0.002	1.9	0.003
unknown 4	1.1	0.001	---	---
Subtotal characterised	12.4	0.010	7.4	0.013
Conventional extracts not analysed	3.9	0.003	3.8	0.006
Total conventional extraction	67.0	0.052	69.0	0.118
<i>Microwave extraction</i>				
BYI 02960 (parent compound)	---	---	6.2	0.011
Subtotal identified	---	---	6.2	0.011
Microwave extracts not analysed	---	---	<0.1	<0.001
Total microwave extraction	---	---	6.3	0.011
Total identified	50.8	0.039	64.2	0.110
Total characterised	12.4	0.010	7.4	0.013
Analysed extract(s)	63.1	0.049	71.5	0.122
Extracts not analysed	3.9	0.003	3.8	0.006
Total extracted	67.0	0.052	75.3	0.129
Unextractable (PES*)	33.0	0.026	24.7	0.042
Accountability	100.0	0.078	100.0	0.171

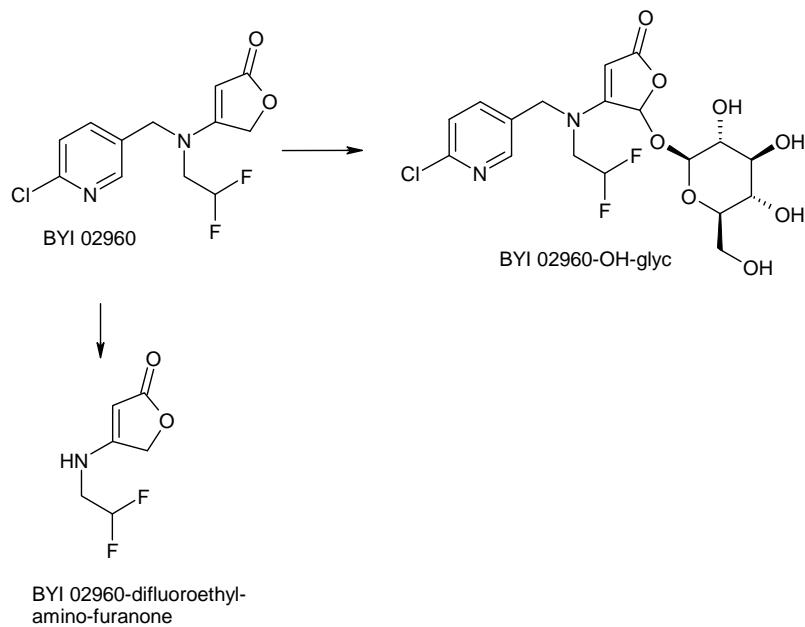
* post extraction solids

III. Conclusions

Based on the metabolites identified the following metabolic routes were deduced:

- hydroxylation of the methylene group of the furanone moiety followed by conjugation with glucose, and
- cleavage of the pyridinylmethylamine bond

Thus, [furanone-4-¹⁴C]BYI 02960 was metabolised moderately in potatoes. On the basis of the results of this study it is concluded that the metabolism of [furanone-4-¹⁴C]BYI 02960 in potatoes is well understood and the following metabolic pathway is proposed.

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)Figure 6.2.1-5: Proposed metabolic pathway of [furanone-4-¹⁴C]BYI 02960 in potatoes



Report:	KIIA 6.2.1/05, Justus, K.; 2011
Title:	Metabolism of [pyridinylmethyl- ¹⁴ C]BYI 02960 in potatoes
Report No & Edition No	MEF-10/710 <u>M-415078-01-2</u>
Guidelines:	OECD 501 Metabolism in Crops US EPA Residue Chemistry Test Guideline OPPTS 860.1300: Nature of the Residue – Plants, Livestock PMRA Regulatory Directive Dir98-02: Residue Chemistry Guidelines Section 2: Nature of the Residue – Plants, Livestock Japanese MAFF, 12 Nousan 8147 European Parliament and Council Regulation (EC) No 1107/2009
GLP	yes

Executive Summary

The metabolism of [pyridinylmethyl-¹⁴C]BYI 02960 in potatoes was investigated according to the maximum envisaged use pattern. Two different methods of application were covered in this study. In one experiment, the seed potato tubers were treated with [pyridinylmethyl-¹⁴C]BYI 02960 formulated as an FS 480 at an application rate of 10.0 g a.s./dt (= 270 g a.s./ha, seed density 27 dt/ha). In the other experiment, [pyridinylmethyl-¹⁴C]BYI 02960, formulated as an SL 200, was sprayed in the furrow onto the soil at an application rate of 626 g a.s./ha (seed density 28 dt/ha) prior to planting of the seed potatoes. The potato tubers were harvested at maturity in both experiments. Concurrently, the leaves and roots as well as the remainders of the seed potatoes were sampled. The TRR values determined are shown in the following table:

Table 6.2.1-15: TRR values in potato tubers and the remaining part of the plant after tuber treatment or in-furrow application of [pyridinylmethyl-¹⁴C]BYI 02960

Matrix	Timing and Application	PHI (days)*	ppm (mg a.s. equiv./kg)
potato tubers	tuber treatment at planting (BBCH 03), 10.0 g a.s./dt	97	0.076
potato leaves and roots		97	8.40
remainders of the seed potatoes		97	33.33
potato tubers	in-furrow application at planting (BBCH 03), 626 g a.s./ha	97	0.115
potato leaves and roots		97	12.44
remainders of the seed potatoes		97	6.91

* PHI: preharvest interval (corresponds to days after last treatment (DAT) at the start of harvest/sampling)

The potato tubers (edible raw agricultural commodity) were extracted conventionally. Four extraction steps with acetonitrile/water mixtures released 93.4% of the TRR in the tuber treatment experiment and 90.4% of the TRR in the in-furrow treatment experiment.

Parent compound and metabolites in the extracts of potato tubers were analysed by HPLC. Major compounds were identified by co-chromatography using two independent chromatographic systems (reversed phase HPLC and normal phase TLC). Minor metabolites were identified by HPLC co-chromatography or by HPLC comparison. Parent compound represented 40.2% and 44.1% of the TRR in potato tubers. Besides parent compound, only 6-CNA was detected as major compound. All other metabolites detected represented less than 10% of the TRR and accounted for less than 0.01 mg/kg.

**Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)**

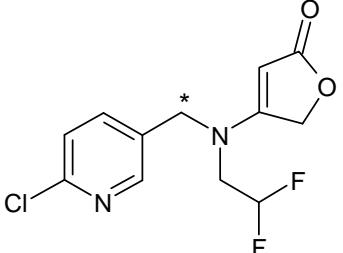
Nevertheless, five metabolites were identified in the tuber extract after in-furrow application and four thereof in the extract after tuber treatment.

[Pyridinylmethyl-¹⁴C]BYI 02960 was moderately metabolised in potatoes. The following metabolic routes were observed:

- hydroxylation of the methylene group of the furanone moiety followed by conjugation with glucose, and
- cleavage of the pyridinylmethylamine bond followed by conjugation with carbohydrates or by oxidation of the methylene group to a carboxylic group.

On the basis of these results, a metabolic pathway of [pyridinylmethyl-¹⁴C]BYI 02960 in potatoes can be proposed.

I. Materials and Methods**A. Materials****1. Test Material:**

Chemical structure		* position of the radiolabel
Radiolabelled test material	[pyridinylmethyl- ¹⁴ C]BYI 02960	
Specific radioactivity	4.37 MBq/mg (118.08 µCi/mg)	
Chemical Purity	> 99% (HPLC)	
Radiochemical purity	> 99% (HPLC and TLC)	

The supplied radiolabelled test compound [pyridinylmethyl-¹⁴C]BYI 02960 was dissolved in acetonitrile. Formulation of the test compound was performed prior to the application. For both formulations (FS 480 and SL 200), adequate parts of this stock solution were transferred into special glass vials and evaporated to dryness. For the preparation of the FS 480 formulation, the blank formulation was added to the test item and was homogenized using a ball mill. Afterwards the mixture was suspended in water by stirring or swirling. For the preparation of the SL 200 formulation, the liquid blank formulation was added to the test item using a magnetic stirrer. The mixture was diluted with water to get the aqueous application solution.

2. Soil: “Monheim 4” (sandy loam soil from Germany), pH (CaCl₂) = 6.8, 69% sand, 18% silt and 13% clay, 1.2% organic carbon, cation exchange capacity (CEC) of 8.3 meq/100 g,

3. Plant Potato, variety “Cilena”, representative for root crops



B. Study Design

Experimental conditions:

Two experiments were performed representing the intended application types (tuber treatment and in-furrow application). Each experiment was conducted with three seed potatoes (variety: Cilena) in a planting container with a surface area of 0.5 m². The plants were cultivated in the glass-roofed vegetation area of the test facility. The plants were grown similar to natural temperature and light conditions, but protected from rainfall. The plants were watered by pouring onto the soil in the planting containers.

Tuber treatment experiment:

The formulated test compound was applied to the seed potatoes at a target rate of 10 g a.s./dt. For this purpose, three seed potatoes were treated with a defined volume of the aqueous application suspension. The potatoes were placed in the furrows before application to prevent any loss of the application suspension. To ensure a regular treatment, the seed potatoes were treated on the upper side, allowed to dry, then turned and treated on the other side. The furrow was closed when the application suspension was dried on the seed potatoes. A total volume of 787.2 µL was applied, corresponding to 59.1 MBq or to 13.5 mg a.s.. The actual seed treatment rate was 10.0 g a.s./dt, corresponding to 270 g a.s./ha. The seed density was 27 dt/ha.

In-furrow treatment experiment:

For the in-furrow treatment experiment, a defined volume of the aqueous application solution was filled into the bottle of a hand pump sprayer. The application solution was sprayed onto the soil in the furrow. The empty pump sprayer was rinsed with 3 mL water, 0.5 mL of the rinse was sampled for radioactivity measurement and the rest of the rinse was sprayed onto the soil in the furrow. Three seed potatoes were placed into the furrow and the furrow was closed. The empty pump sprayer was again rinsed with 3 mL water. The remaining radioactivity in the rinsing solution was determined by LSC. The losses were subtracted from the initial amount of radioactivity. An amount of 136.6 MBq or 31.3 mg a.s. was applied to the three potatoes, corresponding to an actual application rate of 626 g a.s./ha. The seed density was 28 dt/ha.

Sampling:

Potato tubers were sampled as raw agricultural commodity (RAC) from the tuber treatment experiment and from the in-furrow treatment experiment at maturity of the potato plants (BBCH 97). The leaves and roots, as well as the remainders of the seed potatoes were sampled at the same time to determine the TRR by combustion analysis.

The potato tubers were allowed to dry at room temperature for one night. Adhering soil was removed. The potato tuber samples from the two experiments were each washed with 1 L of water and the radioactivity in the wash water was determined. The potato tubers were cut into small cubes. The cubes were randomized by shuffling and stored in a freezer ($\leq -18^{\circ}\text{C}$) in aliquots.

The leaves and roots as well as the remainders of the seed potatoes were cut in pieces, liquid nitrogen was added and the mixtures were homogenised using a high speed blender (Polytron PT6000). The powdered samples were stored in a freezer ($\leq -18^{\circ}\text{C}$).



C. Analytical Procedures

Extraction:

The cut potato tubers of the tuber treatment and the in-furrow treatment experiment were extracted three times with a mixture of acetonitrile/water (8:2, v/v) and one time with a mixture of acetonitrile/water (1:1, v/v) using a high speed blender (Polytron PT6000). The radioactivity in the extracts was determined by LSC, in the solids by combustion followed by LSC. The actual TRR value of the RAC was determined by summing up the radioactivity measured in the extracts and in the remaining solids. The first two extracts were combined and subjected to a clean-up step using an SPE RP 18 cartridge (Phenomenex, Strata C18-E, 20 g), which was conditioned with acetonitrile beforehand. The flow-through fraction (percolate) was collected and the cartridge was rinsed with 100 mL of acetonitrile/water (8:2, v/v) and methanol/dichloromethane (1:1, v/v). The percolate and the acetonitrile/water fraction were combined and concentrated by rotary evaporation in vacuo for HPLC analysis.

Quantification:

Parent compound and metabolites in the extracts were analysed by reversed phase HPLC coupled to a radioactivity detector with a glass scintillator cell. The HPLC chromatograms (= metabolite profiles) were integrated for quantification of compounds.

Identification and characterisation:

Major compounds (parent compound and one metabolite) were identified using two independent chromatographic systems (reversed phase HPLC and normal phase TLC). Minor metabolites (<10% of the TRR and representing <0.01 mg/kg) were identified by HPLC co-chromatography with authentic reference compounds or by HPLC comparison.

The metabolite profiles of the potato tubers of the tuber treatment and the in-furrow application experiments were very similar. Thus, identification of metabolites was performed either in the extract of the in-furrow application experiment or in the tuber treatment experiment. Assignment of the according metabolite in the complementary experiment was completed by comparing the profiles.

Storage stability:

All extraction experiments and the first HPLC analyses were performed within three months after harvest of the potato tubers. The extracts were analysed after two and five days following the start of extraction. According to OECD Guidance for the Testing of Chemicals 501 (2007), storage stability data are not necessary for samples analysed within 6 months of collection. Therefore it was concluded that the data in hand provide adequate evidence to show the stability of the compounds and no additional investigations deemed necessary.

In addition, the extract stability was demonstrated by comparing the HPLC chromatograms recorded at different times during the study. The profiles did not change significantly during the whole course of the study. The stability of extracts was demonstrated for parent and metabolites for at least three months which covers the time period of all analytical investigations including identification and characterisation of metabolites.



II. Results and Discussion

The metabolism of [pyridinylmethyl-¹⁴C]BYI 02960 was investigated in potato tubers following two different soil application methods. In one experiment, tubers of seed potatoes were treated with [pyridinylmethyl-¹⁴C]BYI 02960 formulated as an FS 480 at an application rate of 10.0 g a.s./dt (= 270 g a.s./ha). In the other experiment [pyridinylmethyl¹⁴C]BYI 02960, formulated as an SL 200, was sprayed in the furrow onto the soil prior to planting of the seed potatoes at an application rate of 626 g a.s./ha.

At harvest, the total radioactive residues (TRR) in potato tubers were low after tuber treatment and after in-furrow application and accounted for 0.076 mg/kg and 0.115 mg/kg, respectively. The TRR values in the remaining plant parts (leaves, roots and remainders of the seed potatoes) were considerably higher. These plant parts were only sampled for optional analysis to support identification of metabolites, if needed.

A high portion of radioactivity in the potato tubers was extracted conventionally by acetonitrile/water mixtures (93.4% to 90.4% of the TRR) as shown in Table 6.2.1-16. HPLC analysis of the extracts revealed that the metabolite profiles of the potato tubers after tuber treatment and in-furrow application were nearly identical – the metabolite patterns showed no significant difference, and even the proportions of the compounds were nearly identical. Major components detected in the HPLC profiles (>10% of the TRR and >0.01 mg/kg) were parent compound and the metabolite BYI 02960-6-CNA. Both components were identified by co-chromatography with two different chromatographic systems. They were identified in the tuber extract of each experiment by reversed phase HPLC and in the tuber extracts of the in-furrow treatment experiment by normal phase TLC using radiolabelled reference compounds. The minor, label-specific metabolites BYI 02960-CHMP-glyc and BYI 02960-CHMP were identified by HPLC co-chromatography with non-radiolabelled reference compounds. The minor, label-specific metabolites BYI 02960-6-CNA-glycerol-gluA and BYI 02960-CHMP-di-glyc were identified by HPLC comparison. The HPLC profiles were compared to that of wheat straw of the 1st rotation of the confined rotational crops study and to that of tomato fruits of the tomato metabolism study. The metabolites had been identified with spectroscopic methods in these studies.

The minor metabolite BYI 02960-OH-glyc was identified by HPLC co-chromatography after semi-preparative isolation. Co-chromatography was performed using an acidic reversed phase HPLC method to ensure separation from BYI 02960-acetic acid, a metabolite co-eluting with BYI 02960-OH-glyc using the profiling method, as shown in the apple metabolism and the confined rotational crop studies. The configuration of the conjugated hexose of BYI 02960-OH-glyc was identified unambiguously as D-glucose in the apple metabolism study conducted with [pyridinylmethyl-¹⁴C]BYI 02960.

The TRR and the distribution of parent compound and metabolites in the extracts is shown in Table 6.2.1-17. In total, 80.5% and 80.9% of the TRR were identified in the potato tubers after tuber treatment or in-furrow application, respectively.

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)Table 6.2.1-16: Distribution of radioactivity in the extracts of the potato tubers after tuber treatment or in-furrow application of [pyridinylmethyl-¹⁴C]BYI 02960

	tuber treatment		in-furrow application	
	potato tubers		potato tubers	
TRR [mg/kg] =	0.076		0.115	
	% of TRR	mg/kg	% of TRR	mg/kg
Conventionally extracted	93.4	0.071	90.4	0.104
Extract for analysis	89.8	0.068	86.7	0.100
Losses (not analysed)	3.7	0.003	3.6	0.004
Total extracted	93.4	0.071	90.4	0.104
Unextractable (PES*)	6.6	0.005	9.6	0.011
Accountability	100.0	0.076	100.0	0.115

* post extraction solids

Table 6.2.1-17: TRR values and distribution of parent compound and metabolites in potato tubers after tuber treatment and in-furrow application of [pyridinylmethyl-¹⁴C]BYI 02960

	tuber treatment		in-furrow application	
	potato tubers		potato tubers	
TRR [mg/kg] =	0.076		0.115	
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg
<i>Conventional extraction</i>				
BYI 02960 (parent compound)	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
OH-glyc	6.7	0.005	4.7	0.005
Total identified	80.5	0.061	80.9	0.093
unknown 1	1.9	0.001	---	---
unknown 2	2.3	0.002	2.8	0.003
unknown 3	5.1	0.004	3.0	0.003
Total characterised	9.3	0.007	5.8	0.007
Analysed extract(s)	89.8	0.068	86.7	0.100
Extracts not analysed	3.7	0.003	3.6	0.004
Total extracted	93.4	0.071	90.4	0.104
Unextractable (PES*)	6.6	0.005	9.6	0.011
Accountability	100.0	0.076	100.0	0.115

* post extraction solids

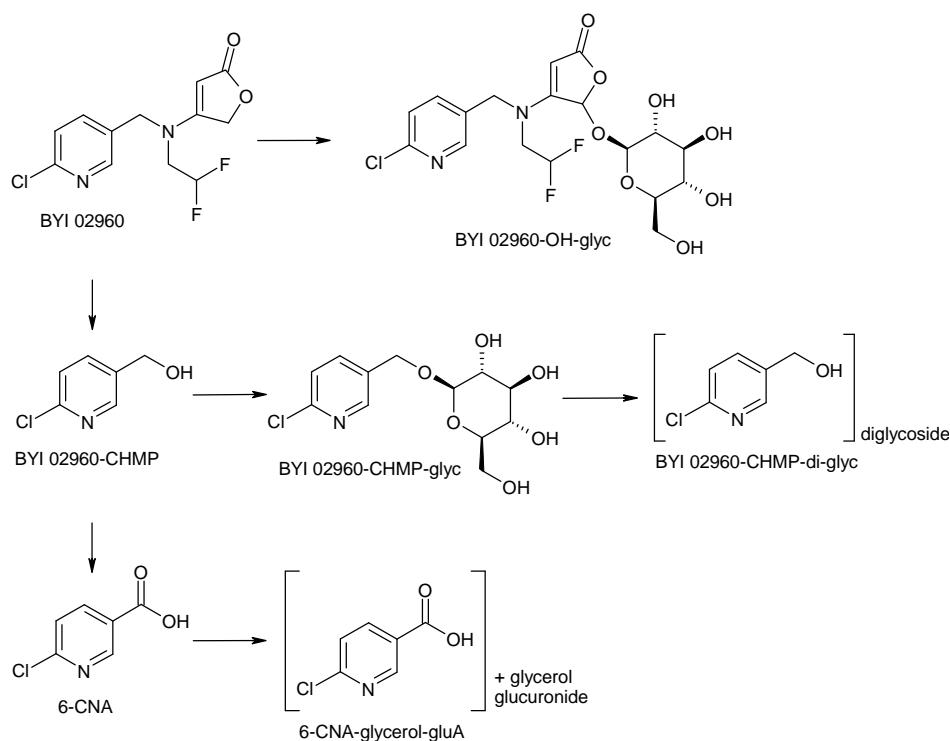
III. Conclusions

[Pyridinylmethyl-¹⁴C]BYI 02960 was moderately metabolised in potatoes. Two metabolic routes were observed:

- cleavage of the pyridinylmethylamine bond followed by conjugation with carbohydrates or by oxidation of the methylene group to a carboxylic group and conjugation with carbohydrates or derivatives, which were the major metabolic routes, and
- hydroxylation of the methylene group of the furanone moiety followed by conjugation with glucose, which was the minor metabolic route.

On the basis of the results of this study it is concluded that the metabolism of [pyridinylmethyl-¹⁴C]BYI 02960 in potatoes is well understood and the following metabolic pathway is proposed.

Figure 6.2.1-6: Proposed metabolic pathway of [pyridinylmethyl-¹⁴C]BYI 02960 in potatoes



Overall Conclusions Potato (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-4-¹⁴C]BYI 02960 or (2) [pyridinylmethyl-¹⁴C]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 cannot be detected with the radiolabels used - revealed even higher concentrations of this metabolite compared to the parent compound.

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

(1) Label 1: [furanone-4-¹⁴C]BYI 02960

Besides parent compound, six minor metabolites were detected, two of them were identified: BYI 02960-OH-glyc, a metabolite common to both radiolabels tested and BYI 02960-difluoroethyl-aminofuranone, a metabolite specific to the furanone-label. The metabolites identified were also detected in tomato fruits after soil application and in the confined rotational crop studies.

BYI 02960 was metabolised moderately in potatoes: Hydroxylation of the methylene group of the furanone moiety was observed followed by conjugation with glucose and cleavage of the pyridinylmethylamine bond. Cleavage of the molecule was also confirmed in the potato study performed with [pyridinylmethyl-¹⁴C]BYI 02960 illustrating that the results of the metabolism studies were in good conformity.

(2) Label 2: [pyridinylmethyl-¹⁴C]BYI 02960

Only one major metabolite (6-CNA) was detected besides parent compound. Additionally, eight minor metabolites were detected, five thereof were identified: BYI 02960-OH-glyc, a metabolite common to both radiolabels tested, and the label-specific metabolites BYI 02960-CHMP, BYI 02960-CHMP-glyc, BYI 02960-CHMP-di-glyc and 6-CNA-glycerol-gluA. These metabolites or at least the aglycons were also detected in tomato fruits after soil application indicating the same metabolic degradation paths.

The major metabolic routes in this study were cleavage of the pyridinylmethylamine bond followed by conjugation with carbohydrates or by oxidation of the methylene group to a carboxylic group.

Cleavage of the molecule was also confirmed in the potato study performed with [furanone-4-¹⁴C]BYI 02960. A minor metabolic route was hydroxylation of the methylene group of the furanone moiety followed by conjugation with glucose. This metabolic route resulted in the non-label specific metabolite BYI 02960-OH-glyc, which was identified in nearly identical concentrations in the potato study performed with [furanone-4-¹⁴C]BYI 02960. Thus, the results of the present metabolism study in potatoes are in good conformity with the results of the corresponding study performed with [furanone-4-¹⁴C]BYI 02960 and as well with all metabolism studies being representative for soil application.

When considering the results from both metabolism studies conducted on potato, it can be concluded that BYI02960 is moderately metabolised in this crop. A total of 2 major and 10 minor metabolites were found, and all major and 6 minor have been identified. The distribution of parent compound and metabolites in the edible commodity potato tuber is summarized in Table 6.2.1-18.

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.2.1-18: TRR values and distribution of parent compound and metabolites in potato tuber after tuber treatment and in-furrow application of BYI 02960

Radiolabel	Potato tuber							
	[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
<i>6-CNA</i>					21.5	0.016	18.4	0.021
<i>6-CNA-glycerol-gluA</i>					---	---	2.3	0.003
<i>CHMP-di-glyc</i>					4.4	0.003	5.3	0.006
<i>CHMP-glyc</i>					3.7	0.003	2.4	0.003
<i>CHMP</i>					3.9	0.003	3.9	0.004
<i>difluoroethyl-amino-furanone</i>	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	0.11	80.5	0.061	80.9	0.093
Total characterised	12.4	0.01	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.

In order to gain information on the fate of the difluoroethane moiety of BYI 02960, the tuber extracts obtained in the potato metabolism study with [pyridinylmethyl-¹⁴C]BYI 02960 were additionally analysed for non-radiolabelled difluoroacetic acid by LC-MS/MS according to residue method 01304 (see KIIA 6.2./12). High levels of this metabolite were found. After tuber treatment and in-furrow application, difluoroacetic acid accounted for 0.39 mg a.s. equiv/kg and 0.54 mg a.s. equiv/kg in potato tubers. These concentrations are by a factor of approx. 10 higher compared to those of the parent compound indicating that difluoroacetic acid is the main residue in potato tubers. Since it is known that BYI 02960 degrades to difluoroacetic acid in soil, the high concentration of difluoroacetic acid is probably the result of both uptake of this metabolite from the soil and degradation of the parent compound BYI 02960 in the plant.

On basis of the metabolites identified, biotransformation of BYI 02960 in potato proceeds by the following pathways:

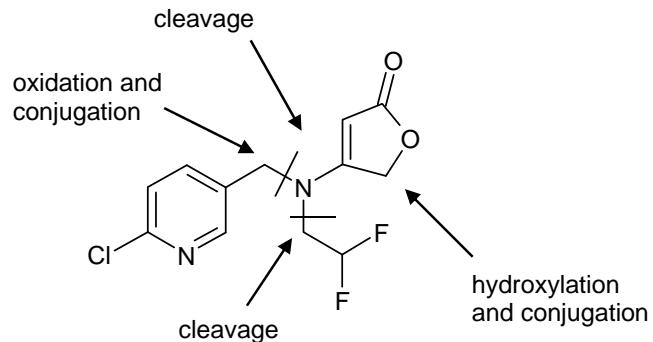
- oxidative cleavage of the difluoroethylamine bond and formation of difluoroacetic acid
- cleavage of the pyridinylmethylamine bond and formation of BYI 02960-difluoroethylaminofuranone and its corresponding counterpart BYI 02960-CHMP, which was either conjugated with carbohydrates or oxidised to 6-chloronicotinic acid (6-CNA), which was conjugated as well
- hydroxylation of the furanone moiety followed by conjugation with glucose



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

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

Figure 6.2.1-7: Positions involved in metabolic degradation of BYI 02960 in potato tubers



Metabolism, distribution and expression of residues in apple (foliar application)

Report:	KIIA 6.2.1/06, Justus, K.; 2011
Title:	Metabolism of [furanone-4- ¹⁴ C]BYI 02960 in apples
Report No & Edition No	MEF-11/499 M-422562-01-1
Guidelines:	OECD 501 Metabolism in Crops US EPA Residue Chemistry Test Guideline OPPTS 860.1300: Nature of the Residue – Plants, Livestock PMRA Regulatory Directive Dir98-02: Residue Chemistry Guidelines Section 2: Nature of the Residue – Plants, Livestock Japanese MAFF, 12 Nousan 8147 European Parliament and Council Regulation (EC) No 1107/2009
GLP	yes

Executive Summary

The metabolism of [furanone-¹⁴-4-C]BYI 02960 formulated as an SL 200 was investigated in apples after foliar spray application. In one experiment (single application experiment), two apple trees were treated at an actual application rate of 86 g a.s. per hectare and per meter canopy height (86 g a.s./ha x m CH) or also referred to 86 g a.s./ha/m CH) at the end of flowering (BBCH 69). In another experiment, one apple tree was treated twice with 86 g a.s./ha x m CH), one time at the end of flowering (BBCH 69) and a second time at 14 days before harvest.

At maturity the apple fruits were harvested. Concurrently, the leaves were sampled to support metabolism investigation. The TRR values are shown in the following table:

Table 6.2.1-19: TRR values in apple fruits and leaves after foliar application of furanone-4-¹⁴C]BYI 02960

Matrix	Timing and Application	PHI (days)*	ppm (mg a.s. equiv./kg)
apple fruits	one foliar spray application at BBCH 69,	98	0.280
apple leaves	86 g a.s./(ha x m CH)	98	38.957
apple fruits ¹	two foliar spray applications, at BBCH 69	14	1.133
apple fruits ²	and 14 days PHI,	14	1.286
apple leaves	2 x 86 g a.s./(ha x m CH)	14	102.919

* PHI: preharvest interval (corresponds to days after last treatment (DAT) at the start of harvest/sampling)

¹ determined from the extraction experiment with prior surface wash

² determined from the extraction experiment without prior surface wash

The apple fruits and leaves were extracted with acetonitrile/water mixtures. The remaining solids after conventional extraction of apple fruits were additionally submitted to exhaustive extraction with microwave assistance and/or to enzymatic treatment with cellulase. In total, 86.5% to 96.5% of the TRR was extracted from the apple matrices. The apple fruits of the double application experiment were additionally surface-washed with dichloromethane and then extracted with acetonitrile/water mixtures. A portion of 7.5% of the TRR was removed by the surface wash, another portion of 81.9% was extracted conventionally and 3.9% of the TRR was extracted after treatment with cellulase.



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Parent compound and metabolites in the extracts of apple fruits and leaves were analysed by HPLC. Identification was performed by comparing the HPLC profiles with those obtained in the apple metabolism study with [pyridinylmethyl-¹⁴C]BYI 02960, or by HPLC co-chromatography with authentic reference compounds. In total, 79.9% to 92.7% of the TRR was identified in apple fruits and leaves. As expected, the residues were dominated by parent compound in the double application experiment due to the late second application. However, irrespective of the amounts, the vast majority of metabolites was identical in the single and the double application experiments.

Glucose, or an isomeric carbohydrate, was the only major metabolite (> 10% of the TRR) in apple fruits, occurring at up to 71.7% of the TRR. The metabolite BYI 02960-OH-glyc was the only major metabolite in apple leaves, occurring at 36.1% and 17.3% of the TRR. All other metabolites were minor. Four basic metabolic routes were detected:

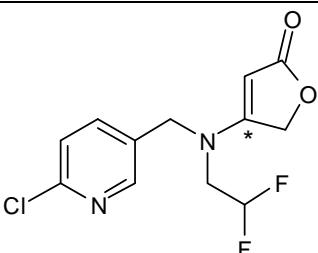
- hydroxylation of the furanone or the difluoroethyl moiety followed by conjugation,
- complete degradation of the furanone moiety and incorporation of carbon atoms into the natural compound pool, i.e. into glucose/carbohydrates,
- cleavage of the pyridinylmethylamine bond, and
- oxidative degradation of the furanone moiety to an acetic acid group followed by conjugation with a carbohydrate.

On the basis of these results, a metabolic pathway of [furanone-4-¹⁴C]BYI 02960 in apples can be proposed.

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	 * position of the radiolabel
Radiolabelled test material	[furanone- ¹⁴ C]BYI 02960
Specific radioactivity (before radiodilution) (after radiodilution)	3.94 MBq/mg (106.46 µCi/mg) 1.97 MBq/mg (53.24 µCi/mg)
Chemical Purity	> 99% (HPLC)
Radiochemical purity	> 99% (HPLC and TLC)

The test compound was formulated as an SL 200. In order to prepare an appropriate stock solution, the supplied radiolabelled test compound [furanone-4-¹⁴C]BYI 02960 was dissolved in acetonitrile. Radiodilution and formulation of the test compound was performed prior to the first and the second application: Therefore, an adequate portion of the non-radiolabelled test compound was dissolved in an adequate volume of the stock solution of the radiolabelled test compound. The solution was evaporated to dryness. The remainder was dissolved in an adequate amount of SL 200 blank



formulation using a magnetic stirrer. Preparation of each application solution was done by dissolving the formulation in water. The specific activity of each application solution was 1.97 MBq/mg (53.24 µCi/mg).

2. Soil: "Monheim 4" (sandy loam soil from Germany), pH (CaCl₂) = 6.8, 69% sand, 18% silt and 13% clay, 1.2% organic carbon, cation exchange capacity (CEC) of 8.3 meq/100 g,

3. Plant Apple, variety "James Grieve", representative for fruiting crops

B. Study Design

Experimental conditions:

Two experiments were performed with a total of three apple trees of the variety "James Grieve". The experiments represented the intended foliar spray application scenarios of BYI 02960 in orchards. In one experiment (single foliar spray application), the apple trees were treated at a target application rate of 75 g a.s./ (ha x m CH) (canopy height) at the end of flowering (BBCH 69). In the other experiment (double foliar spray application), one apple tree was treated two times. The target application rate was 75 g a.s./ (ha x m CH) at each application. The first application was conducted at the end of flowering (BBCH 69) and the second at 14 days before harvest. The total target rate corresponds to the anticipated maximum application rate. The apple trees used in the present study had a canopy height (CH) of 0.5 m and were cultivated in the sandy loam soil "Monheim 4" in a planting container with a surface area of 0.091 m². The plants were grown in the glass-roofed vegetation area of the test facility which allows cultivation similar to natural temperature and light conditions, but protected from rainfall. The plants were watered according to their needs.

Single foliar spray application (BBCH 69):

Two apple trees with a canopy height of 0.5 m were treated each with 10 mL of the aqueous application solution using a hand pump sprayer. Prior to the application, each apple tree was covered with a protective plastic wrap to prevent radioactivity spreading into the vegetation area. After application, the hand pump sprayer was rinsed with water and acetonitrile and the plastic wrap was rinsed with methanol. The radioactivity in the rinse solutions was determined and subtracted from the amount in the application solution. A total amount of 28.2 MBq was applied per tree, corresponding to 14.3 mg a.s. From a planting density of 3000 trees/ha an actual application rate of 86 g a.s./ (ha x m CH) was calculated.

Double foliar spray application (BBCH 69 & 14 days before harvest):

One apple tree with a canopy height of 0.5 m was treated for the first time with 10 mL of the aqueous application solution using a hand pump sprayer at the end of flowering (BBCH 69). A second application was performed at 14 days before harvest. Again, 10 mL of the aqueous application solution was applied. As described above, the hand pump sprayer and the protective plastic wrap were rinsed to determine the losses of radioactivity due to the application. A total amount of 28.2 MBq was applied at the first application and 28.1 MBq were applied at the second application. Thus 14.3 mg a.s. were applied at each treatment. From a planting density of 3000 trees/ha a total application rate (comprising both applications) of 172 g a.s./ (ha x m CH) was calculated.

**Sampling:**

Apple fruits of both experiments were sampled at maturity (BBCH 87- 89). On the same day, the leaves were cut off the trees with scissors. The leaves were stored in a freezer ($\leq -18^{\circ}\text{C}$) on the day of harvest until extraction.

All apple fruits of the single application experiment were cut in pieces. The pieces were mixed and divided into aliquots. The apple aliquots were stored in a freezer ($\leq -18^{\circ}\text{C}$) until needed for extraction. The apple fruits of the double application experiment were separated into two subsamples. Approx. one third of the whole apple fruit sample was stored in a freezer ($\leq -18^{\circ}\text{C}$) on the day of harvest. The remaining whole fruits were stored overnight at room temperature. On the next day the apples were subjected to a surface wash with dichloromethane.

C. Analytical Procedures**Extraction:**Single application experiment (apple fruits):

The apple pieces were extracted three times with a mixture of acetonitrile/water (8:2, v/v) and one time with a mixture of acetonitrile/water (1:1, v/v) using a high speed blender. The radioactivity in the extracts was determined by LSC, in the solids by combustion followed by LSC. The actual TRR value of the RAC was determined by summing up the radioactivity measured in the extracts and in the remaining solids. All extracts were combined and subjected to a clean-up step using an SPE RP 18 cartridge (Phenomenex, Strata C18-E, 20 g), which was conditioned with acetonitrile beforehand. The flow-through fraction (percolate) was collected and the cartridge was rinsed with acetonitrile/water (8:2, v/v) and methanol/dichloromethane (1:1, v/v). The percolate and the acetonitrile/water fraction were combined and concentrated by rotary evaporation in vacuo. The pH value of the concentrate was adjusted to pH 7 with ammonium carbonate before chromatographic analysis by HPLC. The solids remaining after the conventional extraction steps were submitted two times to a microwave-assisted extraction step with acetonitrile/water (1:1, v/v). After each extraction step, extracts and solids were separated by centrifugation. The extracts were combined and concentrated by rotary evaporation in vacuo. Prior to HPLC analysis, an aliquot of the extract was treated with cellulase to reduce viscosity. Additionally, the solids remaining after microwave-assisted extraction were suspended in water and the mixture was concentrated by rotary evaporation in vacuo to remove acetonitrile. Cellulase was added and the mixture was stirred at room temperature for 3 days. The aqueous phase was separated by centrifugation and adjusted to pH 7 with ammonium carbonate for HPLC analysis.

Double application experiment (apple fruits):

The subsample of the whole apple fruits which was stored at room temperature was subjected to a surface wash with dichloromethane. An aliquot of the dichloromethane surface wash was dispersed with 5 mL of water by ultrasonic treatment. The dichloromethane was removed by a nitrogen stream and the remaining concentrate was analysed by HPLC. The washed apples were cut into pieces. The apples pieces were mixed, divided into aliquots and stored in a freezer ($\leq -18^{\circ}\text{C}$). Extraction, purification and concentration of the extract were performed exactly as described for the single application experiment. The resulting sample was adjusted to pH 7 with ammonium carbonate for HPLC analysis.



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Additionally, three whole apples of the double application experiment were extracted without prior surface wash. The frozen apples were crushed with a wooden hammer and homogenised under liquid nitrogen using a high speed blender. An aliquot of the apple mush was extracted for final analysis. Extraction, purification and concentration of the extract were performed exactly as described for the single application experiment. The resulting sample was adjusted to pH 7 with ammonium carbonate for HPLC analysis.

Aliquots of the apple leaves of the single and double application experiment were conventionally extracted as described for the fruits of the single application experiment. The clean-up procedure and the concentration step were also identical with the one applied for the fruit samples. The resulting sample was adjusted to pH 7 with ammonium carbonate for HPLC analysis.

Quantification:

Parent compound and metabolites in the extracts were analysed by reversed phase HPLC coupled to a radioactivity detector with a glass scintillator cell. The HPLC chromatograms (= metabolite profiles) were integrated for quantification of compounds.

Identification and characterisation:

Identification of parent compound and metabolites common to both radiolabels was based on the metabolite profiles obtained in the apple metabolism study with [pyridinylmethyl-¹⁴C]BYI 02960. In this study parent compound and all metabolites common to both radiolabels were identified by two independent chromatographic methods (reversed phase HPLC and normal phase TLC) or by LC-MS/MS after semi-preparative isolation of the compounds. Thus assignment of compounds in the present study was possible by comparing the metabolite profiles.

The only major label-specific metabolite present in the current study was identified by TLC co-chromatography before and after derivatisation, whereas a minor label-specific metabolite was identified by HPLC co-chromatography with an authentic reference compound.

Storage stability:

All extraction and surface wash experiments and the first HPLC analyses of the apple fruits were performed within 4 months after harvest. The extraction experiments and the first HPLC analyses of the apple leaves were also performed within 4 months after harvest. Thus, no additional tests are necessary to prove the stability of the relevant residues in frozen matrices. The extracts were analysed after 1 to 6 days following start of extraction and the surface wash were analysed 14 days after washing.

Comparison of the HPLC chromatograms recorded at different times during the study showed that the profiles of the extracts did not change significantly during the analytical work up, which did not exceed approx. nine months.

It was therefore concluded, that the residues in the matrices and in the extracts were sufficiently stable during the experimental period of the study and that the chromatograms represented the metabolic pattern in the samples at harvest.



II. Results and Discussion

The metabolism of [furanone-4-¹⁴C]BYI 02960 was investigated in apple fruits and leaves following two different spray scenarios. In one experiment (single application experiment), two apple trees were treated at an actual application rate of 86 g a.s./ha x m CH) (CH = meter canopy height) at the end of flowering (BBCH 69). In the other experiment, one apple tree was treated two times with an actual application rate of 86 g a.s./ha x m CH), one time at the end of flowering (BBCH 69) and a second time at 14 days before harvest. The total application rate in the double application experiment was 172 g a.s./ha x m CH). The actual single application rates were slightly above the anticipated maximum rate of 75 g a.s./ha x m CH).

Apple fruits were harvested for analysis from the single application experiment at 98 days after the treatment and from the double application experiment at 14 days after the last treatment. Concurrently, the leaves were sampled to support the analysis of metabolites. The apple fruit samples of the double application experiment were investigated with and without a surface wash step.

The TRR level in the apple fruits, which represent the edible RAC, was 0.280 mg/kg in the single application experiment. In the double application experiment, the apple fruits had a TRR of 1.133 mg/kg (extraction including surface wash) and 1.286 mg/kg (extraction without surface wash). The TRR values of the apple leaves were high: 38.957 mg/kg were found in the leaves of the single application experiment and 102.919 mg/kg in the leaves of the double application experiment. Apple leaves were only sampled to support the identification of metabolites.

The radioactive residues were efficiently extracted with acetonitrile/water mixtures after conventional and exhaustive extraction procedures (86.5% to 96.5% of the TRR) as shown in Table 6.2.1-20 and Table 6.2.1-21. The apple fruits of the double application experiment were additionally surface-washed with dichloromethane and then extracted conventionally with acetonitrile/water mixtures. A portion of 7.5% of the TRR was removed by the surface wash and another portion of 81.9% was extracted conventionally indicating a good uptake of the product and its systemic behaviour. HPLC analysis of the surface wash and the conventional extracts of apple fruits and leaves after both spray scenarios revealed that all metabolite profiles were very well comparable with those of the study conducted with [pyridinylmethyl-¹⁴C]BYI 02960. Thus, identification was performed generally by comparison of profiles.

The main compound in apple fruits of the single application experiment was the natural compound glucose (or a corresponding isomeric carbohydrate). It was also a major component in the in apple fruits of the double application experiment. It was isolated by semi-preparative HPLC and identified by TLC co-chromatography before and after derivatisation. ¹⁴C-glucose and ¹⁴C-pentabenzoyl-D-glucose were used as authentic reference compounds. While correspondence with these reference compounds had clearly been shown, the methods were not considered selective enough to discriminate the configuration of the sugar moiety. Therefore, this label-specific fraction has been assigned more generally as glucose/carbohydrates.

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As expected, parent compound was the main component in the extracts of apple fruits and leaves of the double application experiment. In the surface wash, parent was by far the predominating component, as well. The high amount of parent compound was due to the fact that the product was sprayed on the developed fruits at 14 days before harvest. Assignment of parent compound was confirmed in the extracts and in the surface wash of apple fruits by HPLC co-chromatography.

All other metabolites detected in the raw agricultural commodity fruit were minor metabolites. They did not exceed 10% of the TRR in the single and the double application experiment and were assigned by comparison of profiles, if possible. Metabolites common to both radiolabels tested as BYI 02960-acetic acid-glyc, BYI 02960-OH-glyc, BYI 02960-acetic acid, BYI 02960-difluoroethyl-OH-glyc and BYI 02960-OH had been assigned by this means. The two metabolites BYI 02960-OH-glyc and BYI 02960-acetic acid co-eluted in one peak if analysed with the profiling method. Chromatographic separation has been achieved with an acidic reversed phase HPLC method in the apple metabolism study conducted with [pyridinylmethyl-¹⁴C]-BYI 02960. The ratios which had been determined for the two metabolites in the different matrices were transferred to the profiles of the current study.

The label-specific metabolite BYI 02960-difluoroethyl-amino-furanone was identified in the extract of apple fruits of the double application experiment by HPLC co-chromatography with a non-radiolabelled reference compound. BYI 02960-difluoroethyl-amino-furanone and glucose/carbohydrates were assigned in the other extracts of the apple matrices by comparison of the HPLC profiles.

The distribution of the radioactive residues in the extracts is shown in Table 6.2.1-20 and Table 6.2.1-21. In total, 83.4% to 92.7% of the TRR were identified in the apple fruits and 79.9% to 87.5% in apple leaves, respectively as summarised in Table 6.2.1-22 and Table 6.2.1-23.

Table 6.2.1-20: Distribution of radioactivity in the extracts of apple fruits and leaves after a single foliar application of [furanone-4-¹⁴C]BYI 02960

TRR [mg/kg] =	single application experiment			
	apple fruits		apple leaves	
	% of TRR	mg/kg	% of TRR	mg/kg
Conventionally extracted	59.8	0.168	94.3	36.725
Extract for analysis	58.7	0.164	94.0	36.604
Losses (not analysed)	1.1	0.003	0.3	0.121
Microwave extraction	9.5	0.027	---	---
Extract for analysis	9.4	0.026	---	---
Losses (not analysed)	<0.1	<0.001	---	---
Cellulase extract	17.2	0.048	---	---
Total extracted	86.5	0.242	94.3	36.725
Unextractable (PES*)	13.5	0.038	5.7	2.232
Accountability	100.0	0.280	100.0	38.957

* post extraction solids

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Table 6.2.1-21: Distribution of radioactivity in the surface wash and the extracts of apple fruits and leaves after a double foliar application of [furanone-4-¹⁴C]BYI 02960

	double application experiment					
	apple fruits incl. surface wash		apple fruits without surface wash		apple leaves	
	TRR [mg/kg] =	1.133		1.286		102.919
	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
<i>Surface wash with DCM</i>	7.5	0.085	---	---	---	---
<i>Conventionally extracted</i>	81.9	0.928	91.9	1.182	96.5	99.280
Extract for analysis	81.6	0.925	91.2	1.173	96.0	98.843
Losses (not analysed)	0.3	0.003	0.7	0.009	0.4	0.436
<i>Cellulase extract</i>	3.9	0.044	---	---	---	---
Total extracted	93.3	1.057	91.1	1.182	96.5	99.280
Unextractable (PES*)	6.7	0.076	8.1	0.104	3.5	3.639
Accountability	100.0	1.133	100.0	1.286	100.0	102.919

* post extraction solids

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.2.1-22: TRR values and distribution of parent compound and metabolites in apple fruits and leaves after a single foliar treatment of [furanone-4-¹⁴C]BYI 02960

	single application			
	apple fruits		apple leaves	
TRR [mg/kg] =	0.280			56.715
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg
<i>Conventional extraction</i>				
BYI 02960 (parent compound)	7.4	0.021	26.0	10.138
glucose/carbohydrates	50.3	0.141	2.5	0.991
difluoroethyl-amino-furanone	3.2	0.009	---	---
acetic acid-glyc	0.3	0.001	6.4	2.486
OH-glyc	0.4	0.001	36.1	14.062
acetic acid	0.2	0.001	2.5	0.956
difluoroethyl-OH-glyc	---	---	5.8	2.264
OH	---	---	0.6	0.244
Subtotal identified	58.7	0.164	79.9	31.141
unknown 1	---	---	0.3	0.120
unknown 3	---	---	1.7	0.646
unknown 4	---	---	---	---
unknown 5	---	---	2.6	1.002
unknown 6	---	---	1.6	0.609
unknown 7	---	---	1.5	0.578
unknown 8	---	---	4.9	1.895
Subtotal characterised	---	---	14.0	5.463
Conventional extracts not analysed	1.1	0.003	0.3	0.121
Total conventional extraction	59.8	0.168	94.3	36.725
<i>Microwave extraction</i>				
glucose/carbohydrates	6.4	0.018	---	---
difluoroethyl-amino-furanone	1.0	0.003	---	---
Subtotal identified	7.5	0.021	---	---
unknown 2	2.0	0.005	---	---
Subtotal characterised	2.0	0.005	---	---
Microwave extracts not analysed	<0.1	<0.001	---	---
Total microwave extraction	9.5	0.027	---	---
<i>Cellulase digestion</i>				
glucose/carbohydrates	15.0	0.042	---	---
difluoroethyl-amino-furanone	2.2	0.006	---	---
Subtotal identified	17.2	0.048	---	---
Subtotal characterised	---	---	---	---
Total cellulase extraction	17.2	0.048	---	---
Total identified	83.4	0.234	79.9	31.141
Total characterised	2.0	0.005	14.0	5.463
Analysed extract(s)	85.3	0.239	94.0	36.604
Extracts not analysed	1.1	0.003	0.3	0.121
Total extracted	86.5	0.242	94.3	36.725
Unextractable (PES*)	13.5	0.038	5.7	2.232
Accountability	100.0	0.280	100.0	38.957

* post extraction solids



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Table 6.2.1-23: TRR values and distribution of parent compound and metabolites in apple fruits and leaves after two foliar treatments of [furanone-4-¹⁴C]BYI 02960

	double application experiment					
	apple fruits incl. surface wash		apple fruits without surface wash		apple leaves	
TRR [mg/kg] =	1.133		1.286		102.919	
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
<i>Surface wash</i>						
parent compound	6.7	0.076				
glucose/carbohydrates	0.2	0.002				
OH-glyc	0.2	0.002				
acetic acid	0.1	0.001				
OH	0.1	0.001				
Subtotal identified	7.2	0.082				
unknown 8	<0.1	<0.001				
unknown 9	0.3	0.003				
Subtotal characterised	0.3	0.003				
Total surface wash	7.5	0.085				
<i>Conventional extraction</i>						
parent compound	64.7	0.733	73.6	0.946	57.9	59.547
glucose/carbohydrates	13.6	0.154	14.2	0.182	3.6	3.687
difluoroethyl-amino-furanone			0.2	0.003	0.7	0.736
acetic acid-glyc	0.8	0.009	0.5	0.007	4.2	4.274
OH-glyc	1.0	0.012	1.1	0.014	17.3	17.856
acetic acid	0.6	0.007	0.7	0.009	1.2	1.214
difluoroethyl-OH-glyc	---	---	---	---	2.1	2.118
OH	0.9	0.010	0.8	0.010	0.6	0.630
Subtotal identified	81.6	0.925	91.1	1.171	87.5	90.063
unknown 1	---	---	---	---	0.6	0.646
unknown 2	---	---	---	---	---	---
unknown 3	---	---	---	---	0.5	0.474
unknown 4	---	---	---	---	0.5	0.530
unknown 5	---	---	---	---	1.9	1.960
unknown 6	---	---	---	---	0.8	0.851
unknown 7	---	---	---	---	0.5	0.501
unknown 8	---	---	---	---	2.5	2.609
unknown 9	---	---	0.1	0.002	1.2	1.210
Subtotal characterised	0.3	0.003	0.1	0.002	8.5	8.781
Conv. extracts not analysed	0.3	0.003	0.7	0.009	0.4	0.436
Total conv. extraction	81.9	0.928	91.9	1.182	96.5	99.280
<i>Cellulase digestion</i>						
glucose/carbohydrates	3.3	0.037				
difluoroethyl-amino-furanone	0.6	0.007				
Subtotal identified	3.9	0.044				
Subtotal characterised	---	---				
Total cellulase extraction	3.9	0.044	---	---	---	---
Total identified	92.7	1.051	91.1	1.171	87.5	90.063
Total characterised	0.3	0.003	0.1	0.002	8.5	8.781

Table continued on next page...

	double application experiment					
	apple fruits incl. surface wash		apple fruits without surface wash		apple leaves	
Analysed extract(s)	93.0	1.054	91.2	1.173	96.0	98.843
Extracts not analysed	0.3	0.003	0.7	0.009	0.4	0.436
Total extracted	93.3	1.057	91.9	1.182	96.5	99.280
Unextractable (PES*)	6.7	0.076	8.1	0.104	3.5	3.639
Accountability	100.0	1.133	100.0	1.286	100.0	102.919

* post extraction solids

III. Conclusions

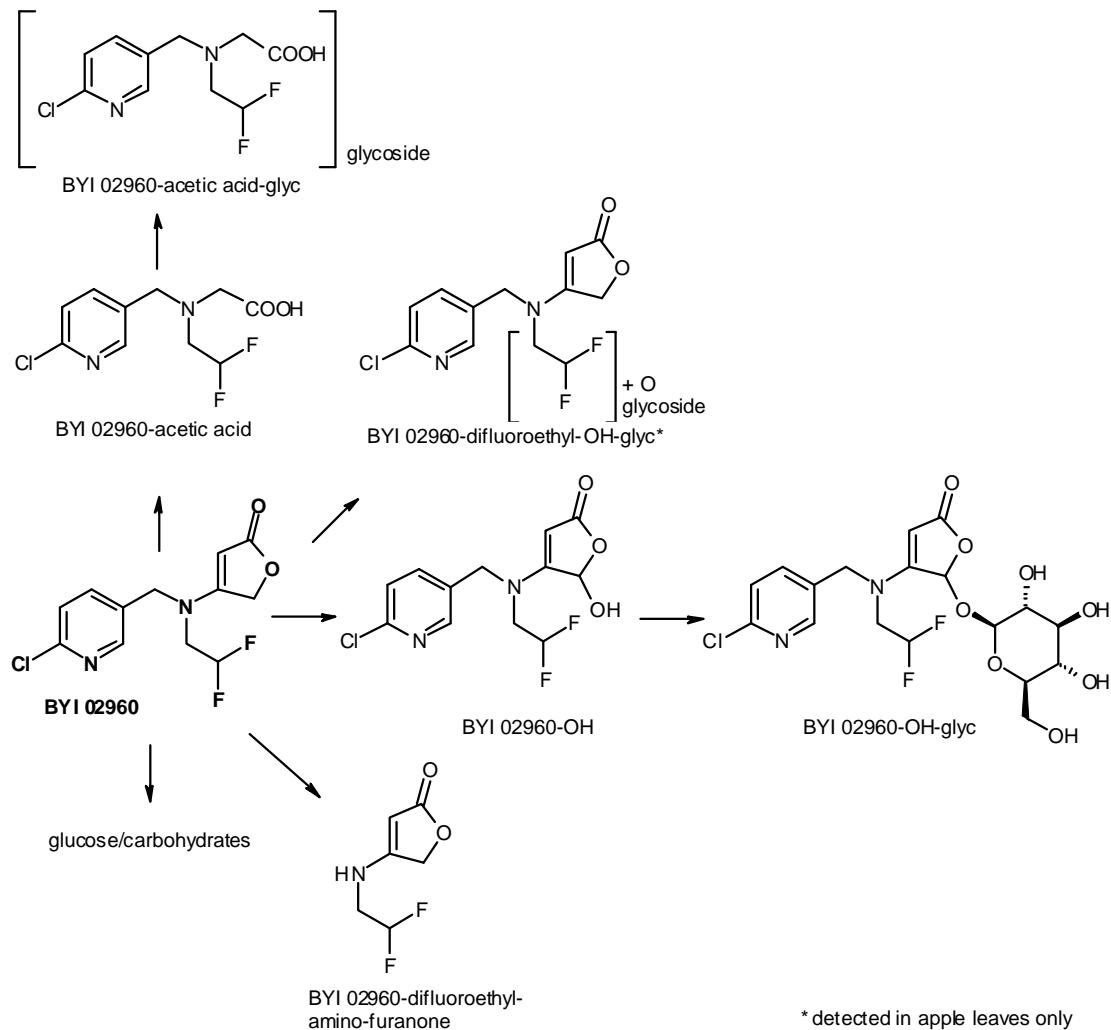
As expected, the residues were dominated by parent compound in the double application experiment, whereas the metabolite pattern was very similar in both experiments.

Four major metabolic routes of [furanone-4-¹⁴C]BYI 02960 were observed in apples:

- hydroxylation of the furanone or the difluoroethyl moiety followed by conjugation with carbohydrates,
- complete degradation of the furanone moiety and incorporation of carbon atoms into the natural compound pool, i.e. into glucose/carbohydrates,
- oxidative degradation of the furanone moiety to an acetic acid group followed by conjugation with a carbohydrate, and
- cleavage of the pyridinylmethylamine bond.

On the basis of the results of this study it is concluded that the metabolism of [furanone-4-¹⁴C]BYI 02960 in apples is well understood and the following metabolic pathway is proposed.

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 Figure 6.2.1-8: Proposed metabolic pathway of [furanone-4-¹⁴C]BYI 02960 in apples




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Report:	KIIA 6.2.1/07, Justus, K.; 2011
Title:	Metabolism of [pyridinylmethyl- ¹⁴ C]BYI 02960 in apples
Report No & Edition No	MEF-11/198 M-414678-01-2
Guidelines:	OECD 501 Metabolism in Crops US EPA Residue Chemistry Test Guideline OPPTS 860.1300: Nature of the Residue – Plants, Livestock PMRA Regulatory Directive Dir98-02: Residue Chemistry Guidelines Section 2: Nature of the Residue – Plants, Livestock Japanese MAFF, 12 Nousan 8147 European Parliament and Council Regulation (EC) No 1107/2009
GLP	yes

Executive Summary

The metabolism of [pyridinylmethyl-¹⁴C]BYI 02960 formulated as an SL 200 was investigated in apples after foliar spray application. In one experiment (single application experiment), two apple trees were treated at an actual application rate of 87 g a.s./ha per meter canopy height (87 g a.s./(ha x m CH)) at the end of flowering (BBCH 69). In another experiment, one apple tree was treated two times, one time at an actual application rate of 87 g a.s./(ha x m CH) at the end of flowering (BBCH 69) and a second time at an application rate of 85 g a.s./(ha x m CH) at 14 days before harvest. The total application rate in the double application experiment was 172 g a.s./(ha x m CH).

At maturity the apple fruits were harvested. Concurrently, the leaves were sampled to support metabolism investigation. The TRR values are shown in the following table:

Table 6.2.1-24: TRR values in apple fruits and leaves after foliar application of [pyridinylmethyl-¹⁴C]BYI 02960

Matrix	Timing and Application	PHI (days)*	ppm (mg a.s. equiv./kg)
apple fruits	one foliar spray application at BBCH 69, 87 g a.s./(ha x m CH)	98	0.079
apple leaves		98	56.715
apple fruits ¹	two foliar spray applications, at BBCH 69	14	1.868
apple fruits ²	and 14 days PHI, 87 and 85 g a.s./(ha x m CH)	14	0.545
apple leaves		14	134.841

* PHI: preharvest interval (corresponds to days after last treatment (DAT) at the start of harvest/sampling)

¹ determined from the extraction experiment with prior surface wash

² determined from the extraction experiment without prior surface wash

The apple fruits and leaves were extracted with acetonitrile/water mixtures releasing 94.2% to 99.2% of the TRR. A subsample of the apple fruits of the double application experiment was surface-washed with dichloromethane (11.0% of the TRR) and then extracted conventionally with acetonitrile/water mixtures (88.2% of the TRR). The high proportion of residues in the conventional extracts indicates a good uptake of the product and demonstrates its systemic properties.

Parent compound and metabolites in the extracts of apple fruits and leaves were analysed by HPLC. Identification of compounds was performed by LC-MS/MS, by HPLC and/or TLC co-chromatography with reference compounds, and as well as by comparison of HPLC profiles. Parent compound, the



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

major metabolites and several minor metabolites were identified. In total, 74.8% to 98.5% of the TRR was identified in apple fruits and leaves.

Parent compound was the main component in all chromatographic profiles and represented 43.1% to 88.4% of the TRR in the fruits and 24.5% and 48.2% of the TRR in the leaves. Nevertheless, a large number of metabolites was detected; 14 metabolites were detected in fruits (all <10% of the TRR) and 24 metabolites in leaves (two metabolites represented >10% of the TRR, all other metabolites were minor). Three basic metabolic routes were detected:

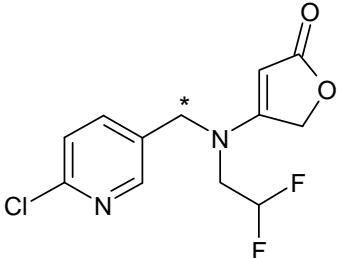
- hydroxylation of the furanone or the difluoroethyl moiety followed by conjugation with carbohydrates,
- cleavage of the pyridinylmethylamine bond followed by conjugation with carbohydrates or by oxidation of the methylene group to a carboxylic group, and
- oxidative degradation of the furanone moiety to an acetic acid group followed either by conjugation with a carbohydrate or by further degradation of the moiety

On the basis of these results, a metabolic pathway of [pyridinylmethyl-¹⁴C]BYI 02960 in apples was proposed.

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	 * position of the radiolabel
Radiolabelled test material	[pyridinylmethyl- ¹⁴ C]BYI 02960
Specific radioactivity (before radiodilution) (after radiodilution)	4.37 MBq/mg (118.08 µCi/mg) 2.18 MBq/mg (58.92 µCi/mg)
Chemical Purity	> 99% (HPLC)
Radiochemical purity	> 98% (HPLC and TLC)

The test compound was formulated as an SL 200. Therefore the supplied radiolabelled test compound [pyridinylmethyl-¹⁴C]BYI 02960 was dissolved in acetonitrile. Radiodilution and formulation of the test compound was performed prior to the first and the second application: Therefore, an adequate portion of the non-radiolabelled test compound was dissolved in an adequate volume of the stock solution of the radiolabelled test compound. The solution was evaporated to dryness. The remainder was dissolved in an adequate amount of SL 200 blank formulation using a magnetic stirrer.

Preparation of each application solution was done by dissolving the formulation in water. The specific activity of each application solution was 2.18 MBq/mg (58.92 µCi/mg).



2. Soil: "Monheim 4" (sandy loam soil from Germany), pH (CaCl_2) = 6.8, 69% sand, 18% silt and 13% clay, 1.2% organic carbon, cation exchange capacity (CEC) of 8.3 meq/100 g,

3. Plant Apple, variety "James Grieve", representative for fruiting crops

B. Study Design

Experimental conditions:

Two experiments were performed with a total of three apple trees of the variety "James Grieve". The experiments represented the intended foliar spray application scenarios of BYI 02960 for fruits. In one experiment (single foliar spray application), the apple trees were treated at a target application rate of 75 g a.s./($\text{ha} \times \text{m CH}$) (canopy height) at the end of flowering (BBCH 69). In the other experiment (double foliar spray application), one apple tree was treated two times. The target application rate was 75 g a.s./($\text{ha} \times \text{m CH}$) at each application. The first application was conducted at the end of flowering (BBCH 69) and the second at 14 days before harvest. The total target rate corresponds to the anticipated maximum application rate. The apple trees had a canopy height (CH) of 0.5 m and were cultivated in the sandy loam soil "Monheim 4" in a planting container with a surface area of 0.091 m^2 . The plants were grown in the glass-roofed vegetation area of the test facility which allows cultivation similar to natural temperature and light conditions, but protected from rainfall. The plants were watered according to their needs.

Single foliar spray application (BBCH 69):

Two apple trees with a canopy height of 0.5 m were treated each with 10 mL of the aqueous application solution using a hand pump sprayer. Prior to the application, each apple tree was covered with a protective plastic wrap to prevent radioactivity spreading into the vegetation area. After application, the hand pump sprayer was rinsed with water and acetonitrile and the plastic wrap was rinsed with methanol. The radioactivity in the rinse solutions was determined and subtracted from the amount in the application solution. A total amount of 31.5 MBq was applied per tree, corresponding to 14.5 mg a.s. From a planting density of 3000 trees/ha an actual application rate of 87 g a.s./($\text{ha} \times \text{m CH}$) was calculated.

Double foliar spray application (BBCH 69 & 14 days before harvest):

One apple tree with a canopy height of 0.5 m was treated for the first time with 10 mL of the aqueous application solution using a hand pump sprayer at the end of flowering (BBCH 69). A second application was performed at 14 days before harvest. Again, 10 mL of the aqueous application solution was applied. As described above, the hand pump sprayer and the protective plastic wrap were rinsed to determine the losses of radioactivity due to the application. A total amount of 31.5 MBq was applied at the first application and 30.9 MBq were applied at the second application. Thus 14.5 mg a.s. and 14.2 mg a.s. were applied. From a planting density of 3000 trees/ha a total application rate (comprising both applications) of 172 g a.s./($\text{ha} \times \text{m CH}$) was calculated.

Sampling:

Apple fruits of both experiments were sampled at maturity (BBCH 87- 89). On the same day, the leaves were cut off the trees with scissors. The leaves were stored in a freezer ($\leq -18^\circ\text{C}$) on the day of harvest until extraction.



All apple fruits of the single application experiment were cut in pieces. The pieces were mixed and divided into aliquots. The apple aliquots were stored in a freezer ($\leq -18^{\circ}\text{C}$) until needed for extraction. The apple fruits of the double application experiment were separated into two subsamples. Approx. one third of the whole apple fruits was stored in a freezer ($\leq -18^{\circ}\text{C}$) on the day of harvest. The remaining whole fruits were stored overnight at room temperature. On the next day the apples were subjected to a surface wash with dichloromethane.

C. Analytical Procedures

Extraction:

Single application experiment (apple fruits):

The apple pieces were extracted three times with a mixture of acetonitrile/water (8:2, v/v) and one time with a mixture of acetonitrile/water (1:1, v/v) using a high speed blender. The radioactivity in the extracts was determined by LSC, in the solids by combustion followed by LSC. The actual TRR value of the RAC was determined by summing up the radioactivity measured in the extracts and in the remaining solids. The first two extracts were combined and subjected to a clean-up step using an SPE RP 18 cartridge (Phenomenex, Strata C18-E, 20 g), which was conditioned with acetonitrile beforehand. The flow-through fraction (percolate) was collected and the cartridge was rinsed with acetonitrile/water (8:2, v/v) and methanol/dichloromethane (1:1, v/v). The percolate and the acetonitrile/water fraction were combined and concentrated by rotary evaporation in vacuo. The pH value of the concentrate was adjusted to pH 7 with ammonium carbonate before chromatographic analysis by HPLC.

Double application experiment (apple fruits):

The subsample of the whole apple fruits which was stored at room temperature was subjected to a surface wash with dichloromethane. An aliquot of the dichloromethane surface wash was dispersed with 1 mL of water by ultrasonic treatment. The dichloromethane was removed by a nitrogen stream and the remaining concentrate was analysed by HPLC. The washed apples were cut into pieces. The apples pieces were mixed, divided into aliquots and stored in a freezer ($\leq -18^{\circ}\text{C}$). Extraction, purification and concentration of the extract were performed exactly as described for the single application experiment. The resulting sample was adjusted to pH 7 with ammonium carbonate for HPLC analysis.

Additionally, three whole apples of the double application experiment were extracted without prior surface wash. The frozen apples were crushed with a wooden hammer and homogenised under liquid nitrogen using a high speed blender. An aliquot of the apple mush was extracted for final analysis. Extraction, purification and concentration of the extract were performed exactly as described for the single application experiment. The resulting sample was adjusted to pH 7 with ammonium carbonate for HPLC analysis.

Aliquots of the apple leaves of the single and double application experiment were extracted as described for the fruits of the single application experiment. The clean-up procedure and the concentration step were also identical with the one applied for the fruit samples. The resulting sample was adjusted to pH 7 with ammonium carbonate for HPLC analysis.

**Quantification:**

Parent compound and metabolites in the extracts were analysed by reversed phase HPLC coupled to a radioactivity detector with a glass scintillator cell. The HPLC chromatograms (= metabolite profiles) were integrated for quantification of compounds.

Identification and characterisation:

Generally, major metabolites were identified by two independent chromatographic methods (reversed phase HPLC and normal phase TLC) or by LC-MS/MS after semi-preparative isolation of the compounds.

Since the profiles of the apple leaves showed higher levels of metabolites than the profiles of apple fruits (in terms of mg/kg), several metabolites were isolated with semi-preparative HPLC from the extract of apple leaves of the single treatment experiment. The isolated compounds were identified with spectroscopic methods, even if they were minor. The isolated and identified metabolites were furthermore used as reference compounds for co-chromatographic identification of metabolites in apple fruits. Other minor metabolites (<10% of the TRR and representing <0.01 mg/kg in edibles) were identified by HPLC co-chromatography with authentic reference compounds or by HPLC comparison.

Storage stability:

All extraction and surface wash experiments and the first HPLC analyses of the apple fruits were performed within 3 months after harvest. The extraction experiments and the first HPLC analyses of the apple leaves were performed within 4 months after harvest. Thus, no additional tests are necessary to prove the stability of the relevant residues in frozen matrices. The extracts were analysed after 1 to 3 days following start of extraction and the surface wash was analysed 13 days after washing.

Comparison of the HPLC chromatograms recorded at different times during the study showed that the profiles of the extracts did not change significantly during the analytical work up, which did not exceed approx. eight months.

It was therefore concluded, that the residues in the matrices and in the extracts were sufficiently stable during the experimental period of the study and that the chromatograms represented the metabolic pattern in the samples at harvest.

II. Results and Discussion

The metabolism of [pyridinylmethyl-¹⁴C]BYI 02960 was investigated in apple fruits and leaves following two different spray scenarios. In one experiment (single application experiment), two apple trees were treated at an actual application rate of 87 g a.s./ha x m CH (CH = meter canopy height) at the end of flowering (BBCH 69). In the other experiment, one apple tree was treated two times, one time at an actual application rate of 87 g a.s./ha x m CH at the end of flowering (BBCH 69) and a second time at an application rate of 85 g a.s./ha x m CH at 14 days before harvest. The total application rate in the double application experiment was 172 g a.s./ha x m CH. The actual single application rates were slightly above the anticipated maximum rate of 75 g a.s./ha x m CH.



Apple fruits were harvested for analysis from the single application experiment at 98 days after the treatment and from the double application experiment at 14 days after the last treatment. Concurrently, the leaves were sampled to support the analysis of metabolites. The apple fruit samples of the double application experiment were investigated with and without a surface wash step.

The TRR level in the apple fruits, which represent the edible RAC, was low in the single application experiment: only 0.079 mg/kg was found. In the double application experiment, the apple fruits had a TRR of 1.868 mg/kg (extraction including surface wash) and 0.545 mg/kg (extraction without surface wash). The difference of the TRR values is presumably due to the low amount of apples harvested and extracted and a partly inhomogeneous spray distribution.

The TRR values of the apple leaves were high: 56.715 mg/kg were found in the leaves of the single application experiment and 134.841 mg/kg in the leaves of the double application experiment. Apple leaves were only sampled to support the identification of metabolites.

The main portion of radioactivity in the apple fruits and leaves was extracted conventionally by acetonitrile/water mixtures (94.2% to 98.7% of the TRR) as shown in Table 6.2.1-25 and Table 6.2.1-26. The apple fruits of the double application experiment were additionally surface-washed with dichloromethane and then extracted conventionally with acetonitrile/water mixtures. A portion of 11.0% of the TRR was removed by the surface wash and another portion of 88.2% was extracted conventionally (see also Table 6.2.1-26).

HPLC analysis of the surface wash and the conventional extracts of apple fruits and leaves after both spray scenarios revealed that all metabolite profiles were very well comparable. Thus, identification was performed generally in the fruit or leaf extract of the single application experiment and metabolites were assigned in the other extracts (including the surface wash) by comparison.

The main compound in apple fruits and leaves of the single and the double application experiment was parent compound. It was identified in the extracts of apple fruits of both experiments and in the extract of apple leaves of the single application experiment by HPLC co-chromatography with a radiolabelled reference compound. For confirmation, parent compound was additionally identified in the extract of apple fruits of the single application experiment by TLC co-chromatography on silica gel.

All other metabolites detected in the raw agricultural commodity fruit were minor metabolites. They did not exceed 10% of the TRR in the single and the double application experiment. However, since all metabolites were detected in higher levels in the extract of leaves, the leaf extract of the single application experiment was fractionated and the fractions were subjected to LC-MS/MS analysis. Thus, metabolites BYI 02960-CHMP-glyc, BYI 02960-difluoroethyl-OH-glyc, BYI 02960-difluoroethanamine, BYI 02960-acetic acid-glyc and BYI 02960-OH-glyc and the corresponding aglycons BYI 02960-acetic acid and BYI 02960-OH were identified. The isolated metabolites were used for HPLC co-chromatography to confirm their occurrence in apple fruits.

Mass spectroscopic investigations of the isolated metabolite BYI 02960-CHMP-glyc showed that the one HPLC peak was represented by two isomers, presumably with different configurations of the conjugated glycoside moiety. The major isomer was assigned to the glucose conjugate of BYI 02960-

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CHMP. This configuration of the carbohydrate was confirmed in the tomato metabolism study and thus it was assumed that it is most probably the preferred configuration in all plants. Another conjugate of BYI 02960-CHMP, the minor metabolite BYI 02960-CHMP-di-glyc was identified by comparison of apple and tomato profiles, too.

LC-MS/MS analysis showed as well that the two metabolites BYI 02960-OH-glyc and BYI 02960-acetic acid co-eluted in one peak using the neutral HPLC profiling method. Chromatographic separation of the compounds was achieved with an acidic reversed phase HPLC method. For sub-quantification the peaks with the two metabolites were isolated from the apple fruits and leaves of the single application experiment by semi-preparative HPLC using the neutral profiling method. Re-analyses of the fraction with the acidic HPLC method separated the two components. The ratios obtained in the extracts of the single application experiment were then used for sub-quantification of the compounds in the double application experiment. The very similar pattern in the profiles of the two experiments (neglecting the amounts of parent compound) justified the transfer of the ratios. The identification of BYI 02960-OH-glyc and BYI 02960-acetic acid was additionally confirmed by comparison with reference compounds isolated and identified in the confined rotational crops study.

Following LC-MS/MS analysis, supportive experiments were performed to fully elucidate the chemical structures of the metabolites BYI 02960-OH-glyc and BYI 02960-OH. Enzymatic treatment with β -glucosidase cleaved the conjugate BYI 02960-OH-glyc. HPLC co-chromatography of the cleavage product with the reference compound BYI 02960-OH located the conjugating hydroxy group at the 5-position of the furanone moiety. Concurrently, the successful enzymatic cleavage revealed D-glucose as the configuration for the conjugated hexose. For the metabolite BYI 02960-difluoroethyl-OH-glyc, the position of the hydroxy group being the link for the conjugation could not be exactly determined by LC-MS/MS. However, the fragmentation pattern indicated hydroxylation and conjugation in the difluoroethyl side chain.

The minor metabolites BYI 02960-6-CNA and BYI 02960-CHMP were identified in apple fruits of the single application experiment by HPLC co-chromatography with authentic reference compounds. Following identification of metabolites by LC-MS/MS analysis and co-chromatography or comparison with reference compounds, assignment to peaks in other profiles was completed by comparison of all profiles

The distribution of the radioactive residues in the extracts is shown in Table 6.2.1-25 and Table 6.2.1-26. In total, 78.9% to 98.5% of the TRR were identified in the apple fruit after foliar application as summarized in Table 6.2.1-27 and Table 6.2.1-28.

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Table 6.2.1-25: Distribution of radioactivity in the extracts of apple fruits and leaves after a single foliar application of [pyridinylmethyl-¹⁴C]BYI 02960

TRR [mg/kg] =	single application experiment			
	apple fruits		apple leaves	
	% of TRR	mg/kg	% of TRR	mg/kg
Conventionally extracted	94.2	0.074	96.7	54.859
Extract for analysis	94.2	0.074	96.5	54.727
Losses (not analysed)	---	---	0.2	0.132
Total extracted	94.2	0.074	96.7	54.859
Unextractable (PES*)	5.8	0.005	3.3	1.855
Accountability	100.0	0.079	100.0	56.715

* post extraction solids

Table 6.2.1-26: Distribution of radioactivity in the extracts of apple fruits and leaves after a double foliar application of [pyridinylmethyl-¹⁴C]BYI 02960

TRR [mg/kg] =	double application experiment					
	apple fruits incl. surface wash		apple fruits without surface wash		apple leaves	
	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Surface wash with DCM	11.0	0.205	---	---	---	---
Conventionally extracted	88.2	1.648	98.7	0.538	98.4	132.635
Extract for analysis	88.1	1.645	98.0	0.534	97.8	131.847
Losses (not analysed)	0.2	0.003	0.6	0.004	0.6	0.788
Total extracted	99.2	1.853	98.7	0.538	98.4	132.635
Unextractable (PES*)	0.8	0.015	1.3	0.007	1.6	2.206
Accountability	100.0	1.868	100.0	0.545	100.0	134.841

* post extraction solids

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Table 6.2.1-27: TRR values and distribution of parent compound and metabolites in apple fruits and leaves after a single foliar treatment of [pyridinylmethyl-¹⁴C]BYI 02960

	single application			
	apple fruits		apple leaves	
TRR [mg/kg] =	0.079		56.715	
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg
<i>Conventional extraction</i>				
BYI 02960 (parent compound)	43.1	0.034	24.5	13.882
6-CNA	5.0	0.004	---	---
CHMP-di-glyc	---	---	0.6	0.342
CHMP-glyc	4.7	0.004	14.4	8.141
CHMP	4.0	0.003	1.3	0.727
acetic acid-glyc	3.5	0.003	5.1	2.891
OH-glyc	4.9	0.004	19.9	11.278
acetic acid	3.0	0.002	1.4	0.767
difluoroethyl-OH-glyc	1.4	0.001	6.4	3.631
AMCP-difluoroethanamine	8.4	0.007	0.4	0.255
OH	0.8	0.001	0.9	0.484
Total identified	78.9	0.062	74.8	42.399
unknown 1	2.7	0.002	---	---
unknown 2	6.6	0.005	---	---
unknown 3	3.5	0.003	1.8	1.000
unknown 4	2.0	0.002	3.6	2.052
unknown 5	---	---	---	---
unknown 6	---	---	1.1	0.634
unknown 7	---	---	0.6	0.322
unknown 8	---	---	---	---
unknown 9	---	---	2.6	1.477
unknown 10	---	---	1.3	0.762
unknown 11	---	---	0.5	0.295
unknown 12	---	---	---	---
unknown 13	---	---	---	---
unknown 14	---	---	0.6	0.353
unknown 15	---	---	0.6	0.315
unknown 16	---	---	1.2	0.682
unknown 17	---	---	0.7	0.403
unknown 18	---	---	2.4	1.386
unknown 19	---	---	1.9	1.088
unknown 20	0.6	<0.001	2.5	1.445
unknown 21	---	---	0.2	0.114
Total characterised	15.3	0.012	21.7	12.328
Analysed extract(s)	94.2	0.074	96.5	54.727
Extracts not analysed	---	---	0.2	0.132
Total extracted	94.2	0.074	96.7	54.859
Unextractable (PES*)	5.8	0.005	3.3	1.855
Accountability	100.0	0.079	100.0	56.715

* post extraction solids



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Table 6.2.1-28: TRR values and distribution of parent compound and metabolites in apple fruits and leaves after two foliar treatments of [pyridinylmethyl-¹⁴C]BYI 02960

	double application experiment					
	apple fruits incl. surface wash		apple fruits without surface wash		apple leaves	
TRR [mg/kg] =	1.868			0.545		134.841
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
<i>Conventional extraction</i>						
parent compound	88.4	1.652	85.6	0.467	48.2	64.981
6-CNA	0.5	0.009	1.5	0.008	0.3	0.436
CHMP-di-glyc	---	---	---	---	0.2	0.327
CHMP-glyc	0.5	0.010	0.9	0.005	7.3	9.842
CHMP	0.7	0.013	0.8	0.004	0.6	0.777
acetic acid-glyc	0.6	0.012	0.8	0.004	5.0	6.799
OH-glyc	1.3	0.024	1.7	0.009	15.4	20.729
acetic acid	0.8	0.015	1.1	0.006	1.0	1.410
difluoroethyl-OH-glyc	---	---	---	---	4.9	6.674
AMCP-difluoroethanamine	4.5	0.085	4.1	0.023	0.8	1.015
OH	1.0	0.020	1.0	0.005	0.7	0.944
Total identified	98.5	1.839	97.6	0.532	84.5	113.933
unknown 1	---	---	---	---	---	---
unknown 2	---	---	---	---	---	---
unknown 3	0.2	0.004	---	---	0.8	1.070
unknown 4	---	---	---	---	2.0	2.652
unknown 5	---	---	---	---	0.2	0.222
unknown 6	---	---	---	---	0.3	0.406
unknown 7	---	---	---	---	0.4	0.601
unknown 8	---	---	---	---	0.4	0.504
unknown 9	---	---	---	---	1.6	2.150
unknown 10	---	---	---	---	0.5	0.721
unknown 11	---	---	---	---	0.5	0.654
unknown 12	---	---	---	---	0.2	0.323
unknown 13	0.3	0.005	---	---	0.4	0.518
unknown 14	---	---	---	---	---	---
unknown 15	---	---	---	---	---	---
unknown 16	---	---	---	---	1.0	1.380
unknown 17	---	---	---	---	0.5	0.669
unknown 18	---	---	---	---	1.7	2.291
unknown 19	---	---	---	---	0.8	1.091
unknown 20	0.1	0.002	0.5	0.002	1.7	2.248
unknown 21	---	---	---	---	0.3	0.413
Total characterised	0.6	0.011	0.5	0.002	13.3	17.914
Analysed extract(s)	99.0	1.850	98.0	0.534	97.8	131.847
Extracts not analysed	0.2	0.003	0.6	0.004	0.6	0.788
Total extracted	99.2	1.853	98.7	0.538	98.4	132.635
Unextractable (PES*)	0.8	0.015	1.3	0.007	1.6	2.206
Accountability	100.0	1.868	100.0	0.545	100.0	134.841

* post extraction solids

III. Conclusions

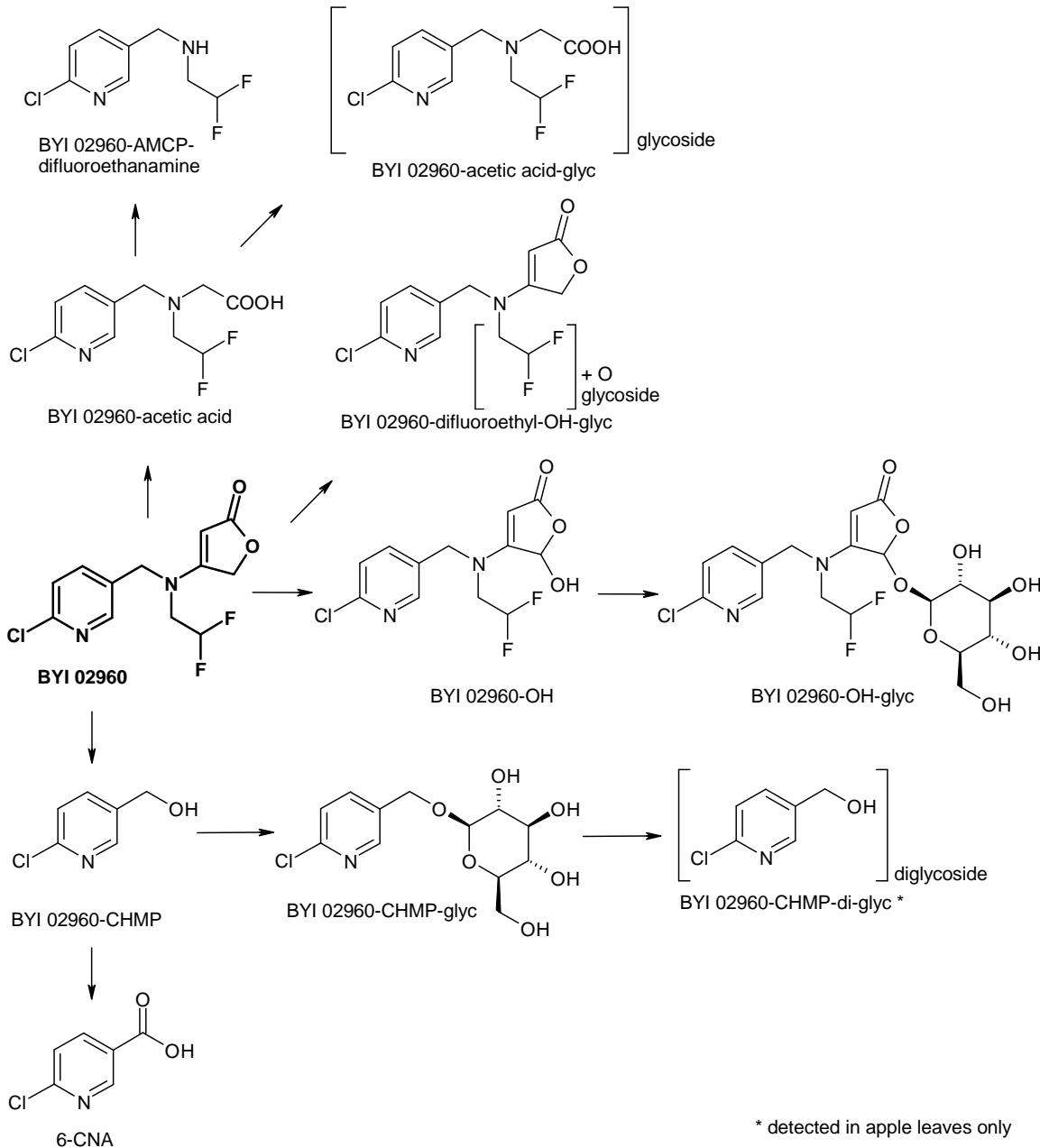
As expected, the residues were dominated by parent compound in the double application experiment, whereas the metabolite pattern was the same in both experiments.

Three major metabolic routes of [pyridinylmethyl-¹⁴C]BYI 02960 were observed in apples:

- hydroxylation followed by conjugation with carbohydrates,
- cleavage of the pyridinylmethylamine bond followed by conjugation with carbohydrates or by oxidation of the methylene group to a carboxylic group, and
- oxidative degradation of the furanone moiety to an acetic acid group followed either by conjugation with a carbohydrate or by further degradation of the moiety.

On the basis of the results of this study it is concluded that the metabolism of [pyridinylmethyl-¹⁴C]BYI 02960 in apples is well understood and the following metabolic pathway is proposed.

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Figure 6.2.1-9: Proposed metabolic pathway of [pyridinylmethyl-¹⁴C]BYI 02960 in apples**Overall Conclusions Apple (foliar application)**

The metabolism of the insecticide BYI 02960 was investigated in apple fruits in two studies following foliar application with (1) [furanone-4-¹⁴C]BYI 02960 or (2) [pyridinylmethyl-¹⁴C]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-4-¹⁴C]BYI 02960 were additionally analysed for non-radiolabelled difluoroacetic acid by LC-MS/MS according to residue method 01304 (see KIIA).



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6.2./12). 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 14 days before harvest. As expected, in both studies the residues in the double application experiment were dominated by parent compound.

Label (1): [furanone-4-¹⁴C]BYI 02960

In the single application experiment a natural compound (glucose/carbohydrates) was by far the main compound in apple fruits, whereas parent compound was predominant in leaves. Nevertheless, the metabolite patterns were very similar in fruits and leaves in both experiments. Besides parent compound and glucose/carbohydrates, each of the other metabolites in apple fruits represented less than 4% of the TRR. In total, 5 and 6 metabolites were identified in the single and the double application experiment, respectively. In apple leaves, BYI 02960-OH-glyc was identified as major metabolite, each of the other metabolites (6 to 7) represented less than 7% of the TRR. Overall, four major metabolic routes were detected: (1) hydroxylation of the parent compound (either the furanone moiety or the difluoroethyl moiety) followed by conjugation with carbohydrates, (2) oxidative degradation of the furanone moiety to an acetic acid group followed either by conjugation with a carbohydrate or by further degradation of the moiety, (3) complete degradation of the furanone moiety and incorporation of carbon atoms into the natural compound pool, i.e. into glucose/carbohydrates, and (4) cleavage of the pyridinylmethylamine bond. The first two routes led to the non label-specific metabolites BYI 02960-acetic acid and BYI 02960-OH, their corresponding glycosides and to BYI 02960-difluoroethyl-OH-glyc, which was detected in apple leaves only. These metabolites were also detected in the apple metabolism study with [pyridinylmethyl-¹⁴C]BYI 02960. Molecule cleavage led to the label-specific metabolite BYI 02960-difluoroethyl-amino-furanone and degradation of the furanone moiety finally to glucose/carbohydrates. The corresponding counterparts BYI 02960-CHMP and BYI 02960-AMCP-difluoroethanamine were detected in the apple metabolism study with [pyridinylmethyl-¹⁴C]BYI 02960. Thus, the results of the present metabolism study in apples are in very good conformity with the results of the corresponding study performed with [pyridinylmethyl-¹⁴C]BYI 02960.

(2) Label 2: [pyridinylmethyl-¹⁴C]BYI 02960

Parent compound was the main compound detected in both experiments in apple fruits at harvest. All metabolites represented less than 10% of the TRR. Nine metabolites were identified in fruits from which BYI 02960-AMCP-difluoroethanamine was the most prominent one, followed by 6-CNA (single application experiment) or BYI 02960-OH-glyc (double application experiment). Overall, three major metabolic routes were detected: (1) Oxidative degradation of the furanone moiety to an acetic acid group followed either by conjugation with a carbohydrate or by further degradation of the moiety; (2) hydroxylation of the parent compound (either the furanone moiety or the difluoroethyl moiety) followed by conjugation with carbohydrates and (3) cleavage of the pyridinylmethylamine bond followed by conjugation with carbohydrates or by oxidation of the methylene group to a carboxylic group. The first two routes led to the non label-specific metabolites BYI 02960-acetic acid and BYI 02960-OH, their corresponding glycosides and to BYI 02960-difluoroethyl-OH-glyc. These metabolites were also detected in the apple metabolism study with [furanone-4-¹⁴C]BYI 02960. Molecule cleavage led to the label-specific metabolites BYI 02960-AMCP-difluoroethaneamine, 6-CNA, BYI 02960-CHMP and its corresponding glycoside. The corresponding counterpart BYI 02960-



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

difluoroethyl-amino-furanone was detected in the apple metabolism study with [furanone-4-¹⁴C]BYI 02960. Thus, the results of the present metabolism study in apples are in good conformity with the results of the corresponding study performed with [furanone-4-¹⁴C]BYI 02960.

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 thereof in fruits) were found. All major and 10 minor metabolites have been identified. The distribution of parent compound and metabolites in the edible commodity apple fruit is summarized in Table 6.2.1-29.

Table 6.2.1-29 TRR values and distribution of parent compound and metabolites in apple fruit after foliar application of BYI 02960

Radiolabel	apple fruits							
	[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
<i>glucose/carbohydrates</i>	<i>71.7</i>	<i>0.201</i>	<i>14.2</i>	<i>0.182</i>				
<i>6-CNA</i>					<i>5.0</i>	<i>0.004</i>	<i>1.5</i>	<i>0.008</i>
<i>CHMP-glyc</i>					<i>4.7</i>	<i>0.004</i>	<i>0.9</i>	<i>0.005</i>
<i>CHMP</i>					<i>4.0</i>	<i>0.003</i>	<i>0.8</i>	<i>0.004</i>
<i>difluoroethyl-amino-furanone</i>	<i>3.2</i>	<i>0.009</i>	<i>0.2</i>	<i>0.003</i>				
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
<i>AMCP-difluoroethanamine</i>					<i>8.4</i>	<i>0.007</i>	<i>4.1</i>	<i>0.023</i>
difluoroethyl-OH-glyc					<i>1.4</i>	<i>0.001</i>	<i>---</i>	<i>---</i>
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 and was thus a main metabolite. Only parent compound and the natural compound glucose were detected in higher concentrations. In leaves quite high concentrations of

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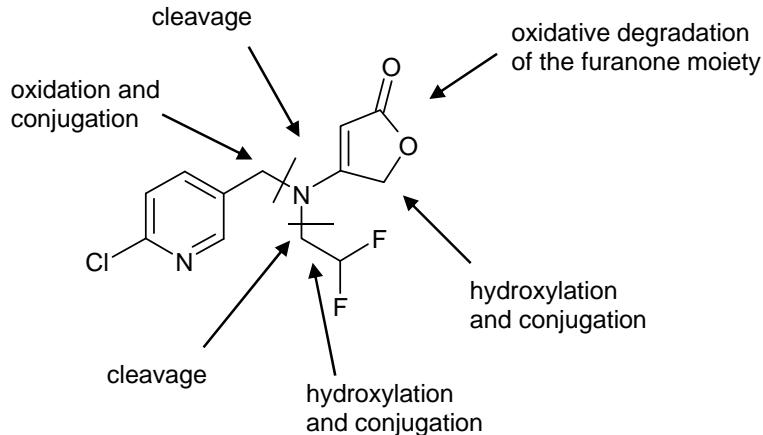
difluoroacetic acid were found (1.86 mg a.s. equiv/kg in the single application experiment and 1.35 mg a.s. equiv./kg in the double application experiment), however the proportions were low compared to the total radioactive residue detected.

On basis of the metabolites identified, biotransformation of BYI 02960 in apple proceeds by the following pathways:

- oxidative cleavage of the difluoroethylamine bond and formation of difluoroacetic acid
- oxidative degradation of the furanone moiety to an acetic acid group followed either by conjugation with a carbohydrate or by further degradation of the moiety
- complete degradation of the furanone moiety and incorporation of carbon atoms into the natural compound pool, e.g. into glucose/carbohydrates
- cleavage of the pyridinylmethylamine bond and formation of BYI 02960-difluoroethylamino-furanone and its corresponding counterpart BYI 02960-CHMP, which was either conjugated with carbohydrates or oxidised to 6-chloronicotinic acid (6-CNA)
- hydroxylation of the furanone or difluoroethane moiety followed by conjugation with glucose or another hexose

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

Figure 6.2.1-10: Positions involved in metabolic degradation of BYI 02960 in apple fruits and leaves



**Metabolism, distribution and expression of residues in cotton (foliar application)**

Metabolism studies in cotton were conducted with [furanone-4-¹⁴C]-, and [pyridinylmethyl-¹⁴C]BYI 02960:

Report:	KIIA 6.2.1/08, Schmeling, S., Weber, E.; 2011
Title:	Metabolism of [furanone-4- ¹⁴ C]BYI 02960 in cotton after spray application
Report No & Edition No	MEF-11/392 M-421625-01-2
Guidelines:	OECD 501 Metabolism in Crops US EPA Residue Chemistry Test Guideline OPPTS 860.1300: Nature of the Residue – Plants, Livestock PMRA Regulatory Directive Dir98-02: Residue Chemistry Guidelines Section 2: Nature of the Residue – Plants, Livestock Japanese MAFF, 12 Nousan 8147 European Parliament and Council Regulation (EC) No 1107/2009
GLP	yes

Executive Summary

The metabolism of [furanone-4-¹⁴C]BYI 02960 formulated as an SL 200 was investigated in cotton after foliar spray application. In one experiment (single application experiment), five cotton plants were treated at an actual application rate of 209 g a.s./ha when the sixth true leaf was unfolded (BBCH 16), i.e. at an early growth stage. In another experiment, one cotton plant was treated two times, one time at an actual application rate of 209 g a.s./ha at BBCH 15 and a second time at an application rate of 176 g a.s./ha at 14 days before harvest of the cotton bolls (when more than 90% of the bolls were open and approx. 50% to 70% of the leaves were discoloured or fallen; BBCH 95 - 97). The total application rate in the double application experiment was 385 g a.s./ha.

Samples were taken from both experiments. Cotton seeds and gin trash were the raw agricultural commodities (RACs) harvested at maturity of the seeds in this study. Lint was collected as additional sample material at the same time. Additionally, one plant of the single application experiment was sampled at an intermediate growth stage (two to four vegetative side shoots were visible; BBCH 22 – 24) to support the identification of metabolites and to possibly obtain additional information on the metabolic degradation behaviour of BYI 02960. The TRR values of all plant matrices determined are shown in the following table:



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.2.1-30: TRR values in cotton (intermediate, gin trash, lint, seeds) after foliar application of [furanone-4-¹⁴C]BYI 02960

Matrix	Timing and Application	PHI (days)*	ppm (mg a.s. equiv./kg)
intermediate	one spray application, 209 g a.s./ha (at BBCH 16)	28	12.391
gin trash		169	0.191
lint		169	0.009
seeds		169	0.013
gin trash	two spray applications, 209 g a.s./ha (at BBCH 15) 176 g a.s./ha (at BBCH 95 – 97)	14	2.767
lint		14	4.993
seeds		14	0.016

* PHI: preharvest interval (corresponds to days after last treatment (DAT) at the start of harvest/sampling)

The radioactive residues were extracted with acetonitrile/water mixtures. In the case of seeds, the samples were extracted with heptane beforehand. When necessary, additional exhaustive extraction steps were applied to the solids of the first extraction. Extraction efficiencies ranged from 23.4% of the TRR for seeds (single application experiment) to 96.6% for lint (double application experiment). The profiles of the extracts comprising a reasonable amount of radioactivity were recorded by HPLC and all major and several minor components were identified. Identification of parent compound and metabolites common to both radiolabels was based on the metabolite profiles obtained in the cotton metabolism study with [pyridinylmethyl-¹⁴C]BYI 02960. Additionally, all assignments made in the present study were confirmed by HPLC co-chromatography using authentic reference compounds. The identification rates ranged from 70.3% of the TRR for gin trash (single application experiment) to 86.0% for lint (double application experiment). [Furanone-4-¹⁴C]BYI 02960 was metabolised moderately in cotton. Parent compound was the most prominent component and represented approx. 40 – 70% of the TRR in all matrices analysed. The sum of metabolites BYI 02960-OH-glyc and BYI 02960-acetic acid represented another major fraction in all matrices and accounted for approx. 14% – 25% of the TRR. In gin trash of the single application experiment, BYI 02960-OH was detected as additional major metabolite representing approx. 13% of the TRR. All other metabolites detected were minor or trace components.

The following metabolic routes of [furanone-4-¹⁴C]BYI 02960 were observed in cotton:

- hydroxylation of the methylene group of the furanone moiety followed by conjugation,
- oxidative opening of the furanone moiety to an acetic acid group followed by further oxidation or conjugation with a carbohydrate (e.g. glycosylation), and
- halogenation (mostly bromination, to a minor extent chlorination) of the furanone moiety

Halogenation of the furanone moiety of the active substance probably occurred in the soil which was supported by the fact that small amounts of halogenated parent compound were identified in the aerobic soil degradation studies. Due to the early timing of the first application, a significant portion of the parent compound could have reached the soil and been subjected to soil metabolism processes.

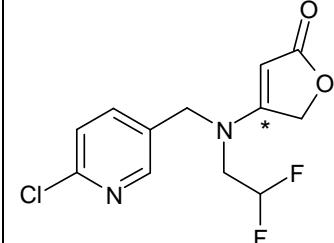
On the basis of these results, a metabolic pathway of [furanone-4-¹⁴C]BYI 02960 in cotton can be proposed.



I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	 * position of the radiolabel
Radiolabelled test material	[furanone-4- ¹⁴ C]BYI 02960
Specific radioactivity	3.94 MBq/mg (106.46 µCi/mg)
Chemical Purity	> 99% (HPLC)
Radiochemical purity	> 98% (HPLC and TLC)

The supplied radiolabelled test compound [furanone-4-¹⁴C]BYI 02960 was dissolved in acetonitrile. Formulation of the test compound was performed prior to each application. For the preparation of the SL 200 formulation, an adequate portion of the stock solution was transferred into a special glass vial and evaporated to dryness. The liquid blank formulation was added to the test item using a magnetic stirrer. The mixture was diluted with water to get the aqueous application solution.

2. Soil: "Einheitserde T" (standard soil containing 2 kg/m³ of a water soluble salt mixture), pH (CaCl₂) = 5.8, 85% white moor peat, 15% clay, containing NO₃⁻, NH₄⁺, P₂O₅, K₂O, Fe, Mn, B, Cu, Mo, Zn; distributor: Balster Einheitserdewerk, Eulenstr. 53; 58730 Froendenberg, Germany

3. Plant Cotton, variety "Carmen", Gossypium hirsutum, representative for oil seeds

B. Study Design

Experimental conditions:

Two experiments were performed with a total of six cotton plants of the variety "Carmen". The experiments represented the intended foliar spray application scenarios of BYI 02960 in cotton. In one experiment (single foliar spray application), the plants were treated at a target application rate of 200 g a.s./ha when the sixth true leaf was unfolded (BBCH 16). In the other experiment (double foliar spray application), one cotton plant was treated two times. The target application rate was 200 g a.s./ha at each application. The first application was conducted at BBCH 15 and the second at 14 days before harvest. The total target rate of 400 g a.s./ha corresponds to the anticipated maximum application rate. The cotton plants were cultivated in the standard soil "Einheitserde T" in planting pots with a surface area of approx. 0.076 m² and a volume of 15 L. The plants were grown in the greenhouse of the test facility which allows cultivation similar to natural temperature and light conditions. The plants were irrigated according to their needs.

**Sampling:**

In both experiments cotton seeds (= undelinted seeds), lint and gin trash were harvested at maturity of the seeds (BBCH 99). The cotton bolls (capsules) were cut from the plants and the seeds were separated manually from the lint. No chemical process followed to delint the seeds. The remaining plants (= gin trash) were cut off just above the soil surface and were shredded into small pieces. The gin trash chaff was combined with the empty capsules which had contained the lint and the seeds. Gin trash and seed samples were further processed using a Polytron homogenizer and liquid nitrogen. The homogenized samples were stored in a freezer ($\leq -18^{\circ}\text{C}$) until analysis. Lint samples of the double application experiment were not submitted to any sample preparation steps and were stored in a freezer ($\leq -18^{\circ}\text{C}$) until analysis.

In the single application experiment, additionally an intermediate was sampled at an early growth stage (two to four vegetative side shoots were visible; BBCH 22 – 24) to support the identification of metabolites. The plant was cut off just above the soil surface and was cut in small pieces prior to extraction and analysis.

C. Analytical Procedures**Extraction:**

Aliquots of all sample matrices were extracted three times with a mixture of acetonitrile/water (8:2, v/v) using a high speed blender. The cotton seed samples were additionally extracted with heptane prior to the first acetonitrile/water extraction step. The radioactivity in the extracts was determined by LSC and in the solids by combustion followed by LSC. The actual TRR value of the RAC was determined by summing up the radioactivity measured in the extracts and in the remaining solids. All extracts were combined. The intermediate and the gin trash extracts were subjected to an SPE clean-up step, whereas the seed and the lint extracts were directly analysed by HPLC analysis after concentration. The clean-up was performed using an SPE RP 18 cartridge (Phenomenex, Strata C18-E, 20 g), which was conditioned with methanol, water and an acetonitrile/water mixture beforehand. The flow-through fraction (percolate) was collected and the cartridge was rinsed with acetonitrile/water (8:2, v/v) and methanol/dichloromethane (1:1, v/v). The percolate and the acetonitrile/water fraction were combined and concentrated by rotary evaporation in vacuo prior to chromatographic analysis by HPLC. The solids remaining after the conventional extraction steps of the gin trash samples were additionally submitted two times to microwave-assisted extraction with acetonitrile/water mixtures (8:2, v/v and 1:1, v/v). After each extraction step, extracts and solids were separated by filtration. The extracts were combined and concentrated by rotary evaporation in vacuo prior to HPLC analysis.

Quantification:

Parent compound and metabolites in the extracts were analysed by reversed phase HPLC coupled to a radioactivity detector with a glass scintillator cell. The HPLC chromatograms (= metabolite profiles) were integrated for quantification of compounds.

Identification and characterisation:

Identification of parent compound and metabolites common to both radiolabels was based on the metabolite profiles obtained in the cotton metabolism study with [pyridinylmethyl- ^{14}C]BYI 02960. In this study parent compound and the major metabolites were identified in at least one matrix either by LC-MS/MS after semi-preparative isolation of the compounds or by HPLC and TLC co-



chromatography with authentic reference compounds. All assignments made in the present study were confirmed by HPLC co-chromatography in gin trash using authentic reference compounds.

Assignment in the other matrices was performed by comparison of HPLC profiles since the metabolite profiles of all matrices were very well comparable. No label-specific metabolite was identified.

Storage stability:

All extraction experiments and the first HPLC analyses were performed within 3 weeks (21 days) after harvest of the cotton plants. The extracts were analysed after 6 days following the start of extraction at the latest. Since the first metabolic analysis of the RACs as well as the last experiments with extracts (HPLC co-chromatography) took place within 6 months, no further stability investigations were necessary according to OECD Guidance for the Testing of Chemicals 501 (2007).

II. Results and Discussion

The metabolism of [furanone-4-¹⁴C]BYI 02960 was investigated in cotton seeds, gin trash and lint following two different spray scenarios. In one experiment (single application experiment), five cotton plants were treated at an actual application rate of 209 g a.s./ha when the sixth true leaf was unfolded (BBCH 16), i.e. at an early growth stage. In another experiment, one cotton plant was treated two times, one time at an actual application rate of 209 g a.s./ha at BBCH 15 and a second time at an application rate of 176 g a.s./ha at 14 days before harvest of the cotton bolls (when more than 90% of the bolls were open and approx. 50% to 70% of the leaves were discoloured or fallen; BBCH 95 - 97). The total application rate in the double application experiment was 385 g a.s./ha.

Cotton seeds, gin trash and lint were harvested for analysis from the single application experiment at 169 days after the treatment and from the double application experiment at 14 days after the last treatment. In the single application experiment, additionally one plant was sampled at an intermediate growth stage (BBCH 22-24) to support the elucidation of metabolites.

TRR levels in the double application experiment were related to the higher application rate and the late application timing. Residues in gin trash and lint were considerably higher compared to the single application experiment. As expected, residues in seeds were not affected by the late second application. The TRR level in the cotton seeds, which represent the edible RAC, was low in the single and in the double application experiment: only 0.013 mg/kg and 0.016 mg/kg were found, respectively. As expected, high residues were detected in gin trash (2.767 mg/kg) and lint (4.993 mg/kg) in the double application experiment where the last treatment was performed 14 days before harvest when more than 90% of the cotton bolls were open. In the single application experiment, the TRR in gin trash accounted for 0.191 mg/kg and the TRR in lint was < 0.01 mg/kg. Due to the negligible residues, lint of the single application experiment was not subjected to extraction.

The main portion of radioactivity in the intermediate (single application experiment), gin trash (single and double application experiment) and lint (double application experiment) was extracted conventionally by acetonitrile/water mixtures. For gin trash, additional exhaustive extraction steps were applied to the solids of the conventional extraction. Exhaustive extraction comprised two extraction steps with acetonitrile/water mixtures at increased temperature (120 °C) under microwave

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

assistance. Extraction efficiencies ranged from 23.4% of the TRR for seeds (single application experiment) to 96.6% for lint (double application experiment) as shown in Table 6.2.1-31 and Table 6.2.1-32. Due to the low radioactivity level in seeds, no further attempts were performed to analyse the extracts or to release additional radioactivity. Analysis of the HPLC analysis of the conventional and exhaustive extracts of the intermediate, gin trash and lint after both spray scenarios revealed that all metabolite profiles were comparable. Additionally, it was shown that the metabolite profiles of all matrices were also nearly identical with those obtained in the cotton metabolism study conducted with [pyridinylmethyl-¹⁴C]BYI 02960. Thus, identification of compounds was based in a first step on the assignments made in the study with the other radiolabel. In a second step, the assignments were confirmed in the gin trash extracts of the present study by HPLC co-chromatography using authentic reference compounds. Corresponding metabolites in other matrices were assigned by comparison with the gin trash profile.

The main compound in all cotton matrices was parent compound, even in the single application experiment. The next prominent fraction comprised BYI 02960-OH-glyc and BYI 02960-acetic acid. These compounds co-eluted with the neutral HPLC method used for metabolite profiling. Therefore, the fraction was isolated from a gin trash extract and re-analysed with an acidic mobile phase. Both compounds were well separated using the acidic method and identification of both compounds became feasible by HPLC co-chromatography with authentic reference compounds. Separation of the compounds revealed that BYI 02960-acetic acid represented approx. 58% of the fraction in gin trash in the single application experiment and approx. 94% of the fraction in the double application experiment. The configuration of the hexose in BYI 02960-OH-glyc was identified as D-glucose in the corresponding apple metabolism study by the specific enzymatic treatment with β -glucosidase.

Besides parent compound and the fraction comprising BYI 02960-OH-glyc and BYI 02960-acetic acid, one additional major compound was detected only in gin trash. It was identified as BYI 02960-OH. All other metabolites detected in gin trash, lint and as well in the intermediate were minor metabolites. They did not exceed 10% of the TRR in the single and the double application experiment. Three of the minor metabolites were identified in gin trash by HPLC co-chromatography using authentic reference compounds: BYI 02960-glyoxylic acid, BYI 02960-acetic acid-glyc and BYI 02960-bromo (probably co-eluting with small amounts of BYI 02960-chloro). Assignment of the metabolites in other matrices was completed by comparison of all metabolite profiles

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Table 6.2.1-31: Distribution of radioactivity in the extracts of cotton matrices (intermediate, gin trash and seeds) after a single foliar application of [furanone-4-¹⁴C]BYI 02960

	single application experiment					
	intermediate		gin trash		seeds	
	TRR [mg/kg] =	12.391	0.191	0.013	% of TRR	mg/kg
Conventional extraction	90.3	11.194	69.4	0.133	23.4	0.003
Extract for analysis	89.9	11.141	66.9	0.128	---	---
Losses (not analysed)	0.4	0.053	2.6	0.005	23.4 [#]	0.003 [#]
Microwave extraction	---	---	10.9	0.021	---	---
Extract for analysis	---	---	10.9	0.021	---	---
Losses (not analysed)	---	---	n.q.	n.q.	---	---
Total extracted	90.3	11.194	80.3	0.153	23.4	0.003
Unextractable (PES*)	9.7	1.197	19.7	0.038	76.6	0.011
Accountability	100.0	12.391	100.0	0.191	100.0	0.013

* post extraction solids

the conventional extract was not further analyzed by HPLC due to the low radioactivity level

n.q. not quantified (< LOQ)

Table 6.2.1-32: Distribution of radioactivity in the extracts of cotton matrices (gin trash, lint and seeds) after two foliar applications of [furanone-4-¹⁴C]BYI 02960

	double application experiment					
	gin trash		lint		seeds	
	TRR [mg/kg] =	2.767	4.993	0.016	% of TRR	mg/kg
Conventional extraction	90.2	2.496	96.6	4.822	57.8	0.009
Extract for analysis	89.7	2.482	96.6	4.822	---	---
Losses (not analysed)	0.5	0.014	n.q.	n.q.	57.8 [#]	0.009 [#]
Microwave extraction	5.6	0.155	---	---	---	---
Extract for analysis	3.8	0.104	---	---	---	---
Losses (not analysed)	1.8	0.051	---	---	---	---
Total extracted	95.8	2.651	96.6	4.822	57.8	0.009
Unextractable (PES*)	4.2	0.116	3.4	0.170	42.2	0.007
Accountability	100.0	2.767	100.0	4.993	100.0	0.016

* post extraction solids

the conventional extract was not further analyzed by HPLC due to the low radioactivity level

n.q. not quantified (< LOQ)

The TRR and distribution of parent and metabolites in the extracts is shown in Table 6.2.1-33 and Table 6.2.1-34. In total, 70.3% to 86.0% of the TRR were identified in the matrices of cotton after foliar application. A summary of the results is given in Table 6.2.1-35.

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Table 6.2.1-33: TRR values and distribution of parent compound and metabolites in cotton matrices after a single foliar treatment of [furanone-4-¹⁴C]BYI 02960

	single application experiment			
	intermediate		gin trash	
	TRR [mg/kg] =	12.391	0.191	0.191
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg
<i>Conventional extraction</i>				
BYI 02960 (parent compound)	42.3	5.237	37.5	0.072
glyoxylic acid	---	---	0.9	0.002
acetic acid-glyc	8.6	1.068	---	---
OH-glyc / acetic acid	24.9	3.082	12.8	0.024
OH	---	---	12.0	0.023
bromo/chloro	0.7	0.089	0.6	0.001
Subtotal identified	76.5	9.476	63.7	0.122
unknown 1	2.2	0.268	2.6	0.005
unknown 2	---	---	---	---
unknown 3	1.0	0.122	---	---
unknown 4	---	---	0.6	0.001
unknown 5	6.6	0.823	---	---
unknown 6	---	---	---	---
unknown 7	2.5	0.310	---	---
unknown 8	---	---	---	---
unknown 9	---	---	---	---
unknown 10	---	---	---	---
unknown 11	1.1	0.141	---	---
unknown 12	---	---	---	---
Subtotal characterised	13.4	1.665	3.2	0.006
Total conventional extraction	89.9	11.141	66.9	0.128
<i>Microwave extraction</i>				
BYI 02960 (parent compound)			2.5	0.005
acetic acid-glyc			---	---
OH-glyc / acetic acid			2.9	0.006
OH			1.2	0.002
Subtotal identified			6.6	0.013
unknown 1			4.2	0.008
unknown 3			---	---
unknown 5			---	---
unknown 7			---	---
Subtotal characterised			4.2	0.008
Total microwave extraction	---	---	10.9	0.021
Total identified	76.5	9.476	70.3	0.134
Total characterised	13.4	1.665	7.4	0.014
Analysed extracts	89.9	11.141	77.7	0.148
Not analysed/losses [#]	0.4	0.053	2.6	0.005
Total extracted	90.3	11.194	80.3	0.153
Unextractable (PES*)	9.7	1.197	19.7	0.038
Accountability	100.0	12.391	100.0	0.191

* post extraction solid

losses during clean-up, concentration, etc

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.2.1-34: TRR values and distribution of parent compound and metabolites in cotton matrices after two foliar treatments of [furanone-4-¹⁴C]BYI 02960

	double application experiment			
	gin trash		lint	
	TRR [%]	mg/kg	% TRR	mg/kg
<i>Conventional extraction</i>				
BYI 02960 (parent compound)	53.3	1.476	70.3	3.512
glyoxylic acid	1.6	0.044	0.2	0.009
acetic acid-glyc	2.2	0.062	---	---
OH-glyc / acetic acid	20.0	0.553	13.9	0.694
OH	0.6	0.016	---	---
bromo/chloro	2.3	0.063	1.6	0.078
Subtotal identified	80.0	2.214	86.0	4.292
unknown 1	4.9	0.135	3.8	0.190
unknown 2	0.5	0.014	0.4	0.018
unknown 3	0.4	0.012	0.9	0.043
unknown 4	2.0	0.056	---	---
unknown 5	0.8	0.023	0.3	0.014
unknown 6	0.5	0.013	---	---
unknown 7	0.2	0.005	0.6	0.028
unknown 8	---	---	0.5	0.024
unknown 9	---	---	2.7	0.135
unknown 10	0.3	0.009	1.2	0.062
unknown 11	---	---	---	---
unknown 12	---	---	0.3	0.016
Subtotal characterised	9.7	0.268	10.6	0.530
Total conventional extraction	89.7	2.482	96.6	4.822
<i>Microwave extraction</i>				
BYI 02960 (parent compound)	1.1	0.029	---	---
acetic acid-glyc	<0.1	0.001	---	---
OH-glyc / acetic acid	0.8	0.023	---	---
OH	---	---	---	---
Subtotal identified	1.9	0.053	---	---
unknown 1	1.7	0.046	---	---
unknown 3	0.1	0.001	---	---
unknown 5	0.1	0.002	---	---
unknown 7	0.1	0.002	---	---
Subtotal characterised	1.8	0.051	---	---
Total microwave extraction	3.8	0.104	---	---
Total identified	81.9	2.268	86.0	4.292
Total characterised	11.5	0.319	10.6	0.530
Analysed extracts	93.4	2.586	96.6	4.822
Not analysed/losses [#]	2.3	0.065	<0.1	<0.001
Total extracted	95.8	2.651	96.6	4.822
Unextractable (PES*)	4.2	0.116	3.4	0.170
Accountability	100.0	2.767	100.0	4.993

* post extraction solid

losses during clean-up, concentration, etc

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.2.1-35: Summary of characterized and identified radioactive residues in cotton matrices after one or two foliar applications of [furanone-4-¹⁴C]BYI 02960

	single application exp.				double application exp.			
	intermediate		gin trash		gin trash		lint	
	TRR [mg/kg] =	12.391		0.191		2.767		4.993
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
BYI 02960 (parent compound)	42.3	5.237	40.0	0.076	54.4	1.505	70.3	3.512
glyoxylic acid	---	---	0.9	0.002	1.6	0.044	0.2	0.009
acetic acid-glyc	8.6	1.068	---	---	2.3	0.063	---	---
OH-glyc / acetic acid	24.9	3.082	15.7	0.030	20.8	0.577	13.9	0.694
OH	---	---	13.1	0.025	0.6	0.016	---	---
bromo/chloro	0.7	0.089	0.6	0.001	2.3	0.063	1.6	0.078
Total identified	76.5	9.476	70.3	0.134	81.9	2.268	86.0	4.292
unknown 1	2.2	0.268	6.8	0.013	6.5	0.181	3.8	0.190
unknown 2	---	---	---	---	0.5	0.014	0.4	0.018
unknown 3	1.0	0.122	---	---	0.5	0.014	0.9	0.043
unknown 4	---	---	0.6	0.001	2.0	0.056	---	---
unknown 5	6.6	0.823	---	---	0.9	0.024	0.3	0.014
unknown 6	---	---	---	---	0.5	0.013	---	---
unknown 7	2.5	0.310	---	---	0.3	0.007	0.6	0.028
unknown 8	---	---	---	---	---	---	0.5	0.024
unknown 9	---	---	---	---	---	---	2.7	0.135
unknown 10	---	---	---	---	0.3	0.009	1.2	0.062
unknown 11	1.1	0.141	---	---	---	---	---	---
unknown 12	---	---	---	---	---	---	0.3	0.016
Total characterised	13.4	1.665	7.4	0.014	11.5	0.319	10.6	0.530
Analysed extracts	89.9	11.141	77.7	0.148	93.4	2.586	96.6	4.822
Not analysed/losses [#]	0.4	0.053	2.6	0.005	2.3	0.065	<0.1	<0.001
Total extracted	90.3	11.194	80.3	0.153	95.8	2.651	96.6	4.822
Unextractable (PES*)	9.7	1.197	19.7	0.038	4.2	0.116	3.4	0.170
Accountability	100.0	12.391	100.0	0.191	100.0	2.767	100.0	4.993

* post extraction solids

losses during clean-up, concentration, etc

III. Conclusions

Metabolism of BYI 02960 was moderate in cotton. The residues were dominated by parent compound in both the single and double application experiments. Cotton gin trash was the matrix showing the highest number of metabolites.

Three major metabolic routes of [furanone-4-¹⁴C]BYI 02960 were observed in cotton:

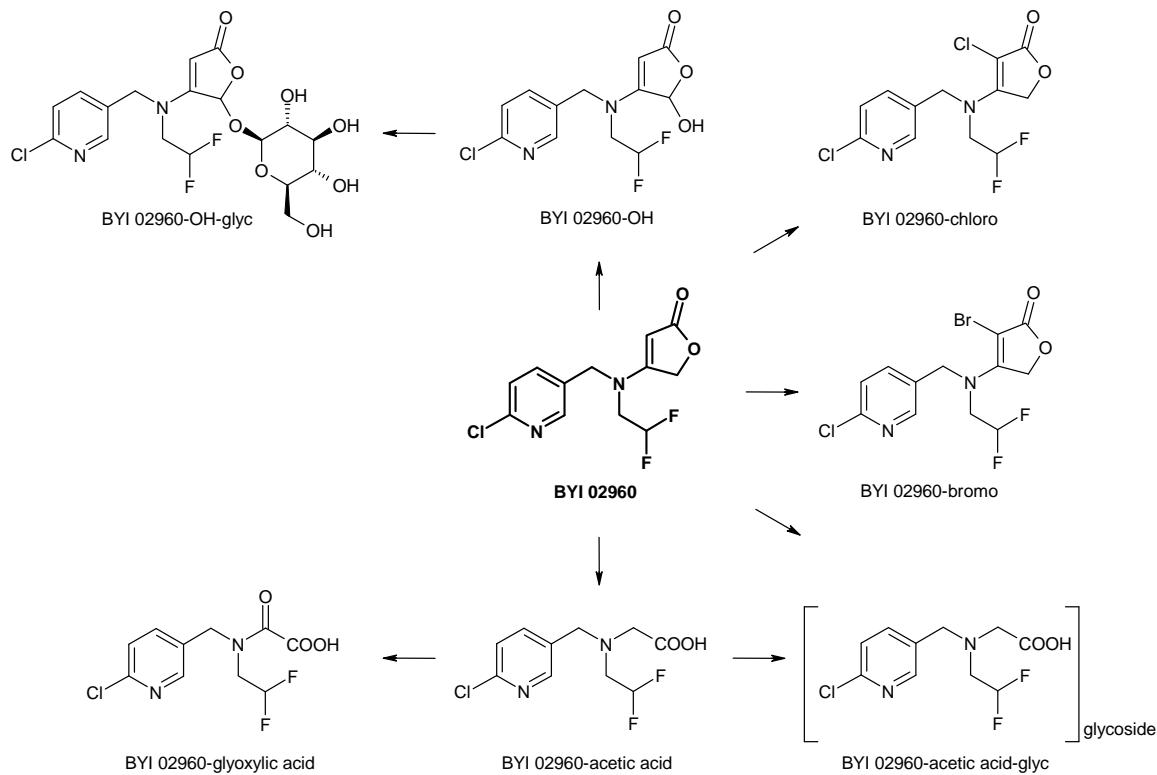
- hydroxylation of the methylene group of the furanone moiety followed by conjugation (i.e. glycosylation),
- oxidative degradation of the furanone moiety to an acetic acid group followed either by conjugation with a carbohydrate or by further oxidation, and
- halogenation (bromination/chlorination) of the furanone moiety.

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Halogenation of the furanone moiety of the active substance probably occurred in the soil which was supported by the fact that small amounts of halogenated parent compound were identified in the aerobic soil degradation studies. Due to the early timing of the first application, a significant portion of the parent compound could have reached the soil and can therefore been subjected to soil metabolism processes.

On the basis of the results of this study it is concluded that the metabolism of [furanone-4-¹⁴C]BYI 02960 in cotton is well understood and the following metabolic pathway is proposed.

Figure 6.2.1-11: Proposed metabolic pathway of [furanone-4-¹⁴C]BYI 02960 in cotton





Report:	KIIA 6.2.1/09, Schmeling, S., Weber, E.; 2011
Title:	Metabolism of [pyridinylmethyl- ¹⁴ C]BYI 02960 in Cotton after Spray Application
Report No & Edition No	MEF-11/393 M-421691-01-2
Guidelines:	OECD 501 Metabolism in Crops US EPA Residue Chemistry Test Guideline OPPTS 860.1300: Nature of the Residue – Plants, Livestock PMRA Regulatory Directive Dir98-02: Residue Chemistry Guidelines Section 2: Nature of the Residue – Plants, Livestock Japanese MAFF, 12 Nousan 8147 European Parliament and Council Regulation (EC) No 1107/2009
GLP	yes

Executive Summary

The metabolism of [pyridinylmethyl-¹⁴C]BYI 02960 formulated as an SL 200 was investigated in cotton after foliar spray application. In one experiment (single application experiment), five cotton plants were treated at an actual application rate of 206 g a.s./ha when the sixth true leaf was unfolded (BBCH 16), i.e. at an early growth stage. In another experiment, one cotton plant was treated two times, one time at an actual application rate of 206 g a.s./ha at BBCH 16 and a second time at an application rate of 177 g a.s./ha at 15 days before harvest of the cotton bolls (when more than 90% of the bolls were open and approx. 50% to 70% of the leaves were discoloured or fallen; BBCH 95 - 97). The total application rate in the double application experiment was 383 g a.s./ha.

Samples were taken from both experiments. Cotton seeds and gin trash were the raw agricultural commodities (RACs) harvested at maturity of the seeds in this study. Lint was collected as additional sample material at the same time. Additionally, one plant of the single application experiment was sampled at an intermediate growth stage (two to four vegetative side shoots were visible; BBCH 22 – 24) to support the identification of metabolites and to possibly obtain additional information on the metabolic degradation behaviour of BYI 02960. The TRR values of all plant matrices determined are shown in the following table:

Table 6.2.1-36: TRR values in cotton (intermediate, gin trash, lint, seeds) after foliar application of [pyridinylmethyl-¹⁴C]BYI 02960

Matrix	Timing and Application	PHI (days)*	ppm (mg a.s. equiv./kg)
intermediate	one spray application, 206 g a.s./ha (at BBCH 16)	28	14.153
gin trash		169	0.310
lint		169	0.007
seeds		169	0.045
gin trash	two spray applications, 206 g a.s./ha (at BBCH 16) 177 g a.s./ha (at BBCH 95 – 97)	15	2.344
lint		15	8.846
seeds		15	0.068

* PHI: preharvest interval (corresponds to days after last treatment (DAT) at the start of harvest/sampling)

The radioactive residues were extracted with acetonitrile/water mixtures. In the case of seeds, the samples were extracted with heptane beforehand. When necessary, additional exhaustive extraction steps were applied to the solids of the first extraction. Extraction efficiencies ranged from 28.3% of the



TRR for seeds (single application experiment) to 99.2% for lint (double application experiment). The profiles of the extracts comprising a reasonable amount of radioactivity were recorded by HPLC, and all major and several minor components were identified. Parent compound and major metabolites were isolated from a representative matrix and identified either by HPLC and TLC co-chromatography (two different chromatographic systems) using authentic reference compounds or by LC-MS/MS. Minor compounds were assigned based on the metabolite profiles obtained in the cotton metabolism study with [furanone-4-¹⁴C]BYI 02960. The identification rates ranged from 16.2% of the TRR for seeds (single application experiment) to 89.7% for lint (double application experiment).

[Pyridinylmethyl-¹⁴C]BYI 02960 was metabolised moderately in cotton. Parent compound was the most prominent component in all matrices, except for seeds of the single application experiment, and accounted for approx. 23% to 73% of the TRR. Additional major metabolites detected in cotton matrices were BYI 02960-acetic acid, BYI 02960-OH and the label-specific metabolite 6-CNA. All other metabolites detected represented minor or trace components.

The following metabolic routes of [pyridinylmethyl-¹⁴C]BYI 02960 were observed in cotton:

- hydroxylation of the methylene group of the furanone moiety followed by conjugation,
- cleavage of the pyridinylmethylamine bond followed by oxidation of the methylene group to 6-CNA
- oxidative opening of the furanone moiety to an acetic acid group followed by further oxidation or conjugation with a carbohydrate (i.e. glycosylation), and
- halogenation (mostly bromination, to a minor extent chlorination) of the furanone moiety

Halogenation of the furanone moiety of the active substance probably occurred in the soil which was supported by the fact that small amounts of halogenated parent compound were identified in the aerobic soil degradation studies. Due to the early timing of the first application, a significant portion of the parent compound could have reached the soil and been subjected to soil metabolism processes.

On the basis of these results, a metabolic pathway of [pyridinylmethyl-¹⁴C]BYI 02960 in cotton can be proposed.



I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	 * position of the radiolabel
Radiolabelled test material	[pyridinylmethyl- ¹⁴ C]BYI 02960
Specific radioactivity	4.37 MBq/mg (118.08 µCi/mg)
Chemical Purity	> 99% (HPLC)
Radiochemical purity	> 98% (HPLC and TLC)

The supplied radiolabelled test compound [pyridinylmethyl-¹⁴C]BYI 02960 was dissolved in acetonitrile. Formulation of the test compound was performed prior to each application. For the preparation of the SL 200 formulation, an adequate portion of the stock solution was transferred into a special glass vial and evaporated to dryness. The liquid blank formulation was added to the test item using a magnetic stirrer. The mixture was diluted with water to get the aqueous application solution.

2. Soil: "Einheitserde T" (standard soil containing 2 kg/m³ of a water soluble salt mixture), pH (CaCl₂) = 5.8, 85% white moor peat, 15% clay, containing NO₃⁻, NH₄⁺, P₂O₅, K₂O, Fe, Mn, B, Cu, Mo, Zn; distributer: Balster Einheitserdewerk, Eulenstr. 53; 58730 Froendenberg, Germany

3. Plant Cotton, variety "Carmen", Gossypium hirsutum, representative for oil seeds

B. Study Design

Experimental conditions:

Two experiments were performed with a total of six cotton plants of the variety "Carmen". The experiments represented the intended foliar spray application scenarios of BYI 02960 in cotton. In one experiment (single foliar spray application), the plants were treated at a target application rate of 200 g a.s./ha when the sixth true leaf was unfolded (BBCH 16). In the other experiment (double foliar spray application), one cotton plant was treated two times. The target application rate was 200 g a.s./ha at each application. The first application was conducted at BBCH 16 and the second at 15 days before harvest. The total target rate of 400 g a.s./ha corresponds to the anticipated maximum application rate. The cotton plants were cultivated in the standard soil "Einheitserde T" in planting pots with a surface area of approx. 0.076 m² and a volume of 15 L. The plants were grown in the greenhouse of the test facility which allows cultivation similar to natural temperature and light conditions. The plants were irrigated according to their needs.

Sampling:

In both experiments cotton seeds (= undelinted seeds), lint and gin trash were harvested at maturity of the seeds (BBCH 99). The cotton bolls (capsules) were cut from the plants and the seeds were



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separated manually from the lint. No chemical process followed to delint the seeds. The remaining plants (= gin trash) were cut off just above the soil surface and were shredded into small pieces. The gin trash chaff was combined with the empty capsules which had contained the lint and the seeds. Gin trash and seed samples were further processed using a Polytron homogenizer and liquid nitrogen. The homogenized samples were stored in a freezer ($\leq -18^{\circ}\text{C}$) until analysis. Lint samples of the double application experiment were not submitted to any sample preparation steps and were stored in a freezer ($\leq -18^{\circ}\text{C}$) until analysis.

In the single application experiment, additionally an intermediate was sampled at an early growth stage (two to four vegetative side shoots were visible; BBCH 22 – 24) to support the identification of metabolites. The plant was cut off just above the soil surface and was cut in small pieces prior to extraction and analysis.

C. Analytical Procedures

Extraction:

Aliquots of all sample matrices were extracted three times with a mixture of acetonitrile/water (8:2, v/v) using a high speed blender. The cotton seed samples were additionally extracted with heptane prior to the first acetonitrile/water extraction step. The radioactivity in the extracts was determined by LSC and in the solids by combustion followed by LSC. The actual TRR value of the RAC was determined by summing up the radioactivity measured in the extracts and in the remaining solids. All extracts were combined. The intermediate, gin trash and seed extracts were subjected to a clean-up step, whereas the lint extract was directly analysed by HPLC analysis after concentration. The clean-up was performed using an SPE RP 18 cartridge (Phenomenex, Strata C18-E, 20 g), which was conditioned with methanol, water and an acetonitrile/water mixture beforehand. The flow-through fraction (percolate) was collected and the cartridge was rinsed with acetonitrile/water (8:2, v/v). Elution of retained compounds was performed with methanol/dichloromethane (1:1, v/v). The percolate and the acetonitrile/water fraction (= rinse) were combined and concentrated by rotary evaporation in vacuo prior to chromatographic analysis by HPLC. The solids remaining after the conventional extraction steps of the gin trash samples were additionally submitted two times to microwave-assisted extraction with acetonitrile/water mixtures (8:2, v/v and 1:1, v/v). After each extraction step, extracts and solids were separated by filtration. The extracts were combined and concentrated by rotary evaporation in vacuo prior to HPLC analysis.

Quantification:

Parent compound and metabolites in the extracts were analysed by reversed phase HPLC coupled to a radioactivity detector with a glass scintillator cell. The HPLC chromatograms (= metabolite profiles) were integrated for quantification of compounds.

Identification and characterisation:

Identification of parent compound and all major metabolites was performed either by HPLC and TLC co-chromatography or by mass spectroscopic means after semi-preparative isolation of the compounds from a representative matrix. Minor metabolites were identified based on the assignments made in the cotton metabolism study with [furanone-4- ^{14}C]BYI 02960. In that study all minor metabolites were identified in a representative matrix by HPLC co-chromatography with an authentic reference compound. Assignment in the other matrices was performed by comparison of HPLC profiles since

the metabolite profiles of all matrices were very well comparable within both cotton metabolism studies.

Storage stability:

Extraction experiments and the first HPLC analyses for quantification were performed within 9 weeks (61 days) after harvest of the cotton plants. The extracts were analysed after 6 days following the start of extraction at the latest. An exception was the analysis of the seed extracts. The extracts of the seeds of the single application experiment (1x) and the seeds of the double application experiment (2x) were analysed 35 days and 49 days following the start of extraction, respectively. The storage stability of the compounds present in the seed extracts was shown by repeating the extraction of seeds of the single application experiment after 5 months. HPLC analysis was done 8 days following the start of extraction. The metabolite profile looked very similar to the one of the first extraction. Hence, the first profile was deemed adequate for quantification despite the prolonged storage period of the extract. Additionally, stability of parent compound, 6-CNA, BYI 02960-acetic acid, BYI 02960-OH-glyc and BYI 02960-OH was proven in a grain extract for at least 21 months in the confined rotational crop study with [pyridinylmethyl-¹⁴C]BYI 02960. Therefore, analysis of the extracts of seeds and quantification of the resulting metabolic profiles was deemed acceptable despite the prolonged storage period.

Since the first metabolic analysis of the RACs as well as the last experiments with extracts (HPLC co-chromatography) took place within 5.5 months, no further stability investigations were necessary according to OECD Guidance for the Testing of Chemicals 501 (2007).

II. Results and Discussion

The metabolism of [pyridinylmethyl-¹⁴C]BYI 02960 was investigated in cotton seeds, gin trash and lint following two different spray scenarios. In one experiment (single application experiment), five cotton plants were treated at an actual application rate of 206 g a.s./ha when the sixth true leaf was unfolded (BBCH 16), i.e. at an early growth stage. In another experiment, one cotton plant was treated two times, one time at an actual application rate of 206 g a.s./ha at BBCH 16 and a second time at an application rate of 177 g a.s./ha at 15 days before harvest of the cotton bolls (when more than 90% of the bolls were open and approx. 50% to 70% of the leaves were discoloured or fallen; BBCH 95 - 97). The total application rate in the double application experiment was 383 g a.s./ha.

Cotton seeds, gin trash and lint were harvested for analysis from the single application experiment at 169 days after the treatment and from the double application experiment at 15 days after the last treatment. In the single application experiment, additionally one plant was sampled at an intermediate growth stage (BBCH 22-24) to support the elucidation of metabolites.

TRR levels in the double application experiment were related to the higher application rate and the late application timing. Residues in gin trash and lint were considerably higher compared to the single application experiment. As expected, residues in seeds were not strongly affected by the late second application. The TRR level in the cotton seeds, which represent the edible RAC, was low in the single and in the double application experiment: only 0.045 mg/kg and 0.068 mg/kg were found, respectively. As expected, high residues were detected in gin trash (2.344 mg/kg) and lint

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(8.846 mg/kg) in the double application experiment where the last treatment was performed 15 days before harvest when more than 90% of the cotton bolls were open. In the single application experiment, the TRR in gin trash accounted for 0.310 mg/kg and the TRR in lint was < 0.01 mg/kg. Due to the negligible residues, lint of the single application experiment was not subjected to extraction.

The main portion of radioactivity in the intermediate (single application experiment), gin trash (single and double application experiment) and lint (double application experiment) was extracted conventionally by acetonitrile/water mixtures. For gin trash, additional exhaustive extraction steps were applied to the solids of the conventional extraction. Exhaustive extraction comprised two extraction steps with acetonitrile/water mixtures at increased temperature (120 °C) under microwave assistance. Extraction efficiencies ranged from 28.3% of the TRR for seeds (single application experiment) to 99.2% for lint (double application experiment) as shown in Table 6.2.1-37 and Table 6.2.1-38. HPLC analysis of the conventional and exhaustive extracts of the intermediate, gin trash, seeds and lint after both spray scenarios revealed that all metabolite profiles were very well comparable. The conventional extracts of gin trash showed the highest number of metabolites along with the extract of the intermediate. Additionally, it was shown that the metabolite profiles of all matrices were nearly identical with those obtained in the cotton metabolism study conducted with [furanone-4-¹⁴C]BYI 02960.

Parent compound and major metabolites were identified either by HPLC and TLC co-chromatography (two independent chromatographic systems) or by mass spectroscopic means (LC-MS/MS) in the gin trash extracts. Minor compounds were assigned by comparison with the corresponding profiles of the cotton metabolism study with [furanone-4-¹⁴C]BYI 02960.

The main compound in all cotton matrices, except for seeds of the single application experiment, was parent compound, even after only one treatment. In seeds of the single application experiment, 6-CNA was identified as main compound. This finding corresponds with the known fact that weak acids have a pronounced phloem mobility and are therefore transported selectively into the seeds as a phloem sink. Initial formation of 6-CNA took most probably place in the leaves, which is in line with the finding that 6-CNA is also the main metabolite detected in gin trash. 6-CNA was also detected in the seeds of the double application experiment, however quite high amounts of parent compound and the co-eluting compounds BYI 02960-OH-glyc and BYI 02960-acetic acid superposed the 6-CNA amounts. Most probably the high amounts of these compounds were caused by “contamination” of the seeds by lint residues. The reason contamination is proposed is that in the cotton bolls (capsules) the seeds are imbedded in the lint. Separation of seeds and lint was performed manually and resulted in undelinted seeds, i.e. the seeds were still covered by lint fibres. Thus it is not uncommon to detect the major residues of lint as well in the seed extract. Since lint showed only high residues in the double application experiment, interfering residues were only detected in seeds of this experiment.

The fraction comprising the metabolites BYI 02960-OH-glyc and BYI 02960-acetic acid represented a major portion of the TRR in all sample matrices except for seeds. These compounds co-eluted when using the neutral HPLC method for metabolite profiling. Therefore, the fraction was isolated from the gin trash extracts and was re-analysed with an acidic mobile phase. Both compounds were very well separated using the acidic method and identification of both compounds became feasible by either

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HPLC co-chromatography with an authentic reference compound (BYI 02960-OH-glyc) or by LC-MS/MS (BYI 02960-acetic acid). The authentic reference compound used for co-chromatography was a metabolite isolated and identified in the apple metabolism study. The hexose in the conjugate was unambiguously assigned to D-glucose and the hydroxy group was located in the 5-position of the molecule. Separation of the compounds revealed that BYI 02960-acetic acid represented approx. 67% of the fraction in gin trash in the single application experiment and approx. 94% of the fraction in the double application experiment.

In gin trash of the first application experiment, BYI 02960-OH was identified as an additional major metabolite. All other metabolites detected in gin trash, seeds and lint and as well in the intermediate were minor, they did not exceed 10% of the TRR in either the single or the double application experiment. In a first step they were identified in gin trash by HPLC comparison. The metabolite profiles of the gin trash extracts were compared with the corresponding profiles obtained in the cotton metabolism study with [furanone-4-¹⁴C]BYI 02960. Assignment of the metabolites in all other matrices was completed by comparison of the gin trash profiles with all other metabolite profiles.

Table 6.2.1-37: Distribution of radioactivity in the extracts of cotton matrices (intermediate, gin trash and seeds) after a single foliar application of [pyridinylmethyl-¹⁴C]BYI 02960

TRR [mg/kg] =	single application experiment					
	intermediate		gin trash		seeds	
	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Conventional extraction	95.0	13.440	81.5	0.253	28.3	0.013
Extract for analysis	94.2	13.336	80.6	0.250	21.9	0.010
Losses (not analysed)	0.7	0.105	0.9	0.003	6.5	0.003
Microwave extraction	---	---	10.4	0.032	---	---
Extract for analysis	---	---	10.4	0.032	---	---
Losses (not analysed)	---	---	n.q.	n.q.	---	---
Total extracted	95.0	13.440	92.0	0.285	28.3	0.013
Unextractable (PES*)	5.0	0.713	8.0	0.025	71.7	0.032
Accountability	100.0	14.153	100.0	0.310	100.0	0.045

* post extraction solids
n.q. not quantified (< LOQ)

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Table 6.2.1-38: Distribution of radioactivity in the extracts of cotton matrices (gin trash, lint and seeds) after two foliar applications of [pyridinylmethyl-¹⁴C]BYI 02960

	double application experiment					
	Gin trash		Lint		Seeds	
	TRR [mg/kg] =	2.344	8.846	0.068		
	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Conventional extraction	89.3	2.094	99.2	8.774	66.1	0.045
Extract for analysis	88.7	2.079	99.2	8.774	38.5	0.026
Losses (not analysed)	0.6	0.014	n.q.	n.q.	27.6	0.019
Microwave extraction	7.9	0.185	---	---	---	---
Extract for analysis	4.7	0.110	---	---	---	---
Losses (not analysed)	3.2	0.075	---	---	---	---
Total extracted	97.2	2.279	99.2	8.774	66.1	0.045
Unextractable (PES*)	2.8	0.065	0.8	0.073	33.9	0.023
Accountability	100.0	2.344	100.0	8.846	100.0	0.068

* post extraction solids

The TRRs and the distribution of parent and metabolites in the extracts is shown in Table 6.2.1-39 and Table 6.2.1-40. In total, 16.2% to 89.7% of the TRR were identified in the matrices of cotton after foliar application. A summary of the results is given in Table 6.2.1-41 and Table 6.2.1-42.

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Table 6.2.1-39: TRR values and distribution of parent compound and metabolites in cotton matrices after a single foliar treatment of [pyridinylmethyl-¹⁴C]BYI 02960

	single application experiment					
	intermediate		gin trash		seeds	
	TRR [%]	mg/kg	TRR [%]	mg/kg	TRR [%]	mg/kg
TRR [mg/kg] =	14.153		0.310		0.045	
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
<i>Conventional extraction</i>						
BYI 02960 (parent compound)	36.9	5.221	24.7	0.077	---	---
6-CNA	2.1	0.298	18.5	0.057	16.2	0.007
glyoxylic acid	1.5	0.209	2.1	0.007	---	---
acetic acid-glyc	6.4	0.899	---	---	---	---
OH-glyc / acetic acid	25.1	3.559	12.0	0.037	---	---
OH	1.2	0.168	13.4	0.042	---	---
bromo/chloro	0.5	0.064	---	---	---	---
Subtotal identified	73.6	10.419	70.7	0.219	16.2	0.007
unknown 1	---	---	2.5	0.008	5.7	0.003
unknown 2	---	---	0.6	0.002	---	---
unknown 3	1.4	0.196	---	---	---	---
unknown 4	2.8	0.403	3.4	0.010	---	---
unknown 5	1.1	0.159	1.0	0.003	---	---
unknown 6	6.6	0.935	0.6	0.002	---	---
unknown 7	3.9	0.551	---	---	---	---
unknown 10	1.9	0.270	---	---	---	---
unknown 11	1.9	0.273	1.2	0.004	---	---
unknown 12	0.9	0.130	0.6	0.002	---	---
Subtotal characterised	20.6	2.917	9.9	0.031	5.7	0.003
Total conventional extraction¹	94.2	13.336	80.6	0.250	21.9	0.010
<i>Microwave extraction</i>						
BYI 02960 (parent compound)			1.6	0.005		
6-CNA			1.7	0.005		
OH-glyc / acetic acid			1.7	0.005		
OH			1.1	0.003		
Subtotal identified			6.1	0.019		
unknown 1			3.8	0.012		
unknown 11			0.5	0.002		
Subtotal characterised			4.3	0.013		
Total microwave extraction	---	---	10.4	0.032	---	---
Total identified	73.6	10.419	76.8	0.238	16.2	0.007
Total characterised	20.6	2.917	14.2	0.044	5.7	0.003
Analysed extracts	94.2	13.336	91.0	0.282	21.9	0.010
Not analysed/losses ²	0.7	0.105	0.9	0.003	6.5	0.003
Total extracted	95.0	13.440	92.0	0.285	28.3	0.013
Unextractable (PES*)	5.0	0.713	8.0	0.025	71.7	0.032
Accountability	100.0	14.153	100.0	0.310	100.0	0.045

* post extraction solid

¹ analysed extracts only

² losses during clean-up, concentration, etc

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Table 6.2.1-40: TRR values and distribution of parent compound and metabolites in cotton matrices after two foliar treatments of [pyridinylmethyl-¹⁴C]BYI 02960

	double application experiment					
	gin trash		lint		seeds	
TRR [mg/kg] =	2.344		8.846		0.068	
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
<i>Conventional extraction</i>						
BYI 02960 (parent compound)	51.3	1.204	73.0	6.455	23.4	0.016
6-CNA	2.0	0.047	0.4	0.031	5.0	0.003
glyoxylic acid	1.5	0.035	0.2	0.015	---	---
acetic acid-glyc	3.6	0.085	---	---	---	---
OH-glyc / acetic acid	20.6	0.484	14.6	1.295	4.9	0.003
OH	1.3	0.030	---	---	---	---
bromo/chloro	2.1	0.049	1.6	0.140	---	---
Subtotal identified	82.6	1.935	89.7	7.936	33.3	0.023
unknown 1	0.4	0.010	---	---	---	---
unknown 2	0.2	0.005	---	---	---	---
unknown 3	0.1	0.004	---	---	---	---
unknown 4	2.2	0.052	---	---	---	---
unknown 5	1.3	0.031	0.3	0.027	---	---
unknown 6	---	---	0.6	0.051	---	---
unknown 7	---	---	0.7	0.064	---	---
unknown 8	---	---	4.9	0.432	---	---
unknown 9	---	---	1.5	0.129	---	---
unknown 10	0.4	0.009	0.3	0.023	---	---
unknown 11	1.4	0.033	0.8	0.070	5.2	0.003
unknown 12	---	---	0.5	0.040	---	---
Subtotal characterised	6.1	0.144	9.5	0.837	5.2	0.003
Total conventional extraction¹	88.7	2.079	99.2	8.774	38.5	0.026
<i>Microwave extraction</i>						
BYI 02960 (parent compound)	1.9	0.044	---	---	---	---
6-CNA	0.2	0.005	---	---	---	---
acetic acid-glyc	0.1	0.002	---	---	---	---
OH-glyc / acetic acid	1.8	0.042	---	---	---	---
Subtotal identified	3.9	0.092	---	---	---	---
unknown 1	0.1	0.004	---	---	---	---
unknown 2	0.1	0.002	---	---	---	---
unknown 5	0.1	0.003	---	---	---	---
unknown 6	0.2	0.004	---	---	---	---
unknown 11	0.2	0.006	---	---	---	---
Subtotal characterised	0.8	0.018	---	---	---	---
Total microwave extraction	4.7	0.110	---	---	---	---
Total identified	86.5	2.028	89.7	7.936	33.3	0.023
Total characterised	6.9	0.162	9.5	0.837	5.2	0.003
Analysed extracts	93.4	2.190	99.2	8.774	38.5	0.026
Not analysed/losses ²	3.8	0.089	<0.1	<0.001	27.6	0.019
Total extracted	97.2	2.279	99.2	8.774	66.1	0.045
Unextractable (PES*)	2.8	0.065	0.8	0.073	33.9	0.023
Accountability	100.0	2.344	100.0	8.846	100.0	0.068

^{*} post extraction solid¹ analysed extracts only² losses during clean-up, concentration, etc

Table 6.2.1-41: Summary of characterized and identified radioactive residues in cotton matrices after one foliar application of [pyridinylmethyl-¹⁴C]BYI 02960

	single application experiment					
	intermediate		gin trash		seeds	
	TRR [mg/kg] =	14.153	0.310	0.045		
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
BYI 02960 (parent compound)	36.9	5.221	26.3	0.082	---	---
6-CNA	2.1	0.298	20.2	0.063	16.2	0.007
glyoxylic acid	1.5	0.209	2.1	0.007	---	---
acetic acid-glyc	6.4	0.899	---	---	---	---
OH-glyc / acetic acid	25.1	3.559	13.7	0.043	---	---
OH	1.2	0.168	14.5	0.045	---	---
bromo/chloro	0.5	0.064	---	---	---	---
Total identified	73.6	10.419	76.8	0.238	16.2	0.007
unknown 1	---	---	6.4	0.020	5.7	0.003
unknown 2	---	---	0.6	0.002	---	---
unknown 3	1.4	0.196	---	---	---	---
unknown 4	2.8	0.403	3.4	0.010	---	---
unknown 5	1.1	0.159	1.0	0.003	---	---
unknown 6	6.6	0.935	0.6	0.002	---	---
unknown 7	3.9	0.551	---	---	---	---
unknown 10	1.9	0.270	---	---	---	---
unknown 11	1.9	0.273	1.7	0.005	---	---
unknown 12	0.9	0.130	0.6	0.002	---	---
Total characterised	20.6	2.917	14.2	0.044	5.7	0.003
Analysed extracts	94.2	13.336	91.0	0.282	21.9	0.010
Not analysed/losses [#]	0.7	0.105	0.9	0.003	6.5	0.003
Total extracted	95.0	13.440	92.0	0.285	28.3	0.013
Unextractable (PES*)	5.0	0.713	8.0	0.025	71.7	0.032
Accountability	100.0	14.153	100.0	0.310	100.0	0.045

* post extraction solids

#: losses during clean-up, concentration, etc

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 Table 6.2.1-42: Summary of characterized and identified radioactive residues in cotton matrices after two foliar applications of [pyridinylmethyl-¹⁴C]BYI 02960

	double application experiment					
	gin trash		lint		seeds	
	TRR [mg/kg] =	2.344	8.846	0.068		
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
BYI 02960 (parent compound)	53.2	1.247	73.0	6.455	23.4	0.016
6-CNA	2.2	0.053	0.4	0.031	5.0	0.003
glyoxylic acid	1.5	0.035	0.2	0.015	---	---
acetic acid-glyc	3.7	0.087	---	---	---	---
OH-glyc / acetic acid	22.4	0.526	14.6	1.295	4.9	0.003
OH	1.3	0.030	---	---	---	---
bromo/chloro	2.1	0.049	1.6	0.140	---	---
Total identified	86.5	2.028	89.7	7.936	33.3	0.023
unknown 1	0.6	0.013	---	---	---	---
unknown 2	0.3	0.007	---	---	---	---
unknown 3	0.1	0.004	---	---	---	---
unknown 4	2.2	0.052	---	---	---	---
unknown 5	1.5	0.034	0.3	0.027	---	---
unknown 6	0.2	0.004	0.6	0.051	---	---
unknown 7	---	---	0.7	0.064	---	---
unknown 8	---	---	4.9	0.432	---	---
unknown 9	---	---	1.5	0.129	---	---
unknown 10	0.4	0.009	0.3	0.023	---	---
unknown 11	1.6	0.039	0.8	0.070	5.2	0.003
unknown 12	---	---	0.5	0.040	---	---
Total characterised	6.9	0.162	9.5	0.837	5.2	0.003
Analysed extracts	93.4	2.190	99.2	8.774	38.5	0.026
Not analysed/losses [#]	3.8	0.089	<0.1	<0.001	27.6	0.019
Total extracted	97.2	2.279	99.2	8.774	66.1	0.045
Unextractable (PES*)	2.8	0.065	0.8	0.073	33.9	0.023
Accountability	100.0	2.344	100.0	8.846	100.0	0.068

* post extraction solids

#: losses during clean-up, concentration, etc

III. Conclusions:

[Pyridinylmethyl-¹⁴C]BYI 02960 is metabolised moderately in cotton. In both the double and single application experiments, residues were dominated by parent compound except for seeds receiving a single foliar application. In total, seven different metabolites were identified in gin trash and the intermediate, which showed the most pronounced metabolite patterns.

Generally, four major metabolic routes of [pyridinylmethyl-¹⁴C]BYI 02960 were observed in cotton:

- oxidative degradation of the furanone moiety to an acetic acid group followed by conjugation with a carbohydrate or by further oxidation,
- cleavage of the pyridinylmethylamine bond followed by oxidation of the methylene group to 6-CNA,

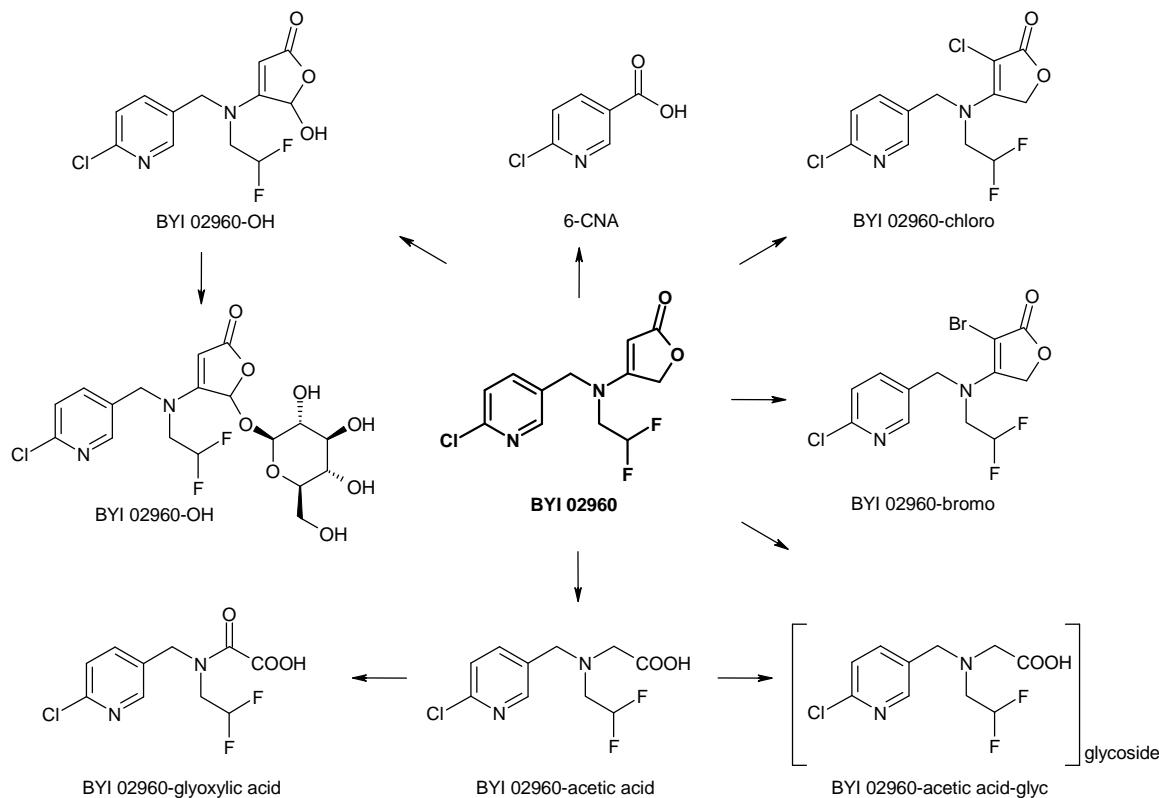
Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

- hydroxylation of the methylene group of the furanone moiety, followed by further conjugation steps (i.e. glycosylation), and
- halogenation (bromination and most probably chlorination, as well) of the furanone moiety.

Halogenation of the furanone moiety of the active substance probably occurred in the soil which is supported by the fact that small amounts of halogenated parent compound were identified in the aerobic soil degradation studies. Due to the early timing of the first application, a significant portion of the parent compound could have reached the soil and been subjected to soil metabolism processes.

On the basis of the results of this study it is concluded that the metabolism of [pyridinylmethyl-¹⁴C]BYI 02960 in cotton is well understood and the following metabolic pathway is proposed.

Figure 6.2.1-12: Proposed metabolic pathway of [pyridinylmethyl-¹⁴C]BYI 02960 in cotton



Overall Conclusions Cotton (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-4-¹⁴C]BYI 02960 or (2) [pyridinylmethyl-¹⁴C]BYI 02960. In the single spray application experiment, cotton plants were treated once at an early growth stage (BBCH 16). In the double spray



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application experiment, one plant was treated at the early growth stage (BBCH 15) and additionally 14 days before harvest. The target rate per application in both experiments was 200 g a.s./ha.

In order to gain information on the fate of the difluoroethane moiety of BYI 02960, the extracts obtained in the cotton metabolism study with [pyridinylmethyl-¹⁴C]BYI 02960 were additionally analysed for non-radiolabelled difluoroacetic acid by LC-MS/MS according to residue method 01304 (see KIIA 6.2./12).

(1) Label 1: [furanone-4-¹⁴C]BYI 02960

At harvest, the radioactive residues in cotton seeds (edible commodity) were very low in both experiments and did not exceed 0.016 mg/kg. Analysis of the extracts was not feasible and therefore, elucidation of the metabolic behaviour of BYI 02960 was based on the gin trash extracts of both experiments, an intermediate extract of the single application experiment and the lint extract of the double application experiment. As expected, the residues in the double spray application experiment were dominated by parent compound. However, even after the single application, parent compound was the main residue in all matrices analysed. One major metabolite fraction was detected in all matrices under investigation, comprising the co-eluting metabolites BYI 02960-acetic acid and BYI 02960-OH-glyc. BYI 02960-acetic acid represented the main part of the fraction in the double application experiment, whereas the dominance was less pronounced in the single application experiment. In gin trash, BYI 02960-OH was detected as additional major metabolite (12% of the TRR). All other metabolites represented less than 10% of the TRR in all matrices, with BYI 02960-acetic acid-glyc showing the highest portion (9% of the TRR) in the intermediate. Overall, three major metabolic routes were detected: (1) Hydroxylation of the methylene group of the furanone moiety followed by conjugation, (2) oxidative degradation of the furanone moiety to an acetic acid group followed either by conjugation with a carbohydrate or by further oxidation, and (3) halogenation (bromination/chlorination) of the furanone moiety. This metabolisation step probably occurred in the soil and halogenated parent compound was then taken up by the plants. No label-specific metabolites were identified. All metabolic routes were also detected in the cotton metabolism study with [pyridinylmethyl-¹⁴C]BYI 02960.

(2) Label 2: [pyridinylmethyl-¹⁴C]BYI 02960.

As expected, the residues in the double spray application experiment were dominated by parent compound. However, even in the single spray application, parent compound was the main residue in all cotton matrices, except for seeds. In seeds, 6-CNA was identified as the only prominent compound. The metabolite patterns of all matrices were very similar in both experiments. Besides parent compound, BYI 02960-acetic acid, BYI 02960-OH-glyc, 6-CNA and BYI 02960-OH were major metabolites detected in the different matrices. Three additional minor metabolites were identified, but none of them exceeded 7% of the TRR. Overall, four major metabolic routes were detected: (1) Oxidative degradation of the furanone moiety to an acetic acid group followed by conjugation with a carbohydrate or by further oxidation, (2) cleavage of the pyridinylmethylamine bond followed by oxidation of the methylene group to 6-CNA; (3) hydroxylation of the furanone moiety of the parent compound followed by conjugation, and (4) halogenation (bromination, to a minor extent chlorination) of the furanone moiety. Only the second route led to a label-specific metabolite, all other routes were also detected in the cotton metabolism study with [furanone-4-¹⁴C]BYI 02960 which showed no label-

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specific metabolites. Thus, the results of the present metabolism study in cotton are in good conformity with the results of the corresponding study performed with [furanone-4-¹⁴C]BYI 02960.

When considering the results from both metabolism studies conducted on cotton, 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, and all major and 3 minor have been identified. The edible commodity cotton seed showed only very low radioactive residues and analysis of the extracts was only feasible in the study conducted with the pyridinylmethyl-label. The distribution of parent compound and metabolites in the edible commodity seeds is shown in Table 6.2.1-43; the distribution in the feed commodity gin trash is summarised in Table 6.2.1-44.

Table 6.2.1-43: TRR values and distribution of parent compound and metabolites in cotton seeds after foliar application of BYI 02960

Radiolabel	cotton seed			
	[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
<i>6-CNA</i>	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

Remark:

The metabolite profile of cotton seeds from the double application experiment shows most probably an interference from the lint profile since undelinted seeds were analysed.

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Table 6.2.1-44: TRR values and distribution of parent compound and metabolites in gin trash after foliar application of BYI 02960

Radiolabel	gin trash							
	[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
<i>6-CNA</i>					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.

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. In gin trash, difluoroacetic acid accounted for 0.12 mg/kg in the single application experiment and exceeded the BYI 02960 concentration. In the double application experiment, parent compound and several metabolites showed higher concentrations than difluoroacetic acid due to the short PHI of the last application.

On basis of the metabolites identified, biotransformation of BYI 02960 in cotton proceeds by the following pathways:

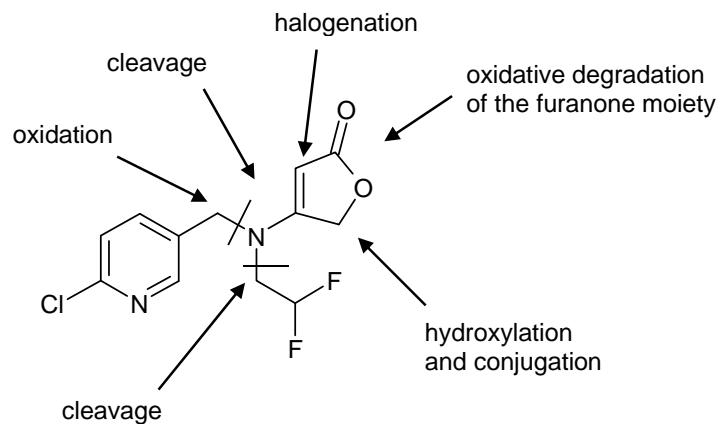
- oxidative cleavage of the difluoroethylamine bond and formation of difluoroacetic acid
- oxidative degradation of the furanone moiety to an acetic acid group followed either by conjugation with a carbohydrate or by further oxidation to BYI 02960-glyoxylic acid
- oxidative cleavage of the pyridinylmethylamine bond and formation of 6-chloronicotinic acid (6-CNA)
- hydroxylation of the furanone moiety followed by conjugation with glucose
- halogenation (bromination and most probably chlorination, as well) of the furanone moiety

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Halogenation of the furanone moiety of the active substance probably occurred in the soil which is supported by the fact that small amounts of halogenated parent compound were identified in the aerobic soil degradation studies. Due to the early timing of the first application, a significant portion of the parent compound could have reached the soil and been subjected to soil metabolism processes.

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

Figure 6.2.1-13: Positions involved in metabolic degradation of BYI 02960 in cotton matrices





Metabolism, distribution and expression of residues in paddy rice (foliar or granular application)

Metabolism studies in paddy rice were conducted with [furanone-4-¹⁴C]- and [pyridinylmethyl-¹⁴C]BYI 02960:

Report:	KIHA 6.2.1/10, Schmeling, S., Weber, E.; 2011
Title:	Metabolism of [furanone-4- ¹⁴ C]BYI 02960 in paddy rice
Report No & Edition No	MEF-11/058 M-414219-01-2
Guidelines:	OECD 501 Metabolism in Crops US EPA Residue Chemistry Test Guideline OPPTS 860.1300: Nature of the Residue – Plants, Livestock PMRA Regulatory Directive Dir98-02: Residue Chemistry Guidelines Section 2: Nature of the Residue – Plants, Livestock Japanese MAFF, 12 Nousan 8147 European Parliament and Council Regulation (EC) No 1107/2009
GLP	yes

Executive Summary

The metabolism of [furanone-4-¹⁴C]BYI 02960 was investigated in paddy rice according to the maximum envisaged use pattern. Two different methods of application were covered in this study. One experiment was a granule treatment (GR) with [furanone-4-¹⁴C]BYI 02960 applied during the transplanting of the rice seedlings at an actual application rate of 409 g a.s./ha. In the second experiment, [furanone-4-¹⁴C]BYI 02960 was formulated as an SL 200 and applied twice as spray treatment (SP) onto plants as well as the water surface. The first spray application took place directly after transplanting of the rice seedlings at a rate of 175 g a.s./ha and the second approx. one month before harvest at a rate of 240 g a.s./ha.

At maturity the rice plants were harvested and separated into straw, kernels and husks. The TRR values of all plant matrices determined are shown in the following table:

Table 6.2.1-45: TRR values in paddy rice (kernels, husks and straw) after granular or spray treatment of [furanone-4-¹⁴C]BYI 02960

Matrix	Timing and Application	PHI (days)*	ppm (mg a.s. equiv./kg)
kernels	one granule application at transplanting of the rice seedlings (BBCH 13-15), 409 g a.s./ha	127	0.140
husks		127	1.404
straw		127	2.879
kernels	two spray applications at transplanting of the rice seedlings (BBCH 13-15) and approx. 30 days before harvest (BBCH 87-89)	29	0.659
husks		29	24.098
straw		29	19.891

* PHI: preharvest interval (corresponds to days after last treatment (DAT) at the start of harvest/sampling)

The radioactive residues were efficiently extracted with acetonitrile/water mixtures. When necessary, additional exhaustive extraction steps were applied to the solids of the first extraction. Extraction efficiencies ranged from 68.7% of the TRR for kernels (GR) to 93.6% for straw (SP) after exhaustive



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extraction. The profiles of the extracts were recorded with HPLC, and all major and several minor components were identified. Identification was performed by LC-MS/MS after isolation of the compounds or by co-chromatography (HPLC or TLC) with reference compounds, as well as by comparison of HPLC profiles. The identification rates ranged from 50.1% of the TRR for kernels (GR) to 85.7% for straw (GR). [Furanone-4-¹⁴C]BYI 02960 was metabolised moderately in paddy rice. Parent compound was the most prominent component and represented approx. 20 – 70% of the TRR in all matrices. Metabolite BYI 02960-chloro/bromo was detected as a major metabolite in straw after granular (GR) and spray treatment (SP) and accounted for approx. 11% of the TRR.

Halogenation of the furanone moiety of the active substance probably occurred in the paddy soil. Soil contact with parent compound resulted in both experiments. Either the active substance was applied as granule in the planting holes or a significant portion was sprayed on the water surface and based on findings in aquatic metabolism studies would be expected to reach the paddy sediment.

Glucose/carbohydrates deriving from BYI 02960 was also detected as major metabolite in kernels (GR) and represented 26.9% of the TRR. All other detected metabolites were minor or trace components.

The following metabolic routes of [Furanone-4-¹⁴C]BYI 02960 were observed in paddy rice:

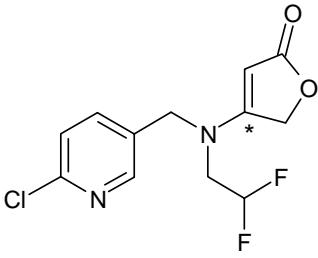
- halogenation (mostly bromination, to a minor extent chlorination) of the furanone moiety,
- hydroxylation of the methylene group of the furanone moiety,
- oxidative opening of the furanone moiety to an acetic acid group followed by further oxidation or conjugation with a carbohydrate (i.e. glycosylation), and
- complete degradation of the furanone moiety and incorporation of the carbon atoms into the natural compound pool, i.e. into glucose/carbohydrates

On the basis of these results, a metabolic pathway of [furanone-4-¹⁴C]BYI 02960 in paddy rice can be proposed.

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure		* position of the radiolabel
Radiolabelled test material	[furanone-4- ¹⁴ C]BYI 02960	
Specific radioactivity	3.94 MBq/mg (106.46 µCi/mg)	
Chemical Purity	> 99% (HPLC)	
Radiochemical purity	> 99% (HPLC and TLC)	

The supplied radiolabelled test compound [furanone-4-¹⁴C]BYI 02960 was dissolved in acetonitrile. Formulation of the test compound was performed prior to each application. For the granule



application, a GR type formulation was simulated. Therefore, an adequate portion of the stock solution was concentrated under a constant flow of nitrogen. The concentrate was then mixed with Sepiolite 30/60, a carrier granule, and the remaining solvent was evaporated yielding the ready-to-use granules. For the preparation of the SL 200 formulation, an adequate portion of the stock solution was transferred into a special glass vial and evaporated to dryness. The liquid blank formulation was added to the test item using a magnetic stirrer. The mixture was diluted with water to get the aqueous application solutions.

2. Soil: “Monheim 4” (sandy loam soil from Germany), pH (CaCl₂) = 6.8, 69% sand, 18% silt and 13% clay, 1.2% organic carbon, cation exchange capacity (CEC) of 8.3 meq/100 g
The soil was submerged and kept under paddy conditions. An adequate water level was maintained for optimal growing conditions.

3. Plant Rice, variety “Nihonbare”, Oryza sativa L., representative for cereals

B. Study Design

Experimental conditions:

Two experiments were performed representing the intended application types in rice (granule application and spray treatment). Each experiment was conducted under paddy conditions in a planting container with a surface area of 0.5 m². The plants were cultivated in a climate chamber in a greenhouse under controlled environmental conditions.

Granule application:

The granules were distributed equally in the planting holes directly before the seedlings (BBCH 13-15) were transplanted into the holes. The target application rate was 400 g a.s./ha. After transplanting of the seedlings, the planting container was flooded with water to simulate paddy conditions. An actual amount of 80.6 MBq or 20.5 mg a.s. was applied, corresponding to 409 g a.s./ha.

Spray application:

For the spray application, a defined volume of the aqueous application solution was filled into the bottle of a hand pump sprayer. The first application was performed after transplanting of the seedlings and flooding of the planting container. The application solution was sprayed equally onto the plants and the water surface. An actual amount of 34.6 MBq or 8.8 mg a.s. was applied at the first application, corresponding to an actual application rate of 175 g a.s./ha. In the second application at 29 days before harvest, an actual amount of 240 g a.s./ha was applied (47.2 MBq or 12.0 g).

Sampling:

In both experiments, the rice was harvested at maturity (BBCH 89-92). The panicles were cut off from the plants and the rice kernels were separated from panicles and husks with an automatic husking machine. The remaining plant parts (straw) were cut off just above the soil surface with scissors. The straw was then cut into pieces and combined with the empty panicles. All plant parts were dried at room temperature for five days. The samples were further processed directly after drying using a Polytron homogenizer and liquid nitrogen. The homogenized samples (kernels, husks and straw) were stored in a freezer ($\leq -18^{\circ}\text{C}$) until analysis.

C. Analytical Procedures

Extraction:

Prior to extraction, the homogenized sample materials were soaked in a solvent mixture of acetonitrile/water (8:2; v/v) for one night. Three extraction steps with acetonitrile/water (8:2; v/v) followed using an Ultraturrax high speed blender. The radioactivity in the extracts was determined by LSC, in the solids by combustion followed by LSC. The actual TRR values of the samples were determined by summing up the radioactivity measured in the extracts and in the remaining solids. All extracts were combined per matrix. The extracts of the sample materials husks and straw were subjected to a clean-up step using an SPE RP 18 cartridge (Phenomenex, Strata C18-E, 20 g), which was conditioned with acetonitrile/water (8:2; v/v) beforehand. The flow-through fraction (percolate) was collected and the cartridge was rinsed four times with 60 mL of acetonitrile/water (8:2; v/v). The percolate and the acetonitrile/water fraction were combined, mixed with a small amount of emulsifier and concentrated by rotary evaporation in vacuo for HPLC analysis. Non-polar contaminations on the cartridge were eluted by rinsing with methanol/dichloromethane (1:1; v/v). Volume and radioactivity of this fraction was also determined. Extracts of kernels were concentrated and analysed by HPLC without any additional purification step.

The post-extraction solids (PES) of kernels and straw of both experiments were subjected to an exhaustive extraction procedure. The solids were extracted in a first step with acetonitrile/water (8:2, v/v) and in a second step with acetonitrile/water (1:1, v/v) under microwave assistance (120 °C for 15 min.). After each extraction step, extracts and solids were separated by filtration. The radioactivity in the extracts was determined by LSC, in the solids by combustion followed by LSC. The two extracts were combined per matrix, purified by SPE and concentrated by rotary evaporation in vacuo for HPLC analysis. The solids of rice kernels of both experiments were subjected to an additional third microwave extraction step with sodium chloride/water (1:99; w/v). This exhaustive extract of the granule treatment was further purified by partitioning with dichloromethane under addition of Celite. The corresponding exhaustive extract of rice kernels from the spray treatment was subjected to a diastase treatment (pH 5, 26 °C, 20 h) before partitioning with dichloromethane and Celite. The resulting organic phases were separated from the aqueous phases, the volumes were measured and aliquots were radioassayed by LSC.

Quantification:

Parent compound and metabolites in the extracts were analysed by reversed phase HPLC coupled to a radioactivity detector with a glass scintillator cell. The HPLC chromatograms (= metabolite profiles) were integrated for quantification of compounds.

Identification and characterisation:

Identification of parent compound and metabolites common to both radiolabels was based on the metabolite profiles obtained in the rice metabolism study with [pyridinylmethyl-¹⁴C]BYI 02960. In this study parent compound and all metabolites common to both radiolabels were identified by LC-MS/MS after semi-preparative isolation of the compounds or by HPLC co-chromatography with authentic reference compounds. Thus assignment of compounds in the present study was possible by comparing the metabolite profiles. Some of these assignments were additionally confirmed by HPLC co-chromatography with reference compounds. The only label-specific metabolite detected was identified by TLC co-chromatography with the polar fraction isolated and identified in the tomato



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metabolism study. Additionally, two non-polar metabolites were identified by LC-MS/MS after isolation of the compounds.

Storage stability:

All extraction experiments and the first HPLC analyses were performed within 3.5 months (104 days) after harvest of the rice plants. The extracts were analysed after 5 days following the start of extraction at the latest. Since the first metabolic analysis of the RACs as well as the last experiments with extracts (HPLC co-chromatography) took place within 6 months, no further stability investigations were necessary according to OECD Guidance for the Testing of Chemicals 501 (2007).

II. Results and Discussion

The metabolism of [furanone-4-¹⁴C]BYI 02960 was investigated in rice kernels, straw and husks following two different application scenarios. In the granule application experiment, the active substance was applied on carrier granules during the transplanting of the seedlings (approx. 3 to 5 leaves unfolded, BBCH 13-15) at an application rate of 409 g a.s./ha. In the spray application experiment, rice was treated two times, one time at an actual application rate of 175 g a.s./ha directly after transplanting of the seedlings (BBCH 13-15) and a second time at an application rate of 240 g a.s./ha at 29 days before harvest. The total application rate in the double application experiment was 415 g a.s./ha. The actual single application rate of the first application was slightly below the anticipated maximum rate of 200 g a.s./ha, thus the second application was slightly overdosed to reach the intended maximum seasonal application rate of 400 g a.s./ha.

The raw agricultural commodities rice kernels and straw were collected in both experiments at maturity (BBCH 89-92), 127 and 29 days after the last application. Rice husks were sampled during the preparation of rice kernels.

The TRR levels in the samples of the granular treatment were approx. five to seventeen times lower compared to those of the spray treatment. The TRR in edible RAC rice kernels accounted for 0.140 mg/kg after granule application and for 0.659 mg/kg after spray application. Straw showed a TRR of 2.879 mg/kg after granule application, whereas the TRR amounts to 19.891 mg/kg in straw from the spray experiment.

The radioactive residues were efficiently extracted with acetonitrile/water mixtures. When necessary, additional exhaustive extraction steps were applied to the solids of the conventional extraction.

Extraction efficiencies ranged from 68.7% of the TRR for kernels (GR) to 93.6% for straw (SP) after exhaustive extraction as shown in Table 6.2.1-46 and Table 6.2.1-47. Exhaustive extraction comprised two extraction steps with acetonitrile/water mixtures and for kernels one additional with an aqueous sodium chloride solution at increased temperature (120 °C) under microwave assistance.

HPLC analysis of the conventional and exhaustive extracts after both application scenarios revealed that all metabolite profiles were comparable among each other and with the profiles obtained in the rice metabolism study performed with [pyridinylmethyl-¹⁴C]BYI 02960.

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

The main compound in all rice matrices, except for rice kernels after granule application, was parent compound. It was identified unambiguously in the extracts of straw in the rice metabolism study performed with [pyridinylmethyl-¹⁴C]BYI 02960. Confirmation of the assignment was achieved in rice kernels and rice straw (RACs) in the present study by HPLC co-chromatography with a radiolabelled reference compound. The main metabolite in rice kernels after granule application was the natural compound glucose/carbohydrates. This metabolite was isolated from the conventional extract of kernels and identified by TLC co-chromatography using the glucose fraction isolated and identified in the tomato metabolism study. Since conventional solvent extraction did not release the total amount of natural compounds, an additional exhaustive extraction step with an aqueous sodium chloride solution under microwave assistance at increased temperature (120 °C) was applied. Partitioning of the extract with dichloromethane under addition of Celite showed that by far the majority of the radioactivity remained in the aqueous phase and indicated the presence of natural compounds. HPLC analysis of the aqueous phase confirmed the assumption. Nearly 90% of the extract was represented by glucose/carbohydrates. In all other matrices, glucose/carbohydrates were assigned by profile comparison.

In rice straw, BYI 02960-bromo was identified as the only major metabolite present. It co-eluted with BYI 02960-chloro, which accounted for significant lower concentrations. Both metabolites were identified by LC-MS/MS analysis in rice straw in the spray application experiment following isolation of the fraction containing both compounds. Most probably, halogenation of the furanone moiety of the active substance occurred already in the paddy soil and the metabolite was taken up by the rice plants. All other metabolites detected in the sample matrices of rice were minor metabolites. They did not exceed 10% of the TRR in both application experiments. In kernels and straw, the presence of metabolites BYI 02960-acetic acid and BYI 02960-OH was confirmed by HPLC co-chromatography and the metabolites BYI 02960-glyoxylic acid and BYI 02960-acetic acid-glyc were identified by comparing the HPLC profiles of the extracts with the corresponding ones obtained in the rice study with the other radiolabel. Co-elution of metabolite BYI 02960-OH-glyc with metabolite BYI 02960-acetic acid was not expected since HPLC-MS/MS analysis of the isolated peak in the corresponding rice metabolism study with [pyridinylmethyl-¹⁴C]BYI 02960 showed only BYI 02960-acetic acid. Compounds in the extracts of husks and in the exhaustive extracts were also assigned by comparison of profiles.

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)Table 6.2.1-46: Distribution of radioactivity in the extracts of rice matrices after one granule application of [furanone-4-¹⁴C]BYI 02960

	granule application experiment					
	kernels		husks		straw	
	TRR [mg/kg] =	0.140	1.404	2.879	% of TRR	mg/kg
Conventionally extracted	20.5	0.029	75.6	1.062	79.7	2.294
Extract for analysis	19.2	0.027	75.6	1.062	79.0	2.275
Losses (not analysed)	1.2	0.002	n.q.	n.q.	0.7	0.020
Exhaustive solvent extr.	6.5	0.009	---	---	11.5	0.331
Extract for analysis	5.6	0.008	---	---	10.9	0.313
Losses (not analysed)	0.8	0.001	---	---	0.6	0.017
Exhaustive NaCl extraction	41.8	0.058	---	---	---	---
aqueous phase	28.6	0.040	---	---	---	---
organic phase	0.8	0.001	---	---	---	---
Losses (not analysed)	12.3	0.017	---	---	---	---
Total extracted	68.7	0.096	75.6	1.062	91.2	2.625
Unextractable (PES*)	31.3	0.044	24.4	0.342	8.8	0.254
Accountability	100.0	0.140	100.0	1.404	100.0	2.879

* post extraction solids

n.q. not quantifiable (residues < LOQ)

Table 6.2.1-47: Distribution of radioactivity in the extracts of rice matrices after two spray applications of [furanone-4-¹⁴C]BYI 02960

	spray application experiment					
	kernels		husks		straw	
	TRR [mg/kg] =	0.659	24.098	19.891	% of TRR	mg/kg
Conventionally extracted	65.8	0.433	90.9	21.917	83.7	16.643
Extract for analysis	65.8	0.433	90.4	21.790	83.3	16.561
Losses (not analysed)	n.q.	n.q.	0.5	0.127	0.4	0.081
Exhaustive solvent extr.	5.6	0.037	---	---	9.9	1.977
Extract for analysis	5.2	0.034	---	---	9.9	1.964
Losses (not analysed)	0.3	0.002	---	---	0.1	0.013
Exhaustive NaCl/diastase extraction ¹	15.8	0.104	---	---	---	---
aqueous phase	13.7	0.090	---	---	---	---
organic phase	0.9	0.006	---	---	---	---
Losses (not analysed)	1.2	0.008	---	---	---	---
Total extracted	87.2	0.574	90.9	21.917	93.6	18.620
Unextractable (PES*)	12.8	0.085	9.1	2.182	6.4	1.271
Accountability	100.0	0.659	100.0	24.098	100.0	19.891

¹ no further HPLC analysis due to high matrix load of the extract

* post extraction solids

n.q. not quantifiable (residues < LOQ)

The TRR values and the distribution of parent compound and metabolites in the extracts is shown in Table 6.2.1-48 and Table 6.2.1-49. In total, 50.1% to 85.7% of the TRR were identified in the raw



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

agricultural commodities of rice after granular and foliar application. A summary of the compounds identified and characterized is given in Table 6.2.1-50 and Table 6.2.1-51.

Table 6.2.1-48: TRR values and distribution of parent compound and metabolites in rice matrices after a single granule application of [furanone-4-¹⁴C]BYI 02960

	granule application experiment					
	kernels		husks		straw	
TRR [mg/kg] =	0.140			1.404		
Compound (BYI 02960-)	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
<i>Conventional extraction</i>						
BYI 02960 (parent comp.)	17.5	0.024	72.3	1.016	57.6	1.658
glucose/carbohydrates	1.7	0.002	1.0	0.014	3.1	0.088
glyoxylic acid	---	---	---	---	1.7	0.050
acetic acid	---	---	0.4	0.006	1.8	0.051
OH	---	---	---	---	0.8	0.024
bromo/chloro	---	---	0.6	0.008	10.6	0.306
Subtotal identified	19.2	0.027	74.3	1.043	75.6	2.176
unknown 1	---	---	---	---	0.8	0.023
unknown 3	---	---	1.3	0.019	1.1	0.031
unknown 5	---	---	---	---	1.1	0.033
unknown 7	---	---	---	---	0.4	0.012
Subtotal characterised	---	---	1.3	0.019	3.4	0.099
Total conventional extr.	19.2	0.027	75.6	1.062	79.0	2.275
<i>Microwave extraction</i>						
BYI 02960 (parent comp.)	5.6	0.008			6.4	0.183
glucose/carbohydrates	---	---			2.3	0.066
glyoxylic acid	---	---			0.2	0.007
acetic acid	---	---			0.2	0.007
OH	---	---			0.2	0.005
bromo/chloro	---	---			0.8	0.022
Subtotal identified	5.6	0.008			10.1	0.290
unknown 3	---	---			0.4	0.011
unknown 4	---	---			0.3	0.007
unknown 5	---	---			0.2	0.005
Subtotal characterized	---	---			0.8	0.023
Total microwave extr.	5.6	0.008	---	---	10.9	0.313
<i>Exhaustive NaCl extraction</i>						
organic phase	0.8	0.001				
aqueous phase	28.6	0.040				
glucose/carbohydrates	25.2	0.035				
Subtotal identified	25.2	0.035				
unknown 2	3.4	0.005				
Subtotal characterized	4.2	0.006				
Total exh. NaCl extraction	29.4	0.041	---	---	---	---

Table continued on next page...

	granule application experiment		
	kernels	husks	straw



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Total identified	50.1	0.070	74.3	1.043	85.7	2.466
Total characterised	4.2	0.006	1.3	0.019	4.2	0.122
Analysed extract(s)	54.3	0.076	75.6	1.062	89.9	2.588
Extracts not analysed	14.4	0.020	n.q.	n.q.	1.3	0.037
Total extracted	68.7	0.096	75.6	1.062	91.2	2.625
Unextractable (PES*)	31.3	0.044	24.4	0.342	8.8	0.254
Accountability	100.0	0.140	100.0	1.404	100.0	2.879

* post extraction solids
n.q. not quantifiable (residues < LOQ)

Table 6.2.1-49: TRR values and distribution of parent compound and metabolites in rice matrices after a two spray application of [furanone-4-¹⁴C]BYI 02960

	spray application experiment					
	kernels		husks		straw	
	TRR [mg/kg] =	0.659	24.098	19.891		
<i>Conventional extraction</i>						
BYI 02960 (parent comp.)	53.3	0.351	74.6	17.972	52.9	10.527
glucose/carbohydrates	1.9	0.012	2.1	0.497	2.0	0.401
glyoxylic acid	---	---	---	---	2.0	0.393
acetic acid-glyc	0.4	0.003	0.2	0.054	1.7	0.329
acetic acid	5.9	0.039	7.0	1.693	7.2	1.432
OH	---	---	---	---	0.4	0.081
bromo/chloro	1.7	0.011	1.6	0.389	10.1	2.016
Subtotal identified	63.1	0.416	85.5	20.605	76.3	15.179
unknown 1	---	---	0.4	0.092	0.6	0.126
unknown 2	---	---	---	---	0.3	0.053
unknown 3	0.9	0.006	0.6	0.138	0.6	0.123
unknown 4	---	---	0.1	0.033	0.1	0.025
unknown 5	---	---	---	---	0.9	0.173
unknown 6	0.9	0.006	0.4	0.103	1.2	0.229
unknown 7	0.8	0.005	2.5	0.608	0.2	0.044
unknown 9	---	---	0.9	0.211	3.1	0.609
Subtotal characterised	2.7	0.017	4.9	1.185	6.9	1.382
Total conventional extr.	65.8	0.433	90.4	21.790	83.3	16.561
<i>Microwave extraction</i>						
BYI 02960 (parent comp.)	3.3	0.021			3.6	0.720
glucose/carbohydrates	1.7	0.011			1.4	0.287
glyoxylic acid	---	---			0.1	0.023
acetic acid-glyc	---	---			0.7	0.132
acetic acid	0.3	0.002			0.8	0.149
OH	---	---			0.1	0.025
bromo/chloro	---	---			0.6	0.116
Subtotal identified	5.2	0.034			7.3	1.451

Table continued on next page...

	kernels		husks		straw	
unknown 1	---	---			0.3	0.062



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

unknown 3	---	---			0.1	0.028
unknown 5	---	---			0.2	0.033
unknown 8	---	---			0.2	0.031
unknown 9	---	---			1.8	0.359
Subtotal characterised	---	---			2.6	0.513
Total microwave extr.	5.2	0.034	---	---	9.9	1.964
<i>Exhaustive NaCl/diastase extraction</i>						
organic phase	0.9	0.006				
aqueous phase	13.7	0.090				
Total exhaustive extr.	14.6	0.096	---	---	---	---
Total identified	68.4	0.450	85.5	20.605	83.6	16.630
Total characterised	17.2	0.113	4.9	1.185	9.5	1.896
Analysed extract(s)	85.6	0.564	90.4	21.790	93.1	18.526
Extracts not analysed	1.6	0.010	0.5	0.127	0.5	0.094
Total extracted	87.2	0.574	90.9	21.917	93.6	18.620
Unextractable (PES*)	12.8	0.085	9.1	2.182	6.4	1.271
Accountability	100.0	0.659	100.0	24.098	100.0	19.891

* post extraction solids

Table 6.2.1-50: Summary of characterization and identification of radioactive residues in rice matrices after one granular application of [furanone-4-¹⁴C]BYI 02960

	granule application experiment					
	kernels		husks		straw	
TRR [mg/kg] =	0.140		1.404		2.879	
Compound (BYI 02960-)	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
<i>Conventional extraction</i>						
BYI 02960 (parent comp.)	23.1	0.032	72.3	1.016	64.0	1.841
glucose/carbohydrates	26.9	0.038	1.0	0.014	5.3	0.153
glyoxylic acid	---	---	---	---	2.0	0.056
acetic acid	---	---	0.4	0.006	2.0	0.058
OH	---	---	---	---	1.0	0.029
bromo/chloro	---	---	0.6	0.008	11.4	0.328
Total identified	50.1	0.070	74.3	1.043	85.7	2.466
unknown 1	---	---	---	---	0.8	0.023
unknown 2	3.4	0.005	---	---	---	---
unknown 3	---	---	1.3	0.019	1.5	0.043
unknown 4					0.3	0.007
unknown 5	---	---	---	---	1.3	0.037
unknown 7	---	---	---	---	0.4	0.012
Total characterised¹	4.2	0.006	1.3	0.019	4.2	0.122
Analysed extract(s)	54.3	0.076	75.6	1.062	89.9	2.588
Extracts not analysed	14.4	0.020	n.q.	n.q.	1.3	0.037
Total extracted	68.7	0.096	75.6	1.062	91.2	2.625
Unextractable (PES*)	31.3	0.044	24.4	0.342	8.8	0.254
Accountability	100.0	0.140	100.0	1.404	100.0	2.879

¹ including characterization by partitioning

* post extraction solids

n.q. not quantifiable (residues < LOQ)



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.2.1-51: Summary of characterization and identification of radioactive residues in rice matrices after two spray applications of [furanone-4-¹⁴C]BYI 02960

	spray application experiment					
	kernels		husks		straw	
	TRR [mg/kg] =	0.659		24.098		19.891
Compound (BYI 02960-)	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
BYI 02960 (parent comp.)	56.6	0.373	74.6	17.972	56.5	11.247
glucose/carbohydrates	3.6	0.023	2.1	0.497	3.5	0.688
glyoxylic acid	---	---	---	---	2.1	0.416
acetic acid-glyc	0.4	0.003	0.2	0.054	2.3	0.461
acetic acid	6.1	0.040	7.0	1.693	7.9	1.581
OH	---	---	---	---	0.5	0.106
bromo/chloro	1.7	0.011	1.6	0.389	10.7	2.132
Total identified	68.4	0.450	85.5	20.605	83.6	16.630
unknown 1	---	---	0.4	0.092	0.9	0.188
unknown 2	---	---	---	---	0.3	0.053
unknown 3	0.9	0.006	0.6	0.138	0.8	0.151
unknown 4	---	---	0.1	0.033	0.1	0.025
unknown 5	---	---	---	---	1.0	0.206
unknown 6	0.9	0.006	0.4	0.103	1.2	0.229
unknown 7	0.8	0.005	2.5	0.608	0.2	0.044
unknown 8	---	---	---	---	0.2	0.031
unknown 9	---	---	0.9	0.211	4.9	0.969
Subtotal characterised	2.7	0.017	4.9	1.185	6.9	1.382
Total characterised¹	17.2	0.113	4.9	1.185	9.5	1.896
Analysed extract(s)	85.6	0.564	90.4	21.790	93.1	18.526
Extracts not analysed	1.6	0.010	0.5	0.127	0.5	0.094
Total extracted	87.2	0.574	90.9	21.917	93.6	18.620
Unextractable (PES*)	12.8	0.085	9.1	2.182	6.4	1.271
Accountability	100.0	0.659	100.0	24.098	100.0	19.891

¹ including characterization by partitioning

* post extraction solids

n.q. not quantifiable (residues < LOQ)

III. Conclusions

[Furanone-4-¹⁴C]BYI 02960 was moderately metabolised in rice. Residues were dominated by parent compound in the foliar application experiment. Parent compound was also the main or the second major compound in all matrices of rice after granule application. Nevertheless, complete degradation of the furanone moiety and incorporation of carbon atoms into the natural compound pool, e.g. into glucose/carbohydrates was observed.

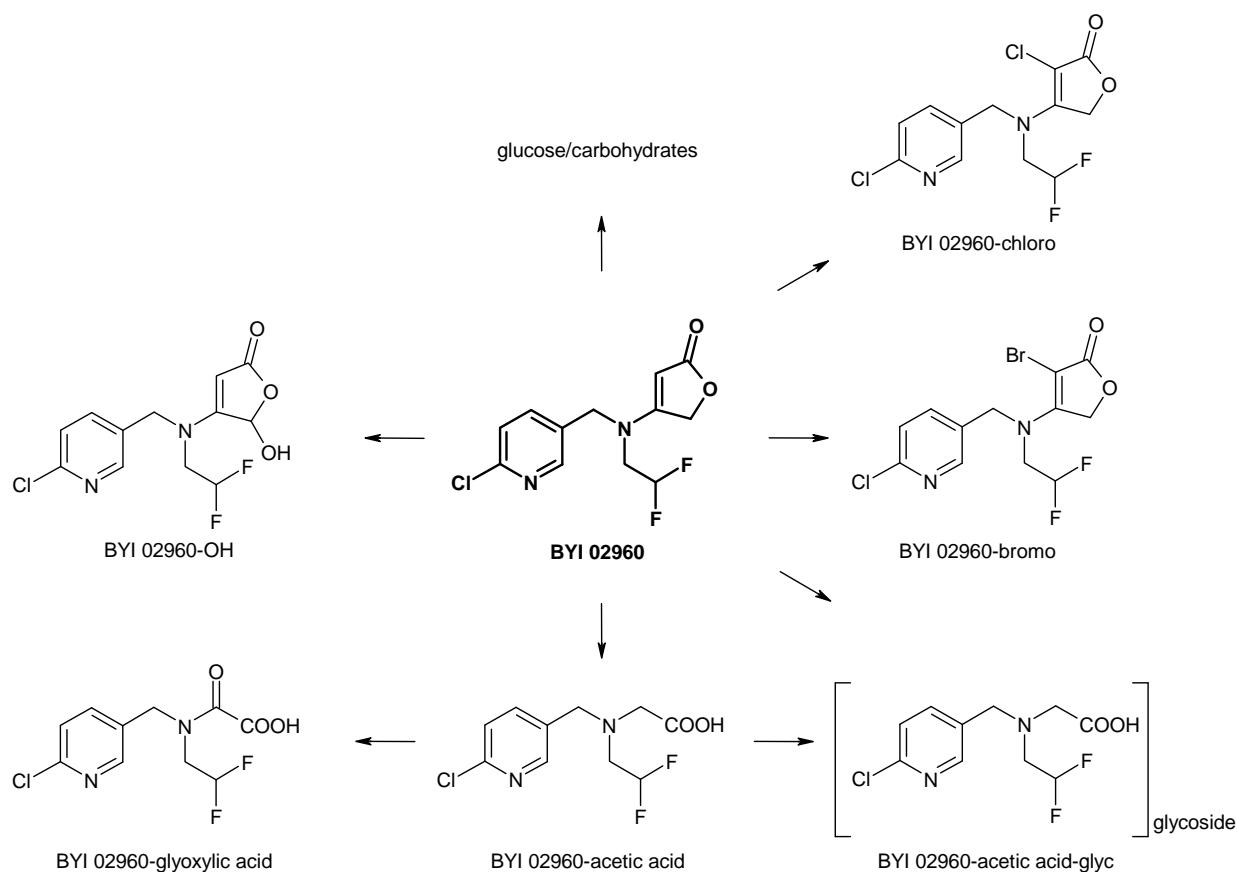
Generally, four major metabolic routes of [furanone-4-¹⁴C]BYI 02960 were observed in rice:

- hydroxylation of the methylene group of the furanone moiety,
- complete degradation of the furanone moiety and incorporation of carbon atoms into the natural compound pool,
- oxidative degradation of the furanone moiety to an acetic acid group followed by conjugation with a carbohydrate or further oxidation, and
- halogenation (mostly bromination, to a minor extent chlorination) of the furanone moiety.

Halogenation of the furanone moiety of the active substance probably occurred in the paddy soil. Soil contact with parent compound resulted in both experiments.

On the basis of the results of this study it is concluded that the metabolism of [furanone-4-¹⁴C]BYI 02960 in rice is well understood and the following metabolic pathway is proposed.

Figure 6.2.1-14: Proposed metabolic pathway of [furanone-4-¹⁴C]BYI 02960 in rice



Report:	KIIA 6.2.1/11, Schmeling, S., Weber, E.; 2011
Title:	Metabolism of [pyridinylmethyl- ¹⁴ C]BYI 02960 in paddy rice
Report No & Edition No	MEF-11/059 M-414328-01-2
Guidelines:	OECD 501 Metabolism in Crops US EPA Residue Chemistry Test Guideline OPPTS 860.1300: Nature of the Residue – Plants, Livestock PMRA Regulatory Directive Dir98-02: Residue Chemistry Guidelines Section 2: Nature of the Residue – Plants, Livestock Japanese MAFF, 12 Nousan 8147 European Parliament and Council Regulation (EC) No 1107/2009
GLP	yes



Executive Summary

The metabolism of [pyridinylmethyl-¹⁴C]BYI 02960 was investigated in paddy rice according to the maximum envisaged use pattern. Two different methods of application were covered in this study. One experiment was a granule treatment (GR) with [pyridinylmethyl-¹⁴C]BYI 02960 during the transplanting of the rice seedlings at an application rate of 434 g a.s./ha. In the other experiment, [pyridinylmethyl-¹⁴C]BYI 02960 was formulated as an SL 200 and applied twice as spray treatment (SP) onto plants and the water surface. The first application took place directly after transplanting of the rice seedlings at a rate of 178 g a.s./ha and the second approx. one month before harvest at a rate of 236 g a.s./ha.

At maturity the rice plants were harvested and separated into straw, kernels and husks. The TRR values of all plant matrices determined are shown in the following table:

Table 6.2.1-52: TRR values in paddy rice (kernels, husks and straw) after granular or spray treatment of [pyridinylmethyl-¹⁴C]BYI 02960

Matrix	Timing and Application	PHI (days)*	ppm (mg a.s. equiv./kg)
kernels	one granule application at transplanting of the rice seedlings (BBCH 13-15), 434 g a.s./ha	127	0.050
husks		127	1.602
straw		127	3.280
kernels	two spray applications at transplanting of the rice seedlings (BBCH 13-15) and approx. 30 days before harvest (BBCH 87-89)	29	0.620
husks		29	23.957
straw		29	24.731

* PHI: preharvest interval (corresponds to days after last treatment (DAT) at the start of harvest/sampling)

The radioactive residues were efficiently extracted with acetonitrile/water mixtures. When necessary, additional exhaustive extraction steps were applied to the solids of the first extraction. Extraction efficiencies ranged from 74.3% of the TRR for kernels (GR) to 97.3% for kernel (SP) after exhaustive extraction. The profiles of the extracts were recorded with HPLC and all major and several minor components were identified. Identification was performed by LC-MS/MS after isolation of the compounds or by co-chromatography (HPLC or TLC) with reference compounds, as well as by comparison of HPLC profiles. The identification rates ranged from 74.3% of the TRR for kernels (GR) to 88.9% for kernels (GR). [Pyridinylmethyl-¹⁴C]BYI 02960 was metabolised moderately in paddy rice. Parent compound was the most prominent component and represented ≥ 60% of the TRR in all matrices. BYI 02960-chloro/bromo was detected as a major metabolite in straw after granular (GR) treatment and represented 12.3% of the TRR. Halogenated parent compound was likely formed in the paddy soil and then taken up by the rice plants. Minor or trace metabolites identified were BYI 02960-6-CNA, BYI 02960-glyoxylic acid, BYI 02960-acetic acid-glyc, BYI 02960-acetic acid and BYI 02960-OH.

The following metabolic routes of [pyridinylmethyl-¹⁴C]BYI 02960 were observed in paddy rice:

- halogenation (mostly bromination, to a minor extent chlorination) of the furanone moiety,
- hydroxylation of the methylene group of the furanone moiety,
- oxidative opening of the furanone moiety to an acetic acid group followed by further oxidation or conjugation with a carbohydrate (e.g. glycosylation), and



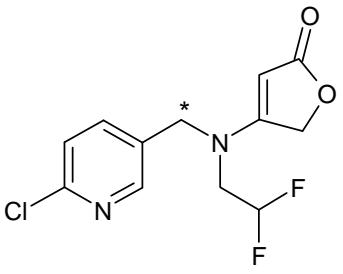
- cleavage of the pyridinylmethylamine bond followed by oxidation of the methylene group to chloronicotinic acid

On the basis of these results, a metabolic pathway of [pyridinylmethyl-¹⁴C]BYI 02960 in paddy rice can be proposed.

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	 * position of the radiolabel
Radiolabelled test material	[pyridinylmethyl- ¹⁴ C]BYI 02960
Specific radioactivity	4.37 MBq/mg (118.08 µCi/mg)
Chemical Purity	> 99% (HPLC)
Radiochemical purity	> 98% (HPLC and TLC)

The supplied radiolabelled test compound [pyridinylmethyl-¹⁴C]BYI 02960 was dissolved in acetonitrile. Formulation of the test compound was performed prior to each application. For the granule application, a GR type formulation was simulated. Therefore, an adequate portion of the stock solution was concentrated under a constant flow of nitrogen. The concentrate was then mixed with Sepiolite 30/60, a carrier granule, and the remaining solvent was evaporated yielding the ready-to-use granules. For the preparation of the SL 200 formulation, an adequate portion of the stock solution was transferred into a special glass vial and evaporated to dryness. The liquid blank formulation was added to the test item and the mixture was homogenized by ultrasonication. Thereafter, the mixture was diluted with water to get the aqueous application solution.

2. Soil: “Monheim 4” (sandy loam soil from Germany), pH (CaCl₂) = 6.8, 69% sand, 18% silt and 13% clay, 1.2% organic carbon, cation exchange capacity (CEC) of 8.3 meq/100 g

The soil was submerged and kept under paddy conditions. An adequate water level was maintained for optimal growing conditions.

3. Plant Rice, variety “Nihonbare”, Oryza sativa L., representative for cereals

B. Study Design

Experimental conditions:

Two experiments were performed representing the intended application types in rice (granule application and spray treatment). Each experiment was conducted under paddy conditions in a planting container with a surface area of 0.5 m². The plants were cultivated in a climate chamber in a greenhouse under controlled environmental conditions.

**Granule application:**

The granules were distributed equally in the planting holes directly before the seedlings (BBCH 13-15) were transplanted into the holes. The target application rate was 400 g a.s./ha. After transplanting of the seedlings, the planting container was flooded with water to simulate paddy conditions. An actual amount of 94.9 MBq or 21.7 mg a.s. was applied, corresponding to 434 g a.s./ha.

Spray application:

For the spray application, a defined volume of the aqueous application solution was filled into the bottle of a hand pump sprayer. The first application was performed after transplanting of the seedlings and flooding of the planting container. The application solution was sprayed equally onto the plants and the water surface. An actual amount of 39.0 MBq or 8.9 mg a.s. was applied at the first application, corresponding to an actual application rate of 178 g a.s./ha. In the second application at 29 days before harvest, an actual amount of 236 g a.s./ha was applied (51.5 MBq or 11.8 g).

Sampling:

In both experiments, the rice was harvested at maturity (BBCH 89-92). The panicles were cut off from the plants and the rice kernels were separated from panicles and husks with an automatic husking machine. The remaining plant parts (straw) were cut off just above the soil surface with scissors. The straw was then cut into pieces and combined with the empty panicles. All plant parts were dried at room temperature for five days. The samples were further processed directly after drying using a Polytron homogenizer and liquid nitrogen. The homogenized samples (kernels, husks and straw) were stored in a freezer ($\leq -18^{\circ}\text{C}$) until analysis.

C. Analytical Procedures**Extraction:**

Prior to extraction, the homogenized sample materials were soaked in a solvent mixture of acetonitrile/water (8:2; v/v) for one night. Three extraction steps with acetonitrile/water (8:2; v/v) followed using an Ultraturrax high speed blender. The radioactivity in the extracts was determined by LSC, in the solids by combustion followed by LSC. The actual TRR values of the samples were determined by summing up the radioactivity measured in the extracts and in the remaining solids. All extracts were combined per matrix. The extracts of the sample materials husks and straw were subjected to a clean-up step using an SPE RP 18 cartridge (Phenomenex, Strata C18-E, 20 g), which was conditioned with acetonitrile/water (8:2; v/v) beforehand. The flow-through fraction (percolate) was collected and the cartridge was rinsed four times with 60 mL of acetonitrile/water (8:2; v/v). The percolate and the acetonitrile/water fraction were combined and concentrated by rotary evaporation in vacuo for HPLC analysis. Non-polar contaminations on the cartridge were eluted by rinsing with methanol/dichloromethane (1:1; v/v). Volume and radioactivity of this fraction was also determined. Conventional extracts of kernels were concentrated and analysed by HPLC without any additional purification step.

The post-extraction solids (PES) of kernels and straw of both experiments were subjected to an exhaustive extraction procedure. The solids were extracted in a first step with acetonitrile/water (8:2, v/v) and in a second step with acetonitrile/water (1:1, v/v) under microwave assistance (120°C for 15 min.). After each extraction step, extracts and solids were separated by filtration. The radioactivity in the extracts was determined by LSC, in the solids by combustion followed by LSC. The two extracts



were combined per matrix, purified by SPE and concentrated by rotary evaporation in vacuo for HPLC analysis. The solids of rice kernels from the spray application experiments were subjected to an additional third microwave extraction step with sodium chloride/water (1:99; w/v). Volume and radioactivity of the resulting extract was determined but it was not combined with the other microwave extracts. It was further subjected to a diastase treatment to characterize the radioactivity incorporated into starch components. Therefore, the extract was buffered with sodium acetate to maintain pH 5.0 and diastase was added. The solution was incubated for 20 h at 26°C while stirring. Afterwards, the pH of the mixture was adjusted to pH 7.0 and the supernatant was separated from the solids by centrifugation. Partitioning with acetonitrile followed after addition of sodium chloride. A second partitioning step with acetonitrile followed after adjusting the aqueous phase to pH 3. HPLC analysis of the aqueous and the organic extracts was not possible due to low radioactivity levels.

Quantification:

Parent compound and metabolites in the extracts were analysed by reversed phase HPLC coupled to a radioactivity detector with a glass scintillator cell. The HPLC chromatograms (= metabolite profiles) were integrated for quantification of compounds.

Identification and characterisation:

Identification of parent compound, the only major metabolite, and the next most prominent metabolite was performed by mass spectrometry after isolation of the compounds from the rice straw extract after spray application. Assignment of the compounds in other matrices was performed by HPLC co-chromatography or by comparison of the HPLC profiles. Additionally, four minor metabolites were identified in rice straw by HPLC co-chromatography using authentic reference compounds.

Confirmation of the assignments by a second chromatographic method was not necessary due to the low residue levels. The presence of the only label-specific metabolite was confirmed in rice kernels by HPLC co-chromatography. Assignment of the other minor compounds in additional matrices was performed by comparison of the HPLC profiles.

Storage stability:

All extraction experiments and the first HPLC analyses were performed within 3.5 months (104 days) after harvest of the rice plants. The extracts were analysed after 4 days following the start of extraction at the latest. Since the first metabolic analysis of the RACs, as well as the last experiments with the extracts (HPLC co-chromatography) took place within 6 months, no further stability investigations were necessary according to OECD Guidance for the Testing of Chemicals 501 (2007).

II. Results and Discussion

The metabolism of [pyridinylmethyl-¹⁴C]BYI 02960 was investigated in rice kernels, straw and husks following two different application scenarios. In the granule application experiment, carrier granules with the active substance were placed in the planting holes during the transplanting of the seedlings (approx. 3 to 5 leaves unfolded, BBCH 13-15). A total amount of 434 g a.s./ha was applied. In the spray application experiment, rice was treated two times, one time at an actual application rate of 178 g a.s./ha directly after transplanting of the seedlings (BBCH 13-15) and a second time at an application rate of 236 g a.s./ha at 29 days before harvest for a total application rate of 414 g a.s./ha. Since the actual single application rate of the first application was below the anticipated maximum rate of 200 g

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a.s./ha, the second application was slightly overdosed to achieve the intended maximum seasonal application rate of 400 g a.s./ha.

The raw agricultural commodities rice kernels and straw were collected in both experiments at maturity (BBCH 89-92), 127 and 29 days after the last application. Rice husks were sampled during the preparation of rice kernels.

The TRR levels in the samples of the granular treatment were approx. seven to fifteen times lower compared to those of the spray treatment. The TRR in edible RAC rice kernels accounted for 0.050 mg/kg after granule application and for 0.620 mg/kg after spray application. Straw showed a TRR of 3.280 mg/kg after granule application, whereas the TRR amounted to 24.731 mg/kg in straw from the spray experiment.

The radioactive residues were efficiently extracted with acetonitrile/water mixtures. When necessary, additional exhaustive extraction steps were applied to the solids after conventional extraction. Extraction efficiencies ranged from 74.3% of the TRR for kernels (GR) to 97.3% for kernels (SP) after exhaustive extraction as shown in Table 6.2.1-53 and Table 6.2.1-54. Exhaustive extraction comprised two extraction steps with acetonitrile/water mixtures and for kernels (SP) one additional with an aqueous sodium chloride solution at increased temperature (120 °C) under microwave assistance. The sodium chloride extract was additionally subjected to a diastase digestion step.

HPLC analysis of the conventional and exhaustive extracts after both application scenarios revealed that all metabolite profiles were comparable among each other and with the profiles obtained in the rice metabolism study performed with [furanone-4-¹⁴C]BYI 02960.

By far, the main compound in all rice matrices was parent compound. It was identified unambiguously in the conventional extract of straw (SP) by LC-MS/MS and in the exhaustive extract of straw (SP) by HPLC co-chromatography. The compound was isolated from the respective extracts and purified prior to identification. In all other matrices, parent was assigned by comparison of the metabolite profiles. In rice kernel (edible RAC), the assignment was additionally confirmed by HPLC co-chromatography with an authentic reference compound.



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One major metabolite (>10% of the TRR) was detected only in rice straw from the granular experiment. The metabolite was identified as BYI 02960-bromo by LC-MS/MS. Co-elution of trace amounts of BYI 02960-chloro are possible since BYI 02960-chloro was identified in the corresponding rice metabolism study with [furanone-4-¹⁴C]BYI 02960 by mass spectrometric means. Halogenation of the furanone moiety of the active substance probably occurred in the paddy soil. Soil contact with parent compound resulted in both experiments. Either the active substance was applied as granule in the planting holes or a significant portion was sprayed on the water surface and based on findings in aquatic metabolism studies BYI 02960 would be expected to reach the paddy sediment.

The most prominent metabolite in rice kernels, straw and husks after spray application was BYI 02960-acetic acid, representing between 6.5% and 7.8% of the TRR. It was isolated from straw and identified by LC-MS/MS. In all other matrices assignment of the metabolite was achieved by comparison of metabolite profiles. Additional minor metabolites identified by HPLC co-chromatography were BYI 02960-acetic acid-glyc, BYI 02960-glyoxylic acid, BYI 02960-OH and the label-specific metabolite 6-CNA

Table 6.2.1-53: Distribution of radioactivity in the extracts of rice matrices after one granule application of [pyridinylmethyl]-¹⁴C]BYI 02960

TRR [mg/kg] =	granule application experiment					
	kernels		husks		straw	
	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Conventionally extracted	62.8	0.032	79.6	1.275	81.6	2.677
Extract for analysis	62.8	0.032	79.6	1.275	80.7	2.648
Losses (not analysed)	n.q.	n.q.	n.q.	n.q.	0.9	0.030
Exhaustive solvent extr.	11.5	0.006	---	---	7.7	0.252
Extract for analysis	11.5	0.006	---	---	7.2	0.237
Losses (not analysed)	n.q.	n.q.	---	---	0.5	0.016
Total extracted	74.3	0.037	79.6	1.275	89.3	2.930
Unextractable (PES*)	25.7	0.013	20.4	0.327	10.7	0.350
Accountability	100.0	0.050	100.0	1.602	100.0	3.280

* post extraction solids

n.q. not quantified (= losses were < LOQ)

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)Table 6.2.1-54: Distribution of radioactivity in the extracts of rice matrices after two spray applications of [pyridinylmethyl-¹⁴C]BYI 02960

	spray application experiment					
	kernels		husks		straw	
	TRR [mg/kg] =	0.620	23.957	24.731		
	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Conventionally extracted	88.4	0.548	90.3	21.625	84.0	20.764
Extract for analysis	88.4	0.548	89.8	21.514	83.7	20.708
Losses (not analysed)	n.q.	n.q.	0.5	0.111	0.2	0.056
Exhaustive solvent extr.	5.6	0.035	---	---	9.7	2.393
Extract for analysis	4.9	0.031	---	---	9.6	2.374
Losses (not analysed)	0.7	0.004	---	---	0.1	0.020
Exhaustive NaCl/ diastase extraction ¹	3.3	0.021	---	---	---	---
aqueous phase	1.8	0.011	---	---	---	---
organic phase	1.2	0.008	---	---	---	---
Losses (not analysed)	0.3	0.003	---	---	---	---
Total extracted	97.3	0.604	90.3	21.625	93.6	23.157
Unextractable (PES*)	2.7	0.017	9.7	2.332	6.4	1.573
Accountability	100.0	0.620	100.0	23.957	100.0	24.731

¹ no further HPLC analysis due to high matrix load of the extract

* post extraction solids

The TRR values and the distribution of parent compound and metabolites in the extracts is shown in Table 6.2.1-55 and Table 6.2.1-56. A summary of the compounds identified and characterized is given in Table 6.2.1-57 and Table 6.2.1-58.



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Table 6.2.1-55: TRR values and distribution of parent compound and metabolites in rice matrices after a single granule application of [pyridinylmethyl-¹⁴C]BYI 02960

	granule application experiment					
	kernels		husks		straw	
	TRR [mg/kg] =	0.050		1.602		3.280
Compound (BYI 02960-)	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
<i>Conventional extraction</i>						
BYI 02960 (parent comp.)	62.8	0.032	77.7	1.244	56.2	1.842
6-CNA	---	---	0.5	0.009	2.9	0.093
glyoxylic acid	---	---	---	---	0.2	0.007
acetic acid	---	---	---	---	1.8	0.058
OH	---	---	---	---	1.1	0.037
bromo/chloro	---	---	0.6	0.010	11.7	0.385
Subtotal identified	62.8	0.032	78.8	1.263	73.9	2.423
unknown 3	---	---	---	---	0.7	0.023
unknown 4	---	---	0.8	0.012	0.7	0.022
unknown 6	---	---	---	---	0.5	0.016
unknown 7	---	---	---	---	1.4	0.047
unknown 9	---	---	---	---	0.3	0.011
unknown 11	---	---	---	---	0.4	0.015
unknown 12	---	---	---	---	0.8	0.027
unknown 13	---	---	---	---	0.9	0.031
unknown 14	---	---	---	---	1.0	0.033
Subtotal characterised	---	---	0.8	0.012	6.9	0.225
Total conventional extr.	62.8	0.032	79.6	1.275	80.7	2.648
<i>Microwave extraction</i>						
BYI 02960 (parent comp.)	6.8	0.003			3.7	0.122
6-CNA	4.7	0.002			1.0	0.031
acetic acid	---	---			0.2	0.006
OH	---	---			0.1	0.004
bromo/chloro	---	---			0.5	0.018
Subtotal identified	11.5	0.006			5.5	0.181
unknown 2	---	---			0.2	0.006
unknown 3	---	---			0.1	0.003
unknown 5	---	---			0.2	0.005
unknown 6	---	---			0.1	0.003
unknown 12	---	---			0.1	0.005
unknown 13	---	---			0.8	0.025
unknown 14	---	---			0.3	0.010
Subtotal characterised	---	---			1.7	0.056
Total microwave extraction	11.5	0.006	---	---	7.2	0.237
Total identified	74.3	0.037	78.8	1.263	79.4	2.603
Total characterised	---	---	0.8	0.012	8.6	0.281
Analysed extract(s)	74.3	0.037	79.6	1.275	87.9	2.884
Losses/extracts not analysed	n.q.	n.q.	n.q.	n.q.	1.4	0.046
Total extracted	74.3	0.037	79.6	1.275	89.3	2.930
Unextractable (PES*)	25.7	0.013	20.4	0.327	10.7	0.350
Accountability	100.0	0.050	100.0	1.602	100.0	3.280

* post extraction solids

n.q. not quantified (= losses were < LOQ)



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Table 6.2.1-56: TRR values and distribution of parent compound and metabolites in rice matrices after two spray applications of [pyridinylmethyl-¹⁴C]BYI 02960

	spray application experiment					
	kernels		husks		straw	
	TRR [mg/kg] =	0.620	% of TRR	23.957	mg/kg	24.731
Compound (BYI 02960-)	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
<i>Conventional extraction</i>						
BYI 02960 (parent comp.)	72.0	0.447	77.3	18.526	56.9	14.071
6-CNA	2.1	0.013	0.4	0.107	0.9	0.227
glyoxylic acid	0.4	0.003	---	---	2.0	0.505
acetic acid-glyc	0.6	0.003	0.2	0.048	1.2	0.308
acetic acid	7.8	0.048	6.5	1.548	6.6	1.631
OH	0.4	0.002	---	---	0.6	0.146
bromo/chloro	1.5	0.009	1.2	0.295	7.9	1.955
Subtotal identified	84.8	0.526	85.7	20.524	76.2	18.844
unknown 1	---	---	---	---	0.1	0.033
unknown 2	---	---	---	---	0.1	0.031
unknown 3	---	---	---	---	0.3	0.079
unknown 4	---	---	0.4	0.085	0.3	0.068
unknown 5	---	---	---	---	0.2	0.047
unknown 6	---	---	0.2	0.044	0.2	0.049
unknown 7	---	---	---	---	0.1	0.022
unknown 8	---	---	---	---	0.2	0.042
unknown 9	0.5	0.003	0.4	0.090	0.8	0.188
unknown 10	1.2	0.008	1.7	0.401	0.3	0.068
unknown 11	---	---	---	---	0.3	0.070
unknown 12	---	---	0.2	0.044	0.7	0.171
unknown 13	1.1	0.007	0.4	0.106	1.1	0.279
unknown 14	0.8	0.005	0.9	0.221	2.9	0.718
Subtotal characterised	3.6	0.022	4.1	0.990	7.5	1.864
Total conventional extr.	88.4	0.548	89.8	21.514	83.7	20.708
<i>Microwave extraction</i>						
BYI 02960 (parent comp.)	3.2	0.020			3.9	0.958
6-CNA	0.9	0.006			0.3	0.073
glyoxylic acid	---	---			0.2	0.052
acetic acid-glyc	---	---			0.7	0.166
acetic acid	---	---			0.7	0.183
OH	---	---			0.2	0.043
bromo/chloro	---	---			0.6	0.139
Subtotal identified	4.1	0.026			6.5	1.615
unknown 1	---	---			<0.1	0.011
unknown 2	---	---			0.1	0.036
unknown 13	0.8	0.005			1.1	0.276
unknown 14	---	---			1.8	0.435
Subtotal characterized	0.8	0.005			3.1	0.758
Total microwave extraction	4.9	0.031	---	---	9.6	2.374

Table continued on next page...



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	kernels		husks		straw	
<i>Exhaustive NaCl / diastase extraction</i>						
organic phase	1.8	0.011				
aqueous phase	1.2	0.007				
Total exhaust. NaCl / diastase extraction	3.1	0.021	---	---	---	---
Total identified	88.9	0.552	85.7	20.524	82.7	20.460
Total characterised	7.4	0.045	4.1	0.990	10.6	2.622
Analysed extract(s)	96.4	0.597	89.8	21.514	93.3	23.082
Extracts not analysed	0.9	0.007	0.5	0.111	0.3	0.075
Total extracted	97.3	0.604	90.3	21.625	93.6	23.157
Unextractable (PES*)	2.7	0.017	9.7	2.332	6.4	1.573
Accountability	100.0	0.620	100.0	23.957	100.0	24.731

* post extraction solids

Table 6.2.1-57: Summary of characterization and identification of radioactive residues in rice matrices after one granular application of [pyridinylmethyl-¹⁴C]BYI 02960

	granule application experiment					
	kernels		husks		straw	
TRR [mg/kg] =	0.050		1.602		3.280	
Compound (BYI 02960-)	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
BYI 02960 (parent comp.)	69.6	0.035	77.7	1.244	59.9	1.964
6-CNA	4.7	0.002	0.5	0.009	3.8	0.125
glyoxylic acid	---	---	---	---	0.2	0.007
acetic acid	---	---	---	---	1.9	0.063
OH	---	---	---	---	1.2	0.040
bromo/chloro	---	---	0.6	0.010	12.3	0.403
Total identified	74.3	0.037	78.8	1.263	79.4	2.603
unknown 2	---	---	---	---	0.2	0.006
unknown 3	---	---	---	---	0.8	0.026
unknown 4	---	---	0.08	0.012	0.7	0.022
unknown 5	---	---	---	---	0.2	0.005
unknown 6	---	---	---	---	0.6	0.019
unknown 7	---	---	---	---	1.4	0.047
unknown 9	---	---	---	---	0.3	0.011
unknown 11	---	---	---	---	0.4	0.015
unknown 12	---	---	---	---	1.0	0.032
unknown 13	---	---	---	---	1.7	0.056
unknown 14	---	---	---	---	1.3	0.044
Total characterised	---	---	0.8	0.012	8.6	0.281
Analysed extract(s)	74.3	0.037	79.6	1.275	87.9	2.884
Losses/extracts not analysed	n.q.	n.q.	n.q.	n.q.	1.4	0.046
Total extracted	74.3	0.037	79.6	1.275	89.3	2.930
Unextractable (PES*)	25.7	0.013	20.4	0.327	10.7	0.350
Accountability	100.0	0.050	100.0	1.602	100.0	3.280

* post extraction solids

n.q. not quantified (< LOQ)



Table 6.2.1-58: Summary of characterization and identification of radioactive residues in rice matrices after two spray applications of [pyridinylmethyl-¹⁴C]BYI 02960

	granule application experiment					
	kernels		husks		straw	
TRR [mg/kg] =	0.620		23.957		24.731	
Compound (BYI 02960-)	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
BYI 02960 (parent comp.)	75.2	0.467	77.3	18.526	60.8	15.029
6-CNA	3.1	0.019	0.4	0.107	1.2	0.301
glyoxylic acid	0.4	0.003	---	---	2.3	0.557
acetic acid-glyc	0.6	0.003	0.2	0.048	1.9	0.475
acetic acid	7.8	0.048	6.5	1.548	7.3	1.814
OH	0.4	0.002	---	---	0.8	0.189
bromo/chloro	1.5	0.009	1.2	0.295	8.5	2.094
Total identified	88.9	0.552	85.7	20.524	82.7	20.460
unknown 1	---	---	---	---	0.2	0.043
unknown 2	---	---	---	---	0.3	0.068
unknown 3	---	---	---	---	0.3	0.079
unknown 4	---	---	0.4	0.085	0.3	0.068
unknown 5	---	---	---	---	0.2	0.047
unknown 6	---	---	0.2	0.044	0.2	0.049
unknown 7	---	---	---	---	0.1	0.022
unknown 8	---	---	---	---	0.2	0.042
unknown 9	0.5	0.003	0.4	0.090	0.8	0.188
unknown 10	1.2	0.008	1.7	0.401	0.3	0.068
unknown 11	---	---	---	---	0.3	0.070
unknown 12	---	---	0.2	0.044	0.7	0.171
unknown 13	1.9	0.012	0.4	0.106	2.2	0.555
unknown 14	0.8	0.005	0.9	0.221	4.7	1.154
Total characterised	7.4	0.045	4.1	0.990	10.6	2.622
Analysed extract(s)	96.4	0.597	89.8	21.514	93.3	23.082
Losses/extracts not analysed	0.9	0.007	0.5	0.111	0.3	0.075
Total extracted	97.3	0.604	90.3	21.625	93.6	23.157
Unextractable (PES*)	2.7	0.017	9.7	2.332	6.4	1.573
Accountability	100.0	0.620	100.0	23.957	100.0	24.731

III. Conclusions

[Pyridinylmethyl-¹⁴C]BYI 02960 is metabolised moderately in rice. Residues were dominated by parent compound in the foliar application experiment, and parent compound was also the main compound in all matrices of rice after granule application. In total, seven different metabolites were identified in rice straw, which shows the most pronounced metabolite pattern. Only one of these metabolites (the halogenated parent compound) represented more than 10% of the TRR (approx. 12%).

Generally, four major metabolic routes of [pyridinylmethyl-¹⁴C]BYI 02960 were observed in rice:

- hydroxylation of the methylene group of the furanone moiety,
- cleavage of the pyridinylmethylamine bond followed by oxidation of the methylene group to 6-CNA,

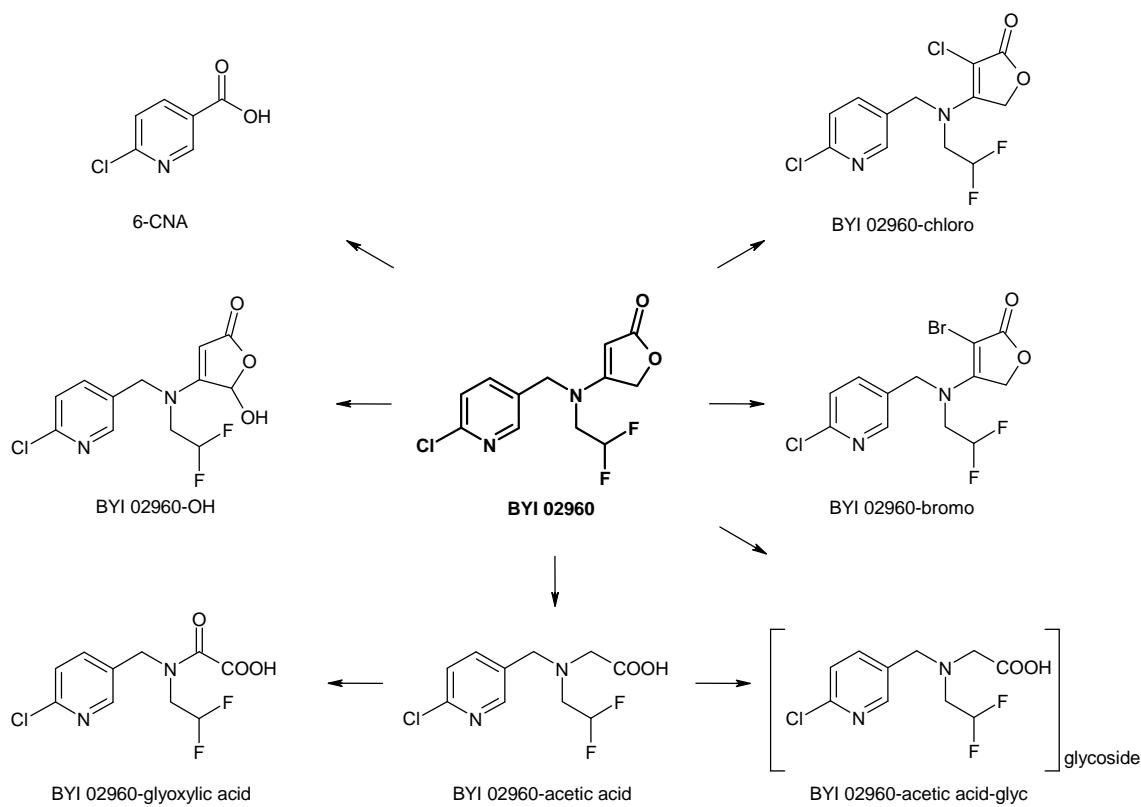
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- oxidative degradation of the furanone moiety to an acetic acid group followed by either conjugation with a carbohydrate or further oxidation, and
- halogenation (mostly bromination, to a minor extent chlorination) of the furanone moiety,

It is considered likely that halogenation of the furanone moiety of the active substance occurred in the paddy soil/sediment.

On the basis of the results of this study it is concluded that the metabolism of [pyridinylmethyl-¹⁴C]BYI 02960 in rice is well understood and the following metabolic pathway is proposed.

Figure 6.2.1-15: Proposed metabolic pathway of [pyridinylmethyl-¹⁴C]BYI 02960 in rice



Overall Conclusions Rice (foliar and granule application)

The metabolism of the insecticide BYI 02960 was investigated in rice kernels, straw and husks following two different application scenarios of (1) [furanone-4-¹⁴C]BYI 02960 or (2) [pyridinylmethyl-¹⁴C]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



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expected, the residues in the spray application experiments were dominated by parent compound in both studies. But, even after the early granule application, parent compound was the main residue in rice husks and straw and, if not the main, a prominent residue in kernels. Subsequent analysis of the extracts on the non-radiolabelled metabolite difluoroacetic acid – which cannot be detected with the radiolabels used – confirmed that parent compound represented always the highest proportion of the residue, except for rice kernels after granular application, where difluoroacetic acid was the main constituent.

(1) Label 1: [furanone-4-¹⁴C]BYI 02960

The lowest residue levels were detected in rice kernels, irrespective from the application scenario. After granule application, the main residue in rice kernels was represented by a natural compound (glucose/carbohydrates), indicating a quite intense degradation of the furanone moiety, whereas parent compound was the main compound after spray application.

The residue levels in straw and husks were significantly higher (by a factor of 10 to 36), but the metabolite patterns were very comparable for all matrices in both experiments. Besides parent compound or glucose, an additional major metabolite (>10% of the TRR) was detected only in rice straw: BYI 02960-bromo, co-eluting with small amounts of BYI 02960-chloro. In total, four additional minor metabolites were identified, but none of them exceeded 8% of the TRR. Overall, four major metabolic routes were detected: (1) Oxidative degradation of the furanone moiety to an acetic acid group followed by further oxidation or conjugation with a carbohydrate, (2) complete degradation of the furanone moiety and incorporation of carbon atoms into the natural compound pool, i.e. into glucose/carbohydrates; (3) hydroxylation of the furanone moiety of the parent compound and (4) halogenation (bromination, to a minor extent chlorination) of the furanone moiety. However, it is considered likely that the halogenation of the furanone moiety of BYI 02960 occurred in the paddy soil/sediment and was not a transformation path in the plant.

Only the second route led to a label-specific metabolite. A corresponding counterpart was detected in the rice study performed with [pyridinylmethyl¹⁴C]BYI 02960. The concentrations of the metabolites common to both radiolabels tested correspond very well when comparing the two metabolism studies conducted in rice. Thus, the results of the present metabolism study are in good conformity with the results of the corresponding study performed with [pyridinylmethyl-¹⁴C]BYI 02960.

(2) Label 2: [pyridinylmethyl-¹⁴C]BYI 02960

As for the study with the furanone-label, the lowest residue levels were detected in rice kernels, irrespective from the application scenario. The residue levels in straw and husks were significantly higher (by a factor of 32 to 66), but the metabolite patterns were very comparable for all matrices in both experiments. Besides parent compound, only in rice straw one additional major metabolite (>10% of the TRR) was detected: BYI 02960-bromo, most probably co-eluting with small amounts of BYI 02960-chloro. These halogenated metabolites were most likely not formed in the plants, but in the paddy soil/sediment and taken up by the plants. Overall, five additional minor metabolites were identified, but none of them exceeded 8% of the TRR. Based on the metabolites identified, four major metabolic routes were detected: (1) Oxidative degradation of the furanone moiety to an acetic acid group followed by either conjugation with a carbohydrate or further oxidation, (2) cleavage of the pyridinylmethylamine bond followed by oxidation of the methylene group to 6-CNA; (3)

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hydroxylation of the furanone moiety of the parent compound and (4) halogenation (bromination, to a minor extent chlorination) of the furanone moiety. The halogenated parent compound was probably formed in the paddy soil and was taken up by the rice plants.

Only the second route led to a label-specific metabolite, all other routes were also detected in the rice metabolism study with [furanone-4-¹⁴C]BYI 02960. The concentrations of the metabolites common to both radiolabels tested correspond very well. Thus, the results of the present metabolism study in rice are in good conformity with the results of the corresponding study performed with [furanone-4-¹⁴C]BYI 02960.

When considering the results from both metabolism studies conducted in rice, it can be concluded that BYI02960 is rather extensively metabolised in this crop. A total of 3 major and approx. 25 minor metabolites were found, and all major and 5 minor have been identified. The edible commodity rice kernel showed low radioactive residues, especially after granule application. The distribution of parent compound and metabolites in the edible commodity rice kernels is shown in Table 6.2.1-59.

Table 6.2.1-59: TRR values and distribution of parent compound and metabolites in rice kernels after granule and foliar application of BYI 02960

Radiolabel	rice kernels							
	[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
<i>glucose/carbohydrates</i>	<i>26.9</i>	<i>0.038</i>	<i>3.6</i>	<i>0.023</i>				
<i>6-CNA</i>					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	0.040	---	---	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. It is also detected in rice husks and straw after spray application, but only as minor metabolite. Difluoroacetic acid accounted for 0.06 mg a.s. equiv./kg and 0.24 mg a.s. equiv./kg in rice

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kernel after granule application and after spray application, respectively. These findings support the assumption that difluoroacetic acid has a pronounced phloem mobility and is therefore transported selectively into the seeds as a phloem sink. In rice straw and husks, the difluoroacetic acid concentrations ranged between 0.36 mg/kg and 1.38 mg/kg. Generally, higher concentrations of difluoroacetic acid were detected after spray application.

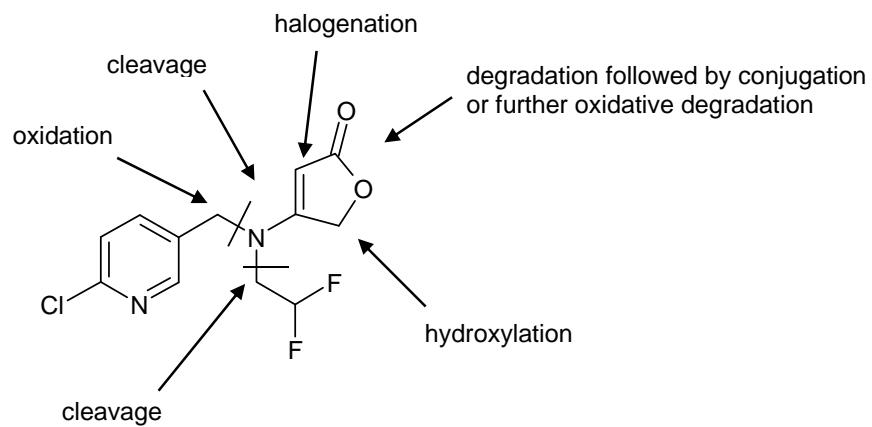
On basis of the metabolites identified, biotransformation of BYI 02960 in rice proceeds by the following pathways:

- oxidative cleavage of the difluoroethylamine bond and formation of difluoroacetic acid
- oxidative degradation of the furanone moiety to an acetic acid group followed either by conjugation with a carbohydrate or by further oxidation to BYI 02960-glyoxylic acid
- complete degradation of the furanone moiety and incorporation of carbon atoms into the natural compound pool, e.g. into glucose/carbohydrates
- oxidative cleavage of the pyridinylmethylamine bond and formation of 6-chloronicotinic acid (6-CNA)
- hydroxylation of the furanone moiety
- halogenation (bromination and most probably chlorination, as well) of the furanone moiety

It is considered likely that halogenation of the furanone moiety of the active substance occurred in the paddy soil/sediment. Uptake of the soil metabolite results in plant residues.

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

Figure 6.2.1-16: Positions involved in metabolic degradation of BYI 02960 in rice matrices



**Analysis on difluoroacetic acid to get information on the fate of the difluoroethane moiety of parent compound BYI 02960**

Extracts of all target and confined rotational crops were analysed for difluoroacetic acid. The extracts originated from the metabolism studies conducted with either [furanone-4-¹⁴C]- or [pyridinylmethyl-¹⁴C]BYI 02960:

Report:	KIIA 6.2.1/12, Schoening, R., Ruhl, S.; 2012
Title:	Determination of residues of difluoroacetic acid in extracts of samples from plant metabolism and confined rotational crops studies after application of BYI 02960
Report No & Edition No	MR-11/050 M-422550-01-1
Guidelines:	OECD 501 Metabolism in Crops US EPA Residue Chemistry Test Guideline OPPTS 860.1300: Nature of the Residue – Plants, Livestock PMRA Regulatory Directive Dir98-02: Residue Chemistry Guidelines Section 2: Nature of the Residue – Plants, Livestock Japanese MAFF, 12 Nousan 8147 European Parliament and Council Regulation (EC) No 1107/2009
GLP	yes

Executive Summary

Due to the fact that only one tomato metabolism study was performed with [ethyl-1-¹⁴C]BYI 02960, additional information on the fate of the difluoroethane moiety of BYI 02960 was provided in the present report. Residues of non-radiolabelled difluoroacetic acid, the most plausible metabolite that can be formed from the difluoroethane moiety due to cleavage of BYI 02960, were determined in extracts of samples of apples, potatoes, cotton, rice, wheat, Swiss chard and turnips originating from plant metabolism and confined rotational crops studies after application of [pyridinylmethyl-¹⁴C]BYI 02960 or [furanone-4-¹⁴C]BYI 02960. To estimate the residue levels of difluoroacetic acid in these crop samples, non-radiolabelled difluoroacetic acid was analyzed according to the provisions of residue analytical method 01304. As expected from the results of the tomato metabolism study performed with [ethyl-1-¹⁴C]BYI 02960, difluoroacetic acid was detected in all crops as a major metabolite accounting for a significant proportion of the BYI 02960 residue.

Material and Methods

Samples of apples, potatoes, cotton, rice, wheat, Swiss chard and turnips were harvested and extracted in different plant metabolism and confined rotational crop studies following application of either [furanone-4-¹⁴C]BYI 02960 or [pyridinylmethyl-¹⁴C]BYI 02960. Aliquots of the crude extracts of all RACs were diluted for analysis of the non-radiolabelled soil and plant metabolite difluoroacetic acid. After dilution an isotopically labelled internal standard solution was added and the extract was analysed by LC-MS/MS according to residue analytical method 01304. No further sample work up was performed.



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The quantification was done by external standardisation in pure solvent using an internal stable labelled standard. During each set of analyses, a calibration curve was established with two measurements on at least five concentration levels for each sample material. The Limit of Quantification (LOQ), defined as the lowest validated fortification level of recovery experiments, was set at 0.01 mg/kg. Difluoroacetic acid was determined as difluoroacetic acid and residues were calculated as difluoroacetic acid.

1. Reference item:

Chemical structure	
Name of Compound	Difluoroacetic acid (BCS-AA56716, DFA)
Certificate of Analysis	AZ 16523, dated 2010-03-31
Chemical name	Difluoroacetic acid
Purity	98.3% (w/w)

2. Internal Standard:

Chemical structure	
Name of Compound	Difluoroacetic acid $^{13}\text{C}_2$ (BCS-AB60481-ISTD, DFA-ISTD)
Certificate of Analysis	KATH 15199-1-4, dated 2010-06-14
Chemical name	Sodium difluoro($^{13}\text{C}_2$)acetat
Purity	> 99%



Findings

The residue levels determined in the samples from the different metabolism studies are summarized in the following tables. The residues are expressed as difluoroacetic acid equivalents.

Table 6.2.1-60: Summary of difluoroacetic acid residues in crop matrices after spray application of BYI 02960

Metabolism study	Crop	Sample material	Use pattern	Residues [mg/kg]	Reference in dossier
Metabolism of [furanone-4- ¹⁴ C]-BYI 02960 in apples	apple	fruits	one foliar spray application at BBCH 69, 86 g a.s./ha x m CH)	0.23	KIIA 6.2.1/06
		leaves		0.62	
		fruits	two foliar spray applications, at BBCH 69 and 14 days PHI, 2 x 86 g a.s./ha x m CH)	0.04	
		leaves		0.45	
Metabolism of [pyridinylmethyl- ¹⁴ C]-BYI 02960 in potatoes	potato	tuber	tuber treatment at planting (BBCH 03), 10.0 g a.s./dt	0.13	KIIA 6.2.1/05
			in-furrow spray application at planting (BBCH 03), 626 g a.s./ha	0.18	
Metabolism of [pyridinylmethyl- ¹⁴ C]-BYI 02960 in Cotton after Spray Application	cotton	gin trash	one spray application, 206 g a.s./ha (at BBCH 16)	0.04	KIIA 6.2.1/09
		seeds		0.03	
		gin trash	two spray applications, 206 g a.s./ha (at BBCH 16), 177 g a.s./ha (at BBCH 95 - 97)	0.02	
		seeds		0.02	
Metabolism of [pyridinylmethyl- ¹⁴ C]-BYI 02960 in paddy rice	rice	straw	two spray applications onto the plants at different growth stages, 178 g a.s./ha (at BBCH 13 - 15) 236 g a.s./ha (at BBCH 87 - 89)	0.39	KIIA 6.2.1/11
		husk		0.46	
		grains		0.08	
		straw	one granular application at the time of transplanting, 434 g a.s./ha (at BBCH 13 - 15)	0.12	
		husk		0.20	
		grains		0.02	



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Table 6.2.1-61 Summary of difluoroacetic acid residues in rotational crop matrices after soil application of BYI 02960

Metabolism study	Rotation	Crop	Sample material	Residues [mg/kg]	Reference in dossier
Metabolism of [furanone-4- ¹⁴ C]BYI 02960 in Confined Rotational Crops	1 st rotation	wheat	forage	0.09	KIIA 6.6.2/01
			hay	0.32	
			straw	0.20	
			grain	1.15	
		Swiss chard	intermediate	0.08	
			mature	0.16	
		turnip	leaves	0.08	
			roots	0.02	
	2 nd rotation	wheat	forage	0.02	
			hay	0.14	
			straw	0.06	
			grain	0.26	
		Swiss chard	intermediate	0.04	
			mature	0.05	
		turnip	leaves	0.03	
			roots	< 0.01	
	3 rd rotation	wheat	forage	< 0.01	
			hay	0.01	
			straw	0.02	
			grain	0.05	
		Swiss chard	intermediate	< 0.01	
			mature	0.01	
		turnip	leaves	< 0.01	
			roots	< 0.01	

Conclusions

Significant levels of difluoroacetic acid (DFA) were detected in most of the primary and confined rotational crops under investigation irrespective of the application technique. High difluoroacetic acid concentrations after foliar spray application indicate that this metabolite is also formed in plants and not only in soil.



Overall Conclusions considering radiolabelled and non-radiolabelled metabolites

Difluoroacetic acid represents the main - or at least a major - proportion of the residue in all edible matrices of primary crops when considering the results of the studies conducted with [¹⁴C]BYI 02960 as shown in Table 6.2.1-62 and Table 6.2.1-63 (see also KIIA 6.11.1).

Table 6.2.1-62: Residues [mg/kg] of BYI 02960 and major metabolites in edible matrices after one application

Compound (BYI 02960-)	potato tubers				apple fruits		rice kernels		cotton seeds	
	tuber treatment		in-furrow appl.		foliar appl.		granule appl.		foliar appl.	
Label	F	P	F	P	F	P	F	P	F	P
TRR	0.078	0.076	0.171	0.115	0.280	0.079	0.140	0.050	0.013²	0.045²
BYI 02960	0.031	0.031	0.097	0.051	0.021	0.034	0.032	0.035		
DFA		0.39 ¹		0.54 ¹	0.69 ¹			0.06 ¹		0.09 ¹
glucose	---		---		0.201		0.038			
6-CNA		0.016		0.021		0.004		0.002		
CHPM-di-glyc		0.003		0.006		---		---		
CHMP-glyc		0.003		0.003		0.004		---		
difluoroethyl-amino-furanone	0.003		0.005		0.009		---			
OH-glyc	0.005	0.005	0.007	0.005	0.001	0.004	---	---		

¹ LC-MS/MS analysis of non-radiolabelled DFA in the extract obtained in the metabolism study

² analysis of the extracts was not feasible due low extraction efficiency and high matrix load in the extracts

F = [furanone-4-¹⁴C]-label

P = [pyridinylmethyl-¹⁴C]-label

Table 6.2.1-63: Residues [mg/kg] of BYI 02960 and major metabolites in edible matrices after two applications

Compound (BYI 02960-)	tomato fruits			apple fruits - with surface wash		apple fruits - w/o surface wash		rice kernels		cotton seeds	
	drench application			spray application				spray appl.		spray appl.	
Label	F	P	E	F	P	F	P	F	P	F	P
TRR	0.096	0.130	0.201	1.133	1.868	1.286	0.545	0.659	0.620	0.016²	0.068²
BYI 02960	0.034	0.031	0.020	0.809	1.652	0.946	0.467	0.373	0.467		
DFA			0.174				0.12 ¹		0.24 ¹		0.06 ¹
glucose	0.026			0.193		0.182		0.023			
6-CNA		0.017			0.009		0.008		0.019		
CHPM-di-glyc		0.048			---		---		---		
CHMP-glyc		0.007			0.010		0.005		---		
difluoroethyl-amino-furanone	0.010		0.004	0.007		0.003		---			
OH-glyc	0.005	0.004	0.001	0.014	0.024	0.014	0.009	---	---		

¹ LC-MS/MS analysis of non-radiolabelled DFA in the extract obtained in the metabolism study

² analysis of the extracts was not feasible due low extraction efficiency and high matrix load in the extracts

F = [furanone-4-¹⁴C]-label

E = [ethyl-1-¹⁴C]

P = [pyridinylmethyl-¹⁴C]-label

In most of the non-edible matrices difluoroacetic acid was also detected at high concentrations, however in lower proportions of the total residue. For example, the non-radioactive DFA residue



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accounted for 0.23 mg/kg in apple fruits after one application of [furanone-4-¹⁴C]BYI 02960, corresponding to 0.69 mg a.s. equiv./kg. Comparing this residue value with the total radioactive residue determined in the fruits of the study (0.28 mg a.s. equiv./kg), it can be estimated that DFA would represent the main portion of the TRR (approx. 90%) in a hypothetical apple metabolism study performed with [ethyl-1-¹⁴C]BYI 02960. The proportion of DFA in apple leaves was much lower: Only approx. 5% of the hypothetical TRR would be represented by DFA, although the absolute residue level was high (0.62 mg/kg, corresponding to 1.86 mg a.s. equiv./kg).

Thus, based on the DFA residue values determined in primary crops, high DFA proportions can be expected in all edible matrices: For apple fruits (single spray treatment), potato tubers (tuber treatment and in-furrow application), cotton seeds (single and double spray experiment) and rice kernels (granular application) it can be estimated that DFA accounts for a higher proportion than parent compound or the main compound detected in the studies with radiolabelled BYI 02960. Thus based upon these findings it can be concluded that DFA is a major plant metabolite in edible crops and should be part of the residue definition for data collection and enforcement.

The difluoroacetic acid residues determined in matrices of confined rotational crops revealed the same picture. For the edible crops wheat grains, Swiss chard and turnip roots, difluoroacetic acid accounted also for a high proportion of the residue, especially in the early rotations. In wheat grains, difluoroacetic acid represented by far the main proportion of a hypothetical TRR (for more details please refer to KIIA 6.11.1) in all three rotations as shown in the following tables.



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Table 6.2.1-64: Residues [mg/kg] of BYI 02960 and major metabolites in the matrices of rotational crops (1st rotation)

Compound (BYI 02960-)	wheat forage		wheat hay		wheat straw		wheat grain	
Label	F	P	F	P	F	P	F	P
TRR	0.783	1.407	2.003	2.409	6.290	9.015	0.478	0.177
BYI 02960	0.365	0.640	0.672	0.676	2.459	3.261	0.002	0.015
DFA	0.27 ¹		0.96 ¹		0.60 ¹		3.45 ¹	
glucose	---		---		---		0.338	
bromo-amino-furanone	0.016		0.033		0.172			---
difluoroethyl-amino-furanone	0.077		0.205		0.374		---	
glyoxylic acid	0.124	0.172	0.227	0.176	0.965	0.615	0.024	0.011
OH-glyc	0.028	0.048	0.067	0.135	0.242	0.296	0.007	0.009
6-CNA-glycerol-gluA (2 + 3)		0.199		0.569		1.900		0.036
OH	0.010	0.019	0.038	0.052	0.161	0.239	0.011	0.019

(2 + 3) isomer 2 and/or isomer 3

F = [furanone-4-¹⁴C]-label P = [pyridinylmethyl-¹⁴C]-label

Compound (BYI 02960-)	Swiss chard intermediate		Swiss chard mature		turnip leaves		turnip roots	
Label	F	P	F	P	F	P	F	P
TRR	0.848	1.358	0.871	1.483	0.679	0.815	0.074	0.072
BYI 02960	0.460	0.779	0.371	0.687	0.437	0.508	0.041	0.042
DFA	0.24 ¹		0.48 ¹		0.24 ¹		0.06 ¹	
glucose	---		---		---		0.003	
bromo-amino-furanone	---		---		---		---	
difluoroethyl-amino-furanone	---		0.010		---		---	
glyoxylic acid	0.031	0.021	0.041	0.039	0.045	0.021	0.009	0.006
OH-glyc	0.072	0.101	0.119	0.162	0.076	0.076	<0.001	0.002
6-CNA-glycerol-gluA (2 + 3)		---		---		---		---
OH	0.017	0.024	0.017	0.023	0.012	0.011	<0.001	<0.001

¹ LC-MS/MS analysis of non-radiolabelled DFA in the extract obtained in the confined rotation crop study performed with [furanone-4-¹⁴C]BYI 02960

(2 + 3) isomer 2 and/or isomer 3

F = [furanone-4-¹⁴C]-label P = [pyridinylmethyl-¹⁴C]-label

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 Table 6.2.1-65: Residues [mg/kg] of BYI 02960 and major metabolites in the matrices of rotational crops (2nd rotation)

Compound (BYI 02960-)	wheat forage		wheat hay		wheat straw		wheat grain	
Label	F	P	F	P	F	P	F	P
TRR	0.193	0.308	1.081	1.009	1.519	2.148	0.103	0.057
BYI 02960	0.124	0.183	0.314	0.283	0.538	0.804	0.001	0.001
DFA	0.06 ¹		0.42 ¹		0.18 ¹		0.78 ¹	
glucose	---		---		---		---	
bromo-amino-furanone	0.006		0.107		0.093		---	
difluoroethyl-amino-furanone	0.016		0.075		0.090		---	
glyoxylic acid	<0.001	---	0.020	0.010	0.018	0.036	0.001	0.001
OH-glyc	0.007	0.008	0.037	0.045	0.08	0.112	0.002	0.003
6-CNA-glycerol-gluA (2 + 3)	---	0.044	---	0.242	---	0.472	---	0.007
OH	0.003	0.005	0.021	0.019	0.047	0.079	0.003	0.004

(2 + 3) isomer 2 and/or isomer 3

 F = [furanone-4-¹⁴C]-label P = [pyridinylmethyl-¹⁴C]-label

Compound (BYI 02960-)	Swiss chard intermediate		Swiss chard mature		turnip leaves		turnip roots	
Label	F	P	F	P	F	P	F	P
TRR	0.311	0.332	0.263	0.438	0.158	0.230	0.014	0.022
BYI 02960	0.171	0.170	0.072	0.108	0.108	0.153	0.004	0.011
DFA	0.12 ¹		0.15 ¹		0.09 ¹		<0.03 ¹	
glucose	---		---		---		0.002	
bromo-amino-furanone	---		---		---		---	
difluoroethyl-amino-furanone	0.032		0.046		0.002		---	
glyoxylic acid	---	---	---	---	0.002	---	<0.001	---
OH-glyc	0.036	0.058	0.047	0.111	0.020	0.025	<0.001	0.001
6-CNA-glycerol-gluA (2 + 3)		---		---	---	---	---	---
OH	0.006	0.013	0.006	0.016	0.002	0.004	---	---

¹ LC-MS/MS analysis of non-radiolabelled DFA in the extract obtained in the confined rotation crop study performed with [furanone-4-¹⁴C]BYI 02960

(2 + 3) isomer 2 and/or isomer 3

 F = [furanone-4-¹⁴C]-label P = [pyridinylmethyl-¹⁴C]-label



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Table 6.2.1-66: Residues [mg/kg] of BYI 02960 and major metabolites in the matrices of rotational crops (3rd rotation)

Compound (BYI 02960-)	wheat forage		wheat hay		wheat straw		wheat grain	
Label	F	P	F	P	F	P	F	P
TRR	0.111	0.117	0.254	0.321	0.462	0.491	0.047	0.017
BYI 02960	0.048	0.053	0.047	0.063	0.096	0.129	0.001	0.002
DFA	<0.03 ¹		0.03 ¹		0.06 ¹		0.15 ¹	
glucose	---		---		---		---	
bromo-amino-furanone	0.006		0.027		0.034		---	
difluoroethyl-amino-furanone	0.013		0.021		0.024		---	
glyoxylic acid	---	0.001	0.001	---	---	0.013	---	---
OH-glyc	0.003	0.004	0.006	0.010	0.016	0.014	0.001	0.001
6-CNA-glycerol-gluA (2 + 3)		0.022		0.095		0.140		0.002
OH	0.001	0.001	0.004	0.005	0.010	0.010	0.001	0.001

(2 + 3) isomer 2 and/or isomer 3

F = [furanone-4-¹⁴C]-label P = [pyridinylmethyl-¹⁴C]-label

Compound (BYI 02960-)	Swiss chard intermediate		Swiss chard mature		turnip leaves		turnip roots	
Label	F	P	F	P	F	P	F	P
TRR	0.180	0.135	0.152	0.130	0.090	0.083	0.008	0.008
BYI 02960	0.066	0.042	0.051	0.036	0.065	0.058	0.006	0.005
DFA	<0.03 ¹		0.03 ¹		<0.03 ¹		<0.03 ¹	
glucose	---		---		---		<0.001	
bromo-amino-furanone	---		---		---		---	
difluoroethyl-amino-furanone	0.025		0.024		0.001		---	
glyoxylic acid	<0.001	0.003	---	0.001	0.002	---	---	---
OH-glyc	0.040	0.033	0.033	0.036	0.009	0.008	<0.001	<0.001
6-CNA-glycerol-gluA (2 + 3)		---		---	---		---	
OH	0.007	0.005	0.005	0.004	0.001	---	---	---

¹ LC-MS/MS analysis of non-radiolabelled DFA in the extract obtained in the confined rotation crop study performed with [furanone-4-¹⁴C]BYI 02960

(2 + 3) isomer 2 and/or isomer 3

F = [furanone-4-¹⁴C]-label P = [pyridinylmethyl-¹⁴C]-label

High difluoroacetic acid concentrations were also detected for the non-edible RACs, but parent compound represented generally the main or at least a comparable residue. In corresponding crop samples from different plant back intervals, the DFA levels generally decreased significantly from the first to the third rotation, showing DFA levels slightly above or below the limit of quantification in all crops of the third rotation. The highest DFA proportion in crops of the third rotation was detected in wheat grains (0.05 mg/kg).

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Thus, based on the difluoroacetic acid residue values determined, high DFA proportions can be expected in confined rotational crops, as well. Based upon these findings it can be concluded that difluoroacetic acid is a major metabolite in edible matrices of rotational crops and should be part of the residue definition for data collection and enforcement.

The analyses on non-radiolabelled difluoroacetic acid has shown that this metabolite can be found at significant residue levels/proportions in fruits, tubers, roots and in seeds. On basis of these results, difluoroacetic acid is expected to have a pronounced phloem mobility and will therefore be transported in these repository parts of plants known as phloem sinks.

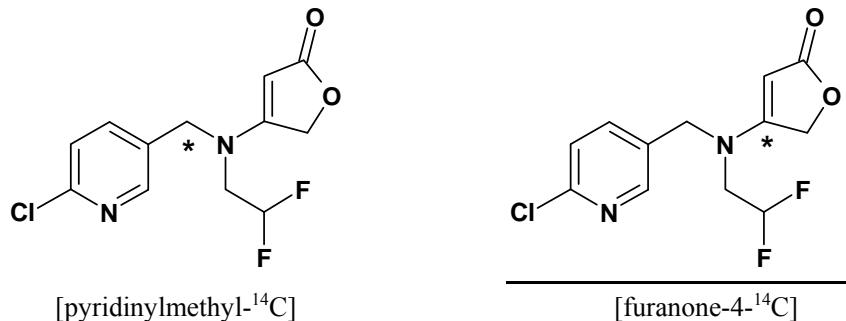
Additional non-GLP studies -initiated to gain information on the systemicity of DFA - confirmed this assumption. In one study BYI 02960 was applied on cucumber leaf sheaths (leaf 1 to 5) and the residue levels of BYI 02960, difluoroacetic acid, BYI 02960-difluoroethyl-amino-furanone and 6-CNA were determined in mature fruits of lower and higher plant parts at different time points (3, 7, 14, 21 and 28 days after application). Only difluoroacetic acid was detected in significant concentrations in fruits of the upper and lower plant parts indicating phloem mobility of this metabolite. Highest DFA levels (approx. 0.5 mg/kg) were found in cucumbers sampled 14 and 21 days after application. BYI 02960-difluoroethyl-amino-furanone was not detected in cucumber fruits, and parent compound BYI 02960 and 6-CNA were detected in trace levels only in some fruit samples. This experiment shows clearly that difluoroacetic acid is phloem mobile and can be transported within the plant.

With this information, an additional experiment was conducted: [Ethyl-1-¹⁴C]BYI 02960 was applied on cucumber leaves (scenario 1: two single droplets on either side of the midrib of leaf 4 and scenario 2: one single droplet on the axil of leaf 4) and the phloem translocation of the radioactivity in lower and upper plant parts was detected at three different time points by employing a Fuji BAS 5000® phosphor-imaging system. The visualized distribution of the radioactivity confirmed phloem mobile compounds. With the radiolabel chosen, it is very likely that metabolite difluoroacetic acid is the phloem mobile compound detected.

The reports of these non-GLP studies are in preparation and will be submitted as soon as they are available.

**IIA 6.2.2 Poultry**

Two studies on the metabolism of BYI02960 in laying hens were conducted with the test compound labelled either in the [pyridinylmethyl-¹⁴C]- or the [furanone-4-¹⁴C]-position as shown by the following structural formulas (* denotes the label position):



Report:	KIIA 6.2.2/01, Authors: [REDACTED], R., [REDACTED], J., 2012
Title:	[Pyridinylmethyl- ¹⁴ C]BYI02960: Metabolism in the Laying Hen
Report No & Document No	MEF-11/199 M-422162-01-2
Date:	10.1.2012
Guidelines:	OECD-Guideline for the Testing of Chemicals No. 503, Metabolism in Livestock US-EPA, Residue Chemistry OPPTS 860.1300; EU Regulation 1107/2009 amended by Directive 96/68/EC, 7030/VI/95/rev.3 Appendix F
GLP	Yes, according to Japan MAFF GLP standard 11 Nousan 6283; US EPA – FIFRA GLP (40CFR Part 160); Principles of GLP, German Chemical Law, current version of Annex 1
Testing Facility and Dates	[REDACTED] [REDACTED], Germany Experimental work: 5.3.2010 – 11.4.2011

Executive Summary

The metabolism and excretion of [pyridinylmethyl-¹⁴C]BYI 02960 (common name: flupyradifurone) were investigated in laying hens as a model for poultry. Six hens were orally dosed once daily in the morning for 14 consecutive days with an aqueous 0.5% Tragacanth® suspension of 1.02 mg/kg body weight which corresponded to 16.18 mg a.s. /kg dry feed/day. The animals were sacrificed six hours after the last administration. Total radioactive residues (TRR) were determined daily in the eggs and excreta, and at sacrifice in the dissected organs and tissues (muscle, fat, liver, kidney, skin and eggs from ovary/oviduct). Eggs, muscle, fat, liver and excreta were extracted and analysed for parent compound and metabolites.

Recovery and Elimination of Radioactivity

The overall recovery rate was 96.11% of the total dose. The remaining radioactivity was expected to still be present in the gastro-intestinal tract at sacrifice, due to the short period between the last administration and sacrifice. On average, only 0.24% of the total dose was measured in the eggs. At sacrifice, the radioactive residues in the organs and tissues were calculated or estimated to be about

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

0.37% of the total dose, half of which was detected in the skeletal muscle (0.19%). Until sacrifice, the excretion of radioactivity accounted for 95.51% of the total dose.

Total Radioactive Residues in Eggs, Organs and Tissues

The concentration of radioactivity in eggs ranged from 0.016 mg/kg at day one to 0.119 mg/kg at sacrifice. Following a rather linear increase, a plateau level of approx. 0.08 mg/kg was reached six days after the first administration.

In the **organs** and **tissues**, the highest radioactivity concentrations were determined in kidney (1.073 mg/kg) and liver (0.435 mg/kg) indicating the significance of these organs for excretion and metabolism. These values corresponded to 0.05% and 0.08% of the total dose, respectively. The residue level of the eggs collected from the ovary and oviduct at sacrifice (0.147 mg/kg) was insignificantly higher than the levels of the laid eggs collected at sacrifice (0.119 mg/kg). This indicated that the egg yolk was not a preferential site for secretion. The residue levels of liver and kidney were followed in decreasing order by those found in the skin (0.094 mg/kg), muscle (0.070 mg/kg) and subcutaneous fat (0.021 mg/kg). The residue level of the total muscle corresponded to about 0.19% of the total dose assuming a value of 40% of the body weight for this tissue. Assuming values of 12% and 4% of the body weight for fat and skin, the residues in both tissues accounted for about 0.04% of the total dose.

Metabolism

For the determination of parent compound and metabolites, eggs, muscle, fat, liver and excreta from day 13 were pooled from all six animals. Eggs, muscle and liver as well as excreta were extracted with mixtures of acetonitrile/water and pure acetonitrile. Fat was extracted with acetonitrile and n-heptane followed by solvent partition. Post-extraction solids of liver were exhaustively extracted using microwave assistance. The resulting extracts of eggs, muscle and liver contained more than 92% of the total radioactive residue. Approx. 80% of the radioactivity was extractable from fat. Unextractable residues in fat were very low and amounted to only 0.004 mg/kg. After purification and concentration, the extracts were analysed using HPLC with radiometric detection.

The metabolic pattern determined in the excreta was similar to those in the eggs, organs and tissues. Therefore, parent compound and metabolites were isolated from excreta and identified by LC-MS/MS or NMR spectroscopy. The identified metabolites were used as reference compounds in extracts of eggs, muscle, fat and liver.

The identification rate was approx. 86% in eggs, 84% in muscle, 78% in fat and 59 % in liver. The remaining residues were characterised by their extraction- and chromatographic behaviour. Generally, the concentrations of parent compound and metabolites were very low. The parent compound was the major compound in eggs, muscle and fat (approx. 10 - 20%) and amounted to less than 0.017 mg/kg. Major metabolites were BYI 02960-acetyl-AMCP in eggs, muscle and fat and BYI 02960-OH-SA in fat and liver. Other metabolites were BYI 02960-lactato-mercaptyl-nicotinic acid, BYI 02960-6-CNA, BYI 02960-des-difluoroethyl and BYI 02960-OH. Minor residues were identified as BYI 02960-acetyl-cysteinyl-nicotinic acid, BYI 02960-des-difluoroethyl-OH-SA and BYI 02960-AMCP-difluoroethanamine-SA. In addition, BYI 02960-acetic acid was detected in the excreta.

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

The metabolite pattern of the extracts of the current hen study and the hen study with the ^{14}C -label in the furanone ring of the molecule (KIIA 6.2.2/02) were comparable, except the label specific metabolites.

The concentration of the total radioactivity (expressed as mg a.s. equiv. /kg), as well as the distribution of the parent compound and metabolites and the identification rates in eggs, liver, muscle and fat are summarised in the following table:

	Eggs (day 3 to 13.25)		Muscle		Fat		Liver	
TRR [mg/kg]	0.084		0.070		0.021		0.435	
Sample/Report name BYI 02960-	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Conventional extraction	96.1	0.081	92.6	0.064	79.7	0.017	74.6	0.324
lactato-mercaptyl-nicotinic acid	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
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
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
OH	18.0	0.015	8.1	0.006	5.5	0.001	1.5	0.007
parent compound	19.8	0.017	9.8	0.007	15.3	0.003	0.9	0.004
Identified in conventional extract	86.2	0.072	84.2	0.059	77.9	0.016	58.6	0.255
Characterised in conventional extract	9.9	0.008	8.4	0.006	1.8	<0.001	16.0	0.070
Exhaustive extraction	n.a.		n.a.		n.a.		19.8	0.086
Total identified	86.2	0.072	84.2	0.059	77.9	0.016	58.6	0.255
Total characterised	9.9	0.008	8.4	0.006	1.8	<0.001	35.8	0.156
Total extracted	96.1	0.081	92.6	0.064	79.7	0.017	94.5	0.411
Solids	3.9	0.003	7.4	0.005	20.3	0.004	5.5	0.024
Accountability	100.0	0.084	100.0	0.070	100.0	0.021	100.0	0.435

The main metabolic reactions of [pyridinylmethyl- ^{14}C]BYI 02960 in the laying hen are:

Hydroxylation in position 5 of the furanone ring forming BYI 02960-hydroxy followed by conjugation with sulfuric acid to BYI 02960-OH-SA

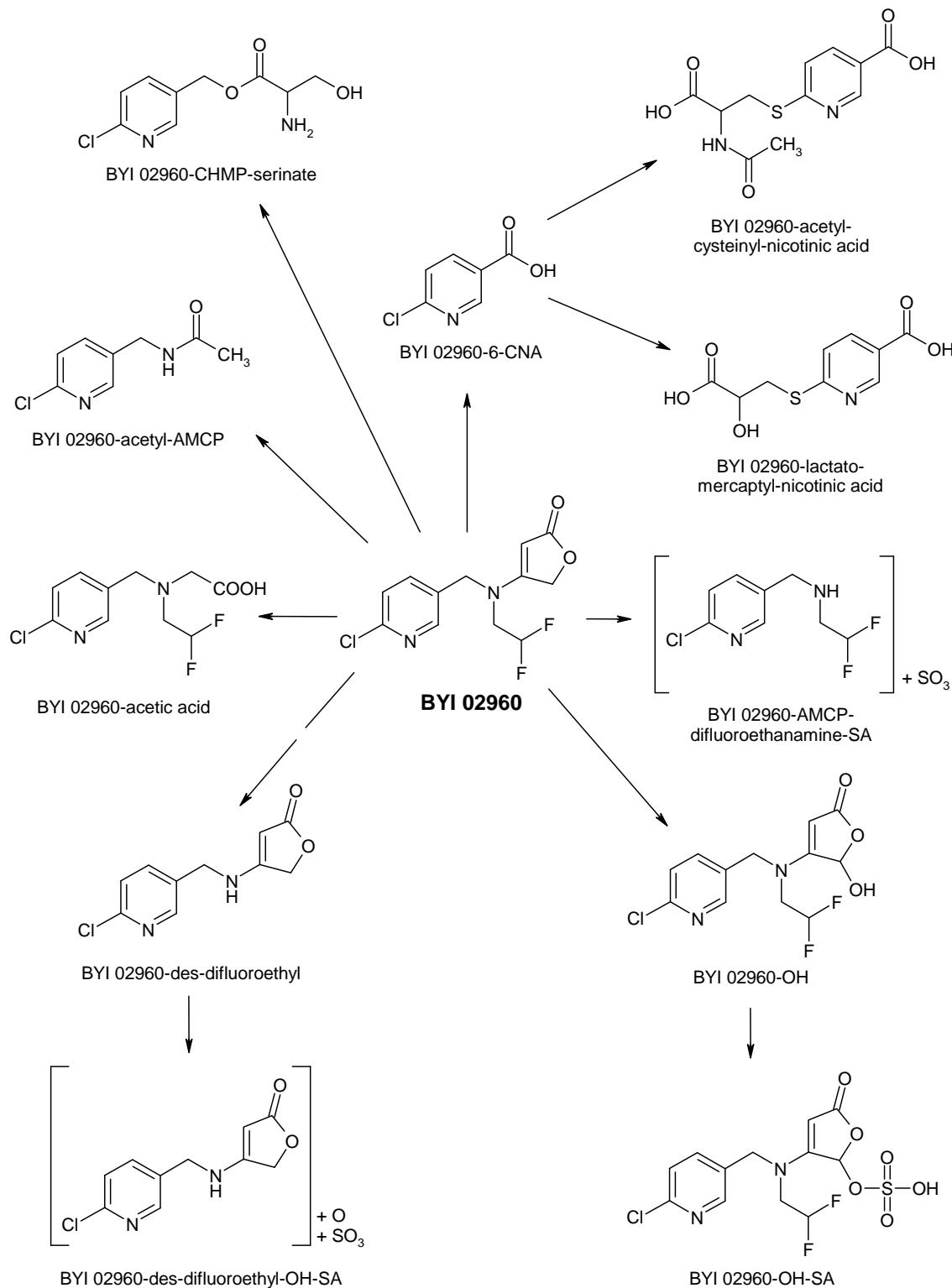
- Oxidative cleavage of the pyridinylmethyl moiety forming BYI 02960-6-CNA
- Substitution of the chloro group of BYI 02960-6-CNA with glutathione followed by degradation resulting in the conjugates BYI 02960-acetyl-cysteinyl-nicotinic acid and BYI 02960-lactato-mercaptyl-nicotinic acid
- Cleavage of 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

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

- Oxidative degradation of the furanone ring forming BYI 02960-acetic acid
- Cleavage of the pyridinylmethyl moiety forming an alcohol conjugated with serine (BYI 02960-CHMP-serinate)
- Cleavage of the furanone ring and the difluoroethyl group forming an amine followed by acetylation to BYI 02960-acetyl-AMCP

Based on these results a metabolic pathway of [pyridinylmethyl-¹⁴C]BYI 02960 in the laying hen is proposed as shown on the next page.

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

 Proposed metabolic Pathway of [pyridinylmethyl-¹⁴C]BYI 02960 in laying hens:


I. Materials and Methods

A. Materials

1. Test Material

IUPAC Name	4-{[(6-chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino}furan-2(5H)-one
Code name	BYI02960
Common name	flupyradifurone (proposed ISO)
Empirical formula	C ₁₂ H ₁₁ ClF ₂ N ₂ O ₂
Molar mass	288.68 g/mol
Labelling position	[pyridinylmethyl- ¹⁴ C]
Specific radioactivity	4.37 MBq/mg = 118.08 µCi/mg (delivered sample before radiodilution) 3.50 MBq/mg = 2.10 x 10 ⁸ dpm/mg = 94.59 µCi/mg = 27.31 Ci/mol (sample after radiodilution)
Radiochemical purity	> 99 % by radio-HPLC and > 98 % by radio-TLC
Nonradioactive test substance	Batch BYI 02960-PU-02
Chemical purity	99.4%
Dose level	14 oral doses of 1.02 mg/kg bw/day by gavage
Vehicle	0.5 % aqueous Tragacanth® suspension

2. Test Animals

Species	Laying hen (<i>Gallus gallus domesticus</i>)
Strain	“White Leghorn”
Breeding facility	Fa. Klaus Mechow, Wuppertal, Germany
Sex and numbers involved	6 out of 18 hens were selected by maximum egg production
Age	6 – 8 months
Body weight	1.63 kg at first administration, 1.60 kg at sacrifice
Acclimatization	35 days
Identification	Cage labelling and wing tags
Housing	Individually in stainless steel metabolism cages for laying hens allowing almost quantitative collection of eggs and excreta (supplier: E. Becker & Co GmbH, Castrop-Rauxel, Germany room temperature 22 - 30°C, relative humidity 30 - 57%. 16 h light / 8 h dark cycle, air change 10 – 15 times per hour
Feed and water	Hens were fed with “RWZ-LegeGold Mehl”, a pulverised chicken feed. This feed was not a certified diet, i.e. it was not checked for contamination according to current standards. The feed was supplemented by eggshells and crushed marine shells during the acclimation period. The feed consumption was recorded by weighing during the experiment (mean consumption 103 g/day/hen), tap water, ad libitum

B. Study Design

Dosing

The radiolabelled test compound was mixed with the non-radiolabelled test compound in order to reduce the specific radioactivity from 4.37 MBq/mg to 3.5 MBq/mg. For the 14 administrations, 4 dosing suspensions in 0.5% aqueous Tragacanth® were prepared and each suspension was applied for three or four administrations. The radiolabelled test substance proved to be stable until the last dose.

The oral administration was carried out with a knob cannula attached to a glass syringe. Immediately after dosage, the swallowing was supported by a gentle massage of the throat towards the crop. The hens received a mean dose of 1.67 mg (3.51×10^8 dpm) per animal and day at a mean body weight of 1.63 kg, corresponding to an actual dose of 1.02 mg a.s./kg bw. The amount of radioactivity of the actually administered amounts served as reference for the calculation of total radioactivity in the biological samples. The administration volume was 1.0 mL/kg body weight. The dose level was tolerated without any observable toxicological symptoms.

Sampling of eggs and excreta

During the test, the cages were inspected for egg production once daily (in the morning before administration) and the number of eggs was recorded for all hens. After sampling, the eggshells were discarded, and the egg white and yolk were weighed and then thoroughly mixed. An aliquot sample of each egg mix was taken for the determination of the total radioactivity in triplicate by LSC. The residual amount of the egg-mix from all animals was stored at ca. -18°C until metabolite analysis.

The excreta of each hen were collected once daily from the collecting tins as quantitatively as possible. The individual samples were homogenised after the addition of water, before recording the total weights. An aliquot of each fraction was processed for radioactivity measurement by combustion/LSC. The remaining samples were stored in a freezer until metabolite analysis.

Sacrifice and sampling of organs and tissues

The treated hens were weighed and sacrificed ca. 6 hours after the last (14th) dosage. The animals were anaesthetised using carbon dioxide, sacrificed by decapitation and exsanguinated. The organs and tissues were prepared immediately after sacrifice. Liver without the gall bladder, kidneys, leg and breast muscle, skin without subcutaneous fat, subcutaneous fat and eggs from the ovary and oviduct were sampled immediately after sacrifice and their fresh weights were recorded. The gall bladders were punctured for the collection of the bile fluid which was then stored frozen for an optional metabolite analysis.

Sample preparation

After dissection, the organs or tissue samples were transferred into ice-cooled vessels. Liver, kidneys, muscle samples and subcutaneous fat as well as eggs dissected from the ovary and oviduct were thoroughly homogenised in half-frozen state. An aliquot of each resulting tissue pulp was weighed, freeze-dried, weighed again and homogenised. The radioactivity was then determined in triplicate using combustion/LSC. Aliquots of subcutaneous fat and skin without fat were weighed and solubilised with tissue solubiliser (BTS-450®) and measured for radioactivity by LSC. All samples were divided into equal portions and stored at ca. -18°C until metabolite analysis.



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To prepare representative samples of eggs, organs or tissues, corresponding samples of all individual animals were pooled before extraction. An egg pool was prepared from day 3 to sacrifice. All egg samples from this collection period were used. Composite samples of muscle (leg and breast), subcutaneous fat, liver and excreta (day 13) from all animals were prepared. The individual samples were thoroughly homogenised and kept frozen until extraction.

The total radioactive residue of each pool was determined by combustion/LSC (solid samples) or directly by LSC (e.g. combined eggs and extracts). Eggs, muscle, liver and excreta were extracted 3 times with acetonitrile/water 80:20 (v/v) and finally with pure acetonitrile. For eggs, muscle and liver, the combined conventional extracts were subjected to a clean-up step using an SPE-cartridge. Post extraction solids of liver were exhaustively extracted twice with acetonitrile/water (1:1; v/v) under microwave assistance. Fat was extracted twice with n-heptane and acetonitrile followed by a solvent partition procedure yielding an acetonitrile - and an n-heptane phase. All extracts were used for quantification of parent compound and metabolites by HPLC.

Radioactivity measurement

Solid samples were combusted prior to radioactivity determination and the formed $^{14}\text{CO}_2$ absorbed in an alkaline trapping solvent. The determination of radioactivity of liquid samples was conducted by liquid scintillation counting (LSC) using sub-samples (1 - 3 replicates). Quenching effects were automatically corrected using an external standard and a quenching library. The instrument background was automatically subtracted. For all samples, the limit of detection (LOD) was established at approximately 10 dpm per aliquot after instrument background correction. The limit of quantification (LOQ) was established as 2 times the background radioactivity (dpm) of each instrument/method.

Metabolite analysis

The prepared extracts were subjected to HPLC using a reversed phase column (C18) and the eluting solvents water/formic acid 99:1 (v/v) and acetonitrile/water/formic acid 97:2:1 (v/v/v) in the gradient mode. Detection was performed by a UV- (254 nm) and a radioisotope detector with a glass bead scintillator. In order to check the completeness of the elution, representative samples of egg, muscle and liver extract were injected, re-collected, and radioassayed by LSC. The recoveries were between 99.1 and 100.5% of the injected radioactivity. Radiolabelled and non-labelled reference compounds were used in co-chromatography for identification of metabolites.

Metabolites were isolated from the excreta, identified by LC-MS/MS or NMR-spectroscopy and used as reference compounds for the identification of metabolites in eggs and liver by HPLC co-chromatography. Other reference compounds were taken from the goat metabolism study also using the pyridinylmethyl- ^{14}C - labelled test compound [KIIA 6.2.3/01] or provided as non-radiolabelled reference compounds. Metabolites in muscle and fat were assigned by comparison of the metabolic patterns and retention times.

II. Results and Discussion

A. Recovery and Elimination of Radioactivity

The recovery of radioactivity in laying hens after administration of an average daily dose of 1.02 mg [pyridinylmethyl-¹⁴C]BYI 02960 per kg bw/day on 14 consecutive days is presented in Table 6.2.2-1. The overall recovery accounted for 96.11% of the totally administered dose. The remaining amount of radioactivity (approx. 4%) was expected to still be present in the gastro-intestinal tract at sacrifice, due to the short period between the last administration and sacrifice.

Table 6.2.2-1: Distribution of residues in eggs, muscle, fat, liver and kidney of laying hens following oral administration of 14 daily doses of [pyridinylmethyl-¹⁴C] BYI02960 at a dose rate of 1.02 mg/kg

Sample	Percent of total dose administered	Concentration of total radioactivity [mg/kg]
Liver	0.08	0.435
Kidney	0.05	1.073
Eggs from ovary/oviduct	0.01	0.147
Muscle, total	0.19	0.070
Skin, total	0.02	0.094
Fat, total	0.02	0.021
Organs/tissues, total	0.37	-----
Eggs	0.24	0.081
Excreta, total	95.51	-----
Total Recovery	96.11	-----

B. Levels and Time Course of Total Radioactive Residues in Eggs

The radioactivity levels measured in the egg samples from all animals are presented in Table 6.2.2-2. The concentration in eggs ranged from 0.016 mg/kg at day one to 0.119 mg/kg at sacrifice. Following a rather linear increase a plateau level of approx. 0.08 mg/kg was reached at day six.

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.2.2-2: Time course of total radioactivity in eggs following oral administration of 14 daily doses of [pyridinylmethyl-¹⁴C] BYI02960 at a dose rate of 1.02 mg/kg

Time after the first admin. [d]	Admin. no.	Cumulative secretion [% of total dose admin.] (mean)	Concentration of total radioactivity [mg/kg]
0	1	----- #	----- #
1	2	0.01	0.016
2	3	0.01	0.044
3	4	0.03	0.061
4	5	0.04	0.068
5	6	0.06	0.075
6	7	0.08	0.080
7	8	0.09	0.083
8	9	0.11	0.083
9	10	0.13	0.087
10	11	0.15	0.090
11	12	0.17	0.087
12	13	0.19	0.085
13	14	0.21	0.088
13.25	---	0.24	0.119

----- # no egg collected

C. Total Radioactive Residues in Dissected Organs and Tissues

The concentration of the total radioactivity in the dissected organs and tissues at sacrifice is shown in Table 6.2.2-1 (last column). The highest concentrations were determined in kidney (1.073 mg/kg) and liver (0.435 mg/kg) reflecting the significance of these organs for excretion and metabolism. In relation to the dose totally administered, these values corresponded to 0.05% and 0.08%, respectively. The residue level of the eggs collected from the ovary and oviduct at sacrifice (0.147 mg/kg) was insignificantly higher (factor 1.2) than the levels of the laid eggs collected at sacrifice (0.119 mg/kg). This indicated that the egg yolk was not a preferential site for secretion of the radioactivity. The residue levels of liver and kidney were followed in decreasing order by those determined in the skin (0.094 mg/kg), muscle (0.070 mg/kg) and subcutaneous fat (0.021 mg/kg). The residue level of the total skeletal muscle corresponded to about 0.19% of the total dose assuming a 40% contribution to the body weight. Assuming values of 12% and 4% of the body weight for fat and skin, the residues in both tissues accounted for about 0.04% of the dose totally administered.

D. Extraction Efficiency of Residues

Eggs (day 3 to sacrifice), muscle and liver pools as well as excreta (day 13) were extracted with acetonitrile/water (8:2; v/v) followed by pure acetonitrile. Additionally, solids of liver were exhaustively extracted with acetonitrile/water (1:1, v/v) under microwave assistance. Fat was extracted with acetonitrile and n-heptane followed by a solvent partition into an acetonitrile- and an n-heptane phase. After purification and concentration, the resulting extracts represented 96.1% of the total



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

radioactivity for eggs (based on the 1st extraction), 92.6% for muscle, 79.7% for fat (based on the 2nd extraction), 94.5% for liver and 97.7% for excreta.

The radioactivity concentrations in the post-extraction solids amounted to 0.003 mg/kg (3.9%) for eggs (based on the 1st extraction), 0.005 mg/kg (7.4%) for muscle, 0.004 mg/kg (20.3%) for fat (based on the 2nd extraction) and 0.024 mg/kg (5.5%) for liver.

A summary of the extraction efficiency is shown in Table 6.2.2-3.

Table 6.2.2-3: Extraction efficiency of eggs, muscle, fat and liver samples following oral administration of 14 daily doses of [pyridinylmethyl-¹⁴C] BYI02960 at a dose rate of 1.02 mg/kg

	Eggs (1 st extraction)		Muscle		Fat (2 nd extraction)		Liver	
TRR [mg/kg]	0.084		0.070		0.021		0.435	
	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Conventional extraction	96.1	0.081	92.6	0.064	79.7	0.017	74.6	0.324
- acetonitrile/water (8/2, v/v)	96.1	0.081	92.6	0.064	---		74.6	0.324
- acetonitrile phase	---		---		79.7	0.017	---	
- n-heptane phase	---		---		n.d.	n.d.	---	
Exhaustive extract:	---		---		---		19.8	0.086
Total extracted	96.1	0.081	92.6	0.064	79.7	0.017	94.5	0.411
Post-extraction solids (PES)	3.9	0.003	7.4	0.005	20.3	0.004	5.5	0.024
Accountability	100.0	0.084	100.0	0.070	100.0	0.021	100.0	0.435

n.d.: not detected

To demonstrate storage stability, a second extraction and sample preparation of eggs and fat were performed approx. 7 months after the first extraction followed by profiling of the metabolites.

E. Quantification, Identification and Characterisation of Residues

Isolation and Identification of Parent Compound and Metabolites in Excreta

The extract of excreta was used to isolate the metabolites using a ternary reversed phase HPLC system. The three eluents employed were:

A: 1L water + 1.54 mL ammonium hydroxide solution (25%) + 0.8 mL formic acid, adjusted to pH 7

B: acetonitrile / eluent A (99:1; v/v)

C: methanol / tetrahydrofuran (1:1; v/v)

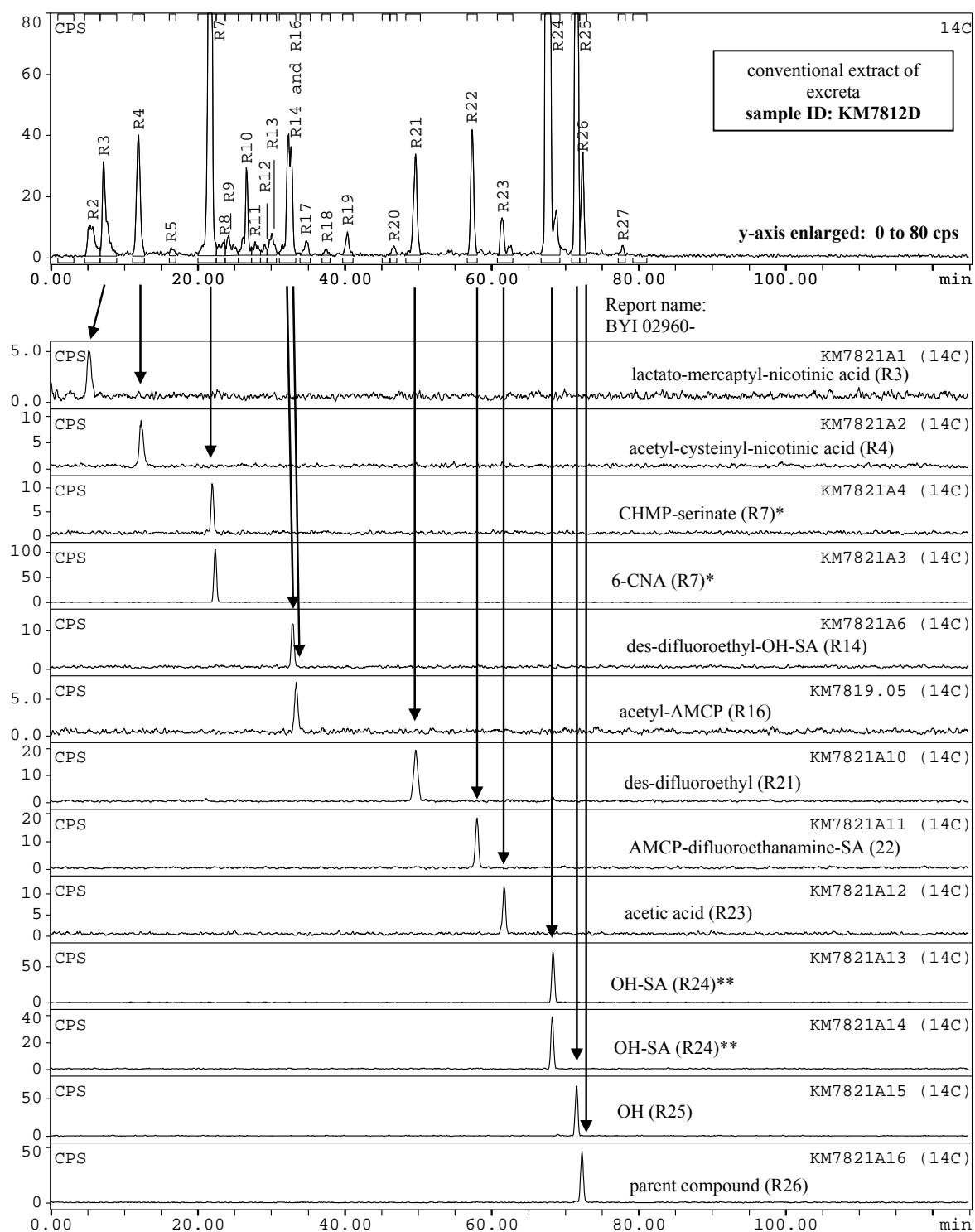
Detailed information can be found in the report.

The identification of the metabolites was achieved by LC-MS/MS. The position of the hydroxy-sulfate group of metabolite BYI 02960-OH-SA was assigned by NMR spectroscopy after multiple purification steps. In case of BYI 02960-OH, the position of the hydroxy group was identified by HPLC co-chromatography with the non-radiolabelled reference compound. An assignment of all identified metabolites to the metabolite profile of excreta is shown in Figure 6.2.2-1.

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Figure 6.2.2-1: Assignment of isolated and identified metabolites in the excreta of laying hens following oral administration of 14 daily doses of [pyridinylmethyl-¹⁴C] BYI02960 at a dose rate of 1.02 mg/kg

Integration G:\ME\M1854569-8\MEF06246\BYI02960TERNÄR\KM7820.07A



* both metabolites can be separated using an acidic RP HPLC system

** BYI 02960-OH-SA was isolated and identified in two neighbouring regions due to a matrix effect

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)Identification and Assignment of Metabolites in Eggs, Organs and Tissues

Identified metabolites from excreta were used as reference compounds for the identification or assignment of metabolites in eggs and liver. Metabolites in other organs and tissues were assigned by comparison of the metabolic profiles and retention times.

F. Distribution of Parent Compound and Metabolites in Eggs, Organs and Tissues

The distribution of the parent compound and metabolites in eggs, organs and tissues is summarised in Table 6.2.2-4. Of the total radioactivity, ca. 86% was identified in eggs, 84% in muscle, 78% in fat and 59% in liver. All other residues were characterised by their extraction and chromatographic behaviour.

Table 6.2.2-4: 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] BYI02960 at a dose rate of 1.02 mg/kg

	Eggs (day 3 to 13.25)		Muscle		Fat		Liver	
TRR [mg/kg]	0.084		0.070		0.021		0.435	
Sample/Report name BYI 02960-	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Conventional extraction	96.1	0.081	92.6	0.064	79.7	0.017	74.6	0.324
lactato-mercaptyl-nicotinic acid	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
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
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
OH	18.0	0.015	8.1	0.006	5.5	0.001	1.5	0.007
parent compound	19.8	0.017	9.8	0.007	15.3	0.003	0.9	0.004
identified in the conventional extract	86.2	0.072	84.2	0.059	77.9	0.016	58.6	0.255
characterised in the conventional extract	9.9	0.008	8.4	0.006	1.8	<0.001	16.0	0.070
Exhaustive extraction	n.a.		n.a.		n.a.		19.8	0.086
Total identified	86.2	0.072	84.2	0.059	77.9	0.016	58.6	0.255
Total characterised	9.9	0.008	8.4	0.006	1.8	<0.001	35.8	0.156
Total extracted	96.1	0.081	92.6	0.064	79.7	0.017	94.5	0.411
Solids	3.9	0.003	7.4	0.005	20.3	0.004	5.5	0.024
Accountability	100.0	0.084	100.0	0.070	100.0	0.021	100.0	0.435

* co-elution with BYI 02960-CHMP-serinate was excluded for eggs, muscle and liver

Metabolites in Eggs

Besides the parent compound (0.017 mg/kg; 19.8%), major metabolites in eggs were BYI 02960-acetyl-AMCP (0.019 mg/kg; 23.1%) and BYI 02960-OH (0.015 mg/kg, 18.0%). Further metabolites



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were BYI 02960-lactato-mercaptyl-nicotinic acid, BYI 02960-6-CNA, BYI 02960-des-difluoroethyl, BYI 02960-OH-SA and two unknown metabolites. They were detected in low amounts ≤ 0.007 mg/kg.

Metabolites in Muscle

The main metabolite in muscle was BYI 02960-acetyl-AMCP, which amounted to 0.028 mg/kg (40.2%). Parent compound, BYI 02960-6-CNA, BYI 02960-des-difluoroethyl and BYI 02960-OH were quantified in amounts between 0.006 to 0.007 mg/kg (approx. 9%). Minor identified metabolites were BYI 02960-lactato-mercaptyl-nicotinic acid, BYI 02960-des-difluoroethyl-OH-SA and BYI 02960-OH-SA (each ≤ 0.002 mg/kg).

Metabolites in Fat

No residues were detected in the n-heptane phase. The main metabolites in the polar phase of fat were parent compound (0.003 mg/kg, 15.3%), BYI 02960-acetyl-AMCP (0.006 mg/kg, 28.5%) and BYI 02960-OH-SA (0.003 mg/kg, 16.2%). All other metabolites were detected in amounts ≤ 0.001 mg/kg.

Metabolites in Liver

The majority of the radioactive residue (approx. 75%) was extracted with acetonitrile/water. The main metabolites in this extract were BYI 02960-OH-SA (0.098 mg/kg, 22.5%), BYI 02960-lactato-mercaptyl-nicotinic acid (0.068 mg/kg, 15.5%), BYI 02960-6-CNA (0.028 mg/kg, 6.4%) and BYI 02960-acetyl-AMCP (0.027 mg/kg, 6.3%). The parent compound was only detected in negligible amounts of 0.004 mg/kg. All other metabolites amounted to ≤ 0.014 mg/kg, except one unknown metabolite (0.027 mg/kg).

Metabolites in the exhaustive extract of liver ranged from 0.002 to 0.027 mg/kg. They were not identical with the metabolites in the acetonitrile/water extract. All unknown metabolites in the exhaustive extract of liver were not further investigated, due to their low amount and the high concentration of matrix. Therefore, they were characterised by their extraction and chromatographic behaviour.

G. Storage Stability of Residues

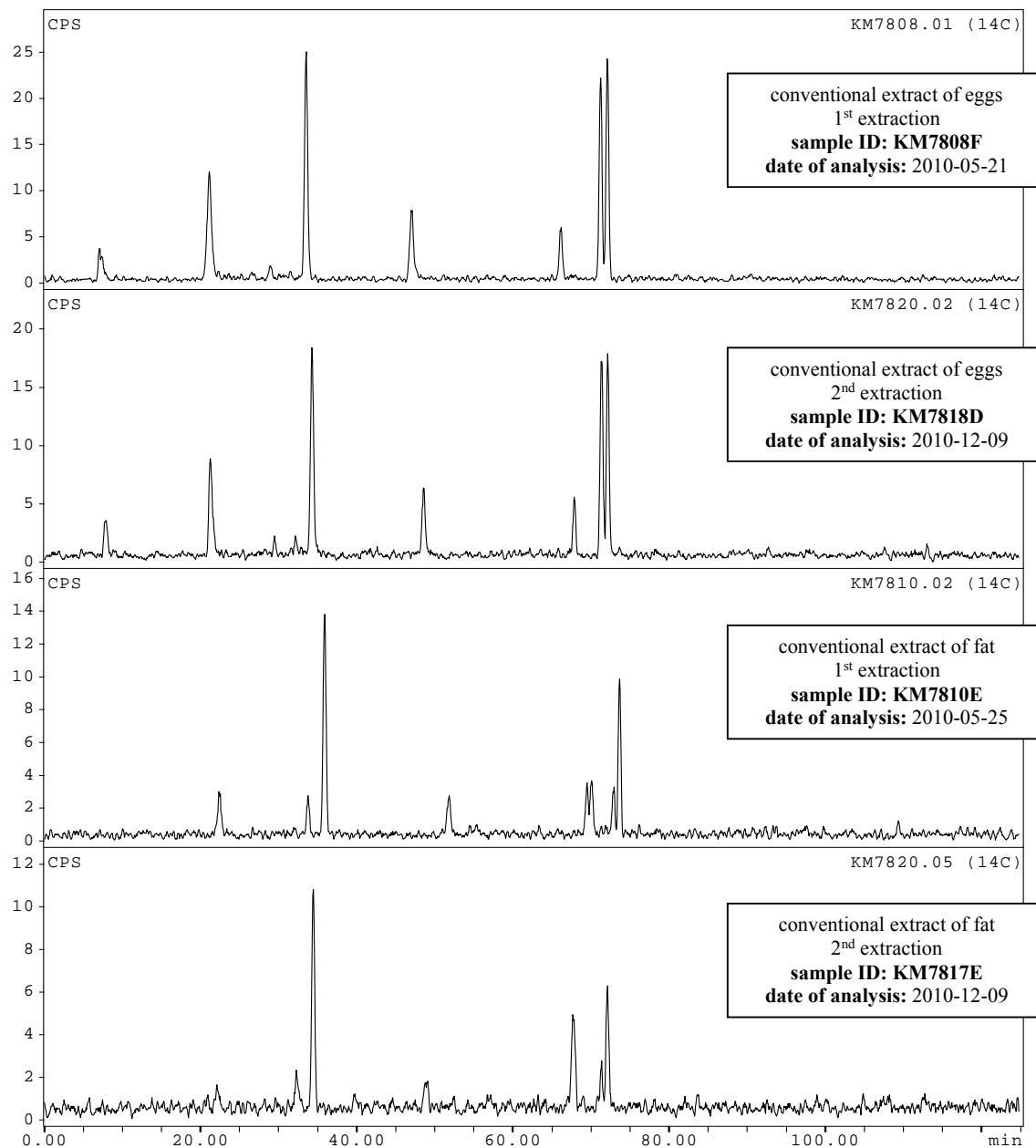
During the study, all samples and extracts were stored below -18°C or for a short time in a refrigerator. All egg samples and edible organs or tissues were extracted within approx. 2 months after sample collection. The first metabolic profile was recorded within six days after the start of the first extraction and sample preparation.

The storage stability of residues in the extracts of eggs and fat was demonstrated for a period of approx. 7 months by a repeated extraction and HPLC profiling (Figure 6.2.2-2).



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Figure 6.2.2-2: Storage stability of aged sample material of eggs and fat of laying hens following oral administration of 14 daily doses of [pyridinylmethyl-14C] BYI 02960 at a dose rate of 1.02 mg/kg



The stability of the extracts of muscle and liver was concluded in analogy to eggs. The pattern of metabolites (fingerprint) in the first profile of the extracts of muscle and liver was similar to the pattern analysed approx. 6 months later. In particular, the distribution of the peak areas was very similar for both analyses. Therefore, it was concluded that as for eggs, also the extracts of muscle and liver were stable for a period of approx. 6 months after extraction.



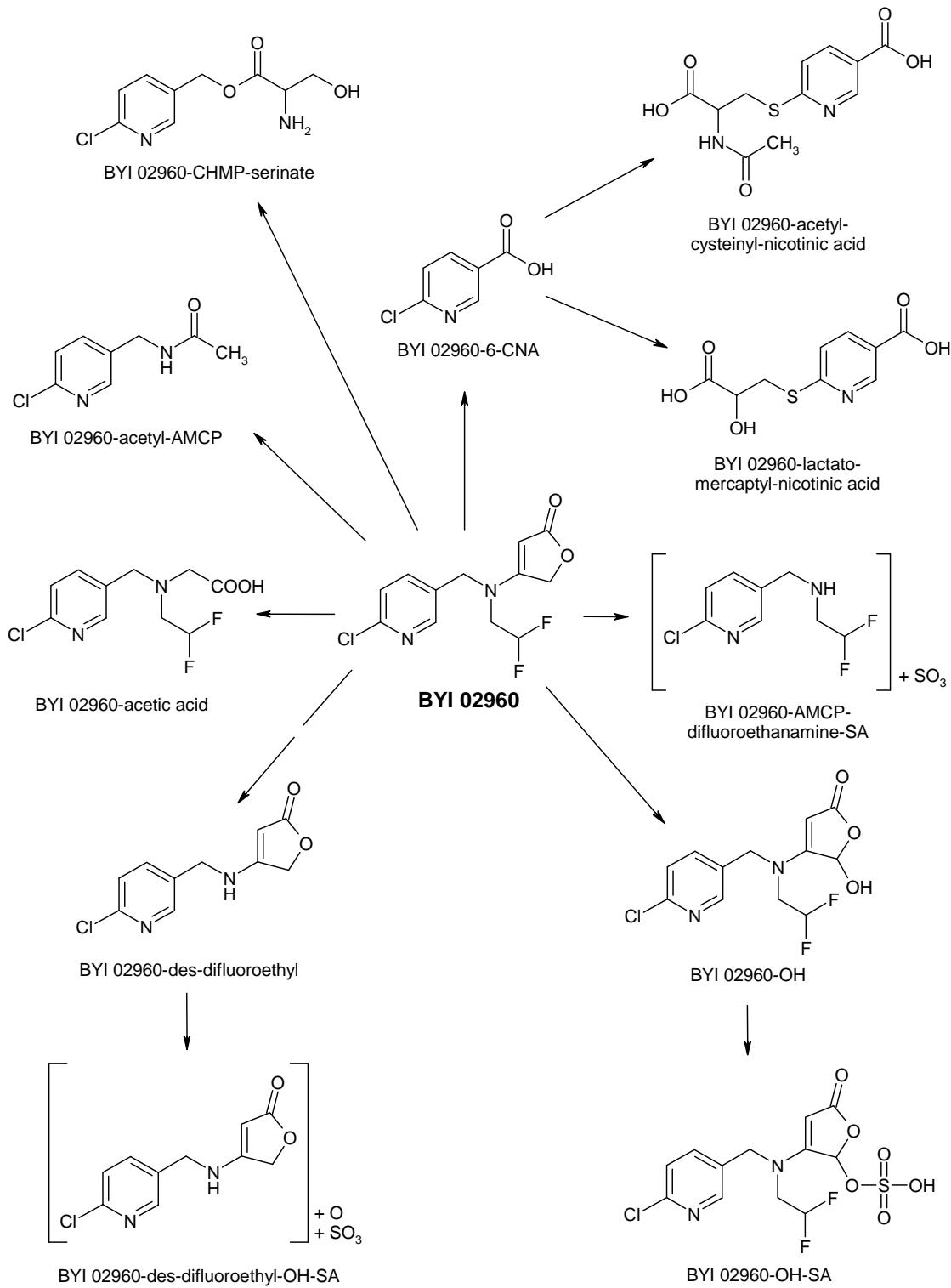
III. Conclusion

The metabolic and excretion behaviour of [pyridinylmethyl-¹⁴C]BYI 02960 in laying hens can be characterised as follows:

- The concentrations of radioactivity in eggs and edible tissues were relatively low if compared to the dose level and the dosing period of 14 days.
- The evaluation of these results should consider the fact that an exaggerated dose level of 16.18 mg/kg feed/day was administered. Furthermore, a significant amount of radioactivity was detected in the excreta and the relatively high radioactivity in kidney and liver at sacrifice 6 hours after the last administration indicate that the residues are further metabolised and finally eliminated.
- A residue plateau level in whole eggs was reached within the test period at the 6th day after the first administration.
- The main portion of residues (ca. 80 to 96%, depending on the matrix) was efficiently extracted from eggs, muscle, fat and liver.
- Only low amounts of parent compound were detected in eggs, muscle, fat and liver.
- Non label specific metabolites are BYI 02960-hydroxy, BYI 02960-OH-SA, BYI 02960-des-difluoroethyl, BYI 02960-des-difluoroethyl-OH-SA and BYI 02960-acetic acid. They were detected in approximately the same low amounts as in the study with the furanone label.
- The cleavage of the molecule was a significant reaction in the metabolism. A prominent portion of label specific metabolites was detected in the edible tissues. Major label specific metabolites were BYI 02960-6-CNA and BYI 02960-acetyl-AMCP.
- The main metabolic reactions in the laying hen are:
 - Hydroxylation in position 5 of the furanone ring forming BYI 02960-OH followed by conjugation with sulfuric acid to BYI 02960-OH-SA
 - Oxidative cleavage of the pyridinylmethyl-bridge forming BYI 02960-6-CNA
 - Substitution of the chloro group of BYI 02960-6-CNA with glutathione followed by degradation resulting in two conjugates BYI 02960-acetyl-cysteinyl-nicotinic acid and BYI 02960-lactato-mercaptyl-nicotinic acid
 - Cleavage of the difluoroethyl group forming BYI 02960-des-difluoroethyl followed by hydroxylation and conjugation with sulfuric acid to BYI 02960-des-difluoroethyl-OH-SA
 - Cleavage of the furanone ring and conjugation with sulfonic acid forming BYI 02960-AMCP-difluoroethanamine-SA
 - Oxidative degradation of the furanone ring forming BYI 02960-acetic acid
 - Cleavage of the pyridinylmethyl-bridge forming an alcohol conjugated with serine (BYI 02960-CHMP-serinate)
 - Cleavage of the furanone ring and the difluoroethyl group forming an amine followed by acetylation (BYI 02960-acetyl-AMCP)

Based on these results a metabolic pathway of [pyridinylmethyl-¹⁴C] BYI02960 in the laying hen is proposed in Figure 6.2.2-3.

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Figure 6.2.2-3: Proposed metabolic pathway of [pyridinylmethyl-¹⁴C] BYI02960 in laying hens



Report:	KIIA 6.2.2/02, Authors: [REDACTED], R., [REDACTED], J., 2012
Title:	[Furanone-4- ¹⁴ C]BYI02960: Metabolism in the Laying Hen
Report No & Document No	MEF-11/200 Date: 10.1.2012 M-422263-01-2
Guidelines:	OECD-Guideline for the Testing of Chemicals No. 503, Metabolism in Livestock US-EPA, Residue Chemistry OPPTS 860.1300; EU Regulation 1107/2009 amended by Directive 96/68/EC, 7030/VI/95/rev.3 Appendix F
GLP	Yes, according to Japan MAFF GLP standard 11 Nousan 6283; US EPA – FIFRA GLP (40CFR Part 160); Principles of GLP, German Chemical Law, current version of Annex 1
Testing Facility and Dates	[REDACTED] [REDACTED], Germany Experimental work: 12.2.2010 - 9.12.2010

Executive Summary

The metabolism and excretion of [furanone-4-¹⁴C]BYI 02960 (common name: flupyradifurone) were investigated in laying hens as a model for poultry. Six hens were orally dosed once daily in the morning for 14 consecutive days with an aqueous 0.5% Tragacanth® suspension of 1.05 mg of the active substance per kg body weight which corresponded to 17.13 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.

Recovery and Elimination of Radioactivity

The overall recovery rate was 82.16% of the total dose. A part of the remaining radioactivity was expected to still be present in the gastro-intestinal tract at sacrifice, due to the short period between the last administration and sacrifice. Another part is probably exhaled as ¹⁴CO₂. The formation of carbon dioxide was also shown in a rat quantitative whole-body autoradiography study using the test compound with the same labelling position (KIIA 5.1.2/02). An average amount of 2.35% of the total dose was determined in the eggs. At sacrifice, the residues in the organs and tissues were calculated or estimated to be about 1.8% of the total dose from which about 28% was detected in the skeletal muscle (0.5%). Until sacrifice, the excretion products accounted for 78.01% of the total dose.

Total Radioactive Residues in Eggs and in Organs and Tissues

The concentration of radioactivity in eggs ranged from 0.024 mg/kg at day one to 1.198 mg/kg at sacrifice. Following a linear increase a plateau level of 1.035 mg/kg was reached nine days after the first administration.

In the organs and tissues, the highest radioactivity concentrations were determined in liver (2.178 mg/kg) and kidney (1.083 mg/kg) indicating the significance of these organs for metabolism and excretion. These values corresponded to 0.37% and 0.05% of the total dose, respectively. The residue level of the eggs collected from the ovary and oviduct at sacrifice (2.774 mg/kg) was by a factor of 2.3 higher than the levels of the laid eggs collected at sacrifice (1.198 mg/kg). This indicated that the egg yolk was a preferential site for secretion of the radioactivity. The residue levels of liver and kidney were followed in decreasing order by those found in the subcutaneous fat (0.427 mg/kg), skin (0.257 mg/kg) and muscle (0.183 mg/kg). The residue level of the total muscle corresponded to about



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

0.50% of the total dose assuming a value of 40% of the body weight for this tissue. Assuming values of 12% and 4% of the body weight for fat and skin, the residues in both tissues accounted for about 0.41% of the total dose.

Metabolism

For metabolism investigations, eggs of day 2 to 7 and 8 to sacrifice, muscle, fat, liver and excreta from day 13 were pooled from all six animals. Eggs, muscle, fat and liver were extracted with acetonitrile and n-heptane followed by solvent partition. In case of eggs, muscle and liver the extraction procedure was continued with a mixture of acetonitrile/water. Excreta were extracted with acetonitrile/water followed by pure acetonitrile. Post-extraction solids of eggs, muscle and liver were exhaustively extracted with mixtures of acetonitrile/water followed by acetonitrile/water/formic acid using microwave assistance. The extraction efficiencies were above 81% of the total radioactivity in all samples. Unextractable residues were low and ranged from 0.006 (eggs day 2 to 7) to 0.036 mg/kg (liver).

The major part of residues was detected in the n-heptane phases of eggs (days 2 to 7 ca. 52% and days 8 to sacrifice ca. 58%), fat (ca. 96%) and liver (ca. 52%). The residues in the n-heptane phase of muscle only amounted to ca. 8%. Residues in the acetonitrile/water extracts of eggs, muscle, fat and liver were detected at the same level as analysed in the laying hen study using the pyridinylmethyl label. The residues ranged from 0.011 mg/kg (2.6%) for fat to 0.450 mg/kg (20.7%) for liver. A significant part of the residues was found in the exhaustive extracted using microwave assistance. Between 4 and 10% of the radioactivity was detected in the neutral exhaustive extracts and between 8 and 40% in the acidic exhaustive extracts of eggs, muscle and liver.

The radioactive residues in the n-heptane phases of eggs, muscle, fat and liver showed the same behaviour in thin layer chromatography. They were identified as fatty acids after saponification. These residues in the n-heptane phases were specific for the furanone label and were caused by the cleavage and subsequent total degradation of the furanone ring to smaller carbon units (C-1- or C-2-fragments), entering the carbon pool of endogenous compounds. A part of these fragments was probably also converted to $^{14}\text{CO}_2$ as can be suggested from the lower overall recovery if compared with the study using the metabolically stable pyridinylmethyl label.

Residues in the acetonitrile/water- and exhaustive extracts were analysed by HPLC with radiometric detection. The metabolite patterns of the current hen study and the corresponding extracts of the hen study with the pyridinylmethyl-label were comparable, except the label specific metabolites. Parent compound, BYI 02960-OH, BYI 02960-OH-SA, BYI 02960-des-difluoroethyl, BYI 02960-des-difluoroethyl-OH-SA were identified in the acetonitrile/water extracts by comparing the retention times and the metabolite patterns found in the current study with those of the laying hen study with the pyridinylmethyl label [KIIA 6.2.2/01]. The metabolites were quantified at almost the same low level as detected in the extracts of the study with the pyridinylmethyl label. Additionally, BYI 02960-acetic acid was detected in excreta.

Metabolites in the polar region of the acetonitrile/water extracts were specific for the furanone label, they ranged from 0.001 to 0.012 mg/kg for muscle and from 0.009 to 0.050 mg/kg for liver. These polar metabolites showed the same behaviour in thin layer chromatography as the metabolites in the

corresponding polar regions of urine from the rat (KIIA 5.1.2/01) and urine, extracts of liver and kidney of the goat (KIIA 6.2.3/02). Metabolites in the neutral and acidic exhaustive extracts were characterised by their extraction behaviour and in some cases by HPLC, depending on the amount of matrix content.

The identification rate, including the parent compound, metabolites and residues identified as fatty acids after saponification, accounted for approx. 59% for eggs day 2 to 7, 63% for eggs day 8 to sacrifice, 17% for muscle, 96% for fat and 59% for liver. All other residues as well as the losses during the concentration procedures were characterised by their extraction- or chromatographic behaviour.

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

The concentration of the total radioactivity (expressed as mg a.s. equiv. /kg) as well as the distribution of the parent compound and metabolites and the identification rates in all edible samples are summarised in the following table:

	Eggs (day 2 to 7)	Eggs (day 8 to 13.25)	Muscle	Fat	Liver
TRR [mg/kg]	0.540	1.048	0.183	0.427	2.178
Sample/Report name BYI 02960-	% of TRR mg/kg	% of TRR mg/kg	% of TRR mg/kg	% of TRR mg/kg	% of TRR mg/kg
Conventional extraction	65.0 0.351	66.8 0.701	38.6 0.071	98.5 0.421	72.1 1.571
n-heptane phase (identified as fatty acids)	52.0 0.281	58.3 0.611	8.1 0.015	95.9 0.410	51.5 1.121
des-difluoroethyl-OH-SA	0.1 0.001	n.d. n.d.	0.5 0.001		0.2 0.004
des-difluoroethyl	1.2 0.006	0.6 0.007	2.6 0.005		0.8 0.017
OH-SA	0.6 0.003	0.5 0.005	n.d. n.d.		5.1 0.112
OH	2.3 0.013	1.6 0.016	2.4 0.004		0.8 0.018
Parent compound	2.3 0.013	1.6 0.016	2.9 0.005		0.5 0.010
identified in the conventional extract	58.5 0.316	62.5 0.656	16.5 0.030	95.9 0.410	58.9 1.282
characterised in the conventional extract	6.4 0.035	4.3 0.045	22.0 0.040	2.6 0.011	13.2 0.288
losses during the conventional extraction	10.7 0.058	10.7 0.112	10.3 0.019	n.d.	n.d.
Exhaustive extraction					
- neutral ACN/water extract	7.0 0.038	3.6 0.038	8.1 0.015	n.a.	9.6 0.209
- 1 st acidic ACN/water extract	8.3 0.045	10.0 0.105	39.5 0.072	n.a.	12.1 0.264
- 2 nd acidic ACN/water extract	1.1 0.006	3.3 0.035	see **	n.a.	n.a.
characterised in the exhaustive extracts	16.5 0.089	16.9 0.177	47.7 0.087	n.a.	21.7 0.473
Total identified	58.5 0.316	62.5 0.656	16.5 0.030	95.9 0.410	58.9 1.282
Total characterised	22.9 0.124	21.2 0.223	69.7 0.128	2.6 0.011	34.9 0.761
Total extracted	81.5 0.440	83.8 0.878	86.2 0.158	98.5 0.421	93.8 2.044
Total losses	17.5 0.094	14.7 0.155	10.3 0.019	n.d. n.d.	4.5 0.098
Solids	1.1 0.006	1.5 0.016	3.5 0.006	1.5 0.006	1.7 0.036
Accountability	100.0 0.540	100.0 1.048	100.0 0.183	100.0 0.427	100.0 2.178

** Both acidic ACN/water extracts of muscle were combined before concentration.



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

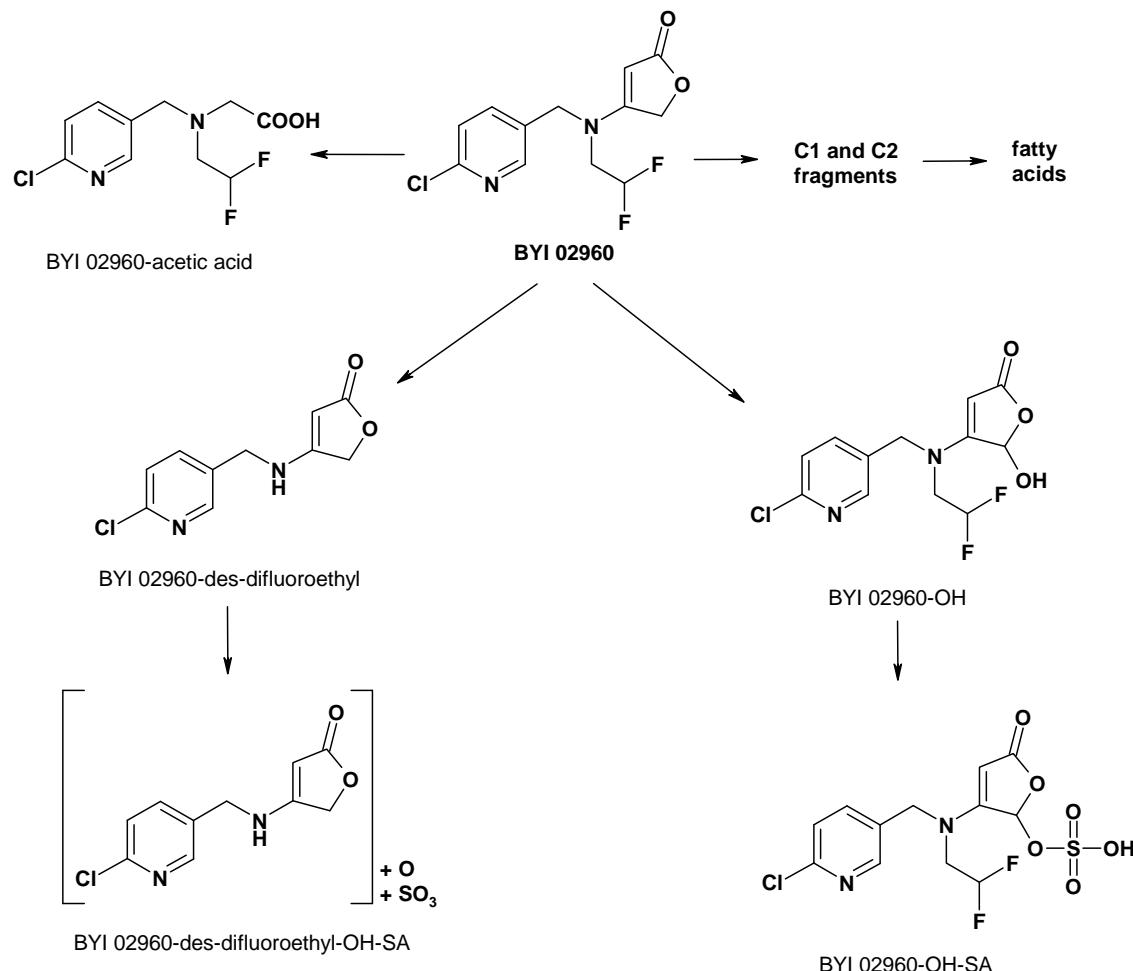
The metabolic and excretion behaviour of [furanone-4-¹⁴C]BYI 02960 in the laying hen can be characterised by the following observations:

- The concentration of the radioactivity in eggs and edible tissues was slightly elevated when compared to the dose level and the dosing period of 14 days. This was mainly caused by the partial instability of the radiolabel used. For a small part of the total dose the furanone ring of the molecule obviously underwent extensive biotransformation to C-1- and C-2-fragments resulting in an accumulation of radioactivity in biomolecules. Some of these fragments were probably also converted to the terminal product ¹⁴CO₂ as can be derived from the lower overall recovery in comparison to the study with the metabolically stable pyridinylmethyl label.
- The relatively high values in liver and kidney at sacrifice 6 hours after the last dose indicated that metabolism and excretion are still ongoing processes.
- A residue plateau level in whole eggs was reached at day nine after the first administration.
- The extraction rates were above 80% for eggs and edible tissues. Unextracted residues were quite low and amounted to ≤ 0.036 mg/kg.
- Parent compound and non label specific metabolites, such as BYI 02960-OH, BYI 02960-OH-SA, BYI 02960-des-difluoroethyl and BYI 02960-des-difluoroethyl-OH-SA were identified in similar amounts as in the study using the pyridinylmethyl label. Additionally, BYI 02960-acetic acid was detected in excreta.
- The main metabolic reactions of [furanone-4-¹⁴C]BYI 02960 in the laying hen are:
 - Cleavage and 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 for the biosynthesis of fatty acids
 - Hydroxylation in position 5 of the furanone ring forming BYI 02960-OH followed by conjugation with sulphuric acid to BYI 02960-OH-SA
 - Cleavage of the difluoroethyl group forming BYI 02960-des-difluoroethyl followed by hydroxylation and conjugation with sulfuric acid to BYI 02960-des-difluoroethyl-OH-SA
 - Oxidative degradation of the furanone ring forming BYI 02960-acetic acid



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Based on these results the following metabolic pathway is proposed:



I. Materials and Methods

A. Materials

1. Test Material

IUPAC Name	4-{[(6-chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino}furan-2(5H)-one
Code name	BYI02960
Common name	flupyradifurone (proposed ISO)
Empirical formula	C ₁₂ H ₁₁ ClF ₂ N ₂ O ₂
Molar mass	288.68 g/mol
Labelling position	[furanone-4- ¹⁴ C]
Specific radioactivity	4.24 MBq/mg = 114.50 µCi/mg (delivered sample before radiodilution) 3.50 MBq/mg = 2.10 x 10 ⁸ dpm/mg = 94.59 µCi/mg = 27.31 Ci/mol (sample after radiodilution)
Radiochemical purity	> 98 % by radio-HPLC and > 99 % by radio-TLC
Nonradioactive test substance	Batch BYI 02960-PU-02
Chemical purity	99.4%
Dose level	14 oral doses of 1.05 mg/kg bw/day by gavage
Vehicle	0.5 % aqueous Tragacanth® suspension

2. Test Animals

Species	Laying hen (<i>Gallus gallus domesticus</i>)
Strain	“White Leghorn”
Breeding facility	Baumeister, Brenscheid 16, 58339 Breckerfeld, Germany
Sex and numbers involved	6 out of 18 hens were selected by maximum egg production
Age	6 – 8 months
Body weight	1.56 kg at first administration, 1.56 kg at sacrifice
Acclimatization	14 days
Identification	Cage labelling and wing tags
Housing	Individually in stainless steel metabolism cages for laying hens allowing almost quantitative collection of eggs and excreta (supplier: E. Becker & Co GmbH, Castrop-Rauxel, Germany room temperature 21 - 26°C, relative humidity 39 - 56%. 16 h light / 8 h dark cycle, air change 10 – 15 times per hour
Feed and water	The hens were fed with “RWZ-LegeGold Mehl”, a pulverised chicken feed. This feed was not a certified diet, i.e. it was not checked for contamination according to current standards. The feed was supplemented by eggshells and crushed marine shells during the acclimation period. The feed consumption was recorded by weighing during the experiment (mean consumption 103 g/day/hen), tap water, ad libitum

B. Study Design

Dosing

The radiolabelled test compound was mixed with the non-radiolabelled test compound in order to reduce the specific radioactivity from 4.24 MBq/mg to 3.5 MBq/mg. For the 14 administrations, 4 dosing suspensions in 0.5% aqueous Tragacanth® were prepared and each suspension was applied for three or four administrations. The radiolabelled test substance proved to be stable until the last dose.

The oral administration was carried out with a knob cannula attached to a glass syringe. Immediately after dosage, the swallowing was supported by a gentle massage of the throat towards the crop. The hens received a mean dose of 1.63 mg (3.42×10^8 dpm) per animal and day at a mean body weight of 1.56 kg, corresponding to an actual dose of 1.05 mg a.s./kg bw. The amount of radioactivity of the actually administered amounts served as reference for the calculation of total radioactivity in the biological samples. The administration volume was 1.0 mL/kg body weight. The dose level was tolerated without any observable toxicological symptoms.

Sampling of eggs and excreta

During the test, the cages were inspected for egg production once daily (in the morning before administration) and the number of eggs was recorded for all hens. After sampling, the eggshells were discarded, and the egg white and yolk were weighed and then thoroughly mixed. An aliquot of each egg mix was taken for the determination of the total radioactivity in triplicate by LSC. The residual amount of the egg-mix from all animals was stored at ca. -18°C until metabolite analysis.

The excreta of each hen were collected once daily from the collecting tins as quantitatively as possible. The individual samples were homogenised after the addition of water, before recording the total weights. An aliquot of each fraction was processed for radioactivity measurement by combustion/LSC. The remaining samples were stored in a freezer until metabolite analysis.

Sacrifice and sampling of organs and tissues

The treated hens were weighed and sacrificed ca. 6 hours after the last (14th) dosage. The animals were anaesthetised using carbon dioxide, sacrificed by decapitation and exsanguinated. The organs and tissues were prepared immediately after sacrifice. Liver without the gall bladder, kidneys, leg and breast muscle, skin without subcutaneous fat, subcutaneous fat, eggs from the ovary and oviduct, were sampled immediately after sacrifice and their fresh weights were recorded. The gall bladders were punctured for the collection of the bile fluid which was then stored frozen for an optional metabolite analysis.

Sample preparation

After dissection, the organs or tissue samples were transferred into ice-cooled vessels. Liver, kidneys, muscle samples, and subcutaneous fat as well as eggs dissected from the ovary and oviduct were thoroughly homogenised in half-frozen state. An aliquot of each resulting tissue pulp was weighed, freeze-dried, weighed again and homogenised. The radioactivity was then determined in triplicate using combustion/LSC. Aliquots of subcutaneous fat and skin without fat were weighed and solubilised with tissue solubiliser (BTS-450®) and measured for radioactivity by LSC. All samples were divided into equal portions and stored at ca. -18°C until metabolite analysis.



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To prepare representative samples of eggs, organs or tissues, corresponding samples of all individual animals were pooled before extraction. Two egg pools were prepared (day 2 - day 7 and day 3 - sacrifice). All egg samples from these collection periods were used. Composite samples of muscle (leg and breast), subcutaneous fat, liver and excreta (day 13) from all animals were prepared. The individual samples were thoroughly homogenised and kept frozen until extraction.

The total radioactive residues of each pool were determined by combustion/LSC (solid samples) or direct LSC (e.g. combined eggs and extracts). Eggs, muscle, fat and liver were successively extracted with acetonitrile and n-heptane followed by a solvent partition procedure. In case of eggs, muscle and liver this extraction procedure was continued using a mixture of acetonitrile/water (7:3; v/v). An aliquot of the excreta of day 13 was extracted with acetonitrile/water (8:2, v/v) followed by pure acetonitrile. All extracts were used for quantification of parent compound and metabolites by HPLC.

Radioactivity measurement

Solid samples were combusted prior to radioactivity determination and the formed $^{14}\text{CO}_2$ absorbed in an alkaline trapping solvent. The determination of radioactivity of liquid samples was conducted by liquid scintillation counting (LSC) using sub-samples (1 - 3 replicates). Quenching effects were automatically corrected using an external standard and a quenching library. The instrument background was automatically subtracted. For all samples, the limit of detection (LOD) was established at approximately 10 dpm per aliquot after instrument background correction. The limit of quantification (LOQ) was established as 2 times the background radioactivity (dpm) of each instrument/method.

Metabolite analysis

The n-heptane phases of eggs, muscle, fat and liver were concentrated and analysed by thin layer chromatography on silica gel plates and radioluminography for detection of radioactive spots. The combined acetonitrile and acetonitrile/water extracts were concentrated and used for the quantification of parent compound and metabolites by HPLC. Although the acetonitrile/water phase was partitioned against n-heptane, a portion of the fatty matrix remained in the acetonitrile/water phase and caused incomplete elution of the radioactivity from the HPLC column and interferences during detection due to chemiluminescence. Therefore a washing step of the HPLC column was implemented and the HPLC run as well as the washing step were quantified by LSC. The eluent of subregion 1 (= analytical run: 0 to 80 min.) and subregion 2 (= washing step: 80 to 120 min.) was collected and the radioactivity was determined by LSC. The sum of both subregions was related to the origin percentage and ppm-value. The completeness of the chromatographic elution for the adapted HPLC profiling method (including the washing step) was shown for extracts from eggs, muscle and liver. The recovery ranged from 97.6 to 104.3% of the injected radioactivity. Parent compound and metabolites were detected and quantified in subregion 1. In case of muscle and liver, the polar region R1 of the subregion 1 was isolated and further investigated by TLC. Post extraction solids of eggs, muscle and liver were exhaustively extracted twice with acetonitrile/water (1:1; v/v) followed by 2 extractions with acetonitrile/water/formic acid (50:50:2.5; v/v/v) using microwave assistance. The concentrated exhaustive extracts were analysed by HPLC, depending on the matrix content.

Basically, the isolation and identification of parent compound and metabolites were performed in the study using the pyridinylmethyl label (KIIA 6.2.2/01). An additional identification of BYI 02960-des-



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difluoroethyl in the extract of muscle was performed by HPLC co-chromatography with the radiolabelled reference compound. The reference compound was isolated and identified in the laying hen study with the pyridinylmethyl label. Metabolites in the polar region R1 of the acetonitrile/water extracts of muscle and liver were quantified and further characterised by thin layer chromatography. Their commonality with polar metabolites in urine and organs of rat and goat was demonstrated by comparison of the TLC profiles (KIIA 5.1.2/01; KIIA 6.2.3/02). Radioactive residues in the n-heptane phases were analysed by TLC and visualised in an iodine vapour chamber. Residues in the n-heptane phase of fat were further identified by investigation of their partition behaviour after saponification and subsequent acidification.

II. Results and Discussion

A. Recovery and Elimination of Radioactivity

The recovery of radioactivity in laying hens after administration of an average daily dose of 1.05 mg [furanone-4-¹⁴C]BYI 02960 per kg bw/day on 14 consecutive days is presented in Table 6.2.2-5. The overall recovery accounted for 82.16% of the totally administered dose. A part of the remaining radioactivity was expected to still be present in the gastro-intestinal tract at sacrifice, due to the short period between the last administration and sacrifice. Another part is probably exhaled as ¹⁴CO₂. The formation of carbon dioxide was also shown in a rat quantitative whole-body autoradiography study using the same labelling position (KIIA 5.1.2/02).

Table 6.2.2-5: Distribution of residues in eggs, muscle, fat, liver and kidney of laying hens following oral administration of 14 daily doses of [furanone-4-¹⁴C] BYI02960 at a dose rate of 1.05 mg/kg

Sample	Percent of total dose administered	Concentration of total radioactivity [mg/kg]
Liver	0.37	2.178
Kidney	0.05	1.083
Eggs from ovary/oviduct	0.46	2.774
Muscle, total	0.50	0.183
Skin, total	0.07	0.257
Fat, total	0.34	0.427
Organs/tissues, total	1.80	-----
Eggs	2.35	0.757
Excreta, total	78.01	-----
Total Recovery	82.16	-----

An average amount of 2.35% of the total dose was measured in the eggs. At sacrifice, the residues in the organs and tissues were calculated or estimated to be about 1.8% of the total dose from which about 28% was detected in the skeletal muscle (0.5%). Until sacrifice, the excreted radioactivity accounted for 78.01% of the total dose.

B. Levels and Time Course of Total Radioactivity in Eggs

The radioactivity levels measured in the egg samples from all animals are presented in Table 6.2.2-6. The concentrations in eggs ranged from 0.024 mg/kg at day one to 1.198 mg/kg at sacrifice. Following

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a linear increase a residue plateau level of 1.035 mg/kg was reached at day nine after the first administration.

Table 6.2.2-6: Time course of total radioactivity in eggs following oral administration of 14 daily doses of [furanone-4-¹⁴C] BYI02960 at a dose rate of 1.05 mg/kg

Time after the first admin. [d]	Admin. no.	Cumulative secretion [% of total dose admin.] (mean)	Concentration of total radioactivity [mg/kg]
0	1	----- #	----- #
1	2	0.01	0.024
2	3	0.04	0.146
3	4	0.10	0.284
4	5	0.20	0.438
5	6	0.40	0.647
6	7	0.56	0.789
7	8	0.72	0.923
8	9	0.94	1.002
9	10	1.20	1.035
10	11	1.44	1.035
11	12	1.74	1.100
12	13	1.97	1.036
13	14	2.04	1.005
13.25	---	2.35	1.198

----- # no egg collected

C. Total Radioactive Residues in Dissected Organs and Tissues

The concentration of the total radioactivity in the dissected organs and tissues at sacrifice is shown in Table 6.2.2-5 (last column). The highest concentrations were determined in liver (2.178 mg/kg) and kidney (1.083 mg/kg) reflecting the significance of these organs for metabolism and excretion. In relation to the dose totally administered, these values corresponded to 0.37% and 0.05%, respectively. The residue level of the eggs collected from the ovary and oviduct at sacrifice (2.774 mg/kg) was higher by a factor of 2.3 than the levels of the laid eggs collected at sacrifice (1.198 mg/kg). This indicated that the egg yolk was a preferential site for the secretion of radioactivity. The residue levels of liver and kidney were followed in decreasing order by those determined in the subcutaneous fat (0.427 mg/kg), skin (0.257 mg/kg) and muscle (0.183 mg/kg). The residue level of the total skeletal muscle corresponded to about 0.50% of the total dose assuming a value of 40% contribution to the body weight. Assuming values of 12% and 4% of the body weight for fat and skin, the residues in both tissues accounted for about 0.41% of the dose totally administered.

D. Extraction Efficiency of Residues

Egg pools (day 2 to 7 and 8 to sacrifice), muscle-, fat- and liver pools were extracted with acetonitrile and n-heptane followed by solvent partition. In case of eggs, muscle and liver the extraction procedure was continued with a mixture of acetonitrile/water (7:3; v/v). Excreta (day 13) were extracted with



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acetonitrile/water (8:2, v/v) and pure acetonitrile. Post-extraction solids of eggs, muscle and liver were extracted with neutral (acetonitrile/water (1:1, v/v)) and acidic (acetonitrile/water/formic acid (50:50:2.5, v/v/v)) solvent mixtures using microwave assistance. Radioactivity concentrations in the post-extraction solids amounted to 0.006 mg/kg (1.1%) for the egg pool 1 (day 2 to 7), 0.016 mg/kg (1.5%) for the egg pool 2 (day 8 to sacrifice), 0.006 mg/kg (3.5%) for muscle, 0.006 mg/kg (1.5%) for fat and 0.036 mg/kg (1.7%) for liver.

The major part of residues was detected in the n-heptane phases of eggs (ca. 52 and 58%), fat (ca. 96%) and liver (ca. 52%). The residues in the n-heptane phase of muscle amounted to only ca. 8%.

Residues in the acetonitrile/water extract of eggs, muscle, fat and liver were detected at the same residue level as analysed in the laying hen study with the pyridinylmethyl label (KIIA 6.2.2/01). The residue concentrationss ranged from 0.011 mg/kg for fat to 0.450 mg/kg for liver. Because of the low residue concentration the acetonitrile phase of fat was not further investigated.

A significant part of residues was exhaustively extracted using microwave assistance. Between 4 and 10% of the total radioactivity was detected in the neutral extracts and between 8 and 40% in the acidic extracts of eggs, muscle and liver. Losses of radioactivity during the extraction procedure of eggs, muscle and liver were between 5 and 18% of the total radioactivity. During the concentration procedure of the extracts losses were between 1.7 and 4.5%. Losses of radioactivity during the extraction and concentration procedures of eggs, muscle and liver were not further investigated. Most probably, the losses of radioactivity were caused by adhesion of radioactive fat or non-polar matrix on the equipment (e.g. Polytron homogenizer, filter etc.). A summary of the extraction efficiency of the samples is presented in Table 6.2.2-7.

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Table 6.2.2-7: Extraction efficiency of eggs, muscle, fat and liver samples following oral administration of 14 daily doses of [furanone-4-¹⁴C] BYI02960 at a dose rate of 1.05 mg/kg

	Eggs (days 2 to 7)		Eggs (days 8 to 13.25)		Muscle		Fat		Liver	
TRR [mg/kg]	0.540		1.048		0.183		0.427		2.178	
	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Conventional extraction	65.0	0.351	66.8	0.701	38.6	0.071	98.5	0.421	72.1	1.571
- n-heptane phase	52.0	0.281	58.3	0.611	8.1	0.015	95.9	0.410	51.5	1.121
- ACN/water extract	13.0	0.070	8.5	0.089	30.4	0.056	2.6	0.011	20.7	0.450
- subregion 1	8.6	0.046	4.9	0.052	27.4	0.050	n.a.	n.a.	19.6	0.426
- subregion 2	2.7	0.015	3.6	0.038	n.d.	n.d.	n.a.	n.a.	1.1	0.024
- losses during the conventional extraction	10.7	0.058	10.7	0.112	10.3	0.019	n.d.	n.d.	n.d.	n.d.
Exhaustive extraction										
- combined neutral ACN/water extract	7.0	0.038	3.6	0.038	8.1	0.015	n.a.	9.6	0.209	
- 1 st acidic ACN/water extract	8.3	0.045	10.0	0.105	39.5	0.072	n.a.	12.1	0.264	
- 2 nd acidic ACN/water extract	1.1	0.006	3.3	0.035	see**	n.a.	n.a.	n.a.		
- losses during the exhaustive extraction	6.8	0.037	4.1	0.043	n.d.	n.d.	n.a.	4.5	0.098	
Total extracted	81.5	0.440	83.8	0.878	86.2	0.158	98.5	0.421	93.8	2.044
Total losses of the extraction procedures	17.5	0.094	14.7	0.155	10.3	0.019	n.d.	n.d.	4.5	0.098
Solids	1.1	0.006	1.5	0.016	3.5	0.006	1.5	0.006	1.7	0.036
Accountability	100.0	0.540	100.0	1.048	100.0	0.183	100.0	0.427	100.0	2.178

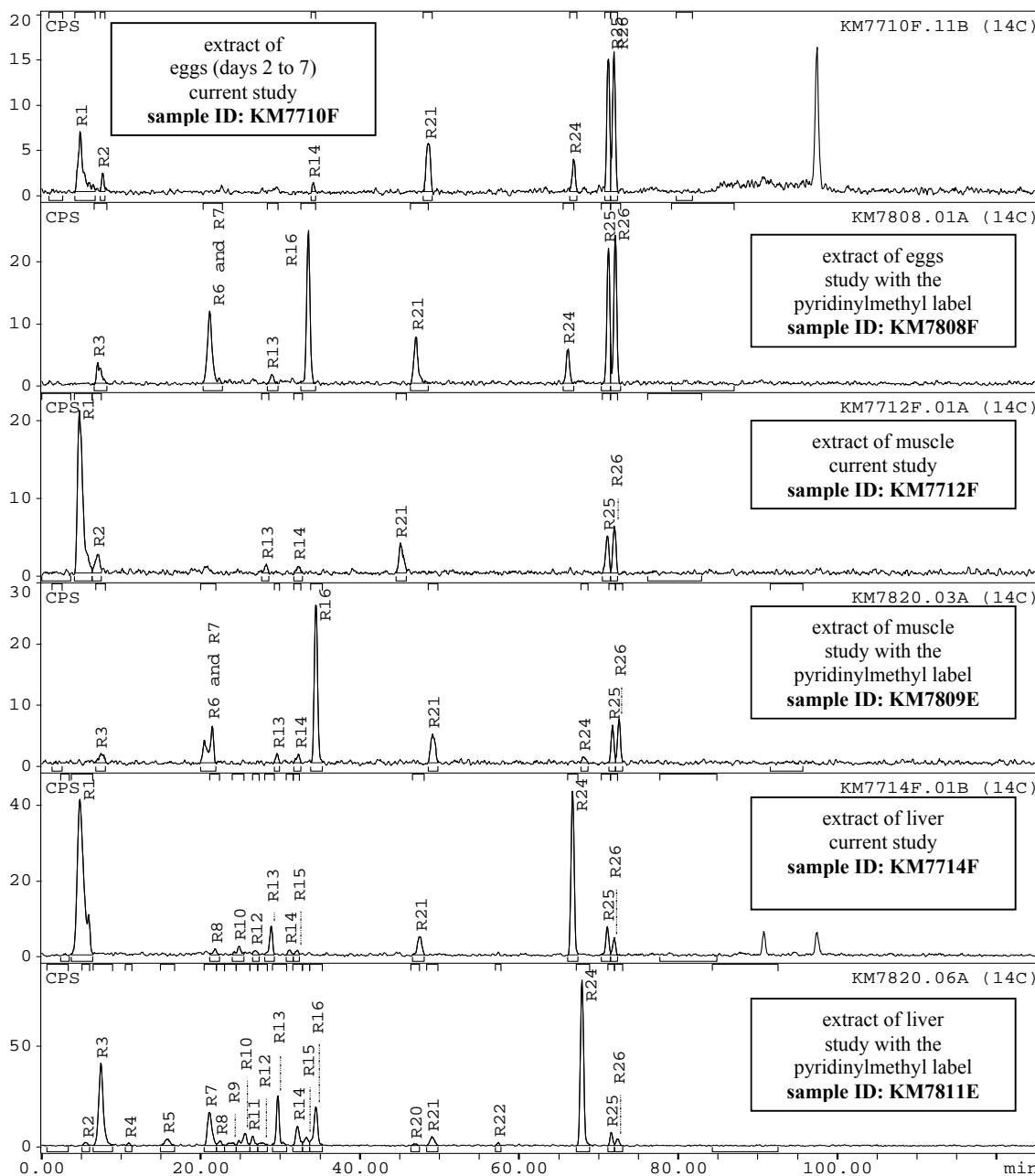
** Both acidic ACN/water extracts of muscle were combined before concentration.

E. Quantification, Identification and Characterisation of Residues

The identification and assignment of parent compound and metabolites in the acetonitrile/water extracts was based on the comparison of the metabolite profiles of the current study with the profiles of the study with the pyridinylmethyl label (KIIA 6.2.2/01) using the HPLC system described there. Additionally, BYI 02960-des-difluoroethyl was identified in the extract of muscle by HPLC co-chromatography with the radiolabelled reference compound. As an example of such a comparison the metabolic profiles in extracts of egg, muscle and liver is shown in Figure 6.2.2-4.

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Figure 6.2.2-4: Comparison of HPLC profiles of the acetonitrile/water extracts from eggs, muscle and liver of the current study and the study with the pyridinylmethyl ^{14}C -label



Metabolites in the Polar Region (R1) of the Conventional Extracts

Metabolites in the polar region R1 of the acetonitrile/water extract of muscle and liver (see Figure 6.2.2-4 above) were further characterised by thin layer chromatography. These metabolites ranged from 0.001 to 0.012 mg/kg for muscle (six metabolites) and from 0.009 to 0.050 mg/kg for liver (nine metabolites). These polar metabolites were specific for the furanone label and were caused by the degradation of the furanone ring. These metabolites were also detected in the corresponding polar region of urine from the rat- (KIIA 5.1.2/01) and urine, liver and kidney from the goat-metabolism



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studies ([KIIA 6.2.3/02]). The polar region R1 of eggs was not investigated due to its low residue level (ca. 0.01 mg/kg) and the high matrix content.

Identification of Residues in the n-Heptane Phases

The thin-layer chromatographic behaviour of the residues in the n-heptane phases of eggs, muscle, fat and liver was identical. As an example, the partition behaviour of the radioactive residues in the n-heptane phase of fat was investigated after saponification and subsequent acidification. The radioactive residues were cleaved by saponification yielding glycerol and salts of fatty acids. After solvent partition, the entire radioactivity was detected in the aqueous phase, where the salts of the fatty acids were dissolved. The radioactivity was transferred back into the n-heptane phase after acidification of the aqueous phase. This partition behaviour is typical for fatty acids, which are building blocks of natural lipids.

The residues in the n-heptane phases were specific for the furanone label and were caused by the cleavage and subsequent degradation of the furanone ring to smaller carbon units (C-1- or C-2-fragments), entering the carbon pool of endogenous compounds.

Distribution of Parent Compound and Metabolites in Eggs, Organs and Tissues

The distribution of the parent compound and metabolites in eggs, organs and tissues is summarised in Table 6.2.2-8. The identification rate was 59% in eggs (days 2 to 7), 63% in eggs (days 8 to sacrifice), 17% in muscle, 96% in fat and 59% in liver. All other residues were characterised by their extraction or chromatographic behaviour.

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 Table 6.2.2-8: Radioactive residues in eggs, muscle, fat and liver samples following oral administration of 14 daily doses of [furanone-4-¹⁴C] BYI02960 at a dose rate of 1.05 mg/kg

	Eggs (day 2 to 7)		Eggs (day 8 to 13.25)		Muscle		Fat		Liver	
TRR [mg/kg]	0.540		1.048		0.183		0.427		2.178	
Sample/Report name BYI 02960-	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Conventional extraction	65.0	0.351	66.8	0.701	38.6	0.071	98.5	0.421	72.1	1.571
n-heptane phase (identified as fatty acids)	52.0	0.281	58.3	0.611	8.1	0.015	95.9	0.410	51.5	1.121
des-difluoroethyl-OH-SA	0.1	0.001	n.d.	n.d.	0.5	0.001			0.2	0.004
des-difluoroethyl	1.2	0.006	0.6	0.007	2.6	0.005			0.8	0.017
OH-SA	0.6	0.003	0.5	0.005	n.d.	n.d.			5.1	0.112
OH	2.3	0.013	1.6	0.016	2.4	0.004			0.8	0.018
Parent compound	2.3	0.013	1.6	0.016	2.9	0.005			0.5	0.010
identified in the conventional extract	58.5	0.316	62.5	0.656	16.5	0.030	95.9	0.410	58.9	1.282
characterised in the conventional extract	6.4	0.035	4.3	0.045	22.0	0.040	2.6	0.011	13.2	0.288
losses during the conventional extraction	10.7	0.058	10.7	0.112	10.3	0.019	n.d.		n.d.	
Exhaustive extraction										
- neutral ACN/water extract	7.0	0.038	3.6	0.038	8.1	0.015	n.a.		9.6	0.209
- 1 st acidic ACN/water extract	8.3	0.045	10.0	0.105	39.5	0.072	n.a.		12.1	0.264
- 2 nd acidic ACN/water extract	1.1	0.006	3.3	0.035	see **		n.a.		n.a.	
characterised in the exhaustive extracts	16.5	0.089	16.9	0.177	47.7	0.087	n.a.		21.7	0.473
Total identified	58.5	0.316	62.5	0.656	16.5	0.030	95.9	0.410	58.9	1.282
Total characterised	22.9	0.124	21.2	0.223	69.7	0.128	2.6	0.011	34.9	0.761
Total extracted	81.5	0.440	83.8	0.878	86.2	0.158	98.5	0.421	93.8	2.044
Total losses	17.5	0.094	14.7	0.155	10.3	0.019	n.d.	n.d.	4.5	0.098
Solids	1.1	0.006	1.5	0.016	3.5	0.006	1.5	0.006	1.7	0.036
Accountability	100.0	0.540	100.0	1.048	100.0	0.183	100.0	0.427	100.0	2.178

** Both acidic ACN/water extracts of muscle were combined before concentration.

Metabolites in Eggs

The main part of the radioactivity in eggs (days 2 to 7 0.281 mg/kg, 52.0%; days 8 to sacrifice 0.611 mg/kg, 58.3%) was detected in the n-heptane phase and identified as natural fats. The parent compound, BYI 02960-OH, BYI 02960-OH-SA, BYI 02960-des-difluoroethyl and BYI 02960-des-difluoroethyl-OH-SA were identified in low amounts (≤ 0.016 mg/kg). Metabolites in the polar region R1 (see Fig. 6.2.2-4) were not further quantified due to the low concentration (≤ 0.010 mg/kg).

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Metabolites in Muscle

Residues in the n-heptane phase of muscle were identified as natural fats and amounted to 0.015 mg/kg (8.1%), only. Very low amounts (≤ 0.005 mg/kg) of parent compound, BYI 02960-OH, BYI 02960-des-difluoroethyl and BYI 02960-des-difluoroethyl-OH-SA were identified in the acetonitrile/water extract. Metabolites in the polar region R1 of the acetonitrile/water extract (see Fig. 6.2.2-4) were further analysed and subquantified by thin layer chromatography. The concentrations ranged from 0.001 to 0.012 mg/kg. The commonality of these metabolites with polar metabolites, which were detected in the urine of rats (KIIA 5.1.2/01) and in the urine, liver and kidney of the goat, (KIIA 6.2.3/02) was demonstrated by comparison of the thin layer chromatograms.

Metabolites in the acidic exhaustive extract represented the major portion of residues in the muscle sample (0.072 mg/kg, 39.5%). They were characterised by their extraction behaviour. An HPLC analysis could not be performed, due to the high matrix burden in the extract.

Metabolites in Fat

The dominating part of the radioactive residues in fat (0.410 mg/kg, 95.9%) was identified as fatty acids after saponification. The fatty acids were formed from smaller carbon units (C-1- or C-2-fragments), which entered the carbon pool of endogenous compounds after cleavage and subsequent degradation of the furanone ring. A small part (0.011 mg/kg, 2.6%) was detected in the acetonitrile phase and was not further investigated.

Metabolites in Liver

More than half of the residues in liver (1.121 mg/kg, 51.5%) was identified as natural fats. Parent compound, BYI 02960-OH, BYI 02960-OH-SA, BYI 02960-des-difluoroethyl and BYI 02960-des-difluoroethyl-OH-SA were identified in the acetonitrile/water extract. The most prominent metabolite was BYI 02960-OH-SA which amounted to 0.112 mg/kg (5.1%). All other identified metabolites were detected in low amounts between 0.004 and 0.018 mg/kg.

Metabolites in the polar region R1 of the acetonitrile/water extract (see Fig. 6.2.2-4) were subquantified by thin layer chromatography. Their concentrations ranged from 0.009 to 0.050 mg/kg. The commonality of these metabolites with polar metabolites, which were detected in the urine of rats (KIIA 5.1.2/01) and in the urine, liver and kidney of the lactating goat, (KIIA 6.2.3/02) was demonstrated by comparison of the thin layer chromatograms.

Metabolites in Excreta

The metabolite profiles of excreta of the current study and the study using the pyridinylmethyl label were very similar, except for the label specific compounds - e.g. in the region of polar metabolites (R1). A comparison of the profiles from both studies is shown in Figure 6.2.2-5. The quantification of metabolites is given in Table 6.2.2-9.

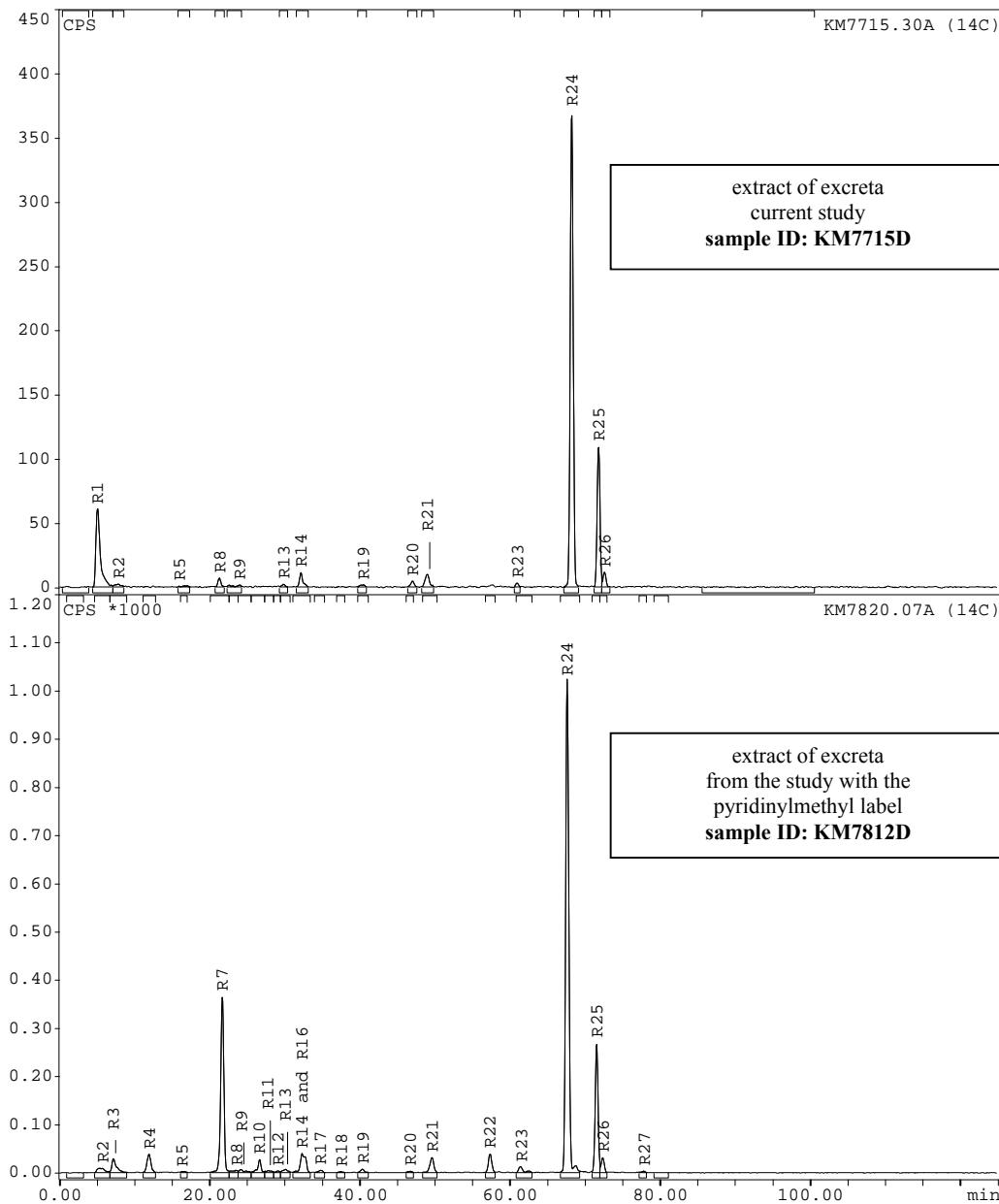
Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.2.2-9: Identified metabolites in the excreta following oral administration of 14 daily doses of [furanone-4-¹⁴C] BYI02960 at a dose rate of 1.05 mg/kg

Report name BYI 02960-	Rt [min]	Area [%]	% of RA in sample
Polar metabolites	5.0	14.28	13.5
des-difluoroethyl-OH-SA	32.1	2.05	1.9
des-difluoroethyl	48.9	2.27	2.2
acetic acid	61.0	0.46	0.4
OH-SA	68.2	58.10	55.1
OH	71.8	16.45	15.6
Parent compound	72.6	1.78	1.7
Total			94.8
Sum identified (w/o polar metabolites)			76.9

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Figure 6.2.2-5: Comparison of HPLC profiles of the extracts from the excreta of laying hens of the current study and the study with the pyridinylmethyl ^{14}C -label



The dominating metabolite was BYI 02960-OH-SA (55.1%) followed by the free aglycone BYI 02960-OH (15.6%). Only traces of parent compound were identified (1.7%). All other identified metabolites were detected in amounts between 0.4 and 2.2%. The label-specific metabolites in the polar region accounted for 13.5% of the radioactivity in the excreta.

F. Storage Stability of Residues

All samples of eggs, and edible organs and tissues were extracted within approx. 2 months after sample collection. The first metabolite profile was recorded latest within about 3 weeks after sample



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preparation and extraction. The stability of the extract of muscle and liver was demonstrated for a period of ca. 7 months by comparison of the HPLC profiles. Further details are provided in the report.

III. Conclusion

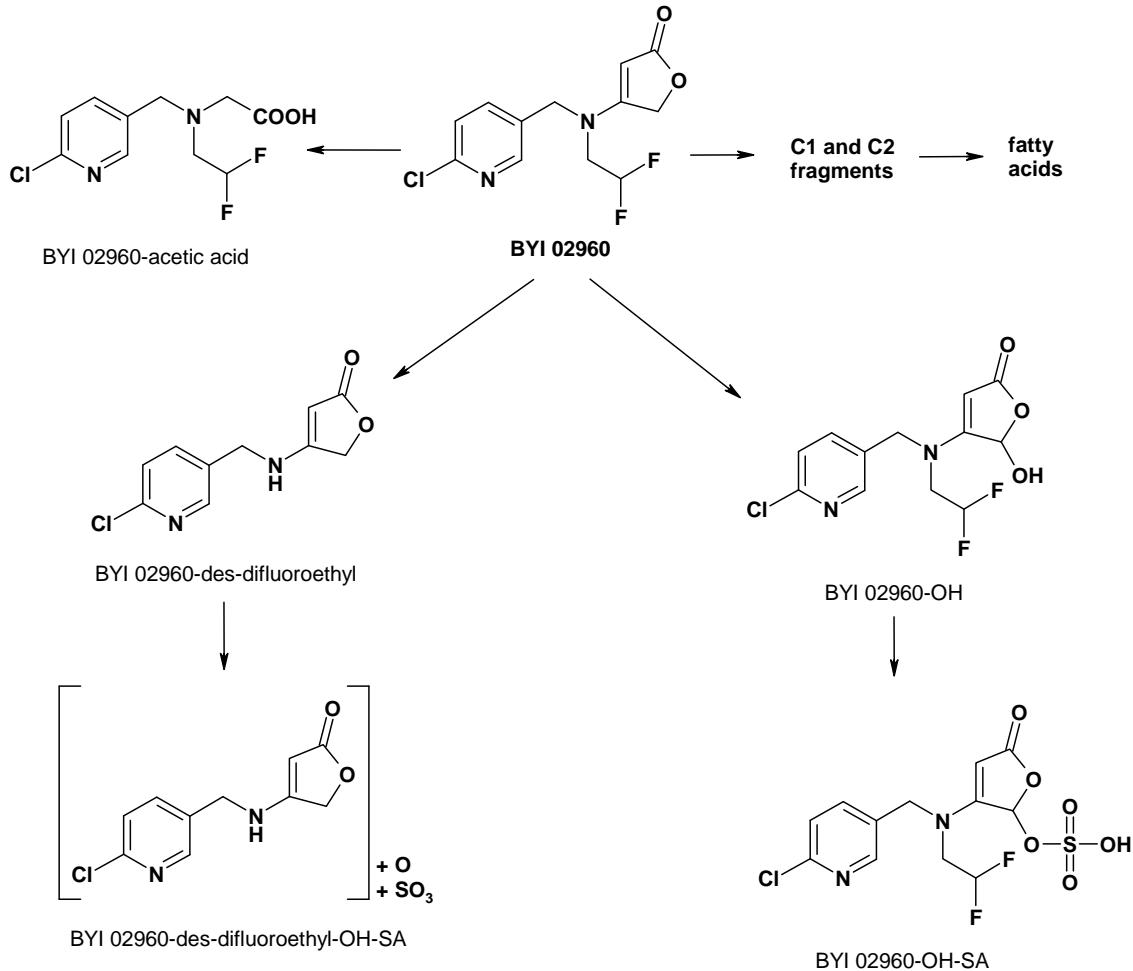
The metabolic and excretion behaviour of [furanone-4-¹⁴C]BYI 02960 in the laying hen can be characterised by the following observations:

- The concentration of the radioactivity in eggs and edible tissues was slightly elevated when compared to the dose level and the dosing period of 14 days. This was mainly caused by the partial instability of the radiolabel used. For a small part of the total dose the furanone ring of the molecule obviously underwent extensive biotransformation to C-1- and C-2-fragments resulting in an accumulation of radioactivity in biomolecules. Some of these fragments were probably also converted to the terminal product ¹⁴CO₂ as can be derived from the lower overall recovery in comparison to the study with the metabolically stable pyridinylmethyl label.
- The relatively high values in liver and kidney at sacrifice 6 hours after the last dose indicated that metabolism and excretion are still ongoing processes.
- A residue plateau level in whole eggs was reached at day nine after the first administration.
- The extraction rates were above 80% for eggs and edible tissues. Unextracted residues were quite low and amounted to ≤0.036 mg/kg.
- Parent compound and non-label specific metabolites, such as BYI 02960-OH, BYI 02960-OH-SA, BYI 02960-des-difluoroethyl and BYI 02960-des-difluoroethyl-OH-SA were identified in similar amounts as in the study using the pyridinylmethyl label. Additionally, BYI 02960-acetic acid was detected in excreta.
- The main metabolic reactions of [furanone-4-¹⁴C]BYI 02960 in the laying hen are:
 - Cleavage and 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 for the biosynthesis of fatty acids
 - Hydroxylation in position 5 of the furanone ring forming BYI 02960-OH followed by conjugation with sulphuric acid to BYI 02960-OH-SA
 - Cleavage of the difluoroethyl group forming BYI 02960-des-difluoroethyl followed by hydroxylation and conjugation with sulfuric acid to BYI 02960-des-difluoroethyl-OH-SA
 - Oxidative degradation of the furanone ring forming BYI 02960-acetic acid

Based on these results the metabolic pathway shown in Figure 6.2.2-6 is proposed.



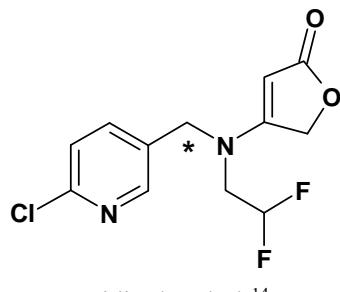
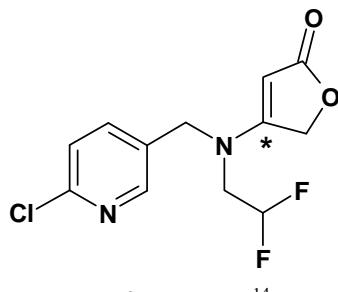
Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Figure 6.2.2-6: Proposed metabolic pathway of [furanone-4-¹⁴C] BYI02960 in laying hens



IIA 6.2.3 Lactating ruminants (goat or cow)

Two studies on the metabolism of BYI 02960 in lactating goats were conducted with the test compound labelled either in the [pyridinylmethyl-¹⁴C]- or the [furanone-4-¹⁴C]-position as shown by the following structural formulas (* denotes the label position):

[pyridinylmethyl-¹⁴C][furanone-4-¹⁴C]

Report:	KIIA 6.2.3/01, Authors: [REDACTED], R., [REDACTED], J., (2011)
Title:	[Pyridinylmethyl- ¹⁴ C]BYI02960: Metabolism in the lactating goat
Report No & Document No:	MEF-11/269 M-419701-01-2
Date:	7.12.2011
Guidelines:	OECD-Guideline for the Testing of Chemicals No. 503, Metabolism in Livestock US EPA Residue Chemistry Test Guideline OPPTS 860.1300 Nature of the Residue – Plants, Livestock European Parliament and Council Regulation (EC) No 1107/2009
GLP:	Yes, according to Japan MAFF GLP standard 11 Nousan 6283; US EPA – FIFRA GLP (40CFR Part 160); Principles of GLP, German Chemical Law, current version of Annex 1
Testing Facility and Dates:	[REDACTED] Experimental work: 9.6.2010 – 6.5.2011

Executive Summary

The metabolism and excretion of [pyridinylmethyl-¹⁴C]BYI 02960 (common name: flupyradifurone) were investigated in the lactating goat as a model for ruminants. The 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 24.4 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 as well as urine were analysed for the unchanged parent compound and metabolites.

Recovery and Elimination of Radioactivity

The overall recovery amounted to 88.75% of the total dose. Much of the remaining radioactivity (ca. 11%) was expected to be still present in the gastro-intestinal tract at sacrifice, due to the short period between the last administration and sacrifice. An amount of 0.78% of the total dose was secreted with the milk. At sacrifice, the residues in the organs and tissues were calculated or estimated to be 2.94% of the total dose from which about 71% was detected in the skeletal muscle.

Until sacrifice, the excretion of radioactivity accounted for 85.02% of the total dose. A portion of 71.74% was found in the urine and 13.28% in the faeces. The urinary and faecal excretion started immediately after the first administration.

Total Radioactive Residues in Milk, Organs and Tissues

The concentration of radioactivity in **milk** samples ranged from 0.053 mg/kg at 96 hours to 1.345 mg/kg at sacrifice, 102 hours after the first administration. The time course in the evening and morning milk pool samples showed a pronounced diurnal pattern. The radioactive residues increased significantly during the eight hour period after each administration followed by a decrease to a very low level of about 0.05 mg/kg measured prior to the next dosing. A stable plateau level of about 0.3 mg/kg was reached already at 8 hours after the first administration.

In the **organs and tissues**, the highest concentrations were determined in kidney (1.869 mg/kg) and liver (1.215 mg/kg) indicating the significance of these organs for excretion and metabolism. These values corresponded to 0.10% and 0.50% of the total dose, respectively. For muscle and fat 0.356 mg/kg and 0.106 mg/kg, respectively, were determined. The radioactivity concentration of the muscle corresponded to 2.10% and that of fat to 0.25% of the total dose assuming a value of 30% and 12% of the body weight for these tissues.

Metabolism

For analysis of parent compound and metabolites, milk, muscle, kidney and liver were extracted with mixtures of acetonitrile/water. For milk an additional extraction step with acetone was performed. Fat was extracted with mixtures of n-heptane and acetonitrile/water followed by a solvent partitioning step yielding an n-heptane and an acetonitrile/water phase. No residues were found in the n-heptane phase of fat. The resulting extracts of milk, muscle, fat, kidney and liver represented between 92.9% and 99.8% of the total radioactive residue. Unextractable residues in milk, muscle and fat were below 0.002 mg/kg. For kidney and liver they were 0.021 mg/kg and 0.086 mg/kg, respectively.

After purification and concentration, the extracts were analysed by HPLC with radiometric detection. The HPLC profiling method was based on the use of a reversed phase column and a neutral water/acetonitrile gradient. The metabolite pattern of the extracts of the current goat study and the goat study with the furanone label (KIIA 6.2.3/02) were comparable with the exception of the label specific metabolites.

All prominent metabolites of the extracts of milk, muscle, fat, kidney and liver were also detected in urine. Therefore, the isolation and identification of metabolites were performed using the urine samples. Isolated metabolites were identified by LC-MS/MS and used as radiolabelled reference compounds for the identification in milk, muscle, fat, kidney and liver by HPLC co-chromatography. The identification of the minor metabolite BYI 02960-AMCP-difluoroethanamine was performed in the liver by HPLC co-chromatography with the non-radiolabelled reference compound.

Approximately 99% of the total radioactivity in milk, muscle, fat and kidney and ca. 93% in liver was identified. All other residues were characterised by their extraction behaviour. The unchanged parent compound was by far the major constituent of the residue in milk, organs and tissues and amounted to 0.165 mg/kg (88.8%) for milk, 0.349 mg/kg (98.0%) for muscle, 0.105 mg/kg (99.2%) for fat, 0.650 mg/kg (34.8%) for kidney and 1.028 mg/kg (84.6%) for liver.

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Extensive metabolism was detected in the kidney, where the main portion of metabolites was found. The major metabolite in the kidney was BYI 02960-OH, with a concentration of 0.299 mg/kg (16.0%). Four prominent glucuronic acid conjugates of the hydroxylated parent compound were detected in the kidney as well as in the urine. The four isomers were named BYI 02960-OH-gluA (isomer 1 to isomer 4); their concentration ranged from 0.112 mg/kg (6.0%) to 0.175 mg/kg (9.3%). BYI 02960-OH-gluA (isomer 2) and BYI 02960-OH-gluA (isomer 3) are diastereomers, which are hydroxylated and conjugated in the 5-position of the furanone ring. BYI 02960-OH-gluA (isomer 4) is hydroxylated and conjugated in the difluoroethyl side chain of the molecule and BYI 02960-OH-gluA (isomer 1) in an unknown position.

A further metabolite was BYI 02960-hippuric acid, which was detected in milk (0.017 mg/kg, 9.1%) and kidney (0.178 mg/kg, 9.5%). Traces of BYI 02960-hippuric acid were also found in the liver (0.010 mg/kg, 0.8%). BYI 02960-cysteinyl-nicotinic acid was identified in the kidney (0.114 mg/kg, 6.1%) and in the liver (0.058 mg/kg, 4.8%). Minor identified metabolites were BYI 02960-methylthio-glyoxylic acid in milk and muscle and BYI 02960-AMCP-difluoroethanamine in kidney and liver. The concentration of each was below 0.020 mg/kg.

The concentration of the total radioactivity (expressed as mg a.s. equiv. /kg) as well as the distribution of the parent compound and metabolites and the identification rates in milk and edible tissues are summarised in the following table:

	Milk (24 to 102 h)		Muscle		Fat		Kidney		Liver	
TRR [mg/kg]	0.186		0.356		0.106		1.869		1.215	
Sample/ Report name BYI 02960-	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
ACN/water extract	99.3	0.184	99.4	0.353	99.2	0.105	98.8	1.847	92.8	1.128
cysteinyl- nicotinic acid	---	---	---	---	---	---	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
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
Total extracted	99.5	0.185	99.5	0.354	99.8	0.106	98.9	1.848	92.9	1.129
Solids	0.5	0.001	0.5	0.002	0.2	<0.001	1.1	0.021	7.1	0.086
Accountability	100.0	0.186	100.0	0.356	100.0	0.106	100.0	1.869	100.0	1.215

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

The metabolic reactions of [pyridinylmethyl-¹⁴C]-BYI 02960 in the lactating goat are:

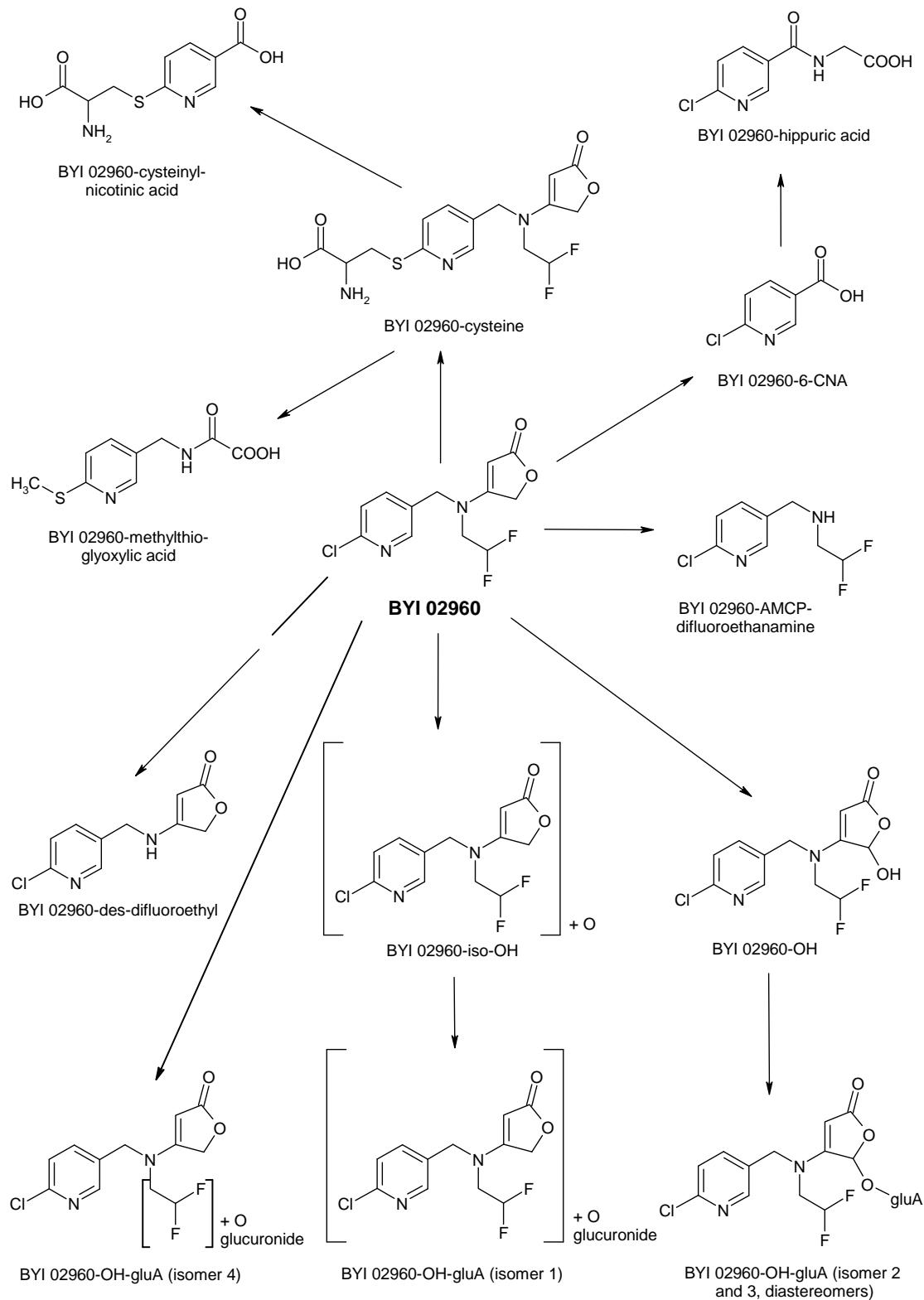
- Hydroxylation followed by conjugation with glucuronic acid forming two diastereomeric conjugates of BYI 02960-OH (BYI 02960-OH-gluA, isomer 2 and 3), the hydroxylation and conjugation being in the 5-position of the furanone ring. One isomer (BYI 02960-OH-gluA, isomer 4) with hydroxylation and conjugation in the difluoroethyl side chain and one isomer (BYI 02960-OH-gluA, isomer 1) with hydroxylation and conjugation in an unknown position
- Oxidative cleavage of the pyridinylmethyl bridge forming BYI 02960-6-CNA followed by conjugation with glycine to form BYI 02960-hippuric acid
- Substitution of the chlorine atom of BYI 02960 with glutathione followed by degradation resulting in the conjugate BYI 02960-cysteine
- Oxidative cleavage of the pyridinylmethyl bridge of BYI 02960-cysteine forming BYI 02960-cysteinyl-nicotinic acid
- Further degradation of BYI 02960-cysteine in the cysteine group and the furanone ring forming BYI 02960-methylthio-glyoxylic acid
- Cleavage of the furanone ring forming BYI 02960-AMCP-difluoroethanamine
- Cleavage of the difluoroethyl group forming BYI 02960-des-difluoroethyl

Based on these results the metabolic pathway of [pyridinylmethyl-¹⁴C]-BYI 02960 in the lactating goat shown on the next page is proposed.



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Proposed metabolic pathway of [pyridinylmethyl-¹⁴C]BYI 02960 in the lactating goat:



I. Materials and Methods

A. Materials

1. Test Material

IUPAC Name	4-{[(6-chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino} furan-2(5H)-one
Code name	BYI02960
Common name	Flupyradifurone (proposed ISO)
Empirical formula	C ₁₂ H ₁₁ ClF ₂ N ₂ O ₂
Molar mass	288.68
Labelling	[pyridinylmethyl- ¹⁴ C]
Specific radioactivity used for administration	3.92 MBq/mg = 105.93 µCi/mg (delivered sample before radiodilution) 3.425 MBq/mg = 2.06 x 10 ⁸ dpm/mg = 92.57 µCi/mg = 26.72 Ci/mol (sample after radiodilution)
Radiochemical purity	> 99 % (HPLC)
Dose level	5 daily oral doses of 1 mg/kg bw by gavage
Vehicle	Capsule

2. Test Animals

Species	Lactating goat (<i>Capra hircus</i>)
Strain	“Weiße Deutsche Edelziege”
Breeding facility	Dr. G. Jux-Straatmann, Halfenslennefe 1, D-51491 Overath, Germany Member of the Landesverband Rheinischer Ziegenzüchter e.V., Halfenslennefe 1, D-51491 Overath, Germany
Sex and numbers involved	1 female animal
Age	ca. 36 months
Body weight	60 kg at first administration, 59 kg at sacrifice
Acclimatization	7 days
Identification	skin marking
Housing	During acclimatization period: raised stall with a metal grid as base and straw and hay as bedding. During the test period: electro-polished stainless steel metabolism cage for farm animals (goat, sheep, and pig), supplied by E. Becker & Co. GmbH “EBECO”, Hermannstr. 2 – 8, 44579 Castrop-Rauxel, Germany. The cage was equipped with a variable-restraining device. room temperature 17 - 24°C, relative humidity 48-93% 12 h light / 12 h dark cycle, air change 10 – 15 times per hour.
Feed and water	The goat was fed <i>ad libitum</i> with hay, hay pellets, apples and supplementary ruminant feed (“Raiffeisen LammGold”, supplied by Raiffeisenmarkt Rhein-Berg eG, Postfach 100565, D-40769 Monheim). During the test period, the average feed consumption was 2.48 kg/day, tap water was offered <i>ad libitum</i>

B. Study Design

Dosing

The test compound was orally dosed once daily for five consecutive days in the morning after milking. Based on the daily feed consumption during the test of 4.11% of the body weight, the dose of 1.0 mg a.s. /kg bw corresponded to 24.36 mg a.s. /kg dry feed per day in the diet.

The radiolabelled test compound was delivered with a specific radioactivity of 3.92 MBq/mg. It was diluted with the non-radiolabelled test compound to a specific radioactivity of 3.425 MBq/mg. In total, five gelatine capsules containing the test compound were prepared. They were stored at $\leq -18^{\circ}\text{C}$ until administration. The remaining test compound was stored in solid form together with the capsules. An aliquot of this sample was dissolved in water (pH 7) and analysed using HPLC after the first administration and after a storage period of about three months in order to demonstrate the stability of the test compound during the administration phase of the study.

The administrations were performed using a capsule applicator. The goat received on each day one gelatine capsule containing on average an amount of 60.41 mg. The totally administered amount and radioactivity accounted for 302.03 mg and 62,067,165,000 dpm, respectively. The total amount of radioactivity administered to the animal served as reference value ($A_0 = 100\%$) for the percentage calculation of the total radioactivity in the biological samples.

Sampling of milk, urine and faeces during the in-life phase

The goat was milked in the morning immediately prior to each administration, about 8 hours later in the afternoon, and directly before sacrifice (8, 24, 32, 48, 56, 72, 80, 96 and 102 hours after the first administration). The milk weights were recorded.

Urine and faeces samples were collected in plastic vessels as quantitatively as possible under dry ice cooling in intervals of 24 hours after the administrations 1 to 4 and 6 hours after the 5th administration. The vessels were changed immediately before the next administration. The collection funnel was rinsed with deionised water into the vessel of the respective collection period. An aliquot of each milk and urine fraction was taken and processed for LSC. The remaining samples were stored at about -18°C for metabolite analysis. For collection of faeces, the collecting grid was cleaned prior to each administration. No samples of the rinsing water were taken for radioactivity measurement. Each faeces fraction was homogenised after addition of water to obtain a wet paste before the total weight was recorded. Aliquots of each sample were weighed and prepared for radioactivity measurement by combustion/LSC. The remainder was stored at room temperature for metabolite analysis.

Sacrifice and dissection of organs and tissues

The animal was sacrificed approx. 6 hours after the last administration. The animal was anaesthetised by an intravenous dose of about 40 mg/kg bw Pentobarbital-Na (Narcoren®), exsanguinated by cannulating the jugular vein and finally terminated by intracardiac injection with approx. 10 mL of the veterinary drug "T 61®". Following sacrifice, the following organs and tissues were sampled: liver without gall bladder, kidneys, two different types of muscle (round and loin), and two different types of fat (perirenal and omental,).

Sample preparation

The organs or tissue samples were weighed and transferred into ice-cooled vessels. Liver, kidneys, muscle, and fat samples were thoroughly homogenised in half-frozen state for several times in a mincing machine. Each resulting tissue pulp was weighed and aliquots were prepared for radioactivity

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

measurement by combustion/LSC. All samples were divided into portions and stored in a freezer until metabolite analysis. The remaining samples of each organ or tissue were stored at $\leq -18^{\circ}\text{C}$.

For metabolism investigations, a pooled sample of milk collected from 24 h until 102 h after the first administration, composite samples of muscle (loin and round) and fat (omental and perirenal), and samples of kidneys and liver were prepared. The samples were homogenised and kept frozen until extraction.

Aliquot samples of milk, muscle, kidney and liver were extracted with acetonitrile/water (8:2; v/v) using an Ultra Turrax homogeniser. In case of milk an additional extraction step with acetone was performed. The fat sample was treated three times with mixtures of n-heptane and acetonitrile/water (8:2; v/v) also using an Ultra Turrax homogeniser followed by a solvent partition yielding single acetonitrile/water phases and a combined n-heptane phase. All acetonitrile/water extracts were subjected to an SPE clean-up step followed by concentration. The final extracts were used for the profiling, quantification and identification of parent compound and metabolites by HPLC.

Radioactivity measurement

The measurement of the radioactivity in the liquid samples was carried out by liquid scintillation counting (LSC). Quenching effects were automatically corrected using an external standard and quenching library. The instrument background of 10 - 32 dpm was subtracted automatically. For all samples, the limit of detection (LOD) was established at approximately 10 dpm measured per aliquot after instrument background correction. The limit of quantification (LOQ) was established as 2 times of the background radioactivity (dpm) of each instrument/method. All solid samples were combusted in an oxygen atmosphere using an oxidiser. The released $^{14}\text{CO}_2$ was trapped in an alkaline scintillation cocktail and the radioactivity was determined by LSC.

Metabolite analysis

The purified extracts of milk and the tissues, and samples of urine were subjected to HPLC using a reversed phase column (C_{18}) and the eluting solvents water/formic acid 99:1 (v/v) and acetonitrile/water/formic acid 97:2:1 (v/v/v) in the gradient mode. Detection was performed by a UV-(254 nm) and a radioisotope detector with a glass bead scintillator. In order to check the completeness of the elution for the HPLC profile, representative samples of milk, muscle, liver, and kidney extract were injected, re-collected, and radioassayed by LSC. The recoveries were between 94.4 and 106.5% of the injected radioactivity. Radiolabelled and non-labelled reference compounds were used in co-chromatography for identification of metabolites.

As a second chromatographic method, thin layer chromatography (TLC) was employed on silica gel plates and radioluminography for detection of radioactive spots. As a solvent system a mixture of acetonitrile/water/formic acid (70:25:5, v/v/v) was used.

The electrospray ionisation mass spectra (ESI) were obtained with an LTQ Orbitrap XL mass spectrometer (Thermo, San Jose, CA, U.S.A.). The chromatographic conditions for the MS experiments are given in the report. The HPLC instrument used for chromatography was an Agilent HP1100 (Agilent, Waldbronn, Germany). The flow from the HPLC column was split between UV-detector followed by a radioactivity detector (Ramona Star, Raytest, Straubenhardt, Germany) and MS spectrometer. $^1\text{H-NMR}$ spectra were obtained using a 600 MHz NMR-spectrometer (BRUKER AV 600, Bruker, Karlsruhe, Germany).

Basically, for the lactating goat studies with the pyridinylmethyl- ^{14}C - and the furanone-4- ^{14}C label (KIIA 6.2.3/02), the isolation and identification of parent compound and metabolites were



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

performed in the current study (pyridinylmethyl label). Metabolites were isolated from urine (24 to 48 h) by liquid/liquid partitioning via Extrelut® cartridge followed by HPLC. The isolated metabolites were identified by LC-MS/MS and served as reference compounds for the identification by HPLC co-chromatography. A further reference compound was BYI 02960-AMCP-difluoroethanamine, which was provided as a non-radiolabelled compound by Bayer CropScience AG, Product Technology-Analytics Frankfurt.

II. Results and Discussion

A. Recovery and Elimination of Radioactivity

The recovery of radioactivity after administration of a daily dose of 1.0 mg [pyridinylmethyl-¹⁴C]BYI 02960 per kg body weight on five consecutive days is presented in Table 6.2.3-1. The overall recovery accounted for 88.75% of the total dose. Much of the remaining radioactivity (ca. 11%) was expected to be still present in the gastro-intestinal tract at sacrifice, due to the short period between the last administration and sacrifice. An amount of 0.78% of the total dose was secreted with the milk. At sacrifice, the residues in the organs and tissues dissected from the body were calculated or estimated to be 2.94% of the total dose from which about 71% was detected in skeletal muscle.

Until sacrifice, the excretion of radioactivity accounted for 85.02% of the total dose. A portion of 71.74% was found in the urine and 13.28% in the faeces. The urinary and faecal excretion started immediately after the first administration.

Table 6.2.3-1: Distribution of residues in milk, muscle, fat, liver and kidney of lactating goats following oral administration of 5 daily doses of [pyridinylmethyl-¹⁴C] BYI02960 at a dose rate of 1.0 mg/kg

Sample	Percent of total dose administered	Concentration of total radioactivity [mg/kg]
Liver	0.50	1.215
Kidney	0.10	1.869
Muscle, total	2.10	0.356
Fat, total	0.25	0.106
Total of organs/tissues	2.94	----
Milk, 0 – 102 h	0.78	0.186*
Urine, 0 – 102 h	71.74	----
Faeces, 0 – 102 h	13.28	----
Total excreted	85.02	----
Total Recovery	88.75	----

* : Milk 24 – 102 h

B. Levels and Time Course of Total Radioactive Residues in Milk

The radioactivity levels and concentrations measured in the milk are presented in Table 6.2.3-2. The concentrations ranged from 0.053 mg/kg at 96 hours to 1.345 mg/kg at sacrifice. The concentrations found in the evening and morning milk samples showed a distinct diurnal pattern. The radioactive



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

residues increased significantly during the eight hour period after each administration followed by a decrease to a very low level of about 0.05 mg/kg measured prior to the administration of the next dose. A stable plateau level of about 0.3 mg/kg was reached already at 8 hours after the first administration.

Table 6.2.3-2: Time course of total radioactivity in the milk of lactating goats following oral administration of 5 daily doses of [pyridinylmethyl-14C] BYI02960 at a dose rate of 1.0 mg/kg

Time after the first admin. [h]	Admin. no.	Cumulative secretion [% of total dose admin.]	Secretion per day [% of total dose admin.]	TRR [mg/kg]
0	1	-----	-----	-----
8		0.12	-----	0.292
24		0.16	0.16	0.060
24	2	-----	-----	-----
32		0.30	-----	0.336
48		0.34	0.18	0.055
48	3	-----	-----	-----
56		0.47	-----	0.318
72		0.50	0.17	0.055
72	4	-----	-----	-----
80		0.64	-----	0.318
96		0.67	0.17	0.053
96	5	-----	-----	-----
102		0.78	0.10	1.345

C. Total Radioactive Residues in the Dissected Organs and Tissues

The concentration of the total radioactivity measured in the dissected organs and tissues collected at sacrifice are presented in Table 6.2.3-1 (last column). The highest concentrations were determined in kidney (1.869 mg/kg) and liver (1.215 mg/kg) indicating the significance of these organs for excretion and metabolism. In relation to the total dose administered, these values corresponded to 0.10% and 0.50%, respectively. For muscle and fat 0.356 mg/kg and 0.106 mg/kg, respectively were determined. The radioactivity concentration of the total body muscle corresponded to 2.10% and of fat to 0.25% of the total dose assuming a value of 30% and 12% of the body weight for these tissues.

D. Extraction Efficiency of Residues

Milk (24 to 102 h), muscle, kidney and liver pools were extracted with a mixture of acetonitrile/water (8:2; v/v). In case of milk an additional extraction step with acetone was performed. Fat was extracted with a mixture of acetonitrile/water (8:2; v/v) and n-heptane followed by a solvent partition yielding an acetonitrile/water phase and a n-heptane phase. Residues were not detected in the n-heptane phase. After purification and concentration steps, the resulting extracts represented 99.3% of the total radioactivity for milk (24 to 102 h), 99.4% for muscle, 99.2% for fat, 98.8% for kidney and 92.8% for liver. Losses were not detected during the sample preparation. There was also no radioactivity in the distillates. Unextractable residues amounted to 0.001 mg/kg (0.5%) for milk, 0.002 mg/kg (0.5%) for



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muscle, <0.001 mg/kg (0.2%) for fat, 0.021 mg/kg (1.1%) for kidney and 0.086 mg/kg (7.1%) for liver.

E. Quantification, Identification and Characterisation of Residues

Quantification of Parent Compound and Metabolites

Parent compound and metabolites were quantified in the extracts as well as in the urine (24 to 48 h) using a ternary reversed phase HPL system. The three eluents employed were:

A: 1L water + 1.54 mL ammonium hydroxide solution (25%) + 0.8 mL formic acid, adjusted to pH 7

B: acetonitrile / eluent A (99:1; v/v)

C: methanol / tetrahydrofuran (1:1; v/v)

Detailed information can be found in the report.

Metabolites in the extracts as well as in the urine (24 to 48 h) were assigned by comparison of the metabolite profiles and their retention times. A summary of the quantification of parent compound and metabolites in milk, muscle, fat, kidney and liver is presented in Table 6.2.3-3.

Isolation and Identification of Parent Compound and Metabolites in Urine

Metabolites were isolated from urine (24 to 48 h) and identified by LC-MS/MS investigations. An assignment of all identified metabolites to the metabolic profile of urine is presented in Figure 6.2.3-1. BYI 02960-OH-gluA (isomer 1, Py6) to BYI 02960-OH-gluA (isomer 4, Py9) were identified as glucuronic acid conjugates of hydroxylated BYI 02960 by LC-MS/MS. The LC-MS/MS fragments of BYI 02960-OH-gluA (isomer 1) and BYI 02960-OH-gluA (isomer 4) were different from each other and were different from BYI 02960-OH-gluA (isomer 2) and BYI 02960-OH-gluA (isomer 3). Most probably BYI 02960-OH-gluA (isomer 2) and BYI 02960-OH-gluA (isomer 3) are two diastereomers because of their identical LC-MS/MS fragments. Therefore, it was concluded that BYI 02960 was hydroxylated and conjugated with glucuronic acid in three different positions of the molecule.

The hydroxylation of the two diastereomers (isomer 2 and 3) was assigned to the 5-position of the furanone ring based on the similarity of the mass spectra with those of BYI 02960-OH-SA. The position of the hydroxylation of BYI 02960-OH-SA was clearly identified by NMR-spectroscopy in the laying hen study with the pyridinylmethyl label (KIIA 6.2.2/01). In the case of isomer 4, the position of the hydroxylation was assigned to the difluoroethyl side chain based on the fragments 225 (ESI+) and 223 (ESI-) proving the presence of the unchanged pyridinylmethyl and furanone moieties. For isomer 1, the hydroxylated position could not be derived from the mass spectra.

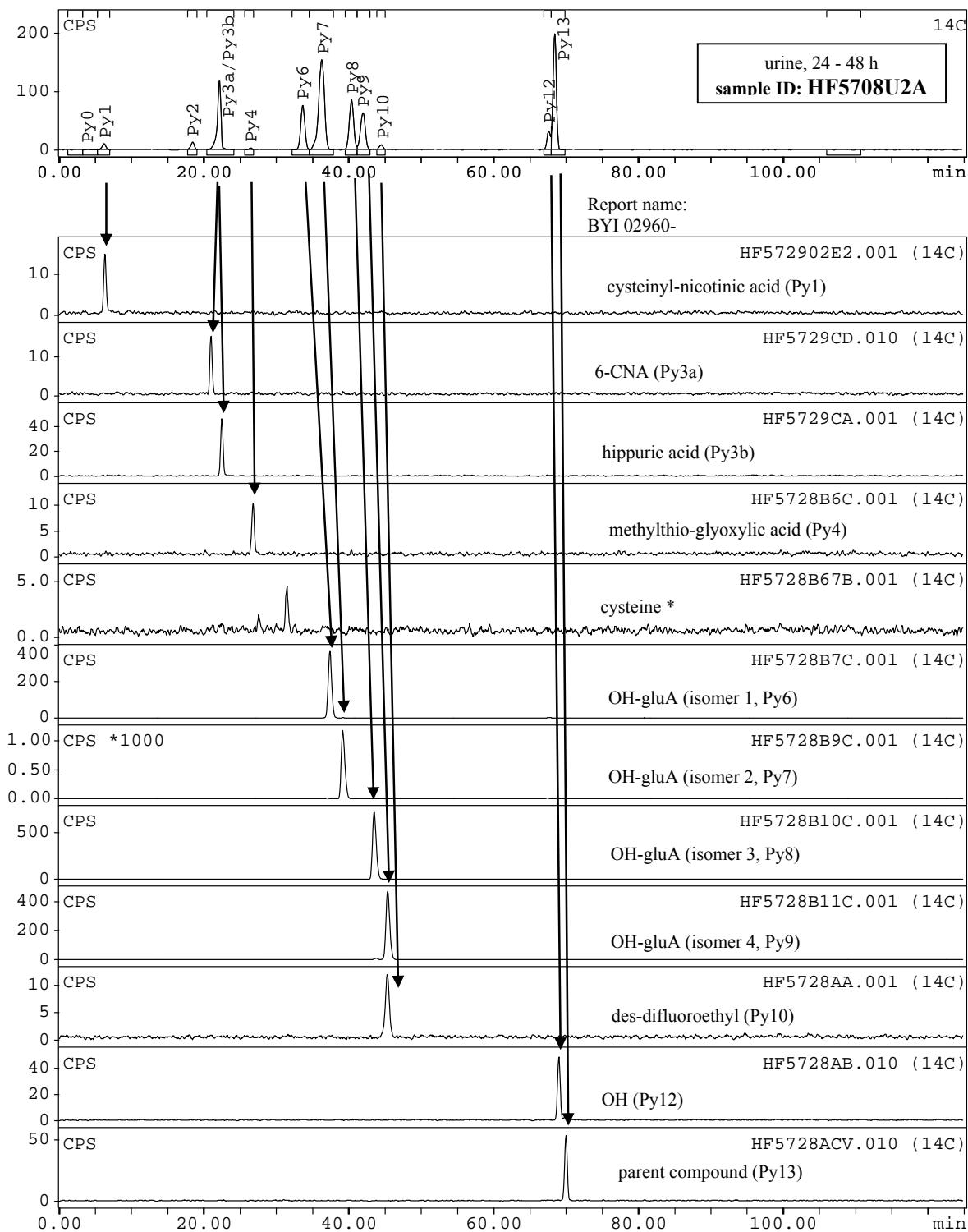
Further evidence for the assignment of the four isomers of BYI 02960-OH-gluA to their corresponding aglycones and decomposition product is presented in the lactating goat study with the furanone label (KIIA 6.2.3/02).



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Figure 6.2.3-1: Assignment of isolated and identified metabolites in urine of lactating goats following oral administration of 5 daily doses of [pyridinylmethyl-¹⁴C] BYI02960 at a dose rate of 1.0 mg/kg.

Integration G:\ME\M1844572-1\MR237_02\BYI02960TERNÄR\HF5708U2A.001M



Note: * BYI 02960-cysteine was isolated from concentrated urine; it was not detected in native urine.

Identification, Assignment and Quantification of Metabolites in Milk, Organs and Tissues

Identified metabolites from urine were used as reference compounds to identify the metabolites in the extracts of milk, muscle, fat, kidney and liver by HPLC or TLC co-chromatography. The identification of BYI 02960-AMCP-difluoroethanamine in the liver was performed with the non-radiolabelled reference compound by HPLC co-chromatography. All other metabolites in milk, organs and tissues were assigned by comparison of the metabolite profiles and retention times based on the profiling method.

The distribution of the parent compound and metabolites in milk, organs and tissues is summarised in Table 6.2.3-3. Approx. 99% of the total radioactivity in milk, muscle, fat and kidney and approx. 93% in liver were identified.

Table 6.2.3-3: Radioactive residues of parent compound and metabolites in milk, urine and edible organs of lactating goats following oral administration of 5 daily doses of [pyridinylmethyl-¹⁴C] BYI02960 at a dose rate of 1.0 mg/kg

	Milk (24 to 102 h)		Muscle		Fat		Kidney		Liver	
TRR [mg/kg]	0.186		0.356		0.106		1.869		1.215	
Sample/ Report name BYI 02960-	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
- ACN/water extract	99.3	0.184	99.4	0.353	99.2	0.105	98.8	1.847	92.8	1.128
cysteinyl-nicotinic acid	---	---	---	---	---	---	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
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
Total extracted	99.5	0.185	99.5	0.354	99.8	0.106	98.9	1.848	92.9	1.129
Solids	0.5	0.001	0.5	0.002	0.2	<0.001	1.1	0.021	7.1	0.086
Accountability	100.0	0.186	100.0	0.356	100.0	0.106	100.0	1.869	100.0	1.215

Parent compound was by far the dominating constituent of the residue in milk, organs and tissues. Its concentration amounted to 0.165 mg/kg (88.8%) for milk, 0.349 mg/kg (98.0%) for muscle, 0.105 mg/kg (99.2%) for fat, 0.650 mg/kg (34.8%) for kidney and 1.028 mg/kg (84.6%) for liver.

The highest number of metabolites was found in the kidney. The major metabolite was BYI 02960-OH (hydroxylation in position 5 of the furanone ring), which amounted to 0.299 mg/kg (16.0%). Four prominent glucuronic acid conjugates of hydroxylated parent compound were detected in the kidney. The four conjugates were named BYI 02960-OH-gluA (isomer 1 to isomer 4), their concentration



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ranged from 0.112 mg/kg (6.0%) to 0.175 mg/kg (9.3%). The hydroxylation of BYI 02960 and conjugation with glucuronic acid resulted into two diastereomers of conjugated BYI 02960-OH (BYI 02960-OH-gluA, isomer 2 and 3) with hydroxylation and conjugation in the 5-position of the furanone ring), one isomer (BYI 02960-OH-gluA, isomer 4) with the hydroxylation and conjugation in the difluoroethyl side chain and one isomer (BYI 02960-OH-gluA, isomer 1) with the hydroxylation and conjugation in an unknown position.

A further major metabolite was BYI 02960-hippuric acid, which was detected in the milk (0.017 mg/kg, 9.1%) and kidney (0.178 mg/kg, 9.5%). Traces of BYI 02960-hippuric acid were also found in liver (0.010 mg/kg,). BYI 02960-cysteinyl-nicotinic acid was identified in kidney (0.114 mg/kg, 6.1%) and in liver (0.058 mg/kg, 4.8%). Minor metabolites were BYI 02960-methylthio-glyoxylic acid in milk and muscle and BYI 02960-AMCP-difluoroethanamine in kidney and liver. Their concentration was below 0.020 mg/kg.

F. Storage Stability of Residues

During the study, all samples and extracts were stored at $\leq -18^{\circ}\text{C}$ or for a short time in a refrigerator. All samples of milk, and edible organs and tissues were extracted within approx. six weeks after sample collection. The first metabolite profile was recorded one day after the start of the extraction and sample preparation. Hence, investigations on storage stability of the residues in the samples were not necessary and it can be concluded that the metabolic profiles represent the residues in the matrices at sacrifice.

III. Conclusion

The metabolic and excretion behaviour of [pyridinylmethyl- ^{14}C]BYI 02960 in the lactating goat can be characterised by the following observations:

- The concentration of radioactivity in milk and edible tissues was rather low compared to the dose level and the dosing period of five days.
- The evaluation of these concentrations should moreover consider the fact that an exaggerated dose level of 24.36 mg/kg feed/day was administered. Furthermore, the significant amount of radioactivity detected in urine and faeces and the relatively high concentration in liver and kidney at sacrifice six hours after the last administration indicate that the residues are further metabolised and finally eliminated
- The residue level in milk showed a pronounced diurnal pattern after the first administration as they declined to a very low level prior to the next dose. A stable residue plateau level was reached already at 8 hours after the first administration.
- The radioactive residues were efficiently extracted from milk as well as from edible organs and tissues; extraction rates were between 92.9% and 99.8%.
- Parent compound was the dominating constituent of the residues in milk (ca. 89%), muscle (ca. 98%), fat (ca. 99%), kidney (ca. 35%) and liver (ca. 85%).
- The biggest number of metabolites was found in the kidney. BYI 02960-OH, four glucuronic acid conjugates of hydroxylated BYI 02960, BYI 02960-hippuric acid and BYI 02960-cysteinyl-nicotinic acid were identified.

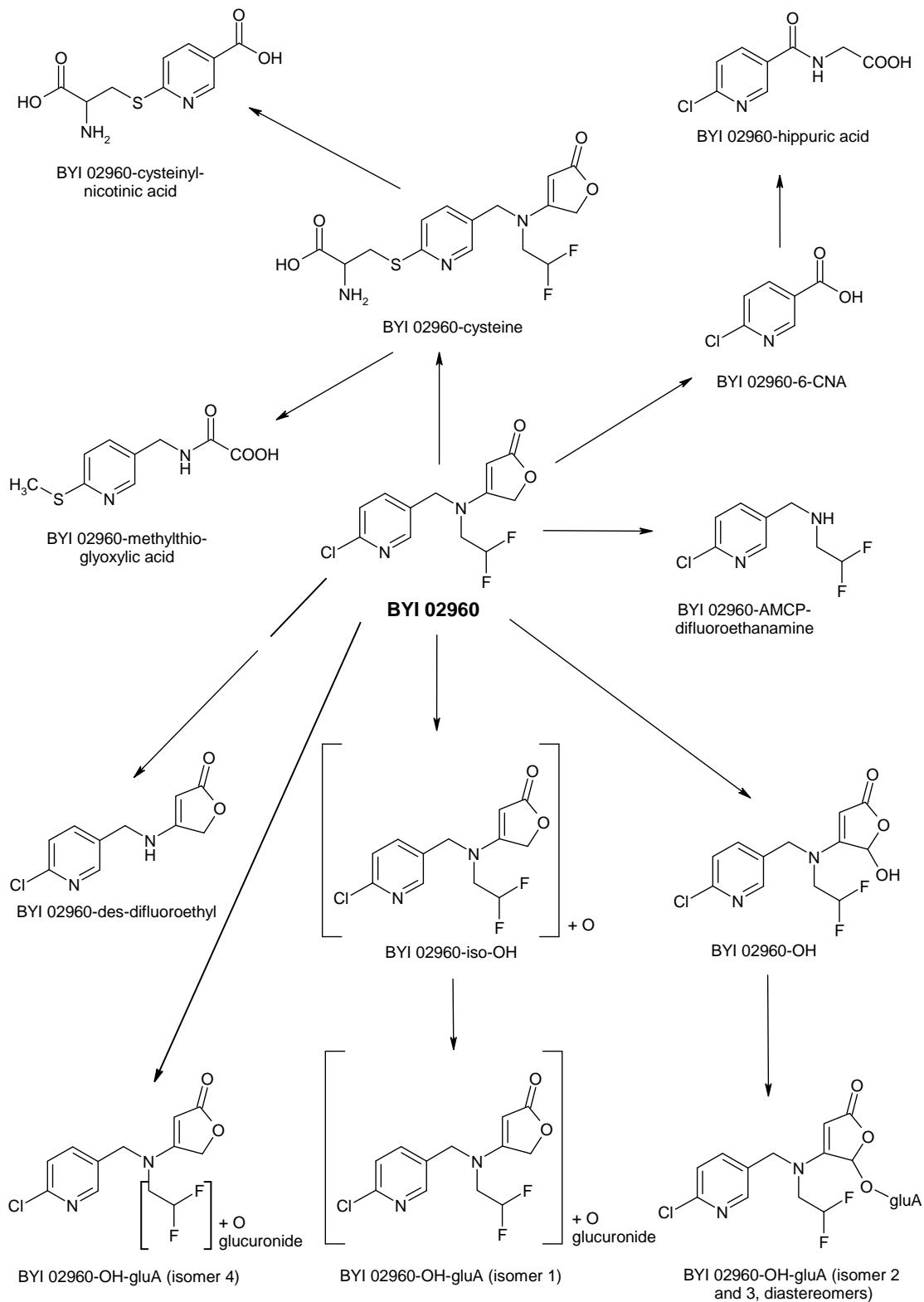


Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

- The cleavage of the molecule was not very pronounced (< 17%). Label specific metabolites were BYI 02960-hippuric acid, BYI 02960-cysteinyl-nicotinic acid, BYI 02960-methylthio-glyoxylic acid and BYI 02960-AMCP-difluoroethanamine. BYI 02960-6-CNA was only found in urine.
- The main metabolic reactions in the lactating goat are:
 - Hydroxylation of BYI 02960 followed by conjugation with glucuronic acid forming two diastereomeric conjugates of BYI 02960-OH (BYI 02960-OH-gluA, isomer 2 and 3) with hydroxylation and conjugation in the 5-position of the furanone ring, one isomer (BYI 02960-OH-gluA, isomer 4) with the hydroxylation and conjugation in the difluoroethyl side chain and one isomer (BYI 02960-OH-gluA, isomer 1) with the hydroxylation and conjugation in an unknown position
 - Oxidative cleavage of the pyridinylmethyl bridge forming BYI 02960-6-CNA followed by conjugation with glycine to BYI 02960-hippuric acid
 - Substitution of the chlorine atom of BYI 02960 with glutathione followed by degradation resulting in BYI 02960-cysteine
 - Oxidative cleavage of the pyridinylmethyl bridge of BYI 02960-cysteine forming BYI 02960-cysteinyl-nicotinic acid
 - Degradation of BYI 02960-cysteine in the cysteine group and furanone ring forming BYI 02960-methylthio-glyoxylic acid
 - Cleavage of the furanone ring forming BYI 02960-AMCP-difluoroethanamine
 - Cleavage of the difluoroethyl group forming BYI 02960-des-difluoroethyl

Based on these results the metabolic pathway of [pyridinylmethyl-¹⁴C] BYI02960 in the lactating goat shown in Figure 6.2.3-2 is proposed.

Figure 6.2.3-2: Proposed metabolic pathway of [pyridinylmethyl-14C] BYI02960 in the lactating goat



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Report:	KIIA 6.2.3/02, Authors: [REDACTED], R., [REDACTED], J., (2011)
Title:	[Furanone-4- ¹⁴ C]BYI02960: Metabolism in the lactating goat
Report No & Document No:	MEF-11/268 Date: 16.12.2011 M-421995-01-2
Guidelines:	OECD-Guideline for the Testing of Chemicals No. 503, Metabolism in Livestock US EPA Residue Chemistry Test Guideline OPPTS 860.1300 Nature of the Residue – Plants, Livestock European Parliament and Council Regulation (EC) No 1107/2009
GLP:	Yes, according to Japan MAFF GLP standard 11 Nousan 6283; US EPA – FIFRA GLP (40CFR Part 160); Principles of GLP, German Chemical Law, current version of Annex 1
Testing Facility and Dates:	[REDACTED] Experimental work: 19.4.2010 – 20.4.2011

Executive Summary

The metabolism and excretion of [furanone-4-¹⁴C]BYI 02960 (common name: flupyradifurone) were investigated in the lactating goat as a model for ruminants. The 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 28.82 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 the unchanged parent compound and metabolites.

Recovery and Elimination of Radioactivity

The overall recovery amounted to 78.94% of the total dose. Much of the remaining radioactivity (ca. 21%) was expected to still be present in the gastro-intestinal tract at sacrifice, due to the short period between the last administration and sacrifice. A significant proportion is probably exhaled as ¹⁴CO₂ since the formation of carbon dioxide had been clearly shown in a rat quantitative whole body autoradiography study using the test compound labelled at the same position (KIIA 5.1.2/02). An amount of 2.58% of the total dose was secreted with the milk. At sacrifice, the residues in the organs and tissues were calculated or estimated to be 4.22% of the total dose from which about 69% was detected in the skeletal muscle.

Until sacrifice, the excretion of radioactivity accounted for 72.14% of the total dose. A portion of 69.15% was found in the urine and 3.0% in the faeces. The urinary and faecal excretion started immediately after the first administration.

Total Radioactive Residues in Milk, Organs and Tissues

The concentration of radioactivity in milk samples ranged from 0.755 mg/kg at 24 hours to 1.213 mg/kg at 56 hours after the first administration. At sacrifice, the residue concentration was 1.165 mg/kg. The time course in the evening and morning milk pool samples showed a pronounced diurnal pattern. The residues increased significantly during the eight hour period after the second and the fifth administration followed by a decrease measured prior to the delivery of the next dose. A plateau level of about 1.1 mg/kg was reached ca. 50 hours after the first administration.

In the organs and tissues, the highest concentrations were determined in liver (1.746 mg/kg) and kidney (1.472 mg/kg) indicating the significance of these organs for excretion and metabolism. These

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values corresponded to 0.65% and 0.09% of the total dose, respectively. For muscle and fat 0.539 mg/kg and 0.265 mg/kg, respectively, were determined. The radioactivity concentration of the muscle corresponded to 2.91% and of fat to 0.57% of the total dose assuming a value of 30% and 12% of the body weight for these tissues.

Metabolism

For analysis of parent compound and metabolites, milk, muscle, kidney and liver were extracted with mixtures of acetonitrile/water. Fat was extracted with mixtures of n-heptane and acetonitrile/water followed by a solvent partition yielding an n-heptane and acetonitrile/water phase. Residues in the n-heptane phase of fat amounted to only 0.014 mg/kg (5.4%). The resulting acetonitrile/water extracts of milk, muscle, fat, kidney and liver represented between 73.3% and 95.0% of the total radioactive residue. Solids of kidney and liver were exhaustively extracted with mixtures of acetonitrile/water, 0.1 N HCl and 0.1 N NaOH using microwave assistance. The exhaustive extracts were not further investigated, due to their low amount of residues and/or their high matrix content. Unextractable residues in milk, muscle and fat were low and amounted to \leq 0.090 mg/kg. The extraction residues of kidney and liver were completely solubilised after the exhaustive extraction with sodium hydroxide. After purification and concentration, the conventional acetonitrile/water extracts were analysed by HPLC with radiometric detection. The HPLC profiling method was based on the use of a reversed phase column and a neutral water/acetonitrile gradient. The metabolite pattern of the extracts of the current goat study and the goat study with the pyridinylmethyl label (KIIA 6.2.3/01) were comparable with the exception of the label specific metabolites.

All prominent metabolites of the extracts of milk, muscle, fat, kidney and liver were also detected in urine, except lactose in the milk. Therefore, the assignment of metabolites in the profiles of the extracts was performed based on the comparison with the urine profiles of the current study and the study with the pyridinylmethyl label. Four isomers of BYI 02960-OH-gluA were isolated and identified by comparison of the LC-MS/MS data. Further identification of the BYI 02960-OH-gluA isomers was achieved by comparison of the degradation rates of isomers with the increasing rates of their aglycones and decomposition product, based on the observed decomposition of the isomers in two urine samples. The radioactive lactose in the milk was identified after isolation and acetylation by HPLC co-chromatography with the radiolabelled reference compound.

Approximately 91% of the total residue in milk, ca. 90% in muscle, 83% in fat, 79% in kidney and ca. 60% in liver was identified. All other residues were characterised by their extraction behaviour. The unchanged parent compound was the main constituent of the residue in organs and tissues and amounted to 0.475 mg/kg (88.1%) for muscle, 0.213 mg/kg (80.5%) for fat, 0.744 mg/kg (50.5%) for kidney and 1.045 mg/kg (59.8%) for liver. Lower amounts of parent compound (0.250 mg/kg, 23.9%) were found in the milk. The major part of residues in the milk was radioactive lactose and amounted to 0.698 mg/kg (66.8%). The lactose was specific for the furanone label and was formed by cleavage and subsequent degradation of the furanone ring to smaller carbon units (C-1- or C-2-fragments), which entered the carbon pool of endogenous compounds.

Extensive metabolism was detected in the kidney, where the main portion of metabolites was found. The main metabolite in kidney was BYI 02960-OH (hydroxylation in the 5-position of the furanone ring), which amounted to 0.215 mg/kg (14.6%). Four prominent glucuronic acid conjugates of hydroxylated parent compound were detected in the kidney and in the urine. The four isomers were named BYI 02960-OH-gluA (isomer 1 to isomer 4). Their concentrations were between 0.032 mg/kg (2.2%) and 0.069 mg/kg (4.7%) in the kidney. BYI 02960-OH-gluA (isomer 2) and BYI 02960-OH-

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

gluA (isomer 3) were diastereomers, which were hydroxylated and conjugated in 5-position of the furanone ring. BYI 02960-OH-gluA (isomer 4) was hydroxylated and conjugated in the difluoroethyl side chain and BYI 02960-OH-gluA (isomer 1) in an unknown position. A minor metabolite was BYI 02960-des-difluoroethyl which amounted to 0.019 mg/kg (1.3%) in the kidney.

Metabolites in the polar region of the acetonitrile/water extracts (≤ 5 min) were specific for the furanone label. Their concentrations ranged from 0.021 to 0.037 mg/kg for kidney and from 0.019 to 0.059 mg/kg for liver. These polar metabolites showed the same thin layer chromatographic behaviour as the metabolites, which were detected in the corresponding polar region of rat urine (KIIA 5.1.2/01) and the extracts of muscle and liver of laying hen (KIIA 6.2.2/02). The polar region of muscle and fat was not further analysed, due to the low amount of radioactivity (≤ 0.024 mg/kg).

The formation of radioactive lactose and polar metabolites was mainly caused by the partial instability of the radiolabel used. For a small portion of the total dose the furanone ring of the molecule obviously underwent extensive biotransformation to C-1- and C-2-fragments resulting in an accumulation of radioactivity in biomolecules during the dosing for five days. A part of these fragments was probably also converted to the terminal product $^{14}\text{CO}_2$, which might explain the lower overall recovery as compared to the study with the metabolically stable pyridinylmethyl label.

The concentration of the total radioactivity (expressed as mg a.s. equiv. /kg) as well as the distribution of the parent compound and metabolites and the identification rates in milk and edible tissues are summarised in the following table:

	Milk (24 to 102 h)		Muscle		Fat		Kidney		Liver	
TRR [mg/kg]	1.046		0.539		0.265		1.472		1.746	
Sample/ Report name BYI 02960-	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
ACN/water extract	90.7	0.948	95.0	0.512	88.4	0.234	89.1	1.311	73.3	1.280
Lactose	66.8	0.698	---	---	---	---	---	---	---	---
Polar metabolites	---	---	4.4	0.024	5.0	0.013	10.0	0.148	12.1	0.211
OH-gluA (isomer 1)	---	---	---	---	---	---	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
Total extracted	91.4	0.956	95.2	0.513	94.1	0.249	100.0	1.472	100.0	1.746
Solids	8.6	0.090	4.8	0.026	5.9	0.016	n.d.	n.d.	n.d.	n.d.
Accountability	100.0	1.046	100.0	0.539	100.0	0.265	100.0	1.472	100.0	1.746

The metabolic and excretion behaviour of [furanone-4- ^{14}C]BYI 02960 in the lactating goat can be characterised by the following observations:

- The concentration of radioactivity in milk and edible tissues were relatively high compared to the dose level and the dosing period of five days. This was mainly caused by the partial instability of the radiolabel used. For a small portion of the total dose the furanone ring obviously underwent



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

extensive biotransformation to C1- and C2-fragments that resulted in an accumulation of radioactivity in biomolecules during dosing for five days.

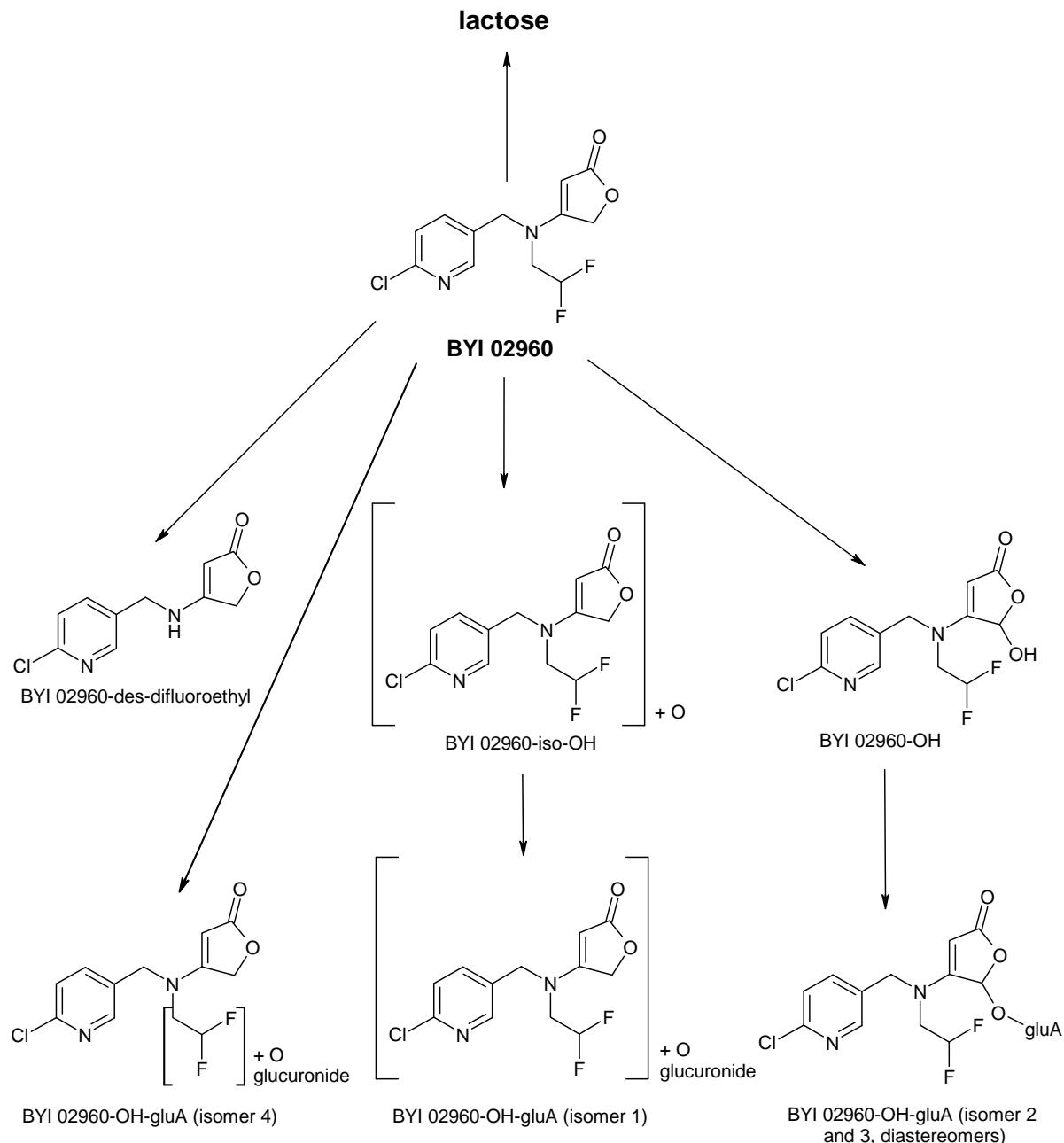
A part of these fragments was probably also converted to the terminal product $^{14}\text{CO}_2$ as can be derived from the lower overall recovery compared to the study with the metabolically stable pyridinylmethyl label.

- However, the fact should be considered that an exaggerated dose level of 28.82 mg/kg feed/day was administered. Furthermore, the significant amount of radioactivity detected in urine and faeces and the relatively high radioactivity in liver and kidney at sacrifice indicate that the residues are further metabolised and finally eliminated.
- The total radioactive residues in milk showed a diurnal pattern after the second administration as they declined significantly prior to the delivery of the next dose. A residue plateau level was reached after the third administration.
- The radioactive residues were efficiently extracted from milk as well as from edible organs and tissues; extraction rates were between 91.4% and 100.0%.
- Parent compound was the main constituent of the residues in muscle (ca. 88%), fat (ca. 81%), kidney (ca. 51%) and liver (ca. 60%). Approximately 24% of the total radioactivity in the milk was identified as parent compound.
- The main residue in the milk was radioactive lactose (approx. 67%)
- Extensive metabolism was detected in the kidney. BYI 02960-OH, four glucuronic acid conjugates of hydroxylated BYI 02960 and BYI 02960-des-difluoroethyl were identified.
- The main metabolic reactions in the lactating goat are:
 - Cleavage and subsequent degradation of the furanone ring forming small carbon units (C-1- or C-2-fragments), entering the carbon pool of endogenous compounds and finally being used for example for the biosynthesis of lactose
 - Hydroxylation followed by conjugation with glucuronic acid forming two diastereomeric conjugates of BYI 02960-OH (BYI 02960-OH-gluA, isomer 2 and 3) with hydroxylation and conjugation in the 5-position of the furanone ring, one isomer (BYI 02960-OH-gluA, isomer 4) with the hydroxylation and conjugation in the difluoroethyl side chain and one isomer (BYI 02960-OH-gluA, isomer 1) with the hydroxylation and conjugation in an unknown position
 - Cleavage of the difluoroethyl group forming BYI 02960-des-difluoroethyl

Based on these results the metabolism of [furanone-4- ^{14}C]BYI 02960 in the lactating goat can be described by the metabolic pathway shown on the next page.

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Proposed metabolic pathway of [furanone-4-¹⁴C]BYI 02960 in the lactating goat:



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)**I. Materials and Methods****A. Materials****1. Test Material**

IUPAC Name	4-{{[(6-chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino} furan-2(5H)-one
Code name	BYI02960
Common name	Flupyradifurone (proposed ISO)
Empirical formula	C ₁₂ H ₁₁ ClF ₂ N ₂ O ₂
Molar mass	288.68
Labelling	[furanone-4- ¹⁴ C]
Specific radioactivity used for administration	4.24 MBq/mg = 114.50 µCi/mg (delivered sample before radiodilution) 3.50 MBq/mg = 2.10 x 10 ⁸ dpm/mg = 94.59 µCi/mg = 27.31 Ci/mol (sample after radiodilution)
Radiochemical purity	> 99 % (HPLC)
Dose level	5 daily oral doses of 1 mg/kg bw by gavage
Vehicle	Capsule

2. Test Animals

Species	Lactating goat (<i>Capra hircus</i>)
Strain	“Weiße Deutsche Edelziege”
Breeding facility	Dr. G. Jux-Straatmann, Halfenslennefe 1, D-51491 Overath, Germany Member of the Landesverband Rheinischer Ziegenzüchter e.V., Halfenslennefe 1, D-51491 Overath, Germany
Sex and numbers involved	1 female animal
Age	24 months
Body weight	47 kg at first administration, 42 kg at sacrifice
Acclimatization	7 days
Identification	skin marking
Housing	During acclimatization period: raised stall with a metal grid as base and straw and hay as bedding. During the test period: electro-polished stainless steel metabolism cage for farm animals (goat, sheep, and pig), supplied by E. Becker & Co. GmbH “EBECO”, Hermannstr. 2 – 8, 44579 Castrop-Rauxel, Germany. The cage was equipped with a variable-restraining device. room temperature 20 - 25°C, relative humidity 40-55% 12 h light / 12 h dark cycle, air change 10 – 15 times per hour.
Feed and water	The goat was fed <i>ad libitum</i> with hay, hay pellets, apples and supplementary ruminant feed (“Raiffeisen LammGold”, supplied by Raiffeisenmarkt Rhein-Berg eG, Postfach 100565, D-40769 Monheim). During the test period, the average feed consumption was 1.63 kg/day, tap water was offered <i>ad libitum</i>

B. Study Design

Dosing

The test compound was orally dosed once daily for five consecutive days in the morning after milking. Based on the daily feed consumption during the test of 3.47% of the body weight, the dose of 1.0 mg a.s. /kg bw corresponded to 28.82 mg a.s. /kg dry feed per day in the diet.

The radiolabelled test compound was delivered with a specific radioactivity of 4.24 MBq/mg. It was diluted with the non-radiolabelled test compound to a specific radioactivity of 3.50 MBq/mg. In total, five gelatine capsules containing the test compound were prepared. They were stored at $\leq -18^{\circ}\text{C}$ until administration. The remaining test compound was stored in solid form together with the capsules. An aliquot of this sample was dissolved in an eluent of the HPLC method (1 L water + 1.54 mL ammonium hydroxide solution (25%) + 0.8 mL formic acid, adjusted to pH 7) and analysed by HPLC after the last administration in order to demonstrate the stability of the test compound during the administration phase.

The administrations were performed using a capsule applicator. The goat received on each day one gelatine capsule containing on average an amount of 47.01 mg. The totally administered amount and radioactivity accounted for 235.07 mg and 49,364,700,000 dpm, respectively. The total amount of radioactivity administered to the animal served as reference-value for the percentage calculation of the total radioactivity in the biological samples.

Sampling of milk, urine and faeces during the in-life phase

The goat was milked in the morning immediately prior to each administration, about 8 hours later in the afternoon, and directly before sacrifice (8, 24, 32, 48, 56, 72, 80, 96 and 102 hours after the first administration). The milk weights were recorded.

Urine and faeces samples were collected in plastic vessels as quantitatively as possible under dry ice cooling in intervals of 24 hours after the administrations 1 to 4 and 6 hours after 5th administration. The vessels were changed immediately before the next administration. The collection funnel was rinsed with deionised water into the vessel of the respective collection period. An aliquot of each milk and urine fraction was taken and processed for LSC. The remaining samples were stored at about -18°C for metabolite analysis. For collection of faeces, the collecting grid was cleaned prior to each administration. No samples of the rinsing water were taken for radioactivity measurement. Each faeces fraction was homogenised after addition of water to obtain a wet paste before the total weight was recorded. Aliquots of each sample were weighed and prepared for radioactivity measurement by combustion/LSC. The remainder was stored at room temperature for metabolite analysis.

Sacrifice and dissection of organs and tissues

The animal was sacrificed approx. 6 hours after the last administration. The animal was anaesthetised by an intravenous dose of about 40 mg/kg bw Pentobarbital-Na (Narcoren®), exsanguinated by cannulating the jugular vein and finally terminated by intracardiac injection with approx. 10 mL of the veterinary drug "T 61®". Following sacrifice, the following organs and tissues were sampled: liver without gall bladder, kidneys, two different types of muscle (round and loin), and two different types of fat (perirenal and omental.). The gall bladder was punctured for the collection of the bile fluid that was then stored in frozen for an optional metabolite analysis.

Sample preparation

The organs or tissue samples were weighed and transferred into ice-cooled vessels. Liver, kidneys, muscle, and fat samples were thoroughly homogenised in half-frozen state for several times in a mincing machine. Each resulting tissue pulp was weighed and aliquots were prepared for radioactivity measurement by combustion/LSC. All samples were divided into portions and stored in a freezer until start of metabolite analysis. The remaining samples of each organ or tissue were stored at -18 °C.

For metabolism investigations, a pooled sample of milk collected from 24 h until 102 h after the first administration and composite samples of muscle (loin and round) and fat (omental and perirenal), as well as samples of kidneys and liver were prepared. The samples were homogenised and kept frozen until extraction.

Aliquot samples of milk, muscle, kidney and liver were extracted with acetonitrile/water (8:2; v/v) using an Ultra Turrax homogeniser. The fat sample was extracted with mixtures of n-heptane and acetonitrile/water (8:2; v/v) also using an Ultra Turrax homogeniser followed by a solvent partition yielding single acetonitrile/water phases and a combined n-heptane phase. Solids of kidney and liver were in addition exhaustively extracted with mixtures of acetonitrile/water, 0.1 N HCl and 0.1 N NaOH using microwave assistance. All acetonitrile/water extracts were subjected to an SPE clean-up step followed by concentration. The final extracts were used for the profiling, quantification and identification of parent compound and metabolites by HPLC.

Radioactivity measurement

The measurement of the radioactivity in the liquid samples was carried out by liquid scintillation counting (LSC). Quenching effects were automatically corrected using an external standard and quenching library. The instrument background of 10 - 32 dpm was subtracted automatically. For all samples, the limit of detection (LOD) was established at approximately 10 dpm measured per aliquot after instrument background correction. The limit of quantification (LOQ) was established as 2 times of the background radioactivity (dpm) of each instrument/method. All solid samples were combusted in an oxygen atmosphere using an oxidiser. The released ¹⁴CO₂ was trapped in an alkaline scintillation cocktail and the radioactivity was determined by LSC.

Metabolite analysis

The purified extracts and samples of urine were subjected to HPLC using a reversed phase column (C18) and a ternary elution gradient. Detection was performed by a UV- (254 nm) and a radioisotope detector with a glass bead scintillator. In order to check the completeness of the elution for the HPLC profile, representative samples of milk, muscle, liver, and kidney extract were injected, re-collected, and radioassayed by LSC. The recoveries were between 95.3 and 105.9% of the injected radioactivity. Radiolabelled and non-labelled reference compounds were used for co-chromatography for identification of metabolites.

As a second chromatographic method thin layer chromatography (TLC) was employed on silica gel plates and radioluminography for detection of radioactive spots. As a solvent system a mixture of acetonitrile/water/formic acid (70:25:5, v/v/v) was used:

The electrospray ionisation mass spectra (ESI) were obtained with an LTQ Orbitrap XL mass spectrometer (Thermo, San Jose, CA, U.S.A.). The chromatographic conditions for the MS experiments are given in the report. The HPLC instrument used for chromatography was an Agilent HP1100 (Agilent, Waldbronn, Germany). The flow from the HPLC column was split between UV-detector followed by a radioactivity detector (Ramona Star, Raytest, Straubenhardt, Germany) and MS

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spectrometer. ^1H -NMR spectra were obtained using a 600 MHz NMR-spectrometer (BRUKER AV 600, Bruker, Karlsruhe, Germany).

The identification of parent compound, BYI 02960-OH and BYI 02960-des-difluoroethyl was performed in isolated fractions of a urine sample of the goat study with the pyridinylmethyl label (KIIA 6.2.3/01) by spectroscopic methods. The metabolites were assigned in the extracts of milk, organs and tissues of the current study based on the comparison of the metabolite pattern of a representative urine of the current study and of the study with the pyridinylmethyl label.

Four isomers of the glucuronic acid conjugate of hydroxylated BYI 02960 were isolated from the urine (24 to 48 h) of the current study by liquid/liquid partitioning via Extrelut® cartridge followed by HPLC. The isolated metabolites were identified by LC-MS/MS. The position of hydroxylation and conjugation for three isomers was assigned based on the LC-MS/MS fragments. Confirmation of the identification was based on the degradation to aglycones and other metabolites observed in two urine samples by comparison of the decrease of glucuronic acid conjugates with the increase of the degradation products in relation to non-degraded urine samples.

Metabolites in the polar region of the acetonitrile/water extracts of kidney and liver were quantified and further characterised by TLC (solvent system: acetonitrile/water/formic acid, 70:25:5; v/v/v). Their commonality with polar metabolites in urine of rat and organs of hen was demonstrated by comparison of the TLC profiles. The polar regions of muscle and fat were not investigated, due to their low amount of residues and high matrix content.

The polar residue in the milk was isolated and identified after acetylation. The identification was performed by HPLC co-chromatography with acetylated radioactive lactose as reference compound. Acetylated radioactive lactose was synthesised from radioactive lactose.

II. Results and Discussion

A. Recovery and Elimination of Radioactivity

The recovery of radioactivity after administration of a daily dose of 1.0 mg [furanone-4- ^{14}C]BYI 02960 per kg body weight on five consecutive days is presented in Table 6.2.3-4. The overall recovery accounted for 78.94% of the total dose. Much of the remaining radioactivity (ca. 21%) was expected to still be present in the gastro-intestinal tract at sacrifice, due to the short period between the last administration and sacrifice. An amount of 2.58% of the total dose was secreted with the milk. A significant proportion is probably exhaled as $^{14}\text{CO}_2$ since the formation of carbon dioxide had been clearly shown in a rat quantitative whole body autoradiography study using the test compound labelled at the same position (KIIA 5.1.2/02). At sacrifice, the residues in the organs and tissues dissected from the body were calculated or estimated to be 4.22% of the total dose from which about 69% was detected in skeletal muscle.

Until sacrifice, the excretion of radioactivity accounted for 72.14% of the total dose. A portion of 69.15% was found in the urine and 3.0% in the faeces. The urinary and faecal excretion started immediately after the first administration.

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Table 6.2.3-4: Distribution of residues in milk, muscle, fat, liver and kidney of lactating goats following oral administration of 5 daily doses of [furanone-4-14C] BYI02960 at a dose rate of 1.0 mg/kg

Sample	Percent of total dose administered	Concentration of total radioactivity [mg/kg]
Liver	0.65	1.746
Kidney	0.09	1.473
Muscle, total	2.91	0.539
Fat, total	0.57	0.265
Total of organs/tissues	4.22	----
Milk, 0 – 102 h	2.58	0.961
Urine, 0 – 102 h	69.15	----
Faeces, 0 – 102 h	3.00	----
Total excreted	72.14	----
Total Recovery	78.94	----

B. Levels and Time Course of Total Radioactive Residues in Milk

The radioactivity levels and concentrations measured in the milk are presented in Table 6.2.3-5. The concentrations ranged from 0.755 mg/kg at 24 hours to 1.213 mg/kg at 56 hours after the first administration. At sacrifice, the residue concentration was 1.165 mg/kg. The time course in the evening and morning milk pool samples showed a pronounced diurnal pattern. The residues increased significantly during the eight hour period after the second and the fifth administration followed by a decrease measured prior to the delivery of the next dose. A plateau level of about 1.1 mg/kg was reached ca. 50 hours after the first administration.

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Table 6.2.3-5: Time course of total radioactivity in the milk of lactating goats following oral administration of 5 daily doses of [furanone-4-14C] BYI02960 at a dose rate of 1.0 mg/kg

Time after the first admin. [h]	Admin. no.	Cumulative secretion [% of total dose admin.]	Secretion per day [% of total dose admin.]	TRR [mg/kg]
0	1	----	----	----
8		0.30	0.68	0.820
24		0.68	0.68	0.755
24	2	----	----	----
32		1.10	0.72	1.130
48		1.40	0.72	0.814
48	3	----	----	----
56		1.67	0.51	1.213
72		1.91	0.51	0.997
72	4	----	----	----
80		2.14	0.53	1.205
96		2.44	0.53	0.992
96	5	----	----	----
102		2.58	0.14	1.165

C. Total Radioactive Residues in the Dissected Organs and Tissues

The concentration of the total radioactivity measured in the dissected organs and tissues collected at sacrifice are presented in Table 6.2.3-4 (last column). The highest concentrations were determined in liver (1.746 mg/kg) and kidney (1.472 mg/kg) indicating the significance of these organs for excretion and metabolism. In relation to the total dose administered, these values corresponded to 0.65% and 0.09%, respectively. For muscle and fat 0.539 mg/kg and 0.265 mg/kg, respectively were determined. The radioactivity concentration of the total body muscle corresponded to 2.91% and of fat to 0.57% of the total dose assuming a value of 30% and 12% of the body weight for these tissues.

D. Extraction Efficiency of Residues

Milk, muscle, kidney and liver pools were extracted with a mixture of acetonitrile/water (8:2; v/v). In case of milk an additional extraction step with acetone was performed. Fat was extracted with a mixture of acetonitrile/water (8:2,v/v) and n-heptane followed by solvent partition yielding an acetonitrile/water phase and an n-heptane phase. Residues in the n-heptane phase amounted to only 0.014 mg/kg (5.4%).

After purification and concentration steps, the resulting extracts represented 91.4% of the total radioactivity for milk (24 to 102 h), 95.2% for muscle, 94.1% for fat, 89.8% for kidney and 73.9% for liver. Post-extraction residues of kidney and liver were extracted exhaustively with mixtures of acetonitrile/water, 0.1 N HCl and 0.1 N NaOH using microwave assistance. The residues in the exhaustive extracts ranged from 0.039 mg/kg to 0.059 mg/kg for the kidney and from 0.053 mg/kg to 0.209 mg/kg for the liver. The exhaustive extracts were not investigated, due to their low amount of residues or their high matrix content. Negligible amounts of radioactivity were found in the distillates. Unextractable residues amounted to 0.0901 mg/kg (8.6%) for milk, 0.026 mg/kg (4.8%) for muscle



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and 0.016 mg/kg (5.9%) for fat. Solids of kidney and liver were completely solubilised after exhaustive extraction with sodium hydroxide

E. Quantification, Identification and Characterisation of Residues

Quantification of Parent Compound and Metabolites

Parent compound and metabolites were quantified in the extracts as well as in the urine (24 to 102 h) using a ternary reversed phase HPL system. The three eluents employed were:

A: 1L water + 1.54 mL ammonium hydroxide solution (25%) + 0.8 mL formic acid, adjusted to pH 7

B: acetonitrile / eluent A (99:1; v/v)

C: methanol / tetrahydrofuran (1:1; v/v)

Detailed information can be found in the report.

Metabolites in the extracts as well as in the urine (24 to 102 h) were assigned by comparison of the metabolite profiles and their retention times. A summary of the quantification of parent compound and metabolites in milk, muscle, fat, kidney and liver is presented in Table 6.2.3-6.

Isolation and Identification of Parent Compound and Metabolites in Urine

The identification of parent compound, BYI 02960-OH (hydroxylation in 5-position of the furanone ring) and BYI 02960-des-difluoroethyl was performed in the urine of the goat study with the pyridinylmethyl label (KIIA 6.2.3/01) by spectroscopic methods. Four isomers of the glucuronic acid conjugate of hydroxylated BYI 02960 were isolated and identified in the urine of the current study and the study with the pyridinylmethyl label. Spectra of the corresponding isomers of both studies were identical. The assignment of the four isomers to the metabolic profile of urine is presented in Figure 6.2.3-3. Identification of the four BYI 02960-OH-gluA isomers was achieved by comparison of the LC-MS/MS spectra and by comparison of the decomposition rates of the four single isomers with the increasing rates of their aglycones and a decomposition product based on the observations in the urine 72 to 96 h.

BYI 02960-OH-gluA (isomer 1) to BYI 02960-OH-gluA (isomer 4) were identified as glucuronic acid conjugates of hydroxylated BYI 02960 by LC-MS/MS. The LC-MS/MS fragments of BYI 02960-OH-gluA (isomer 1) and BYI 02960-OH-gluA (isomer 4) were different from each other and were different from BYI 02960-OH-gluA (isomer 2) and BYI 02960-OH-gluA (isomer 3). Most probably BYI 02960-OH-gluA (isomer 2) and BYI 02960-OH-gluA (isomer 3) are two diastereomers, due to their identical LC-MS/MS spectra. Therefore, it was concluded that BYI 02960 was hydroxylated and conjugated with glucuronic acid in three different positions of the molecule. The hydroxylation of the two diastereomers (isomer 2 and 3) was assigned to the 5-position of the furanone ring based on the similarity of the mass spectra with those of BYI 02960-OH-SA. The position of the hydroxylation of BYI 02960-OH-SA was clearly identified by NMR-spectroscopy in the laying hen study with the pyridinylmethyl label (KIIA 6.2.2/01). For isomer 4, the position of the hydroxylation was assigned to the difluoroethyl side chain based on the fragments 225 (ESI+) and 223 (ESI-) which prove the presence of the unchanged pyridinylmethyl and furanone moieties. For isomer 1, the position of the hydroxylation could not be derived from the mass spectra, therefore the position remains unknown. The assignment of the BYI 02960-OH-gluA isomers could be further confirmed based on the observed decomposition in two urine samples, mainly by comparison of the profiles of the urine samples collected between 72 and 96 h and between 96 and 102 h. Basically, the amounts of the BYI 02960-OH-gluA isomers as well as of the other metabolites were almost the same during the entire test period. This was observed for the urine samples of the goat study with the pyridinylmethyl label (KIIA

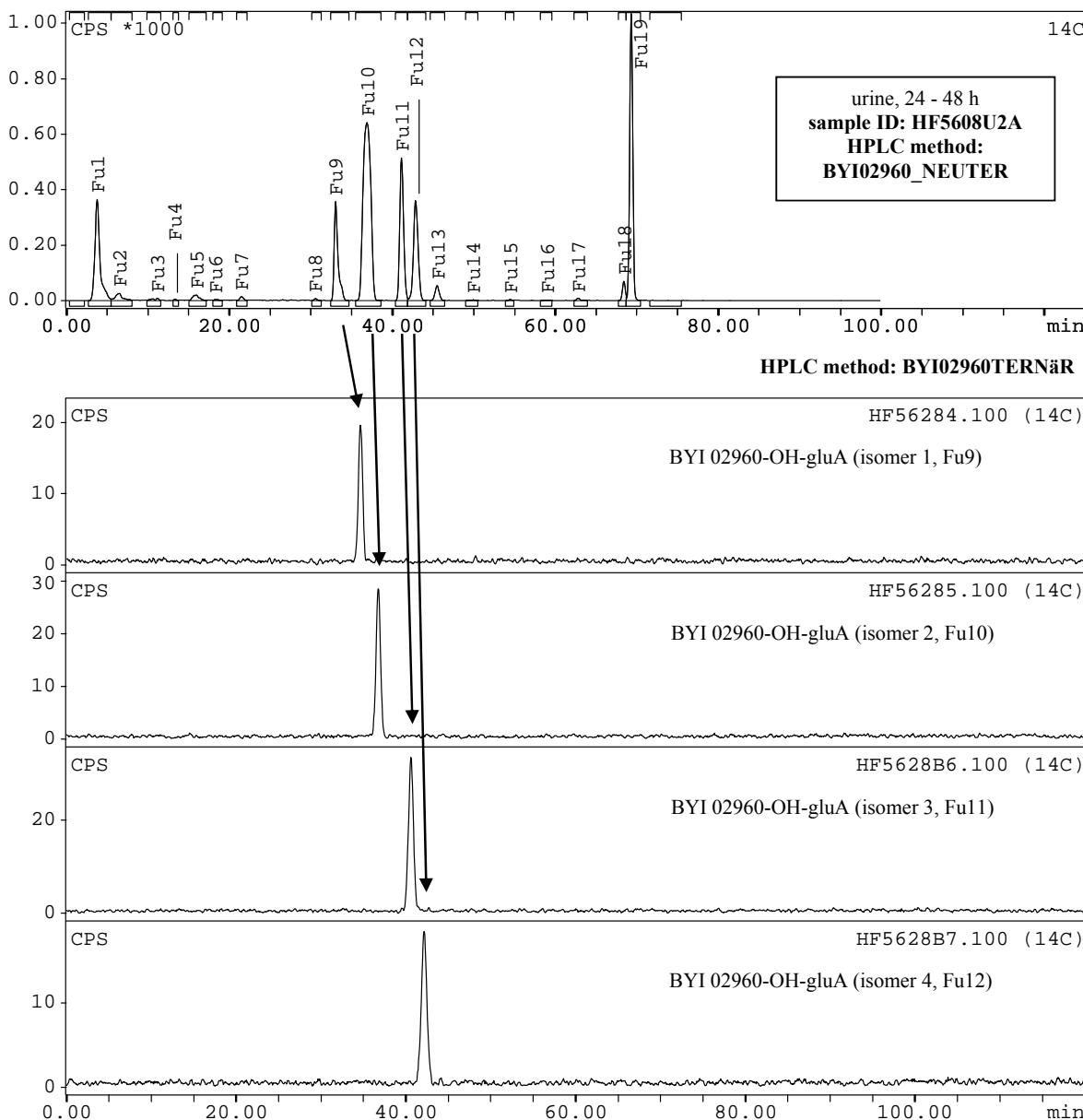


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6.2.3/01) and also for urine samples 0 to 24 h, 24h to 48 h and 96 to 102 h of the current study. By comparison of the decrease of the conjugates with the increase of other compounds, the aglycones of the isomers could be identified.

Figure 6.2.3-3: Assignment of isolated and identified glucoronic acid conjugates in urine of lactating goats following oral administration of 5 daily doses of [furanone-4-¹⁴C] BYI02960 at a dose rate of 1.0 mg/kg

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Identification, Assignment and Quantification of Metabolites in Milk, Organs and Tissues

Metabolites in the extracts of milk, organs and tissues of the current study were identified based on the comparison of the metabolite pattern in the extracts with the urines of the current study and the study

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with the pyridinylmethyl label. A comparison of the urine profiles from both labels is shown in Figure 6.2.3-4.

The distribution of the parent compound and metabolites in milk, organs and tissues is summarised in Table 6.2.3-6. Approximately 91% of the total radioactivity in milk, ca. 90% in muscle, ca. 83% in fat, ca. 79% in kidney and ca. 60% in liver were identified. All other residues were characterised by their extraction and chromatographic behaviour.

The unchanged parent compound was a major compound in milk (0.250 mg/kg, 23.9%). The main part of residues in milk was radioactive lactose and amounted to 0.698 mg/kg (66.8%). Parent compound was also the major constituent of the residue in organs and tissues and amounted to 0.475 mg/kg (88.1%) for muscle, 0.213 mg/kg (80.5%) for fat, 0.744 mg/kg (50.5%) for kidney and 1.045 mg/kg (59.8%) for liver.

Extensive metabolism was detected in the kidney, where the main portion of metabolites was found. The main metabolite in kidney was BYI 02960-OH (hydroxylation in position 5 of the furanone ring), which amounted to 0.215 mg/kg (14.6%). BYI 02960-OH was detected in lower amounts (≤ 0.010 mg/kg) in muscle and fat. Four prominent glucuronic acid conjugates of hydroxylated parent compound were detected in the kidney. The four conjugates were named BYI 02960-OH-gluA (isomer 1 to 4) and amounted between 0.032 mg/kg (2.2%) and 0.069 mg/kg (4.7%). The hydroxylation of BYI 02960 and conjugation with glucuronic acid resulted in two diastereomers (BYI 02960-OH-gluA, isomer 2 and 3) with hydroxylation and conjugation in the 5-position of the furanone ring, one isomer (BYI 02960-OH-gluA, isomer 4) with the hydroxylation and conjugation in the difluoroethyl side chain and one isomer (BYI 02960-OH-gluA, isomer 1) with the hydroxylation and conjugation in an unknown position of the molecule. Metabolites in the exhaustive extracts of kidney and liver were characterised by their extraction behaviour. They were not further investigated due to the high matrix content.

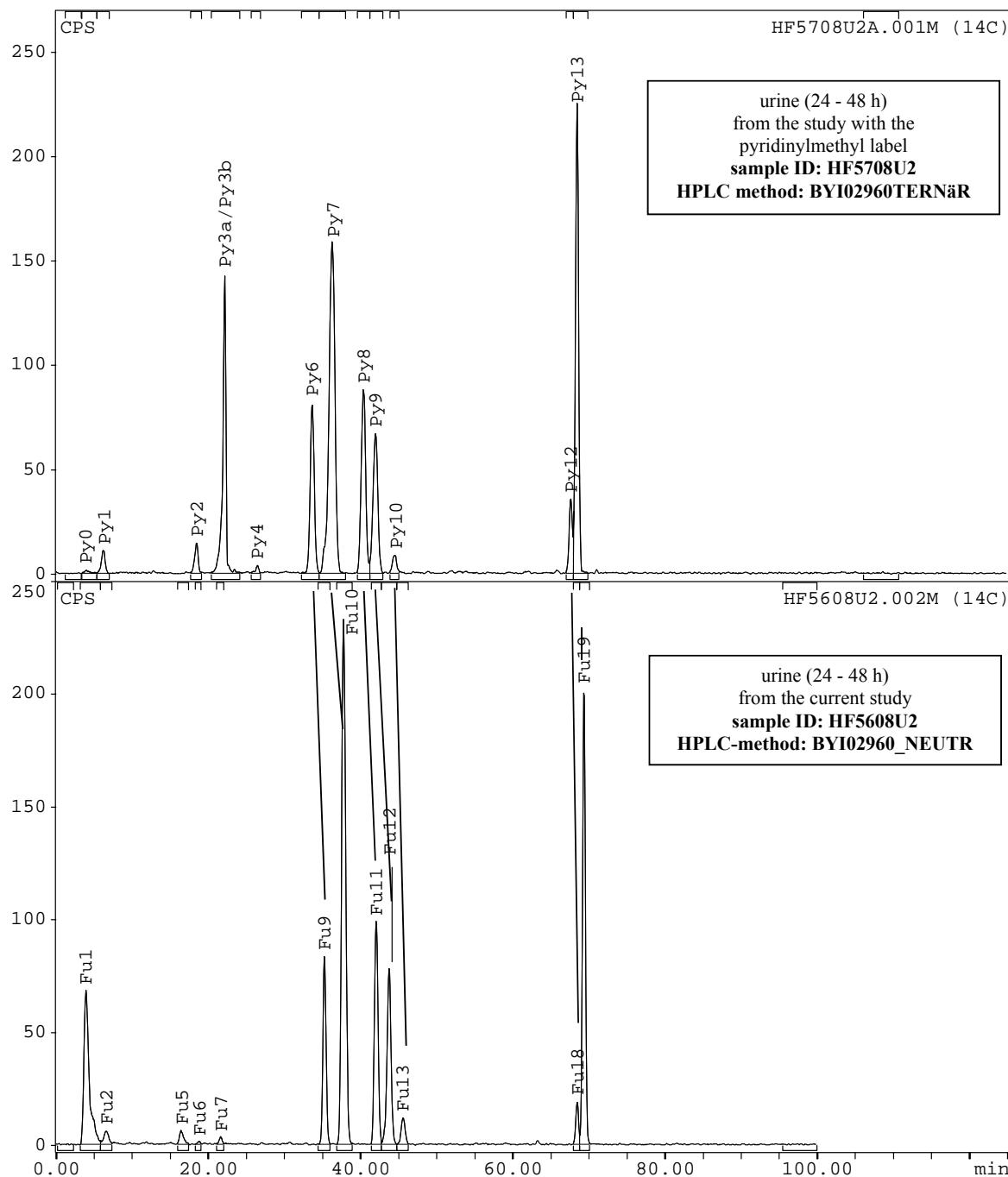
Table 6.2.3-6: Radioactive residues of parent compound and metabolites in milk, urine and edible organs of lactating goats following oral administration of 5 daily doses of [furanone-4- ^{14}C] BYI02960 at a dose rate of 1.0 mg/kg

	Milk (24 to 102 h)		Muscle		Fat		Kidney		Liver	
TRR [mg/kg]	1.046		0.539		0.265		1.472		1.746	
Sample/ Report name BYI 02960-	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
- ACN/water extract	90.7	0.948	95.0	0.512	88.4	0.234	89.1	1.311	73.3	1.280
Lactose	66.8	0.698	---	---	---	---	---	---	---	---
Polar metabolites	---	---	4.4	0.024	5.0	0.013	10.0	0.148	12.1	0.211
OH-gluA (isomer 1)	---	---	---	---	---	---	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
Total extracted	91.4	0.956	95.2	0.513	94.1	0.249	100.0	1.472	100.0	1.746

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Solids	8.6	0.090	4.8	0.026	5.9	0.016	n.d.	n.d.	n.d.	n.d.
Accountability	100.0	1.046	100.0	0.539	100.0	0.265	100.0	1.472	100.0	1.746

Figure 6.2.3-4: Comparison of the HPLC profiles of urine of lactating goats with the pyridinylmethyl ^{14}C - (top) and the furanone-4- ^{14}C -label (bottom)



Metabolites in the polar region (Fu1 in Figure 6.2.3-4) of the acetonitrile/water extract of kidney, liver and urine were further characterised by thin-layer chromatography. Their concentration ranged from 0.021 to 0.037 mg/kg for kidney and from 0.019 to 0.059 mg/kg for liver. These polar metabolites were specific for the furanone label and were formed by the degradation of the furanone ring. The

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metabolites showed the same retention in TLC as the metabolites, which were detected in the corresponding polar region of urine from the rat (KIIA 5.1.2/01) and muscle and liver from the hen (KIIA 6.2.2/02). The corresponding polar regions of muscle and fat were not investigated, due to their low amount of residues (≤ 0.013 mg/kg) and the high matrix content.

The residues in the corresponding polar region of milk were identified as radioactive lactose. The presence of the radioactive lactose in the milk was specific for the furanone label and was caused by the cleavage and subsequent degradation of the furanone ring to smaller carbon units (C-1- or C-2-fragments), entering the carbon pool of endogenous compounds. The identification was performed after isolation and acetylation by HPLC co-chromatography with acetylated radioactive lactose as reference compound. The acetylated radioactive lactose was synthesised from the radioactive lactose. The identity of the radioactive lactose and the acetylated radioactive lactose was confirmed by LC-MS/MS.

F. Storage Stability of Residues

During the study, all samples and extracts were stored in at ≤ -18 °C or for a short time in a refrigerator. All samples of milk, and edible organs and tissues were extracted within approx. seven weeks after sample collection. The first metabolite profile was recorded not later than two days after the start of the extraction and sample preparation. The first metabolic profiles were used for the quantification of metabolites, except for the profile of milk. The quantification of metabolites in the extract of milk was performed approx. four weeks later. The stability of the extract of milk was demonstrated by comparison of the first profile with the profile after storage of the extract.

For these reasons investigations on storage stability of the residues in the sample extracts were rendered unnecessary. It can be concluded that the metabolic profiles represent the residues in the matrices at sacrifice.

III. Conclusion

The metabolic and excretion behaviour of [furanone-4- ^{14}C]BYI 02960 in the lactating goat can be characterised by the following observations:

- The concentration of radioactivity in milk and edible tissues were relatively high compared to the dose level and the dosing period of five days. This was mainly caused by the partial instability of the radiolabel used. For a small portion of the total dose the furanone ring obviously underwent extensive biotransformation to C-1- and C-2-fragments that resulted in an accumulation of radioactivity in biomolecules during dosing for five days.
A part of these fragments was probably also converted to the terminal product $^{14}\text{CO}_2$ as can be derived from the lower overall recovery compared to the study with the metabolically stable pyridinylmethyl label.
- However, the fact should be considered that an exaggerated dose level of 28.82 mg/kg feed/day was administered. Furthermore, the significant amount of radioactivity detected in urine and faeces and the relatively high radioactivity in liver and kidney at sacrifice indicate that the residues are further metabolised and finally eliminated.
- The total radioactive residues in milk showed a diurnal pattern after the second administration as they declined significantly prior to the delivery of the next dose. A residue plateau level was reached after the third administration.
- The radioactive residues were efficiently extracted from milk as well as from edible organs and tissues; extraction rates were between 91.4% and 100.0%.



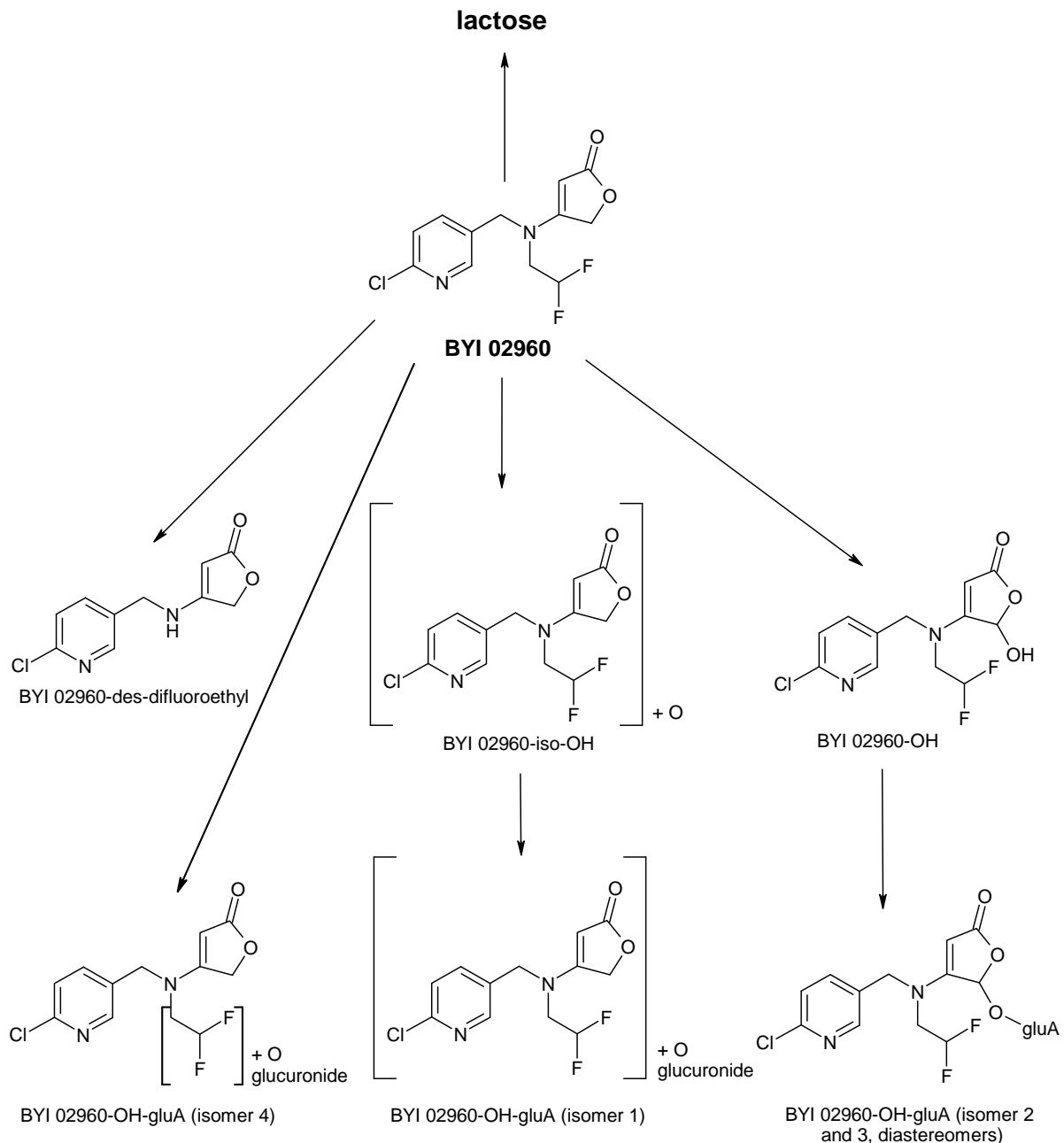
Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

- Parent compound was the main constituent of the residues in muscle (ca. 88%), fat (ca. 81%), kidney (ca. 51%) and liver (ca. 60%). Approximately 24% of the total radioactivity in the milk was identified as parent compound.
- The main residue in the milk was radioactive lactose (approx. 67%)
- Extensive metabolism was detected in the kidney. BYI 02960-OH, four glucuronic acid conjugates of hydroxylated BYI 02960 and BYI 02960-des-difluoroethyl were identified.
- The main metabolic reactions in the lactating goat are:
 - Cleavage and subsequent degradation of the furanone ring forming small carbon units (C-1- or C-2-fragments), entering the carbon pool of endogenous compounds and finally being used for example for the biosynthesis of lactose
 - Hydroxylation followed by conjugation with glucuronic acid forming two diastereomeric conjugates of BYI 02960-OH (BYI 02960-OH-gluA, isomer 2 and 3) with hydroxylation and conjugation in the 5-position of the furanone ring, one isomer (BYI 02960-OH-gluA, isomer 4) with the hydroxylation and conjugation in the difluoroethyl side chain and one isomer (BYI 02960-OH-gluA, isomer 1) with the hydroxylation and conjugation in an unknown position
 - Cleavage of the difluoroethyl group forming BYI 02960-des-difluoroethyl

Based on these results the metabolism of [furanone-4-¹⁴C] BYI02960 in the lactating goat can be described by the metabolic pathway shown in Figure 6.2.3-5.

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Figure 6.2.3-5: Proposed metabolic pathway of [furanone-4-14C] BYI02960 in the lactating goat





IIA 6.2.4 Pigs

The draft European data requirements (SANCO/11802/2010 Rev. 3) state that “metabolism studies on pigs shall be provided where the plant protection product is used in crops whose parts or products, also after processing, are fed to pigs and where it becomes apparent that metabolic pathways differ significantly in the rat as compared to ruminants.” The OECD Test Guideline 503 (Metabolism in Livestock) further specifies that these significant differences may occur in the extent of the metabolism or the nature of the observed residue or the appearance of metabolites with sub-structures, which are of known potential toxicological concern.

The dominating constituent of the residue in the milk and the edible organs and tissues of the goat is the unchanged parent compound. As the main metabolite, the hydroxylated derivative (BYI 02960-OH) was identified mainly in the kidney. Further metabolites in the kidney and partly also in the liver were determined as glucuronide conjugates of BYI 02960-OH and as BYI 02960 hippuric acid which is the glycine conjugate of 6-chloronicotinic acid. All these metabolites also do occur in the rat in significant amounts (KIIA 5.1). A few minor metabolites were found exclusively in the goat. These are BYI 02960 methylthio-glyoxylic acid which occurred in milk and muscle at concentrations below 0.005 mg/kg and BYI 02960 AMCP-difluoroethanamine which was found in kidney and liver also at low concentrations (< 0.020 mg/kg).

The only major goat metabolite which was not found in the rat is BYI 02960 cysteinyl-nicotinic acid. This was found in the kidney (0.114 mg/kg) and the liver (0.058 mg/kg). This compound is formed as a product of a typical detoxification reaction by conjugation of 6-chloronicotinic acid with cysteine under loss of the chlorine atom. The compound is very polar and easily excreted via urine which is supported by the fact that it was only found in kidney and liver but not in the peripheral compartments of muscle or fat. In this context it should also be noted that the lactating goat was dosed at an exaggerated dose level of ca. 24 mg/kg dry feed which is approximately 6 times higher as the 1X dose level in the cattle feeding study.

Taking all these results into consideration, it can be concluded that the ruminant and rat metabolism follow the same metabolic pathway and thus are neither different in extent nor in nature. Furthermore, no sub-structures of known potential toxicological concern became apparent. Thus a pig metabolism study was rendered unnecessary.

IIA 6.2.5 Nature of residue in fish

The draft European data requirements (SANCO/11802/2010 Rev. 3) state that “metabolism studies on freshwater fish shall be provided where the plant protection product is used in crops whose parts or products, also after processing, are fed to fish and where significant residues in feed may occur from the intended applications. Taking into account that, according to the experience gained so far, uptake, metabolism and residues in fish are more likely associated with fat-soluble residues, tests shall be provided for residues where the log Pow of the active substance or the residue of concern, that is to say all components of the residue definition, is greater than or equal to three.”

According to EPA Pesticide Assessment Guideline §165-4, a fish bioaccumulation study including investigation of metabolism will not normally required if a compound has a relatively low potential for



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accumulation in fish as indicated by an octanol/water partition coefficient less than approximately 1000.

BYI 02960 has a log Pow of 1.2 in the range of pH 4 - 9 which is clearly below this threshold. Furthermore, all results of ADME and livestock studies demonstrate that BYI 02960 and its metabolites are quickly excreted and not retained or accumulated in the body of animals. Therefore, a fish metabolism study is not required for BYI 02960.

IIA 6.2.6 Chemical identity

Not required by Regulation 1107/2009.



IIA 6.3 Residue trials (supervised field trials)

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

General remark:

In this summary section (KIIA 6.3), the name DFEAF will be used for the metabolite BYI 02960-difluoroethyl-amino-furanone, which is relevant to the tested residue definition:

Name	Metab. No.	Standard "dossier name"
DFEAF	M34	BYI 02960-difluoroethyl-amino-furanone

IIA 6.3.1 Residue Trials in the EU

IIA 6.3.1.1 Lettuce

BYI 02960 (common name: flupyradifurone) is to be registered in Europe for use in lettuce. European residue data in lettuce crops are therefore presented below to support the intended "safe use". Use pattern (GAP) information, including the European "agricultural use" as well as the "home & garden use" to be supported, is summarized in Table 6.3.1-1.

Table 6.3.1-1: Use patterns (GAPs) for the spray application of BYI 02960-containing formulations in/on lettuce in European fields (northern and southern residue regions) and greenhouses

Description	F/G	No. of appls.	Application rate per treatment (g a.s./ha)	Application rate per season (g a.s./ha)	Water volume (L/ha)	Interval (days)	PHI (days)
"agricultural" use*	F†	1	125	125	200-800	--	10
	G	2	125	250	200-800	10	3
"home & garden"**	F†	2	125	250	200-800	10	3

* agricultural use based on an SL 200 formulation

** "home & garden" uses with an SL 50 formulation (available to the general public via retail sale)

† uses in both the northern and southern residue regions (EU-N and EU-S)

In order to support the EU "safe use" of BYI 02960, sets of GLP trials were conducted in northern and southern European fields and in greenhouses in 2010 and 2011. In northern and in southern European field-grown lettuce, BYI 02960 was applied twice as an SL formulation (BYI 02960 SL 200, containing 200 g/L BYI 02960 a.s.), at 10-day intervals. For the envisaged agricultural use, samples were taken immediately prior to the second application, thus representing a 1-application, 10-day PHI use pattern. Further samples were taken subsequent to the 2nd application, with an envisaged PHI of 3 days, reflecting the intended use of a retail-sale formulation for private home and garden use.

**Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)**

In the greenhouse trials, BYI 02960 was applied twice as an SL formulation (BYI 02960 SL 200), at 10-day intervals, with an envisaged PHI of 3 days.

Residue levels of BYI 02960 and its metabolites DFA and DFEAF were analyzed individually and summed to yield the calculated "total residue of BYI 02960". The most critical residue levels were observed in the greenhouse trials, in which a highest total residue value (HR) of 6.0 mg/kg was determined. The STMR in these trials was also the highest for any set, at 2.2 mg/kg.

The number of trials conducted for each use described above (incl. information on geographical region and vegetation period) is summarized below in table 6.3.1-2.

Table 6.3.1-2: Overview of European residue trials conducted in lettuce per geographical "residue region" and vegetation period, including key results

Use description (cf. table 6.3.1-1)	Region	No. of trials			Residue levels (mg/kg)		Report No.	Dossier ref.: IIA 6.3.1/...
		Veget. period 2010	2011	Σ	HR	STMR		
<i>trials in EUROPE</i>								
"agricultural" use*	EU-N	5	4	18	0.83	0.23	10-2223, 11-2082	01, 02
	EU-S	5	4		0.83	0.32	10-2213, 11-2071	03, 04
	G	5	4	9	6.0	2.2	10-2212, 11-2070	05, 06
"home & garden"**	EU-N	5	4	18	3.0	0.71	10-2223, 11-2082	01, 02
	EU-S	5	4		3.2	1.2	10-2213, 11-2071	03, 04

EU-N = northern EU field, EU-S = southern EU field, G = greenhouse

* residue levels shown based on total residues in lettuce head samples taken at a PHI of 10 days (field uses) or 3 days (greenhouse)

** residue levels shown based on total residues in lettuce head samples taken at a PHI of 3 days.

Northern Europe (residue region)

Report:	KIIA 6.3.1.1/01, Noss, G.; Bauer, J. 2012
Title:	Determination of the residues of BYI 02960 in/on lettuce after spraying of BYI 02960 SL 200 in the field in the Netherlands, Belgium, France (North) and Germany
Report No. & Document No.:	10-2223, dated February 8, 2012 M-424742-01-1

Report:	KIIA 6.3.1.1/02, Uceda, L. 2012
Title:	Determination of the residues of BYI 02960 in/on lettuce after spray application of BYI 02960 SL 200 in the field in Germany, northern France and Belgium
Report No. & Document No.:	11-2082, dated February 23, 2012 M-425941-01-2

Guidelines (applies to both studies):	Directive 91/414/EEC, residues in or on treated products, food and feed
GLP (applies to both studies):	yes (certified laboratory); Deviations: none

I. Materials and Methods



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Nine field residue trials were conducted in the northern European residue region, as follows:

In 2010, 5 trials (Netherlands, Belgium, France, and Germany [2]) were conducted to support the use of BYI 02960 SL 200 in lettuce (Noss & Bauer, 2012, KIIA 6.3.1/01). The lettuce varieties used were either closed-head (3 trials) or leafy (2) varieties, as per the prevailing EU guidance at the time. Two applications were made at intervals of 10 days (9 in one trial) at a nominal rate of 0.625 L/ha, corresponding to 125 g/ha BYI 02960 a.s.; the water rate was 300-600 L/ha, reflecting local practice in the trial regions. All treatments were made at the scheduled rates.

Four further trials were carried out in 2011, in France, Belgium, and Germany (2), to complete the data package (Uceda, 2012, KIIA 6.3.1/02). All lettuce varieties used were leafy (open-head) varieties, in order to comply with the upcoming revision of the EU guidance for this crop. The basic application parameters were as in 2010; water rates ranged from 500-750 L/ha. Again, all treatments were made at the scheduled rates.

Samples of lettuce heads were taken immediately prior and subsequent to the final application, and at several intervals thereafter (up to 7 or 14 days after treatment in 2010 and 2011 trials, respectively). The envisaged PHI was 3 days.

The samples were analyzed for the parent compound and its metabolites DFA and DFEAF using methods 01304 (2010 trials; for method details, cf. KIIA 4.3/03) or 01212 (2011 trials; cf. KIIA 4.3/05). The respective LOQs for the 3 analytes were 0.01, 0.02, and 0.01 mg/kg (all in parent equivalents).

II. Findings

Concurrent recoveries of BYI 02960 and its metabolites DFA and DFEAF were obtained from samples of lettuce heads. This sample material is representative of all sample materials collected in these trials.

The recovery samples for parent and DFEAF were spiked at levels of 0.01 mg/kg and 0.10 mg/kg, as well as 0.50, 1.0, and 5.0 mg/kg (expressed in BYI 02960 equivalents). Mean recoveries were all within acceptable ranges (91-104%, RSDs of the larger validations sets [$n > 2$] 2.2-10.7%, $n = 2-15$).

Fortification levels for DFA were 0.02 mg/kg, 0.05 mg/kg, and 0.50 mg/kg, as well as 0.20, 1.0, and 5.0 mg/kg (expressed in BYI 02960 equivalents). Mean recoveries were all within acceptable ranges (90-98%, RSDs of the larger validations sets [$n > 2$] 4.3-10.2%, $n = 2-12$).

Details of recovery data are shown in table 6.3.1-4. All trial data are summarised below in table 6.3.1-3a & b and in greater detail in the Tier 1 summary forms. (Residues of parent BYI 02960 as well as its metabolites DFA and DFEAF are expressed in BYI 02960 equivalents. From these individual values, the "total residue of BYI 02960" was calculated as the sum of these three analytes, expressed in parent equivalents.)

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Relevant residues of BYI 02960 were determined in lettuce head samples taken 10 days subsequent to the first application (immediately prior to the 2nd treatment) as well as at various intervals after the final application. Analyses showed that total residue levels declined with time.

"Agricultural" use

Lettuce heads were taken 9-10 days after the first treatment (before the final treatment) in order to represent a 1-application use with a 10-day PHI, as is envisaged for general agricultural use in northern European fields. Total residue levels ranged from 0.07-0.83 mg/kg (n=9, median: 0.23 mg/kg).

"Home & garden" use

On day 0, immediately following the 2nd and final treatment, residue levels in lettuce heads were between 1.5 and 4.1 mg/kg (median 2.6 mg/kg). By day 3 — the PHI for home & garden use — the levels had declined to 0.14-3.0 mg/kg (n=9), with a median value of 0.71 mg/kg. Residues continued to decrease until day 14, the final sampling event, when levels ranged from 0.047-1.0 mg/kg (n=4, median 0.10 mg/kg).

III. Conclusions (lettuce, northern Europe)

In order to support the use in the EU of BYI 02960 in lettuce, 9 valid trials were conducted in the northern 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 9-10 days. All applications were at the required rates, and all trials were conducted according to GLP.

The envisaged "agricultural use" nominally calls for 1 spray at 125 g/ha and a PHI of 10 days. To evaluate this use, samples were taken just prior to the 2nd application, i.e. 10 days after the first treatment. For the "home & garden use", samples were taken immediately after the 2nd application and at several intervals thereafter, including the envisaged PHI of 3 days.

Samples were analyzed for the relevant residues of BYI 02960, comprising the parent compound and its metabolites DFA and DFEAF. The residues of all three analytes were summed to yield a calculated "total residue of BYI 02960". The results of the trials presented above demonstrate that:

- total residues of BYI 02960 dissipated rapidly in lettuce heads, from levels of 1.5-4.1 mg/kg on day 0 after the final treatment to 0.14-3.0 mg/kg on day 3 (PHI for the "home & garden" use). The respective median values were 2.6 and 0.71 mg/kg.
- ten days after a single application of BYI 02960 SL 200 – representing the envisaged "agricultural" use – total residue levels ranged from 0.11-0.83 mg/kg, with a median value of 0.23 mg/kg.

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

 Table 6.3.1-3a: Application scenario in residue trials conducted in/on **lettuce** after spraying with BYI 02960 SL 200 in the field (*northern EU residue region*)

Study No. (Trial No.) Country Location	Crop Variety	FL	No.	Application		GS	PHI (days)
				kg/ha (a.s.)	kg/hl (a.s.)		
10-2223 (10-2223-01) Netherlands 1681 ND Zwaagdijk-Oost EU-N 2010	lettuce Gisela, Butterhead variety	200 SL	2	0.125	0.0417	48	3
10-2223 (10-2223-02) Belgium 6210 Villers-Perwin EU-N 2010	lettuce Lucan, Butterhead variety	200 SL	2	0.125	0.0250	48	3
10-2223 (10-2223-03) France 95000 Cergy EU-N 2010	lettuce Abago, Butterhead variety	200 SL	2	0.125	0.0208	48	3
10-2223 (10-2223-04) Germany 40764 Langenfeld-Reusrath EU-N 2010	lettuce Cavernet Lollo rosso, loose leaf variety	200 SL	2	0.125	0.0417	48	3
10-2223 (10-2223-05) Germany 67125 Schauernheim EU-N 2010	lettuce Chloe Lollo rosso, loose leaf variety	200 SL	2	0.125	0.0313	48	3

FL = formulation

GS = growth stage (BBCH-code) at last treatment

EU-N = northern Europe

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Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

 Table 6.3.1-3a (cont.): Application scenario in residue trials conducted in/on **lettuce** after spraying with BYI 02960 SL 200 in field (*northern EU residue region*)

Study No. (Trial No.) Country Location Region Year	Crop Variety	FL	No.	Application		GS	PHI (days)
				kg/ha (a.s.)	kg/hl (a.s.)		
II-2082 (11-2082-01) Germany 40764 Langenfeld EU-N 2011	lettuce Aleppo Lollo bionda, loose leaf variety	200 SL	2	0.125	0.0208	48	3
II-2082 (11-2082-02) Germany 59457 Werl-Westönnen EU-N 2011	lettuce Kitara Lollo bionda, loose leaf variety	200 SL	2	0.125	0.0250	48	3
II-2082 (11-2082-03) France 37230 Fondettes EU-N 2011	lettuce Quenty Feuille de chene (oak leaf lettuce)	200 SL	2	0.125	0.0208	48	3
II-2082 (11-2082-04) Belgium 6210 Villers-Perwin EU-N 2011	lettuce Funnas, leafy variety curly	200 SL	2	0.125	0.0167- 0.0167	48	3

FL = formulation GS = growth stage (BBCH-code) at last treatment

EU-N = northern Europe

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.3.1-3b: Results of residue trials conducted in/on **lettuce** after spraying with BYI 02960 SL 200 in the field (*northern EU residue region*)

Study No. (Trial No.) Country GLP	Portion analyzed	DALT (days)	Residues (mg/kg) expressed as BYI 02960			total residue of BYI 02960 calc.
			BYI 02960	difluoroacetic acid	BYI 02960- difluoro- ethylamino- furanone	
<i>10-2223 (10-2223-01) Netherlands GLP: yes</i>	head	0*	0.20	<0.02	<0.01	0.23
		0	1.9	<0.02	<0.01	2.0
		1	1.8	<0.02	<0.01	1.8
		3	0.58	<0.02	<0.01	0.61
		5	0.34	<0.02	<0.01	0.37
		7	0.22	<0.02	<0.01	0.25
<i>10-2223 (10-2223-02) Belgium GLP: yes</i>	head	0*	0.08	<0.02	<0.01	0.11
		0	1.7	<0.02	<0.01	1.7
		1	0.43	<0.02	<0.01	0.46
		3	0.37	<0.02	<0.01	0.40
		5	0.34	<0.02	<0.01	0.37
		7	0.21	<0.02	<0.01	0.24
<i>10-2223 (10-2223-03) France GLP: yes</i>	head	0*	0.13	<0.02	<0.01	0.16
		0	1.5	<0.02	<0.01	1.5
		1	1.3	<0.02	<0.01	1.3
		3	0.68	<0.02	<0.01	0.71
		5	0.52	0.02	<0.01	0.55
		7	0.46	0.03	<0.01	0.50
<i>10-2223 (10-2223-04) Germany GLP: yes</i>	head	0*	0.37	<0.02	<0.01	0.40
		0	1.7	<0.02	<0.01	1.8
		1	1.1	<0.02	<0.01	1.1
		3	1.0	<0.02	0.01	1.0
		5	0.87	<0.02	<0.01	0.90
		7	0.66	<0.02	<0.01	0.69
<i>10-2223 (10-2223-05) Germany GLP: yes</i>	head	0*	0.80	<0.02	0.01	0.83
		0	4.1	<0.02	0.02	4.1
		1	1.0	<0.02	0.01	1.1
		3	0.83	<0.02	0.01	0.87
		5	0.83	<0.02	0.01	0.86
		7	0.65	<0.02	<0.01	0.68
<i>11-2082 (11-2082-01) Germany GLP: yes</i>	head	0*	0.19	<0.02	<0.01	0.22
		0	2.7	0.020	<0.01	2.7
		3	1.5	0.023	0.017	1.6
		7	0.52	0.028	<0.01	0.56
		10	0.13	0.020	<0.01	0.16
		14	0.073	0.024	<0.01	0.11
<i>11-2082 (11-2082-02) Germany GLP: yes</i>	head	0*	0.11	<0.02	<0.01	0.14
		0	2.6	<0.02	<0.01	2.6
		3	0.11	<0.02	<0.01	0.14
		7	0.033	0.022	<0.01	0.065
		10	0.023	0.027	<0.01	0.060
		14	0.011	0.027	<0.01	0.047

DALT = days after last treatment

* prior to last treatment

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Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.3.1-3b (cont'd): Results of residue trials conducted in/on **lettuce** after spraying with BYI 02960 SL 200 in the field (*northern EU residue region*)

Study No. (Trial No.) Country GLP:	Portion analyzed	DALT (days)	Residues (mg/kg) expressed as BYI 02960			
			BYI 02960	difluoroacetic acid	BYI 02960- difluoro- ethylamino- furanone	total residue of BYI 02960 calc.
11-2082 (11-2082-04) Belgium GLP: yes	head	-10	5.6	<0.02	0.011	5.6
		-8	2.0	<0.02	0.015	2.0
		-5	0.63	0.028	<0.01	0.67
		-2	0.35	0.030	<0.01	0.39
		0*	0.25	0.026	<0.01	0.28
		0	2.8	0.022	<0.01	2.8
		3	0.43	0.028	<0.01	0.47
		7	0.22	0.030	<0.01	0.26
		10	0.12	0.026	<0.01	0.15
		14	0.058	0.025	<0.01	0.093

DALT = days after last treatment

* prior to last treatment

Table 6.3.1-4: Recovery data for BYI 02960 in **lettuce**

Study No. Trial No. GLP Year	Crop	Portion analysed	a.s./ metabolite	n	Fortifi- cation level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
11-2082 11-2082-01 to 11-2082-04 GLP: yes 2011	lettuce	head	BYI 02960	2	0.01	106;111	106	111	109	5.4
				2	0.10	104;105	104	105	105	
				1	2.0	119	119	119	119	
				1	8.0	114	114	114	114	
				6	overall	104	119	110	110	
			difluoroacetic acid	2	0.02	89;109	89	109	99	6.9
				2	0.20	98;106	98	106	102	
				1	4.0	102	102	102	102	
				1	16	102	102	102	102	
				6	overall	89	109	101	101	
			BYI 02960- difluoroethyl- aminofuranone	2	0.01	114;123	114	123	119	5.4
				2	0.10	108;109	108	109	109	
				1	2.0	107	107	107	107	
				1	8.0	116	116	116	116	
				6	overall	107	123	113	113	

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Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.3.1-4 (cont'd): Recovery data for BYI 02960 in lettuce

Study No. Trial No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
10-2223 10-2223-01 to 10-2223-05 GLP: yes 2010	lettuce, head	head	BYI 02960	15	0.01	79;87;102; 106; 107;109;110; 116;92;97;107; 108;114;116; 117	79	117	104	10.7
				5	0.10	88;90;90;92;93	88	93	91	2.2
				2	0.50	103;106	103	106	105	
				2	1.0	92;94	92	94	93	
				2	5.0	90;98	90	98	94	
			difluoroacetic acid	26	overall	79	117	100	10.5	
				12	0.02	90;93;94;95;97; 112;112;116; 86;89;93;95	86	116	98	10.2
				3	0.05	90;94;98	90	98	94	4.3
				2	0.20	92;94	92	94	93	
				5	0.50	93;101;90;91; 92	90	101	93	4.7
			BYI 02960-difluoroethyl-aminofuranone	2	1.0	90;92	90	92	91	
				2	5.0	90;89	89	90	90	
				26	overall	86	116	95	7.9	

Southern Europe

Report:	KIIA 6.3.1.1/03, Schoening, R.; Bauer, J. 2012
Title:	Determination of the residues of BYI 02960 in/on lettuce, head after spray application of BYI 02960 SL 200 in the field in France (South), Spain and Italy - Amendment no. 0001 to report no. 10-2213
Report No. & Document No.:	10-2213, dated February 27, 2012 M-425913-02-1

Report:	KIIA 6.3.1.1/04, Uceda, L. 2012
Title:	Determination of the residues of BYI 02960 in/on lettuce after spray application of BYI 02960 SL 200 in the field in Spain, Italy, southern France and Portugal
Report No. & Document No.:	11-2071, dated February 23, 2012 M-425784-02-1

Guidelines (applies to both studies):	Directive 91/414/EEC, residues in or on treated products, food and feed
GLP (applies to both studies):	yes (certified laboratory); Deviations: none

I. Materials and Methods

Nine field residue trials were conducted in southern Europe, as follows:

In 2010, 5 trials (France, Italy [2], and Spain [2]) were conducted to support the use of BYI 02960 SL 200 in lettuce (Schoening & Bauer, 2012, KIIA 6.3.1/03). The lettuce varieties used were either closed-head (3 trials) or leafy (2) varieties, as per the prevailing EU guidance at the time. Two applications were made at intervals of 10 days (11 in one trial) at a nominal rate of 0.625 L/ha, corresponding to 125 g/ha BYI 02960 a.s.; the water rate was 500-700 L/ha, reflecting local practice in the trial regions. All treatments were made at the scheduled rates.

Four further trials were carried out in 2011, in France, Spain, Portugal, and Italy, to complete the data package (Uceda, 2012, KIIA 6.3.1/04). All lettuce varieties used were leafy (open-head) varieties, in order to comply with the upcoming revision of the EU guidance for this crop. The basic application parameters were as in 2010 (interval in one trial: 9 day); water rates ranged from 500-800 L/ha. Again, all treatments were made at the scheduled rates.

Samples of lettuce heads were taken immediately prior and subsequent to the final application, and at several intervals thereafter (up to 7 or 14 days after treatment in 2010 and 2011 trials, respectively). The envisaged PHI was 3 days.

The samples were analyzed for the parent compound and its metabolites DFA and DFEAF using methods 01304 (2010 trials; for method details, cf. KIIA 4.3/03) or 01212 (2011 trials; cf. KIIA 4.3/05). The respective LOQs for the 3 analytes were 0.01, 0.02, and 0.01 mg/kg (all in parent equivalents).



II. Findings

During the conduct of the complete set of lettuce studies in 2010-2011, concurrent recoveries of BYI 02960 and its metabolites DFA and DFEAF were obtained from samples of lettuce heads. This sample material is representative of all sample materials collected in these trials.

The recovery samples for parent and DFEAF were spiked at levels of 0.01 mg/kg and 0.10 mg/kg, as well as 0.50, 1.0, and 5.0 mg/kg (expressed in BYI 02960 equivalents). Mean recoveries were all within acceptable ranges (91-104%, RSDs of the larger validations sets [n > 2] 2.2-10.7%, n=2-15).

Fortification levels for DFA were or 0.02 mg/kg, 0.05 mg/kg, and 0.50 mg/kg, as well as 0.20, 1.0, and 5.0 mg/kg (expressed in BYI 02960 equivalents). Mean recoveries were all within acceptable ranges (90-98%, RSDs of the larger validations sets [n > 2] 4.3-10.2%, n=2-12).

Details of recovery data are shown in table 6.3.1-6. All trial data are summarised below in table 6.3.1-5a & b and in greater detail in the Tier 1 summary forms. (Residues of parent BYI 02960 as well as its metabolites DFA and DFEAF are expressed in BYI 02960 equivalents. From these individual values, the "total residue of BYI 02960" was calculated as the sum of these three analytes, expressed in parent equivalents.)

Relevant residues of BYI 02960 were determined in lettuce head samples taken 10 days subsequent to the first application (immediately prior to the 2nd treatment) as well as at various intervals after the final application. Analyses showed that total residue levels declined with time.

"Agricultural" use

Lettuce heads were taken 9-11 days after the first treatment (before the final treatment) in order to represent a 1-application use with a 10-day PHI, as is envisaged for general agricultural use in southern European fields. Total residue levels ranged from 0.07-0.83 mg/kg (n=9, median: 0.32 mg/kg).

"Home & garden" use

On day 0, immediately following the 2nd and final treatment, residue levels in lettuce heads were between 1.9 and 7.4 mg/kg (median 2.9 mg/kg). By day 3 — the PHI for home & garden use — the levels had declined to 0.39-3.2 mg/kg (n=9), with a median value of 1.2 mg/kg. Residues continued to decrease until day 14, the final sampling event, when levels ranged from 0.094-0.30 mg/kg (n=4, median 0.17 mg/kg).

III. Conclusions (lettuce, southern Europe)

In order to support the use in the EU of BYI 02960 in lettuce, 9 valid trials were conducted in southern Europe 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. All applications were at the required rates, and all trials were conducted according to GLP.

The envisaged "agricultural use" nominally calls for 1 spray at 125 g/ha and a PHI of 10 days. To evaluate this use, samples were taken just prior to the 2nd application, i.e. 10 days after the first treatment. For the "home & garden use", samples were taken immediately after the 2nd application and at several intervals thereafter, including the envisaged PHI of 3 days.

Samples were analyzed for the relevant residues of BYI 02960, comprising the parent compound and its metabolites DFA and DFEAF. The residues of all three analytes were summed to yield a calculated "total residue of BYI 02960". The results of the trials presented above demonstrate that:

- total residues of BYI 02960 dissipated rapidly in lettuce heads, from levels of 1.9-7.4 mg/kg on day 0 after the final treatment to 0.39-3.2 mg/kg on day 3 (PHI for the "home & garden" use). The respective median values were 2.9 and 1.2 mg/kg.
- ten days after a single application of BYI 02960 SL 200 – representing the envisaged "agricultural" use – total residue levels ranged from 0.07-0.83 mg/kg, with a median value of 0.32 mg/kg.

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.3.1-5a: Application scenario in residue trials conducted in/on **lettuce** after spraying with BYI 02960 SL 200 in the field (*southern EU residue region*)

Study No. (Trial No.) Country Location Region Year	Crop Variety	FL	No.	Application		GS	PHI (days)
				kg/ha (a.s.)	kg/hl (a.s.)		
10-2213 (10-2213-01) France 86380 Marigny brizay EU-S 2010	lettuce Madita Head	200 SL	2	0.125	0.0250	48	3
10-2213 (10-2213-02) Spain 46440 Almussafes EU-S 2010	lettuce Dauair Trocadero	200 SL	2	0.125	0.0208	49	3
10-2213 (10-2213-03) Italy 45020 Lusia Rovigo EU-S 2010	lettuce Ballerina butterhead	200 SL	2	0.125	0.0208	46	3
10-2213 (10-2213-04) Spain 08850 Gava EU-S 2010	lettuce Murai Lollo Rosso, loose leaf variety	200 SL	2	0.125	0.0208	49	4
10-2213 (10-2213-05) Spain 70031 Andria EU-S 2010	lettuce Bergamo Blond lollo, loose leaf variety	200 SL	2	0.125	0.0179	49	3
11-2071 (11-2071-01) Spain 46230 Alginet EU-S 2010	lettuce Livigna RZ, loose leaf variety	200 SL	2	0.125	0.0250	49	3

FL = formulation

GS = growth stage (BBCH-code) at last treatment

EU-S = southern Europe

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Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

 Table 6.3.1-5a (cont.): Application scenario in residue trials conducted in/on **lettuce** after spraying with BYI 02960 SL 200 in field (*southern EU residue region*)

Study No. (Trial No.) Country Location Region Year	Crop Variety	FL	No.	Application		GS	PHI (days)
				kg/ha (a.s.)	kg/hl (a.s.)		
II-2071 (11-2071-02) Italy 95100 Catania EU-S 2010	lettuce Lollo Rosso, loose leaf variety	200 SL	2	0.125	0.0179	46	3
II-2071 (11-2071-03) France 31200 Toulouse - Croix daurade EU-S 2010	lettuce Pitice, loose leaf variety	200 SL	2	0.125	0.0156	48	3
II-2071 (11-2071-04) Portugal 2715-252 Almargem do Bispo EU-S 2010	lettuce Caypira, loose leaf variety	200 SL	2	0.125	0.0156	49	3

FL = formulation

GS = growth stage (BBCH-code) at last treatment

EU-S = southern Europe

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.3.1-5b: Results of residue trials conducted in/on **lettuce** after spraying with BYI 02960 SL 200 in the field (*southern EU residue region*)

Study No. (Trial No.) Country GLP	Portion analyzed	DALT (days)	Residues (mg/kg) expressed as BYI 02960			
			BYI 02960	difluoroacetic acid	BYI 02960- difluoro- ethylamino- furanone	total residue of BYI 02960 calc.
<i>10-2213 (10-2213-01) France</i>	head	0*	0.04	<0.02	<0.01	0.07
		0	2.6	<0.02	<0.01	2.6
		1	0.57	<0.02	<0.01	0.60
		3	0.40	0.02	<0.01	0.43
		5	0.29	0.02	<0.01	0.32
		7	0.15	0.02	<0.01	0.19
<i>10-2213 (10-2213-02) Spain</i>	head	0*	0.80	0.02	<0.01	0.83
		0	3.8	0.02	0.02	3.8
		1	3.5	0.03	0.02	3.5
		3	2.7	0.03	0.02	2.7
		4	2.3	0.03	0.02	2.4
		7	1.3	0.03	0.01	1.4
<i>10-2213 (10-2213-03) Italy</i>	head	0*	0.05	0.03	<0.01	0.09
		0	2.7	0.04	0.01	2.7
		1	2.2	0.04	0.02	2.2
		3	0.48	0.05	<0.01	0.53
		5	0.21	0.05	<0.01	0.27
		7	0.09	0.05	<0.01	0.15
<i>10-2213 (10-2213-04) Spain</i>	head	0*	0.38	0.03	<0.01	0.41
		0	3.6	0.03	0.02	3.7
		1	3.0	0.04	0.02	3.1
		4	2.1	0.05	0.02	2.2
		5	2.0	0.05	0.02	2.1
		7	1.3	0.05	0.02	1.3
<i>10-2213 (10-2213-05) Spain</i>	head	0*	0.04	<0.02	<0.01	0.07
		0	2.9	<0.02	<0.01	2.9
		1	1.9	<0.02	0.01	2.0
		3	1.1	<0.02	0.02	1.2
		5	0.21	<0.02	<0.01	0.24
		7	0.17	0.02	<0.01	0.20
<i>11-2071 (11-2071-01) Spain</i>	head	0*	0.43	0.046	0.021	0.49
		0	2.6	0.062	0.031	2.7
		3	1.5	0.077	0.046	1.6
		7	0.77	0.097	0.034	0.90
		10	0.57	0.11	0.024	0.71
		14	0.033	0.15	<0.01	0.19
<i>11-2071 (11-2071-02) Italy</i>	head	0*	0.55	0.051	0.011	0.61
		0	7.3	0.039	0.023	7.4
		3	3.1	0.083/0.032**	0.045	3.2/0.052**
		7	1.4	0.13	0.025	1.5
		10	0.24	0.11	<0.01	0.37
		14	0.17	0.12	<0.01	0.30
<i>11-2071 (11-2071-03) France</i>	head	-9	5.3	<0.02	0.015	5.4
		-7	3.4	<0.02	0.024	3.5
		-4	0.69	<0.02	0.014	0.72
		-1	0.39	<0.02	<0.01	0.42
		0*	0.29	<0.02	<0.01	0.32
		0	3.5	0.020	0.022	3.6
		3	0.72	0.035	0.020	0.78
		7	0.25	0.032	0.011	0.29
		10	0.20	0.047	0.012	0.26
		14	0.094	0.046	<0.01	0.15

DALT = days after last treatment

* prior to last treatment

**residues in control

Continued on next page...

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.3.1-5b (cont'd): Results of residue trials conducted in/on **lettuce** after spraying with BYI 02960 SL 200 in the field (*southern EU residue region*)

Study No. (Trial No.) Country GLP	Portion analyzed	DALT (days)	Residues (mg/kg) expressed as BYI 02960			total residue of BYI 02960 calc.
			BYI 02960	difluoroacetic acid	BYI 02960- difluoro- ethylamino- furanone	
BYI 02960 SL 200						
11-2071 (11-2071-04) Portugal GLP: yes	head	-10	<0.01	<0.02	<0.01	<0.04
		-8	1.8	0.022	0.021	1.8
		-5	0.92	0.031	0.015	0.97
		-2	0.27	0.027	<0.01	0.31
		0*	0.11	0.029	<0.01	0.15
		0	1.9	0.026	<0.01	1.9
		3	0.35	0.030	<0.01	0.39
		7	0.16	0.031	<0.01	0.20
		10	0.067	0.034	<0.01	0.11
		14	0.046	0.038	<0.01	0.094

DALT = days after last treatment

* prior to last treatment

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.3.1-6: Recovery data for BYI 02960 in lettuce

Study No. Trial No. GLP Year	Crop	Portion analysed	a.s./ metabolite	n	Fortifi- cation level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
10-2213 10-2213-01 to 10-2213-05 GLP: yes 2010	Lettuce, head	BYI 02960		15	0.01	79;87;102;106; 107;109;110;116; 92;97;107;108; 114;116;117	79	117	104	10.7
				5	0.10	88;90;90;92;93	88	93	91	2.2
				2	0.50	103;106	103	106	105	
				2	1.0	92;94	92	94	93	
				2	5.0	90;98	90	98	94	
				26	overall	79	117	100	10.5	
		difluoroacetic acid		12	0.02	90;93;94;95;97; 112;112;116;86; 89;93;95	86	116	98	10.2
				3	0.05	90;94;98	90	98	94	4.3
				2	0.20	92;94	92	94	93	
				5	0.50	93;101;90;91;92	90	101	93	4.7
				2	1.0	90;92	90	92	91	
				2	5.0	90;89	89	90	90	
		BYI 02960- difluoroethyl-a minofuranone		15	0.01	87; 93; 95; 100; 100; 104; 105; 107; 83; 83; 86; 88; 90; 92; 96	83	107	94	8.4
				5	0.10	85; 98; 97; 97; 99	85	99	95	6.1
				2	0.50	97; 109	97	109	103	
				2	1.0	86; 101	86	101	94	
				2	5.0	97; 96	96	97	97	
				26	overall	83	109	95	7.7	
11-2071 11-2071-01 to 11-2072-04 GLP: yes 2011	Lettuce	BYI 02960		1	0.01	88	88	88	88	
				3	0.10	98;111;95	95	111	101	8.4
				1	10	104	104	104	104	
				5	overall	88	111	99	8.8	
		difluoroacetic acid		1	0.02	87	87	87	87	
				3	0.20	113;95;95	95	113	101	10.3
				4	overall	87	113	98	11.3	
				1	0.01	105	105	105	105	
		BYI 02960- difluoroethyl- aminofuranone		3	0.10	98;105;99	98	105	101	3.8
				4	overall	98	105	102	3.7	

Greenhouse

Report:	KIIA 6.3.1.1/05, Schulte, G. 2012
Title:	Determination of the residues of BYI 02960 in/on lettuce after spraying of BYI 02960 SL 200 in the greenhouse in France (North), Germany, the Netherlands and Italy
Report No. & Document No.:	10-2212, dated February 22, 2012 M-425829-01-1

Report:	KIIA 6.3.1.1/06, Uceda, L. 2012
Title:	Determination of the residues of BYI 02960 in/on lettuce after spray application of BYI 02960 SL 200 in the greenhouse in northern France, Italy, Spain and Germany
Report No. & Document No.:	11-2070, dated February 23, 2012 M-425786-01-1

Guidelines (applies to both studies):	Directive 91/414/EEC, residues in or on treated products, food and feed
GLP (applies to both studies):	yes (certified laboratory); Deviations: none

I. Materials and Methods

Nine residue trials were conducted in European greenhouses, as follows:

In 2010, 5 trials (France, Germany [2], the Netherlands, and Italy) were conducted to support the use of BYI 02960 SL 200 in lettuce (Schulte, 2012, KIIA 6.3.1/05). The lettuce varieties used were either closed-head (3 trials) or leafy (2) varieties, as per the prevailing EU guidance at the time. Two applications were made at intervals of 10 days (11 in one trial) at a nominal rate of 0.625 L/ha, corresponding to 125 g/ha BYI 02960 a.s.; the water rate was 400-600 L/ha, reflecting local practice in the trial regions. All treatments were made at the scheduled rates.

Four further trials were carried out in 2011, in France, Spain, Germany, and Italy, to complete the data package (Uceda, 2012, KIIA 6.3.1/06). In 3 of the 4 trials, the lettuce varieties used were leafy (open-head) varieties. The basic application parameters were as in 2010 (interval in one trial: 9 day); water rates ranged from 400-600 L/ha. Again, all treatments were made at the scheduled rates.

Samples of lettuce heads were taken immediately prior and subsequent to the final application, and at several intervals thereafter (up to 7 or 14 days after treatment in 2010 and 2011 trials, respectively). The envisaged PHI was 3 days.

The samples were analyzed for the parent compound and its metabolites DFA and DFEAF using methods 01304 (2010 trials; for method details, cf. KIIA 4.3/03) or 01212 (2011 trials; cf. KIIA 4.3/05). The respective LOQs for the 3 analytes were 0.01, 0.02, and 0.01 mg/kg (all in parent equivalents).



II. Findings

During the conduct of the complete set of lettuce studies in 2010-2011, concurrent recoveries of BYI 02960 and its metabolites DFA and DFEAF were obtained from samples of lettuce heads. This sample material is representative of all sample materials collected in these trials.

The recovery samples for parent and DFEAF were spiked at levels of 0.01 mg/kg and 0.10 mg/kg, as well as 0.50, 1.0, and 5.0 mg/kg (expressed in BYI 02960 equivalents). Mean recoveries were all within acceptable ranges (91-104%, RSDs of the larger validations sets [n > 2] 2.2-10.7%, n=2-15).

Fortification levels for DFA were or 0.02 mg/kg, 0.05 mg/kg, and 0.50 mg/kg, as well as 0.20, 1.0, and 5.0 mg/kg (expressed in BYI 02960 equivalents). Mean recoveries were all within acceptable ranges (90-98%, RSDs of the larger validations sets [n > 2] 4.3-10.2%, n=2-12).

Details of recovery data are shown in table 6.3.1-8. All trial data are summarised below in table 6.3.1-7a &b and in greater detail in the Tier 1 summary forms. (Residues of parent BYI 02960 as well as its metabolites DFA and DFEAF are expressed in BYI 02960 equivalents. From these individual values, the "total residue of BYI 02960" was calculated as the sum of these three analytes, expressed in parent equivalents.)

Relevant residues of BYI 02960 were determined in lettuce head samples taken 10 days subsequent to the first application (immediately prior to the 2nd treatment) as well as at various intervals after the final application. Analyses showed that total residue levels declined with time.

On day 0, immediately following the final treatment, residue levels in lettuce heads were between 1.5 and 7.7 mg/kg (median 3.9 mg/kg). By day 3 — the envisaged PHI — the levels had declined to 0.80-6.0 mg/kg (n=9), with a median value of 2.2 mg/kg. Residues continued to decrease until day 14, the final sampling event, when levels ranged from 0.21-2.7 mg/kg (n=4, median 0.28 mg/kg).

III. Conclusions (lettuce, greenhouse)

In order to support the use in the EU of BYI 02960 in lettuce, 9 valid trials were conducted in European greenhouses 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. All applications were at the required rates, and all trials were conducted according to GLP.

The greenhouse use calls for 2 sprays at 125 g/ha and a PHI of 3 days. To evaluate this use, samples were taken at several intervals after the final application, including the envisaged PHI of 3 days.

Samples were analyzed for the relevant residues of BYI 02960, comprising the parent compound and its metabolites DFA and DFEAF. The residues of all three analytes were summed to yield a calculated "total residue of BYI 02960". The results of the trials presented above demonstrate that:

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

- total residues of BYI 02960 dissipated rapidly in lettuce heads, from levels of 1.5-7.7 mg/kg on day 0 after the final treatment to 0.80-6.0 mg/kg on day 3 (envisaged PHI). The respective median values were 3.9 and 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.

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 Table 6.3.1-7a: Application scenario in residue trials conducted in/on **lettuce** after spraying with BYI 02960 SL 200 in European greenhouses

Study No. (Trial No.) Country Location Region Year	Crop Variety	FL	No.	Application		GS	PHI (days)
				kg/ha (a.s.)	kg/hl (a.s.)		
10-2212 (10-2212-01) France 95000 Cergy EU-N 2010	lettuce Kitonia, leafy variety	200 SL	2	0.125	0.0208	49	3
10-2212 (10-2212-02) Germany 42799 Leichlingen EU-N 2010	lettuce Antoni, leafy variety	200 SL	2	0.125	0.0208	48	3
10-2212 (10-2212-03) Germany 53332 Bornheim EU-N 2010	lettuce Torpedo, butterhead variety	200 SL	2	0.125	0.0313	48	3
10-2212 (10-2212-04) Netherlands 2988 DA Ridderkerk EU-N 2010	lettuce Gardia, butterhead variety	200 SL	2	0.125	0.0208	45	3
10-2212 (10-2212-05) Italy 97019 Vittoria EU-S 2010	lettuce Cappuc- cina, butterhead variety	200 SL	2	0.125	0.0250	45	3
11-2070 (11-2070-01) France 95000 Cergy EU-N 2011	lettuce Quenty, open leaf variety	200 SL	2	0.125	0.0208	48	3

FL = formulation

GS = growth stage (BBCH-code) at last treatment

EU-N = northern Europe

EU-S = southern Europe

Continued on next page...

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.3.1-7a (cont.): Application scenario in residue trials conducted in/on **lettuce** after spraying with BYI 02960 SL 200 in European greenhouses

Study No. (Trial No.) Country Location Region Year	Crop Variety	FL	No.	Application		GS	PHI (days)
				kg/ha (a.s.)	kg/hl (a.s.)		
<i>II-2070</i> (11-2070-02) Italy 76011 Bisceglie EU-S 2011	lettuce Expedition RZ, Green incised-leaf variety	200 SL	2	0.125	0.0208	47	3
<i>II-2070</i> (11-2070-03) Spain 08415 Bigues i Riells EU-N 2011	lettuce Oak Leaf, leaf variety	200 SL	2	0.125	0.0250-0.0313	47	3
<i>II-2070</i> (11-2070-04) Germany 69121 HD-Handschuhsheim EU-N 2011	lettuce Judita, head lettuce	200 SL	2	0.125	0.0208	49	3

FL = formulation

GS = growth stage (BBCH-code) at last treatment

EU-N = northern Europe

EU-S = southern Europe



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.3.1-7b: Results of residue trials conducted in/on lettuce after spraying with BYI 02960 SL 200 in European greenhouses

Study No. (Trial No.) Country GLP	Portion analyzed	DALT (days)	BYI 02960	Residues (mg/kg) expressed as BYI 02960		
				difluoroacetic acid	BYI 02960- difluoro- ethylamino- furanone	total residue of BYI 02960 calc.
10-2212 (10-2212-01) France GLP: yes	head	0*	0.23	<0.02	<0.01	0.26
		0	1.5	<0.02	<0.01	1.5
		1	1.3	<0.02	<0.01	1.4
		3	1.4	<0.02	<0.01	1.4
		5	1.2	0.02	<0.01	1.3
		7	1.4	0.02	<0.01	1.4
		0*	2.7	<0.02	0.01	2.7
10-2212 (10-2212-02) Germany GLP: yes	head	0	6.7	<0.02	0.02	6.7
		1	2.3	<0.02	<0.01	2.4
		3	2.0	<0.02	0.01	2.0
		5	1.5	<0.02	<0.01	1.5
		7	1.3	<0.02	<0.01	1.4
		0*	0.13	<0.02	<0.01	0.16
		0	5.2	<0.02	0.01	5.3
10-2212 (10-2212-03) Germany GLP: yes	head	1	4.6	<0.02	0.01	4.7
		3	3.5	<0.02	0.01	3.5
		5	2.4	0.02	0.01	2.4
		7	1.7	0.02	0.01	1.8
		0*	0.29	0.02	0.01	0.32
		0	3.1	0.02	0.02	3.1
		1	2.5	0.02	0.02	2.5
10-2212 (10-2212-04) Netherlands GLP: yes	head	3	2.5	0.03	0.02	2.5
		5	2.0	0.03	0.03	2.0
		7	0.99	0.04	0.02	1.0
		0*	0.65	<0.02	0.01	0.68
		0	3.8	<0.02	0.02	3.9
		1	2.7	<0.02	0.02	2.7
		3	1.8	0.02	0.02	1.8
10-2212 (10-2212-05) Italy GLP: yes	head	5	1.3	0.02	0.01	1.3
		7	0.74	0.03	0.01	0.78
		0	3.0	<0.02	<0.01	3.0
		3	2.1	0.022	0.011	2.2
		7	0.58	0.026	<0.01	0.62
		10	0.39	0.028	<0.01	0.43
		14	0.18	0.026	<0.01	0.21
11-2070 (11-2070-01) France GLP: yes	head	0	4.1	0.040	<0.01	4.1
		3	0.73	0.059	<0.01	0.80
		7	0.43	0.073	<0.01	0.52
		10	0.32	0.098	<0.01	0.42
		14	0.13	0.13	<0.01	0.27
		0	7.6	0.029	0.024	7.7
		3	6.0	0.035	0.027	6.0
11-2070 (11-2070-03) Spain GLP: yes	head	7	4.6	0.055	0.037	4.7
		11	2.9	0.061	0.038	3.0
		13	2.6	0.069	0.049	2.7

DALT = days after last treatment

* prior to last treatment

Continued on next page...

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Table 6.3.1-7b (cont'd): Results of residue trials conducted in/on **lettuce** after spraying with BYI 02960 SL 200 in European greenhouses

Study No. (Trial No.) Country GLP	Portion analyzed	DALT (days)	BYI 02960	Residues (mg/kg) expressed as BYI 02960		
				difluoroacetic acid	BYI 02960- difluoro- ethylamino- furanone	total residue of BYI 02960 calc.
11-2070 (11-2070-04) Germany GLP: yes	head	0	3.7	0.027	0.015	3.8
		3	2.6	0.036	0.014	2.7
		7	0.87	0.043	0.010	0.93
		10	0.46	0.053	<0.01	0.52
		14	0.23	0.041	<0.01	0.28

DALT = days after last treatment

* prior to last treatment

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Table 6.3.1-8: Recovery data for BYI 02960 in lettuce

Study No. Trial No. GLP Year	Crop	Portion analysed	a.s./ metabolite	n	Fortifi- cation level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
10-2213 10-2213-01 to 10-2213-05 GLP: yes 2010	Lettuce, head	BYI 02960		15	0.01	79;87;102;106; 107;109;110;116; 92;97;107;108; 114;116;117	79	117	104	10.7
				5	0.10	88;90;90;92;93	88	93	91	2.2
				2	0.50	103;106	103	106	105	
				2	1.0	92;94	92	94	93	
				2	5.0	90;98	90	98	94	
				26	overall	79	117	100	10.5	
		difluoroacetic acid		12	0.02	90;93;94;95;97; 112;112;116;86; 89;93;95	86	116	98	10.2
				3	0.05	90;94;98	90	98	94	4.3
				2	0.20	92;94	92	94	93	
				5	0.50	93;101;90;91;92	90	101	93	4.7
				2	1.0	90;92	90	92	91	
				2	5.0	90;89	89	90	90	
		BYI 02960- difluoroethyl- aminofuranone		15	0.01	87; 93; 95; 100; 100; 104; 105; 107; 83; 83; 86; 88; 90; 92; 96	83	107	94	8.4
				5	0.10	85; 98; 97; 97; 99	85	99	95	6.1
				2	0.50	97; 109	97	109	103	
				2	1.0	86; 101	86	101	94	
				2	5.0	97; 96	96	97	97	
				26	overall	83	109	95	7.7	
11-2071 11-2071-01 to 11-2072-04 GLP: yes 2011	Lettuce	BYI 02960		1	0.01	88	88	88	88	
				3	0.10	98;111;95	95	111	101	8.4
				1	10	104	104	104	104	
				5	overall	88	111	99	8.8	
		difluoroacetic acid		1	0.02	87	87	87	87	
				3	0.20	113;95;95	95	113	101	10.3
				4	overall	87	113	98	11.3	
				1	0.01	105	105	105	105	
		BYI 02960- difluoroethyl- aminofuranone		3	0.10	98;105;99	98	105	101	3.8
				4	overall	98	105	102	3.7	

IIA 6.3.1.2 Hops



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BYI 02960 (common name: flupyradifurone) is to be registered in northern Europe for use in hops. Thus, European residue data in hops are presented below to support the intended "safe" use. Use pattern (GAP) information is summarized in Table 6.3.2-1.

Table 6.3.2-1: Use patterns (GAPs) for the spray application of BYI 02960-containing formulations in/on hops in European fields

Description	Reg.	No. of appls.	Application rate per treatment (g a.s./ha)	per season (g a.s./ha)	Water volume (L/ha)	Interval (days)	PHI (days)
"safe use"*	EU-N	1	120	120	2000-3000	-	21

EU-N = northern EU residue region

* use based on an SL 200 formulation

In order to support the EU "safe use" of BYI 02960, sets of GLP trials were conducted in northern European fields in 2010 and 2011. BYI 02960 SL 200 (containing 200 g/L BYI 02960 a.s.) was applied once. Samples were taken at various intervals subsequent to the application. The envisaged PHI was 21 days.

Residue levels of BYI 02960 and its metabolites DFA and DFEAF were analyzed individually and summed to yield the calculated "total residue of BYI 02960". Total residue levels determined in the trials reached a maximum of 2.4 mg/kg in dried cones, with an STMR of 1.1 mg/kg.

The number of trials conducted for each use described above (incl. information on geographical region and vegetation period) is summarized below in table 6.3.2-2.

Table 6.3.2-2: Overview of European residue trials conducted in hops per geographical "residue region" and vegetation period, including key results

Use description (cf. table 6.3.2-1)	Region	No. of trials			Residue levels* (mg/kg)		Report No.	Dossier ref.: IIA 6.3.2/...
		Veget. period 2010	2011	Σ	HR	STMR		
<i>trials in EUROPE</i>								
"safe use"	EU-N	4	4	8	<i>green cone:</i> 0.87 0.47 <i>dried cone:</i> 2.4 1.1	10-2225, 11-2076	01, 02	

EU-N = northern EU residue region

* residue results based on total residues in samples taken on day 21 (= envisaged PHI)

Northern Europe (residue region)

Report:	KIIA 6.3.1.2/01, Noss, G.; Ballmann, A. 2012
Title:	Determination of the residues of BYI 02960 in/on hop after spraying of BYI 02960 SL 200 in the field in Germany
Report No. & Document No.:	10-2225, dated February 13, 2012 M-425351-01-1

Report:	KIIA 6.3.1.2/02, Noss, G.; Ballmann, A. 2012
Title:	Determination of the residues of BYI 02960 in/on hop after spray application of BYI 02960 SL 200 in Germany
Report No. & Document No.:	11-2076, dated February 13, 2012 M-425339-01-1

Guidelines (applies to both studies):	Directive 91/414/EEC, residues in or on treated products, food and feed
GLP (applies to both studies):	yes (certified laboratory); Deviations: none

I. Materials and Methods

Eight residue trials were conducted in the northern European residue region, as follows:

In 2010 and 2011, 8 trials (4 trials per year, all in Germany) were conducted to support the use of BYI 02960 SL 200 in hops (Noss & Ballmann, 2012, KIIA 6.3.2/01 and /02). A single application was made 21 days before the projected harvest at a nominal rate of 0.6 L/ha, corresponding to 120 g/ha BYI 02960 a.s. Water rates were 2000-3000 L/ha, reflecting local practice in the trial regions. All treatments were made at the scheduled rates.

Samples of green hop cones were taken immediately subsequent to the final application and at several intervals thereafter (up to 28 days after treatment). The envisaged PHI was 21 days. (In two trials, the PHI samples were taken on day 20; in one other, on day 22.) In addition to the green cone samples, additional cones were taken at the later sampling intervals (nominally days 14, 21, and 28) and dried according to standard practice, as dry cones are the primary traded commodity from the grower to the market.

The samples were analyzed for the parent compound and its metabolites DFA and DFEAF using method 01304 (cf. KIIA 4.3/03). The respective LOQs for the 3 analytes were 0.10, 0.20, and 0.10 mg/kg (all in parent equivalents), yielding a calculated total-residue LOQ of 0.40 mg/kg.

II. Findings

During the conduct of the 2010 studies, both validation and concurrent recoveries of BYI 02960 and its metabolites DFA and DFEAF were obtained from samples of hop cones (green and dried). (The validation work was done due to the fact that hops are considered "difficult to analyze" but were not included in the original validation set for method 01304. Details of the validation recoveries are



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presented in chapter 4.3 of this dossier with method 01304.) In 2011, samples were analyzed for concurrent recoveries.

Concurrent recovery samples for parent compound and DFEAF were spiked at levels of 0.10 mg/kg and 1.0 mg/kg, as well as 5.0 mg/kg (expressed in BYI 02960 equivalents). Mean recoveries in green cones in 2010 were 80-94%, with RSDs of the larger validations sets ($n > 2$) of 6.0-13.9%; $n=1-6$. In 2011, mean recoveries were 85-91%, with RSDs of the larger validation sets (1.0 mg/kg) of 0.7-2.3%; $n=1-3$. All values were within acceptable ranges.

Mean recoveries in dried cones in 2010 were 103-112%, with RSDs (of the larger validations sets [$n > 2$]) of 1.4-7.2%; $n=1-6$. All of these values were considered to be acceptable because, even in the case of values over 110%, they were only marginally higher and the RSD values were very low; also, in the cases of the exceptions, the overall means of all recovery analyses for the given matrices with each individual analyte were 107% and 108%, with overall RSDs of 6.2% and 4.2%. In 2011, recoveries were 79-91%; $n=1$ for each concentration.

For DFA, concurrent recovery samples were spiked at levels of 0.20 mg/kg and 1.0 mg/kg, as well as 5.0 mg/kg (expressed in BYI 02960 equivalents). Mean recoveries in green cones in 2010 were 83-99%, with RSDs (of the larger validations sets [$n > 2$]) of 8.2% and 8.9%; $n=1-6$. In 2011, mean recoveries were 79% and 88%, with an RSD of the larger validation set (1.0 mg/kg) of 3.2%; $n=1-3$. All values were within acceptable ranges.

In dried cones, mean DFA recoveries in 2010 were 98-106%, with RSDs of the larger validations sets ($n > 2$) of 3.1 and 8.9%; $n=1-6$. In 2011, recoveries were 70 and 73%; $n=1$ for each concentration. The values were all within acceptable ranges.

Details of recovery data are shown in table 6.3.2-4. All trial data are summarised below in table 6.3.2-3a & b and in greater detail in the Tier 1 summary forms. (Residues of parent BYI 02960 as well as its metabolites DFA and DFEAF are expressed in BYI 02960 equivalents. From these individual values, the "total residue of BYI 02960" was calculated as the sum of these three analytes, expressed in parent equivalents.)

Relevant residues of BYI 02960 were determined in hop cone samples taken at various intervals after application.

Analyses of green cones showed that total residue levels generally declined with time. On day 0, immediately following treatment, residue levels in green hop cones were between 0.79 and 2.7 mg/kg (median 1.3 mg/kg). By day 21 — the envisaged PHI (samples were taken on day 20 in two trials and day 22 in one further trial) — the levels had declined to <0.40-0.87 mg/kg ($n=8$), with a median value of 0.47 mg/kg. Residues continued to decrease until day 26-28, the final sampling event, when levels ranged from <0.40-0.69 mg/kg ($n=8$, median 0.41 mg/kg).

The residue behaviour was somewhat less predictable in dried cones. Whereas a decline was generally evident over time, in three of the trials, residue levels at the final sampling interval (28 days) were higher than at the PHI (day 21). Residue levels on day 21 (20 in two trials, 22 in one other) ranged

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from 0.56-2.4 mg/kg, with a median value of 1.1 mg/kg. On day 28 (day 26 and 27 in one trial each), they were generally lower, at <0.40-2.3 mg/kg (median 0.71 mg/kg). Taking the highest residues at relevant sampling intervals into consideration (either day 21 or 28), residues ranged from 0.61-2.4 mg/kg; the median value was 1.2 mg/kg.

III. Conclusions (hops)

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. All applications were at the required rates, and all trials were conducted according to GLP.

To evaluate this use, samples of both green and dried hop cones were taken at several intervals after the final application, including the envisaged PHI of 21 days. Samples were analyzed for the relevant residues of BYI 02960, comprising the parent compound and its metabolites DFA and DFEAF. The residues of all three analytes were summed to yield a calculated "total residue of BYI 02960". The results of the trials presented above demonstrate that:

- total residues of BYI 02960 dissipated rapidly in green hop cones, from levels of 0.79-2.7 mg/kg on day 0 after the treatment to <0.40-0.87 mg/kg on day 21 (envisaged PHI). The respective median values were 1.3 and 0.47 mg/kg.
- in dried cones, residue levels also tended to decline with time. When evaluating the highest residues at relevant sampling intervals (either day 21 or, in three trials, day 28), residues ranged from 0.61-2.4 mg/kg, with a median of 1.2 mg/kg.

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 Table 6.3.2-3a: Application scenario in residue trials conducted in/on **hops** after spraying with BYI 02960 SL 200 in the field

Study No. (Trial No.) Country Location Region Year	Crop Variety	FL	No.	Application		GS	PHI (days)
				kg/ha (a.s.)	kg/hl (a.s.)		
10-2225 (10-2225-01) Germany 91792 Ellingen EU-N 2010	hop Hallertauer Gold	200 SL	1	0.12	0.004	BBCH: 73-74	21
10-2225 (10-2225-02) Germany 99955 Luetzensoemmern EU-N 2010	hop Magnum	200 SL	1	0.12	0.0055	75	21
10-2225 (10-2225-03) Germany 04769 Muegeln EU-N 2010	hop Hallertauer Magnum	200 SL	1	0.12	0.0055	75	20
10-2225 (10-2225-04) Germany 88069 Tettnang EU-N 2010	hop Hallertauer Tradition	200 SL	1	0.12	0.0055	85	21
11-2076 (11-2076-01) Germany 91792 Ellingen EU-N 2011	hop Hallertauer mittelfrüh	200 SL	1	0.15	0.006	75	21

FL = formulation

GS = growth stage (BBCH-code) at last treatment

EU-N = northern Europe

Continued on next page...

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Table 6.3.2-3a (cont.): Application scenario in residue trials conducted in/on **hops** after spraying with BYI 02960 SL 200 in the field

Study No. (Trial No.) Country Location Region Year	Crop Variety	FL	No.	Application		GS	PHI (days)
				kg/ha (a.s.)	kg/hl (a.s)		
11-2076 (11-2076-02) Germany 99955 Luetzensoemmern EU-N 2011	hop Magnum	200 SL	1	0.15	0.006	BBCH: 73	21
11-2076 (11-2076-03) Germany 04703 Meinitz EU-N 2011	hop Hallertauer Tradition	200 SL	1	0.15	0.006	86	20
11-2076 (11-2076-04) Germany 88069 Tettnang EU-N 2011	hop Tettnanger	200 SL	1	0.15	0.0075	BBCH: 78	22

FL = formulation

GS = growth stage (BBCH-code) at last treatment

EU-N = northern Europe



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Table 6.3.2-3b: Results of residue trials conducted in/on hops after spraying with BYI 02960 SL 200 in the field

Study No. (Trial No.) Country GLP	Portion analyzed	DALT (days)	Residues (mg/kg) expressed as BYI 02960			
			BYI 02960	difluoroacetic acid	BYI 02960- difluoro- ethylamino- furanone	total residue of BYI 02960 calc.
10-2225 (10-2225-01) Germany GLP: yes	cone, green	0	1.3	<0.2	<0.1	1.6
		7	0.62	<0.2	<0.1	0.92
		14	0.29	<0.2	<0.1	0.59
		21	0.52	<0.2	<0.1	0.82
		28	0.16	<0.2	<0.1	0.46
	cone, kiln-dried	14	1.5	0.27	<0.1	1.9
		21	0.81	0.20	<0.1	1.1
		28	1.1	0.40	<0.1	1.6
	cone, green	0	0.49	<0.2	<0.1	0.79
		8	0.27	<0.2	<0.1	0.57
		13	0.19	<0.2	<0.1	0.49
		20	<0.1	<0.2	<0.1	<0.4
		27	<0.1	<0.2	<0.1	<0.4
10-2225 (10-2225-02) Germany GLP: yes	cone, kiln-dried	13	0.54	<0.2	<0.1	0.84
		20	0.48	<0.2	<0.1	0.78
		27	<0.1	<0.2	<0.1	<0.4
		0	1.4	<0.2	<0.1	1.7
		7	0.54	<0.2	<0.1	0.84
	cone, green	14	0.36	<0.2	<0.1	0.66
		21	0.20	<0.2	<0.1	0.50
		28	<0.1	<0.2	<0.1	<0.4
		14	1.4	0.25	<0.1	1.7
		21	0.77	0.28	<0.1	1.1
10-2225 (10-2225-03) Germany GLP: yes	cone, kiln-dried	28	0.32	<0.2	<0.1	0.62
		0	0.56	<0.2	<0.1	0.86
		8	0.27	<0.2	<0.1	0.57
		14	0.17	<0.2	<0.1	0.47
		21	0.14	<0.2	<0.1	0.44
	cone, green	28	<0.1	<0.2	<0.1	<0.4
		14	0.54	<0.2	<0.1	0.84
		21	0.90	0.21	<0.1	1.2
		28	0.49	<0.2	<0.1	0.79

DALT = days after last treatment

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Table 6.3.2-3b (cont.): Results of residue trials conducted in/on **hops** after spraying with BYI 02960 SL 200 in the field

Study No. (Trial No.) Country GLP	Portion analyzed	DALT (days)	Residues (mg/kg) expressed as BYI 02960			
			BYI 02960	difluoroacetic acid	BYI 02960- difluoro- ethylamino- furanone	total residue of BYI 02960 calc.
BYI 02960 SL 200						
11-2076 (11-2076- 01) Germany GLP: yes	cone, green	0	2.4	<0.2	<0.1	2.7
		14	0.47	<0.2	<0.1	0.77
		21	0.51	<0.2	<0.1	0.81
		28	0.39	<0.2	<0.1	0.69
	cone, kiln-dried	21	1.0	0.36	<0.1	1.5
		28	1.8	0.5	<0.1	2.3
	cone, green	0	0.55	<0.2	<0.1	0.85
		14	0.21	<0.2	<0.1	0.51
		21	<0.1	<0.2	<0.1	<0.4
		28	0.10	<0.2	<0.1	0.40
11-2076 (11-2076- 02) Germany GLP: yes	cone, kiln-dried	21	0.26	<0.2	<0.1	0.56
		28	0.31	<0.2	<0.1	0.61
	cone, green	0	2.1	<0.2	<0.1	2.4
		13	0.78	<0.2	<0.1	1.1
		20	0.57	<0.2	<0.1	0.87
		26	0.23	<0.2	<0.1	0.53
	cone, kiln-dried	20	2.0	0.27	<0.1	2.4
		26	0.49	<0.2	<0.1	0.79
11-2076 (11-2076- 04) Germany GLP: yes	cone, green	0	0.61	<0.2	<0.1	0.91
		13	0.11	<0.2	<0.1	0.41
		22	<0.1	<0.2	<0.1	<0.4
		28	0.11	<0.2	<0.1	0.41
	cone, kiln-dried	22	0.43	<0.2	<0.1	0.73
		28	0.29	<0.2	<0.1	0.59

DALT = days after last treatment



Table 6.3.2-4: Recovery data for BYI 02960 on hops

Study No. Trial No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
10-2225 10-2225-01 to 10-2225-04 GLP: yes 2010	hop	cone, green	BYI 02960	6	0.10	89;89;91;94; 95;107	89	107	94	7.2
				5	1.0	85;86;87;92;98	85	98	90	6.0
				1	5.0	87	87	87	87	
				12	overall		85	107	92	6.8
			difluoroacetic acid	6	0.20	91;92;95;99; 100;115	91	115	99	8.9
				5	1.0	76;79;83;84;94	76	94	83	8.2
				1	5.0	86	86	86	86	
				12	overall		76	115	91	11.7
			BYI 02960- difluoroethyl- aminofuranone	6	0.10	68;73;79;85; 95;96	68	96	83	13.9
		cone, kiln-dried		5	1.0	76;77;78;84;91	76	91	81	7.8
				1	5.0	80	80	80	80	
				12	overall		68	96	82	10.6
			BYI 02960	6	0.10	102;103;103; 104;105;106	102	106	104	1.4
				5	1.0	107;108;111; 114;115	107	115	111	3.2
				1	5.0	112	112	112	112	
				12	overall		102	115	108	4.2
			difluoroacetic acid	6	0.20	82;96;97;103; 103;106	82	106	98	8.9
				5	1.0	101;105;106; 107;110	101	110	106	3.1
				1	5.0	98	98	98	98	
				12	overall		82	110	101	7.3
			BYI 02960- difluoroethyl- aminofuranone	6	0.10	89;100;106; 107;107;108	89	108	103	7.2
				5	1.0	108;109;110; 112;114	108	114	111	2.2
				1	5.0	112	112	112	112	
				12	overall		89	114	107	6.2

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Table 6.3.2-4 (cont'd): Recovery data for BYI 02960 on hops

Study No. Trial No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
11-2076 11-2076-01 to 11-2076-04 GLP: yes 2011	hop	cone, green	BYI 02960	1	0.10	90	90	90	90	
				3	1.0	84;85;85	84	85	85	0.7
				4	overall		84	90	86	3.1
		difluoroacetic acid		1	0.20	88	88	88	88	
				3	1.0	77;79;82	77	82	79	3.2
				4	overall		77	88	82	5.9
		BYI 02960- difluoroethyl- aminofuranone		1	0.10	91	91	91	91	
				3	1.0	87;90;91	87	91	89	2.3
				4	overall		87	91	90	2.1
11-2076 11-2076-01 to 11-2076-04 GLP: yes 2011	hop	cone, kiln-dried	BYI 02960	1	0.10	90	90	90	90	
				1	1.0	87	87	87	87	
				2	overall		87	90	89	
		difluoroacetic acid		1	0.20	73	73	73	73	
				1	1.0	70	70	70	70	
				2	overall		70	73	72	
		BYI 02960- difluoroethyl- aminofuranone		1	0.10	79	79	79	79	
				1	1.0	91	91	91	91	
				2	overall		79	91	85	

IIA 6.3.2 Residue trials from the Global Joint Review partner countries Australia, Brazil, Canada, and the USA

These trial results will be submitted along with the remaining results from the EU in a separate document at a later point in time.



IIA 6.4 Livestock feeding studies

In chapter 6.3 of this dossier, residue trials are presented in which the residue behavior of BYI 02960 in two "EU safe use" crops (lettuce and hops) and in three rotational crop groups (root, leafy, and cereal crops) is described. However, as also mentioned there, additional crops will be presented in subsequent documents, including primary programs in vegetable, fruit, and cereal crops, etc., many of which will yield feed-relevant residues. The primary crops tested thus far in the EU include fruiting vegetables (tomatoes, peppers, cucumbers, melons), brassica vegetables (head, leafy, and flowering, as well as Brussels sprouts and kohlrabi), pome and stone fruit, grapes, strawberries and raspberries, peas, potatoes, and cereals, with additional import-tolerance relevant crops such as coffee, cocoa, blueberries, soybeans, and citrus fruit being tested in North and South America and in Africa. These trials will be submitted in batches, with the first large batch to be submitted later in 2012. Also, a large package of rotational crop tests in crops representing seven further groups has been conducted in the EU for 2012 submission.

Due to the nature of BYI 02960, measurable residues have been determined in virtually every crop tested. Many of these crops are relevant as feed items, either in the EU or in Australia or NAFTA. Thus, based on the results of metabolism studies which showed that animal matrices will also likely contain measurable residues, livestock feeding studies were conducted. Multi-region livestock diet calculations were conducted in order to conduct the studies in a manner appropriate to the entire scope of BYI 02960 use, allowing data to be generated in a fashion such that, for animal welfare considerations, a low number of animals will be used, while yielding valid data to evaluate expected residue levels in all key animal tissues and products.

The test substance used in the 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 might 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 + DFA, as well as separately for DFA alone.

While the nature of the calculations/evaluations for the total residue and for parent compound itself are relatively straightforward, using the study data to evaluate the metabolite DFA required careful consideration. As agreed with the 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.



In the following sections (KIIA 6.4.1 and 6.4.2), the basic study data will be presented first, followed by the specific calculations for the transfer factors for total residues and for DFA alone.

Calculation of dietary burden

The estimated dietary burden of total BYI 02960 residues in livestock commodities, based on EU crop residue data and the European dietary burden calculator, are presented for poultry and cattle below in tables 6.4-1 and 6.4-2, respectively.

Table 6.4-1: Anticipated dietary burden for BYI 02960 residues in poultry diet based on EU residue data and guideline

Feedstuff	Dry matter (DM)		Residue level		Dietary burden / dose	
	content (%)	intake (%) ¹	fresh weight basis (mg/kg) ²	dry weight basis (mg/kg) ³	in diet (mg/kg feed)	per animal (mg a.s./kg b.w./day)
Wheat grain ⁴	86	70	0.78	0.91	0.635	
Kale ⁴	14	5	1.36	9.71	0.486	
Turnips	10	20	0.14	1.40	0.280	
<i>Total:</i>		95			1.401	0.0885

1: Percentage of feedstuff in the diet for poultry in EU

2: Highest residue value (BYI 02960 + DFA) from European field trials

3: Corrected residue = residue level ÷ % dry matter × 100

4: Residue trials in these crops will be submitted at a later date

Table 6.4-2: Anticipated dietary burden for BYI 02960 residues in cattle diet based on EU residue data and guideline

Feedstuff	Dry matter (DM)		Residue level		Dietary burden / dose	
	content (%)	intake (%) ¹	fresh weight basis (mg/kg) ²	dry weight basis (mg/kg) ³	in diet (mg/kg feed)	per animal (mg a.s./kg b.w./day)
Kale ⁴	14	35	1.36	9.714	3.40	0.146
Turnips	10	60	0.14	1.400	0.84	0.036
Wheat grain ⁴	86	5	0.78	0.907	0.05	0.002
<i>Total:</i>		100			4.29	0.184

1: Percentage of feedstuff in the diet for cattle in EU

2: Highest residue value (BYI 02960 + DFA) from European field trials

3: Corrected residue = residue level ÷ % dry matter × 100

4: Residue trials in these crops will be submitted at a later date

As stated above, the feeding studies were designed to meet the necessary criteria in several regions, including NAFTA and Australia. The OECD calculator was also used but it was found that the current regional guidelines lead to more critical dietary burden values, thus these needed to be taken into consideration when designing the studies. The NAFTA base anticipated dietary burden for BYI 02960 residues in livestock feed was calculated using the Revisions of Feedstuffs in Table 1 of OPPTS Test Guideline 860.1000, the Guidance on Constructing Maximum Reasonably Balanced Diets (MRBD) that provide adequate nutrition and are consistent with modern feeding practices, and the anticipated

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tolerances based on field crop residue data generated from residue studies conducted in USA and Canada. The results are presented below in tables 6.4-3 and 6.4-4.

Table 6.4-3: NAFTA dietary burden based on NAFTA crop residue data and maximum reasonably balanced diet for poultry

Feedstuff	Type ¹	Residue level		% of diet ⁴	Dietary burden / dose in diet (mg a.s./kg feed)	per animal (mg a.s./kg b.w./day)
		Tolerance ² (mg/kg)	Corrected ³ (mg/kg)			
Alfalfa meal	PC	40.0	40.0	5	2.0	
Soybean seed	PC	2.0	2.0	20	0.4	
Rye grain	CC	4.0	4.0	35	1.4	
Wheat, milled by-products	CC	4.0	4.0	40	1.6	
<i>Total:</i>				100	5.4	0.3411

1: PC=Protein Concentrate, CC=Carbohydrate Concentrate

2: Proposed tolerance values (BYI 02960 + DFA)

3: Poultry diet residues are on as-fed basis (not adjusted to % dry matter)

4: Poultry livestock diet as listed in Table 1 of the EPA OPPTS 860.1000, based on 0.12 kg feed consumption and 1.9 kg body weight

Table 6.4-4: NAFTA dietary burden based on NAFTA crop residue data and maximum reasonably balanced diet for cattle

Feedstuff	Type ¹	Dry Matter		Residue levels		Dietary burden / dose	
		Dietary Intake (%)	Content (%)	fresh weight basis (mg/kg) ²	dry weight basis (mg/kg) ³	in diet (mg/kg feed)	per animal (mg/kg bw/day) ⁴
Soybean forage/silage	R	20	35	20.0	57.14	11.43	
Wheat, hay	R	25	88	40.0	45.46	11.36	
Corn, sweet, cannery waste	CC	10	30	3.96	13.20	1.32	
Rye, grain	CC	20	88	4.0	4.55	0.91	
Wheat, milled byproducts	CC	15	88	4.0	4.55	0.68	
Alfalfa, meal	PC	10	89	40.0	44.94	4.49	
<i>Total:</i>			100			30.20	1.01

1: R= Roughage, PC=Protein Concentrate, CC=Carbohydrate Concentrate

2: Proposed tolerance values (BYI 02960 + DFA)

3: Corrected residue = residue level ÷ % dry matter × 100

4: Based on 20 kg feed consumption and 550 kg body weight

**IIA 6.4.1 Poultry**

Report:	KIHA 6.4.1/01, [REDACTED] J.M., & [REDACTED], D.J.; 2012
Title:	BYI 02960 – Magnitude of the residue in laying hens
Report No. & Document No.:	RARVP041 M-428933-01-1
Guidelines:	– OPPTS 860.1480 – Meat/milk/poultry/eggs – OECD Guideline 505 – APVMA Residue Guideline No. 23 – DACO 7.5 – Meat/milk/poultry/eggs – OPPTS 860.1340 – Residue Analytical Method
GLP:	yes (certified laboratory)

I. Materials and MethodsTest system, dosing

Eighty-four mature laying hens (*Gallus gallus domesticus*) were dosed orally, via capsule, for 29 consecutive days with BYI 02960 at dose rates of 0 mg/kg feed/day (control; 24 hens, 6 subgroups), 1.5 mg/kg feed/day (1X EU dose group; 12 hens, 3 subgroups), 6.5 mg/kg feed/day (4.3X EU dose group; 12 hens, 3 subgroups), 19.4 mg/kg feed/day (13X EU dose group; 12 hens, 3 subgroups), and 65.1 mg/kg feed/day (43X EU dose group; 24 hens, 6 subgroups). These levels were approximately 0.3X, 1.3X, 3.3X and 11X the anticipated maximum dietary burden of BYI 02960 residues in livestock feed based on NAFTA residue data and livestock diet.

Dose rates used in this study were calculated according to both EU (Appendix G, Livestock feeding studies, 70.1/VI/95 rev4 [1996] and in Annex 4 of the OECD Guidance Document ENV/JM/MONO [2006]32, European Food Safety Authority [EFSA]) and NAFTA (Revisions of Feedstuffs in Table 1 of OPPTS Test Guideline 860.1000 and Guidance on Constructing Maximum Reasonably Balanced Diets [MRBD], Table 1 Feedstuffs [June 2008]) guidance.

The target and actual dose rates employed in the study are summarized below in table 6.4.1-1. The dose rates were adjusted weekly, based on the actual weekly feed consumption by the hens in each dose group during the previous week.

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Table 6.4.1-1: Summary of target and actual BYI 02960 dose administration.

EU ¹	Dose groups NAFTA ^{2,3}	Number of hens	Dose levels in feed		per animal actual ⁶ (mg a.s./kg b.w./day)
			target ⁴ (mg/kg feed)	actual ⁵ (mg/kg feed)	
	control	24	0	0	0
1.0X dose	0.3X dose	12	1.4	1.5	0.10
4.3X dose	1.3X dose	12	6.0	6.5	0.45
13X dose	3.3X dose	12	18	19.4	1.31
43X dose	11.1X dose	24	60	65.1	4.54

Footnotes:

1: EU dose rate exaggerations are based on EU dietary burden of 1.4 mg a.s./kg feed (see table 6.4-1)

2: NAFTA dose rate exaggerations are based on NAFTA dietary burden of 5.4 mg a.s./kg feed (see table 6.4-3)

3: Report RARVP041 uses the NAFTA exaggerations, reported there as 0.3X, 1X, 3X, and 11X

4: Target dose was calculated based on NAFTA and EU dietary burdens (tables 6.4-1 and 6.4-3)

5: Actual dose based on average feed consumption data collected from the study and average amount (mg) test substance for each dose group over the entire dosing period

6: Actual dose based on average amount (mg) test substance and the average body weight for each dose over the entire dosing period

The hens were dosed orally once per day each morning after collection of eggs and feeding. The control animal received a placebo (empty capsule) concurrently with the treated animals.

Sampling

Eggs were collected twice daily (afternoon and morning prior to the day's dosing). The eggs collected in the afternoon from each sub-group were combined with the eggs collected the following morning from the same sub-group. The egg contents were combined (shells discarded) by sub-group into a labeled container, weighed, and thoroughly mixed by vigorous shaking. Composite egg samples collected on days 0, 2, 4, 7, 10, 14, 17, 21, 24, 28, 35, 42 and 49 were shipped to Bayer CropScience for analysis for the 43X dose group; for all other doses, only the egg samples from days 24 and 28 were analyzed.

On day 29 of the study, twelve hens from the control and 43X dose groups and all hens in the 1X, 4.3X dose, and the 13X dose groups were sacrificed by CO₂ asphyxiation within 24 hours of the administration of the final dose. Liver (entire), muscle (thigh and breast), and fat (abdominal and subcutaneous) were collected, homogenized in the presence of dry ice, and the samples were shipped to Bayer CropScience.

Twenty four hens (12 from the control group and 12 from the 43X group) entered into a 21-day depuration phase, as required for Australia, following the administration of the final dose. Egg and tissue samples were collected on study days 35, 42, and 49 for analysis.

To estimate the extent of exposure of laying hens to DFA following the oral administration of BYI 02960, excreta was also collected daily from all the subgroups of the 4.3X and from the A, B and C subgroups of the 43X group during the entire dosing period and composited by sub-group. The

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entire sample from each sub-group was mixed at the end of the dosing period, and a subsample was homogenized for analysis.

Analysis

BYI 02960 and its metabolites were analytically determined using analytical method RV-004-A11-04 (supplied as an appendix to the main study report; cf. KIIA 4.3/08 for details relating to the method), which was validated prior to and parallel to the residue analysis of the samples. The LOQ was 0.01 mg/kg for all analytes (parent compound, DFA, BYI 02960-acetyl-AMCP, and BYI 02960-OH), expressed in BYI 02960 equivalents.

II. Findings

Main study

The mean values of the concurrent recovery rates per compound, sample material, and spiking level were in the range of 72-116%, with relative standard deviations in the range of 1-16%. (In the few cases of mean recoveries outside of the desired range -- BYI 02960-OH in fat at 4.0 mg/kg, BYI 02960-acetyl-AMCP in muscle at the LOQ – the RSD values were low [2.1 and 11.6%, respectively] and thus the values were considered to be acceptable.) Details of recovery data are shown in table 6.4.1-5.

Feed consumption, body weights, and egg production were not adversely affected by treatment with BYI 02960. In fact, feed consumption increased significantly relative to increases in body weight during the dosing period. Subsequently, the dose levels (mg a.s./hen in a given dose group), which were calculated using the feed consumption data and the corresponding dose rates based on mg a.s./kg body weight, also increased during the 29-day dosing period, as shown in table 6.4.1-2.

In the groups representing the nominal worst-case EU and NAFTA dietary burden (EU 1X and 4.3X groups, respectively), the total residues of BYI 02960 – comprising parent compound, DFA, BYI 02960-acetyl-AMCP, and BYI 02960-OH – were measured at sacrifice in poultry tissues and were as follows: 0.0883 and 0.3038 mg/kg in muscle, 0.0330 and 0.1213 mg/kg in fat, and 0.1077 and 0.4335 mg/kg in liver, respectively. In eggs taken on days 24 and 28, total residues in the 1X group were 0.057 and 0.053 mg/kg, respectively; the parallel values in the 4.3X group were 0.166 and 0.183 mg/kg. These values, as shown in the study report ([REDACTED] & [REDACTED], 2012; KIIA 6.4.1/01), reflect the standard practice in the USA of using all residue values as shown, even if they are below the nominal LOQs.

The proposed residue definition for both enforcement and risk assessment will include only parent BYI 02960 plus DFA, which are by far the two major components of the residue. The following values were determined for the nominal worst-case EU and NAFTA dietary burden groups (EU 1X and 4.3X groups, respectively) at sacrifice for the combined residue of the two components (calculated in the "traditional" manner, i.e. if one component is <LOQ, it is calculated as being *at* the LOQ):

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0.093 and 0.30 mg/kg in muscle, 0.039 and 0.13 mg/kg in fat, and 0.11 and 0.43 mg/kg in liver, respectively. In eggs taken on days 24 and 28, total residues in the 1X group were 0.061 and 0.057 mg/kg, respectively; the parallel values in the 4.3X group were 0.17 mg/kg at both days.

The residues found in the eggs, tissues, and excreta collected from the laying hens during dosing, at the end of the dosing period, and during the depuration phase are presented in tables 6.4.1-3 and 6.4.1-4.

With respect to eggs, the highest total BYI 02960 residues were found in the day-28 eggs from the 43X dose group. However, as stated previously, dose rates calculated with respect to mg a.s./kg bw increased during the dosing period (cf. table 6.4.1-2). The 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 (for details, cf. table 6.4.1-6); increases in residue levels appear only to be due to increased dose rates. This estimation is in line with the results of the poultry metabolism studies, in which the plateau level in whole eggs was reached at day 6 (pyridinylmethyl label, cf. KIIA 6.2.2/01) or day 9 (furanone label, cf. KIIA 6.2.2/02).

In the depuration phase, total BYI 02960 residues in eggs, fat, liver, and muscle from the 43X dose group hens declined from 1.722, 1.230, 3.480, and 2.410 mg/kg, respectively, to <LOQ at 14 days after cessation of dose administration (=day 42 of the study). The residue data provided in this study are suitable for regulatory purposes. (Depuration data are also presented in tables 6.4.1-3 and 6.4.1-4.)

The levels of DFA residues found in the excreta are summarized in table 6.4.1-7. DFA residues represented 65% to 74% of the total residues of BYI 02960 in the excreta samples, suggesting significant exposure of the laying hens to DFA following the daily oral administration of BYI 02960 to laying hens over 29 days.

Transfer factors for total residues and for DFA alone

Additional calculations were conducted to describe the transfer of both total residues and of DFA alone into poultry tissues and eggs following exposure to BYI 02960 and DFA via the diet. As they were not part of the main study and are not included in the study report RARVP041 (█████ & █████, 2012; KIIA 6.4.1/01), they are not presented here, but rather later in this section.

III. Conclusions (main study)

A feeding study was conducted with BYI 02960 on poultry in order to elucidate the levels of relevant residues in poultry tissues and in eggs. The study was 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.

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

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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 + 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 at various intervals in the high-dose group, and on days 24 and 28 in the three lower dose groups. In the EU 1X dose group, residues (BYI 02960 + DFA) in eggs amounted to 0.061 and 0.057 mg/kg, respectively.

Residue analysis of the high-dose eggs showed that levels reached a plateau in eggs. Though residues were highest at day 28, residue data from the 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 (pyridinylmethyl 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, 1.230, 3.480, and 2.410 mg/kg, respectively, to <LOQ at 14 days after cessation of dose administration (=day 42 of the study). The residue data provided in this study are suitable for regulatory purposes.

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



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Table 6.4.1-2: Dosing regime used in the poultry feeding study

Dose Group	Timing	Dose rate* (mg/kg feed)	Feed intake fresh (g/bird/d)	Feed intake dry (g/bird/d)	Administered dose (mg)	Average body weight** (kg)	Average BYI 02960 a.s. (mg/kg bw)
Control	4 Weeks		127.8		0	1.47	0
1X (US 0.3X)	Week 1	1.5	110.6	95.0	0.14	1.49	0.09
	Week 2	1.5	131.3	112.8	0.16	1.50	0.10
	Week 3	1.4	127.9	109.9	0.14	1.51	0.11
	Week 4	1.6	140.4	120.6	0.17	1.52	0.13
Overall Average		1.5	134.1	109.6	0.15	1.50	0.10
4.3X (US 1.3X)	Week 1	6.6	108.4	93.6	0.62	1.41	0.43
	Week 2	6.4	121.9	106.8	0.67	1.47	0.46
	Week 3	6.5	116.5	102.5	0.65	1.47	0.45
	Week 4	6.4	134.2	116.6	0.74	1.45	0.51
Overall Average		6.5	125.4	104.9	0.67	1.45	0.46
13X (US 3X)	Week 1	19.6	108.3	93.1	1.83	1.44	1.27
	Week 2	19.8	119.0	103.4	2.03	1.48	1.37
	Week 3	19.7	116.2	100.5	1.96	1.49	1.32
	Week 4	18.3	137.1	117.5	2.16	1.49	1.45
Overall Average		19.4	126.6	103.6	1.99	1.47	1.35
43X (US 11X)	Week 1	64.6	109.2	93.8	6.05	1.45	4.18
	Week 2	67.4	120.4	103.4	6.97	1.48	4.72
	Week 3	65.6	117.1	100.6	6.6	1.49	4.42
	Week 4	62.9	134.8	115.7	7.28	1.50	4.84
Overall Average		65.1	125.9	103.4	6.72	1.48	4.54

* dose rate in feed calculated on a dry weight basis

** these weights reflect those determined at the end of the given study week

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Table 6.4.1-3: Levels of the relevant residues of BYI 02960 in eggs

group	sampling day	Residue levels of individual analytes (mg/kg)				Total residue levels (mg/kg)	
		BYI 02960 LOD = 0.004 LOQ = 0.01	DFA LOD = 0.003 LOQ = 0.01	-AMCP* LOD = 0.003 LOQ = 0.01	-OH LOD = 0.003 LOQ = 0.01	sum of 4†	BYI 02960 + DFA‡
1X	24	<LOD/LOQ	0.051	<LOD/LOQ	<LOD/LOQ	0.057	0.061
	28	<LOD/LOQ	0.047	<LOD/LOQ	<LOD/LOQ	0.053	0.057
4.3X	24	<LOD/LOQ	0.155	<LOD/LOQ	<LOD/LOQ	0.166	0.165
	28	<LOD/LOQ	0.163	<LOD/LOQ	<LOD/LOQ	0.183	0.173
13X	24	0.019	0.497	0.017	0.014	0.545	0.516
	28	0.023	0.508	0.015	0.018	0.565	0.532
43X [▲]	0	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	<0.02
	2	0.048	0.334	0.019	0.024	0.424	0.382
	4	0.068	0.898	0.045	0.027	1.037	0.966
	7	0.054	1.022	0.042	0.026	1.143	1.076
	10	0.065	1.211	0.052	0.041	1.368	1.276
	14	0.063	0.972	0.038	0.038	1.110	1.035
	17	0.080	1.170	0.043	0.055	1.347	1.250
	21	0.071	1.202	0.043	0.050	1.366	1.273
	24	0.082	1.486	0.059	0.050	1.676	1.568
	28	0.173	1.414	0.051	0.084	1.722	1.587
	35**	<LOD/LOQ	0.130	<LOD/LOQ	<LOD/LOQ	0.140	0.140
	42**	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	0.013	<0.02
	49**	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	<0.02

All metabolite residues expressed in parent compound equivalents

* AMCP = BYI 02960-acetyl-AMCP

** depuration phase, no dosing (sampling days 35-49)

† this value, as shown in the study report, includes values below the LOQ calculated at the apparent residue value

‡ this value reflects the proposed residue definition, and as such calculates each component at or above the respective LOQ

▲ day 0-28 values shown for the high-dose group reflect an average of all animals, including those designated for depuration



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Table 6.4.1-4: Levels of the relevant residues of BYI 02960 in poultry tissues

group	sampling day	Residue levels of individual analytes (mg/kg)				Total residue levels (mg/kg)	
		BYI 02960	DFA	-AMCP*	-OH	sum of 4†	BYI 02960 + DFA‡
<i>POULTRY FAT</i>							
1X	29	<LOD/LOQ	0.029	<LOD/LOQ	<LOD/LOQ	0.033	0.039
4.3X	29	<LOD/LOQ	0.117	<LOD/LOQ	<LOD/LOQ	0.121	0.127
13X	29	<LOD/LOQ	0.272	<LOD/LOQ	<LOD/LOQ	0.281	0.282
43X^	29	0.192	1.006	0.021	0.010	1.230	1.198
	35**	<LOD/LOQ	0.041	<LOD/LOQ	<LOD/LOQ	0.056	0.051
	42**	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	0.009	<0.02
	49**	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	0.013	<0.02
<i>POULTRY LIVER</i>							
1X	29	<LOD/LOQ	0.104	<LOD/LOQ	<LOD/LOQ	0.108	0.114
4.3X	29	0.006/0.011	0.413	0.011	0.006/0.011	0.444	0.423
13X	29	>LOD/<LOQ	1.012	0.025	>LOD/<LOQ	1.043	1.022
43X^	29	0.032	3.313	0.083	0.051	3.480	3.345
	35**	<LOD/LOQ	0.085	<LOD/LOQ	<LOD/LOQ	0.089	0.088
	42**	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	0.012	<0.02
	49**	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	0.006	<0.02
<i>POULTRY MUSCLE</i>							
1X	29	<LOD/LOQ	0.083	<LOD/LOQ	<LOD/LOQ	0.0883	0.086
4.3X	29	>LOD/<LOQ	0.290	0.010	>LOD/<LOQ	0.304	0.300
13X	29	>LOD/<LOQ	0.719	0.024	>LOD/<LOQ	0.750	0.729
43X^	29	0.039	2.27	0.069	0.032	2.410	2.309
	35**	<LOD/LOQ	0.051	<LOD/LOQ	<LOD/LOQ	0.055	0.061
	42**	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	0.011	<0.02
	49**	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	<LOD/LOQ	0.007	<0.02

All metabolite residues expressed in parent compound equivalents

* AMCP = BYI 02960-acetyl-AMCP

** depuration phase, no dosing (sampling days 35-49)

† this value, as shown in the study report, includes values below the LOQ calculated at the apparent residue value

‡ this value reflects the proposed residue definition, and as such calculates each component at or above the respective LOQ

^ values shown for the high-dose group reflect an average of all animals, including those designated for depuration



Table 6.4.1-5: Concurrent recovery data for the relevant residues of BYI 02960 in poultry matrices

Study No.	Matrix	a.s./metabolite	n	Spike level (mg/kg)	Individual recoveries	Recovery (%)			
						Min	Max	Mean	RSD
RARVP041	eggs	BYI 02960	17	0.01	113,115,118,103, 109,94,98,87,84, 77,93,76,115, 94,84,94,84	76	118	96	14.2
			3	4.0	100,81,95	81	100	92	10.7
		DFA	17	0.01	100,112,109,78, 87,74,81,76,78, 68,77,70,83,73, 79,76,71	68	112	82	15.9
			3	4.0	75,70,71	70	75	72	3.7
		BYI 02960-acetyl-AMCP	16	0.01	84,97,89,120,92, 103,100,76,86, 100,70,89,99, 104,91,90	70	120	93	12.7
			3	4.0	93,81,90	81	93	88	7.1
		BYI 02960-OH	17	0.01	102,109,101,89, 99,90,96,83,90, 72,95,89,113, 107,73,96,99	72	113	94	12.0
			3	4.0	102,89,96	89	102	96	6.8
	fat	BYI 02960	12	0.01	87,88,77,85, 95,103,96,94, 88,93,75,105	75	105	91	9.5
			3	4.0	110,104,106	104	110	107	2.6
		DFA	12	0.01	97,91,94,97, 84,80,88,96, 83,85,75,74	74	97	87	9.4
			3	4.0	100,96,92	92	100	96	4.2
		BYI 02960-acetyl-AMCP	12	0.01	119,81,96,85,99, 77,100,92,95, 107,95,87	77	119	94	12.2
			3	4.0	109,111,108	108	111	109	1.4
		BYI 02960-OH	12	0.01	95,106,100,87, 100,88,90,93, 87,86,90,102	86	106	94	7.2
			3	4.0	117,113,118	113	118	116	2.1
	liver	BYI 02960	12	0.01	93,104,121,114, 102,114,90,94, 110,95,84,92	84	121	101	11.4
			3	4.0	112,102,100	100	112	104	6.2
		DFA	12	0.01	75,90,83,97, 89,93,81,103, 84,77,78,82	75	103	86	10.2
			3	4.0	91,85,82	82	91	86	5.2
		BYI 02960-acetyl-AMCP	12	0.01	108,112,116,117, 96,116,96,114, 115,89,96,94	89	117	106	10.1
			3	4.0	115,100,99	99	115	105	8.4
		BYI 02960-OH	12	0.01	84,85,120,83, 74,88,89,97, 101,72,91,71	71	120	88	15.6
			3	4.0	108,106,107	106	108	107	0.7

All concentrations are expressed in parent compound equivalents.

Continued next page...

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Table 6.4.1-5 (cont'd): Concurrent recovery data for the relevant residues of BYI 02960 in poultry matrices

Study No.	Matrix	a.s./metabolite	n	Spike level (mg/kg)	Recovery (%)				RSD
					Individual recoveries	Min	Max	Mean	
RARVP041	muscle	BYI 02960	12	0.01	117,106,115,119,96,119,82,81,116,94,115,98	81	119	105	13.5
			3	4.0	108,112,108	108	112	109	2.1
		DFA	12	0.01	91,95,101,85,82,92,88,118,98,87,86,104	82	118	95	10.4
			3	4.0	86,83,80	80	86	83	3.6
		BYI 02960-acetyl-AMCP	12	0.01	114,116,117,135,103,119,118,116,110,101,92,88	88	135	111	11.6
			3	4.0	108,107,101	101	108	105	3.5
		BYI 02960-OH	12	0.01	100,112,114,97,105,93,120,119,111,107,107,81	81	120	106	10.6
			3	4.0	108,112,110	108	112	110	1.8
	excreta	BYI 02960	8	0.01	87,110,104,91,112,109,88,111	87	112	102	10.8
			3	4.0	98,96,93	93	98	95	2.6
		DFA	8	0.01	118,110,103,114,112,121,99,92	92	121	109	9.1
			3	4.0	86,86,88	86	88	87	1.3
		BYI 02960-acetyl-AMCP	8	0.01	112,106,98,101,99,108,109,109	98	112	105	5.0
			3	4.0	98,91,91	91	98	93	4.3
		BYI 02960-OH	8	0.01	102,104,100,110,102,105,101,91	91	105	102	5.3
			3	4.0	97,99,91	91	99	95	4.4

All concentrations are expressed in parent compound equivalents.

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Table 6.4.1-6: Weekly dose rates versus total residues of BYI 02960 residues in eggs from 43X dose group

Dose group	Dose week	Sampling interval	Dose administered (mg)	Average body weight (kg)	Dose rate (mg a.s./kg bw)	Total residues* of BYI 02960 (mg/kg)
43X	1	day Zero	6.05	1.45	4.17	<LOD
		day 2				0.424
		day 4				1.037
	2	day 7	6.97	1.48	4.71	1.143
		day 10				1.368
	3	day 14	6.60	1.49	4.43	1.110
		day 17				1.347
	4	day 21	7.28	1.50	4.85	1.366
		day 24				1.676
		day 28				1.722

* total residues here are the sum of BYI 02960, DFA, BYI 02960-acetyl-AMCP, and BYI 02960-OH, expressed as parent cpd.

Table 6.4.1-7: Proportion of DFA in the total residues of BYI 02960 in excreta

Matrix	Dose group	Composite sample*	Total BYI 02960 residue** (mg/kg)	DFA residues† (mg/kg)	Proportion of DFA in total residue (%)
excreta	4.3X	Subgroup A	1.628	1.097	67
		Subgroup B	1.587	1.077	68
		Subgroup C	1.824	1.247	68
	43X	Subgroup A	12.647	8.253	65
		Subgroup B	14.445	9.493	66
		Subgroup C	15.374	11.300	74

* Composite sample from entire 29-day dosing period per subgroup

** "total BYI 02960 residue" is the sum of BYI 02960, BYI 02960-OH, BYI 02960-acetyl-AMCP, and DFA residues, expressed in BYI 02960 equivalents

† expressed in BYI 02960 equivalents

► Transfer factors calculated for DFA and the total residue

As agreed with the EU Rapporteur (Ctgb, NL), separate transfer factors for DFA (difluoroacetic acid) were estimated from the poultry feeding study on the basis of the data available after dosage of the active substance BYI 02960 to laying hens. In order to accomplish this, a theoretical dose of DFA must be estimated. For this estimation, the amounts of DFA in all organs/tissues and particularly in excreta must be considered. The absolute amount of DFA formed in the animals, and which has thus been systemically available, can then be equated with a minimum dose theoretically fed to the animals.

The estimation of the dose of DFA theoretically fed to the animals was conducted on the basis of the data collected for the highest dose group (43X in EU, corresponding to 11X in NAFTA) ([Wade & Netzband, 2012; KIIA 6.4.1/01](#)), using the animals of the subgroups A to C (actual mean dose of BYI 02960 = 65.1 mg/kg feed).



For a better understanding, the data used in the different calculations are summarized separately at the end of this section in tables, as listed here:

- Table 6.4.1-12: Calculation of the mean body weight of the laying hens in the 43X dose group, subgroups A to C
- Table 6.4.1-13: Liver weights of laying hens in the 43X dose group, subgroups A to C
- Table 6.4.1-14: Estimation of the average weight of excreta collected from white leghorn hens in a 24-h period based on data from the metabolism studies
- Table 6.4.1-15: Calculation of the average weight of an egg
- Table 6.4.1-16: Individual egg production of laying hens in the 43X dose group, subgroups A to C
- Table 6.4.1-17: Calculation of the weighted residue level in eggs
- Table 6.4.1-18: Residue levels in animal matrices after dosing of BYI 02960 to laying hens
- Table 6.4.1-19: Total residues calculated for animal matrices after dosing of BYI 02960 to laying hens

Minimum dose of DFA theoretically fed to poultry:

The residue levels in excreta, eggs, tissues, and organs, as well as the weights of the animal matrices (total weight of 12 birds) used for the calculation of the minimum theoretical dose are summarized in the Table 6.4.1-8.

Table 6.4.1-8: Calculation of absolute DFA amounts in animal matrices on basis of the sample weights and the residues in the samples (43X dose group)

Animal matrix	Weight [kg]	DFA residue [mg a.s. equiv./kg]	Absolute DFA amounts [mg a.s. equiv.]
eggs*	16.695 ¹	1.130 ⁵	18.863
liver*	0.487	3.313	1.614
muscle*	7.058 ²	2.27	16.023
fat*	2.118 ³	1.006	2.131
excreta	73.08 ⁴	9.682	707.577
total (12 birds)			746.208

* animals of subgroups A to C were used to calculate the average/weighted residues (*without "depuration" animals*)

¹ the total weight of the eggs was calculated based on the total number of eggs collected during the in-life phase of the study (day 1 to 29) and the average egg weight determined from the eggs used for analysis; see Table 6.4.1-15 and Table 6.4.1-16

² total muscle weight was calculated assuming a value of 30% of the mean body weight (1.47 kg) for this tissue

³ total fat weight was calculated assuming a value of 12% of the mean body weight (1.47 kg) for this tissue

⁴ the total weight of excreta was estimated based on the mean excreta weight (210 g/day/hen) determined from the 12 white leghorn hens used in the BYI 02960 poultry metabolism studies (mean body weight: 1.60 kg); see Table 6.4.1-14

⁵ weighted DFA residue value of eggs collected at day 2, 4, 7, 10, 14, 17, 21, 24 and 28; see Table 6.4.1-17

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According to the balance established, the minimum amount of DFA systemically available was 746.2 mg a.s. equiv. for twelve animals (animals of subgroups A, B & C), corresponding to **62.2 mg a.s. equiv. per animal**.

Calculation of transfer factors:

The total amount of BYI 02960 administered in the feeding study accounted for 193.5 mg per animal (4.54 mg/kg bw/day \times 1.47 kg bw \times 29 days). Thus the total amount of systemically available DFA represented 32.1% of the amount administered as BYI 02960 (62.2 mg/193.5 mg = 32.1%).

Expressing the dose of BYI 02960 in [mg/kg dry feed], the mean dose level in the 43Xdose group accounted for 65.1 mg a.s./kg dry feed. The theoretical dose of DFA can thus be calculated to be **20.9 mg a.s. equiv./kg dry feed** ($65.1 \text{ mg a.s./kg feed} \times 32.1\% = 20.9 \text{ mg a.s. equiv./kg dry feed}$).

On the basis of the DFA residues in eggs and organs/tissues and the theoretical dose of DFA, transfer factors have been calculated, according to the following equation:

$$\text{TF} = \frac{\text{residue level in edible commodity}}{\text{residue level in the diet}}$$

The following tables summarise the residue values determined in the animal matrices and the corresponding transfer factors derived from them.

DFA transfer:

Assuming that the theoretical dose of DFA is reflected by the same proportion (32.1% of the parent dose) for all dose levels, transfer factors for DFA can be estimated at all levels.

It has to be mentioned that, except for the highest dose level, no composite samples of eggs were collected which covered the whole administration phase. Thus for the 1X, 4.3X, and 13X dose levels, only egg samples from day 28 (the plateau level had been reached by that time) were considered when calculating the transfer.



Table 6.4.1-9: Transfer factors calculated for DFA

Animal matrix	DFA residue [mg a.s equiv./kg]	Transfer factor
Theoretical dose administered: 20.9 mg DFA/kg dry feed (expressed in parent equivalents)		
egg composite* (days 1 to 29)	1.130	0.054
muscle*	2.270	0.108
fat*	1.006	0.048
liver*	3.313	0.158
Theoretical dose administered: 6.2 mg DFA/kg dry feed (expressed in parent equivalents)		
egg (day 28)	0.508	0.082
muscle	0.719	0.115
fat	0.272	0.044
liver	1.012	0.163
Theoretical dose administered: 2.1 mg DFA/kg dry feed (expressed in parent equivalents)		
egg (day 28)	0.163	0.078
muscle	0.290	0.139
fat	0.117	0.056
liver	0.413	0.198
Theoretical dose administered: 0.5 mg DFA/kg dry feed (expressed in parent equivalents)		
egg (day 28)	0.047	0.097
muscle	0.083	0.172
fat	0.029	0.060
liver	0.104	0.216

* animals of subgroups A to C were used to calculate the average/weighted residues (*without "depuration" animals*)

Comparing the transfer factors estimated for the animal matrices at different dose levels, it is evident that the transfer of DFA into animal matrices is less pronounced at higher doses. The residue levels in the animal matrices do not increase linearly with the dose levels. The residues detected in eggs and organs/tissues are slightly higher at lower dose levels indicating even more pronounced excretion of DFA at higher dose levels.

Transfer of BYI 02960 residues:

The transfer of the relevant residues of BYI 02960 into edible matrices was also calculated on the basis of the dose administered to the animals in [mg/kg dry feed] and the total residues of BYI 02960 detected in the animal matrices in [mg/kg].

Since the total residue for data collection is different to the relevant residue for enforcement and risk assessment, separate transfer factors were calculated for all animal matrices in all dose groups tested. The total residue as defined for data collection comprises the compounds BYI 02960, BYI 02960-OH, BYI 02960-acetyl-AMCP, and DFA, whereas only parent compound BYI 02960 and DFA were proposed as relevant residue for enforcement and risk assessment (cf. KIIA 6.7.1).

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The following tables refer to calculations on the basis of the total residue for data collection (BYI 02960, BYI 02960-OH, BYI 02960-acetyl-AMCP and DFA):

Table 6.4.1-10: Transfer factors calculated for the total residue as defined in the data collection method (parent compound BYI 02960, BYI 0296-acetyl-AMCP, BYI 02960-OH and DFA)

Animal matrix	Total residue [mg a.s equiv./kg]	Transfer factor
Average dose administered: 65.1 mg a.s./kg dry feed (43X)		
egg composite* (days 1 to 29)	1.308	0.020
muscle*	2.409	0.037
fat*	1.229	0.019
liver*	3.480	0.053
Average dose administered: 19.4 mg a.s./kg dry feed (13X)		
egg (day 28)	0.565	0.029
muscle	0.759	0.039
fat	0.302	0.016
liver	1.054	0.055
Average dose administered: 6.5 mg a.s./kg dry feed (4.3X)		
egg (day 28)	0.193	0.030
muscle	0.320	0.049
fat	0.147	0.023
liver	0.444	0.068
Average dose administered: 1.5 mg a.s./kg dry feed (1X)		
egg (day 28)	0.077	0.051
muscle	0.113	0.075
fat	0.059	0.039
liver	0.134	0.089

* animals of subgroups A to C were used to calculate the average/weighted residues (*without "depuration" animals*)



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In the subsequent table, the transfer factors were elucidated for the combined residues of BYI 02960 + DFA (*without* the other analytes), as these two compounds have been proposed as the relevant residues of BYI 02960 in poultry matrices for enforcement and risk assessment (cf. KIIA 6.7.1).

Table 6.4.1-11: Transfer factors calculated for the compounds relevant for enforcement/risk assessment (parent compound BYI 02960 and DFA)

Animal matrix	Total residue [mg a.s. equiv./kg]	Transfer factor
Average dose administered: 65.1 mg a.s./kg dry feed (43X)		
egg composite* (days 1 to 29)	1.212	0.019
muscle*	2.309	0.035
fat*	1.199	0.018
liver*	3.346	0.051
Average dose administered: 19.4 mg a.s./kg dry feed (13X)		
egg (day 28)	0.532	0.027
muscle	0.729	0.038
fat	0.282	0.015
liver	1.022	0.053
Average dose administered: 6.5 mg a.s./kg dry feed (4.3X)		
egg (day 28)	0.173	0.027
muscle	0.300	0.046
fat	0.127	0.020
liver	0.423	0.065
Average dose administered: 1.5 mg a.s./kg dry feed (1X)		
egg (day 28)	0.057	0.038
muscle	0.093	0.062
fat	0.039	0.026
liver	0.114	0.076

* animals of subgroups A to C were used to calculate the average/weighted residues (*without* "depuration" animals)

As was the case for the transfer factors calculated for DFA, the transfer factors for the total residue (either as defined for data collection or for enforcement/risk assessment) are not independent of the dose fed. Higher doses result in a lower transfer of the compounds into the animal matrices. This is in line with the findings that DFA represents the predominant component of the BYI 02960 residues in poultry matrices. Moreover, the "total residue" transfer factors calculated for the lower doses overestimate the transfer of the residues into the animal matrices since all/most components, except for DFA, are well below the LOD in all/most matrices. Although the compounds were not detectable, they were considered to be *at* the LOQ level of 0.01 mg/kg when calculating the total residues. Therefore it is more appropriate to use the transfer factors calculated for parent compound + DFA (relevant residues of BYI 02960 for risk assessment and enforcement) rather than using the transfer factors calculated for the total residue as defined for data collection.

It is evident that parent compound BYI 02960 was metabolized rather quickly to DFA after feeding of BYI 02960 to poultry, and quite high amounts of DFA were systemically available. As expected, the

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highest DFA concentration – and therefore the highest transfer factor – was detected in the metabolizing organ liver, followed by muscle and eggs. However, the transfer of total BYI 02960 residues into all edible matrices is rather low at all dose levels tested.

Feeding of DFA only would thus result in approx. threefold higher transfer factors, as demonstrated above (cf. Table 6.4.1-9). The highest transfer was also determined for liver, followed by muscle and eggs. The lowest transfer factor was determined for fat.

Using these transfer factors derived for the combined residue of BYI 02960 + DFA as well as for DFA alone, estimations of the residue levels reasonably expected to be determined in poultry tissues and eggs after feeding of a mixture of BYI 02960 and DFA (as would realistically be anticipated in the feed crops) can be calculated.

Supporting information:**Estimation of the total weight of muscle and fat of hens and determination of the total liver weight (subgroups A to C)**

Table 6.4.1-12: Calculation of the mean body weight of the laying hens in the 43X dose group; subgroups A to C

Group	Animal No.	Weight at Study Day [g]						Average Weight [g]
		-7	1	8	15	22	29	
11X-A	954	1467.3	1449.5	1481.5	1455.5	1473.1	1469.6	1466.1
11X-A	955	1624.6	1639.7	1603.2	1657.8	1669.7	1714.5	1651.6
11X-A	952	1422.2	1393.5	1449.2	1428.2	1428.9	1440.1	1427.0
11X-A	951	1516.5	1540.7	1556.4	1595.4	1613.3	1590.9	1568.9
11X-B	967	1393.3	1418.4	1388.5	1444.8	1469.8	1463.2	1429.7
11X-B	968	1325.7	1320.1	1371.3	1367.6	1377.3	1367.7	1355.0
11X-B	970	1477.3	1536.9	1481.3	1543.8	1560.7	1561.1	1526.9
11X-B	969	1346.5	1407.9	1404.9	1438.4	1463.0	1442.8	1417.3
11X-C	823	1438.6	1459.2	1324.7	1498.2	1501.2	1505.9	1454.6
11X-C	821	1521.1	1535.3	1555.7	1593.1	1618.4	1516.4	1556.7
11X-C	825	1389.7	1425.3	1391.3	1439.3	1456.5	1450.9	1425.5
11X-C	822	1338.2	1329.7	1367.7	1426.3	1362.9	1366.5	1365.2
Overall average weight								1470.5

Based on the average body weight of 1470.5 g, the average weights of muscle (40% of the total body weight) and fat (12% of the total body weight) were estimated to be 588.2 g and 325.8 g, respectively. Thus the total **muscle** weight amounts to **7058 g** for **twelve animals** and the total **fat** weight amounts to **2118 g** for **twelve animals**.

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Table 6.4.1-13: Liver weights of laying hens in the 43X dose group; subgroups A to C

Dose Group-Subgroup	Animal No.	Liver weight [g]
11X-A	954	44.2
11X-A	955	59.1
11X-A	952	39.8
11X-A	951	35.0
11X-B	967	34.3
11X-B	968	36.3
11X-B	970	50.5
11X-B	969	33.4
11X-C	823	41.2
11X-C	821	40.2
11X-C	825	34.6
11X-C	822	38.6
Total liver weight of 12 hens		487.2

At necropsy the entire liver of each animal was collected, weighed, and pooled per subgroup. The total liver weight amounts to **487.2 g** for twelve animals.

Estimation of the total excreta weight of hens (subgroup A to C)

Table 6.4.1-14: Estimation of the average weight of excreta collected from white leghorn hens in a 24-h period based on data from the metabolism studies

Time after the 1 st admin. [d]	Weight of excreta [g]					
Poultry metabolism study conducted with [furanone-4-¹⁴C]BYI 02960 (see KIIA 6.2.2/02)						
	animal 956	animal 957	animal 958	animal 959	animal 960	animal 961
0	-----	-----	-----	-----	-----	-----
1	183.23	220.76	217.46	192.94	185.40	185.40
2	208.07	197.27	244.21	178.59	174.99	174.99
3	229.33	209.54	230.93	209.24	180.52	180.52
4	207.48	225.63	229.07	166.95	207.94	207.94
5	227.77	225.91	251.07	161.09	199.49	199.49
6	221.91	218.23	215.58	221.74	211.72	211.72
7	245.23	214.06	216.23	169.87	202.52	202.52
8	241.05	293.95	236.26	200.62	219.34	219.34
9	217.93	303.16	239.60	230.71	228.64	228.64
10	216.23	278.29	253.51	228.34	220.73	220.73
11	242.78	296.54	219.67	239.19	218.84	218.84
12	213.44	222.15	184.08	229.71	235.84	235.84
13	226.80	216.55	208.81	229.64	221.67	221.67
13.25	97.48	183.36	131.27	141.64	122.07	122.07
average weight day 1 to 13	221.63	240.16	226.65	204.51	208.28	208.28

Table continued on next page...

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Time after the 1 st admin. [d]	Weight of excreta [g]					
Poultry metabolism study conducted with [pyridinylmethyl-¹⁴C]BYI 02960 (see KIIA 6.2.2/01)						
	animal 962	animal 963	animal 964	animal 965	animal 966	animal 967
0	-----	-----	-----	-----	-----	-----
1	221.63	208.24	180.33	122.69	227.60	211.21
2	219.64	227.64	151.96	119.05	193.77	204.10
3	213.68	217.76	126.35	186.00	208.25	178.51
4	236.13	193.85	135.26	160.08	198.49	203.95
5	231.74	215.88	167.17	166.01	213.74	186.55
6	250.23	198.97	173.20	188.65	244.92	186.90
7	368.86	194.35	208.94	175.82	263.03	201.91
8	178.52	178.89	191.19	169.49	174.85	165.08
9	222.68	212.98	176.45	221.20	230.97	225.28
10	227.84	191.95	163.88	187.79	225.24	176.40
11	250.38	195.19	193.51	170.27	246.21	187.62
12	210.87	177.87	159.08	175.45	183.33	190.20
13	258.11	174.14	154.96	153.46	230.41	197.32
13.25	72.36	72.67	63.92	59.71	101.60	70.15
average weight day 1 to 13	237.72	199.05	167.87	168.92	218.52	193.46
overall average						207.92

----- no excreta collected

Body weights were determined at the day before the first administration.

Based on the average weight of excreta collected in the poultry metabolism studies, the average excreta weight in the present study was estimated to be **210 g in a 24-h period**. Thus the total **excreta** weight was estimated to be **73080 g for twelve hens**.

**Estimation of the total egg weight of hens (subgroups A to C)**

Table 6.4.1-15: Calculation of the average weight of an egg

Dose Group-Subgroup	Number of birds	Day of treatment	Weight of eggs [g]	Number of eggs	Average weight of one egg [g]
11X-A	4	2	202.8	4	50.7
11X-B	4	2	149.8	3	49.9
11X-C	4	2	140.2	3	46.7
11X-A	4	4	200.1	4	50.0
11X-B	4	4	242.6	5	48.5
11X-C	4	4	235.1	5	47.0
11X-A	4	7	228.2	4	57.1
11X-B	4	7	287.0	6	47.8
11X-C	4	7	189.6	4	47.4
11X-A	4	10	154.6	3	51.5
11X-B	4	10	145.3	3	48.4
11X-C	4	10	186.0	4	46.5
11X-A	4	14	214.0	4	53.5
11X-B	4	14	203.7	4	50.9
11X-C	4	14	194.2	4	48.6
11X-A	4	17	215.2	4	53.8
11X-B	4	17	201.7	4	50.4
11X-C	4	17	151.9	3	50.6
11X-A	4	21	211.4	2	105.7
11X-B	4	21	251.7	5	50.3
11X-C	4	21	198.4	4	49.6
11X-A	4	24	209.9	4	52.5
11X-B	4	24	210.7	4	52.7
11X-C	4	24	200.3	4	50.1
11X-A	4	28	219.4	4	54.9
11X-B	4	28	211.7	4	52.9
11X-C	4	28	96.0	2	48.0
Total			5351.5	104	
Average weight of one egg					52.5



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.4.1-16: Individual egg production of laying hens in the 43X dose group; subgroups A to C

Dose Group-Subgroup	Study day [week 1]							Total # of eggs per week
	1	2	3	4	5	6	7	
11X-A	3	4	4	4	3	4	4	26
11X-B	1	3	4	5	3	4	6	26
11X-C	1	3	4	5	4	4	4	25
	Study day [week 2]							
	8	9	10	11	12	13	14	
11X-A	4	4	3	4	4	4	4	27
11X-B	4	4	3	5	2	4	4	26
11X-C	3	4	4	4	4	5	4	28
	Study day [week 3]							
	15	16	17	18	19	20	21	
11X-A	3	4	4	8	0	4	2	25
11X-B	6	4	4	5	2	4	5	30
11X-C	4	4	3	6	2	4	4	27
	Study day [week 4]							
	22	23	24	25	26	27	28	
11X-A	4	4	4	3	5	4	4	28
11X-B	1	6	4	1	5	4	4	25
11X-C	2	5	4	3	5	4	2	25
Total number of eggs							318	

Based on the average weight of an egg and the total number of the eggs laid during the study phase (day 1 to 28), the total egg weight in the present study was estimated to be **16695 g** for **twelve hens** ($52.5 \text{ g} \times 318 = 16695 \text{ g}$).

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.4.1-17: Calculation of the weighted residue level in eggs

Dose Group-Subgroup	Number of birds	Day of treatment	Weight of eggs [kg]	Total residue level [mg a.s. equiv./kg]	Absolute residue level [mg a.s. equiv.]
11X-A	4	2	0.2028	0.381	0.0773
11X-B	4	2	0.1498	0.345	0.0517
11X-C	4	2	0.1402	0.342	0.0479
11X-A	4	4	0.2001	0.808	0.1617
11X-B	4	4	0.2426	1.000	0.2426
11X-C	4	4	0.2351	0.952	0.2238
11X-A	4	7	0.2282	1.07	0.2442
11X-B	4	7	0.2870	1.10	0.3157
11X-C	4	7	0.1896	1.03	0.1953
11X-A	4	10	0.1546	1.35	0.2087
11X-B	4	10	0.1453	1.26	0.1831
11X-C	4	10	0.1860	1.27	0.2362
11X-A	4	14	0.2140	1.03	0.2204
11X-B	4	14	0.2037	1.12	0.2281
11X-C	4	14	0.1942	0.982	0.1907
11X-A	4	17	0.2152	1.02	0.2195
11X-B	4	17	0.2017	1.32	0.2662
11X-C	4	17	0.1519	1.09	0.1656
11X-A	4	21	0.2114	1.22	0.2579
11X-B	4	21	0.2517	1.2	0.3020
11X-C	4	21	0.1984	1.29	0.2559
11X-A	4	24	0.2099	1.43	0.2991
11X-B	4	24	0.2107	1.63	0.3434
11X-C	4	24	0.2003	1.50	0.2994
11X-A	4	28	0.2194	1.41	0.3094
11X-B	4	28	0.2117	1.68	0.3557
11X-C	4	28	0.0960	1.51	0.1450
Total			5.3515		6.0466
Weighted residue [mg a.s. equiv./kg]			1.130		

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)**Residue levels detected in animal matrices**

Table 6.4.1-18: Residue levels in animal matrices after dosing of BYI 02960 to laying hens

Dose level [mg/kg feed]	Residues [mg a.s. equiv./kg]				
	Animal matrix	BYI 02960	DFA	BYI 02960-acetyl-AMCP	BYI 02960-OH
1X (1.5 mg/kg dry feed)	eggs (day 28)	< 0.004 (LOD)	0.047	< 0.003 (LOD)	< 0.003 (LOD)
	fat	< 0.003 (LOD)	0.029	< 0.003 (LOD)	< 0.002 (LOD)
	liver	< 0.003 (LOD)	0.104	< 0.003 (LOD)	< 0.002 (LOD)
	muscle	< 0.004 (LOD)	0.083	< 0.003 (LOD)	< 0.003 (LOD)
4.3X (6.5 mg/kg dry feed)	eggs (day 28)	< 0.004 (LOD)	0.163	< 0.003 (LOD)	< 0.003 (LOD)
	fat	< 0.003 (LOD)	0.117	< 0.003 (LOD)	< 0.002 (LOD)
	liver	0.006	0.413	0.011	0.006
	muscle	< 0.004 (LOD)	0.290	0.010	< 0.003 (LOD)
13X (19.4 mg/kg dry feed)	eggs (day 28)	0.023	0.508	0.015	0.018
	fat	< 0.003 (LOD)	0.272	< 0.003 (LOD)	< 0.002 (LOD)
	liver	< 0.003 (LOD)	1.012	0.025	0.005
	muscle	0.004	0.719	0.024	0.005
43X (65.1 mg/kg dry feed)	egg composite	0.082 ¹	1.130 ¹	0.048 ¹	0.048 ¹
	fat	0.192	1.006	0.021	0.010
	liver	0.032	3.313	0.083	0.052
	muscle	0.039	2.270	0.068	0.032

¹ weighted residue values, according to Table 6.4.1-16

Table 6.4.1-19: Total residues calculated for animal matrices after dosing of BYI 02960 to laying hens

Dose level [mg/kg feed]	Residues [mg a.s. equiv./kg]		
	Animal matrix	total residue for data collection ¹	total residue for enforcement / risk assessment ²
1X (1.5 mg/kg dry feed)	eggs	0.077	0.057
	fat	0.059	0.039
	liver	0.134	0.114
	muscle	0.113	0.093
4.3X (6.5 mg/kg dry feed)	eggs	0.193	0.173
	fat	0.147	0.127
	liver	0.444	0.423
	muscle	0.320	0.300
13X (19.4 mg/kg dry feed)	eggs	0.565	0.532
	fat	0.302	0.282
	liver	1.054	1.022
	muscle	0.759	0.729
43X (65.1 mg/kg dry feed)	eggs	1.308	1.212
	fat	1.229	1.199
	liver	3.480	3.346
	muscle	2.409	2.309

¹ total residue for data collection: BYI 02960, DFA, BYI 02960-acetyl-AMCP and BYI 02960-hydroxy² total residue for enforcement / risk assessment: BYI 02960 and DFA**Remark:** All values <LOQ of 0.01 mg/kg were considered as being at 0.01 mg/kg to calculate the total residue

**IIA 6.4.2 Lactating ruminants (goat or cow)**

Report:	KIHA 6.4.2/01, Moore, S.M., & [REDACTED], A.M.; 2012
Title:	BYI 02960 – Magnitude of the residue in dairy cows
Report No. & Document No.:	RARVP050 M-428416-01-1
Guidelines:	– OPPTS 860.1480 – Meat/milk/poultry/eggs – OECD Guideline 505 – APVMA Residue Guideline No. 23 – DACO 7.5 – Meat/milk/poultry/eggs
GLP:	yes (certified laboratory)

I. Materials and MethodsTest system, dosing

Twenty Holstein dairy cows (*Bos Taurus*, approximately 2.5 to 3.5 years of age) purchased from a local dairy were transferred to Southwest Bio-Labs SWB's Animal Care Facility fourteen days prior to the initiation of the study (study day -14). Following randomization, the animals were labeled by study number, pen number, dose group (when assigned) and animal identification. The animals were allowed *ad libitum* access to water and feed throughout the study.

Dose rates used in this study were calculated according to both EU (Appendix G, Livestock feeding studies, 70.1/VI/95 rev4 [1996] and in Annex 4 of the OECD Guidance Document ENV/JM/MONO [2006]32, European Food Safety Authority [EFSA]) and NAFTA (Revisions of Feedstuffs in Table 1 of OPPTS Test Guideline 860.1000 and Guidance on Constructing Maximum Reasonably Balanced Diets [MRBD], Table 1 Feedstuffs [June 2008]) guidance.

Individual cows were weighed within 24 hours of initial dosing and the dose rate (mg BYI 02960/kg body weight) was calculated, based on this weight, and administered daily each study day. The target and actual dose rates employed in the study are summarized below in table 6.4.2-1.

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Table 6.4.2-1: Summary of target and actual BYI 02960 dose administration.

EU ¹	Dose groups NAFTA ²	Number of cows	Dose levels in feed		per animal actual ⁵ (mg a.s./kg b.w./day)
			target ³ (mg/kg feed)	actual ⁴ (mg/kg feed)	
	control	2	0	0	0
1.3X dose	0.2X dose	4	5.5	4.8	0.184
6.3X dose	0.9X dose	3	27	23	0.898
13X dose	1.8X dose	3	55	50	1.838
34X dose	4.9X dose	7	147	135	4.90

Footnotes:

1: EU dose rate exaggerations are based on EU dietary burden of 4.3 mg a.s./kg feed (see table 6.4-2)

2: NAFTA dose rate exaggerations are based on NAFTA dietary burden of 30.2 mg a.s./kg feed (see table 6.4-4). These are the values shown in the report RARVP050.

3: Target dose was calculated based on NAFTA and EU dietary burdens (tables 6.4-2 and 6.4-4) and est. 20 kg/d food consumption

4: Actual dose based on average feed consumption data collected from the study and amount (mg) test substance for each dose group over the entire dosing period

5: Actual dose based on average amount (mg) test substance and the average body weight for each dose over the entire dosing period

Cows were randomly assigned to the five dose groups: control, EU 1.3X, 6.3X (=0.9X for the NAFTA calculation), 13X, and 34X. Two cows were assigned as controls, four cows were assigned to the 1.3X treatment group (one of which was designated as a urine-collection cow), three cows were each assigned to the 6.3X and to the 13X treatment groups, and seven cows were assigned to the 34X group (three of which were designated as depuration cows and one as a urine collection cow.) The animals were dosed orally once per day each morning after milking and feeding. The control animal received a placebo (empty capsule) concurrently with the treated animals.

Sampling

Milk was collected twice daily (afternoon and morning), and the milk was weighed after each milking. On study days 0, 2, 4, 7, 10, 14, 17, 19, 25, 28, 29, 30, 31, 35, 38, and 42, a subsample of the evening milk was retained (refrigerated) and then composited with the morning milk proportional to the amount collected at each milking. Samples from all of these sampling events were analyzed for the 34X group; only day-28 samples were analyzed from the other dose groups. Additional 25-day milk was collected for processing into whey (skim milk) and cream (milk fat) from the one control cow and three of the 34X dose group cows.

On study day 29, all animals except the four depuration cows were humanely sacrificed at least three hours following the last dose. The depuration cows (34X) were sacrificed on study days 32, 36, and 43 (a control cow was also sacrificed on day 43).

Representative samples of liver (each lobe), kidney (center and ends), fat (approximately equal composite of omental, renal, and subcutaneous), and muscle (composite of loin, round, and flank) were collected. The tissue samples were homogenized in the presence of dry ice and the homogenized samples were placed in a freezer prior to shipping.

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To estimate the levels of exposure of dairy cows to DFA residues following administration of BYI 02960, urine was collected from two cows (one each from 1.3X and 34X dose groups). Starting on study day 1, prior to morning dosing, the 24-hour collected sample from each cow was weighed and a 50-g aliquot was subsampled. (Unfortunately, these samples were not always composited in a manner proportional to the amounts collected. However, since a 24-hour period was always covered, the "inaccuracy" will be negligible.) These subsamples were pooled by week, resulting in one sample per week for four weeks. The samples were frozen prior to shipping.

All samples were shipped frozen to Bayer CropScience for analysis.

Analysis

BYI 02960 and its metabolites were analytically determined using analytical method RV-004-A11-04 (supplied as an appendix to the main study report; cf. KIIA 4.3/08 for details relating to the method), which was validated prior to and parallel to the residue analysis of the samples. The LOQ was 0.01 mg/kg for all analytes (parent compound, BYI 02960-acetyl-AMCP, and BYI 02960-OH) except DFA, for which it was 0.02 mg/kg (0.05 mg/kg in whey). All values are expressed in BYI 02960 equivalents.

II. Findings

Main study

The mean values of the concurrent recovery rates per compound, sample material, and spiking level were generally in the range of 70-110%, with very few (and minor) exceptions. As the relative standard deviations were always low – in the range of 0-13% – all concurrent recoveries were considered to be acceptable. Details of recovery data are shown in table 6.4.2-4.

Feed consumption and milk production were not adversely affected by treatment with BYI 02960. Although some animals gained weight while others lost weight, average body weights decreased slightly in all groups, including the control, but somewhat more in the highest dose group. Thus it appears that 34X dosing may have had an effect on body weight. One cow in the 34X group suffered health complications (chronic mastitis, metritis) for which she was treated, but which could not be resolved during the study period. Her condition affected both her feed consumption and body weight, although no effect was evident with respect to milk production.

In the groups representing the nominal worst-case EU and NAFTA dietary burden (EU 1.3X and 6.3X groups, respectively), the total residues of BYI 02960 – comprising parent compound, DFA, BYI 02960-acetyl-AMCP, and BYI 02960-OH – were measured at sacrifice in bovine tissues and were as follows: 0.057 and 0.307 mg/kg in muscle, 0.061 and 0.151 mg/kg in fat, 0.159 and 0.824 mg/kg in liver, and 0.193 and 0.893 mg/kg in kidney, respectively. In milk taken on day 28, total residues in the 1.3X and 6.3X groups were 0.063 and 0.125 mg/kg, respectively. These values, as

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

shown in the study report (████ & █████, 2012; KIIA 6.4.2/01), reflect the standard practice in the USA of using all residue values as shown, even if they are below the nominal LOQs.

As the proposed residue definition for both enforcement and risk assessment will include only parent BYI 02960 + DFA, which are by far the two major components of the residue, the critical values for this study may not reflect the total of all components as listed above. In the nominal worst-case EU and NAFTA dietary burden groups (EU 1.3X and 6.3X groups, respectively), the combined residues of the two components (calculated in the "traditional" manner, i.e. if one component is <LOQ, it is calculated as being *at* the LOQ) were as follows: 0.063 and 0.304 mg/kg in muscle, 0.041 and 0.147 mg/kg in fat, 0.165 and 0.812 mg/kg in liver, and 0.180 and 0.867 mg/kg in kidney, respectively. In milk taken on day 28, total residues in the 1.3X and 6.3X groups were 0.043 and 0.129 mg/kg, respectively.

In addition to the general testing, day-25 milk samples from the 34X dose group were separated into cream and whey, in order to determine if the residues preferentially collect in more aqueous or fatty compartments. The processing/transfer factors (PF) for the total residue (four components) indicated a very slight preference for more aqueous conditions, with a PF of 1.15 to whey and 0.79 to cream. These factors were also representative for the 2 analytes of primary concern, BYI 02960 parent compound and DFA.

The residues found in the milk, tissues, and excreta collected from the dairy cows during dosing, at the end of the dosing period, and during the depuration phase are presented in tables 6.4.2-2 and 6.4.2-3.

With respect to milk, the highest total BYI 02960 residues were found in the day-17 sample from the 34X dose group. Thus, BYI 02960 residues reached a plateau between at that time and remained at that approximate level for the remainder of the dosing period. The highest total residues in milk at any time in the study in this group were 0.979 mg/kg (total of 4 components); when evaluating only the two analytes of import, BYI 02960 and DFA, the level was 0.974 mg/kg. This was only slightly higher than 0.886 and 0.806 mg/kg, the values determined in the 34X group on days 28 and 29, respectively.

In the depuration phase, total BYI 02960 residues in milk, fat, liver, kidney, and muscle from the 34X dose group cows declined from 0.81, 1.40, 1.59, 5.39, and 1.91 mg/kg, respectively, to <LOQ at 6-7 days after cessation of dose administration (=day 35-36 of the study). The residue data provided in this study are suitable for regulatory purposes. (Depuration data are also presented in tables 6.4.2-2 and 6.4.2-3.)

The levels of DFA residues found in urine are summarized in table 6.4.2-5. DFA residues represented 6% to 8% of the total residues of BYI 02960 in the urine samples, suggesting low but significant exposure of the dairy cattle to DFA following the daily oral administration of BYI 02960 over 29 days.

Transfer factors for total residues and for DFA alone

Additional calculations were conducted to describe the transfer of both total residues and of DFA alone into bovine tissues and milk following exposure to BYI 02960 and DFA via the diet. As they were not part of the main study and are not included in the study report RARVP050 (████████, 2012; KIIA 6.4.2/01), they are not presented here, but rather later in this section.

III. Conclusions (main study)

A feeding study was conducted with BYI 02960 on dairy cattle in order to elucidate the levels of relevant residues in bovine tissues and in milk. The study was 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.

BYI 02960 was administered orally (via capsule) to dairy cows for 29 consecutive days at average *actual* dose rates of 4.8 mg/kg feed (1.3X EU dose), 23 mg/kg feed (6.3X, which approximated a 0.9X NAFTA dose), 50 mg/kg feed (13X), and 135 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 at various intervals in the high-dose group, and on day 28 in the three lower dose groups. In the EU 1.3X dose group, the residues (BYI 02960 + DFA) amounted to 0.043 mg/kg.

Residue levels reached a plateau in milk. Highest residue were determined at day 17 in the 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.

DFA residues represented 6% to 8% of the total BYI 02960 residues in the urine samples, suggesting low but significant exposure of the dairy cows to DFA residues following the administration of BYI 02960 for 29 days.

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Table 6.4.2-2: Levels of the relevant residues of BYI 02960 in milk

group	sampling day	Residue levels of individual analytes (mg/kg)				Total residue levels (mg/kg)	
		BYI 02960	DFA	-AMCP*	-OH	sum of 4**	BYI 02960 + DFA
1.3X [▲]	28	0.023	<0.02	<0.01	<0.01	0.028	0.043
6.3X	28	0.108	0.021	<0.01	<0.01	0.125	0.129
13X	28	0.267	0.041	<0.01	<0.01	0.310	0.308
34X [▲]	2	0.759	0.081	<0.01	<0.01	0.831	0.840
	4	0.869	0.105	<0.01	<0.01	0.978	0.973
	7	0.688	0.138	<0.01	<0.01	0.830	0.826
	10	0.763	0.137	<0.01	<0.01	0.906	0.900
	14	0.783	0.151	<0.01	<0.01	0.940	0.935
	17	0.831	0.143	<0.01	<0.01	0.979	0.974
	19	0.825	0.130	<0.01	<0.01	0.960	0.955
	25	0.651	0.115	<0.01	<0.01	0.770	0.765
	25 [†]	0.553	0.050	<0.01	<0.01	0.607	0.603
	25 [‡]	0.758	0.123	<0.01	<0.01	0.887	0.881
	28	0.748	0.138	<0.01	<0.01	0.892	0.886
	29***	0.667	0.140	<0.01	<0.01	0.811	0.806
	30***	0.059	0.078	<0.01	<0.01	0.138	0.137
	31***	<0.01	0.043	<0.01	<0.01	0.050	0.053
	35***	<0.01	<0.02	<0.01	<0.01	<0.05	<0.03

* AMCP = BYI 02960-acetyl-AMCP

** this value, as shown in the study report, includes values below the LOQ calculated at the apparent residue value

*** depuration phase, no dosing (sampling days 35-49)

† values for cream

‡ values for whey

▲ day-28 values for the 1.3X and 34X groups include samples from all animals (including "urine" and "depuration" animals)



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Table 6.4.2-3: Levels of the relevant residues of BYI 02960 in bovine tissues

group	sampling day	Residue levels of individual analytes (mg/kg)				Total residue levels (mg/kg)	
		BYI 02960	DFA	-AMCP*	-OH	sum of 4 [‡]	BYI 02960 + DFA
<i>BOVINE FAT</i>							
1.3X [†]	29	0.021	<0.02	<0.01	<0.01	0.032	0.041
6.3X	29	0.109	0.038	<0.01	<0.01	0.151	0.147
13X	29	0.285	0.099	<0.01	<0.01	0.388	0.384
34X [†]	29	0.977	0.392	<0.01	0.020	1.390	1.369
	32**	<0.01	0.100	<0.01	<0.01	0.111	0.110
	36**	<0.01	<0.02	<0.01	<0.01	0.019	<0.03
	43**	<0.01	<0.02	<0.01	<0.01	<LOD	<0.03
<i>BOVINE KIDNEY</i>							
1.3X [†]	29	0.159	0.017	<0.01	0.019	0.193	0.176
6.3X	29	0.786	0.081	<0.01	0.026	0.893	0.867
13X	29	1.789	0.203	<0.01	0.045	2.037	1.992
34X [†]	29	4.720	0.558	<0.01	0.103	5.380	5.279
	32**	0.045	0.141	<0.01	<0.01	0.187	0.186
	36**	<0.02	<0.01	<0.01	<0.01	0.010	<0.03
	43**	<0.02	<0.01	<0.01	<0.01	<LOD	<0.03
<i>BOVINE LIVER</i>							
1.3X [†]	29	0.145	<0.02	<0.01	0.011	0.159	0.165
6.3X	29	0.755	0.057	<0.01	0.011	0.824	0.812
13X	29	1.680	0.132	<0.01	0.020	1.842	1.812
34X [†]	29	3.451	0.390	<0.01	0.035	3.890	3.841
	32**	0.033	0.106	<0.01	<0.01	0.140	0.139
	36**	<0.02	<0.01	<0.01	<0.01	0.012	<0.03
	43**	<0.02	<0.01	<0.01	<0.01	<LOD	<0.03
<i>BOVINE MUSCLE</i>							
1.3X [†]	29	0.043	<0.02	<0.01	<0.01	0.057	0.063
6.3X	29	0.250	0.054	<0.01	<0.01	0.307	0.304
13X	29	0.597	0.136	<0.01	<0.01	0.739	0.733
34X [†]	29	1.505	0.385	<0.01	0.014	1.901	1.890
	32**	0.017	0.095	<0.01	<0.01	0.114	0.112
	36**	<0.02	<0.01	<0.01	<0.01	0.018	<0.03
	43**	<0.02	<0.01	<0.01	<0.01	<LOD	<0.03

* AMCP = BYI 02960-acetyl-AMCP

** depuration phase, no dosing (sampling days 35-49)

† day-29 values for the 1.3X and 34X groups include samples from all animals (including "urine" and "depuration" animals)

‡ this value, as shown in the study report, includes values below the LOQ calculated at the apparent residue value



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Table 6.4.2-4: Concurrent recovery data for the relevant residues of BYI 02960 in bovine matrices

Study No.	Matrix	a.s./metabolite	n	Spike Level (mg/kg)	Individual recoveries	Recovery (%) ^a			
						Min	Max	Mean	RSD
RARVP050	milk	BYI 02960	7	0.010	109,109,108, 110,108,107,102	102	110	108	2
			1	0.0250	107	107	107		
			5	0.050	10,102,97, 105, 105	97	105	102	3
			3	0.10	103, 109 ,104	103	109	105	3
			1	0.250	103	103	103		
			3	2.0	10, 102 ,103	10	103	102	2
		difluoroacetic acid	7	0.020	85,88,80,88,92, 76, 84	76	88	85	6
			7	0.050	81,77,78,89,91, 93, 93	77	93	86	8
			3	0.20	91, 92 ,94	91	94	92	2
			3	0.40	85, 85 ,93	85	93	88	5
		BYI 02960-acetyl-AMCP	7	0.010	103,105,104,104, 109 ,106, 101	101	109	105	2
			1	0.0250	112	112	112		
			5	0.050	103, 102 ,103, 109, 107	102	109	105	3
			3	0.10	108, 112 ,107	107	112	109	2
			1	0.250	103	103	103		
			3	2.0	98, 99 ,99	98	99	99	1
		BYI 02960-OH	7	0.010	115,110,111,105, 113 ,110, 108	105	115	110	3
			1	0.0250	106	106	106		
			5	0.050	101, 104 ,10, 106, 106	10	106	103	3
			3	0.10	110, 108 ,105	105	110	108	2
			1	0.250	105	105	105		
			3	2.0	101, 105 ,101	101	105	102	2
cream	cream	BYI 02960	7	0.010	109,112,107,118, 105 ,110, 106	105	118	110	4
			3	0.10	109, 107 ,113	107	113	110	3
			3	1.0	111, 109 ,111	109	111	110	1
		difluoroacetic acid	7	0.020	103, 95 ,89, 93, 85 ,103, 96	85	103	95	7
			3	0.20	101, 102 ,106	101	106	103	3
		BYI 02960-acetyl-AMCP	7	0.010	106, 108 ,102, 113, 93 ,98, 93	93	113	102	8
			3	0.10	111, 111 ,114	111	114	112	2
			3	1.0	114, 109 ,115	109	115	113	3
		BYI 02960-OH	7	0.010	116,115,113,124, 107 ,109, 111	107	124	114	5
			3	0.10	110, 111 ,113	110	113	111	1
			3	1.0	114, 111 ,114	111	114	113	2

All concentrations are expressed in parent compound equivalents.

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Table 6.4.2-4 (cont'd): Concurrent recovery data for the relevant residues of BYI 02960 in bovine matrices

Study No.	Matrix	a.s./metabolite	n	Spike Level (mg/kg)	Individual recoveries	Recovery (%) ^a			
						Min	Max	Mean	RSD
RARVP050	whey	BYI 02960	7	0.010	98, 99, 10, 98, 105, 101, 104	98	105	101	3
			7	0.0250	104, 106, 102, 102, 101, 102, 106	101	106	103	2
			3	0.10	101, 102, 102	101	102	102	1
			3	0.250	105, 103, 104	103	105	104	1
			3	1.0	102, 10, 104	10	104	102	2
		difluoroacetic acid	7	0.050	96, 94, 95, 95, 87, 96, 89	87	96	93	4
			3	0.50	102, 94, 99	94	102	98	4
		BYI 02960-acetyl-AMCP	7	0.010	99, 10, 102, 103, 104, 103, 106	99	106	102	2
			3	0.10	102, 102, 103	102	103	102	1
		BYI 02960-OH	7	0.010	104, 104, 104, 104, 102, 10, 103	10	104	103	1
			7	0.0250	107, 112, 104, 109, 107, 108, 107	104	112	108	2
			3	0.10	101, 10, 102	10	102	101	1
			3	0.250	106, 112, 107	106	112	108	3
	fat	BYI 02960	7	0.010	104, 101, 105, 96, 107, 106, 103	96	107	103	4
			2	0.050	93, 10	93	10	97	
			3	0.10	97, 10, 101	97	101	99	2
			3	1.50	92, 95, 99	92	99	95	4
		difluoroacetic acid	7	0.020	81, 96, 88, 95, 92, 86, 87	81	96	89	6
			2	0.050	89, 93	89	93	91	
			3	0.60	88, 89, 90	88	90	89	1
		BYI 02960-acetyl-AMCP	7	0.010	94, 97, 10, 98, 99, 97, 96	94	10	97	2
			2	0.050	97, 98	97	98	98	
			3	0.10	96, 98, 101	96	101	98	3
			3	1.50	101, 102, 106	101	106	103	3
		BYI 02960-OH	7	0.010	106, 107, 109, 101, 108, 107, 105	101	109	106	2
			2	0.050	98, 105	98	105	102	
			3	0.10	97, 101, 106	97	106	101	4
			3	1.50	94, 97, 98	94	98	96	2

All concentrations are expressed in parent compound equivalents.

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Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.4.2-4 (cont'd): Concurrent recovery data for the relevant residues of BYI 02960 in bovine matrices

Study No.	Matrix	a.s./metabolite	n	Spike Level (mg/kg)	Individual recoveries	Recovery (%) ^a			
						Min	Max	Mean	RSD
RARVP050	kidney	BYI 02960	7	0.010	98,99,95,91,94,98, 95	91	99	96	3
			3	0.050	93, 87, 98	87	98	93	6
			3	0.10	96, 96, 94	94	96	95	1
			3	6.0	90, 92, 97	90	97	93	4
		difluoroacetic acid	7	0.020	74, 73, 74, 79, 66, 69, 66	66	79	72	7
			2	0.050	69, 72	69	72	71	
			3	0.80	84, 79, 84	79	84	82	4
		BYI 02960-acetyl-AMCP	7	0.010	102, 103, 105, 99, 99, 99, 96	96	105	10	3
			3	0.050	10, 94, 90	90	10	95	5
			3	0.10	98, 99, 97	97	99	98	1
			3	6.0	91, 93, 95	91	95	93	2
		BYI 02960-OH	7	0.010	101, 90, 99, 94, 90 , 105, 98	90	105	97	6
			3	0.050	97, 93, 95	93	97	95	2
			3	0.10	98, 98, 98	98	98	98	0
			3	6.0	95, 94, 10	94	10	96	3
	liver	BYI 02960	7	0.010	98, 90, 89, 93, 91, 85, 84	84	98	90	5
			3	0.050	96, 98, 96	96	98	97	1
			3	0.10	87, 88, 91	87	91	89	2
			3	4.0	90, 93, 91	90	93	91	2
		difluoroacetic acid	7	0.020	87, 75, 97, 68, 73, 73, 82	68	97	79	13
			2	0.050	64, 72	64	72	68	
			3	0.60	79, 80, 89	79	89	83	7
		BYI 02960-acetyl-AMCP	7	0.010	99, 85, 104, 93, 98 , 86, 80	80	104	92	9
			3	0.050	103, 109, 95	95	109	102	7
			3	0.10	93, 92, 98	92	98	94	3
			3	4.0	93, 94, 94	93	94	94	1
		BYI 02960-OH	7	0.010	93, 88, 88, 90, 95, 90, 87	87	95	90	3
			3	0.050	98, 10, 93	93	10	97	4
			3	0.10	91, 91, 92	91	92	91	1
			3	4.0	97, 98, 95	95	98	97	2

All concentrations are expressed in parent compound equivalents.

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Table 6.4.2-4 (cont'd): Concurrent recovery data for the relevant residues of BYI 02960 in bovine matrices

Study No.	Matrix	a.s./metabolite	n	Spike Level (mg/kg)	Individual recoveries	Recovery (%) ^a			
						Min	Max	Mean	RSD
RARVP050	muscle	BYI 02960	7	0.010	92, 97, 92, 10, 96, 98, 10	92	10	96	3
			2	0.050	95, 99	95	99	97	
			3	0.10	93, 98, 93	93	98	95	3
			3	2.0	92, 90, 89	89	92	90	2
		difluoroacetic acid	7	0.020	66, 71, 76, 76, 77, 78, 73	66	78	74	6
			3	0.050	71, 78, 70	70	78	73	6
			3	0.50	67, 65, 73	65	73	68	6
		BYI 02960-acetyl-AMCP	7	0.010	89, 101, 92, 98, 98, 101, 99	89	101	97	5
			2	0.050	95, 103	95	103	99	
			3	0.10	93, 103, 91	91	103	96	7
			3	2.0	96, 96, 95	95	96	96	1
		BYI 02960-OH	7	0.010	93, 10, 106, 95, 101, 101, 99	93	106	99	4
			2	0.050	117, 96	96	117	107	
			3	0.10	96, 101, 94	94	101	97	4
			3	2.0	92, 92, 92	92	92	92	0
	urine	BYI 02960	7	0.010	102, 104, 103, 103, 104, 103, 101	101	104	103	1
			3	0.10	102, 102, 102	102	102	102	0
			3	40.0	109, 103, 104	103	109	105	3
		difluoroacetic acid	7	0.020	78, 85, 67, 73, 79, 70, 76	67	85	75	8
			3	0.20	92, 91, 92	91	92	92	1
			3	5.0	106, 102, 101	101	106	103	3
		BYI 02960-acetyl-AMCP	7	0.010	112, 119, 106, 109, 113, 111, 110	106	119	111	4
			3	0.10	108, 109, 107	107	109	108	1
			3	40.0	109, 105, 107	105	109	107	2
		BYI 02960-OH	7	0.010	109, 116, 110, 108, 113, 113, 112	108	116	112	2
			3	0.10	107, 105, 105	105	107	106	1
			3	40.0	110, 106, 105	105	110	107	2

All concentrations are expressed in parent compound equivalents.



Table 6.4.2-5: Proportion of DFA in the total residues of BYI 02960 in urine

Matrix	Dose group	Sampling week	Urine collected (kg)*	Total BYI 02960 residue** (mg/kg)	DFA residues† (mg/kg)	Proportion of DFA in total residue (%)
urine	1.3X	week 1	146	1.85	0.112	6
		week 2	122	2.18	0.185	8
		week 3	125	2.24	0.184	8
		week 4	142	1.81	0.142	8
	34X	week 1	226	29.1	1.92	7
		week 2	218	30.8	2.33	8
		week 3	159	46.0	3.71	8
		week 4	152	40.9	3.33	8

* Daily average (determined for each of the four study weeks) was multiplied by 7 days to determine the weekly urine output. Both the 1.3X and 34X animals experienced some hose blockages; the urine lost during these events was estimated by the in-life phase facility but is not included

** "total BYI 02960 residue" is the sum of BYI 02960, BYI 02960-OH, BYI 02960-acetyl-AMCP, and DFA residues, expressed in BYI 02960 equivalents

† expressed in BYI 02960 equivalents

► Transfer factors calculated for DFA and the total residue

As agreed with the EU Rapporteur (Ctgb, NL), separate transfer factors for DFA were estimated from the cattle feeding study on the basis of the data available after dosage of the active substance BYI 02960 to lactating cows. In order to accomplish this, a theoretical dose of DFA must be estimated. For this estimation, the amounts of difluoroacetic in all organs/tissues and particularly in urine must be considered. In the rat ADME study, the major amount of the total radioactivity, i.e. > 80% of the dose administered, was excreted via the urine. DFA is a polar compound with a low molecular weight, so it can be assumed that is excreted mainly via the urine. Thus the role of excretion via faeces will be minimal, and this matrix was not considered in the dairy cow balance. The absolute amount of DFA formed in the cows and which was thus systemically available can then be equated with a minimum dose theoretically fed to the animals.

The estimation of the dose of DFA theoretically fed to the animals was conducted on the basis of the data collected for the 34X dose group (4.9X dose group in NAFTA), using the animals 5152, 5155, 5158, and animal 5153 for collection of urine (actual mean dose of BYI 02960 = 135 mg/kg feed), and additionally on the basis of the data collected for the 1.3X dose group using animals 5150, 5159, 5163, and animal 5151 for collection of urine (actual mean dose of BYI 02960 = 4.8 mg/kg feed).

For a better understanding, the data used in the different calculations are summarized separately at the end of this section in tables, as listed here:

- Table 6.4.2-10: Calculation of the mean body weight of cattle in the 34X and 1.3X dose group
- Table 6.4.2-11: Estimation of the kidney weight as percent of the body weight
- Table 6.4.2-12: Estimation of the liver weight as percent of the body weight
- Table 6.4.2-13: Weight of organs and tissues of the lactating cows in the 34X and 1.3X dose group



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- Table 6.4.2-14: Residue levels of DFA in organs and tissues of the lactating cows (34X dose group)
- Table 6.4.2-15: Residue levels of DFA in organs and tissues of the lactating cows (1.3X dose group)
- Table 6.4.2-16: Individual urine production of lactating cows (34X dose group)
- Table 6.4.2-17: Individual urine production of lactating cows (1.3X dose group)
- Table 6.4.2-18: Calculation of the weighted DFA concentration in urine of lactating cows (34X dose group)
- Table 6.4.2-19: Calculation of the weighted DFA concentration in urine of lactating cows (1.3X dose group)
- Table 6.4.2-20: Individual milk production of lactating cows (34X dose group)
- Table 6.4.2-21: Calculation of the weighted DFA concentration in milk (34X dose group)
- Table 6.4.2-22: Residue levels in animal matrices after dosing of BYI 02960 to lactating cows
- Table 6.4.2-23: Total residues calculated for animal matrices after dosing of BYI 02960 to lactating cows

Minimum dose theoretically fed to lactating cows:

The residue levels in urine, milk, tissues and organs and the weights of the animal matrices (average weight for one animal) used for the calculation of the minimum theoretical dose are summarized in Table 6.4.2-6 (34X and 1.3X dose).

Table 6.4.2-6: Calculation of absolute DFA amounts in cattle matrices on the basis of the sample weights and the residues in the samples

Animal matrix	Weight [kg]	DFA residue* [mg a.s. equiv./kg]	Absolute DFA amounts [mg a.s. equiv.]
Actual dose administered: 135 mg/kg dry feed (34X)			
milk composite (day 1-28)	874	0.118 ⁵	103.13
muscle	162 ¹	0.381	61.72
fat	64.9 ²	0.335	21.74
liver	8.1 ³	0.380	3.078
kidneys	1.4 ⁴	0.512	0.717
urine	754	2.70 ⁵	2035.8
total (one animal)			2226.2
Actual dose administered: 4.8 mg/kg dry feed (1.3X)			
milk (day 28)	845	0.004	3.50
muscle	165 ¹	0.013	2.09
fat	65.9 ²	0.011	0.75
liver	8.2 ³	0.012	0.10
kidneys	1.4 ⁴	0.018	0.02
urine	535	0.153 ⁵	82.10
total (one animal)			88.56

* milk and organs/tissues:

average/weighted residues were calculated for the "residue animals" using samples from animals 5152, 5155, and 5158 for the 34X dose group and from animals 5150, 5159, and 5158 for the 1.3X dose group

urine:

residues were calculated from samples of the "urine collection" animals 5153 (34X) and 5151 (1.3X)

¹ total muscle weight was calculated assuming a value of 30% of the mean body weight for this tissue

² total fat weight was calculated assuming a value of 12% of the mean body weight for this tissue

³ total liver weight was calculated assuming a value of 1.5% of the mean body weight for this tissue

⁴ total kidney weight was calculated assuming a value of 0.25% of the mean body weight for this tissue

⁵ weighted DFA residue value, see Table 6.4.2-18 (urine 34X), Table 6.4.2-19 (urine 1.3X) and Table 6.4.2-20 (milk 34X)

According to the balance established, the minimum amount of DFA systemically available was **2226.2 mg a.s. equiv. per animal** in the 34X dose experiment and **88.6 mg a.s. equiv. per animal** in the 1.3X dose experiment.

Calculation of transfer factors:**34X dose:**

The total amount of BYI 02960 administered in the 34X dose experiment of the feeding study accounted for 76876 mg per animal (4.9 mg/kg bw/day × 541 kg bw × 29 days). Thus the total amount of DFA being systemically available represented 2.9% of the amount administered as BYI 02960.



Expressing the dose of BYI 02960 in [mg/kg dry feed], the mean dose level in the 34X dose group accounted for 135 mg a.s./kg dry feed. The theoretical dose of DFA can thus be calculated as being **3.9 mg a.s. equiv./kg dry feed** ($135 \text{ mg a.s./kg feed} \times 2.9\% = 3.9 \text{ mg a.s. equiv./kg dry feed}$).

1.3X dose:

The total amount of BYI 02960 administered in the 1.3X dose experiment of the feeding study accounted for 2929.5 mg per animal ($0.184 \text{ mg/kg bw/day} \times 549 \text{ kg bw} \times 29 \text{ days}$). Thus the total amount of DFA being systemically available represented 3.0% of the amount administered as BYI 02960. This proportion fits very well with the percentage calculated for the 34X dose (2.9% of the BYI 02960 dose administered).

Expressing the dose of BYI 02960 in [mg/kg dry feed], the mean dose level in the 1.3X dose group accounted for 4.8 mg a.s./kg dry feed. The theoretical dose of DFA can thus be calculated as being **0.14 mg a.s. equiv./kg dry feed** ($4.8 \text{ mg a.s./kg feed} \times 3.0\% = 0.14 \text{ mg a.s. equiv./kg dry feed}$).

On the basis of the DFA residues in milk and organs/tissues and the theoretical dose of DFA, transfer factors have been calculated, according to the following equation:

$$\text{TF} = \frac{\text{residue level in edible commodity}}{\text{residue level in the diet}}$$

The following tables summarise the residue values determined in the animal matrices and the corresponding transfer factors derived from them.

DFA transfer:

Assuming that the theoretical dose of DFA is reflected by the same proportion for all dose levels (as shown for the 34X and the 1.3X dose), transfer factors for DFA were estimated at all levels. In contrast to the 34X dose experiment (where weighted residues in milk have been calculated), residues in milk were estimated in all other dose groups on the basis of the 28-day sample, i.e. at a point in time at which the plateau had clearly been reached.

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Table 6.4.2-7: Transfer factors calculated for DFA

Animal matrix	DFA residue* [mg a.s equiv./kg]	Transfer factor
Theoretical DFA dose administered: 3.9 mg/kg dry feed; expressed in parent equivalents (34X)		
milk composite (days 1 to 28)	0.118	0.030
muscle	0.381	0.097
fat	0.335	0.086
liver	0.380	0.097
kidney	0.512	0.131
Theoretical DFA dose administered: 1.45 mg/kg dry feed; expressed in parent equivalents (13X)		
milk (day 28)	0.041	0.028
muscle	0.136	0.094
fat	0.099	0.068
liver	0.132	0.091
kidney	0.203	0.140
Theoretical dose administered: 0.67 mg/kg dry feed; expressed in parent equivalents (6.3X)		
milk (day 28)	0.016	0.024
muscle	0.054	0.081
fat	0.038	0.057
liver	0.057	0.085
kidney	0.081	0.121
Theoretical dose administered: 0.14 mg/kg dry feed; expressed in parent equivalents (1.3X)		
milk (day 28)	0.004	0.028
muscle	0.013	0.088
fat	0.011	0.079
liver	0.012	0.086
kidney	0.018	0.125

* average/weighted residues were calculated using samples from animals 5152, 5155, 5158 ("residue" animals) for the 34X dose group and from animals 5150, 5159 and 5158 for the 1.3X dose group, in agreement with calculation of residues in the samples of all other dose groups which consisted of "residue" animals, only. Samples of the "urine collection" (34X and 1.3X) and "depuration" animals (34X) were not included.

Comparing the transfer factors estimated for the animal matrices at different dose levels, it is obvious that the transfer factors correspond very well. The transfer factors for all matrices were in a very narrow range, e.g. the transfer factor for milk ranged from 0.024 to 0.030 and the one for muscle from 0.081 to 0.097. The residue levels in the animal matrices increase linearly with increasing dose levels.

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Transfer of BYI 02960 residues:

The transfer of BYI 02960 residues into edible matrices was also calculated on the basis of the dose administered to the animals in [mg/kg dry feed] and the total residue of BYI 02960 detected in the animal matrices in [mg/kg].

Since the total residue for data collection is different from the relevant residue for enforcement and risk assessment, separate transfer factors were calculated for all animal matrices in all dose groups tested. The total residue as defined for data collection comprises the compounds BYI 02960, BYI 02960-OH, BYI 02960-acetyl-AMCP, and DFA, whereas only parent compound BYI 02960 + DFA were proposed as relevant residue for enforcement and risk assessment (cf. KIIA 6.7.1).

The following tables refer to calculations on the basis of the total residue for data collection (BYI 02960, BYI 02960-OH, BYI 02960-acetyl-AMCP and DFA):

Table 6.4.2-8: Transfer factors calculated for the total residue consisting of parent compound BYI 02960, BYI 02960-acetyl-AMCP, BYI 02960-OH and DFA

Animal matrix	Total residue* [mg a.s equiv./kg]	Transfer factor
Average dose administered: 135 mg/kg dry feed (34X)		
milk composite (days 1 to 28)	0.942 (0.928)	0.007 (0.007)
muscle	1.784 (1.776)	0.013 (0.013)
fat	1.206 (1.197)	0.009 (0.009)
liver	3.903 (3.896)	0.029 (0.029)
kidney	5.027 (5.018)	0.037 (0.037)
Average dose administered: 50 mg/kg dry feed (13X)		
milk (day 28)	0.328 (0.310)	0.007 (0.006)
muscle	0.753 (0.740)	0.015 (0.015)
fat	0.404 (0.388)	0.008 (0.008)
liver	1.841 (1.834)	0.037 (0.037)
kidney	2.046 (2.037)	0.041 (0.041)

Table continued on next page...

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Table 6.4.2-9 (cont'd): Transfer factors calculated for the total residue consisting of parent compound BYI 02960, BYI 0296-acetyl-AMCP, BYI 02960-OH and DFA

Animal matrix	Total residue* [mg a.s equiv./kg]	Transfer factor
Average dose administered: 23 mg/kg dry feed (6.3X)		
milk (day 28)	0.148 (0.126)	0.006 (0.005)
muscle	0.324 (0.308)	0.014 (0.013)
fat	0.167 (0.151)	0.007 (0.007)
liver	0.833 (0.825)	0.036 (0.036)
kidney	0.903 (0.894)	0.039 (0.039)
Average dose administered: 4.8 mg/kg dry feed (1.3X)		
milk (day 28)	0.065 (0.030)	0.013 (0.006)
muscle	0.085 (0.060)	0.018 (0.013)
fat	0.062 (0.035)	0.013 (0.007)
liver	0.195 (0.180)	0.041 (0.037)
kidney	0.209 (0.198)	0.043 (0.041)

* average/weighted residues were calculated using samples from animals 5152, 5155, 5158 ("residue" animals) for the 34X dose group and from animals 5150, 5159 and 5158 for the 1.3X dose group, in agreement with calculation of the residues in the samples of all other dose groups which consisted of "residue" animals, only. Samples of the "urine collection" (34X and 1.3X) and "depuration" animals (34X) were not included.

Remark re. two values shown for total residue and transfer factors:

In a first step, all values below the LOQ of 0.01 mg/kg (BYI 02960, BYI 02960-acetyl-AMCP and BYI 02960-OH) or below the LOQ of 0.02 mg/kg (DFA) were considered as being *at* 0.01 or 0.02 mg/kg, respectively, to calculate the total residue.

Values in parentheses: In a second step, all values above the LOD but below the LOQ were considered "as reported"; values below the LOD were calculated as being *at* the LOD (values in brackets); see Table 6.4.2-18



In the subsequent table, the transfer factors were elucidated for the combined residues of BYI 02960 + DFA (*without* the other analytes), as these two compounds have been proposed as the relevant residues of BYI 02960 in poultry matrices for enforcement and risk assessment (cf. KIIA 6.7.1).

Table 6.4.2-10: Transfer factors calculated for the total residue consisting of parent compound BYI 02960 + DFA

Animal matrix	Total residue [mg a.s equiv./kg]	Transfer factor
Average dose administered: 135 mg/kg dry feed (34X)		
milk composite (days 1 to 28)	0.922 (0.922)	0.007
muscle	1.760 (1.760)	0.013
fat	1.182 (1.182)	0.009
liver	3.851 (3.851)	0.029
kidney	4.919 (4.919)	0.036
Average dose administered: 50 mg/kg dry feed (13X)		
milk (day 28)	0.308 (0.308)	0.006
muscle	0.733 (0.733)	0.015
fat	0.384 (0.384)	0.008
liver	1.811 (1.811)	0.036
kidney	1.992 (1.992)	0.040
Average dose administered: 23 mg/kg dry feed (6.3X)		
milk (day28)	0.128 (0.124)	0.006 (0.005)
muscle	0.304 (0.304)	0.013
fat	0.147 (0.147)	0.006
liver	0.812 (0.812)	0.035
kidney	0.867 (0.867)	0.038
Average dose administered: 4.8 mg/kg dry feed (1.3X)		
milk (day 28)	0.045 (0.028)	0.009 (0.006)
muscle	0.065 (0.057)	0.013 (0.012)
fat	0.042 (0.033)	0.009 (0.007)
liver	0.174 (0.166)	0.036 (0.035)
kidney	0.179 (0.178)	0.037 (0.037)

* average/weighted residues were calculated using samples from animals 5152, 5155, 5158 ("residue" animals) for the 34X dose group and from animals 5150, 5159 and 5158 for the 1.3X dose group, in agreement with calculation of the residues in the samples of all other dose groups which consisted of "residue" animals, only. Samples of the "urine collection" (34X and 1.3X) and "depuration" animals (34X) were not included.

Remark re. two values shown for total residue and transfer factors:

In a first step, all values below the LOQ of 0.01 mg/kg (BYI 02960) or below the LOQ of 0.02 mg/kg (DFA) were considered as being *at* 0.01 or 0.02 mg/kg, respectively, to calculate the total residue.

Values in parentheses: In a second step, all values above the LOD but below the LOQ were considered "as reported"; values below the LOD were calculated as being *at* the LOD (values in brackets); see Table 6.4.2-18

The predominant component of the residues in milk, edible tissues, and organs was parent compound BYI 02960, followed by DFA. The metabolite BYI 02960-acetyl-AMCP was below the LOD in all dose groups, and BYI 02960-OH was detected in the high dose groups only. When calculating the



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total residue, all constituents of the residue definition are considered. Compounds detected below the LOQ were estimated to be at the LOQ level. Thus the total residue overestimates the proportion of BYI 02960-acetyl-AMCP and BYI 02960-OH, especially at the 1X dose where neither of the two metabolites were detected. Therefore, calculation of transfer factors should be restricted to parent compound + DFA, the constituents of the residue definition for enforcement and risk assessment.

In milk, DFA concentrations were lower than those for parent by a factor of approx. 6 to 7, as soon as the plateau was reached in milk. DFA concentrations in muscle and fat were lower by a factor of approx. 2 to 4, and by a factor of approx. 9 in the metabolizing organs liver and kidney. Clearly, parent compound BYI 02960 was metabolized quite slowly to DFA after feeding BYI 02960 to lactating cows. As soon as DFA was formed, it was distributed equally within the body. Transfer into milk was rather low.

Feeding of DFA only would result in approx. three to tenfold higher transfer factors, as demonstrated above (cf. Table 6.4.2-7). The highest transfer was also detected into the metabolizing organs liver and kidney, followed by muscle and fat. The lowest transfer factor was determined for milk.

Using these transfer factors derived for the combined residue of BYI 02960 + DFA as well as for DFA alone, estimations of the residue levels reasonably expected to be determined in ruminant tissues and milk after feeding of a mixture of BYI 02960 and DFA (as would realistically be anticipated in the feed crops) can be calculated.

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Supporting information:**Estimation of the total weight of muscle, fat, liver and kidney of cattle**

Only subsamples of muscle, fat, liver and kidney were collected at necropsy from all animals of all dose groups tested. Therefore the total weight of the tissues and organs was estimated based on the average body weight of the animals of the 4.9X dose group determined on the day before administration (cows designated for depuration and urine collection were not considered).

Table 6.4.2-11: Calculation of the mean body weight of cattle in the 34X and 1.3X dose group

Dose Group	Animal No.	Body weight at study start [kg]
34X	5152	553
34X	5155	591
34X	5158	479
Average body weight		541
1.3X	5150	595
1.3X	5159	556
1.3X	5163	496
Average body weight		549

Based on the average body weight, the average weights of

- muscle (30% of the total body weight for total muscle),
- fat (12% of the total body weight for dissectable fat),
- liver (1.5% of the total body weight), and
- kidneys (0.25% of the total body weight)

were estimated.

Since no literature values were available for kidney, an average kidney weight was estimated based on the values from a recently conducted cattle feeding study (Bomke, S.; Mefenpyr-diethyl: Feeding study with dairy cows, Bayer CropScience internal report, 2012-01-11). The liver weights determined in that study confirmed the percentage cited in literature. The literature value was used for the estimation of the average liver weight as a worst-case assumption.

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Table 6.4.2-12: Estimation of the kidney weight as a percentage of the body weight
(data collected in a feeding study recently performed¹)

Body weight [kg]	Weight of kidneys [g]			Percent of body weight [%]
	left	right	sum	
589	611.0	654.4	1265.4	0.21
460	601.8	587.2	1189.0	0.26
538	816.7	807.1	1623.8	0.30
572	592.7	591.3	1184.0	0.21
545	637.8	592.8	1230.6	0.23
504	658.6	645.9	1304.5	0.26
516	743.3	646.1	1389.4	0.27
481	673.4	633.9	1307.3	0.27
541	874.1	827.1	1701.2	0.31
531	588.5	609.9	1198.4	0.23
546	623.4	659.1	1282.5	0.23
505	544.6	579.9	1124.5	0.22
516	725.3	683.3	1408.6	0.27
572	669.3	722.9	1392.2	0.24
524	603.5	603.3	1206.8	0.23
540	697.8	659.3	1357.1	0.25
623	682.9	752.2	1435.1	0.23
511	619.6	610.2	1229.8	0.24
534 (average)	664.7	659.2	1323.9	0.25 (average)

¹ Bomke, S.: Mefenpyr-diethyl: Feeding study with dairy cows, Bayer CropScience Report No. MR-11/040, 2012-01-11

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 Table 6.4.2-13: Estimation of the liver weight in proportion to the body weight
 (data collected in a feeding study recently performed²)

body weight [kg]	weight of liver [g]	percent of body weight [%]
589	8571.3	1.46
460	7812.7	1.70
538	8113.3	1.51
572	8232.8	1.44
545	8183.3	1.50
504	8251.0	1.64
516	9533.1	1.85
481	7619.6	1.58
541	8438.9	1.56
531	7955.5	1.50
546	8497.9	1.56
505	8849.1	1.75
516	7280.7	1.41
572	8828.3	1.54
524	8400.3	1.60
540	8946.5	1.66
623	8679.3	1.39
511	8434.4	1.65
534 (average)	8686.7 (average)	1.57 (average)

Table 6.4.2-14: Weight of organs and tissues of the lactating cows in the 34X and 1.3X dose group

Animal matrix	percentage of body weight [%]	average weight [kg] 34X dose	average weight [kg] 1.3X dose
muscle	30	162.3	164.7
fat	12	64.9	65.9
liver	1.5	8.1	8.2
kidney	0.25	1.4	1.4

At necropsy, only subsamples of muscle, fat, liver and kidneys were collected. The subsamples were weighed and pooled per subgroup for analysis. The total weight of the tissues and organs was estimated on the basis of the average body weight of the cows of the 34X and 1.3X dose groups and accounted for **162.3 kg / 164.7kg** for **muscle**, **64.9 kg / 65.9 kg** for **fat**, **8.1 kg / 8.2 kg** for **liver** and **1.4 kg / 1.4 kg** for **kidney** per animal.

² Bomke, S.: Mefenpyr-diethyl: Feeding study with dairy cows, Bayer CropScience Report No. MR-11/040, 2012-01-11



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Table 6.4.2-15: Residue levels of DFA in organs and tissues of the lactating cows (34X dose group)

Animal number	DFA residues [mg a.s. equiv./kg]			
	muscle	fat	liver	kidney
5152	0.302	0.204	0.284	0.379
5155	0.368	0.427	0.348	0.528
5158	0.473	0.375	0.507	0.630
Average	0.381	0.335	0.380	0.512

Table 6.4.2-16: Residue levels of DFA in organs and tissues of the lactating cows (1.3X dose group)

Animal number	DFA residues [mg a.s. equiv./kg]			
	muscle	fat	liver	kidney
5150	0.013	0.012	0.014	0.022
5159	0.011	0.009	0.011	0.016
5163	0.014	0.013	0.012	0.016
Average	0.013	0.011	0.012	0.018

Total urine weight and calculation of weighted residues in urine

Table 6.4.2-17: Individual urine production of lactating cows (34X dose group)

Animal number	Study day [week 1]							Total weight of urine per week [g]
	1	2	3	4	5	6	7	
5153	16009	22937	24561	13937	20937	66603	60778	225762
Study day [week 2]								
	8	9	10	11	12	13	14	
5153	63517	31041	27111	25937	20545	18232	31136	217519
Study day [week 3]								
	15	16	17	18	19	20	21	
5153	24732	27801	20771	24801	23722	19152	17531	158510
Study day [week 4]								
	22	23	24	25	26	27	28	
5153	21804	25560	25552	21831	19835	22739	14731	152052
Total weight of urine collected (animal 5153)								753843

On study day 1, animal 5153 lost approx. 5-6 liters of urine due to hose blockage

On study day 2, animal 5153 had mucus and blood plugging the urine hose leading to urine loss

On study day 13, animal 5153 lost approx. 2-3 liters of urine due to hose blockage

On study day 15, animal 5153 lost approx. 4-6 liters of urine due to urine device displacement

On study day 19, animal 5153 lost approx. 1 liter of urine

On study day 21, animal 5153 lost approx. 4.5 liters of urine

On study day 26, animal 5153 lost approx. 2-4 liters of urine

On study day 27, animal 5153 lost approx. 2 liters of urine

Animal 5153 had several incidences of anorexia / depression / lethargy and mastitis during the study. Further, upon necropsy she was found to have signs of metritis. Since she was under increased stress, which may have complicated her condition, urine collection was rather difficult and several liters of urine were lost. Nevertheless, no correction of the total urine amount was performed and thus a slightly lower systemic exposure to DFA has been calculated, which will lead to slightly overestimated transfer factors (worst-case consideration).



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The total **urine** weight was approx. **754 kg** for animal 5153.

Table 6.4.2-18: Individual urine production of lactating cows (1.3X dose group)

Animal number	Study day [week 1]							Total weight of urine per week [g]
	1	2	3	4	5	6	7	
5151	20541	20972	21856	23705	19941	20939	18040	145994
Study day [week 2]								
	8	9	10	11	12	13	14	
5151	19546	18018	12887	18810	16694	15727	20130	121812
Study day [week 3]								
	15	16	17	18	19	20	21	
5151	16416	20920	17998	16082	16097	18724	19037	125274
Study day [week 4]								
	22	23	24	25	26	27	28	
5151	22491	21558	20714	20168	20101	19607	17426	142065
Total weight of urine collected (animal 5151)								535145

On study day 3, animal 5151 lost approx. 1 liter of urine during dosing

The total **urine** weight was approx. **535 kg** for animal 5151.

Table 6.4.2-19: Calculation of the weighted DFA concentration in urine of lactating cows (34X dose group)

Animal number	Week of treatment	Weight of urine composite [kg]	DFA residue level [mg a.s. equiv./kg]	Absolute DFA level [mg a.s. equiv.]
5153	1	225.8	1.92	433.5
5153	2	217.5	2.33	506.8
5153	3	158.5	3.71	588.0
5153	4	152.1	3.33	506.5
5153		753.8	2.70	2034.8

Table 6.4.2-20: Calculation of the weighted DFA concentration in urine of lactating cows (1.3X dose group)

Animal number	Week of treatment	Weight of urine composite [kg]	DFA residue level [mg a.s. equiv./kg]	Absolute residue level [mg a.s. equiv.]
5151	1	146.0	0.112	16.4
5151	2	121.8	0.185	22.5
5151	3	125.3	0.184	23.1
5151	4	142.1	0.142	20.2
5151		535.1	0.153	82.1



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Total milk weight and calculation of weighted residue level in milk

Table 6.4.2-21: Individual milk production of lactating cows (34X dose group)

Animal number	Study day [week 1]							Total weight of milk per week [g]
	1	2	3	4	5	6	7	
5152	33263	30378	32333	32682	31758	32653	30268	223335
5155	35194	36404	36579	37549	38042	37595	36085	257448
5158	27781	27787	27430	26392	25779	26845	28959	190973
	Study day [week 2]							
	8	9	10	11	12	13	14	
5152	31961	30587	31401	29270	30247	30769	31062	215297
5155	35842	35224	37570	35058	35863	36608	35857	252022
5158	27146	27705	27157	26013	27081	26309	27037	188448
	Study day [week 3]							
	15	16	17	18	19	20	21	
5152	30574	30346	30299	29744	31796	29495	29777	212031
5155	36220	34145	33875	33813	34465	35924	34468	242910
5158	26401	26905	27605	28679	27533	28822	27160	193105
	Study day [week 4]							
	22	23	24	25	26	27	28	
5152	30289	31028	31594	30925	30623	29482	31424	215365
5155	32803	34031	35980	31826	34131	36530	36333	241634
5158	25566	24924	27203	27729	26123	28550	28369	188464
Total weight of milk collected								2621032

The total weight of the milk collected during the study phase (days 1 to 28) was 2621.0 kg for three animals, corresponding to approx. **874 kg per cow**.



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Table 6.4.2-22: Calculation of the weighted DFA concentration in milk (34X dose group)

Animal number	Day of treatment	Weight of milk sample [kg]	DFA residue level [mg a.s. equiv./kg]	Absolute DFA level [mg a.s. equiv.]
5152	2	30.378	0.080	2.430
5152	4	32.682	0.114	3.726
5152	7	30.268	0.105	3.178
5152	10	31.401	0.112	3.517
5152	14	31.062	0.126	3.914
5152	17	30.299	0.121	3.666
5152	19	31.796	0.112	3.561
5152	25	30.925	0.093	2.867
5152	28	31.424	0.099	3.124
5152		280.235	0.107	29.982
5155	2	36.404	0.087	3.182
5155	4	37.549	0.108	4.055
5155	7	36.085	0.106	3.825
5155	10	37.570	0.107	4.020
5155	14	35.857	0.129	4.626
5155	17	33.875	0.119	4.031
5155	19	34.465	0.109	3.757
5155	25	31.826	0.092	2.922
5155	28	36.333	0.094	3.419
5155		319.964	0.106	33.836
5158	2	27.787	0.092	2.556
5158	4	26.392	0.128	3.378
5158	7	28.959	0.135	3.909
5158	10	27.157	0.159	4.318
5158	14	27.037	0.166	4.488
5158	17	27.605	0.156	4.306
5158	19	27.533	0.149	4.102
5158	25	27.729	0.141	3.910
5158	28	28.369	0.136	3.858
5158		248.568	0.140	34.827
Average weighted residue level			0.118	

Residue levels detected in animal matrices

Table 6.4.2-23: Residue levels in animal matrices after dosing of BYI 02960 to lactating cows

Dose level [mg/kg feed]	Residues [mg a.s. equiv./kg]				
	Animal matrix	BYI 02960	DFA	BYI 02960- acetyl-AMCP	BYI 02960-OH
1.3X (4.8 mg/kg dry feed)	milk	0.025	0.004 ¹	<0.0008 (LOD)	<0.0008 (LOD)
	muscle	0.045	0.013 ¹	<0.0015 (LOD)	<0.0014 (LOD)
	fat	0.022	0.011 ¹	<0.0007 (LOD)	<0.0008 (LOD)
	liver	0.154	0.012 ¹	<0.0027 (LOD)	<0.0009 (LOD)
	kidney	0.159	0.018 ¹	<0.0010 (LOD)	<0.0017 (LOD)
6.3X (23 mg/kg dry feed)	milk	0.108	0.016 ¹	<0.0008 (LOD)	<0.0008 (LOD)
	muscle	0.250	0.054	<0.0015 (LOD)	0.0021 ²
	fat	0.109	0.038	<0.0007 (LOD)	0.0037 ²
	liver	0.755	0.057	<0.0027 (LOD)	0.011
	kidney	0.786	0.081	<0.0010 (LOD)	0.026
13X (50 mg/kg dry feed)	milk	0.267	0.041	<0.0008 (LOD)	0.001 ²
	muscle	0.597	0.136	<0.0015 (LOD)	0.005 ²
	fat	0.285	0.099	<0.0007 (LOD)	0.003 ²
	liver	1.680	0.132	<0.0027 (LOD)	0.020
	kidney	1.789	0.203	<0.0010 (LOD)	0.045
34X (135 mg/kg dry feed)	milk	0.804 ³	0.118 ³	<0.0008 (LOD)	0.006 ³
	muscle	1.379	0.381	<0.0015 (LOD)	0.014
	fat	0.846	0.335	<0.0007 (LOD)	0.015
	liver	3.472	0.380	<0.0027 (LOD)	0.042
	kidney	4.407	0.512	<0.0010 (LOD)	0.098

¹ > LOD, but < LOQ (0.02 mg/kg)

² > LOD, but < LOQ (0.01 mg/kg)

³ weighted residue value

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Table 6.4.2-24: Total residues calculated for animal matrices after dosing of BYI 02960 to lactating cows

Dose level [mg/kg feed]	Residues [mg a.s. equiv./kg]		
	Animal matrix	total residue for data collection ¹	total residue for enforcement / risk assessment ²
1.3X (4.8 mg/kg dry feed)	milk	0.065 (0.030 ³)	0.045 (0.028 ³)
	muscle	0.085 (0.060 ³)	0.065 (0.057 ³)
	fat	0.062 (0.035 ³)	0.042 (0.033 ³)
	liver	0.195 (0.180 ³)	0.174 (0.166 ³)
	kidney	0.209 (0.198 ³)	0.179 (0.178 ³)
6.3X (23 mg/kg dry feed)	milk	0.148 (0.126 ³)	0.128 (0.124 ³)
	muscle	0.324 (0.308 ³)	0.304 (0.304 ³)
	fat	0.167 (0.151 ³)	0.147 (0.147 ³)
	liver	0.833 (0.825 ³)	0.812 (0.812 ³)
	kidney	0.903 (0.894 ³)	0.867 (0.867 ³)
13X (50 mg/kg dry feed)	milk	0.328 (0.310 ³)	0.308 (0.308 ³)
	muscle	0.753 (0.740 ³)	0.733 (0.733 ³)
	fat	0.404 (0.388 ³)	0.384 (0.384 ³)
	liver	1.841 (1.834 ³)	1.811 (1.811 ³)
	kidney	2.046 (2.037 ³)	1.992 (1.992 ³)
34X (135 mg/kg dry feed)	milk	0.942 (0.928 ³)	0.922 (0.922 ³)
	muscle	1.784 (1.776 ³)	1.760 (1.760 ³)
	fat	1.206 (1.197 ³)	1.182 (1.182 ³)
	liver	3.903 (3.896 ³)	3.851 (3.851 ³)
	kidney	5.027 (5.018 ³)	4.919 (4.919 ³)

¹ total residue for data collection: BYI 02960, DFA, BYI 02960-acetyl-AMCP and BYI 02960-hydroxy

² total residue for enforcement / risk assessment: BYI 02960 + DFA

³ values above the LOD were considered as measured, for values below the LOD the LOD was taken for calculation

Remark re. two values shown for total residue and transfer factors:

In a first step, all values below the LOQ of 0.01 mg/kg (BYI 02960, BYI 02960-acetyl-AMCP and BYI 02960-OH) or below the LOQ of 0.02 mg/kg (DFA) were considered as being at 0.01 or 0.02 mg/kg, respectively, to calculate the total residue.

Values in parentheses: In a second step, all values above the LOD but below the LOQ were considered "as reported"; values below the LOD were calculated as being at the LOD (values in brackets); see Table 6.4.2-18

IIA 6.4.3 Pigs

No feeding study in pigs is required because metabolic pathways in the rat, in ruminants and in poultry are similar (cf IIA 6.2.4).

IIA 6.4.4 Fish

No feeding study in fish is required due to the low potential for accumulation in fish as indicated by the log Pow of 1.2 in the range of pH 4 – 9 (cf IIA 6.2.5).



IIA 6.5 Effects of industrial processing and/or household preparation on residues

Numerous processing studies have been conducted to support the use of BYI 02960 in various crops. In this Annex II dossier, only the so-called "safe uses" are described (lettuce and hops), thus only the processing studies relevant to those crops are presented. In order to enable risk assessment, further data on other crops will be submitted in a separate document with the respective field residue studies.

IIA 6.5.1 The nature of residue

Report:	KIIA 6.5.1/01, Weber, E.; 2011
Title:	Nature of the residues of [pyridinylmethyl- ¹⁴ C]BYI02960 in processed commodities - high temperature hydrolysis
Report No. & Document No.:	MEF-10/856, dated February 9, 2011 M-402311-01-2
Guidelines:	– EU Council Directive 91/414/EEC amended by Commission Directive 96/68/EC Section 6.5, Subsection 6.5.1 – OECD Guideline for the Testing of Chemicals No. 507, Nature of the Pesticide Residues in Processed Commodities - High Temperature Hydrolysis, adopted 2007-10-16
GLP:	yes (certified laboratory)

Executive Summary

The behaviour of BYI 02960 was studied in buffered drinking water under conditions of processing. The radiolabelled test compound [pyridinylmethyl-¹⁴C]BYI02960 was used for the hydrolysis investigations.

One concentration (approx. 1.0 mg/L) of the analyte was prepared in sterilized buffered drinking water and incubated under three representative sets of hydrolysis conditions:

Pasteurisation: 90°C at pH 4 for 20 min

Baking, brewing, boiling: 100°C at pH 5 for 60 min

Sterilisation: 120°C at pH 6 for 20 min

At test termination, the material balances in all tests were in the range of 97.3 to 104.9% of the applied radioactivity, indicating that no radioactivity and no volatile degradation products dissipated from the test system.

HPLC profiling of samples before and after processing proved that the test compound BYI 02960 was stable under the test conditions. The test compound amounted to 100.0% in all test solutions before and after hydrolysis. No hydrolysis products were detected above an estimated LOD of 0.5%.



I. Materials and Methods

A. Materials

Table 6.2.1-1 Test material

	Position of radiolabel	Radiochemical purity (%)	Specific Activity (MBq/mg)
BYI 02960	[pyridinylmethyl- ¹⁴ C]	> 99% by HPLC > 99% by TLC	4.37

B. Study Design

Experimental conditions: One sample of the test solution was prepared for each of the three tests. An appropriate amount of the aqueous stock solution was diluted with buffer solution to give a theoretical concentration of approx. 1 mg/L in the test solution. The pH value of all samples was measured and three aliquots of each test solution were subjected to LS-measurement to determine the actual radioactivity in the test solution before starting the treatment. A further aliquot from each sample was taken for chromatographic analysis of the zero-time purity.

The test compound was incubated in buffered drinking water at the following three representative sets of conditions to investigate the effects of hydrolysis as appropriate for the relevant processing operations:

pH	Temperature [°C]	Test period [min]	Process
4 ± 0.1	90 ± 5	20 ± 1	pasteurisation
5 ± 0.1	100 ± 5	60 ± 1	baking, brewing and boiling
6 ± 0.1	120 ± 5	20 ± 1	sterilisation

The tests at 90°C and 100°C were carried out by using a dry block heater. The test at 120°C was performed in an autoclave. For the experiments at 90°C and 100°C, the actual temperature was recorded in a control vial filled with blank buffer solution. For the autoclave experiment at 120°C, the programmed figures were used as temperature data.

After the application procedure, the test vessels were closed with a septum and a crimp top and were subjected to the intended incubation conditions. Samples for hydrolysis were weighed before and after hydrolysis to correct for possible losses by evaporation of water.

Sampling: After termination of each test and cooling to room temperature, the pH value was measured. Three aliquots were again taken from each test solution for the determination of the radioactivity content by Liquid Scintillation Counting (LSC).



C. Analytical Procedure

Processing: The radioactivity content of each test solution was determined by LSC before starting and after termination the hydrolysis. Aliquots of all samples were analysed by HPLC for detection of possible hydrolysis products. All analyses were performed within approx. one day after sampling.

Quantitation: Parent compound was quantified by LSC.

Identification and characterisation: The identity of the test compound was confirmed by LC-MS/MS. In the test solutions, parent compound was identified by HPLC comparison.

II. Results and Discussion

pH, temperatures and test periods: The pH values of the test solutions were adjusted to pH 4, pH 5, and pH 6 (each ± 0.1) and remained as required. The temperatures were in the ranges of $90 \pm 5^\circ\text{C}$, $100 \pm 5^\circ\text{C}$ and $120 \pm 5^\circ\text{C}$ during the test periods.

Material balance: The applied radioactivity was defined as the amount of radioactivity measured in the samples taken at the beginning of the incubation period. Based on the results of LSC measurements immediately after test termination, a radioactivity balance was established for each experiment.

All material balances were in the range between 97.3 to 104.9%. The material balances demonstrate that no radioactivity dissipated from the test systems.

Test compound and hydrolysis products in test samples: The radiochemical purity of the test compound was checked in the stock solution by HPLC and amounted to 100%. Aliquots of all tests solutions were analysed by HPLC before and after hydrolysis. All analyses were performed within approx. one day after sampling. No hydrolysis products were detected in any sample. The LOD was estimated as being approx. 0.5% of the total radioactivity.

III. Conclusions

The test compound BYI 02960 was stable under all conditions of high temperature hydrolysis for simulation of food processing. The following conditions were tested:

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

No hydrolysis products of BYI 02960 were detected above an estimated LOD of 0.5%.



IIA 6.5.2 Distribution of the residue in peel/pulp

As the "safe use" crops supported in this dossier, lettuce and hops, are not peeled before use, no data was necessary to cover this point. .

IIA 6.5.3 Residue levels - balance studies on set of representative processes

As the newest OECD guidelines no longer specifically require balance studies, all processing trials on lettuce and hops are presented below under point 6.5.4.

IIA 6.5.4 Residue levels - follow-up studies: concentration or dilution factors

General remark:

In this summary section (KIIA 6.5.4), the name DFEAF will be used for the metabolite BYI 02960-difluoroethyl-amino-furanone, which is relevant to the tested residue definition:

Name	Metab. No.	Standard "dossier name"
DFEAF	M34	BYI 02960-difluoroethyl-amino-furanone

Lettuce

Report:	KIIA 6.5.4/01, Schulte, G. & Bauer, J.; 2012
Title:	Determination of the residues of BYI 02960 in/on lettuce and head lettuce and the processed fractions (head, inner parts; leaf, outer; leaf, inner; leaf, inner, washed and washings) after spraying of BYI 02960 SL 200 in the field in the Netherlands, Belgium and Germany
Report No. & Document No.:	10-3223, dated March 7, 2012 <u>M-426982-01-1</u>
Guidelines:	– EU Council Directive 91/414/EEC amended by Commission Directive 96/68/EC – EU Guidance Working Document 7035/VI/95 rev. 5 – OECD Guideline for the Testing of Chemicals No. 508, Magnitude of the Pesticide Residues in Processed Commodities – EPA Ref. OPPTS 860.1500, Crop Field Trials
GLP:	yes (certified laboratory)

I. Materials and Methods

Due to the presence of measurable residues of BYI 02960 on harvested lettuce determined in samples from field residue trials performed according to the intended commercial use conditions (see point IIA 6.3.1) as well as to the nature of lettuce use (preparation and consumption patterns) and the highly variable residue levels commonly seen in this crop, investigations on the effects of basic processing have been conducted. Four trials were conducted in the northern European residue region, in

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Germany (2), the Netherlands, and Belgium, in order to determine the total residues of BYI 02960 in unprocessed lettuce heads and then in washed and unwashed commodities including external and internal leaves (Schulte & Bauer, 2012; KIIA 6.5.4/01).

The field trials were conducted as part of study 10-2223 (cf. KIIA 6.3.1/01 for details). Two of the trials were conducted on open-headed lettuces, two on closed-head varieties. BYI 02960 SL 200 was sprayed twice at an application rate of approx. 125 g a.s./ha and a water volume of 300-600 L/ha. The final application was conducted at a pre-harvest interval of 3 days.

After processing (described below), residue analysis was performed according to method 01304 as used in the RAC trials themselves (for more information, cf. points IIA 6.3.1 and IIA 4.3). The limits of quantitation were 0.01 mg/kg (BYI 02960 and DFEAF) and 0.02 mg/kg (DFA), expressed in parent compound equivalents, yielding a calculated total-residue LOQ of 0.04 mg/kg, in all matrices. Prior and parallel to the residue analysis, the method was validated by recovery experiments.

Leaf removal, washing, etc.:

Lettuce "processing" was designed to simulate typical household kitchen practices in the use and preparation of lettuce for salads.

The complete outer layer of leaves were removed from the heads, yielding outer leaves and inner head parts. From the inner head parts, leaves were separated from the stalk (inner leaves), then washed in standing water (washed inner leaves and washing water). The process is illustrated in flow diagram 6.5.4-1.

II. Findings

Limited validation sets and concurrent recoveries of BYI 02960 and its metabolites DFA and DFEAF were obtained from samples of lettuce matrices (head and washings). In head samples, recovery samples for parent compound and DFEAF were spiked at levels of 0.01 mg/kg and 0.10 mg/kg, as well as at 0.50, 1.0, and 5.0 mg/kg (expressed in BYI 02960 equivalents); in the washing water, spiking levels were 0.01 and 0.50 mg/kg. Mean recoveries for all matrices were 90-107%, with RSDs in the larger validations sets ($n > 2$) of 2.2-10.7%; $n=2-15$.

For DFA, concurrent recovery samples for lettuce head were spiked at levels of 0.02, 0.05, and 0.50 mg/kg, as well as at 0.20, 1.0, and 5.0 mg/kg (expressed in BYI 02960 equivalents); fortification levels in the washing water were 0.02 and 0.50 mg/kg. Mean recoveries in all matrices were 90-98%, with RSDs in the larger validations sets ($n > 2$) of 3.2-10.2%; $n=2-12$.

Although very similar to lettuce head and thus technically covered by head-sample validation, additional individual concurrent recoveries were also run with samples of inner head parts and of outer leaves. Recovery values were very similar to those seen for head.

A tabular summary of the recovery values is presented below in table 6.5.4-3.

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

The total residues of BYI 02960 (parent compound plus DFA and DFEAF) in the harvested heads at day 3 ranged from 0.40-1.0 mg/kg, as summarized previously (cf. KIIA 6.3.1/01). These values were used for the calculation of "processing" factors.

Outer leaves and inner head parts:

The outer leaves were analyzed separately after their removal; the residue levels were from 0.71-1.8 mg/kg. In the remaining inner head parts, residue levels were considerably lower, at 0.30-0.74 mg/kg.

Based on these values, it is evident that a large proportion of the residues result from surface deposits. The measured residue levels lead to mean "processing" factors of 1.8 for outer leaves and 0.73 for the inner head parts.

Inner leaves and washed inner leaves:

After separation from the stalks, inner leaves samples prior and subsequent to washing. Residue values ranged from 0.08-0.83 mg/kg and 0.29-0.52 mg/kg in the unwashed and washed inner leaves, respectively. In the intermediate product washing water, they were very low, ranging from <0.04-0.05 mg/kg.

These findings indicate that the remainder of the residues are absorbed to a large extent. Washing does not affect them; indeed, the residues are virtually the same in both washed and unwashed leaves (and also essentially as in the inner head parts above). Based on these values, mean "processing" factors of 0.76 and 0.67 can be calculated from lettuce head to unwashed and washed inner leaves, respectively.

The transfer factors for the total residues of BYI 02960 are summarized below in table 6.5.4-1. All trial data are summarised further below in table 6.5.4-2a & b and in greater detail in the Tier 1 summary forms.

Table 6.5.4-1: Summary of the total residues of BYI 02960 in mg/kg and transfer factors (*in italics and parentheses*) in lettuce RACs and processed products following application of BYI 02960 SL 200

Trial number	lettuce head*	outer leaves	inner head parts	inner leaves	washed inner leaves	washing water
10-3223-01	0.61	1.3 (2.1)	0.49 (0.80)	0.48 (0.79)	0.51 (0.84)	<0.04 (<0.07)
10-3223-02	0.40	0.71 (1.8)	0.30 (0.75)	0.46 (1.2)	0.29 (0.73)	0.05 (0.13)
10-3223-04	1.0	1.8 (1.8)	0.74 (0.74)	0.08 (0.08)	0.50 (0.50)	0.05 (0.05)
10-3223-05	0.87	1.4 (1.6)	0.55 (0.63)	0.83 (0.95)	0.52 (0.60)	0.05 (0.06)
<i>Mean transfer factors:</i>		<i>1.8</i>	<i>0.73</i>	<i>0.76</i>	<i>0.67</i>	<i>0.07</i>

* residue levels for "head" samples taken from study 10-2223 (cf. KIIA 6.3.1/01)

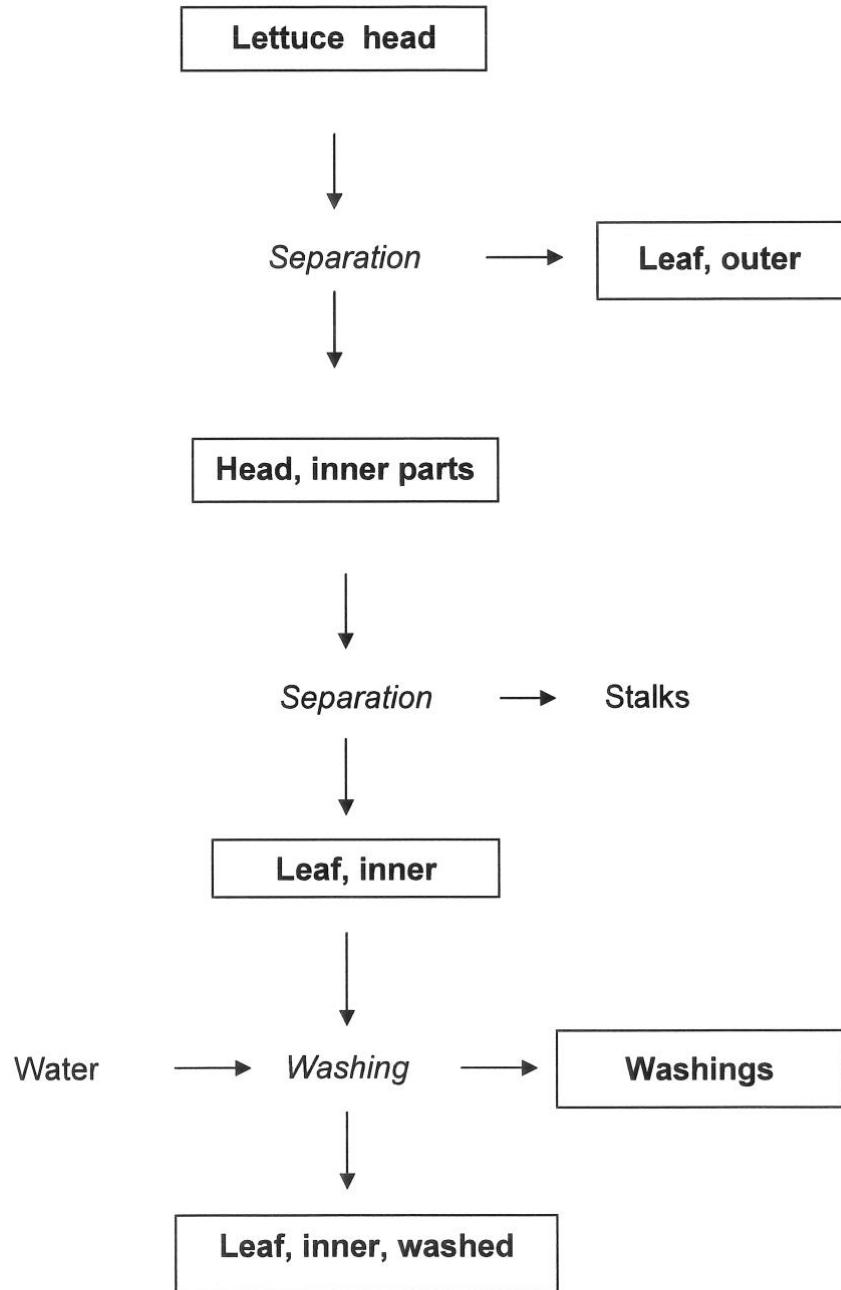


III. Conclusions

In order to determine "processing" factors for total residues of BYI 02960 from lettuce heads to parts thereof, processing studies have been conducted. 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.



Diagram 6.5.4-1: Household "processing" of lettuce heads



Samples or fractions to be analysed



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Table 6.5.4-2a: Application scenario in residue processing trials conducted in/on lettuce after spraying with BYI 02960 SL 200 in European fields

Study No. (Trial No.) Country Location	Crop Variety	FL	No.	Application		GS	PHI (days)
				kg/ha (a.s.)	kg/hl (a.s.)		
10-2223 (10-2223-01) Netherlands 1681 ND Zwaagdijk-Oost EU-N 2010	lettuce, head Gisela, Butterhead variety	200 SL	2	0.125	0.0417	48	3
10-2223 (10-2223-02) Belgium 6210 Villers-Perwin EU-N 2010	lettuce, head Lucan, Butterhead variety	200 SL	2	0.125	0.0250	48	3
10-2223 (10-2223-04) Germany 40764 Langenfeld-Reusrath EU-N 2010	lettuce Cavernet Lollo rosso, Loose leaf variety	200 SL	2	0.125	0.0417	48	3
10-2223 (10-2223-05) Germany 67125 Schauernheim EU-N 2010	lettuce Chloe Lollo rosso, Loose leaf variety	200 SL	2	0.125	0.0313	48	3

FL = formulation

GS = growth stage (BBCH-code) at last treatment

EU-N = northern Europe

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.5.4-2b: Results of residue processing trials conducted in/on lettuce after spraying with BYI 02960 SL 200 in European fields

Study No. (Trial No.) Country GLP	Portion analyzed	DALT (days)	Residues (mg/kg) expressed as BYI 02960			total residue of BYI 02960 calc.
			BYI 02960	difluoroacetic acid	BYI 02960- difluoro- ethylamino- furanone	
10-2223 (10-2223-01) Netherlands GLP: yes	head	3	0.58	<0.02	<0.01	0.61
	head, inner parts	3	0.46	0.02	<0.01	0.49
	leaf, outer	3	1.3	0.02	0.02	1.3
	leaf, inner	3	0.45	0.02	<0.01	0.48
	leaf, inner, washed	3	0.48	<0.02	<0.01	0.51
	washings	3	<0.01	<0.02	<0.01	<0.04
10-2223 (10-2223-02) Belgium GLP: yes	head	3	0.37	<0.02	<0.01	0.40
	head, inner parts	3	0.27	<0.02	<0.01	0.30
	leaf, outer	3	0.68	<0.02	0.01	0.71
	leaf, inner	3	0.43	<0.02	<0.01	0.46
	leaf, inner, washed	3	0.26	<0.02	<0.01	0.29
	washings	3	0.02	<0.02	<0.01	0.05
10-2223 (10-2223-04) Germany GLP: yes	head	3	1.0	<0.02	0.01	1.0
	head, inner parts	3	0.71	<0.02	<0.01	0.74
	leaf, outer	3	1.8	0.02	0.01	1.8
	leaf, inner	3	0.05	<0.02	<0.01	0.08
	leaf, inner, washed	3	0.47	<0.02	<0.01	0.50
	washings	3	0.02	<0.02	<0.01	0.05
10-2223 (10-2223-05) Germany GLP: yes	head	3	0.83	<0.02	0.01	0.87
	head, inner parts	3	0.52	<0.02	<0.01	0.55
	leaf, outer	3	1.3	0.02	0.01	1.4
	leaf, inner	3	0.80	<0.02	0.01	0.83
	leaf, inner, washed	3	0.49	<0.02	<0.01	0.52
	washings	3	0.02	<0.02	<0.01	0.05

DALT = days after last treatment

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.5.4-3: Recovery data for BYI 02960 in lettuce and lettuce matrices

Study No. Trial No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
10-3223 10-3223-01, 10-3223-02, 10-3223-04 and 10-3223-05 GLP: yes 2010	Lettuce, head	head	BYI 02960	15	0.01	79;87;102;106; 107;109;110; 116;92;97;107; 108;114;116; 117	79	117	104	10.7
				5	0.10	88;90;90;92;93	88	93	91	2.2
				2	0.50	103;106	103	106	105	
				2	1.0	92;94	92	94	93	
				2	5.0	90;98	90	98	94	
				26	overall	79	117	100	10.5	
		difluoroacetic acid		12	0.02	90;93;94;95;97 ;112;112;116; 86;89;93;95	86	116	98	10.2
				3	0.05	90;94;98	90	98	94	4.3
				2	0.20	92;94	92	94	93	
				5	0.50	93;101;90;91; 92	90	101	93	4.7
				2	1.0	90;92	90	92	91	
				2	5.0	90;89	89	90	90	
				26	overall	86	116	95	7.9	
		BYI 02960- difluoroethyl- aminofuranone		15	0.01	87;93;95;100; 100;104;105; 107;83;83;86; 88;90;92;96	83	107	94	8.4
				5	0.10	85;98;97;97;99	85	99	95	6.1
				2	0.50	97;109	97	109	103	
				2	1.0	86;101	86	101	94	
				2	5.0	97;96	96	97	97	
				26	overall	83	109	95	7.7	
		head, inner parts	BYI 02960	1	0.01	114	114	114	114	
				1	overall	114	114	114	114	
		difluoroacetic acid		1	0.02	93	93	93	93	
				1	overall	93	93	93	93	
		BYI 02960- difluoroethyl- aminofuranone		1	0.01	93	93	93	93	
				1	overall	93	93	93	93	

Continued on next page...



Table 6.5.4-3 (cont'd): Recovery data for BYI 02960 in lettuce and lettuce matrices

Study No. Trial No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
10-3223 10-3223-01, 10-3223-02, 10-3223-04 and 10-3223-05 GLP: yes 2010 (cont.)	leaf, outer washings	BYI 02960	1	0.01	115	115	115	115		
			1	10	94	94	94	94		
			2	overall	94	115	115	105		
		difluoroacetic acid	1	0.02	94	94	94	94		
			1	10	90	90	90	90		
			2	overall	90	94	94	92		
		BYI 02960-difluoroethyl-aminofuranone	1	0.01	90	90	90	90		
			1	10	95	95	95	95		
			2	overall	90	95	95	93		
		BYI 02960	5	0.01	99;102;106;109; 117	99	117	107	6.5	
			3	0.50	95;97;111	95	111	101	8.6	
			8	overall	95	117	105	7.3		
		difluoroacetic acid	5	0.02	95;96;97;97; 103	95	103	98	3.2	
			3	0.50	91;94;97	91	97	94	3.2	
			8	overall	91	103	96	3.5		
		BYI 02960-difluoroethyl-aminofuranone	5	0.01	95;99;100;100; 107	95	107	100	4.3	
			3	0.50	86;88;96	86	96	90	5.9	
			8	overall	86	107	96	7.1		

Hops

Report:	KIIA 6.5.4/02, Schulte, G. & Bauer, J.; 2012
Title:	Determination of the residues of BYI 02960 in/on hop (cone, green and cone, kiln-dried) and the processed fractions (hops draff, brewer's yeast and beer) after spraying of BYI 02960 SL 200 in the field in Germany
Report No. & Document No.:	10-3407, dated February 13, 2012 <u>M-425311-01-1</u>
Guidelines:	– EU Council Directive 91/414/EEC amended by Commission Directive 96/68/EC – EU Guidance Working Document 7035/VI/95 rev. 5 – OECD Guideline for the Testing of Chemicals No. 508, Magnitude of the Pesticide Residues in Processed Commodities
GLP:	yes (certified laboratory)

I. Materials and Methods

In view of the presence of measurable residues of BYI 02960 on harvested hop cones determined in samples from field residue trials performed according to the intended commercial use conditions (see point IIA 6.3.2), investigations on the effects of processing have been conducted. Two trials were conducted in Germany in order to determine the total residues of BYI 02960 in green and dried hop cones and then, after processing, in beer as well as the additional processing intermediates hops draff and brewer's yeast (Schulte & Bauer, 2012; KIIA 6.5.4/02).

The field trials were conducted at approx. 2½ times the normal application rate for hops, in an attempt to ensure that quantifiable residues will be present in the RAC samples. BYI 02960 SL 200 was sprayed once at an application rate of approx. 360 g a.s./ha and a water volume of 3000 L/ha. The application was conducted at a pre-harvest interval of 21 days.

After processing (described below), residue analysis was performed according to method 01304 as used in the RAC trials themselves (for more information, cf. points IIA 6.3.2 and IIA 4.3). The limits of quantitation were 0.1 mg/kg (BYI 02960 and DFEAF) and 0.2 mg/kg (DFA), expressed in parent compound equivalents, yielding a calculated total-residue LOQ of 0.4 mg/kg, in all matrices except beer, in which the LOQs were 10 times lower (0.01 and 0.02 mg/kg; total residue: 0.04 mg/kg). Prior and parallel to the residue analysis, the method was validated by recovery experiments.

Preparation of beer:

Beer processing simulated industrial brewing practices. Prior to the addition of hops to the wort, ground malted barley and water were mixed in a mash tun ("mashing"), then the wort was separated from the insoluble malt components ("lautering").

Green hop cones were dried in hop driers, according to standard industrial procedures, then ground and pelleted. Hop pellets were added to the wort, which was then boiled. Subsequently, the flocs were separated via a whirlpool in which the sludge (hops draff) deposited at the bottom of the cone-shaped vessel. After addition of yeast, the mixture was allowed to ferment in bottom-fermentation containers ("primary fermentation", temp. 9°C). As soon as the extract content of the young beer was sufficient, storing began. Before maturation, however, the young beer was cooled.



During the main fermentation, the yeast deposits on the bottom of the tank; this material is sampled (brewer's yeast). The beer is stored under pressure at approx. 2°C for 3-4 weeks. All further sludge-like materials settle to the bottom. The matured liquid is filtered and then the final product, beer, is sampled. The process is illustrated in flow diagram 6.5.4-2.

II. Findings

Limited validation sets and concurrent recoveries of BYI 02960 and its metabolites DFA and DFEAF were obtained from samples of hop matrices (green and dried cones, hops draff, brewer's yeast, and beer). In all matrices except for beer, recovery samples for parent compound and DFEAF were spiked at levels of 0.10 mg/kg and 1.0 mg/kg, as well as 5.0 mg/kg for hop cones (expressed in BYI 02960 equivalents); in beer, spiking levels were 0.01 and 0.10 mg/kg. Mean recoveries in the larger validations sets ($n > 2$) for all matrices were 81-111%, with RSDs of 1.4-13.9%; $n=3-6$. Even in the case of values over 110%, these values were considered to be acceptable because they were only marginally higher and the RSD values were very low; also, in the cases of the exceptions, the overall means of all recovery analyses for the given matrices with each individual analyte were 107-108%, with overall RSDs of 4.2-7.1%.

For DFA, concurrent recovery samples for all matrices except beer were spiked at levels of 0.20 mg/kg and 1.0 mg/kg, as well as 5.0 mg/kg for hop cones (expressed in BYI 02960 equivalents); fortification levels in beer were 0.02 and 0.20 mg/kg. Mean recoveries in the larger validations sets ($n > 2$) in all matrices were 83-109%, with RSDs of 2.6-14.1%; $n=3-6$. All values were within acceptable ranges.

A tabular summary of the recovery values is presented below in [table 6.5.4-2](#).

Beer:

The total residues of BYI 02960 (parent compound plus DFA and DFEAF) in the harvested green cones at day 21 were 0.73 and 1.5 mg/kg. After drying, the values were 3.0 and 5.0 mg/kg. As the dried cone is generally considered to be the "RAC" in the case of hops, all processing factors are based on the residues in the dried commodity.

Residue values in beer were 0.04 and 0.05 mg/kg. Based on these values, a mean transfer factor of 0.01 to beer can be calculated from dried hop cones (0.04 from green cones). Residue levels in all sampled intermediate products (hops draff and brewer's yeast) were below the LOQ of 0.4 mg/kg. All relevant data

The transfer factors for the total residues of BYI 02960 are summarized below in [table 6.5.4-4](#). All trial data are summarised further below in [table 6.5.4-5](#) and in greater detail in the Tier 1 summary forms.

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Table 6.5.4-4: Summary of total residues of BYI 02960 in mg/kg and transfer factors (*in italics and parentheses*) in hop RACs and processed products following application of BYI 02960 SL 200

Trial number	Green cone	Dried cone (RAC)	Hops draf ^f	Brewer's yeast	Beer
10-3407-01	0.73	3.0	<0.4 (<0.13)	<0.4 (<0.13)	0.04 (0.01)
10-3407-02	1.5	5.0	<0.4 (<0.08)	<0.4 (<0.08)	0.05 (0.01)
<i>Mean transfer factors:</i>			<0.1	<0.1	0.01

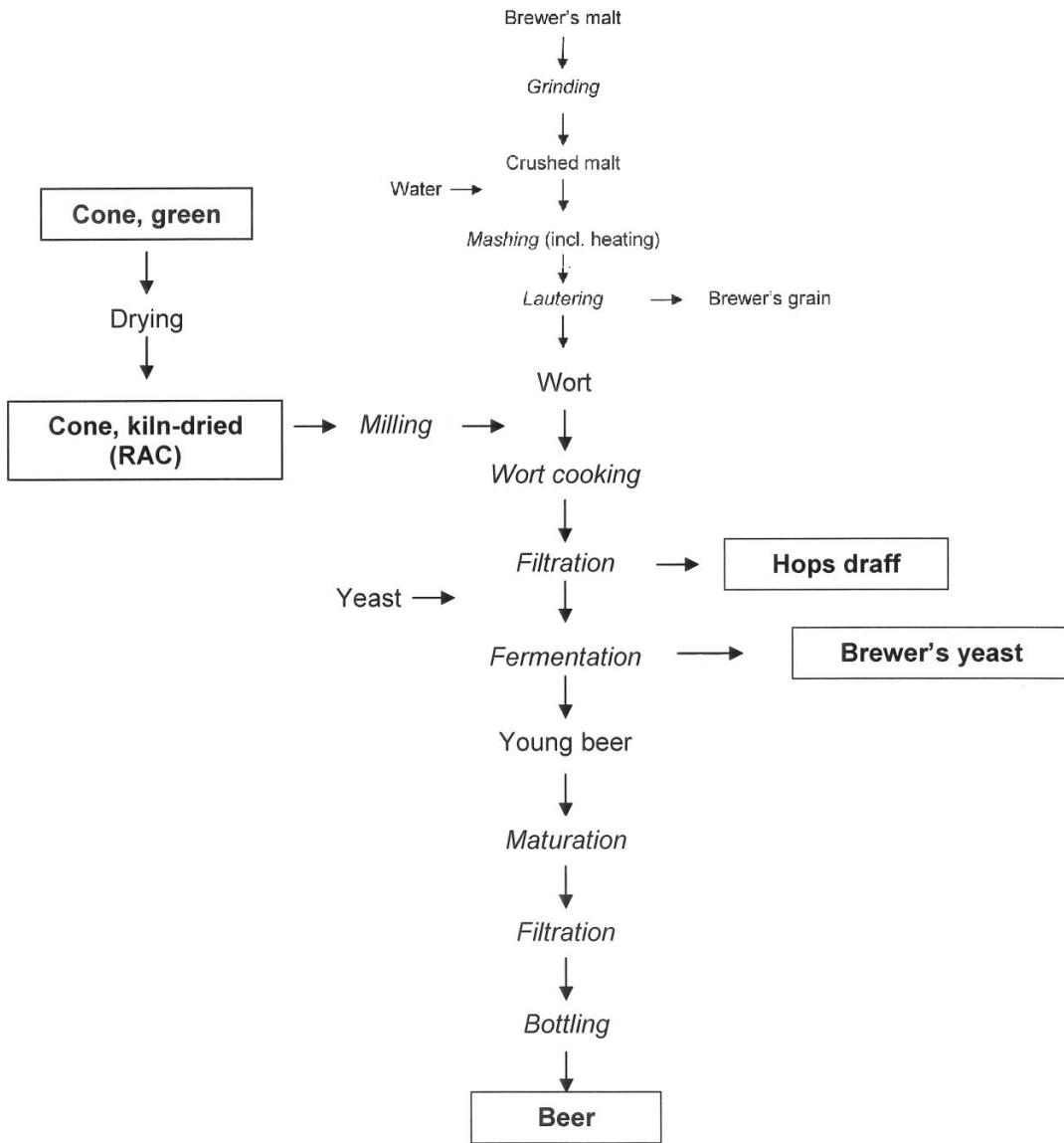
III. Conclusions

In order to determine transfer factors for total residues of BYI 02960 from hops (dried cones) to beer, processing studies have been conducted.

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.



Diagram 6.5.4-2: Processing of hop cones to beer



: Samples or fractions to be analysed



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Table 6.5.4-5a: Application scenario in residue processing trials conducted in/on **hops** after spraying with BYI 02960 SL 200 in European fields

Study No. (Trial No.) Country Location Region Year	Crop Variety	FL	No	Application		GS	PHI (days)
				kg/ha (a.s.)	kg/hl		
10-3407 (10-3407-01) Germany 04685 Golzern EU-N 2010	hop Nugget	200 SL	1	0.36	0.012	71	21
10-3407 (10-3407-02) Germany 99706 Hohene-bra EU-N 2010	hop Nordischer Brauer	200 SL	1	0.36	0.012	75	21

FL = formulation

GS = growth stage (BBCH-code) at last treatment

EU-N = northern Europe

Table 6.5.4-5b: Results of residue processing trials conducted in/on **hops** after spraying with BYI 02960 SL 200 in European fields

Study No. (Trial No.) Country GLP	Portion analyzed	DALT (days)	Residues (mg/kg) expressed as BYI 02960			
			BYI 02960	difluoroacetic acid	BYI 02960- difluoro- ethylamino- furanone	total residue of BYI 02960 calc.
10-3407 (10-3407- 01) Germany GLP: yes	cone, green	21	0.43	<0.2	<0.1	0.73
	cone, kiln-dried	21	2.2	0.72	<0.1	3.0
	hops draff	21	<0.1	<0.2	<0.1	<0.4
	brewer's yeast	21	<0.1	<0.2	<0.1	<0.4
	beer	21	0.01	<0.02	<0.01	0.04
10-3407 (10-3407- 02) Germany GLP: yes	cone, green	21	1.1	0.37	<0.1	1.5
	cone, kiln-dried	21	4.2	0.76	<0.1	5.0
	hops draff	21	<0.1	<0.2	<0.1	<0.4
	brewer's yeast	21	<0.1	<0.2	<0.1	<0.4
	beer	21	0.02	<0.02	<0.01	0.05

DALT = days after last treatment



Table 6.5.4-6: Recovery data for BYI 02960 in hops and processed hop matrices

Study No. Trial No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
10-3407 10-3407-01 and 10-3407-02 GLP: yes 2010	Hop	cone, green	BYI 02960	6	0.10	89;89;91;94;95; 107	89	107	94	7.2
				5	1.0	85;86;87;92;98	85	98	90	6.0
				1	5.0	87	87	87	87	
		difluoroacetic acid		12	overall		85	107	92	6.8
				6	0.20	91;92;95;99;100; 115	91	115	99	8.9
				5	1.0	76;79;83;84;94	76	94	83	8.2
				1	5.0	86	86	86	86	
		BYI 02960- difluoroethyl- aminofuranone		12	overall		76	115	91	11.7
				6	0.10	68;73;79;85;95; 96	68	96	83	13.9
				5	1.0	76;77;78;84;91	76	91	81	7.8
				1	5.0	80	80	80	80	
		cone, kiln-dried	BYI 02960	12	overall		68	96	82	10.6
				6	0.10	102;103;103; 104;105;106	102	106	104	1.4
				5	1.0	107;108;111; 114;115	107	115	111	3.2
				1	5.0	112	112	112	112	
		difluoroacetic acid		12	overall		102	115	108	4.2
				6	0.20	82;96;97;103; 103;106	82	106	98	8.9
				5	1.0	101;105;106; 107;110	101	110	106	3.1
				1	5.0	98	98	98	98	
		BYI 02960- difluoroethyl- aminofuranone		12	overall		82	110	101	7.3
				6	0.10	89;100;106;107; 107;108	89	108	103	7.2
				5	1.0	108;109;110; 112;114	108	114	111	2.2
				1	5.0	112	112	112	112	
				12	overall		89	114	107	6.2

Continued on next page...



Table 6.5.4-6 (cont'd): Recovery data for BYI 02960 in hops and processed hop matrices

Study No. Trial No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
		hops draff	BYI 02960	5	0.10	84;94;96;105; 108	84	108	97	9.8
				3	1.0	101;102;105	101	105	103	2.0
				8	overall	84	108	99	7.8	
			difluoroacetic acid	5	0.20	98;99;108;109; 111	98	111	105	5.8
				3	1.0	97;101;102	97	102	100	2.6
				8	overall	97	111	103	5.3	
			BYI 02960-difluoroethylaminofuranone	5	0.10	103;105;105; 105;109	103	109	105	2.1
				3	1.0	100;104;107	100	107	104	3.4
				8	overall	100	109	105	2.5	
		brewer's yeast	BYI 02960	5	0.10	98;109;111;111; 113	98	113	108	5.5
				3	1.0	77;99;102	77	102	93	14.7
				8	overall	77	113	103	11.5	
			difluoroacetic acid	5	0.20	99;107;109;115; 116	99	116	109	6.3
				3	1.0	76;90;101	76	101	89	14.1
				8	overall	76	116	102	13.2	
			BYI 02960-difluoroethylaminofuranone	5	0.10	97;107;107;110; 111	97	111	106	5.2
				3	1.0	85;99;109	85	109	98	12.3
				8	overall	85	111	103	8.6	
		beer	BYI 02960	5	0.01	95;100;110;114; 115	95	115	107	8.3
				3	0.10	105;112;116	105	116	111	5.0
				8	overall	95	116	108	7.1	
			difluoroacetic acid	5	0.02	93;100;110;110; 113	93	113	105	8.0
				3	0.20	108;108;110	108	110	109	1.1
				8	overall	93	113	107	6.2	
			BYI 02960-difluoroethylaminofuranone	5	0.01	92;94;102;111; 115	92	115	103	9.9
				3	0.10	102;107;112	102	112	107	4.7
				8	overall	92	115	104	8.0	



IIA 6.6 Residues in succeeding crops

The nature and level of residues in succeeding crops (confined rotational crops, field rotational crops) is influenced by the amount of active ingredient applied to the soil, by the degradation behaviour in soil, and by the uptake of parent compound and soil metabolites by the roots. Additionally, parent compound and soil metabolites can be metabolized by the plants, where hydroxylation reactions and formation of conjugates are often observed.

The aerobic degradation of BYI 02960 in soil was investigated in laboratory studies (see KIIA 7.1.1/01-06). The primary metabolic pathway of BYI 02960 in soil is molecular cleavage resulting in the metabolites difluoroacetic acid (DFA) and 6-chloronicotinic acid (6-CNA). DFA has been identified in all soils tested with a maximum percentage of 34%. 6-CNA was identified in several soils tested with a maximum percentage of 17%. Additionally, the minor metabolites BYI 02960-desdifluoroethyl (maximum level 0.4%) and BYI 02960-chloro (maximum level 1.8%) have been identified. Further microbial breakdown led to the formation of significant amounts of carbon dioxide and soil bound residues.

Since the exposure of following crops to BYI 02960 soil residues cannot be excluded, the metabolism of BYI 02960 was investigated in representative rotational crops (wheat, Swiss chard and turnips) following soil application of either [pyridinylmethyl-UL-¹⁴C] or [furanone-4-¹⁴C] radiolabelled active substance. The application rates were slightly above the anticipated maximum field rate of 400 g a.s./ha (for US uses). [Ethyl-1-¹⁴C]BYI 02960 was not available for a confined rotational crop study when the studies were started. Therefore, no detection of ¹⁴C-difluoroacetic acid was possible. To estimate the residue levels of difluoroacetic acid in the crop samples, non-radiolabelled difluoroacetic acid was analysed in the samples originating from the CRC studies with the other radiolabels by LC-MS/MS according to the conditions of residue analytical method 01304 (see KIIA 6.2.1/12).

IIA 6.6.1 Theoretical consideration of the nature and level of the residue

Confined rotational crop studies were planned as part of standard procedure. The results of the studies strongly indicated that residues are also to be expected under true field conditions.



IIA 6.6.2 Metabolism and distribution studies on representative crops

Report:	KIIA 6.6.2/01, Klempner, A.; 2011
Title:	Metabolism of [furanone-4- ¹⁴ C]BYI 02960 in confined rotational crops
Report No & Edition No	MEF-11/365 M-421861-01-2
Guidelines:	OECD 502 Metabolism in Rotational Crops US EPA Residue Chemistry Test Guideline OPPTS 860.1850: Confined Accumulation in Rotational Crops European Parliament and Council Regulation (EC) No 1107/2009
GLP	yes

Executive Summary

The metabolism of the insecticide BYI 02960 was investigated in the representative rotational crops wheat, Swiss chard and turnips from three consecutive rotations. [Furanone-4-¹⁴C]BYI 02960 was formulated as an SL 300 and sprayed onto the soil of a planting container (approx. 1 m²). The actual application rate corresponded to 436 g a.s./ha, slightly above the anticipated maximum seasonal field rate of 400 g a.s./ha. The crops were each sown at 29, 135 and 296 days after the soil application, representing the first, second and third rotation.

Intermediate raw agricultural commodities (RACs) investigated were Swiss chard immature, wheat forage and wheat hay. All other RACs (wheat straw, wheat grain, Swiss chard, turnip leaves and turnip roots) were harvested at maturity.

The TRR values for all RACs are given in the following table.

Table 6.6.2-1 TRR values in the different RACs of the three rotations after soil application of [furanone-4-¹⁴C]BYI 02960

TRR [mg/kg]	Wheat				Swiss chard		Turnips	
	forage	hay	straw	grain	imm.	mature	leaves	roots
1 st rotation	0.783	2.003	6.290	0.478	0.848	0.871	0.679	0.074
2 nd rotation	0.193	1.081	1.519	0.103	0.311	0.263	0.158	0.014
3 rd rotation	0.111	0.254	0.462	0.047	0.180	0.152	0.090	0.008

Except for wheat grain, extraction efficiencies ranged from 70% to 97% for all commodities of all rotations after conventional extraction with acetonitrile/water mixtures. Subsequent exhaustive extraction steps (microwave and/or digestive extraction steps), applied to the post extraction solids in wheat grain and wheat straw, released additional significant amounts of radioactivity and increased the extraction efficiencies to a range of 83% to 98% of the TRR. Thus overall, it was shown that the exhaustive extraction steps applied were able to release high amounts of radioactivity if conventional extraction was not sufficient.

Parent compound and metabolites in the extracts were analysed by reversed phase HPLC.

Identification was achieved by spectroscopic evidence (LC-MS/MS) after isolation and purification of the respective compounds from the conventional extracts of wheat straw or Swiss chard of the 1st rotation. HPLC co-chromatography with the isolated and identified radiolabelled metabolites allowed



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the assignment of the compounds in other extracts. Additionally, HPLC comparison of the metabolite profiles of the different extracts among each other and with the ones of the confined rotational crop study performed with [pyridinylmethyl-¹⁴C]BYI 02960, completed the picture. The polar fraction detected in wheat grains was identified by TLC co-chromatography using the natural compound (glucose/carbohydrates) identified in the tomato metabolism study conducted with [furanone-4-¹⁴C]BYI 02960, as reference compound. Other polar metabolites/fractions were also analysed by normal phase TLC

Parent compound was the most prominent compound in all RACs of all rotations, except in wheat grains, where it was detected as a minor compound only. The main compound detected in grains accounted for approx. 70% of the TRR in the sample of the 1st rotation and was identified as a natural compound (glucose/carbohydrates). Major metabolites in other wheat RACs, Swiss chard and turnips were BYI 02960-OH-glyc, -glyoxylic acid, -difluoroethyl-amino-furanone, -difluorethyl-amino-furanone-OH-glyc and BYI 02960-bromo-amino-furanone which were detected in a range of approx. 10% - 22% of the TRR in numerous samples. All other identified metabolites were detected at minor or trace levels. In total, fourteen metabolites were identified; six of them were specific to the radiolabel tested.

[Furanone-4-¹⁴C]BYI 02960 was rather extensively metabolised in confined rotational crops. The following metabolic routes were observed:

- cleavage of the pyridinylmethylamine bond and formation of metabolites based on difluoroethyl-amino-furanone and amino-furanone structures,
- BYI 02960-difluorethyl-amino-furanone conjugation with either mercapto-lactic acid or conjugation with carbohydrates after hydroxylation,
- hydroxylation of the methylene group of the furanone moiety followed by conjugation with carbohydrates and sulphate,
- oxidative degradation of the furanone moiety to BYI 02960-acetic acid, which was either subjected to conjugation with glucose or to a further oxidation step
- complete degradation of the furanone moiety and incorporation of carbon atoms into the natural compound pool, i.e. into glucose/carbohydrates, and
- halogenation of the furanone ring with bromine or chlorine.

Halogenation of the furanone moiety of the active substance probably occurred in the soil which is supported by the fact that small amounts of halogenated parent compound have been identified in the aerobic soil degradation studies. Metabolite BYI 02960-glyoxylic acid, the oxidation product of BYI 02960-acetic acid, was probably a transient soil metabolite that was taken up by the plants, since it was only prominent in the samples of the 1st rotation.

On the basis of these results, a metabolic pathway of [furanone-4-¹⁴C]BYI 02960 in confined rotational crops can be proposed.



I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure		* position of the radiolabel
Radiolabelled test material	[furanone-4- ¹⁴ C]BYI 02960	
Specific radioactivity	3.97 MBq/mg (106.46 µCi/mg)	
Chemical Purity	> 99% (HPLC)	
Radiochemical purity	> 99% (HPLC and TLC)	

The supplied radiolabelled test compound [furanone-4-¹⁴C]BYI 02960 was dissolved in acetonitrile. Formulation of the test compound was performed prior to the application: An appropriate amount of this stock solution was evaporated to dryness. The respective amount of blank formulation SL 300 was added and the mixture was homogenised using an ultrasonic bath (radioactive formulation). The sample was adjusted to a final volume of approx. 100 mL with water and homogenised by stirring to get the ready-to-use spray dilution.

2. Soil: “Monheim 4” (sandy loam soil from Germany), pH (CaCl₂) = 6.9, 58% sand, 28% silt and 14% clay, 2.1% organic carbon, cation exchange capacity (CEC) of 8.1 meq/100 g

3. Plants:

rotational crop	variety	representative for crop group
spring wheat	Thasos	small grain
Swiss chard	Lukullus	leafy vegetable
turnips	Rondo	root crop

B. Study Design

Experimental conditions:

BYI 02960 was applied as an SL formulation with a computer controlled track sprayer onto the bare soil of a planting container (surface area of approx. 1 m²). The application rate was 436 g a.s./ha and was slightly above the anticipated maximum seasonal rate of BYI 02960. The treated soil remained undisturbed for an aging period of 29 days. After this period, the upper layer of the soil (approx. 15 cm) was intensively mixed and wheat, Swiss chard and turnips of the first rotation were sown. Wheat was sown on approx. 50% (0.5 m²) of the soil area of the planting container. Swiss chard and turnips were each sown on 25% (0.25 m²) of the soil area of the container. At day 135 and 296 after the application (after harvest of the mature wheat - the crop with the longest vegetation period) the soil was cultivated again and the crops of the second and third rotation were sown, respectively. With each rotation the plots of the crops in the container were changed. Wheat was sown on the plot where Swiss



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chard and turnips had been sown in the preceding rotation and Swiss chard and turnips were sown on the plot where wheat was sown before.

Sampling:

Raw agricultural commodities (RAC) sampled for this study included the immature samples forage and hay from wheat, and an immature sample of Swiss chard. All other samples (wheat straw and wheat grain, mature Swiss chard, turnip leaves and turnip roots) were harvested at maturity of each rotation.

About 20% of the wheat plants were cut shortly above the ground as forage sample at BBCH growth stage 29-31 (stem elongation). At BBCH 79-83 (late milk to early dough stage) again 20% of the wheat plants were cut as hay sample and dried at room temperature for three to five days. At BBCH 89-92 (maturity) the remaining wheat plants were cut and grains were separated by hand. The remaining ears and chaffs were combined with the straw. A part of the Swiss chard plants were sampled at an immature stage (BBCH 44-46) and the remaining plants at maturity (BBCH 49). The Swiss chard plants were cut above the roots. The mature turnips plants were removed from soil, separated into leaves and roots. The plant materials were cut in pieces before homogenization with liquid nitrogen using a Polytron homogenizer.

Aliquots of the homogenized samples were used to estimate the TRR in the sample material by combustion and were used for extraction. Remaining homogenized sample material was stored in a freezer at approx. -18 °C.

C. Analytical Procedures**Extraction:**

An aliquot of each homogenized RAC was extracted conventionally three to four times with ACN/water (8:2, v/v). The extracts were combined, purified using a pre-conditioned SPE RP 18 cartridge, concentrated and analysed by HPLC. The radioactivity in the extracts was determined by LSC, in the solids by combustion followed by LSC. The actual TRR values of the samples were determined by summing up the radioactivity measured in the extracts and in the remaining solids. If needed, solids were further extracted exhaustively using microwave conditions. Post-extraction solids of wheat straw (1st and 2nd rotation) were subjected to a sequential extraction procedure after one exhaustive extraction step with ACN/water (8:2, v/v) at increased temperature (60 °C) under microwave conditions. The solids of the microwave extraction step were subjected to a treatment with sodium chloride solution (2 h at 100 °C under microwave assistance), a diastase incubation (approx. 20 h at 26 °C), a treatment with EDTA solution (3 h at 100 °C under microwave assistance) and a cellulase treatment (approx. 20 h at 40 °C) at adjusted temperatures to break down the plant cell walls and liberate residues bound to cell walls or in the cells. The remaining solids of the sequential extraction were extracted finally in two steps with a 5N HCl and a 5N NaOH solution (each 2 h at 100 °C). Extracts of the first three exhaustive extraction steps were concentrated and analysed separately by HPLC. Extracts of the EDTA, the cellulase and the HCl treatment were combined and adjusted to pH 7. The combined extract was concentrated, centrifuged and analysed by HPLC. The NaOH extract was not submitted to HPLC due to its high viscosity.



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Post-extraction solids of grains from the 1st and the 2nd rotation were subjected to three subsequent exhaustive extraction steps: Microwave extraction with ACN/water at 100 °C followed by a digestion step with diastase (20 h at 26 °C) and subsequent microwave extraction with a sodium chloride solution (2 h at 100 °C). Only the first exhaustive extract was analysed by HPLC, the two following extracts were highly viscous due to a high matrix load and could not be injected in the HPLC system. Therefore a further aliquot of the post extraction solids of grains from the 1st rotation was subjected to digestion with diastase over 14 days at ambient temperature. The residues in the diastase digestion solution were further characterized by partitioning against dichloromethane.

Quantification:

Parent compound and metabolites in the extracts were analysed by reversed phase HPLC coupled to a radioactivity detector with a glass scintillator cell. The HPLC chromatograms (= metabolite profiles) were integrated for quantification of compounds.

Identification and characterisation:

Identification of parent compound and metabolites was based on the metabolite profiles of the conventional extracts of Swiss chard and wheat straw of the first rotation. These extracts showed all major and minor metabolites also detected in the other extracts. Therefore, parent compound, all major and most minor metabolites were isolated from these extracts, purified and identified by HPLC-MS/MS. Two metabolites were co-eluting in the extract of wheat straw when analysed with the profiling method. To ensure the right assignment in other extracts, the respective peak was isolated by semi-preparative HPLC and re-analysed with an acidic method which was able to separate the two compounds, if present. Based on these assignments, the compounds in all conventional and exhaustive extracts of all rotations were identified by comparison with the metabolite patterns of wheat straw and Swiss chard. The presence of several metabolites was additionally confirmed by HPLC co-chromatography using the isolated compounds as reference compounds. Confirmation of assignments by HPLC co-chromatography became important since variations in retention times of some metabolites and distortion of peaks was observed when analysing extracts of wheat forage (1st rotation) and Swiss chard (2nd rotation) at different time points. The retention shift was most probably caused by an interaction between matrix components and metabolites. Natural label-specific compounds detected in wheat grains were identified by TLC co-chromatography subsequent to a diastase digestion step using a metabolite isolated and identified in the tomato metabolism study performed with [furanone-4-¹⁴C]BYI 02960. The same natural compound was identified in turnip roots by TLC comparison of the isolated and purified metabolites.

Storage stability:

All samples were conventionally extracted and analysed by HPLC within a few days after sampling. Quantitative ¹⁴C-HPLC analysis of the extracts was performed with the profiling method BYI02960_NEUTR within one week after harvest. Only for wheat forage and hay, Swiss chard and turnips from the 1st rotation, the metabolic profile was measured with the preliminary profiling method BYI02960_CRC directly after extraction. The preliminary method was based on the same type of column and the same eluents but had a slightly shorter gradient. The metabolite profiles obtained from the two methods were comparable, however profiling method BYI02960_NEUTR showed an improved separation for some metabolites. For better comparison of metabolic pattern over all RACs of all rotations, the profiles of wheat forage and hay, Swiss chard and turnips of the 1st rotation were



re-analysed with method BYI02960_NEUTR about 1 – 2 months after extract preparation. These new metabolic profiles were then considered as basis for quantification. Extract stability has been demonstrated for this time period.

Exhaustive extractions were started for wheat straw and wheat grain within approx. two to seven months after harvest. Since the compounds identified in the exhaustive extracts were comparable with the compounds of the conventional extracts, it was concluded that the residues in the solids were stable and reflected the released residues at the time of exhaustive extraction.

Thus it can be concluded that the residues in all matrices were sufficiently stable during the experimental period of the study and that the first quantified profiles represented the metabolic pattern in the samples at harvest. Nevertheless, storage stability of BYI 02960 residues in frozen sample material was additionally demonstrated in wheat straw (1st and 2nd rotation) and in Swiss chard (1st rotation) for a storage period of about 18 to 24 months. The stored sample material was subjected to a second extraction. Analysis of the new extracts showed the same metabolite patterns as the first extracts and confirmed the storage stability of the residues in the stored RACs.

Extract stability has been demonstrated for the time of the sample preparation and the identification process of metabolites, as well: The metabolite patterns of stored extracts remained stable when re-analysing extracts, e.g. for isolation of metabolites or identification purposes. Neither significant degradation nor transformation of metabolites in extracts was observed.

Hence, it was concluded that the results of the present study were not impacted by the storage of the samples and that no further storage stability investigations are required.

II. Results and Discussion

The metabolism of [furanone-4-¹⁴C]BYI 02960 was investigated in the rotational crops spring wheat, Swiss chard and turnips following application on the soil. The active substance was applied as an SL formulation on the bare soil at a rate of 436 g/ha at 29 days before sowing of the crops representing the first rotation. Crops of the 2nd and 3rd rotation were sown 135 and 296 days after application. Immature Swiss chard, wheat forage and wheat hay were harvested as intermediate raw agricultural commodities (RACs). All other RACs (wheat straw, wheat grain, Swiss chard, turnip leaves and turnip roots) were harvested at maturity.

The TRR values of all RACs declined significantly (by a factor of 5 to 13) from the first to the third rotation. Highest residues were detected in the non-edible commodities wheat straw and wheat hay, as shown above in Table 6.6.2-1.

Radioactive residues were efficiently extracted from all commodities of all rotations with acetonitrile/water mixtures, except for wheat grain and wheat straw (only 14.6% to 20.4% and 70.3% to 77.6% of the TRR and was detected after conventional extraction, respectively). For wheat grain and wheat straw additional exhaustive extraction steps were applied for the samples of the 1st and 2nd rotation. Exhaustive extraction comprised one extraction step with acetonitrile/water (8:2; v/v) at increased temperature (60 °C) under microwave assistance and additional sequential extraction steps



including enzymatic digestion steps. Exhaustive extraction steps released an additional portion of 68% – 73% of the TRR from the solids of grains and about 21% – 27% of the TRR from the solids of straw resulting in total extraction efficiencies ranging between 83% and 98% of the TRR.

Parent compound BYI 02960 and about 30 metabolites were detected in the conventional and exhaustive extracts of the various samples of the three rotations. Of these the active substance and 14 metabolites were identified by LC-MS/MS. The other metabolites, none of them exceeding 5% of the TRR or 0.05 mg/kg., were characterised by their extraction behaviour and their retention in radio-HPLC or TLC. The amounts of active substance and metabolites in all RACs are summarized in Table 6.6.2-2 to Table 6.6.2-7 for the three rotations.

Parent compound was by far the main component detected in all matrices of all rotations, except for wheat grain. Parent accounted for 34% to 64% of the TRR in the commodities of the 1st rotation, for 28% to 68% in the 2nd rotation and covered 18% to 72% in the 3rd rotation, not considering grains. In wheat grains, only trace amounts of parent compound were detected (<1% and 2% of the TRR). The highest proportion of parent compound was always detected in turnip leaves.

Six of the identified metabolites were specific to the radiolabel used: BYI 02960-difluoroethyl-amino-furanone, its conjugate BYI 02960-mercaptopo-lactic acid, BYI 02960-difluoroethyl-amino-furanone-OH-glyc, BYI 02960-amino-furanone, BYI 02960-bromo-amino-furanone, and the natural compound glucose (or probably an isomeric carbohydrate). The natural compound was identified as the main component in wheat grain after subjecting the post extraction solids of the conventional extraction (1st rotation) to a diastase digestion step. The released radioactivity in the aqueous digestion solution was partitioned against dichloromethane. The majority of the radioactivity remained in the aqueous phase indicating that the metabolite was probably a polar natural compound. Isolation of the polar fraction by semi-preparative HPLC and following analysis by normal phase TLC showed one spot indicating that the fraction is represented most probably by one compound. TLC co-chromatography with the natural compound isolated and identified in the tomato metabolism study with [furanone-4-¹⁴C]BYI 02960 confirmed the correspondance of the metabolites. TLC analysis of the isolated polar fractions detected in the conventional and exhaustive extracts of wheat straw and in the conventional extracts of Swiss chard and turnip roots revealed that these fractions were represented by several compound (up to eight). Only minor percentages of the fractions appear identical with glucose. Based upon TLC, for turnip roots, a trace amount was assigned to the natural compound.

Label unspecific metabolites (= metabolites common to both radiolabels tested) were BYI 02960-OH and its conjugates, BYI 02960-acetic acid, its conjugate BYI 02960-acetic acid-glyc and its successor molecule BYI 02960-glyoxylic acid and the chlorinated/brominated parent compound. These metabolites were also detected in the CRC study conducted with [pyridinylmethyl-¹⁴C]BYI 02960. The metabolites BYI 02960-acetic acid and BYI 02960-OH-glyc were not separated by the HPLC profiling method and co-eluted in one peak or one peak zone. Separation and sub-quantification of the metabolites was shown for wheat straw (1st rotation), Swiss chard (1st rotation) and turnip leaves (1st rotation) as representative crops. Therefore the corresponding peak zones were isolated by semi-preparative HPLC, concentrated and re-analysed with an acidic HPLC method which separated the two metabolites. The ratios of BYI 02960-acetic acid and BYI 02960-OH-glyc obtained for wheat straw were assigned to all wheat samples, the ratio of Swiss chard to immature and mature Swiss

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

chard, and the ratio of turnip leaves to turnip roots of all rotations. BYI 02960-OH-glyc was the prominent compound in all samples of all rotations.

Overall, identification rates were high for all samples of all rotations. For some RACs (e.g., wheat straw and wheat grain), high identification rates were only shown for the 1st and 2nd rotation (or in the case of grains for the 1st rotation only) since exhaustive extraction and following analysis of the exhaustive extracts was only conducted exemplarily for these samples. Thus, lower identification rates in this study were generally linked to higher bound residues (wheat matrices) and no subsequent exhaustive extraction step. In the case of turnip roots of the 2nd rotation, the identification rate was also quite low (53%) since one unknown compound represented a high percentage of the TRR, but at a very low residue level (0.004 mg/kg).

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Table 6.6.2-2 Distribution of parent compound and metabolites in wheat matrices after a 29 day plant back interval (1st rotation, [furanone-4-¹⁴C]BYI 02960)

1 st Rotation	wheat forage		wheat hay		wheat straw		wheat grains	
TRR [mg/kg]	0.783		2.003		6.290		0.478	
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
<i>Conventional extraction</i>								
BYI 02960 (parent comp.)	46.6	0.365	33.6	0.672	33.9	2.135	0.4	0.002
glucose/carbohydrates	---	---	---	---	---	---	---	---
amino-furanone	1.9	0.015	3.8	0.076	1.9	0.120	0.5	0.002
mercapto-lactic acid	1.6	0.013	0.4	0.008	1.1	0.068	---	---
bromo-amino-furanone	2.0	0.016	1.6	0.033	2.6	0.161	---	---
difluoroethyl-amino-furanone-OH-glyc	3.2	0.025	5.8	0.116	3.5	0.217	---	---
difluoroethyl-amino-furanone	9.9	0.077	10.2	0.205	5.3	0.333	---	---
glyoxylic acid	15.8	0.124	11.3	0.227	14.7	0.925	5.1	0.024
acetic acid-glyc	---	---	1.2	0.024	0.7	0.043	---	---
acetic acid	1.7	0.013	1.6	0.031	1.7	0.106	0.6	0.003
OH-glyc	3.6	0.028	3.4	0.067	3.6	0.228	1.4	0.007
OH	1.3	0.010	1.9	0.038	2.3	0.147	2.3	0.011
bromo / chloro	0.3	0.003	---	---	0.2	0.011	---	---
Subtotal identified	88.0	0.689	74.7	1.496	71.4	4.493	10.3	0.049
unknown 1a ¹	1.5	0.012	3.7	0.075	3.6	0.227	2.3	0.011
unknown 2	---	---	---	---	0.3	0.018	---	---
unknown 3	---	---	---	---	0.1	0.008	---	---
unknown 4	---	---	---	---	0.2	0.011	---	---
unknown 5	---	---	---	---	0.2	0.010	---	---
unknown 6	---	---	---	---	0.3	0.020	---	---
unknown 7	---	---	0.3	0.007	0.5	0.032	---	---
unknown 8	---	---	---	---	---	---	---	---
unknown 9	---	---	---	---	---	---	1.2	0.006
unknown 10	---	---	---	---	---	---	---	---
unknown 11	---	---	---	---	---	---	0.7	0.003
unknown 12	---	---	---	---	---	---	---	---
unknown 13	---	---	---	---	---	---	---	---
unknown 14	---	---	---	---	---	---	---	---
unknown 15	---	---	---	---	---	---	---	---
unknown 16	---	---	---	---	0.3	0.022	---	---
unknown 17	---	---	2.0	0.041	---	---	---	---
Subtotal characterised	1.5	0.012	6.1	0.122	5.5	0.347	4.2	0.020
Total conventional extr.	89.5	0.701	80.8	1.619	76.9	4.840	14.5	0.069

Table continued on next page...



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

1 st Rotation	wheat forage	wheat hay	wheat straw	wheat grains			
<i>Microwave extraction I (ACN/water)</i>							
BYI 02960 (parent comp.)				1.6	0.102		
amino-furanone				0.2	0.013		
bromo-amino-furanone				0.2	0.011		
difluoroethyl-amino-furanone-OH-glyc				0.2	0.012		
difluoroethyl-amino-furanone				0.2	0.013		
glyoxylic acid				0.5	0.029		
acetic acid				0.1	0.003		
OH-glyc				0.1	0.007		
OH				0.1	0.007		
Subtotal identified				3.1	0.196		
unknown 1a ¹				1.4	0.085		
Subtotal characterised				1.4	0.085		
Total microwave extr. I				4.5	0.282		
<i>Microwave extraction II (1% NaCl)</i>							
BYI 02960 (parent comp.)				2.0	0.124		
amino-furanone				0.7	0.044		
difluoroethyl-amino-furanone				0.3	0.019		
glyoxylic acid				0.1	0.008		
acetic acid				<0.1	0.003		
OH-glyc				0.1	0.006		
OH				0.1	0.006		
Subtotal identified				3.3	0.209		
unknown 1a ¹				1.4	0.086		
Subtotal characterised				1.4	0.086		
Total 1% NaCl extraction				4.7	0.295		
<i>Diastase digestion</i>							
BYI 02960 (parent comp.)				0.7	0.042	---	---
glucose/carbohydrates				---	---	70.5	0.338
amino-furanone				0.3	0.016	---	---
difluoroethyl-amino-furanone				0.1	0.009	---	---
glyoxylic acid				0.1	0.003	---	---
OH				<0.1	0.001	---	---
Subtotal identified				1.1	0.072	70.5	0.338
unknown 1a ¹				0.8	0.050	---	---
Subtotal characterised				0.8	0.050	---	---
Total diastase digestion				1.9	0.122	70.5	0.338
<i>EDTA + cellulase + 5N HCl extraction</i>							
BYI 02960 (parent comp.)				0.9	0.057		
amino-furanone				0.5	0.032		
Subtotal identified				1.4	0.089		
unknown 1a ¹				3.6	0.229		
Subtotal characterised				3.6	0.229		

Table continued on next page...



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

1 st Rotation	wheat forage		wheat hay		wheat straw		wheat grains	
Total EDTA + cellul.+ 5N HCl extraction					5.1	0.318		
Total identified	88.0	0.689	74.7	1.496	80.4	5.059	80.8	0.387
Total characterised	1.5	0.012	6.1	0.122	12.7	0.798	4.2	0.020
Total extractable	89.7	0.703	81.6	1.634	98.4	6.190	88.0	0.421
Not analysed/losses	0.3	0.002	0.7	0.015	0.7	0.042	3.0	0.014
Unextractable (PES*)	10.3	0.081	18.4	0.369	1.6	0.099	12.0	0.058
Accountability	100.0	0.783	100.0	2.003	100.0	6.290	100.0	0.478

* post extraction solids

¹ polar unknown peak 1a in the conventional extract of wheat straw of the 1st rotation consisted of 4 different metabolites, all of them were minor according to TLC subquantification

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.6.2-3 Distribution of parent compound and metabolites in wheat matrices after a 135 day plant back interval (2nd rotation, [furanone-4-¹⁴C]BYI 02960)

2 nd Rotation	wheat forage		wheat hay		wheat straw		wheat grains	
TRR [mg/kg]	0.193		1.081		1.519		0.103	
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
<i>Conventional extraction</i>								
BYI 02960 (parent comp.)	63.9	0.124	29.1	0.314	28.5	0.433	0.5	0.001
glucose/carbohydrates	---	---	---	---	---	---	---	---
amino-furanone	2.0	0.004	6.2	0.067	4.1	0.062	1.3	0.001
mercapto-lactic acid	1.6	0.003	3.5	0.037	4.4	0.067	---	---
bromo-amino-furanone	3.3	0.006	9.9	0.107	6.1	0.093	---	---
difluoroethyl-amino-furanone-OH-glyc	1.3	0.003	7.3	0.078	4.2	0.064	---	---
difluoroethyl-amino-furanone	8.2	0.016	6.9	0.075	5.3	0.081	---	---
glyoxylic acid	0.3	<0.001	1.9	0.020	1.2	0.018	0.8	0.001
acetic acid-glyc	---	---	0.5	0.005	0.9	0.013	---	---
acetic acid	1.6	0.003	1.6	0.017	2.3	0.035	1.0	0.001
OH-glyc	3.4	0.007	3.4	0.037	5.0	0.076	2.1	0.002
OH	1.8	0.003	1.9	0.021	2.7	0.042	3.4	0.003
bromo / chloro	---	---	---	---	---	---	---	---
Subtotal identified	87.3	0.169	71.9	0.778	64.7	0.982	9.2	0.009
unknown 1a ¹	1.3	0.002	4.3	0.047	5.1	0.078	2.5	0.003
unknown 2	---	---	---	---	---	---	---	---
unknown 3	---	---	---	---	---	---	---	---
unknown 4	0.4	0.001	0.6	0.006	0.6	0.009	---	---
unknown 5	---	---	---	---	---	---	---	---
unknown 6	---	---	---	---	---	---	---	---
unknown 7	0.2	<0.001	0.5	0.006	---	---	---	---
unknown 8	---	---	---	---	---	---	---	---
unknown 9	---	---	---	---	---	---	0.7	0.001
unknown 10	---	---	---	---	---	---	---	---
unknown 11	---	---	---	---	---	---	0.9	0.001
unknown 12	0.3	0.001	---	---	---	---	---	---
unknown 13	---	---	---	---	---	---	---	---
unknown 14	---	---	---	---	---	---	---	---
unknown 15	---	---	---	---	---	---	---	---
unknown 16	---	---	---	---	---	---	---	---
unknown 17	---	---	---	---	---	---	---	---
Subtotal characterised	2.2	0.004	5.4	0.059	5.8	0.087	4.2	0.004
Total conventional extr.	89.6	0.173	77.4	0.837	70.4	1.070	13.4	0.014

Table continued on next page...



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

2 nd Rotation	wheat forage	wheat hay	wheat straw	wheat grains				
<i>Microwave extraction I (ACN/water)</i>								
BYI 02960 (parent comp.)					2.0	0.031	---	---
amino-furanone					0.7	0.011	---	---
difluoroethyl-amino-furanone-OH-glyc					0.1	0.002	---	---
difluoroethyl-amino-furanone					0.3	0.005	---	---
glyoxylic acid					0.1	0.001	---	---
acetic acid					0.1	0.001	---	---
OH-glyc					0.2	0.003	---	---
OH					0.2	0.004	---	---
Subtotal identified					3.8	0.057	---	---
unknown 1a ¹					1.0	0.015	---	---
Subtotal characterised					1.0	0.015	---	---
Total microwave extr. I	---	---	---	---	4.8	0.073	2²2.5	2²0.003
<i>Microwave extraction II (1% NaCl)</i>								
BYI 02960 (parent comp.)					2.3	0.035	---	---
amino-furanone					0.8	0.012	---	---
difluoroethyl-amino-furanone					0.3	0.004	---	---
acetic acid					<0.1	0.001	---	---
OH-glyc					0.1	0.001	---	---
OH					0.1	0.002	---	---
Subtotal identified					3.6	0.055	---	---
unknown 1a ¹					1.5	0.023	---	---
Subtotal characterised					1.5	0.023	---	---
Total 1% NaCl extraction	---	---	---	---	5.2	0.078	2²41.7	2²0.043
<i>Diastase digestion</i>								
BYI 02960 (parent comp.)					1.0	0.015	---	---
amino-furanone					0.4	0.006	---	---
Subtotal identified					1.4	0.021	---	---
unknown 1a ¹					0.9	0.014	---	---
Subtotal characterised					0.9	0.014	---	---
Total diastase digestion	---	---	---	---	2.3	0.035	2²23.7	2²0.024
<i>EDTA + cellulase + 5N HCl extraction</i>								
BYI 02960 (parent comp.)					1.6	0.024		
amino-furanone					1.3	0.019		
Subtotal identified					2.9	0.043		
unknown 1a ¹					3.3	0.050		
Subtotal characterised					3.3	0.050		

Table continued on next page...

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

2 nd Rotation	wheat forage		wheat hay		wheat straw		wheat grains	
Total EDTA + cellul.+ 5N HCl extraction	---	---	---	---	6.2	0.094		
<i>5N NaOH extraction</i>								
Subtotal characterised					8.7	0.131		
Total identified	87.3	0.169	71.9	0.778	76.4	1.159	9.2	0.009
Total characterised	2.2	0.004	5.4	0.059	12.5	0.102	4.2	0.004
Total extractable	89.6	0.173	77.9	0.842	97.8	1.485	82.6	0.085
Not analysed/losses	---	---	0.5	0.006	0.3	0.005	69.2	0.071
Unextractable (PES*)	10.4	0.020	22.1	0.239	2.2	0.033	17.4	0.018
Accountability	100.0	0.193	100.0	1.081	100.0	1.431	100.0	0.103

* post extraction solids

¹ polar unkown peak 1a in the conventional extract of wheat straw of the 1st rotation consisted of 4 different metabolites, all of them were minor according to TLC subquantification² no analysis performed, but presumably glucose/carbohydrates as identified in grains of 1st rotationTable 6.6.2-4 Distribution of parent compound and metabolites in wheat matrices after a 296 day plant back interval (3rd rotation, [furanone-4-¹⁴C]BYI 02960)

3 rd Rotation	wheat forage		wheat hay		wheat straw		wheat grains	
TRR [mg/kg]	0.111		0.254		0.462		0.047	
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
<i>Conventional extraction</i>								
BYI 02960 (parent comp.)	43.0	0.048	18.3	0.047	20.7	0.096	2.0	0.001
glucose/carbohydrates	---	---	---	---	---	---	---	---
amino-furanone	3.6	0.004	7.4	0.019	4.9	0.023	---	---
mercapto-lactic acid	2.6	0.003	5.1	0.013	1.5	0.007	---	---
bromo-amino-furanone	5.7	0.006	10.7	0.027	7.4	0.034	---	---
difluoroethyl-amino-furanone-OH-glyc	5.5	0.006	8.6	0.022	8.7	0.040	---	---
difluoroethyl-amino-furanone	12.1	0.013	8.3	0.021	5.2	0.024	---	---
glyoxylic acid	---	---	0.5	0.001	---	---	---	---
acetic acid	1.2	0.001	1.1	0.003	1.6	0.007	0.8	<0.001
OH-glyc	2.5	0.003	2.3	0.006	3.4	0.016	1.7	0.001
OH	0.8	0.001	1.6	0.004	2.1	0.010	2.8	0.001
bromo / chloro	---	---	---	---	---	---	---	---
Total identified	77.1	0.085	64.0	0.163	55.6	0.257	7.4	0.003
unknown 1a ¹	2.4	0.003	4.7	0.012	12.0	0.055	3.2	0.001
unknown 4	0.7	0.001	0.8	0.002	0.9	0.004	---	---
unknown 9	---	---	---	---	---	---	1.1	0.001
Total characterised	3.1	0.003	5.5	0.014	12.9	0.060	4.3	0.002
Total extractable	80.2	0.089	69.5	0.177	70.3	0.325	20.4	0.010
Not analysed/losses	---	---	---	---	1.8	0.008	8.8	0.004
Unextractable (PES*)	19.8	0.022	30.5	0.077	29.7	0.137	² 79.6	² 0.037
Accountability	100.0	0.111	100.0	0.254	100.0	0.462	100.0	0.047

* post extraction solids

¹ polar unkown peak 1a in the conventional extract of wheat straw of the 1st rotation consisted of 4 different metabolites, all of them were minor according to TLC subquantification² no analysis performed, but presumably glucose/carbohydrates as identified in grains of 1st rotation

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Table 6.6.2-5 Distribution of parent compound and metabolites in Swiss chard and turnip matrices after a 29 day plant back interval (1st rotation, [furanone-4-¹⁴C]BYI 02960)

1 st Rotation	Swiss chard immature		Swiss chard mature		turnip leaves		turnip roots	
TRR [mg/kg]	0.848		0.871		0.679		0.074	
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
<i>Conventional extraction</i>								
BYI 02960 (parent comp.)	54.3	0.460	42.6	0.371	64.3	0.437	55.9	0.041
glucose/carbohydrates	---	---	---	---	---	---	3.4	0.003
amino-furanone	1.8	0.015	3.1	0.027	0.9	0.006	4.1	0.003
difluoroethyl-amino-furanone-OH-glyc	---	---	1.2	0.010	---	---	---	---
difluoroethyl-amino-furanone	16.6	0.141	16.6	0.145	1.0	0.007	---	---
glyoxylic acid	3.7	0.031	4.7	0.041	6.6	0.045	12.2	0.009
OH-glyc-SA, isomer 1	1.2	0.010	2.5	0.022	---	---	---	---
acetic acid-glyc	0.5	0.004	1.6	0.014	3.5	0.024	---	---
OH-glyc-SA, isomer 2	3.3	0.028	3.2	0.028	---	---	---	---
acetic acid	0.4	0.003	0.6	0.005	1.2	0.008	0.2	<0.001
OH-glyc	8.5	0.072	13.6	0.119	11.2	0.076	2.1	<0.001
OH	2.0	0.017	1.9	0.017	1.7	0.012	0.4	<0.001
bromo / chloro	0.6	0.005	0.4	0.003	1.1	0.007	1.3	0.001
Total identified	92.8	0.787	92.0	0.802	91.5	0.621	79.6	0.058
unknown 1a ¹	2.5	0.021	3.5	0.031	1.4	0.010	---	---
unknown 1b	---	---	---	---	---	---	6.0	0.004
unknown 6	---	---	---	---	0.8	0.006	---	---
unknown 7	---	---	---	---	0.8	0.005	---	---
unknown 8	---	---	---	---	0.5	0.003	---	---
unknown 10	---	---	---	---	0.5	0.003	---	---
unknown 11	---	---	---	---	0.2	0.002	---	---
unknown 14	---	---	0.3	0.002	0.8	0.006	---	---
unknown 17	---	---	---	---	---	---	2.4	0.002
Total characterised	2.5	0.021	13.8	0.033	5.1	0.035	8.4	0.006
Total extractable	95.7	0.812	96.1	0.838	96.6	0.656	88.1	0.065
Not analysed/losses	0.4	0.003	0.3	0.002	---	---	---	---
Unextractable (PES*)	4.3	0.037	3.9	0.034	3.4	0.023	11.9	0.009
Accountability	100.0	0.848	100.0	0.871	100.0	0.679	100.0	0.073

* post extraction solids

¹ polar unknown peak 1a in Swiss chard consisted of 8 different metabolites, all of them were minor according to TLC subquantification

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 Table 6.6.2-6 Distribution of parent compound and metabolites in Swiss chard and turnip matrices after a 135 day plant back interval (2nd rotation, [furanone-4-¹⁴C]BYI 02960)

2 nd Rotation	Swiss chard immature		Swiss chard mature		turnip leaves		turnip roots	
TRR [mg/kg]	0.311		0.263		0.158		0.014	
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
<i>Conventional extraction</i>								
BYI 02960 (parent comp.)	55.0	0.171	27.5	0.072	68.1	0.108	31.0	0.004
glucose/carbohydrates	---	---	---	---	---	---	16.0	0.002
amino-furanone	8.8	0.027	8.8	0.023	0.6	0.001	---	---
difluoroethyl-amino-furanone-OH-glyc	---	---	---	---	---	---	---	---
difluoroethyl-amino-furanone	10.3	0.032	17.4	0.046	1.0	0.002	---	---
glyoxylic acid	---	---	---	---	1.5	0.002	1.8	<0.001
OH-glyc-SA, isomer 1	2.9	0.009	3.5	0.009	---	---	---	---
acetic acid-glyc	1.8	0.006	1.1	0.003	0.8	0.001	---	---
OH-glyc-SA, isomer 2	2.0	0.006	3.2	0.008	---	---	---	---
acetic acid	0.5	0.002	0.8	0.002	1.3	0.002	0.2	<0.001
OH-glyc	11.7	0.036	18.0	0.047	12.7	0.020	2.3	<0.001
OH	2.0	0.006	2.4	0.006	1.1	0.002	---	---
bromo / chloro	0.5	0.001	---	---	1.3	0.002	1.6	<0.001
Total identified	95.5	0.297	82.7	0.218	88.5	0.140	53.0	0.007
unknown 1a ¹	0.9	0.003	9.3	0.025	1.4	0.002	---	---
unknown 1b	---	---	---	---	---	---	28.0	0.004
unknown 6	---	---	---	---	0.8	0.001	---	---
unknown 7	---	---	---	---	0.6	0.001	---	---
unknown 8	---	---	---	---	0.5	0.001	---	---
unknown 14	0.4	0.001	---	---	2.8	0.004	1.3	<0.001
unknown 15	0.4	0.001	---	---	---	---	---	---
Total characterised	1.6	0.005	9.3	0.025	6.0	0.010	29.3	0.004
Total extractable	97.1	0.302	92.5	0.243	94.5	0.150	82.3	0.012
Not analysed/losses	---	---	0.5	0.001	---	---	---	---
Unextractable (PES*)	2.9	0.009	7.5	0.020	5.5	0.009	17.7	0.003
Accountability	100.0	0.311	100.0	0.263	100.0	0.158	100.0	0.014

* post extraction solids

¹ polar unknown peak 1a in Swiss chard consisted of 8 different metabolites, all of them were minor according to TLC subquantification

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Table 6.6.2-7 Distribution of parent compound and metabolites in Swiss chard and turnip matrices after a 296 day plant back interval (3rd rotation, [furanone-4-¹⁴C]BYI 02960)

3 rd Rotation	Swiss chard immature		Swiss chard mature		turnip leaves		turnip roots	
TRR [mg/kg]	0.180		0.152		0.090		0.008	
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
<i>Conventional extraction</i>								
BYI 02960 (parent comp.)	36.7	0.066	33.4	0.051	72.4	0.065	69.9	0.006
glucose/carbohydrates	---	---	---	---	---	---	5.3	<0.001
amino-furanone	1.3	0.002	1.8	0.003	0.5	<0.001	5.0	<0.001
difluoroethyl-amino-furanone-OH-glyc	2.1	0.004	1.9	0.003	---	---	---	---
difluoroethyl-amino-furanone	13.7	0.025	15.6	0.024	1.6	0.001	---	---
glyoxylic acid	0.2	<0.001	---	---	1.7	0.002	---	---
OH-glyc-SA, isomer 1	1.7	0.003	2.4	0.004	---	---	---	---
acetic acid-glyc	0.2	<0.001	0.2	<0.001	0.5	<0.001	---	---
OH-glyc-SA, isomer 2	4.2	0.008	2.4	0.004	---	---	---	---
acetic acid	0.9	0.002	0.9	0.001	1.1	0.001	0.3	<0.001
OH-glyc	22.2	0.040	21.9	0.033	10.2	0.009	3.1	<0.001
OH	3.6	0.007	3.1	0.005	0.6	0.001	---	---
bromo / chloro	0.5	0.001	0.6	0.001	1.8	0.002	2.5	<0.001
Total identified	87.3	0.157	84.2	0.128	90.2	0.081	86.2	0.007
unknown 1a ¹	3.4	0.006	1.8	0.003	1.1	0.001	---	---
unknown 1b	---	---	---	---	---	---	9.2	0.001
unknown 3	0.5	0.001	0.6	0.001	---	---	---	---
unknown 4	0.8	0.001	0.8	0.001	---	---	---	---
unknown 5	0.6	0.001	0.2	<0.001	---	---	---	---
unknown 6	---	---	---	---	1.1	0.001	---	---
unknown 11	0.3	<0.001	0.2	<0.001	---	---	---	---
unknown 13	---	---	0.6	0.001	---	---	---	---
unknown 14	0.2	<0.001	---	---	---	---	---	---
unknown 15	0.8	0.001	0.8	0.001	---	---	---	---
Total characterised	6.5	0.012	4.9	0.008	2.3	0.002	9.2	0.001
Total extractable	93.8	0.169	89.1	0.135	92.5	0.083	95.5	0.008
Not analysed/losses	---	---	---	---	---	---	---	---
Unextractable (PES*)	6.2	0.011	10.9	0.017	7.5	0.007	4.5	<0.001
Accountability	100.0	0.180	100.0	0.152	100.0	0.090	100.0	0.008

* post extraction solids

¹ polar unknown peak 1a in Swiss chard of 1st rotation consisted of 8 different metabolites, all of them were minor according to TLC subquantification



III. Conclusions

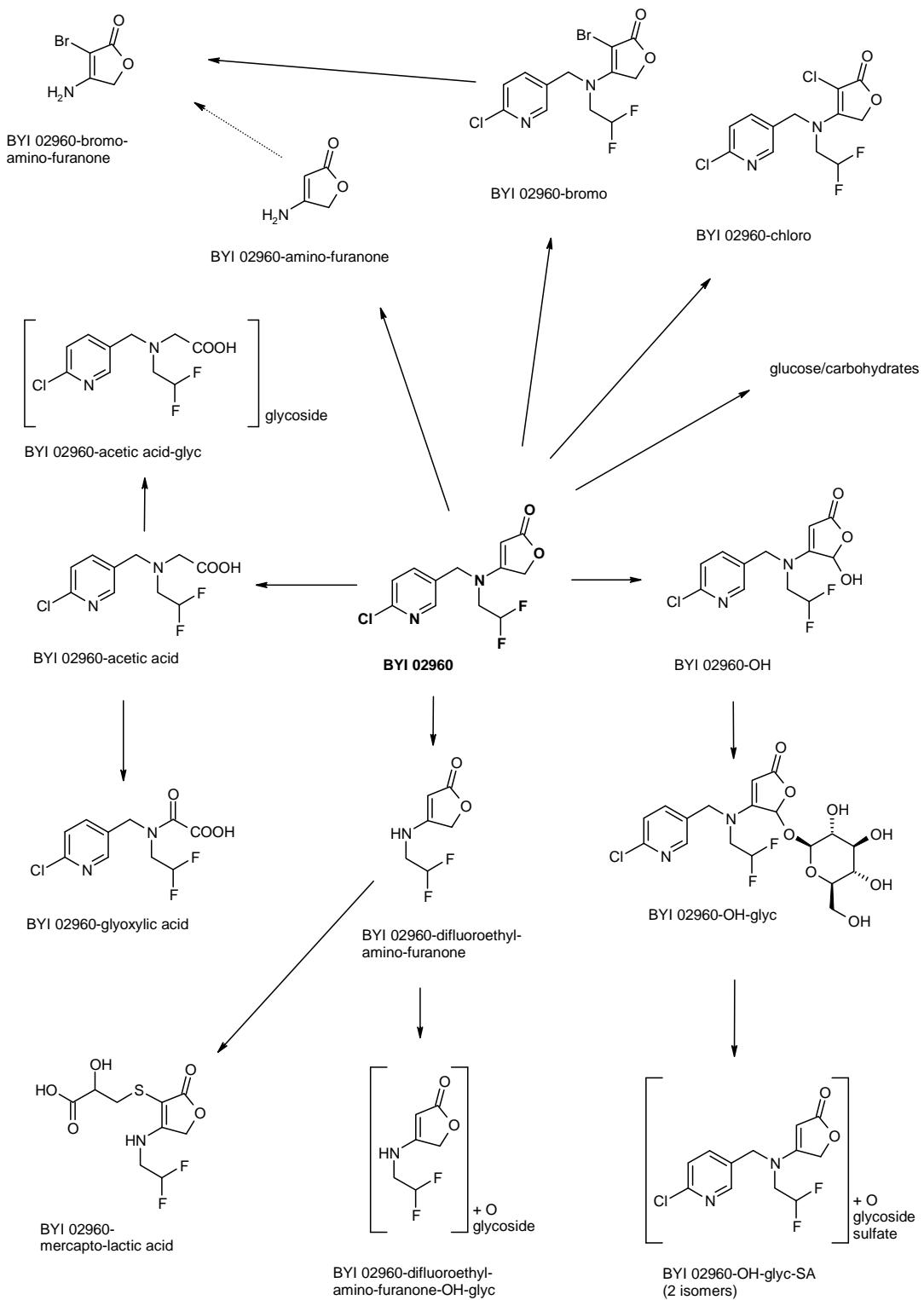
[Furanone-4-¹⁴C] BYI 02960 was rather extensively metabolised in confined rotational crops. Parent compound and fourteen metabolites were identified. Parent compound was the main component in all samples of the three rotations, except for wheat grains. Four general metabolic transformation reactions were observed (cleavage, hydroxylation, conjugation and halogenation):

- cleavage of the pyridinylmethylamine bond and formation of metabolites based on difluoroethyl-amino-furanone and amino-furanone structures,
- BYI 02960-difluorethyl-amino-furanone was either conjugated with mercapto-lactic acid or hydroxylated and conjugated with carbohydrates,
- hydroxylation of the furanone moiety followed by conjugation with carbohydrates and sulphate,
- oxidative degradation of the furanone moiety to BYI 02960-acetic acid, which was further oxidized or conjugated with glucose
- complete degradation of the furanone moiety and incorporation of carbon atoms into the natural compound pool, i.e. into glucose/carbohydrates, and
- halogenation of the furanone ring with bromine or chlorine.

BYI 02960-glyoxylic acid, the oxidation product of BYI 02960-acetic acid, was presumably a transient soil metabolite that was taken up by the plants, since it was prominent in the samples of the 1st rotation, only. Halogenation of the furanone moiety of the active substance occurred most probably also in the soil which was supported by the fact that small amounts of halogenated parent compound were identified in the aerobic soil degradation studies.

On the basis of the results of this study it is concluded that the metabolism of [furanone-4-¹⁴C]BYI 02960 in confined rotational crops is well understood and the following metabolic pathway is proposed:

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 Figure 6.6.2-1 Proposed metabolic pathway of [furanone-4-¹⁴C]BYI 02960 in confined rotational crops




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Report:	KIIA 6.6.2/02, Breuer-Rehm, M.; 2011
Title:	Metabolism of [pyridinylmethyl- ¹⁴ C]BYI 02960 in confined rotational crops
Report No & Edition No	MEF-10/892 M-419853-02-1
Guidelines:	OECD 502 Metabolism in Rotational Crops US EPA Residue Chemistry Test Guideline OPPTS 860.1850: Confined Accumulation in Rotational Crops European Parliament and Council Regulation (EC) No 1107/2009
GLP	yes

Executive Summary

The metabolism of the insecticide BYI 02960 was investigated in the representative rotational crops spring wheat, Swiss chard and turnips from three consecutive rotations. [Pyridinylmethyl-¹⁴C]BYI 02960 was formulated as an SL 300 and used for one spray application onto the soil of a planting container (approx. 1 m²). The actual application rate corresponded to 433 g a.s./ha, slightly above the anticipated maximum seasonal field rate of 400 g a.s./ha. The crops were each sown at 29, 135 and 296 days after the soil application, representing the first, the second and the third rotation.

Intermediate raw agricultural commodities (RACs) investigated were Swiss chard immature, wheat forage and wheat hay. All other RACs (wheat straw, wheat grain, Swiss chard, turnip leaves and turnip roots) were harvested at maturity.

The TRR values for all RACs are given in the following table.

Table 6.6.2-8 TRR values in the different RACs of the three rotations after soil application of [pyridinylmethyl-¹⁴C]BYI 02960

TRR [mg/kg]	Wheat				Swiss chard		Turnips	
	forage	hay	straw	grain	imm.	mature	leaves	roots
1 st rotation	1.407	2.409	9.015	0.177	1.358	1.483	0.815	0.072
2 nd rotation	0.308	1.009	2.148	0.057	0.332	0.438	0.230	0.022
3 rd rotation	0.117	0.321	0.491	0.017	0.135	0.130	0.083	0.008

The samples were conventionally extracted four times with acetonitrile/water mixtures releasing 35.5% (wheat grain, 2nd rotation) to 98.1% (immature Swiss chard and turnip leaves, 2nd rotation) of the TRR. To increase the extraction efficiency in wheat matrices additional exhaustive extraction steps were applied. Finally, the extraction efficiency in wheat grain was increased significantly from 52.2% to 83.8% of the TRR (1st rotation) and from 35.4% to 83.8% of the TRR (2nd rotation). In wheat forage, hay and straw, the additionally extracted portions ranged from 3.5% to 20.5% of the TRR, depending on the extraction steps applied.

Parent compound and metabolites in the extracts were analysed by HPLC. Identification was achieved by spectroscopic evidence (LC-MS/MS) after isolation and purification of the respective compounds from the conventional extracts of wheat straw or Swiss chard of the 1st rotation. HPLC co-chromatography with the isolated and identified radiolabelled metabolites and HPLC comparison of metabolic profiles of the different extracts allowed the assignment of the compounds in all other extracts.



Parent compound was the most prominent compound in all RACs of all rotations, except in wheat grains of the 1st and the 2nd rotation, where it was detected as a minor compound. Seventeen metabolites were identified. Major metabolites were BYI 02960-6-CNA-glycerol-gluA, BYI 02960-glyoxylic acid and BYI 02960-OH in wheat matrices and BYI 02960-OH-glyc in Swiss chard and in turnip matrices. Compared to the confined rotational crop study performed with [furanone-4-¹⁴C]BYI 02960, eight common metabolites were found.

BYI 02960 was rather extensively metabolised in confined rotational crops. The following metabolic routes were observed:

- cleavage of the pyridinylmethylamine bond followed by conjugation with carbohydrates or by oxidation of the methylene group to a carboxylic group,
- hydroxylation of the methylene group of the furanone moiety followed by conjugation with carbohydrates and sulphate,
- cleavage or loss of the furanone moiety followed by several oxidation and conjugation reactions, and
- halogenation of the furanone moiety with bromine or chlorine.

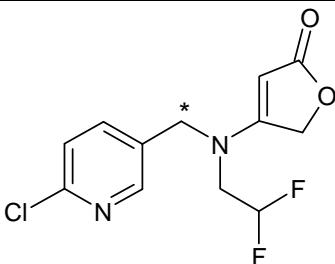
Halogenation of the furanone moiety of the active substance probably occurred in the soil. This assumption is supported by the fact that small amounts of halogenated parent compound were identified in the aerobic soil degradation studies.

On the basis of these results, a metabolic pathway of [pyridinylmethyl-¹⁴C]BYI 02960 in confined rotational crops can be proposed.

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	 * position of the radiolabel
Radiolabelled test material	[pyridinylmethyl- ¹⁴ C]BYI 02960
Specific radioactivity (before radiodilution)	4.37 MBq/mg (118.08 µCi/mg)
Chemical Purity	> 99% (HPLC)
Radiochemical purity	> 99% (HPLC and TLC)

The supplied radiolabelled test compound [pyridinylmethyl-¹⁴C]BYI 02960 was dissolved in acetonitrile. Formulation of the test compound was performed prior to the application: An appropriate amount of this stock solution was evaporated to dryness. The respective amount of blank formulation



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SL 300 was added and the mixture was homogenised using an ultrasonic bath (radioactive formulation). The sample was adjusted to a final volume of approx. 100 mL with water and homogenised by stirring (spray dilution).

2. Soil: “Monheim 4” (sandy loam soil from Germany), pH (CaCl₂) = 6.9, 58% sand, 28% silt and 14% clay, 2.1% organic carbon, cation exchange capacity (CEC) of 8.1 meq/100 g

3. Plants:

rotational crop	variety	representative for crop group
spring wheat	Thasos	small grain
Swiss chard	Lukullus	leafy vegetable
turnips	Rondo	root crop

B. Study Design

Experimental conditions:

BYI 02960 was applied as an SL formulation with a computer controlled track sprayer onto the bare soil of a planting container (surface area of approx. 1 m²). The application rate was 433 g a.s./ha and was slightly above the anticipated maximum seasonal rate of BYI 02960. The treated soil remained undisturbed for an aging period of 29 days. After this period, the upper layer of the soil (approx. 15 cm) was intensively mixed and wheat, Swiss chard and turnips of the first rotation were sown. Wheat was sown on approx. 50% (0.5 m²) of the soil area of the planting container. Swiss chard and turnips were each sown on 25% (0.25 m²) of the soil area of the container. At day 135 and 296 after the application (after harvest of the mature wheat - the crop with the longest vegetation period) the soil was cultivated again and the crops of the second and third rotation were sown, respectively. With each rotation the plots of the crops in the container were changed. Wheat was sown on the plot where Swiss chard and turnips had been sown in the preceding rotation and Swiss chard and turnips were sown on the plot where wheat was sown before.

Sampling:

Raw agricultural commodities (RAC) sampled for this study included the immature samples forage and hay from wheat, and the immature samples from Swiss chard. All other samples (wheat straw and wheat grain, Swiss chard, turnip leaves and turnip roots) were harvested of each rotation at maturity.

About 20% of the wheat plants were cut shortly above the ground as forage sample at BBCH growth stage 29-31 (stem elongation). At BBCH 79-83 (“medium dough stage”) again 20% of the wheat plants were cut as hay sample and dried at room temperature for three to five days. At BBCH 89-92 (maturity) the remaining wheat plants were cut and grains were separated by hand. The remaining ears and chaffs were combined with the straw. A part of the Swiss chard plants were sampled at an immature stage (BBCH 44-46) and the remaining plants at maturity (BBCH 49). The Swiss chard plants were cut above the roots. The mature turnips plants were removed from soil, separated into leaves and roots. The plant materials were cut in pieces before homogenization with liquid nitrogen using a Polytron homogenizer.



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Aliquots of the homogenized samples were used to estimate the TRR in the sample material by combustion and were used for extraction. Remaining homogenized sample material was stored in a freezer at approx. -18 °C.

C. Analytical Procedures

Extraction:

An aliquot of each homogenized RAC was extracted conventionally three to four times with ACN/water (8:2, v/v). The extracts were combined, purified using a pre-conditioned SPE RP 18 cartridge, concentrated and analysed by HPLC. The radioactivity in the extracts was determined by LSC, in the solids by combustion followed by LSC. The actual TRR values of the samples were determined by summing up the radioactivity measured in the extracts and in the remaining solids. If needed, solids were further extracted exhaustively using microwave conditions. Post-extraction solids of forage and hay (1st rotation) were extracted two times with an ACN/water mixture under microwave assistance at increased temperature. Post-extraction solids of wheat straw (1st rotation) were subjected to a sequential extraction procedure after one exhaustive extraction step with ACN/water (8:2, v/v) at increased temperature (60 °C) under microwave conditions. The solids of the microwave extraction step were subjected to a treatment with sodium chloride solution (2 h at 100 °C under microwave assistance), a diastase incubation (approx. 6 h at 26 °C), a treatment with EDTA solution (1 h at 100 °C under microwave assistance) and a cellulase treatment (approx. 12 h at 40 °C) at adjusted temperatures to break down the plant cell walls and liberate residues bound to cell walls or in the cells. The remaining solids of the sequential extraction were extracted finally in two steps with a 5N HCl and a 0.1N NaOH solution. Extracts of the EDTA, the cellulase and the HCl treatment were combined and adjusted to pH 7. The extract was concentrated, centrifuged and analysed by HPLC. The NaOH extract was not submitted to HPLC due to its high viscosity. Post-extraction solids of grains (1st rotation) were subjected to four subsequent exhaustive extraction steps under microwave conditions at increased temperature. Extraction was performed with ACN/water mixtures, 0.1N HCl and 0.1N NaOH. The ACN/water extracts were combined, concentrated and analysed by HPLC. The post-extraction solids of grains of the 2nd rotation were subjected to one exhaustive extraction step with ACN/water (8:2, v/v) at increased temperature under microwave assistance, followed by a diastase digestion (20 h at 26 °C) and a treatment with sodium chloride solution at 100 °C. HPLC analysis of the extracts was not performed due to the low radioactivity levels in the extracts.

Quantification:

Parent compound and metabolites in the extracts were analysed by reversed phase HPLC coupled to a radioactivity detector with a glass scintillator cell. The HPLC chromatograms (= metabolite profiles) were integrated for quantification of compounds.

Identification and characterisation:

Identification of parent compound and metabolites was based on the metabolite profiles of the conventional extracts of Swiss chard and wheat straw of the first rotation. These extracts showed all major and minor metabolites also detected in the other extracts. Therefore, parent compound, all major and most minor metabolites were isolated from these extracts, purified and identified by HPLC-MS/MS. Two metabolites were co-eluting in the extract of wheat straw when analysed with the profiling method. To ensure the right assignment in other extracts, the respective peak was isolated by semi-preparative HPLC and re-analysed with an acidic method which was able to separate the two

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compounds, if present. Based on these unambiguous assignments, the compounds in all conventional and exhaustive extracts of all rotations were identified by comparison with the metabolite patterns of Swiss chard and wheat straw. The presence of several metabolites was additionally confirmed by HPLC co-chromatography using the isolated compounds of Swiss chard or wheat straw as reference compounds.

Storage stability:

All conventional extraction experiments and the first HPLC analyses were performed within 9 days after harvest of the plants, with the exception for wheat grain of the second rotation which was extracted within 7 days after harvest, but analysed only 154 days after harvest. Therefore, the extract stability was investigated by re-analysing the grain extract after a storage time of more than 21 months in the freezer at $\leq -18^{\circ}\text{C}$. No change in the metabolite pattern was detected indicating that the residues in the extracts are stable for at least 21 months.

Quantitative ^{14}C -HPLC analysis of the extracts was performed with the profiling method BYI02960_NEUTR. Only for wheat forage and hay, Swiss chard and turnips from the 1st rotation, the metabolic profile was measured with the preliminary profiling method BYI02960_CRC directly after extraction. The preliminary method was based on the same type of column and the same eluents but had a slightly shorter gradient. The metabolite profiles obtained from the two methods were comparable, however profiling method BYI02960_NEUTR showed an improved separation for some metabolites. For better comparison of metabolic pattern over all RACs of all rotations, the profiles of wheat forage and hay, Swiss chard and turnips of the 1st rotation were re-analysed with method BYI02960_NEUTR about 1 – 2 months after extract preparation. These new metabolic profiles were then considered as basis for quantification. Extract stability has been demonstrated for this time period.

Exhaustive extractions were started within approx. four to seven months after harvest. Since the compounds identified in the exhaustive extracts were identical with the compounds of the conventional extracts, it was concluded that the residues in the solids were stable and reflected the released residues at the time of exhaustive extraction.

Thus it can be concluded that the residues in all matrices were sufficiently stable during the experimental period of the study and that the first quantified profiles represented the metabolic pattern in the samples at harvest. Nevertheless, storage stability of BYI 02960 residues in frozen sample material was additionally demonstrated for Swiss chard and wheat straw for at least 20 or 18 months, respectively. Swiss chard and wheat straw samples were extracted and analysed for a second time after storage of the samples at $\leq -18^{\circ}\text{C}$ for at least 18 months. Comparison of the HPLC profiles showed no changes in the metabolite patterns.

As well, extract stability was proven for selected matrices (wheat forage, wheat grain, Swiss chard, turnip leaves and turnip roots) by re-analysis of stored extracts. All extracts were stable for at least 4 months (period tested for turnip roots). For wheat grain an extract stability of at least 21 months was demonstrated, as mentioned above.



II. Results and Discussion

The metabolism of [pyridinylmethyl-¹⁴C]BYI 02960 was investigated in the representative rotational crops spring wheat, Swiss chard and turnips following one soil application. The active substance was applied as an SL formulation on the bare soil at a rate of 433 g/ha at 29 days before sowing of the crops representing the first rotation. Crops of the 2nd and 3rd rotation were sown 135 and 296 days after application. Immature Swiss chard, wheat forage and wheat hay were harvested as intermediate raw agricultural commodities (RACs). All other RACs (wheat straw, wheat grain, Swiss chard, turnip leaves and turnip roots) were harvested at maturity.

The TRR values of all RACs declined significantly (by a factor of 7 to 18) from the first to the third rotation. Highest residues were detected in the non-edible commodities of wheat and in the edible commodities of Swiss chard, as shown above in Table 6.6.2-8.

The radioactive residues were efficiently extracted from all commodities of all rotations with acetonitrile/water mixtures, except for wheat grain where only 35.4% to 52.2% of the TRR was detected after conventional extraction. For all wheat matrices additional exhaustive extraction steps were applied to the solids after conventional extraction. Exhaustive extraction comprised one to two extraction steps with acetonitrile/water mixtures at increased temperature (60 °C) under microwave assistance and in the case of wheat straw (1st and 2nd rotation) and wheat grain (2nd rotation) additional sequential extraction steps including enzymatic digestion steps. For wheat grain, exhaustive extraction released additional high amounts of radioactivity from the post-extraction solids. The extracted portions increased from 52.2% to 83.8% of the TRR (1st rotation) and from 35.4% to 89.4% of the TRR (2nd rotation). For the other wheat matrices, the additionally extracted portions ranged between 3.5% and 20.5% of the TRR depending on the exhaustive extraction steps applied. Thus, extraction efficiencies were increased significantly by exhaustive extraction steps, if applied – and extraction efficiencies above 80% resulted, even for wheat grain.

Parent compound BYI 02960 and about 50 metabolites were detected in the conventional and exhaustive extracts of the various samples of the three rotations. Of these the active substance and 17 metabolites were identified by LC-MS/MS. The other metabolites, none exceeding 5% of the TRR or 0.05 mg/kg, were characterised by their extraction behaviour and their retention time in radio-HPLC. The amounts of active substance and metabolites in all RACs are summarized in Table 6.6.2-9 to Table 6.6.2-14 for the three rotations.

Except in wheat grain, parent compound was by far the main component detected in all matrices of all rotations, accounting for 8% to 62% of the TRR in the commodities of the 1st rotation, 1% to 67% in the 2nd rotation and 14% to 69% in the 3rd rotation. The lowest percentage was always detected in wheat grain and the highest in turnip leaves.

Eight of the identified metabolites were specific to the radiolabel used: BYI 02960-CHMP, three different conjugates of this metabolite, the corresponding oxidation product 6-CNA and its glycerol-glucuronic acid conjugates (3 isomers) and two oxidation products of the postulated cleavage product BYI 02960-AMCP-difluoroethanamine. The isomers of 6-CNA-glycerol-gluA were detected as major metabolites in all matrices of wheat. In wheat grains they represented the highest proportion of the TRR. It is probable that the weak acid 6-CNA with its pronounced phloem mobility was transported

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into the seeds as a phloem sink, with conjugation occurring after transport. The 6-CNA conjugates were not identified in the matrices of Swiss chard or turnips.

Identified metabolites common to both radiolabels were BYI 02960-OH and its conjugates, BYI 02960-acetic acid, its conjugates and its successor molecule BYI 02960-glyoxylic acid and the chlorinated/brominated parent compound.

Overall, identification rates were high and ranged from 68% - 87% of the TRR in all RACs apart from wheat grains. In grains the identification rate was lower (29% - 58% of the TRR), but at least additional significant portions of the TRR were characterized by the extraction behaviour of the residues. The solids remaining after the conventional extraction of the grains of the 2nd rotation were subjected in a first step to microwave extraction with ACN/water (8:2, v/v) at increased temperature (15 min at 100 °C). An additional portion of approx. 5% of the TRR was released. Analysis of this extract showed the known major compounds of the conventional extract besides a high amount of a polar unknown compound. Enzymatic treatment of the post-extraction solids of the microwave extraction with diastase (approx. 20 h at 26 °C) solubilised a significant higher portion of the TRR (approx. 21% of the TRR), indicating that a quite polar residue was released. Subsequent extraction of the remaining solids with a sodium chloride solution (2 h at 100 °C) released another significant portion of the TRR (approx. 28% of the TRR). These extraction steps indicate that a major part of the residues in grain is of polar nature – and can be most probably assigned to natural compounds.

Table 6.6.2-9 Distribution of parent compound and metabolites in wheat matrices after a 29 day plant back interval (1st rotation, [pyridinylmethyl-¹⁴C]BYI 02960)

1 st Rotation	wheat forage		wheat hay		wheat straw		wheat grains	
TRR [mg/kg]	1.407		2.409		9.015		0.177	
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
<i>Conventional extraction</i>								
BYI 02960 (parent comp.)	43.8	0.619	25.0	0.601	31.4	2.834	1.8	0.003
6-CNA	2.0	0.029	3.0	0.072	2.8	0.249	3.8	0.007
6-CNA-glycerol-gluA (1)	3.2	0.045	3.4	0.083	2.1	0.193	2.3	0.004
6-CNA-glycerol-gluA (2 + 3)	14.1	0.200	23.6	0.569	19.2	1.733	15.8	0.028
CHMP-glyc	1.7	0.024	5.0	0.120	2.4	0.213	---	---
CHMP	1.2	0.016	1.5	0.036	1.2	0.107	---	---
glyoxylic acid	12.3	0.173	7.3	0.176	5.6	0.508	6.0	0.011
acetic acid-glyc	0.6	0.009	---	---	0.8	0.072	---	---
OH-glyc	3.4	0.048	4.0	0.095	3.2	0.284	5.0	0.009
acetic acid	1.2	0.017	1.4	0.033	1.1	0.100	1.7	0.003
OH	1.4	0.020	2.2	0.052	2.4	0.212	6.8	0.012
bromo/chloro	---	---	0.9	0.021	0.4	0.036	---	---
Subtotal identified	84.9	1.200	77.2	1.858	72.6	6.542	43.3	0.077

Table continued on next page...



1 st Rotation	wheat forage		wheat hay		wheat straw		wheat grains	
unknown 1	0.8	0.011	---	---	---	---	---	---
unknown 2	---	---	---	---	0.3	0.024	---	---
unknown 3	---	---	0.7	0.017	---	---	---	---
unknown 5	---	---	0.5	0.013	0.2	0.020	---	---
unknown 6	---	---	1.1	0.026	---	---	---	---
unknown 7	---	---	---	---	0.2	0.022	---	---
unknown 9	---	---	---	---	0.6	0.056	---	---
unknown 10	---	---	---	---	0.6	0.050	2.2	0.004
unknown 14	0.7	0.010	---	---	0.5	0.042	---	---
unknown 16	---	---	0.9	0.023	0.5	0.044	---	---
unknown 17	---	---	---	---	0.3	0.028	---	---
unknown 18	---	---	---	---	0.5	0.042	---	---
unknown 21	---	---	0.9	0.022	---	---	4.1	0.007
unknown 24	---	---	---	---	0.4	0.034	---	---
unknown 25	0.7	0.010	0.8	0.018	0.3	0.023	2.6	0.005
unknown 26	---	---	---	---	0.6	0.052	---	---
unknown 27	---	---	1.0	0.023	0.7	0.060	---	---
unknown 28	1.8	0.025	0.6	0.014	0.9	0.077	---	---
unknown 29	1.0	0.014	0.8	0.019	0.6	0.055	---	---
unknown 33	---	---	---	---	1.4	0.123	---	---
Subtotal characterised	5.0	0.071	7.3	0.176	8.4	0.754	8.9	0.016
Total conventional extr.	89.9	1.271	84.5	2.034	81.0	7.296	52.2	0.093
<i>Microwave extraction I (ACN/water)</i>								
BYI 02960 (parent comp.)	1.6	0.023	3.1	0.075	2.4	0.214	6.5	0.012
6-CNA	---	---	---	---	0.1	0.012	---	---
6-CNA-glycerol-gluA (2 + 3)	---	---	---	---	1.4	0.123	4.4	0.008
glyoxylic acid	---	---	---	---	0.6	0.058	---	---
OH-glyc	---	---	1.6	0.040	0.1	0.012	---	---
acetic acid	---	---	0.6	0.014	0.1	0.004	---	---
OH	---	---	---	---	0.2	0.017	3.6	0.006
Subtotal identified	1.6	0.023	5.3	0.129	4.9	0.440	14.5	0.026
unknown 1	0.5	0.007	2.7	0.065	0.4	0.036	4.9	0.009
unknown 29	1.3	0.019	---	---	0.1	0.011	---	---
Subtotal characterised	1.8	0.026	2.7	0.065	0.5	0.047	4.9	0.009
Total microwave extr. I	3.5	0.049	8.0	0.193	5.4	0.487	19.4	0.034
<i>Microwave extraction II (1% NaCl)</i>								
BYI 02960 (parent comp.)					1.0	0.087		
6-CNA					0.8	0.076		
6-CNA-glycerol-gluA (1)					0.2	0.016		
6-CNA-glycerol-gluA (2 + 3)					0.2	0.020		
OH					0.1	0.006		
Subtotal identified					2.3	0.205		
unknown 1					0.4	0.040		
unknown 29					0.2	0.015		
Subtotal characterised					0.6	0.055		

Table continued on next page...

1 st Rotation	wheat forage	wheat hay	wheat straw	wheat grains



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Total microwave extr. II	---	---	---	---	2.9	0.260	---	---
<i>Diastase digestion</i>								
BYI 02960 (parent comp.)					0.7	0.064		
6-CNA					0.6	0.055		
6-CNA-glycerol-gluA (2 + 3)					0.1	0.005		
glyoxylic acid					0.2	0.020		
OH					<0.1	0.004		
Subtotal identified					1.6	0.148		
unknown 1					0.3	0.024		
unknown 5					0.1	0.006		
unknown 9					0.1	0.008		
unknown 29					0.1	0.013		
Subtotal characterised					0.6	0.051		
Total diastase digestion	---	---	---	---	2.2	0.199	---	---
<i>EDTA + cellulase + 5N HCl extraction (wheat straw) or 0.1N HCl (wheat grain)</i>								
BYI 02960 (parent comp.)					0.7	0.062		
6-CNA					0.7	0.066		
6-CNA-glycerol-gluA (2 + 3)					0.2	0.019		
glyoxylic acid					0.3	0.028		
Subtotal identified					1.9	0.176		
unknown 1					0.8	0.071		
unknown 29					1.0	0.088		
Subtotal characterised					1.8	0.159		
Total exhaustive extraction	---	---	---	---	3.7	0.335	¹ 2.3	0.004
<i>5N NaOH extraction</i>								
Total NaOH extraction							¹ 9.9	0.018
Total identified	86.5	1.220	82.5	1.987	83.4	7.511	57.8	0.103
Total characterised	6.9	0.097	10.0	0.240	11.8	1.066	13.8	0.025
Total extractable	93.5	1.316	92.5	2.227	95.2	8.578	83.8	0.149
Not analysed/losses	0.3	0.004	---	---	4.5	0.403	12.2	0.022
Unextractable (PES*)	6.2	0.087	7.5	0.181	0.4	0.035	16.2	0.029
Accountability	100.0	1.407	100.0	2.409	100.0	9.015	100.0	0.177

(1) isomer 1

(2 + 3) isomer 2 and/or isomer 3

* post extraction solids

¹ no analysis was feasible



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.6.2-10 Distribution of parent compound and metabolites in wheat matrices after a 135 day plant back interval (2nd rotation, [pyridinylmethyl-¹⁴C]BYI 02960)

2 nd Rotation	wheat forage		wheat hay		wheat straw		wheat grains	
TRR [mg/kg]	0.308		1.009		2.148		0.057	
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
<i>Conventional extraction</i>								
BYI 02960 (parent comp.)	59.6	0.183	28.1	0.283	31.9	0.684	1.1	0.001
6-CNA	1.6	0.005	3.2	0.032	4.0	0.086	---	---
6-CNA-glycerol-gluA (1)	4.4	0.014	2.4	0.024	2.4	0.051	2.5	0.001
6-CNA-glycerol-gluA (2 + 3)	14.4	0.044	23.9	0.242	22.0	0.472	11.9	0.007
CHMP-glyc	0.7	0.002	0.8	0.008	2.2	0.048	---	---
CHMP	---	---	0.5	0.005	---	---	---	---
glyoxylic acid	---	---	1.0	0.010	1.7	0.036	0.9	0.001
acetic acid-glyc	0.7	0.002	---	---	---	---	---	---
OH-glyc	2.6	0.008	4.5	0.045	5.3	0.112	4.6	0.003
acetic acid	0.9	0.003	1.6	0.016	1.8	0.039	1.6	0.001
OH	1.7	0.005	1.9	0.019	3.4	0.072	6.4	0.004
Subtotal identified	86.5	0.266	67.9	0.685	74.6	1.600	29.0	0.017
unknown 1	0.3	0.001	0.4	0.004	1.3	0.028	---	---
unknown 2	1.2	0.004	---	---	---	---	---	---
unknown 3	---	---	0.5	0.005	---	---	---	---
unknown 5	---	---	0.4	0.004	---	---	---	---
unknown 7	---	---	0.6	0.006	---	---	---	---
unknown 10	---	---	0.5	0.005	---	---	1.1	0.001
unknown 14	---	---	2.3	0.023	1.1	0.024	---	---
unknown 17	---	---	1.2	0.012	---	---	---	---
unknown 18	---	---	0.6	0.006	---	---	---	---
unknown 20	---	---	0.9	0.009	---	---	---	---
unknown 21	---	---	---	---	---	---	2.1	0.001
unknown 24	0.5	0.001	0.8	0.008	---	---	---	---
unknown 25	---	---	---	---	1.8	0.039	2.3	0.001
unknown 27	0.4	0.001	0.8	0.008	---	---	---	---
unknown 28	0.3	0.001	0.2	0.002	---	---	---	---
unknown 29	0.6	0.002	0.5	0.005	---	---	---	---
Subtotal characterised	3.3	0.010	9.8	0.099	4.2	0.091	5.6	0.003
Total conventional extr.	89.9	0.276	77.6	0.784	78.8	1.692	34.6	0.020
<i>Microwave extraction I (ACN/water)</i>								
BYI 02960 (parent comp.)					3.4	0.072		
6-CNA					1.5	0.032		
OH					0.3	0.007		
Subtotal identified					5.2	0.111		
unknown 1					1.6	0.034		
Subtotal characterised					1.6	0.034		

Table continued on next page...



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

2 nd Rotation	wheat forage		wheat hay		wheat straw		wheat grains	
Total microwave extr. I					6.7	0.145		
<i>Microwave extraction II (1% NaCl)</i>								
BYI 02960 (parent comp.)					0.9	0.020		
6-CNA					0.9	0.019		
Subtotal identified					1.8	0.040		
unknown 1					0.3	0.006		
Subtotal characterised					0.3	0.006		
Total NaCl extraction					2.1	0.046		
<i>Diastase digestion</i>								
BYI 02960 (parent comp.)					0.6	0.013		
6-CNA					0.5	0.011		
Subtotal identified					1.1	0.024		
unknown 1					0.3	0.006		
unknown 29					0.1	0.002		
Subtotal characterised					0.4	0.008		
Total diastase digestion					1.5	0.032		
<i>EDTA + cellulase + 5N HCl extraction</i>								
BYI 02960 (parent comp.)					0.7	0.013		
6-CNA					0.5	0.011		
Subtotal identified					1.1	0.024		
unknown 1					0.7	0.014		
unknown 29					0.8	0.017		
Subtotal characterised					1.5	0.032		
Total EDTA + cellul.+ 5N HCl extraction					2.6	0.056		
Total identified	86.5	0.266	67.9	0.685	83.8	1.799	29.0	0.017
Total characterised	3.3	0.010	9.8	0.099	8.0	0.171	5.6	0.003
Total extractable	89.9	0.276	77.6	0.784	91.8	1.970	34.6	0.020
Not analysed/losses	---	---	---	---	7.9	0.169	54.8	0.032
Unextractable (PES*)	10.1	0.031	22.4	0.226	0.3	0.007	10.5	0.006
Accountability	100.0	0.308	100.0	1.009	100.0	2.148	100.0	0.057

(1) isomer 1

(2 + 3) isomer 2 and/or isomer 3

* post extraction solids

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

 Table 6.6.2-11 Distribution of parent compound and metabolites in wheat matrices after a 296 day plant back interval (3rd rotation, [pyridinylmethyl-¹⁴C]BYI 02960)

3 rd Rotation	wheat forage		wheat hay		wheat straw		wheat grains	
TRR [mg/kg]	0.117		0.321		0.491		0.017	
Compound (BYI 02960-)	% TRR	mg/kg						
<i>Conventional extraction</i>								
BYI 02960 (parent comp.)	45.3	0.053	19.6	0.063	26.2	0.129	13.9	0.002
6-CNA	2.7	0.003	4.3	0.014	4.8	0.024	---	---
6-CNA-glycerol-gluA (1)	3.1	0.004	3.6	0.011	4.5	0.022	---	---
6-CNA-glycerol-gluA (2 + 3)	19.1	0.022	29.7	0.095	28.4	0.140	11.2	0.002
CHMP-glyc	3.1	0.004	4.9	0.016	2.9	0.014	---	---
CHMP	1.4	0.002	2.9	0.009	---	---	---	---
glyoxylic acid	0.8	0.001	---	---	2.6	0.013	---	---
OH-glyc	3.2	0.004	3.0	0.010	2.9	0.014	5.2	0.001
acetic acid	1.1	0.001	1.1	0.003	1.0	0.005	1.8	<0.001
OH	1.0	0.001	1.7	0.005	2.1	0.010	5.5	0.001
Total identified	80.7	0.094	70.8	0.227	75.4	0.370	37.7	0.006
unknown 1	---	---	0.7	0.002	---	---	---	---
unknown 7	---	---	0.5	0.002	---	---	---	---
unknown 10	---	---	1.1	0.003	---	---	---	---
unknown 16	---	---	1.3	0.004	---	---	---	---
unknown 21	---	---	---	---	---	---	2.4	<0.001
unknown 24	1.5	0.002	---	---	---	---	---	---
unknown 25	---	---	---	---	---	---	1.8	<0.001
unknown 27	---	---	0.6	0.002	---	---	---	---
unknown 28	---	---	---	---	---	---	2.8	0.001
Total characterised	1.5	0.002	4.2	0.014	---	---	7.1	0.001
Total extractable	82.2	0.096	75.0	0.241	75.4	0.370	44.8	0.007
Not analysed/losses	---	---	---	---	1.2	0.006	---	---
Unextractable (PES*)	17.8	0.021	25.0	0.080	23.4	0.115	55.2	0.009
Accountability	100.0	0.117	100.0	0.321	100.0	0.491	100.0	0.017

(1) isomer 1

(2 + 3) isomer 2 and/or isomer 3

* post extraction solids

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.6.2-12 Distribution of parent compound and metabolites in Swiss chard and turnip matrices after a 29 day plant back interval (1st rotation, [pyridinylmethyl-14C]BYI 02960)

1 st Rotation	Swiss chard immature		Swiss chard mature		turnip leaves		turnip roots	
TRR [mg/kg]	1.358		1.483		0.815		0.072	
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
<i>Conventional extraction</i>								
BYI 02960 (parent comp.)	57.4	0.779	46.3	0.687	62.4	0.508	57.8	0.042
CHMP-glyc-tri-SA	1.8	0.025	2.3	0.034	---	---	---	---
6-CNA	7.3	0.099	7.0	0.103	1.0	0.008	8.5	0.006
CHMP-glyc-di-SA	2.5	0.035	3.0	0.045	---	---	---	---
CHMP-glyc	5.5	0.075	5.4	0.080	4.5	0.037	---	---
CHMP	---	---	---	---	---	---	4.1	0.003
glyoxylic acid	1.5	0.021	2.6	0.039	2.6	0.021	8.6	0.006
acetic acid-glyc	1.6	0.022	---	---	0.4	0.003	---	---
acetic acid-glyc/ OH-glyc-SA (2)	---	---	2.5	0.037	---	---	---	---
OH-glyc	7.4	0.101	10.9	0.162	9.4	0.076	2.5	0.002
OH-glyc-SA (1)	1.1	0.015	1.7	0.025	0.6	0.005	---	---
OH-glyc-SA (2)	0.5	0.006	---	---	1.7	0.014	---	---
N-formyl- /N-acetyl-AMCP- difluoroethanamine	4.0	0.055	2.9	0.043	3.6	0.030	3.5	0.003
OH	1.8	0.024	1.6	0.023	1.4	0.011	0.5	<0.001
bromo/chloro	0.7	0.009	0.5	0.008	1.3	0.011	1.4	0.001
Total identified	93.2	1.266	86.6	1.285	88.9	0.724	86.8	0.063
unknown 1	---	---	0.3	0.005	0.4	0.004	2.2	0.002
unknown 2	---	---	0.5	0.007	---	---	---	---
unknown 6	---	---	---	---	1.3	0.011	---	---
unknown 7	---	---	---	---	---	---	1.1	0.001
unknown 11	0.7	0.010	1.1	0.016	---	---	---	---
unknown 12	---	---	0.4	0.006	0.6	0.005	---	---
unknown 13	3.0	0.041	5.0	0.075	---	---	---	---
unknown 14	---	---	0.4	0.006	---	---	---	---
unknown 15	0.8	0.011	0.3	0.004	---	---	---	---
unknown 16	---	---	1.2	0.018	2.0	0.016	---	---
unknown 17	---	---	---	---	---	---	---	---
unknown 18	---	---	---	---	0.9	0.007	---	---
unknown 25	---	---	---	---	0.5	0.004	---	---
unknown 26	---	---	---	---	1.1	0.009	---	---
unknown 27	---	---	---	---	<0.1	<0.001	---	---
unknown 28	---	---	---	---	0.4	0.004	---	---
unknown 29	---	---	---	---	---	---	---	---
unknown 30	---	---	0.3	0.004	1.6	0.013	---	---
unknown 31	---	---	0.2	0.003	---	---	---	---
unknown 32	---	---	0.3	0.004	---	---	---	---
Total characterised	4.5	0.062	9.9	0.146	8.8	0.071	3.2	0.002

Table continued on next page...

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

1 st Rotation	Swiss chard immature		Swiss chard mature		turnip leaves		turnip roots	
Total extractable	97.7	1.327	96.5	1.431	97.7	0.796	90.0	0.065
Not analysed/losses	0.3	0.004	0.5	0.008	---	---	---	---
Unextractable (PES*)	2.1	0.028	2.9	0.043	2.3	0.019	10.0	0.007
Accountability	100.0	1.358	100.0	1.483	100.0	0.815	100.0	0.072

(1) isomer 1

(2) isomer 2

* post extraction solids

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

 Table 6.6.2-13 Distribution of parent compound and metabolites in Swiss chard and turnip matrices after a 135 day plant back interval (2nd rotation, [pyridinylmethyl-¹⁴C]BYI 02960)

2 nd Rotation	Swiss chard immature		Swiss chard mature		turnip leaves		turnip roots	
TRR [mg/kg]	0.332		0.438		0.230		0.022	
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
<i>Conventional extraction</i>								
BYI 02960 (parent comp.)	51.2	0.170	24.6	0.108	66.8	0.153	48.4	0.011
CHMP-glyc-tri-SA	2.8	0.009	2.0	0.009	---	---	---	---
6-CNA	2.9	0.010	2.9	0.013	0.8	0.002	7.7	0.002
CHMP-glyc-di-SA	1.8	0.006	2.4	0.010	---	---	---	---
CHMP-glyc	5.1	0.017	1.3	0.006	4.9	0.011	2.0	<0.001
CHMP	---	---	---	---	---	---	1.1	<0.001
acetic acid-glyc	---	---	3.0	0.013	---	---	---	---
OH-glyc	17.4	0.058	25.3	0.111	11.1	0.025	2.4	0.001
OH-glyc-SA (1)	1.6	0.005	2.9	0.013	---	---	---	---
OH-glyc-SA (2)	2.5	0.008	6.3	0.028	---	---	---	---
N-formyl- /N-acetyl-AMCP-difluoroethanamine	1.6	0.005	---	---	1.2	0.003	---	---
OH	3.9	0.013	3.6	0.016	1.6	0.004	---	---
bromo/chloro	0.5	0.002	0.2	0.001	1.6	0.004	2.0	<0.001
Total identified	91.4	0.303	74.6	0.327	88.0	0.202	63.6	0.013
unknown 1	1.4	0.005	6.8	0.030	---	---	5.6	0.001
unknown 5	---	---	---	---	---	---	1.1	<0.001
unknown 11	---	---	1.5	0.006	---	---	---	---
unknown 13	3.6	0.012	4.3	0.019	---	---	0.6	<0.001
unknown 14	---	---	1.2	0.005	0.7	0.002	---	---
unknown 15	0.9	0.003	0.7	0.003	---	---	---	---
unknown 16	---	---	1.1	0.005	---	---	---	---
unknown 18	---	---	---	---	1.7	0.004	---	---
unknown 19	---	---	4.8	0.021	---	---	---	---
unknown 25	---	---	---	---	1.9	0.004	---	---
unknown 30	0.8	0.003	0.6	0.003	5.7	0.013	6.5	0.001
unknown 31	---	---	0.6	0.002	---	---	---	---
Total characterised	6.7	0.022	14.7	0.064	10.0	0.023	13.9	0.003
Total extractable	98.1	0.325	96.1	0.421	98.1	0.225	77.5	0.015
Not analysed/losses	---	---	0.7	0.003	---	---	0.6	<0.001
Unextractable (PES*)	1.9	0.006	3.2	0.014	1.9	0.004	21.9	0.005
Accountability	100.0	0.332	100.0	0.438	100.0	0.230	100.0	0.022

(1) isomer 1

(2) isomer 2

* post extraction solids



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.6.2-14 Distribution of parent compound and metabolites in Swiss chard and turnip matrices after a 296 day plant back interval (3rd rotation, [pyridinylmethyl-¹⁴C]BYI 02960)

3 rd Rotation	Swiss chard immature		Swiss chard mature		turnip leaves		turnip roots	
TRR [mg/kg]	0.135		0.130		0.083		0.008	
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
<i>Conventional extraction</i>								
BYI 02960 (parent comp.)	31.6	0.042	27.4	0.036	69.2	0.058	64.8	0.005
CHMP-glyc-tri-SA	2.5	0.003	0.4	0.001	---	---	---	---
6-CNA	5.3	0.007	5.1	0.007	0.8	0.001	4.7	<0.001
CHMP-glyc-di-SA	2.6	0.003	2.8	0.004	---	---	---	---
CHMP-glyc	5.1	0.007	6.2	0.008	5.9	0.005	---	---
CHMP	---	---	---	---	---	---	1.4	<0.001
glyoxylic acid	2.1	0.003	1.0	0.001	---	---	---	---
acetic acid-glyc/ OH-glyc-SA (2)	---	---	2.8	0.004	0.9	0.001	---	---
OH-glyc	24.6	0.033	28.1	0.036	9.6	0.008	3.8	<0.001
OH-glyc-SA (1)	5.3	0.007	2.9	0.004	0.5	<0.001	---	---
OH-glyc-SA (2)	0.5	0.001	---	---	---	---	---	---
N-formyl- /N-acetyl-AMCP- difluoroethanamine	0.8	0.001	1.8	0.002	2.2	0.002	---	---
OH	3.8	0.005	3.4	0.004	---	---	---	---
bromo/chloro	0.7	0.001	0.4	0.001	1.6	0.001	2.5	<0.001
Total identified	84.9	0.114	82.4	0.107	90.7	0.075	77.1	0.005
unknown 1	---	---	0.8	0.001	0.4	<0.001	8.4	0.001
unknown 2	---	---	---	---	0.2	<0.001	---	---
unknown 4	0.4	0.001	---	---	---	---	---	---
unknown 5	0.4	0.001	0.6	0.001	---	---	---	---
unknown 6	0.5	0.001	0.5	0.001	---	---	---	---
unknown 7	---	---	0.5	0.001	0.3	<0.001	---	---
unknown 11	1.0	0.001	1.4	0.002	---	---	---	---
unknown 12	---	---	---	---	1.2	0.001	---	---
unknown 13	4.4	0.006	---	---	---	---	3.2	<0.001
unknown 14	0.6	0.001	5.7	0.007	---	---	---	---
unknown 15	0.5	0.001	---	---	0.4	<0.001	---	---
unknown 16	0.2	<0.001	1.0	0.001	---	---	---	---
unknown 18	---	---	---	---	1.8	0.001	---	---
unknown 30	1.1	0.001	---	---	---	---	1.1	<0.001
unknown 32	---	---	1.2	0.002	---	---	---	---
Total characterised	9.0	0.012	11.6	0.016	4.2	0.002	12.7	0.001
Total extractable	93.9	0.126	94.0	0.123	94.9	0.078	89.8	0.006
Not analysed/losses	---	---	---	---	---	---	3.3	<0.001
Unextractable (PES*)	6.1	0.008	6.0	0.008	5.1	0.004	6.9	0.001
Accountability	100.0	0.135	100.0	0.130	100.0	0.083	100.0	0.008

(1) isomer 1

(2) isomer 2

* post-extraction solids



III. Conclusions

[Pyridinylmethyl-¹⁴C]BYI 02960 was rather extensively metabolised in confined rotational crops. However, parent compound was detected as main component in all RACs of all rotations, except in wheat grain of the first and the second rotation. Considering all samples, BYI 02960-6-CNA-glycerol-gluA (a mixture of two isomers) was the main metabolite in the wheat matrices and BYI 02960-OH-glyc was the main metabolite detected in Swiss chard and turnips. The following main metabolic routes were observed:

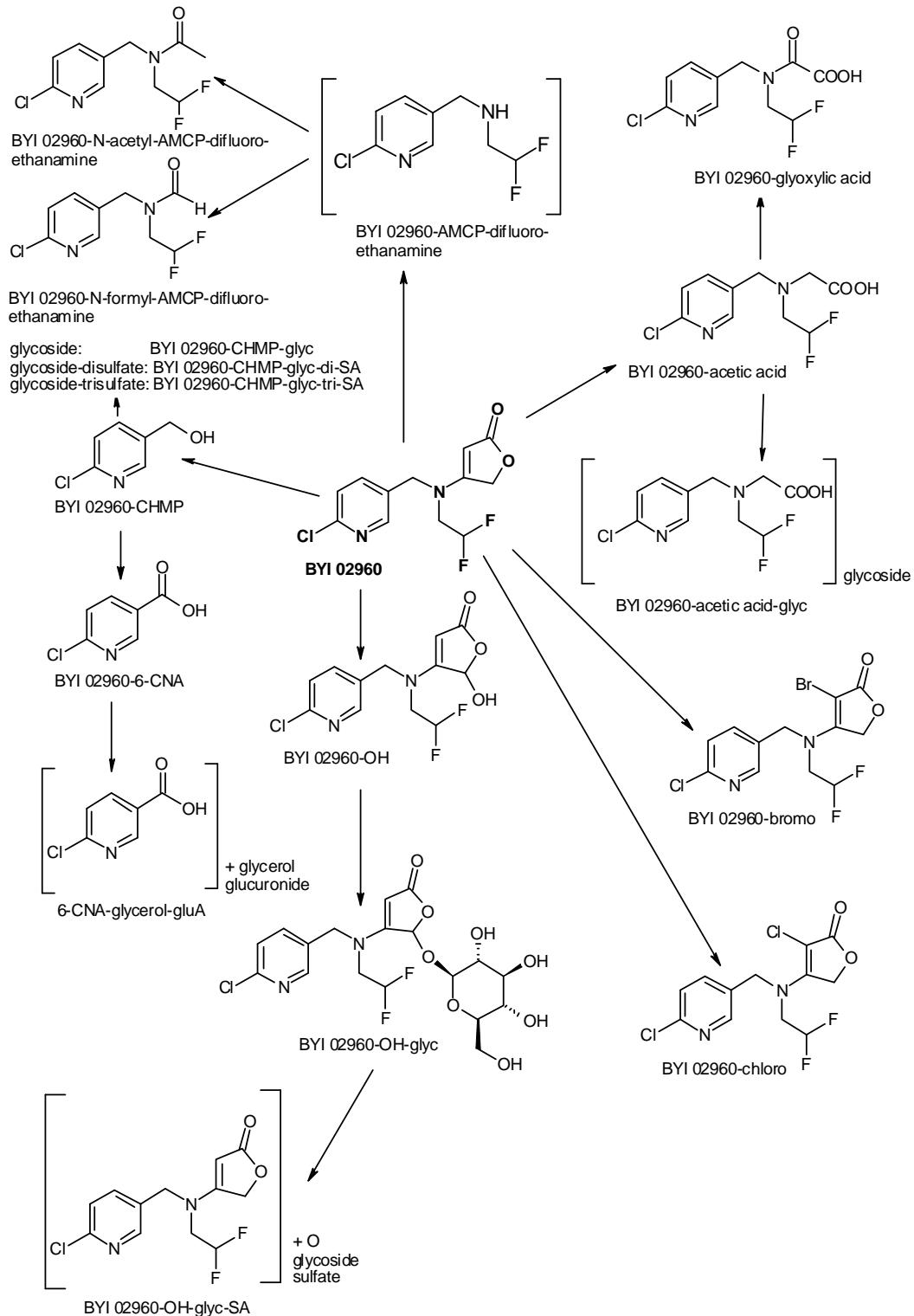
- cleavage of the pyridinylmethylamine bond followed by conjugation with carbohydrates and sulphate or by oxidation of the methylene group to a carboxylic group,
- hydroxylation of the methylene group of the furanone moiety followed by conjugation with carbohydrates and sulphate,
- cleavage or complete degradation of the furanone moiety followed by several oxidation and conjugation reactions, and
- halogenation of the furanone moiety with bromine or chlorine.

Halogenation of the furanone moiety of the active substance probably occurred in the soil which is supported by the fact that small amounts of halogenated parent compound were identified in the aerobic soil degradation studies.

On the basis of the results of this study it is concluded that the metabolism of [pyridinylmethyl-¹⁴C]BYI 02960 in confined rotational crops is well understood and the following metabolic pathway is proposed:

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Figure 6.6.2-2 Proposed metabolic pathway of [pyridinylmethyl-¹⁴C]BYI 02960 in confined rotational crops





Overall Conclusions for CRC

The metabolism of the insecticide BYI 02960 was investigated in the rotational crops wheat, Swiss chard and turnips from three consecutive rotations in two separate experiments with (1) [furanone-4-¹⁴C]BYI 02960 or (2) [pyridinylmethyl-¹⁴C]BYI 02960. In each study, parent compound was applied to the bare soil 29 days before sowing of the crops of the first rotation. The plant back intervals were 29, 135 and 296 days after the soil application, representing the first, second and third rotation. Sample materials under investigation were the immature raw agricultural commodities wheat forage, wheat hay and Swiss chard at an intermediate growth stage and the mature raw agricultural commodities wheat straw and wheat grain, Swiss chard and turnip leaves and roots.

(1) Label 1: [furanone-4-¹⁴C]BYI 02960

The total radioactive residues (TRR) in all RACs declined significantly from the first to the third rotation. Parent compound was the main component detected in all RACs in all rotations (except for wheat grain), indicating that no major metabolites were formed in the soil and taken up by the plants. These results are in line with the aerobic soil degradation studies which show a quite high mineralization rate of BYI 02960, with only two major soil metabolites detected, both of them being label-specific and not possible to detect in the present study. The formation of BYI 02960-chloro and BYI 02960-bromo in soil was detected to a minor extent. Subsequent uptake of these soil metabolites is most probably the explanation for the trace levels detected in the different RACs. Further degradation of these halogenated compounds in the plant was probably the cause of metabolites such as BYI 02960-bromo-amino-furanone.

The main metabolite in wheat grains was assigned to a natural compound. Diastase digestion of the post extraction solids of the conventional extraction led to one polar metabolite which was identified as glucose by TLC co-chromatography. Radiolabelled glucose was also found in trace levels in turnip roots. Other label-specific metabolites identified were BYI 02960-difluoroethyl-amino-furanone, its conjugates BYI 02960-mercapto-lactic acid and BYI 02960-difluoroethyl-amino-furanone-OH-glyc, as well as the metabolites BYI 02960-amino-furanone and BYI 02960-bromo-amino-furanone. All other identified metabolites were not label specific and were detected at comparable residue levels in the CRC study performed with [pyridinylmethyl-¹⁴C]BYI 02960.

On basis of the metabolites identified, the metabolic pathway was deduced. One major metabolic route was the cleavage of the pyridinylmethylamine bond and the formation of BYI 02960-difluoroethylamino-furanone and BYI 02960-amino-furanone after loss of the difluoroethyl moiety. The cleavage of the molecule was also detected in the CRC study conducted with [pyridinylmethyl-¹⁴C]BYI 02960. BYI 02960-CHMP, 6-CNA and conjugates of these metabolites were identified as the corresponding counterparts. Oxidative degradation of the furanone moiety was also an important degradation path resulting in the metabolites BYI 02960-acetic acid, BYI 02960-glyoxylic and corresponding conjugates. Hydroxylation of the furanone moiety and subsequent conjugation with carbohydrates was an additional metabolic route resulting in metabolites common to both radiolabels tested. Complete degradation of the furanone moiety and incorporation of the carbon atoms into the natural compound pool, i.e. most probably into glucose was also observed. Halogenation of the furanone moiety of the active substance was most probably a process which occurred in the soil.



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

The results of the present metabolism study in confined rotation crops are in good conformity with the results of the corresponding study performed with [pyridinylmethyl-¹⁴C]BYI 02960.

(2) Label 2: [pyridinylmethyl-¹⁴C]BYI 02960

The total radioactive residues (TRR) in all RACs declined significantly from the first to the third rotation. Parent compound was the main component detected in most of the RACs in all rotation (not in wheat grain of the 1st rotation, not in wheat grain and mature Swiss chard in the 2nd rotation, and not in wheat hay, wheat straw and mature Swiss chard of the 3rd rotation). Other major metabolites were the glucuronic acid conjugates of 6-CNA-glycerol (which were detected in quite high amounts in all wheat matrices) and BYI 02960-OH-glyc (which were detected in significant amounts in Swiss chard). These results are in line with the aerobic soil degradation studies which show a quite high mineralization rate of BYI 02960, with only two major soil metabolites detected, one of them being label-specific and not possible to detect in the present study, and the other 6-CNA, which would be expected to be taken up by the plants and further metabolized (conjugated). The formation of 6-CNA is expected to also occur in the plant, supported by the fact that the preceding metabolite BYI 02960-CHMP - or conjugates of it - were also identified in several plant matrices. Metabolite 6-CNA and its preceding metabolite BYI 02960-CHMP, as well as their corresponding conjugates were the only label specific metabolites detected. All other metabolites identified were not label specific and were detected at comparable residue levels in the CRC study performed with [furanone-4-¹⁴C]BYI 02960.

On basis of the metabolites identified, the metabolic pathway was deduced. Overall, three major metabolic routes were detected: (1) Cleavage of the pyridinylmethylamine bond followed by conjugation with carbohydrates and sulphate or by oxidation of the methylene group to a carboxylic group and further conjugation, (2) hydroxylation of the methylene group of the furanone moiety followed by conjugation with carbohydrates and sulphate, and (3) cleavage or complete degradation of the furanone moiety followed by several oxidation and conjugation reactions. Halogenation of the furanone moiety of the active substance was most probably a process which occurred in the soil. Most probably the metabolites were taken up by the roots and were not formed in the plants. This is in line with the finding of the aerobic soil degradation studies summarized in KAII 7.1.

The cleavage of the molecule was also detected in the CRC study conducted with [furanone-4-¹⁴C]BYI 02960. BYI 02960-difluoroethyl-amino-furanone and BYI 02960-amino-furanone and conjugates of these metabolites were identified as the corresponding counterparts to BYI 02960-CHMP and 6-CNA or their conjugates. Thus, the results of the present metabolism study in confined rotation crops are in good conformity with the results of the corresponding study performed with [furanone-4-¹⁴C]BYI 02960.

When considering the results from both confined rotational crop studies conducted, it can be concluded that BYI02960 is rather extensively metabolised in rotational crops. A total of 6 major and approx. 40 minor metabolites were found, and all major and 14 minor have been identified. The distribution of parent compound and metabolites in the edible matrices of confined rotational crops are shown in Table 6.6.2-15 to Table 6.6.2-20.

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

 Table 6.6.2-15 TRR values and distribution of parent compound and metabolites in the edible matrix
 Swiss chard (rotational crop; 1st rotation)

	Confined rotational crops							
	Swiss chard, immature				Swiss chard, mature			
	[furanone-4- ¹⁴ C]	[pyridinylmethyl- ¹⁴ C]	[furanone-4- ¹⁴ C]	[pyridinylmethyl- ¹⁴ C]				
TRR [mg/kg] =		0.848		1.358		0.871		1.483
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
BYI 02960 (parent comp.)	54.3	0.460	57.4	0.779	42.6	0.371	46.3	0.687
<i>glucose/carbohydrates</i>	---	---			---	---		
<i>amino-furanone</i>	1.8	0.015			3.1	0.027		
<i>CHMP-glyc-tri-SA</i>			1.8	0.025			2.3	0.034
<i>6-CNA</i>			7.3	0.099			7.0	0.103
<i>6-CNA-glycerol-gluA (1)</i>			---	---			---	---
<i>6-CNA-glycerol-gluA (2+3)</i>			---	---			---	---
<i>CHMP-glyc-di-SA</i>			2.5	0.035			3.0	0.045
<i>CHMP-glyc</i>			5.5	0.075			5.4	0.080
<i>CHMP</i>			---	---			---	---
<i>difluoroethyl-amino-furanone-OH-glyc</i>	---	---			1.2	0.010		
<i>difluoroethyl-amino-furanone</i>	16.6	0.141			16.6	0.145		
glyoxylic acid	3.7	0.031	1.5	0.021	4.7	0.041	2.6	0.039
OH-glyc-SA (1)	1.2	0.010	1.1	0.015	2.5	0.022	1.7	0.025
acetic acid-glyc	0.5	0.004	1.6	0.022	1.6	0.014	---	---
OH-glyc-SA (2)	3.3	0.028	0.5	0.006	3.2	0.028	¹ 2.5	¹ 0.037
OH-glyc	8.5	0.072	7.4	0.101	13.6	0.119	10.9	0.162
acetic acid	0.4	0.003	---	---	0.6	0.005	---	---
<i>N-formyl-/N-acetyl-AMCP-difluoroethanamine</i>			4.0	0.055			2.9	0.043
OH	2.0	0.017	1.8	0.024	1.9	0.017	1.6	0.023
chloro/ bromo	0.6	0.005	0.7	0.009	0.4	0.003	0.5	0.008
Total identified	92.8	0.787	93.2	1.266	92.0	0.802	86.6	1.285
Total characterised	2.5	0.021	4.5	0.062	3.8	0.033	9.9	0.146
Analysed extract(s)	95.3	0.809	97.7	1.327	95.9	0.835	96.5	1.431
Extract(s) not analysed	0.4	0.003	0.3	0.004	0.3	0.002	0.5	0.008
Total extracted	95.7	0.812	97.7	1.327	96.1	0.838	96.5	1.431
Unextractable (PES*)	4.3	0.037	2.1	0.028	3.9	0.034	2.9	0.043
Accountability	100.0	0.848	100.0	1.359	100.0	0.871	100.0	1.483

* post extraction solids

¹ co-elution with BYI 02960-acetic acid-glyc

(1) isomer 1

(2) isomer 2

(2 + 3) isomer 2 and/or isomer 3

Label specific metabolites are printed in italic.



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.6.2-16 TRR values and distribution of parent compound and metabolites in the edible matrix Swiss chard (rotational crop; 2nd rotation)

	Confined rotational crops							
	Swiss chard, immature				Swiss chard, mature			
	[furanone-4- ¹⁴ C]	[pyridinylmethyl- ¹⁴ C]	[furanone-4- ¹⁴ C]	[pyridinylmethyl- ¹⁴ C]				
TRR [mg/kg] =		0.311		0.332		0.263		0.438
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
BYI 02960 (parent comp.)	55.0	0.171	51.2	0.170	27.5	0.072	24.6	0.108
<i>glucose/carbohydrates</i>	---	---			---	---		
<i>amino-furanone</i>	8.8	0.027			8.8	0.023		
<i>CHMP-glyc-tri-SA</i>			2.8	0.009			2.0	0.009
<i>6-CNA</i>			2.9	0.010			2.9	0.013
<i>6-CNA-glycerol-gluA (1)</i>			---	---			---	---
<i>6-CNA-glycerol-gluA (2+3)</i>			---	---			---	---
<i>CHMP-glyc-di-SA</i>			1.8	0.006			2.4	0.010
<i>CHMP-glyc</i>			5.1	0.017			1.3	0.006
<i>CHMP</i>			---	---			---	---
<i>difluoroethyl-amino-furanone-OH-glyc</i>	---	---			---	---		
<i>difluoroethyl-amino-furanone</i>	10.3	0.032			17.4	0.046		
glyoxylic acid	---	---	---	---	---	---	---	---
OH-glyc-SA (1)	2.9	0.009	1.6	0.005	3.5	0.009	2.9	0.013
acetic acid-glyc	1.8	0.006	---	---	1.1	0.003	3.0	0.013
OH-glyc-SA (2)	2.0	0.006	2.5	0.008	3.2	0.008	6.3	0.028
OH-glyc	11.7	0.036	17.4	0.058	18.0	0.047	25.3	0.111
acetic acid	0.5	0.002	---	---	0.8	0.002	---	---
<i>N-formyl-/N-acetyl-AMCP-difluoroethanamine</i>			1.6	0.005			---	---
OH	2.0	0.006	3.9	0.013	2.4	0.006	3.6	0.016
chloro/ bromo	0.5	0.001	0.5	0.002	---	---	0.2	0.001
Total identified	95.5	0.297	91.4	0.303	82.7	0.218	74.6	0.327
Total characterised	1.6	0.005	6.7	0.022	9.3	0.025	14.7	0.064
Analysed extract(s)	97.1	0.302	91.4	0.303	92.1	0.242	96.1	0.421
Extract(s) not analysed	---	---	---	---	0.5	0.001	0.7	0.003
Total extracted	97.1	0.302	98.1	0.325	92.5	0.243	96.1	0.421
Unextractable (PES*)	2.9	0.009	1.9	0.006	7.5	0.02	3.2	0.014
Accountability	100.0	0.311	100.0	0.332	100.0	0.263	100.0	0.438

* post extraction solids

(1) isomer 1

(2) isomer 2

(2 + 3) isomer 2 and/or isomer 3

Label specific metabolites are printed in italic.

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.6.2-17 TRR values and distribution of parent compound and metabolites in the edible matrix Swiss chard (rotational crop; 3rd rotation)

		Confined rotational crops						
		Swiss chard, immature		Swiss chard, mature				
		[furanone-4- ¹⁴ C]	[pyridinylmethyl- ¹⁴ C]	[furanone-4- ¹⁴ C]		[pyridinylmethyl- ¹⁴ C]		
TRR [mg/kg] =		0.180		0.135		0.152		0.130
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
BYI 02960 (parent comp.)	36.7	0.066	31.6	0.042	33.4	0.051	27.4	0.036
<i>glucose/carbohydrates</i>	---	---			---	---		
<i>amino-furanone</i>	1.3	0.002			1.8	0.003		
<i>CHMP-glyc-tri-SA</i>			2.5	0.003			0.4	0.001
<i>6-CNA</i>			5.3	0.007			5.1	0.007
<i>6-CNA-glycerol-gluA (1)</i>			---	---			---	---
<i>6-CNA-glycerol-gluA (2+3)</i>			---	---			---	---
<i>CHMP-glyc-di-SA</i>			2.6	0.003			2.8	0.004
<i>CHMP-glyc</i>			5.1	0.007			6.2	0.008
<i>CHMP</i>			---	---			---	---
<i>difluoroethyl-amino-furanone-OH-glyc</i>	2.1	0.004			1.9	0.003		
<i>difluoroethyl-amino-furanone</i>	13.7	0.025			15.6	0.024		
glyoxylic acid	0.2	<0.001	2.1	0.003	---	---	1.0	0.001
OH-glyc-SA (1)	1.7	0.003	5.3	0.007	2.4	0.004	2.9	0.004
acetic acid-glyc	0.2	<0.001	---	---	0.2	<0.001	---	---
OH-glyc-SA (2)	4.2	0.008	0.5	0.001	2.4	0.004	¹ 2.8	¹ 0.004
OH-glyc	22.2	0.04	24.6	0.033	21.9	0.033	28.1	0.036
acetic acid	0.9	0.002	---	---	0.9	0.001	---	---
<i>N-formyl-/N-acetyl-AMCP-difluoroethanamine</i>			0.8	0.001			1.8	0.002
OH	3.6	0.007	3.8	0.005	3.1	0.005	3.4	0.004
chloro/ bromo	0.5	0.001	0.7	0.001	0.6	0.001	0.4	0.001
Total identified	87.3	0.157	84.9	0.114	84.2	0.128	82.4	0.107
Total characterised	6.5	0.012	9.0	0.012	4.9	0.008	11.6	0.016
Analysed extract(s)	93.8	0.169	84.9	0.114	89.1	0.135	82.4	0.107
Extract(s) not analysed	---	---	---	---	---	---	---	---
Total extracted	93.8	0.169	93.9	0.126	89.1	0.135	94.0	0.123
Unextractable (PES*)	6.2	0.011	6.1	0.008	10.9	0.017	6.0	0.008
Accountability	100.0	0.180	100.0	0.134	100.0	0.152	100.0	0.130

* post extraction solids

¹ co-elution with BYI 02960-acetic acid-glyc

(1) isomer 1

(2) isomer 2

(2 + 3) isomer 2 and/or isomer 3

Label specific metabolites are printed in italic.

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.6.2-18 TRR values and distribution of parent compound and metabolites in edible matrices wheat grains and turnip roots (rotational crops; 1st rotation)

	Confined rotational crops							
	wheat grains				turnip roots			
	[furanone-4- ¹⁴ C]	[pyridinylmethyl- ¹⁴ C]	[furanone-4- ¹⁴ C]	[pyridinylmethyl- ¹⁴ C]				
TRR [mg/kg] =		0.478		0.177		0.074		0.072
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
<i>BYI 02960</i>	0.4	0.002	8.3	0.015	55.9	0.041	57.8	0.042
<i>glucose/carbohydrates</i>	70.5	0.338			3.4	0.003		
<i>amino-furanone</i>	0.5	0.002			4.1	0.003		
<i>CHMP-glyc-tri-SA</i>			---	---			---	---
<i>6-CNA</i>			3.8	0.007			8.5	0.006
<i>6-CNA-glycerol-gluA (1)</i>			2.3	0.004			---	---
<i>6-CNA-glycerol-gluA (2+3)</i>			20.2	0.036			---	---
<i>CHMP-glyc-di-SA</i>			---	---			---	---
<i>CHMP-glyc</i>			---	---			---	---
<i>CHMP</i>			---	---			4.1	0.003
<i>difluoroethyl-amino-furanone-OH-glyc</i>	---	---			---	---		
<i>difluoroethyl-amino-furanone</i>	---	---			---	---		
glyoxylic acid	5.1	0.024	6.0	0.011	12.2	0.009	8.6	0.006
OH-glyc-SA (1)	---	---	---	---	---	---	---	---
acetic acid-glyc	---	---	---	---	---	---	---	---
OH-glyc-SA (2)	---	---	---	---	---	---	---	---
OH-glyc	1.4	0.007	5.0	0.009	2.1	<0.001	2.5	0.002
acetic acid	0.6	0.003	1.7	0.003	0.2	<0.001	---	---
<i>N-formyl-/N-acetyl-AMCP-difluoroethanamine</i>			---	---			3.5	0.003
OH	2.3	0.011	10.4	0.019	0.4	<0.001	0.5	<0.001
chloro/ bromo	---	---	---	---	1.3	0.001	1.4	0.001
Total identified	80.8	0.387	57.8	0.103	79.6	0.058	86.8	0.063
Total characterised	4.2	0.020	13.8	0.025	8.4	0.006	3.2	0.002
Analysed extract(s)	85.0	0.407	71.6	0.127	88.1	0.065	90.0	0.065
Extract(s) not analysed	3.0	0.014	12.2	0.022	---	---	---	---
Total extracted	88.0	0.421	83.8	0.149	88.1	0.065	90.0	0.065
Unextractable (PES*)	12.0	0.058	16.2	0.029	11.9	0.009	10.0	0.007
Accountability	100.0	0.478	100.0	0.177	100.0	0.074	100.0	0.072

* post extraction solids

(1) isomer 1

(2) isomer 2

(2 + 3) isomer 2 and/or isomer 3

Label specific metabolites are printed in italic.

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.6.2-19 TRR values and distribution of parent compound and metabolites in edible matrices wheat grains and turnip roots (rotational crops; 2nd rotation)

	Confined rotational crops							
	wheat grains				turnip roots			
	[furanone-4- ¹⁴ C]	[pyridinylmethyl- ¹⁴ C]	[furanone-4- ¹⁴ C]	[pyridinylmethyl- ¹⁴ C]				
TRR [mg/kg] =		0.103		0.057		0.014		0.022
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
BYI 02960	0.5	0.001	1.1	0.001	31.0	0.004	48.4	0.011
glucose/carbohydrates	---	---			16.0	0.002		
amino-furanone	1.3	0.001			---	---		
CHMP-glyc-tri-SA			---	---			---	---
6-CNA			---	---			7.7	0.002
6-CNA-glycerol-gluA (1)			2.5	0.001			---	---
6-CNA-glycerol-gluA (2+3)			11.9	0.007			---	---
CHMP-glyc-di-SA			---	---			---	---
CHMP-glyc			---	---			2.0	<0.001
CHMP			---	---			1.1	<0.001
difluoroethyl-amino-furanone-OH-glyc	---	---			---	---		
difluoroethyl-amino-furanone	---	---			---	---		
glyoxylic acid	0.8	0.001	0.9	0.001	1.8	<0.001	---	---
OH-glyc-SA (1)	---	---	---	---	---	---	---	---
acetic acid-glyc	---	---	---	---	---	---	---	---
OH-glyc-SA (2)	---	---	---	---	---	---	---	---
OH-glyc	2.1	0.002	4.6	0.003	2.3	<0.001	2.4	0.001
acetic acid	1.0	0.001	1.6	0.001	0.2	<0.001	---	---
N-formyl-/N-acetyl-AMCP-difluoroethanamine			---	---			---	---
OH	3.4	0.003	6.4	0.004	---	---	---	---
chloro/ bromo	---	---	---	---	1.6	<0.001	2.0	<0.001
Total identified	9.2	0.009	29.0	0.017	53.0	0.007	63.6	0.013
Total characterised	4.2	0.004	5.6	0.003	29.3	0.004	13.9	0.003
Analysed extract(s)	13.4	0.014	34.6	0.02	82.3	0.012	77.5	0.015
Extract(s) not analysed	¹ 69.2	¹ 0.071	54.8	0.032	---	---	0.6	<0.001
Total extracted	82.6	0.085	34.6	0.020	82.3	0.012	77.5	0.015
Unextractable (PES*)	17.4	0.018	10.5	0.006	17.7	0.003	21.9	0.005
Accountability	100.0	0.103	100.0	0.057	100.0	0.014	100.0	0.022

* post extraction solids

¹ no analysis performed, but presumably glucose/carbohydrates as identified in grains of 1st rotation post extraction solids

Label specific metabolites are printed in italic.

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.6.2-20 TRR values and distribution of parent compound and metabolites in edible matrices wheat grains and turnip roots (rotational crops; 3rd rotation)

	Confined rotational crops							
	wheat grains				turnip roots			
	[furanone-4- ¹⁴ C]	[pyridinylmethyl- ¹⁴ C]	[furanone-4- ¹⁴ C]	[pyridinylmethyl- ¹⁴ C]				
TRR [mg/kg] =		0.047		0.017		0.008		0.008
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
BYI 02960	2.0	0.001	13.9	0.002	69.9	0.006	64.8	0.005
<i>glucose/carbohydrates</i>	---	---			5.3	<0.001		
<i>amino-furanone</i>	---	---			5.0	<0.001		
<i>CHMP-glyc-tri-SA</i>			---	---			---	---
<i>6-CNA</i>			---	---			4.7	<0.001
<i>6-CNA-glycerol-gluA (1)</i>			---	---			---	---
<i>6-CNA-glycerol-gluA (2+3)</i>			11.2	0.002			---	---
<i>CHMP-glyc-di-SA</i>			---	---			---	---
<i>CHMP-glyc</i>			---	---			---	---
<i>CHMP</i>			---	---			1.4	<0.001
<i>difluoroethyl-amino-furanone-OH-glyc</i>	---	---			---	---		
<i>difluoroethyl-amino-furanone</i>	---	---			---	---		
<i>glyoxylic acid</i>	---	---	---	---	---	---	---	---
<i>OH-glyc-SA (1)</i>	---	---	---	---	---	---	---	---
<i>acetic acid-glyc</i>	---	---	---	---	---	---	---	---
<i>OH-glyc-SA (2)</i>	---	---	---	---	---	---	---	---
<i>OH-glyc</i>	1.7	0.001	5.2	0.001	3.1	<0.001	3.8	<0.001
<i>acetic acid</i>	0.8	<0.001	1.8	<0.001	0.3	<0.001	---	---
<i>N-formyl-/N-acetyl-AMCP-difluoroethanamine</i>			---	---			---	---
<i>OH</i>	2.8	0.001	5.5	0.001	---	---	---	---
<i>chloro/ bromo</i>	---	---	---	---	2.5	<0.001	2.5	<0.001
Total identified	7.4	0.003	37.7	0.006	86.2	0.007	77.1	0.005
Total characterised	4.3	0.002	7.1	0.001	9.2	0.001	12.7	0.001
Analysed extract(s)	11.7	0.005	44.8	0.007	95.5	0.007	89.8	0.006
Extract(s) not analysed	8.8	0.004	---	---	---	---	3.3	<0.001
Total extracted	20.4	0.01	44.8	0.007	95.5	0.008	89.8	0.006
Unextractable (PES*)	¹ 79.6	¹ 0.037	55.2	0.009	4.5	<0.001	6.9	0.001
Accountability	100.0	0.047	100.0	0.017	100.0	0.008	100.0	0.008

* post extraction solids

¹ no analysis performed but presumably glucose/carbohydrates as identified in grains of 1st rotation pose extraction solids

(1) isomer 1 (2) isomer 2 (2 + 3) isomer 2 and/or isomer 3

Label specific metabolites are printed in italic.



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

In order to gain information on the fate of the difluoroethane moiety of BYI 02960, the extracts obtained in the CRC study with [furanone-4-¹⁴C]BYI 02960 were additionally analysed for non-radiolabelled difluoroacetic acid by LC-MS/MS according to residue method 01304 (see KIIA 6.2./12). Significant levels of difluoroacetic acid were detected in all plant matrices of the first and second rotation. DFA represented the main proportion of the residues in the edible crops wheat grain, Swiss chard and turnip roots and as well as in the feed item wheat hay. In wheat forage and straw and turnip leaves, DFA was less prominent than parent, but it was still a major compound.

In corresponding crop samples from different plant back intervals, the DFA levels generally decreased significantly from the first to the third rotation, showing DFA levels slightly above or below the limit of quantification in all crops of the third rotation. The highest DFA concentrations were detected in wheat grains ranging from 4.45 mg a.s. equiv./kg in the first rotation to 0.15 mg a.s. equiv./kg in the third rotation.

On basis of the metabolites identified, biotransformation of BYI 02960 in confined rotational crops proceeds by the following main pathways:

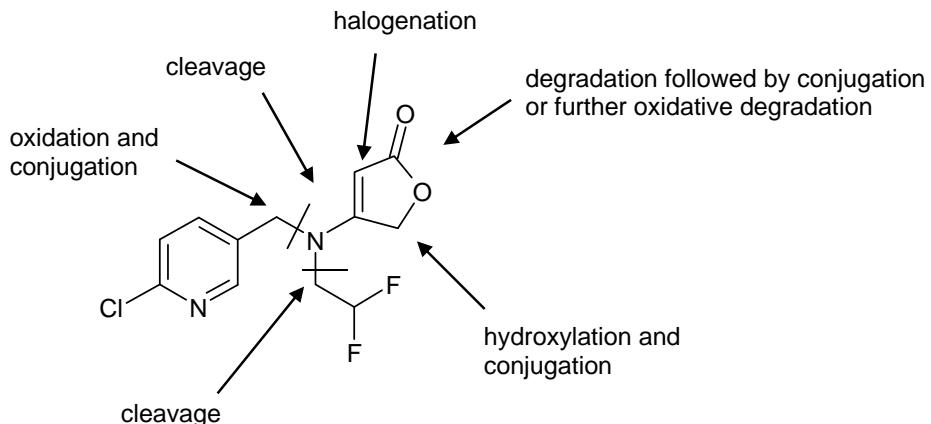
- oxidative cleavage of the difluoroethylamine bond and formation of difluoroacetic acid
- cleavage of the pyridinylmethylamine bond and formation of BYI 02960-difluoroethyl-amino-furanone and the corresponding counterparts BYI 02960-CHMP and 6-CNA followed by several conjugation reactions
- cleavage of the furanone moiety followed by several oxidation and conjugation reactions
- complete degradation of the furanone moiety and incorporation of carbon atoms into the natural compound pool, e.g. into glucose/carbohydrates
- hydroxylation of the methylene group of the furanone moiety followed by conjugation with carbohydrates and sulphate
- halogenation (bromination and most probably chlorination, as well) of the furanone moiety

Halogenation of the furanone moiety of the active substance probably occurred in the soil which is supported by the fact that small amounts of halogenated parent compound were identified in the aerobic soil degradation studies.



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

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



IIA 6.6.3 Field trials on representative crops

Numerous field rotational crop trials have been conducted to support the use of BYI 02960. In this Annex II dossier, only the "main" field study will be described (containing data for three rotations with three crop groups: leafy, root, and cereal crops). In order to enable MRL-setting, further data on other crops will be submitted in a separate document.

General remark:

In this summary section (KIIA 6.6.3), the name DFEAF will be used for the metabolite BYI 02960-difluoroethyl-amino-furanone, which is relevant to the tested residue definition:

Name	Metab. No.	Standard "dossier name"
DFEAF	M34	BYI 02960-difluoroethyl-amino-furanone

Both EU residue regions

Report:	KIIA 6.6.3/01, Schoening, R.; Bauer, J. 2012
Title:	Determination of the residues of BYI 02960 in/on the field rotational crops barley, carrot, lettuce and turnip after spray application of BYI 02960 SL 200 on lettuce and soil in the field in Germany, the Netherlands, France (South) and Spain
Report No. & Document No.:	10-2503, dated April 11, 2012 M-429091-01-1
Guidelines:	– EU Council Directive 91/414/EEC amended by Commission Directive 96/68/EC – EU Guidance Working Document 7029/VI/95 rev. 5 – OECD Guideline for the Testing of Chemicals No. 504, Residues in Rotational Crops
GLP:	yes (certified laboratory)



I. Materials and Methods

Multi-crop, multi-plot/plantback field rotational crop residue trials were conducted in Europe, two in the northern and two in the southern residue region, as follows:

In 2010-11, 4 trials (one each in Germany, the Netherlands, Spain, and southern France) were conducted to support the use of BYI 02960 SL 200 in field-grown, non-perennial crops (Schoening & Bauer, 2012, KIIA 6.6.3/01). A single application was made at a nominal rate of 1 L/ha, corresponding to 200 g/ha BYI 02960 a.s., which reflected the projected rate for soil treatment with BYI 02960 (via irrigation) in arable, non-perennial crops, such as lettuce. Water rates were 300-400 L/ha. Applications were either made to bare soil or to lettuce ("target crop"); in the latter case, the crop was then harvested from the field before further crops were planted. All treatments were made at the scheduled rates. All data pertaining to the application/use pattern are presented in table 6.6.3-1.

At various intervals, crops were planted back onto the test area in order to simulate a crop failure ("rotation 1", plant-back interval [PBI] 25-33 days), a second use of the plot in the same year ("rotation 2", PBI 60-200 days), or use of the same plot in the succeeding year ("rotation 3", PBI 260-330 days). In each rotation, 3 different crops representing different botanical groups were planted: a root crop (carrots or turnips), a leafy crop (lettuce), or a hard cereal (barley).

Samples of the rotational crops were taken at their respective harvest times, as well as at one earlier interval (immature RACs for lettuce and root crops, or fodder ["green material"] for barley). The samples were analyzed for the parent compound and its metabolites DFA and DFEAF using method 01304 (cf. KIIA 4.3/03). The respective LOQs for the 3 analytes were 0.01, 0.02, and 0.01 mg/kg (all in parent equivalents), yielding a calculated total-residue LOQ of 0.04 mg/kg.

II. Findings

Concurrent recoveries of BYI 02960 and its metabolites DFA and DFEAF were obtained from samples of carrots, turnips, lettuce, and barley. (Validation recoveries were conducted separately. Details of the validation recoveries are presented in chapter 4.3 of this dossier with method 01304.)

Root crops:

Concurrent recovery samples for parent compound and DFEAF were spiked at levels of 0.01 mg/kg as well as at 0.10 mg/kg, 0.50, and 1.0 mg/kg (expressed in BYI 02960 equivalents). Mean recoveries in root crop samples (carrot or turnip roots, turnip leaves) were 74-108%, with RSDs of the larger validations sets ($n > 2$, at the LOQ) of 7.1-16.0%; $n=1-6$. All values were within acceptable ranges.

For DFA, concurrent recovery samples were spiked at levels of 0.02 mg/kg, as well as at 0.20, 0.50, and 1.0 mg/kg (expressed in BYI 02960 equivalents). Mean recoveries were 84-109%, with RSDs (of the larger validations sets [$n > 2$]) of 7.4-17.1%; $n=1-6$.

Details of recovery data are shown in table 6.6.3-5. All trial data are summarised below in table 6.6.3-2 and in greater detail in the Tier 1 summary forms. (Residues of parent BYI 02960 as well as its metabolites DFA and DFEAF are expressed in BYI 02960 equivalents.)



The total residues of BYI 02960 (parent compound plus DFA and DFEAF) in the harvested roots of carrots or turnips were generally highest in the first rotation, i.e. after the shortest plant-back interval (PBI) of 25-30 days, when they ranged at an "intermediate" growth stage (including "early harvest", BBCH 47-49) from 0.05-0.14 mg/kg, and at typical harvest ripeness (BBCH 49) from 0.05-0.12 mg/kg. In one trial, however, the residues in the 3rd and final rotation (PBI 284 days) were slightly higher than in the first rotation, at 0.06 mg/kg compared to 0.05 mg/kg. Thus, highest residues in roots in the entire study ranged from 0.06-0.14 mg/kg (median 0.08 mg/kg).

By the third rotation, residues in harvestable roots ranged from <0.04-0.06 mg/kg, with a median value of 0.05 mg/kg.

In turnip tops (=leaves; one trial only), highest residue levels were seen in the first rotation (0.24 mg/kg), but these were not significantly different from those seen in the third rotation (0.21 mg/kg).

Leafy crops:

Concurrent recovery samples for parent compound and DFEAF were spiked at levels of 0.01 mg/kg and 0.10 mg/kg, as well as at 0.50 mg/kg, 1.0, and 5.0 mg/kg (expressed in BYI 02960 equivalents). Mean recoveries in root crop samples (lettuce heads) were 91-105%, with RSDs of the larger validations sets ($n > 2$) of 2.2-10.7%; $n=2-15$.

For DFA, concurrent recovery samples were spiked at levels of 0.02, 0.05, and 0.50 mg/kg, as well as at 0.20, 1.0, and 5.0 mg/kg (expressed in BYI 02960 equivalents). Mean recoveries were 90-98%, with RSDs (of the larger validations sets [$n > 2$]) of 4.3-10.2%; $n=2-12$.

Details of recovery data are shown in table 6.6.3-5. All trial data are summarised below in table 6.6.3-3 and in greater detail in the Tier 1 summary forms. (Residues of parent BYI 02960 as well as its metabolites DFA and DFEAF are expressed in BYI 02960 equivalents.)

The total residues of BYI 02960 (parent compound plus DFA and DFEAF) in the harvested heads of lettuce were highest in the first rotation, i.e. after the shortest plant-back interval (PBI) of 25-30 days, when they ranged at an "intermediate" growth stage (from BBCH 41-47, including "early harvest", BBCH 46-47) from 0.06-0.21 mg/kg, and at typical harvest ripeness (BBCH 49) from <0.04-0.16 mg/kg. Thus, highest residues in "marketable" heads in the entire study ranged from <0.04-0.16 mg/kg, with a median value of 0.08 mg/kg.

By the third rotation, total residues in lettuce heads ranged from <0.04-0.10 mg/kg (median <0.04 mg/kg).

Cereal crops:

Concurrent recovery samples for parent compound and DFEAF were spiked at levels of 0.01 mg/kg as well as at 0.10 mg/kg and 1.0 mg/kg (expressed in BYI 02960 equivalents). Mean recoveries in cereal crop samples (green material, grain, and straw) were 76-110%, with RSDs of the larger validations sets ($n > 2$, at the LOQ) of 5.6-9.2%; $n=1-4$. All values were within acceptable ranges.



For DFA, concurrent recovery samples were spiked at levels of 0.02 mg/kg, as well as at 0.05, 0.20, 0.50, and 1.0 mg/kg (expressed in BYI 02960 equivalents). Mean recoveries were 71-108%, with RSDs (of the larger validations sets [$n > 2$]) of 3.0-7.1%; $n=1-3$.

Details of recovery data are shown in table 6.6.3-5. All trial data are summarised below in table 6.6.3-4 and in greater detail in the Tier 1 summary forms. (Residues of parent BYI 02960 as well as its metabolites DFA and DFEAF are expressed in BYI 02960 equivalents.)

The total residues of BYI 02960 (parent compound plus DFA and DFEAF) in the harvested barley grain were highest in the first rotation, i.e. after the shortest plant-back interval (PBI) of 25-33 days, when they ranged from 0.11-0.65 mg/kg (median 0.35 mg/kg). In one trial, however, no grain samples could be taken due to damage by geese (trial 10-2503-02, Netherlands). Based on the data from straw, it is clear that the missing trial would have yielded residues well below the highest residues and probably below the current median. Residues in straw from the first rotation ranged from <0.07-0.39 mg/kg, with a median value of 0.12 mg/kg. (The level of residues in straw in the Dutch trial were the lowest of any trial.)

By the third rotation, residues in grain ranged from 0.08-0.39 mg/kg, with a median value of 0.12 mg/kg. In straw, they had dropped to <0.07-0.19 mg/kg (median <0.07 mg/kg).

Early in the respective growing periods of each rotation, samples were also taken of green material (forage). Again, residues were highest in the first rotation, in which they ranged from 0.05-0.41 mg/kg, with a median of 0.10 mg/kg. (Residue levels in green material in the Dutch trial were 0.06 mg/kg, below the median and the 2nd-lowest value in this study.)

III. Conclusions

In order to support the use in the EU of BYI 02960 in non-perennial crops, four multi-plantback multi-crop rotational crop trials were conducted in Europe (2 each in the northern and southern residue regions) in the years 2010-2011. BYI 02960 was applied once as an SL 200 formulation either to bare soil or to a target crop (lettuce) at an active substance rate of 200 g/ha, the target crop was then harvested, and crops representing 3 different botanical groups (roots, leafy veg., cereals) were planted on the plots at 3 intervals thereafter.. All applications were at the required rates, and all trials were conducted according to GLP.

To evaluate the potential residues in following crops, samples of the rotated crops were taken at an intermediate stage and at usual full harvest ripeness. Samples were analyzed for the relevant residues of BYI 02960, comprising the parent compound and its metabolites DFA and DFEAF. The residues of all three analytes were summed to yield a calculated "total residue of BYI 02960". The results of the trials presented above demonstrate that:

- total residues of BYI 02960 in all rotational crops tended to be highest in the first, earliest rotation, i.e. grown after a plant-back interval (PBI) of 25-33 days.

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- 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).

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Table 6.6.3-1: Application scenario in field rotational crop trials (study 10-2503): Spray treatment with BYI 02960 SL 200 to soil or a target crop

Trial No. Plot No Country Location Region Year	Target Crop, Variety	FL	No.	Application		GS
				kg/ha (a.s.)	kg/hl (a.s.)	
10-2503-01 10-2503-01-T-1A Germany 51399 Burscheid EU-N 2010	soil	200 SL	1	0.20	0.067	
10-2503-01 10-2503-01-T-2A Germany 51399 Burscheid EU-N 2010	lettuce Argentines, Butterhead variety	200 SL	1	0.20	0.067	19
10-2503-01 10-2503-01-T-3A Germany 51399 Burscheid EU-N 2010	lettuce Argentines, Butterhead variety	200 SL	1	0.20	0.067	19
10-2503-02 10-2503-02-T-1A Netherlands 1681 ND Zwaagdijk EU-N 2010	soil	200 SL	1	0.20	0.050	
10-2503-02 10-2503-02-T-2A Netherlands 1681 ND Zwaagdijk EU-N 2010	lettuce Gisela, Butterhead variety	200 SL	1	0.20	0.050	19
10-2503-02 10-2503-02-T-3A Netherlands 1681 ND Zwaagdijk EU-N 2010	lettuce Gisela, Butterhead variety	200 SL	1	0.20	0.050	19

FL = formulation

GS = growth stage (BBCH-code) at last treatment

EU-N = northern Europe

Continued on next page...

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.6.3-1 (cont'd): Application scenario in field rotational crop trials (study 10-2503): Spray treatment with BYI 02960 SL 200 to soil or a target crop

Trial No. Plot No Country Location Region Year	Target Crop, Variety	FL	No.	Application		GS
				kg/ha (a.s.)	kg/hl (a.s.)	
10-2503-03 10-2503-03-T-1A France 31200 Toulouse EU-S 2010	soil	200 SL	1	0.20	0.050	
10-2503-03 0-2503-03-T-2A France 31200 Toulouse EU-S 2010	lettuce Pitice, Loose leaf	200 SL	1	0.20	0.050	19
10-2503-03 10-2503-03-T-3A France 31200 Toulouse EU-S 2010	lettuce Pitice, Loose leaf	200 SL	1	0.20	0.050	19
10-2503-04 10-2503-04-T-1A Spain 08520 Llerona EU-S 2010	soil	200 SL	1	0.20	0.067	
10-2503-04 10-2503-04-T-2A Spain 08520 Llerona EU-S 2010	lettuce Maravilla, Butterroot	200 SL	1	0.212	0.0667	19
10-2503-04 10-2503-04-T-3A Spain 08520 Llerona EU-S 2010	lettuce Maravilla, Butterroot	200 SL	1	0.20	0.067	19

FL = formulation

GS = growth stage (BBCH-code) at last treatment

EU-S = southern Europe



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Table 6.6.3-2: Results of field rotational crop trials following spray treatment with BYI 02960 SL 200 to soil or a target crop and then planting back and sampling of various rotational crops
Here: **root crops**

Study No. (Trial No.) Plot No Country GLP	Rotational Crop, Variety (rotation information)	Portion analysed	GS	DALT (days)	Residues (mg/kg) expressed as BYI 02960				total residue of BYI 02960 calc.
					BYI 02960	difluoro- acetic acid	BYI 02960- difluoro- ethylamino- furanone		
10-2503 (10-2503-01) 10-2503-01-T-1A Germany GLP: yes	Carrot, Cestas F1 (Rotation 1) PBI 25 days	root	47 49	95 109	<0.01 <0.01	0.03 0.03	<0.01 <0.01	0.05 0.05	
10-2503 (10-2503-01) 10-2503-01-T-2A Germany GLP: yes	Carrot, Cestas F1 (Rotation 2) PBI 70 days	root	48 49	150 164	<0.01 <0.01	<0.02 <0.02	<0.01 <0.01	<0.04 <0.04	
10-2503 (10-2503-01) 10-2503-01-T-3A Germany GLP: yes	Carrot, Cestas (Rotation 3) PBI 284 days	root	48 49	396 410	<0.01 <0.01	0.03 0.04	<0.01 <0.01	0.05 0.06	
10-2503 (10-2503-02) 10-2503-02-T-1A Netherlands GLP: yes	Carrot, Nerja (Rotation 1) PBI 25 days	root	48 49	92 106	<0.01 <0.01	0.03 0.05	<0.01 <0.01	0.05 0.07	
10-2503 (10-2503-02) 10-2503-02-T-2A Netherlands GLP: yes	Carrot, Nerja (Rotation 2) PBI 61 days	root	48 49	151 165	<0.01 <0.01	0.03 0.03	<0.01 <0.01	0.05 0.05	
10-2503 (10-2503-02) 10-2503-02-T-3A Netherlands GLP: yes	Carrot, Nerja (Rotation 3) PBI 328 days	root	47 49	406 420	<0.01 <0.01	<0.02 <0.02	<0.01 <0.01	<0.04 <0.04	

DALT = days after last treatment

GS = growth stage (BBCH-code) at sampling

PBI = plant-back interval

Continued on next page...

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.6.3-2 (cont'd): Results of field rotational crop trials following spray treatment with BYI 02960 SL 200 to soil or a target crop and then planting back and sampling of various rotational crops
Here: **root crops**

Study No. (Trial No.) Plot No Country GLP	Rotational Crop, Variety (rotation information)	Portion analysed	GS	DALT (days)	Residues (mg/kg) expressed as BYI 02960	BYI 02960- difluoro- acetic acid	BYI 02960- difluoro- ethylamino- furanone	total residue of BYI 02960 calc.
10-2503 (10-2503-03) 10-2503-03-T-1A France GLP: yes	Turnip, edible Aramis (Rotation 1) PBI 25 days	body	49 49	82 96	<0.01 <0.01	0.12 0.10	<0.01 <0.01	0.14 0.12
		leaf	49 49	82 96	0.03 0.02	0.20 0.07	<0.01 <0.01	0.24 0.10
	Turnip, edible Aramis (Rotation 2) PBI 70 days	body	49 49	127 141	<0.01 <0.01	0.05 0.04	<0.01 <0.01	0.07 0.06
		leaf	49 49	127 141	0.02 <0.01	0.08 0.03	<0.01 <0.01	0.11 0.05
10-2503 (10-2503-03) 10-2503-03-T-3A France GLP: yes	Carrot, Turnip, edible Aramis (Rotation 3) PBI 314 days	body	47 47	380 394	<0.01 <0.01	0.04 0.03	<0.01 <0.01	0.06 0.05
		leaf	49 49	380 394	0.03 0.04	0.11 0.16	<0.01 <0.01	0.15 0.21
	Carrot, Coral Nantesa (Rotation 1) PBI 30 days	root	47 49	100 114	<0.01 <0.01	0.05 0.06	<0.01 <0.01	0.07 0.08
		root	49 49	314 328	<0.01 <0.01	0.02 0.03	<0.01 <0.01	0.04 0.05
10-2503 (10-2503-04) 10-2503-04-T-1A Spain GLP: yes	Carrot, Coral Nantesa (Rotation 2) PBI 145 days	root	49 49	349 363	<0.01 <0.01	<0.02 0.03	<0.01 <0.01	<0.04 0.05
		root	47 49	349 363	<0.01 <0.01	<0.02 0.03	<0.01 <0.01	<0.04 0.05

DALT = days after last treatment

GS = growth stage (BBCH-code) at sampling

PBI = plant-back interval

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.6.3-3: Results of field rotational crop trials following spray treatment with BYI 02960 SL 200 to soil or a target crop and then planting back and sampling of various rotational crops
 Here: leafy crops

Study No. (Trial No.) Plot No Country GLP	Rotational Crop, Variety (rotation information)	Portion analysed	GS	DALT (days)	Residues (mg/kg) expressed as BYI 02960			
					BYI 02960	difluoro- acetic acid	BYI 02960- difluoro- ethylamino- furanone	total residue of BYI 02960 calc.
10-2503 (10-2503-01) 10-2503-01-T-1B Germany GLP: yes	Lettuce, Argentines, Butterhead variety (Rotation 1) PBI 25 days	head	46 49	63 77	0.01 <0.01	0.06 0.05	<0.01 <0.01	0.09 0.07
10-2503 (10-2503-01) 10-2503-01-T-2B Germany GLP: yes	Lettuce, Argentines, Butterhead variety (Rotation 2) PBI 77 days	head	46 49	124 138	<0.01 <0.01	<0.02 <0.02	<0.01 <0.01	0.04 0.04
10-2503 (10-2503-01) 10-2503-01-T-3B Germany GLP: yes	Lettuce, Aleppo, Loose leaf variety (Rotation 3) PBI 320 days	head	46 49	354 368	<0.01 <0.01	<0.02 <0.02	<0.01 <0.01	<0.04 <0.04
10-2503 (10-2503-02) 10-2503-02-T-1B Netherlands GLP: yes	Lettuce, Lucan, Butterhead variety (Rotation 1) PBI 25 days	head	41 49	46 60	0.03 <0.01	<0.02 <0.02	<0.01 <0.01	0.06 <0.04
10-2503 (10-2503-02) 10-2503-02-T-2B Netherlands GLP: yes	Lettuce, Lucan, Butterhead variety (Rotation 2) PBI 64 days	head	46 49	95 109	<0.01 <0.01	<0.02 <0.02	<0.01 <0.01	<0.04 <0.04
10-2503 (10-2503-02) 10-2503-02-T-3B Netherlands GLP: yes	Lettuce, Lucan, Butterhead variety (Rotation 3) PBI 329 days	head	45 49	358 372	<0.01 <0.01	<0.02 <0.02	<0.01 <0.01	<0.04 <0.04

DALT = days after last treatment

GS = growth stage (BBCH-code) at sampling

PBI = plant-back interval

Continued on next page...



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.6.3-3 (cont'd): Results of field rotational crop trials following spray treatment with BYI 02960 SL 200 to soil or a target crop and then planting back and sampling of various rotational crops
Here: leafy crops

Study No. (Trial No.) Plot No Country GLP	Rotational Crop, Variety (rotation information)	Portion analysed	GS	DALT (days)	Residues (mg/kg) expressed as BYI 02960			total residue of BYI 02960 calc.
					BYI 02960	difluoro- acetic acid	BYI 02960- difluoro- ethylamino- furanone	
10-2503 (10-2503-03) 10-2503-03-T-1B France GLP: yes	Lettuce, Pitice, Loose leaf (Rotation 1) PBI 28 days	head	41 49	55 69	0.08 0.03	0.12 0.11	<0.01 <0.01	0.21 0.16
10-2503 (10-2503-03) 10-2503-03-T-2B France GLP: yes	Lettuce, Pitice, Loose leaf (Rotation 2) PBI 70 days	head	46 49	107 121	0.03 0.01	0.08 0.09	<0.01 <0.01	0.12 0.11
10-2503 (10-2503-03) 10-2503-03-T-3B France GLP: yes	Lettuce, Pitice, Loose leaf (Rotation 3) PBI 314 days	head	46 49	357 371	0.02 <0.01	0.08 0.05	<0.01 <0.01	0.10 0.07
10-2503 (10-2503-04) 10-2503-04-T-1B Spain GLP: yes	Lettuce, Murai, (leafy variety) (Rotation 1) PBI 30 days	head	47 49	72 85	0.03 0.01	0.03 0.03	<0.01 <0.01	0.07 0.06
10-2503 (10-2503-04) 10-2503-04-T-2B Spain GLP: yes	Lettuce, Pelican, Butterhead (Rotation 2) PBI 145 days	head	48 49	291 305	<0.01 <0.01	<0.02 <0.02	<0.01 <0.01	<0.04 <0.04
10-2503 (10-2503-04) 10-2503-04-T-3B Spain GLP: yes	Lettuce, Murai (leafy variety) (Rotation 3) PBI 279 days	head	47 49	321 334	<0.01 <0.01	<0.02 <0.02	<0.01 <0.01	<0.04 <0.04

DALT = days after last treatment

GS = growth stage (BBCH-code) at sampling

PBI = plant-back interval

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.6.3-4: Results of field rotational crop trials following spray treatment with BYI 02960 SL 200 to soil or a target crop and then planting back and sampling of various rotational crops
Here: cereal crops

Study No. (Trial No.) Plot No Country GLP	Rotational Crop, Variety (rotation information)	Portion analysed	DALT (days)	Residues (mg/kg) expressed as BYI 02960			
				BYI 02960	difluoroacetic acid	BYI 02960- difluoro- ethylamino- furanone	total residue of BYI 02960 calc.
10-2503 (10-2503-01) 10-2503-01-T-1C Germany GLP: yes	Barley, Simba (Rotation 1) PBI 25 days	green material	56	0.01	0.11	<0.01	0.14
		grain	116	<0.01	0.33	<0.01	0.35
		straw	116	0.02	0.13	<0.01	0.15
10-2503 (10-2503-01) 10-2503-01-T-2C Germany GLP: yes	Barley, Leibnitz (Rotation 2) PBI 116 days	green material	305	<0.01	0.03	<0.01	0.05
		grain	406	<0.01	0.10	<0.01	0.12
		straw	406	<0.01	<0.05	<0.01	<0.07
10-2503 (10-2503-01) 10-2503-01-T-3C Germany GLP: yes	Barley, Simba (Rotation 3) PBI 284 days	green material	333	<0.01	<0.02	<0.01	<0.04
		grain	420	<0.01	0.10	<0.01	0.12
		straw	420	<0.01	<0.05	<0.01	<0.07
10-2503 (10-2503-02*) 10-2503-02-T-1C Netherlands GLP: yes	Barley, Cervoise (Rotation 1) PBI 33 days	green material	226	<0.01	0.04	<0.01	0.06
		straw	329	<0.01	<0.05	<0.01	<0.07
10-2503 (10-2503-02) 10-2503-02-T-2C Netherlands GLP: yes	Barley, Cervoise (Rotation 2) PBI 137 days	green material	330	<0.01	0.03	<0.01	0.05
		grain	446	<0.01	0.04	<0.01	0.06
		straw	446	<0.01	<0.05	<0.01	<0.07
10-2503 (10-2503-02) 10-2503-02-T-3C Netherlands GLP: yes	Barley, Tripple (Rotation 3) PBI 295 days	green material	350	<0.01	0.02	<0.01	0.04
		grain	459	<0.01	0.06	<0.01	0.08
		straw	459	<0.01	<0.05	<0.01	<0.07

DALT = days after last treatment

PBI = plant-back interval

* grain could not be taken in the 1st rotation of this trial due to geese feeding on the field

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Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.6.3-4 (cont'd): Results of field rotational crop trials following spray treatment with BYI 02960 SL 200 to soil or a target crop and then planting back and sampling of various rotational crops
 Here: cereal crops

Study No. (Trial No.) Plot No Country GLP	Rotational Crop, Variety (rotation information)	Portion analysed	DALT (days)	Residues (mg/kg) expressed as BYI 02960			
				BYI 02960	difluoroacetic acid	BYI 02960- difluoro- ethylamino- furanone	total residue of BYI 02960 calc.
10-2503 (10-2503-03) 10-2503-03-T-1C France GLP: yes	Barley, Attrction (Rotation 1) PBI 27 days	green material	126	0.02	0.37	<0.01	0.41
		grain	218	<0.01	0.63	<0.01	0.65
		straw	218	0.04	0.34	<0.01	0.39
10-2503 (10-2503-03) 10-2503-03-T-2C France GLP: yes	Barley, Attrction (Rotation 2) PBI 195 days	green material	294	0.01	0.10	<0.01	0.13
		grain	386	<0.01	0.26	<0.01	0.28
		straw	386	0.02	0.10	<0.01	0.12
10-2503 (10-2503-03) 10-2503-03-T-3C France GLP: yes	Barley, Attrction (Rotation 3) PBI 290 days	green material	324	0.03	0.15	<0.01	0.19
		grain	419	<0.01	0.37	<0.01	0.39
		straw	419	<0.01	0.17	<0.01	0.19
10-2503 (10-2503-04) 10-2503-04-T-1C Spain GLP: yes	Barley, Graphic (Rotation 1) PBI 27 days	green material	133	<0.01	0.03	<0.01	0.05
		grain	233	<0.01	0.09	<0.01	0.11
		straw	233	0.01	0.05	<0.01	0.08
10-2503 (10-2503-04) 10-2503-04-T-2C Spain GLP: yes	Barley, Graphic (Rotation 2) PBI 143 days	green material	249	<0.01	0.03	<0.01	0.05
		grain	349	<0.01	0.09	<0.01	0.11
		straw	349	<0.01	<0.05	<0.01	<0.07
10-2503 (10-2503-04) 10-2503-04-T-3C Spain GLP: yes	Barley, Graphic (Rotation 3) PBI 266 days	green material	306	<0.01	0.02	<0.01	0.04
		grain	363	<0.01	0.09	<0.01	0.11
		straw	363	<0.01	<0.05	<0.01	<0.07

DALT = days after last treatment

PBI = plant-back interval

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.6.3-5: Recovery data for BYI 02960 in rotational crop matrices (root, leafy, and cereal crops)

Study No. Trial No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
10-2503 (10-2503-01), (10-2503-02) and (10-2503-04) Plots T-1A to T-3A GLP: yes 2010	carrot	root	BYI 02960	6	0.01	90;99;110;111; 120;120	90	120	108	11.0
				1	0.10	87	87	87	87	
				2	0.50	99;107	99	107	103	
				1	1.0	80	80	80	80	
				10	overall	80	120	102	102	13.4
			difluoroacetic acid	6	0.02	91;99;109;114; 114;114	91	114	107	9.1
				1	0.20	86	86	86	86	
				2	0.50	97;97	97	97	97	
				1	1.0	84	84	84	84	
				10	overall	84	114	101	101	11.6
			BYI 02960- difluoroethyl- aminofuranone	6	0.01	90;92;106;113; 118;119	90	119	106	12.0
				1	0.10	95	95	95	95	
				2	0.50	101;105	101	105	103	
				1	1.0	91	91	91	91	
				10	overall	90	119	103	103	10.7
10-2503 (10-2503-03) Plots T-1A to T-3A GLP: yes 2010	turnip, edible	body	BYI 02960	3	0.01	77;95;100	77	100	91	13.3
				1	0.10	74	74	74	74	
				1	0.50	92	92	92	92	
				5	overall	74	100	88	88	13.1
			difluoroacetic acid	3	0.02	83;112;115	83	115	103	17.1
				1	0.20	85	85	85	85	
				1	0.50	86	86	86	86	
				5	overall	83	115	96	96	16.5
			BYI 02960- difluoroethyl- aminofuranone	3	0.01	84;105;116	84	116	102	16.0
				1	0.10	97	97	97	97	
				1	0.50	99	99	99	99	
				5	overall	84	116	100	100	11.7
				leaf	BYI 02960	94;104;108	94	108	102	7.1
			difluoroacetic acid	3	0.01	81	81	81	81	
				1	0.10	103	103	103	103	
				1	0.50	81	108	98	98	11.0
				5	overall	87	115	101	101	13.2

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Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.6.3-5 (cont'd): Recovery data for BYI 02960 in rotational crop matrices (root, leafy, and cereal crops)

Study No. Trial No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Individual recoveries	Recovery (%)			
							Min	Max	Mean	RSD
10-2503 (10-2503-03) Plots T-3A GLP: yes 2010 (cont.)	turnip, edible	leaf	BYI 02960-difluoroethylaminofuranone	3 1 1 5	0.01 0.10 0.50 overall	90;99;108 92 99 90	90 92 99 108	108 92 99 98	99 92 99 98	9.1 7.3
10-2503 (10-2503-01) to (10-2503-04) Plots T-1B to T-3B GLP: yes 2010	lettuce	head	BYI 02960 difluoroacetic acid BYI 02960-difluoroethylaminofuranone	15 5 2 2 26 12 3 2 5 2 2 26 15 5 2 2 26	0.01 0.10 0.50 1.0 5.0 overall 0.02 0.05 0.20 0.50 1.0 5.0 overall 0.01 0.10 0.50 1.0 5.0 overall	79;87;102;106;107 109;110;116;92; 97;107;108;114; 116;117 88;90;90;92;93 103;106 92;94 90;98 79 90;93;94;95;97; 112;112;116;86; 89;93;95 90;98 92;94 90;101 90;92 89;90 86 87;93;95;100;100; 104;105;107;83;83 86;88;90;92;96 85;98;97;97;99 97;109 86;101 97;96 83 87;93;95;100;100; 104;105;107;83;83 86;88;90;92;96 85;98;97;97;99 97;109 101;103 94 97 95 83	79 88 103 92 79 86 90 92 94 90 89 86 83	117 93 106 94 117 116 98 94 93 90 92 90 116 107 99 109 101 97 109 94 97 95	104 91 105 93 100 98 93 91 90 92 90 95 8.4 6.1	10.7 2.2 10.5 10.2 4.3 4.7 7.9 8.4 6.1

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Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.6.3-5 (cont'd): Recovery data for BYI 02960 in rotational crop matrices (root, leafy, and cereal crops)

Study No. Trial No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Individual recoveries	Recovery (%)			
							Min	Max	Mean	RSD
10-2503 (10-2503-01) to (10-2503-04) Plots T-1C to T-3C GLP: yes 2010	barley	green material	BYI 02960	2	0.01	92;104	92	104	98	
				1	0.10	88	88	88	88	
				1	1.0	85	85	85	85	
				4	overall		85	104	92	9.0
			difluoroacetic acid	2	0.02	88;97	88	97	93	
				1	0.20	87	87	87	87	
				1	1.0	82	82	82	82	
				4	overall		82	97	89	7.1
			BYI 02960- difluoroethyl- aminofuranone	2	0.01	91;93	91	93	92	
				1	0.10	99	99	99	99	
				1	1.0	95	95	95	95	
				4	overall		91	99	95	3.6
		grain	BYI 02960	4	0.01	86;91;98;89	86	98	91	5.6
				2	0.10	90;88	88	90	89	
				1	1.0	105	105	105	105	
				7	overall		86	105	92	7.3
			difluoroacetic acid	3	0.02	94;99;99	94	99	97	3.0
				1	0.05	82	82	82	82	
				1	0.20	72	72	72	72	
				1	0.50	74	74	74	74	
				1	1.0	71	71	71	71	
				7	overall		71	99	84	15.0
			BYI 02960- difluoroethyl- aminofuranone	4	0.01	92;95;95;82	82	95	91	6.8
				2	0.10	92;90	90	92	91	
				1	1.0	110	110	110	110	
				7	overall		82	110	94	9.0
		straw	BYI 02960	4	0.01	95;100;116;98	95	116	102	9.2
				2	0.10	65;92	65	92	79	
				1	1.0	76	76	76	76	
				7	overall		65	116	92	18.2
			difluoroacetic acid	3	0.02	101;106;116	101	116	108	7.1
				1	0.05	95	95	95	95	
				1	0.20	66	66	66	66	
				1	0.50	95	95	95	95	
				1	1.0	71	71	71	71	
				7	overall		66	116	93	19.6

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Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.6.3-5 (cont'd): Recovery data for BYI 02960 in rotational crop matrices (root, leafy, and cereal crops)

Study No. Trial No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
10-2503 (10-2503-01) to (10-2503-04) Plots T-1C to T-3C GLP: yes 2010 contd.			BYI 02960-difluoroethyl-aminofuranone	4 2 1 7	0.01 0.10 1.0 overall	81;83;96;80 71;95 82	80 71 82 71	96 95 82 96	85 83 82 84	8.8 10.5



IIA 6.7 Proposed residue definition and maximum residue levels

IIA 6.7.1 Proposed residue definition

► PLANT MATRICES

– Data collection, risk assessment

For BYI 02960 residues in *target plants* (primary crops) and *rotational crops* (succeeding crops), the proposed residue definition for **data collection** and **risk assessment** is the sum of parent compound BYI 02960 and its metabolites DFA and BYI 02960-difluoroethyl-amino-furanone (DFEAF), expressed in BYI 02960 equivalents.

Metabolism studies have been conducted in five diverse crops (tomato, apple, potato, cotton, and rice) for foliar and soil applications and in confined rotational crops (Swiss chard, turnip, and wheat) after application of BYI 02960 onto bare soil and cultivating of succeeding crops at three plant-back intervals. The plant metabolism studies have shown a reasonably consistent metabolic profile across both foliar and soil application.

The only residues of BYI 02960 that were consistently observed at significant levels across all primary and succeeding crops were the parent compound BYI 02960 and DFA, both of which are specific to BYI 02960 use. In primary crops, all other major metabolites were detected in individual RACs only, and generally at low concentrations. Only the natural class of compounds glucose/carbohydrates, which was formed from the degradation of the furanone moiety and incorporation of ¹⁴C into natural plant constituents, was detected in relatively high concentrations.

In confined rotational crops, nine major metabolites were detected besides the parent compound and DFA. However, all of them were detected in individual RACs only, mainly in feed commodities. The major metabolites detected in food commodities were generally at low concentrations, except for glucose/carbohydrates, which are natural compounds, and the metabolites BYI 02960-OH and BYI 02960-OH-glyc, which were also observed in the rat. The metabolite BYI 02960-difluoroethyl-amino-furanone (=DFEAF) was also a major metabolite found in Swiss chard RACs in all rotations. Since it was not observed in the rat and the metabolism studies indicated that no other appropriate marker compound was present, BCS included this metabolite in the residue definition for the data collection method.

BYI 02960-difluoroethyl-amino-furanone appeared to be a suitable marker compound for estimating residue levels of other metabolites, if deemed necessary. For the sake of consistency, BCS analysed the samples of all supervised residue trials on target plants and rotational crops for these three compounds. Thus the residue definition for risk assessment for target plants and rotational crops has three constituents: BYI 02960 (parent compound), DFA, and BYI 02960-difluoroethyl-amino-furanone (DFEAF).

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No other metabolite need be included in the residue definition for risk assessment, since it has been shown that none of the metabolites present a risk for the consumer (cf KIIA 5.10/03). All metabolites identified in the metabolism studies show either no consumer exposure under realistic conditions or a consumer exposure below the agreed Threshold level of Toxicological Concern (TTC; <1.5 µg/kg bw), or it has been shown that the metabolites are covered by the endpoints derived for the parent compound BYI 02960 (either via additional toxicity testing or their presence in the rat ADME studies).

– Enforcement

Results from supervised residue trials show that BYI 02960 and DFA are, by far, the most prominent components of the residues in target plants and rotational crops. The residues of metabolite BYI 02960-difluoroethyl-amino-furanone (DFEAF) were below the LOQ of 0.01 mg/kg in virtually all samples.

Considering these results as well as those from the metabolism studies, parent compound BYI 02960 and DFA are the proposed constituents (expressed as their sum, in BYI 02960 equivalents) for the residue definition for **enforcement** for *target plants and rotational crops*.

► ANIMAL MATRICES

– Data collection

For *animal matrices* the sum of BYI 02960, DFA, BYI 02960-OH, and BYI 02960-acetyl-AMCP, expressed as BYI 02960 equivalents, is proposed as residue definition for **data collection**.

The proposal is based on the results of the livestock metabolism studies (cf KIIA 6.2). In these studies, parent compound BYI 02960 is a significant, if not the dominant constituent of the residue in milk, eggs, and edible tissues of both tested species. Other metabolites determined in comparable concentrations are the natural compound lactose in goat's milk after administration of [furanone-4-¹⁴C]BYI 02960, BYI 02960-acetyl-AMCP in eggs and tissues of laying hens after administration of [pyridinylmethyl-¹⁴C]BYI 02960, and BYI 02960-OH in eggs.

DFA was determined in selected livestock samples by high-resolution LC-MS subsequent to the metabolism studies, since rat studies conducted with [ethyl-1-¹⁴C]BYI 02960 showed major amounts of this metabolite in organs and tissues. Extrapolation of rat data suggested high DFA levels in livestock tissues as well, which was confirmed by the non-radioactive LC-MS analyses. Based on these findings, it was concluded that difluoroacetic acid is a major livestock metabolite and should be a constituent of the residue definition for data collection.

– Risk assessment, enforcement

On the basis of the results of the feeding studies conducted in poultry and cattle (cf KIIA 6.4.1 and KIIA 6.4.2), it has been shown that parent BYI 02960 and metabolite DFA were by far the predominant compounds detected. The residues of the metabolite BYI 02960-acetyl-AMCP were well

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below the LOQ of 0.01 mg/kg in all animal matrices at the corresponding 1X dose levels; the metabolite BYI 02960-OH was detected in cattle liver and kidney, only (0.011-0.019 mg/kg). However, this metabolite is also a major rat metabolite and therefore its toxicity is covered by the endpoints derived for the parent compound.

Considering these results in combination with those from livestock metabolism, BYI 02960 parent compound and its metabolite DFA (expressed as their sum, in BYI 02960 equivalents) are the proposed constituents for the residue definitions for **risk assessment** and **enforcement** in *animal matrices*.

No other metabolite need be included in the residue definition for risk assessment, since it has been shown that none of the metabolites pose a risk for the consumer (cf KIIA 5.10/03). All metabolites identified in the metabolism studies show either no consumer exposure under realistic conditions or a consumer exposure below the agreed Threshold level of Toxicological Concern (TTC; <1.5 µg/kg bw), or it has been shown that the metabolites are covered by the endpoints derived for the parent compound BYI 02960 (either via additional toxicity testing or their presence in the rat ADME studies).



IIA 6.7.2 Proposed maximum residue levels (MRLs) and justification

General remark:

In this summary section (KIIA 6.7.2), the name DFEAF will be used for the metabolite BYI 02960-difluoroethyl-amino-furanone, which is relevant to the tested residue definition:

Name	Metab. No.	Standard "dossier name"
DFEAF	M34	BYI 02960-difluoroethyl-amino-furanone

► PLANT MATRICES

Based on the proposed residue definition for risk assessment in plant materials — the calculated total residues of BYI 02960, consisting of the sum of the residues of the parent compound and its metabolites DFA and DFEAF — and on the studies presented in this dossier, MRL proposals are presented below for the use of BYI 02960 in lettuce and hops.

The proposed residue definition for enforcement is slightly different than the one for risk assessment, as it does not include DFEAF. However, DFEAF 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 are valid for MRL calculations.

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.

– MRLs resulting from primary uses

The studies described in this dossier reflect only the "safe uses", i.e. lettuce and hops. Further uses will be submitted separately to cover numerous other crops.

• Lettuce

Complete sets of field residue trials were conducted in lettuce in both the northern and southern European field residue regions, as well as in greenhouses. When comparing the results, it is evident that final residue levels are generally highest in the greenhouse, with the highest HR value of any trial set and considerably higher median residues. Total residue levels of BYI 02960 in all lettuce head samples taken at 3 days after the final application (3 days represents the envisaged PHI for this crop, in the field "home & garden use" as well as in the "agricultural use" in greenhouses) were 0.14-3.0 mg/kg in the north (median 0.71 mg/kg), 0.39-3.2 mg/kg in the south (median 1.2 mg/kg), and 0.80-6.0 mg/kg in the greenhouse (median 2.2 mg/kg). Residues for the outdoor "agricultural use" (1 application, 10-day PHI) were markedly lower.

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(The total residue values include the metabolite DFEAF, which is not proposed to be a constituent of the enforcement residue definition. However, residues of DFEAF did not play a major role in any of the trials, having been determined at levels of 0.01-0.09 mg/kg in each residue result reported [0.01 mg/kg is the LOQ for the analyte, expressed in parent compound equivalents]. As such, it is a very minor contributor to the total residue levels determined in these trials; its effect on the MRL calculation is negligible.)

Thus, the greenhouse use can be defined as critical "region" for the calculation of the MRL. A summary of the calculation is shown below in table 6.7.2-1. (More details of the calculations are shown in table 6.7.2-6, later in this chapter.)

Table 6.7.2-1: Total residues of BYI 02960/lettuce — maximum residue values for a pre-harvest interval of 3 days (from greenhouse trials)

EU Method I (all values)	7.12 mg/kg
EU Method II (75% quantile)	6.20 mg/kg
OECD Unrounded MRL Estimate	8.58 mg/kg

0.80; 1.4; 1.8; 2.0; 2.2; 2.5; 2.7; 3.5; 6.0 mg/kg
STMR: 2.2 mg/kg
HR: 6.0 mg/kg

Based on the actual trial results, in which the highest residue levels were 6.0 mg/kg, combined with the calculated results using the EU system, the proposed EU MRL for BYI 02960 in lettuce is 7.0 mg/kg.

Remark 1:

If based on the OECD calculator, the proposal would be 9.0 mg/kg.

Remark 2:

The residue behaviour was similar in both open and closed-headed varieties of lettuce. In the greenhouse trials with closed-head varieties, total residues on day 3 ranged from 1.8-3.5 mg/kg (n=4). In trials with open-headed (leafy) varieties, the range was 0.80-6.0 mg/kg (n=5). In the field trials in section 6.3.1, residues in closed-head varieties ranged from 0.40-2.7 mg/kg (n=6) while those in open-head varieties ranged from 0.14-3.2 mg/kg (n=12).

Thus, these trials also provide valid data for the establishment of MRLs, despite the stipulation in the new guidance, soon to be effective (2013/14), that only open-headed varieties should be used.)

• Hops

Eight field residue trials were conducted in hops in the northern European field residue region. Total residue levels of BYI 02960 in all green cone samples taken at 21 days after the final application (representing the envisaged PHI for this crop) were <0.40-0.87 mg/kg, with a median value of 0.47 mg/kg.

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In general, MRLs in Europe are set on the dried hop cones. In the kiln-dried commodity, total residue levels of BYI 02960 21 days after the final application were 0.56-2.4 mg/kg, with a median value of 1.1 mg/kg. However, in three trials, residues were higher on day 28 than on day 21; when taking these higher values into consideration, residues ranged from 0.61-2.4 mg/kg, with a median value of 1.2 mg/kg.

(The total residue values include the metabolite DFEAF, which is not proposed to be a constituent of the enforcement residue definition. However, residues of DFEAF were below its LOQ [0.1 mg/kg in BYI 02960 equivalents] in each residue result reported, and, as such, it is a very minor contributor to the total residue levels calculated and reported in these trials. Its effect on the MRL calculation is negligible.)

A summary of the calculation is shown below in table 6.7.2-2. (More details of the calculations are shown in table 6.7.2-7, later in this chapter.)

Table 6.7.2-2: Total residues of BYI 02960/dried hop cones — maximum residue values for a pre-harvest interval of 21 days

EU Method I (all values)	3.56 mg/kg
EU Method II (75% quantile)	4.25 mg/kg
OECD Unrounded MRL Estimate	4.13 mg/kg

0.61; 0.73; 0.78; 1.1; 1.2; 1.6; 2.3; 2.4

STMR: 1.2 mg/kg

HR: 2.4 mg/kg

Based on the actual trial results and using both the EU and the OECD calculation systems, the proposed EU MRL for BYI 02960 in hop (dried cone) is 4.0 mg/kg.

– MRLs in rotational crops resulting from earlier uses on a plot

The field rotational crop data described in this 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 separately.

• Leafy vegetables (based on crop rotation)

Although primary residue trials were conducted on lettuce, residues in other botanically related leafy vegetable plants can arise by re-use of a particular field following a previous application of BYI 02960. In rotational crop trials, levels of the total residues of BYI 02960 in lettuce were highest in the first rotation, i.e. after the shortest plant-back interval of 25-30 days, representing field re-use after crop failure. Total residue levels in "marketable" lettuce heads ranged from <0.04-0.16 mg/kg, with a median value of 0.08 mg/kg.

(The total residue values include the metabolite DFEAF, which is not proposed to be a constituent of the enforcement residue definition. However, residues of DFEAF did not play a major role in any of

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the trials, having been determined at levels of <0.01 mg/kg in each residue result reported [0.01 mg/kg is the LOQ for the analyte, expressed in parent compound equivalents]. As such, it is a very minor contributor to the total residue levels determined in these trials; its effect on the MRL calculation is negligible.)

A summary of the calculation is shown below in table 6.7.2-3. (More details of the calculations are shown in table 6.7.2-8, later in this chapter.)

Table 6.7.2-3: Total residues of BYI 02960/rotational leafy crops — maximum residue values for a plant-back interval of 25-30 days

EU Method I (all values)	0.35 mg/kg
EU Method II (75% quantile)	0.29 mg/kg
OECD Unrounded MRL Estimate	0.29 mg/kg

<0.04; 0.07; 0.09; 0.16 mg/kg

STMR: 0.08 mg/kg

HR: 0.16 mg/kg

Based on the these trial results and using the EU and OECD calculation systems, the proposed EU MRL for BYI 02960 in leafy vegetable crops as following crops (i.e. as a result of field re-use) is 0.3 mg/kg.

• Root vegetables (based on crop rotation)

No primary residue trials were conducted on root crops, but residues in these plants can arise by re-use of a particular field following a previous application of BYI 02960. In rotational crop trials, levels of the total residues of BYI 02960 in roots (carrot or turnip) were generally highest in the first rotation, i.e. after the shortest plant-back interval of 25-30 days, representing field re-use after crop failure. In one case, residues were higher in the third rotation (PBI 284 days). Highest total residue levels in "marketable" root vegetables ranged from 0.06-0.14 mg/kg, with a median value of 0.08 mg/kg.

(The total residue values include the metabolite DFEAF, which is not proposed to be a constituent of the enforcement residue definition. However, residues of DFEAF did not play a major role in any of the trials, having been determined at levels of <0.01 mg/kg in each residue result reported [0.01 mg/kg is the LOQ for the analyte, expressed in parent compound equivalents]. As such, it is a very minor contributor to the total residue levels determined in these trials; its effect on the MRL calculation is negligible.)

A summary of the calculation is shown below in table 6.7.2-4. (More details of the calculations are shown in table 6.7.2-9, later in this chapter.)

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Table 6.7.2-4: Total residues of BYI 02960/rotational root crops — maximum residue values for a plant-back interval of 25-30 days

EU Method I (all values)	0.27 mg/kg
EU Method II (75% quantile)	0.25 mg/kg
OECD Unrounded MRL Estimate	0.26 mg/kg

0.06; 0.07; 0.08; 0.14 mg/kg

STMR: 0.08 mg/kg

HR: 0.14 mg/kg

Based on the these trial results and using both the EU and OECD MRL calculations, the proposed EU MRL for BYI 02960 in root vegetable crops as following crops (i.e. as a result of field re-use) is 0.3 mg/kg.

• **Cereals (based on crop rotation)**

In rotational crop trials, levels of the total residues of BYI 02960 in grain were highest in the first rotation, i.e. after the shortest plant-back interval of 25-33 days, representing field re-use after crop failure. Residue levels were 0.11-0.65 mg/kg, with a median value of 0.35 mg/kg.

(The total residue values include the metabolite DFEAF, which is not proposed to be a constituent of the enforcement residue definition. However, residues of DFEAF did not play a major role in any of the trials, having been determined at levels of <0.01 mg/kg in each residue result reported [0.01 mg/kg is the LOQ for the analyte, expressed in parent compound equivalents]. As such, it is a very minor contributor to the total residue levels determined in these trials; its effect on the MRL calculation is negligible.)

A summary of the calculation is shown below in table 6.7.2-5. (More details of the calculations are shown in table 6.7.2-10, later in this chapter.)

Table 6.7.2-5: Total residues of BYI 02960/rotational cereal crops — maximum residue values for a plant-back interval of 25-33 days

EU Method I (all values)	2.44 mg/kg
EU Method II (75% quantile)	1.30 mg/kg
OECD Unrounded MRL Estimate	1.45 mg/kg

0.11; 0.35; 0.65 mg/kg

STMR: 0.23 mg/kg

HR: 0.65 mg/kg

Based on the actual trial results, in which the highest residue levels were 0.65 mg/kg, combined with the calculated results using both the EU and OECD calculation systems, the proposed EU MRL for BYI 02960 in cereal grain as following crops (i.e. as a result of field re-use) is 1.5 mg/kg.

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Table 6.7.2-6: Calculation of MRL proposals for lettuce according to EU and OECD guidelines

BASIC DATA

Residue :	total residue of BYI 02960 calc.	Crop group :	Leaf and stem vegetables
Portion analysed :	head	Commodity :	Lettuce (greenhouse)
Target value :	MRL	PHI :	3 d

No .	Crop	Days after application	Residue value (mg/kg)	Trial No./ Study No.	No. of appl.	FL-Type	Product	Country	Area of appl .
1	Lettuce	7 ²	1.4	10-2212-01/10-2212	2	SL 200	BYI 02960 SL 200	France	G
2	Lettuce	3	2.0	10-2212-02/10-2212	2	SL 200	BYI 02960 SL 200	Germany	G
3	Lettuce, head	3	3.5	10-2212-03/10-2212	2	SL 200	BYI 02960 SL 200	Germany	G
4	Lettuce, head	3	2.5	10-2212-04/10-2212	2	SL 200	BYI 02960 SL 200	Netherlands	G
5	Lettuce, head	3	1.8	10-2212-05/10-2212	2	SL 200	BYI 02960 SL 200	Italy	G
6	Lettuce	3	2.2	11-2070-01/11-2070	2	SL 200	BYI 02960 SL 200	France	G
7	Lettuce	3	0.80	11-2070-02/11-2070	2	SL 200	BYI 02960 SL 200	Italy	G
8	Lettuce	3	6.0	11-2070-03/11-2070	2	SL 200	BYI 02960 SL 200	Spain	G
9	Lettuce, head	3	2.7	11-2070-04/11-2070	2	SL 200	BYI 02960 SL 200	Germany	G

* - this value was used as it was higher than the value at the PHI (3 days)

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Table 6.7.2-6 (cont'd): Calculation of MRL proposals for lettuce according to EU and OECD guidelines

EVALUATION SUMMARIES

- EU guideline 7039/VI/95 of 22 July 1997

Results (Lettuce)

Method I (Weinmann/Nolting) (all values)	R	2.544
	s	1.508
	k	3.032
	Rmax=R+k*s	7.118
Method II (Wilkening) (75 % quantile)	R (0.75)	3.100
	Rber=2*R(0.75)	6.200

Summary of results:

Maximum residue values for a pre-harvest interval of: 3 days

Method I (all values)	7.12 mg/kg
Method II (75% quantile)	6.20 mg/kg

- OECD Calculator

Results (Lettuce)

Total number of data (n)	9	Standard deviation (SD)	1.508
Lowest residue	0.8	Percentage of censored data	0
Highest residue	6	Number of non-censored data	9
Median residue	2.200	Correction factor for censoring (CF)	1.000
Mean	2.544		

Proposed MRL estimate

Highest residue	6
Mean + 4 SD	8.578
CF x 3 mean	7.633
Unrounded MRL	8.578
Rounded MRL	9

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.7.2-7: Calculation of MRL proposals for hops according to EU and OECD guidelines

BASIC DATA

Residue :	total residue of BYI 02960 calc.	Crop group :	Stimulant plants
Portion analysed :	cone, kiln-dried	Commodity :	Hop
Target value :	MRL	PHI :	21 d

No.	Crop	Days after application	Residue value (mg/kg)	Trial No./ Study No.	No. of applic.	FL-Type	Product	Country	Area of applic.
1	Hop	28*	1.6	10-2225-01 / 10-2225	1	SL 200	BYI 02960 SL 200	Germany	F
2	Hop	20	0.78	10-2225-02 / 10-2225	1	SL 200	BYI 02960 SL 200	Germany	F
3	Hop	21	1.1	10-2225-03 / 10-2225	1	SL 200	BYI 02960 SL 200	Germany	F
4	Hop	21	1.2	10-2225-04 / 10-2225	1	SL 200	BYI 02960 SL 200	Germany	F
5	Hop	28*	2.3	11-2076-01 / 11-2076	1	SL 200	BYI 02960 SL 200	Germany	F
6	Hop	28*	0.61	11-2076-02 / 11-2076	1	SL 200	BYI 02960 SL 200	Germany	F
7	Hop	20	2.4	11-2076-03 / 11-2076	1	SL 200	BYI 02960 SL 200	Germany	F
8	Hop	22	0.73	11-2076-04 / 11-2076	1	SL 200	BYI 02960 SL 200	Germany	F

* - this value was used as it was higher than the value at the PHI (21 days)

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Table 6.7.2-7 (cont'd): Calculation of MRL proposals for hops according to EU and OECD guidelines

EVALUATION SUMMARIES

- EU guideline 7039/VI/95 of 22 July 1997

Results (Hop)

Method I (Weinmann/Nolting) (all values)	R	1.340
	s	0.698
	k	3.188
	Rmax=R+k*s	3.565
Method II (Wilkening) (75 % quantile)	R (0.75)	2.125
	Rber=2*R(0.75)	4.250

Summary of results:

Maximum residue values for a pre-harvest interval of: 21 days

Method I (all values)	3.56 mg/kg
Method II (75% quantile)	4.25 mg/kg

- OECD Calculator

Results (Hop)

Total number of data (n)	8	Standard deviation (SD)	0.698
Lowest residue	0.61	Percentage of censored data	0
Highest residue	2.4	Number of non-censored data	8
Median residue	1.150	Correction factor for censoring (CF)	1.000
Mean	1.340		

Proposed MRL estimate

Highest residue	2.4
Mean + 4 SD	4.131
CF x 3 mean	4.020
Unrounded MRL	4.131
Rounded MRL	5

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.7.2-8: Calculation of MRL proposals for rotational root crops according to EU and OECD guidelines

BASIC DATA

Residue:	total residue of BYI 02960 calc.	Crop group :	rotational root vegetables
Portion analysed :	root (=body)	Commodity :	carrot, turnip
Target value :	MRL	PBI :	25-30 days

No.	Crop	Days after application *	Residue value (mg/kg)	Plot No./ Study No.	No. of applic.	FL-Type	Product	Country	Area of applic.
1	Carrot	410	0.06	10-2503-01-T-3A / 10-2503	1	SL 200	BYI 02960 SL 200	Germany	F
2	Carrot	106	0.07	10-2503-02-T-1A / 10-2503	1	SL 200	BYI 02960 SL 200	Netherlands	F
3	Turnip, edible	82	0.14	10-2503-03-T-1A / 10-2503	1	SL 200	BYI 02960 SL 200	France	F
4	Carrot	114	0.08	10-2503-04-T-1A / 10-2503	1	SL 200	BYI 02960 SL 200	Spain	F

* - PBI (plant-back interval) for these, relevant trials: 25-30 days

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Table 6.7.2-8 (cont'd): Calculation of MRL proposals for rotational root crops according to EU and OECD guidelines

EVALUATION SUMMARIES

- EU guideline 7039/VI/95 of 22 July 1997

Results (rotational root crops, represented by carrot/turnip)

Method I (Weinmann/Nolting) (all values)	R	0.088
	s	0.036
	k	5.144
	Rmax=R+k*s	0.272
Method II (Wilkening) (75 % quantile)	R (0.75)	0.125
	Rber=2*R(0.75)	0.250

Summary of results:

Maximum residue values for a plant-back interval of: 25-30 days

Method I (all values)	0.27 mg/kg
Method II (75% quantile)	0.25 mg/kg

- OECD Calculator

Results (rotational root crops, represented by carrot/turnip)

Total number of data (n)	4	Standard deviation (SD)	0.036
Lowest residue	0.06	Percentage of censored data	0
Highest residue	0.14	Number of non-censored data	4
Median residue	0.075	Correction factor for censoring (CF)	1.000
Mean	0.088		

Proposed MRL estimate

Highest residue	0.14
Mean + 4 SD	0.231
CF x 3 mean	0.263
Unrounded MRL	0.263
Rounded MRL	0.3

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.7.2-9: Calculation of MRL proposals for rotational leafy vegetable crops according to EU and OECD guidelines

BASIC DATA

Residue :	total residue of BYI 02960 calc.	Crop group :	rotational leafy vegetables
Portion analysed :	head	Commodity :	lettuce
Target value :	MRL	PBI :	25-30 days

No.	Crop	Days after application *	Residue value (mg/kg)	Plot No./ Study No.	No. of applic.	FL-Type	Product	Country	Area of applic.
1	Lettuce	63	0.09	10-2503-01-T-1B / 10-2503	1	SL 200	BYI 02960 SL 200	Germany	F
2	Lettuce	60	<0.04	10-2503-02-T-1B / 10-2503	1	SL 200	BYI 02960 SL 200	Netherlands	F
3	Lettuce	69	0.16	10-2503-03-T-1B / 10-2503	1	SL 200	BYI 02960 SL 200	France	F
4	Lettuce	72	0.07	10-2503-04-T-1B / 10-2503	1	SL 200	BYI 02960 SL 200	Spain	F

* - PBI (plant-back interval) for these, relevant trials: 25-30 days

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Table 6.7.2-9 (cont'd): Calculation of MRL proposals for rotational leafy vegetable crops according to EU and OECD guidelines

EVALUATION SUMMARIES

- EU guideline 7039/VI/95 of 22 July 1997

Results (rotational leafy crops, represented by lettuce)

Method I (Weinmann/Nolting) (all values)	R s k Rmax=R+k*s	0.090 0.051 5.144 0.352
Method II (Wilkening) (75 % quantile)	R (0.75) Rber=2*R(0.75)	0.143 0.285

Summary of results:

Maximum residue values for a plant-back interval of: 25-30 days

Method I (all values)	0.35 mg/kg
Method II (75% quantile)	0.29 mg/kg

- OECD Calculator

Results (rotational leafy crops, represented by lettuce)

Total number of data (n)	4	Standard deviation (SD)	0.051
Lowest residue	0.04	Percentage of censored data	25
Highest residue	0.16	Number of non-censored data	3
Median residue	0.080	Correction factor for censoring (CF)	0.833
Mean	0.090		

Proposed MRL estimate

Highest residue	0.16
Mean + 4 SD	0.294
CF x 3 mean	0.225
Unrounded MRL	0.294
Rounded MRL	0.3

Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.7.2-10: Calculation of MRL proposals for rotational cereal crops according to EU and OECD guidelines

BASIC DATA

Residue:	total residue of BYI 02960 calc.	Crop group :	rotational cereals
Portion analysed :	grain	Commodity :	barley
Target value :	MRL	PBI :	25-33 days

No.	Crop	Days after application *	Residue value (mg/kg)	Plot No./ Study No.	No. of applic.	FL-Type	Product	Country	Area of applic.
1	Barley	116	0.35	10-2503-01-T-1C / 10-2503	1	SL 200	BYI 02960 SL 200	Germany	F
2	Barley	218	0.65	10-2503-03-T-1C / 10-2503	1	SL 200	BYI 02960 SL 200	France	F
3	Barley	233	0.11	10-2503-04-T-1C / 10-2503	1	SL 200	BYI 02960 SL 200	Spain	F

* - PBI (plant-back interval) for these, relevant trials: 25-33 days

Continued on next page...

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Table 6.7.2-10 (cont'd): Calculation of MRL proposals for rotational cereal crops according to EU and OECD guidelines

EVALUATION SUMMARIES

- EU guideline 7039/VI/95 of 22 July 1997

Results (rotational cereal crops, represented by barley)

Method I (Weinmann/Nolting) (all values)	R s k Rmax=R+k*s	0.370 0.271 7.656 2.441
Method II (Wilkening) (75 % quantile)	R (0.75) Rber=2*R(0.75)	0.650 1.300

Summary of results:

Maximum residue values for a plant-back interval of: 25-33 days

Method I (all values)	2.44 mg/kg
Method II (75% quantile)	1.30 mg/kg

- OECD Calculator

Results (rotational cereal crops, represented by barley)

Total number of data (n)	3	Standard deviation (SD)	0.271
Lowest residue	0.11	Percentage of censored data	0
Highest residue	0.65	Number of non-censored data	3
Median residue	0.350	Correction factor for censoring (CF)	1.000
Mean	0.370		

Proposed MRL estimate

Highest residue	0.65
Mean + 4 SD	1.452
CF x 3 mean	1.110
Unrounded MRL	1.452
Rounded MRL	1.5

► ANIMAL MATRICES

Based on the proposed residue definition for risk assessment in animal matrices — the calculated total residues of BYI 02960, consisting of the sum of the residues of the parent compound and its metabolite DFA — and on the studies presented in this dossier, MRL proposals are presented below for animal tissues and products, resulting from exposure of livestock to feed crops treated with BYI 02960.

Note: The proposed residue definitions for both risk assessment and enforcement are different than the one used in the feeding studies for data collection, as while the former include only two components, the data gathering method determined 2 additional analytes, BYI 02960-acetyl-AMCP and BYI 02960-OH. However, as explained previously (section 6.7.1), these did not play a major role in any of the trials and thus they are not included in the MRL calculations summarized below.

The crop studies described in this dossier reflect only the "safe uses", i.e. lettuce and hops, as well as the rotational crop groups covered in the "major" study. Further uses will be submitted separately to cover numerous other crops; these uses are reflected in the crops affecting the dietary burden and thus the residue levels in animal matrices.

• Poultry

When chickens and other poultry are exposed to relevant residues of BYI 02960 via ingestion of plant materials, they are primarily exposed to two substances, the parent compound itself and its main metabolite, DFA. A feeding study was conducted by feeding parent compound to laying hens (KIIA 6.4.1/01), and, by way of "material balancing" including determination of residues in the excreta, transfer factors for DFA were calculated on the basis of the systemically available amount of DFA, and thus the residue values obtained in the study can be re-calculated to yield realistic worst-case MRLs based on exposure to both parent and DFA.

In the EU 1X animal group, representing the maximum proposed dietary burden of total residues of BYI 02960 in crops/matrices relevant to poultry feed, the following combined residue levels, comprising BYI 02960 and DFA, were observed in relevant poultry matrices taken at sacrifice:

Table 6.7.2-11: Combined residues of BYI 02960 and DFA in poultry matrices as determined in the feeding study (1X dose group)

Commodity	combined residue	Residue levels (mg/kg)	
		individual components	
		BYI 02960	DFA
eggs	0.061 [†]	<0.01* [†]	0.051 [†]
muscle	0.093	<0.01*	0.083
fat	0.039	<0.01*	0.029
liver	0.114	<0.01*	0.104

* LOQ for BYI 02960 = 0.01 mg/kg

† milk value based on the day-24 egg sample (cf. Table 6.4.1-3)



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The dietary residues fed to the chickens were based on residue values in crops covered in this dossier (rotational turnips and cereals) as well as preliminary data from additional uses to be submitted later (kale and primary cereal uses). The contributions to the diet were as follows:

Table 6.7.2-12: Key data re. the make-up of the diet fed to chickens in the feeding study

Crop	Residue levels (mg/kg)		Levels in dry matter		Dietary burden (mg/kg feed)	Residue-to-burden factor
	total res.*	BYI 02960	DFA	% in crop	residue (mg/kg)	
wheat	0.78	0.01	0.77	86	0.91	0.635
kale	1.36	1.10	0.26	14	9.71	0.486
turnip	0.14	0.01	0.13	10	1.40	0.280
<i>Totals:</i>		1.12	1.16		1.401	

* "total residue" of BYI 02960 + DFA

Based on the feeding study results, transfer factors were calculated both for the *combined residue* of BYI 02960 and DFA derived from the feeding of BYI 02960 as well as for *DFA alone* derived from the exposure levels of the chickens to DFA in the feeding studies. (For details, cf. section 6.4.1 of this dossier.) Using these transfer factors, estimations of the residue levels reasonably expected to be determined after feeding of a *mixture* of BYI 02960 and DFA, as would be anticipated in the feed crops, can be calculated.

To do this, the residues fed must first be separated into the individual components. Using the residue-to-dietary-burden factors determined for the study, each component can be calculated as an individual contributor to the diet, as shown in table 6.7.2-13:

Table 6.7.2-13: Re-calculation of dietary burdens for the individual components of the relevant residue in poultry feed (BYI 02960 and DFA)

Crop	Residue levels (mg/kg)		Levels in dry matter		Dietary burden (mg/kg feed)	Residue-to-burden factor
	total res.*	BYI 02960	DFA	% in crop	residue (mg/kg)	
wheat	0.78	0.01		86	0.01	0.008
kale	1.36	1.10		14	7.86	0.393
turnip	0.14	0.01		10	0.10	0.020
<i>Subtotal BYI 02960:</i>		1.12			0.421	
wheat	0.78		0.77	86	0.90	0.627
kale	1.36		0.26	14	1.86	0.093
turnip	0.14		0.13	10	1.30	0.260
<i>Subtotal DFA:</i>		1.16			0.980	
<i>Totals:</i>		1.12	1.16		1.401	

* "total residue" of BYI 02960 + DFA

Using these dietary burden levels (0.421 mg/kg feed of BYI 02960 and 0.980 mg/kg feed of DFA) and the transfer factors derived in the feeding study (for the low-dose group re. the total residue, and for the most relevant exposure scenario – again, the lowest, 0.5 mg/kg feed – for DFA), the following theoretical residue levels can be calculated for the feeding of BYI 02960 and DFA individually in

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ratios relevant to the worst-case residues expected in animal feed. They are summarized in the table below.

Table 6.7.2-14: Theoretical residues resulting from feeding BYI 02960 and DFA to poultry in a ratio relevant to the actual residues expected in feed commodities

Dietary burden (mg/kg feed)	Matrix	Transfer factor*	Resulting residue (mg/kg)
<i>BYI 02960</i>			
0.421	egg	0.038	0.016
	muscle	0.062	0.026
	fat	0.026	0.011
	liver	0.076	0.032
<i>DFA</i>			
0.980	egg	0.097	0.095
	muscle	0.172	0.169
	fat	0.060	0.059
	liver	0.216	0.212

* for derivation of transfer factors, cf. section 6.4.1

Table 6.7.2-15 summarizes the results of the theoretical residues calculated above, matrix for matrix. The following levels of total residues of BYI 02960 and DFA (expressed in parent compound equivalents) can be expected after feeding of realistic worst-case residues in feed crops to poultry.

Table 6.7.2-15: Levels of the relevant residue of BYI 02960 (comprising BYI 02960 + DFA) in poultry tissues and eggs expected after feeding a worst-case diet containing residues due to treatment of crops with BYI 02960

Matrix	Residue levels* (mg/kg) based on feeding of			Proposed MRL
	BYI 02960	DFA	Sum	
egg	0.016	0.095	0.111	0.15
muscle	0.026	0.169	0.195	0.20
fat	0.011	0.059	0.070	0.07
liver	0.032	0.212	0.244	0.30

* all residue levels expressed in parent compound equivalents

Thus, on the basis of the feeding study in poultry, the following MRLs are proposed for the total residue of BYI 02960 and DFA in edible matrices: 0.15 mg/kg in eggs, 0.20 mg/kg in muscle, 0.07 mg/kg in fat, and 0.30 mg/kg in liver and edible offal.

• Ruminants

When cattle and similar animals are exposed to relevant residues of BYI 02960 via ingestion of plant materials, they are primarily exposed to two substances, the parent compound itself and its main metabolite, DFA. A feeding study was conducted by feeding parent compound to dairy cows (KIIA 6.4.2/01), and, by way of "material balancing" including determination of residues in the urine, transfer factors for DFA were calculated on the basis of the systemically available amount of DFA,

and thus the residue values obtained in the study can be re-calculated to yield realistic worst-case MRLs based on exposure to both parent and DFA.

In the EU 1.3X animal group, representing slightly more than the maximum proposed dietary burden of total residues of BYI 02960 in crops/matrices relevant to ruminant feed, the following combined residue levels, comprising BYI 02960 and DFA, were observed in relevant cattle matrices taken at sacrifice:

Table 6.7.2-16: Combined residues of BYI 02960 and DFA in cattle matrices as determined in the feeding study (1.3X dose group)

Commodity	Residue levels (mg/kg)		
	combined residue	individual components	
		BYI 02960	DFA
milk	0.043 [†]	0.023 [†]	<0.02* [†]
muscle	0.063	0.043	<0.02*
fat	0.041	0.021	<0.02*
liver	0.165	0.145	<0.02*
kidney	0.180	0.159	0.021

* LOQ for DFA = 0.02 mg/kg, expressed in BYI 02960 equivalents

† milk value based on the day-28 milk sample (cf. Table 6.4.2-2)

The dietary residues fed to the cows were based on residue values in crops covered in this dossier (rotational turnips and cereals) as well as preliminary data from additional uses to be submitted later (kale and primary cereal uses). The contributions to the diet were as follows:

Table 6.7.2-17: Key data re. the make-up of the diet fed to dairy cows in the feeding study

Crop	Residue levels (mg/kg)			Levels in dry matter		Dietary burden (mg/kg feed)	Residue-to-burden factor
	total res.*	BYI 02960	DFA	% in crop	residue (mg/kg)		
wheat	0.78	0.01	0.77	86	0.91	0.050	18.140
kale	1.36	1.10	0.26	14	9.71	3.400	2.857
turnip	0.14	0.01	0.13	10	1.40	0.840	1.667
<i>Totals:</i>	1.12	1.16			4.290		

* "total residue" of BYI 02960 + DFA

Based on the feeding study results, transfer factors were calculated both for the *combined residue* of BYI 02960 and DFA derived from the feeding of BYI 02960 as well as for *DFA alone* derived from the exposure levels of the cows to DFA in the feeding studies. (For details, cf. section 6.4.2 of this dossier.) Using these transfer factors, estimations of the residue levels reasonably expected to be determined after feeding of a *mixture* of BYI 02960 and DFA, as would be anticipated in the feed crops, can be calculated.

To do this, the residues fed must first be separated into the individual components. Using the residue-to-dietary-burden factors determined for the feeding study, each component can be calculated as an individual contributor to the diet, as shown in table 6.7.2-18:



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Table 6.7.2-18: Re-calculation of dietary burdens for the individual components of the relevant residue in ruminant feed (BYI 02960 and DFA)

Crop	Residue levels (mg/kg)		Levels in dry matter		Dietary burden (mg/kg feed)	Residue-to-burden factor	
	total res.*	BYI 02960	DFA	% in crop	residue (mg/kg)		
wheat	0.78	0.01		86	0.01	0.001	18.140
kale	1.36	1.10		14	7.86	2.750	2.857
turnip	0.14	0.01		10	0.10	0.060	1.667
<i>Subtotal BYI 02960:</i>	1.12					2.811	
wheat	0.78		0.77	86	0.90	0.049	18.140
kale	1.36		0.26	14	1.86	0.650	2.857
turnip	0.14		0.13	10	1.30	0.780	1.667
<i>Subtotal DFA:</i>		1.16				1.479	
<i>Totals:</i>	1.12	1.16				4.290	

* "total residue" of BYI 02960 + DFA

Using these dietary burden levels (2.811 mg/kg feed of BYI 02960 and 1.479 mg/kg feed of DFA) and the transfer factors derived in the feeding study (for the low-dose group re. the total residue, and for the most relevant exposure scenario – 1.45 mg/kg feed – for DFA), the following theoretical residue levels can be calculated for the feeding of BYI 02960 and DFA individually in ratios relevant to the worst-case residues expected in animal feed. They are summarized in the table below.

Table 6.7.2-19: Theoretical residues resulting from feeding BYI 02960 and DFA to cows in a ratio relevant to the actual residues expected in feed commodities

Dietary burden (mg/ kg feed)	Matrix	Transfer factor*		Resulting residue (mg/kg)	
		using apparent residues**	using values "at LOQ" [†]	using apparent residues**	using values "at LOQ" [†]
<i>BYI 02960</i>					
2.811	milk	0.006	0.009	0.017	0.025
	muscle	0.012	0.013	0.034	0.037
	fat	0.007	0.009	0.020	0.025
	liver	0.035	0.036	0.098	0.101
	kidney	0.037	0.037	0.104	0.104
<i>DFA</i>					
1.479 [‡]	milk		0.028		0.041
	muscle		0.094		0.139
	fat		0.068		0.101
	liver		0.091		0.135
	kidney		0.140		0.207

* for derivation of transfer factors, cf. section 6.4.2

** "apparent residues" used to derive factors include measured values below the tested LOQ

† when deriving factors, residue values of <LOQ were calculated as being at the LOQ, thus representing a worst case for exposure

‡ based on this dietary burden, the transfer factor chosen for DFA reflects the closest exposure rate, 1.45 mg/kg feed



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Table 6.7.2-20 summarizes the results of the theoretical residues calculated above, matrix for matrix. The following levels of total residues of BYI 02960 and DFA (expressed in parent compound equivalents) can be expected after feeding of realistic worst-case residues in feed crops to cattle.

Table 6.7.2-20: Levels of the relevant residue of BYI 02960 (comprising BYI 02960 + DFA) in bovine tissues and milk expected after feeding a worst-case diet containing residues due to treatment of crops with BYI 02960

Matrix	Residue levels* (mg/kg) based on feeding of BYI 02960			Sum		Proposed MRL*
	"app."** * "at LOQ"†		DFA	"app."**	"at LOQ"†	
milk	0.017	0.025	0.041	0.058	0.067	0.07
muscle	0.034	0.037	0.139	0.173	0.176	0.20
fat	0.020	0.025	0.101	0.120	0.126	0.15
liver	0.098	0.101	0.135	0.233	0.236	0.30
kidney	0.104	0.104	0.207	0.311	0.311	0.40

* all residue levels expressed in parent compound equivalents

** total reflects "apparent" residue levels (even if a component is below the LOQ), as shown in table 6.7.2-19

† total reflects residue levels of all components at or above the LOQ, as shown in table 6.7.2-19

Thus, on the basis of the feeding study in dairy cows, the following MRLs are proposed for the total residue of BYI 02960 and DFA in edible matrices: 0.07 mg/kg in milk, 0.20 mg/kg in muscle, 0.15 mg/kg in fat, 0.30 mg/kg in liver, and 0.40 mg/kg in kidney and other edible offal.

• Summary of MRL proposals

The following MRLs for the total residue of BYI 02960 plus DFA have been proposed in this dossier based on uses described above in section 6.3, the related feeding and rotational crop studies (sections 6.4 and 6.6.3, respectively) and the MRL calculations described above:

Table 6.7.2-21: Proposed MRLs based on envisaged uses of BYI 02960

Commodity	MRL proposals* (mg/kg)	Commodity	MRL proposals* (mg/kg)
lettuce and similar plants**	7.0	eggs	0.15
hops (dried cone)	4.0	poultry meat (muscle)	0.20
rotational root crops	0.30	poultry fat	0.07
rotational leafy veg. crops	0.30	poultry liver/offal	0.30
rotational cereal crops	1.5	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

IIA 6.8 Proposed pre-harvest intervals, re-entry or withholding periods**IIA 6.8.1 Pre-harvest interval (in days) for each relevant crop**

The envisaged pre-harvest intervals are as described above in the field residue trials section of this chapter (point KIIA 6.3). For:

- lettuce grown in greenhouses, the critical PHI is 3 days.
- lettuce grown outdoors, the critical PHI is 3 days based on the "home & garden" use. For the agricultural use label, a PHI of 10 days will be stipulated.
- hops, the PHI is 21 days.

IIA 6.8.2 Re-entry period (in days) for livestock, to areas to be grazed

BYI 02960-containing products are not intended for use in areas to be grazed by livestock. Therefore, a re-entry period does not need to be established.

IIA 6.8.3 Re-entry period for man to crops, buildings or spaces treated

Under practical conditions there is no reason to enter a crop shortly after treatment. Even if done, one would wait until the spray solution has dried on the plant surface, at least. Under these circumstances, re-entry exposure was evaluated based on measured dislodgeable foliar residues as well as on conservative model assumptions. Exposure was estimated to be within acceptable levels and no unacceptable risk is anticipated for workers entering the treated crop and performing re-entry activities when standard work clothing is worn (shoes, socks, long pants, and long sleeves).

Therefore, setting a specific re-entry period is not indicated.

b) Buildings

Not relevant.

c) Spaces

Not relevant

IIA 6.8.4 Withholding period (in days) for animals feedingstuffs

Lettuce:

Due to the time between last treatment and harvest, as defined by the GAPs, it is not necessary to set a withholding period for use of treated plants as animal feedingstuffs. The withholding period is covered by the vegetation period of the crop. However, a poultry feeding study with parent BYI 02960 was conducted at dose rates which reflect maximum possible exposure to livestock based



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on uses in numerous crops, many of which will be submitted in a separate document. That study covers all aspects of residues in livestock and thus no specific withholding period is required.

Hops:

Not relevant for hops since they are not used as animal feedingstuffs.

IIA 6.8.5 Waiting period between last application and sowing or planting

Not applicable, because BYI 02960-containing products are not intended for use on lettuce or hops prior to sowing or planting.

IIA 6.8.6 Waiting period between application and handling treated products

The use of BYI 02960-containing products is intended in lettuce or hops prior to harvest. The proposed pre-harvest interval is 3 days for lettuce and 21 days for hops. There is no need to handle treated crops before harvest.

IIA 6.8.7 Waiting period before sowing/planting 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.

IIA 6.9 Estimation of exposure through diet and other means**IIA 6.9.1 TMDI calculations**

The assessment of the chronic uptake of BYI 02960 residues with food is made based on the proposed MRLs as shown in this dossier (cf. section 6.7.2) and the Acceptable Daily Intake (ADI) of 0.078 mg/kg bw/day, which was established based on the lowest NOAELs obtained in a chronic toxicity study in the most sensitive species. The lowest NOAELs has been observed in the rat 2-generation reproduction study based on body weight effects in females and in the 1-year dog study based on histopathological findings in the skeletal muscle (cf. section 3, point 5.11).

In order to evaluate the potential chronic exposure to BYI 02960 residues through the diet, the Theoretical Maximum Daily Intakes (TMDI) of residues was estimated using the EFSA PRIMO model. The MRLs as summarized in table 6.7.2-21 were used as the basis for calculation.

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For agricultural and "home & garden" uses, the calculated TMDI values according to the relevant modules of the EFSA Primo consumer exposure model are summarized in table 6.9.1-1 below. (Details of the individual TMDI calculations are shown in table 6.9.1-2.)

Table 6.9.1-1: BYI 02960 – Summary of the TMDI calculation (EFSA PRIMo model rev. 2.0)
Top ten ADI usage results including top contributors

TMDI (% ADI)	Diet	Highest contributor to the diet (% ADI)	Commodity	2 nd -highest contributor to the diet (% ADI)	Commodity
29.4	WHO Cluster diet B	22.9	Cereals	3.2	Lettuce
23.8	DK child	20.0	Cereals	1.3	Lettuce
20.3	IT children/toddlers	16.1	Cereals	2.6	Lettuce
18.8	NL child	10.8	Cereals	2.6	Milk/cream
18.0	WHO cluster diet D	16.2	Cereals	0.5	Milk/cream
16.9	ES child	10.0	Cereals	3.7	Lettuce
16.6	IE adult	12.7	Cereals	0.8	Lettuce
15.4	WHO cluster diet E	11.7	Cereals	0.8	Lettuce
15.4	DE child	10.7	Cereals	1.3	Milk/cream
15.3	WHO cluster diet F	10.5	Cereals	2.7	Lettuce

The TMDI, calculated according to the EFSA PRIMo 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.

(It should be noted that these calculations represent an overestimate of the true residue intake. All models fail to consider the fact that the percentage of the total area of a crop treated with BYI 02960 is far below 100%; most treated crops contain residues well below the MRL at harvest; residues are usually reduced during storage, preparation, commercial processing, and cooking; and, it is not realistic to assume that every commodity for which an MRL is proposed or exists has been treated with BYI 02960 over the lifetime of the consumer — in fact, most commodities will not have been treated with the compound and will not contain any BYI 02960 residues at all.)

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Table 6.9.1-2: BYI 02960 – Details of the TMDI calculation (EFSA PRIMo model rev. 2.0)

BYI 02960		
Status of the active substance:	new	Code no.
LOQ (mg/kg bw):	0.04	proposed LOQ:
Toxicological end points		
ADI (mg/kg bw/day):	0.078	ARfD (mg/kg bw): 0.35
Source of ADI:	KIIA 5	Source of ARfD: KIIA 5
Year of evaluation:	2012	Year of evaluation: 2012

Explain choice of toxicological reference values.

The risk assessment has been performed on the basis of the MRLs collected from Member States in April 2006. For each pesticide/commodity the highest national MRL was identified (proposed temporary MRL = pTMRL). The pTMRLs have been submitted to EFSA in September 2006.

Chronic risk assessment						
		TMDI (range) in % of ADI minimum - maximum 1 29				
		No of diets exceeding ADI: ---				
Highest calculated TMDI values in % of ADI	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)
29.2	WHO Cluster diet B	22.9	CEREALS	3.2	Lettuce	0.4
23.8	DK child	20.0	CEREALS	1.3	Lettuce	1.1
20.2	IT kids/toddler	16.1	CEREALS	2.6	Lettuce	1.0
18.0	WHO cluster diet D	16.2	CEREALS	0.5	Milk and cream,	0.2
17.4	NL child	10.8	CEREALS	2.6	Milk and cream,	0.9
16.9	ES child	10.0	CEREALS	3.7	Lettuce	1.1
16.6	IE adult	12.7	CEREALS	0.8	Lettuce	0.7
15.3	WHO Cluster diet F	10.5	CEREALS	2.7	Lettuce	0.4
15.3	DE child	10.7	CEREALS	1.3	Milk and cream,	0.7
15.0	WHO cluster diet E	11.7	CEREALS	0.8	Lettuce	0.3
15.0	IT adult	9.7	CEREALS	3.4	Lettuce	1.4
14.2	UK Infant	8.7	CEREALS	3.5	Milk and cream,	0.6
13.2	UK Toddler	8.8	CEREALS	1.9	Milk and cream,	1.2
13.0	WHO regional European diet	7.1	CEREALS	3.4	Lettuce	0.4
12.6	ES adult	6.1	CEREALS	4.8	Lettuce	0.4
12.5	FR toddler	5.7	CEREALS	3.6	Milk and cream,	1.1
12.2	SE general population 90th percentile	9.6	CEREALS	1.1	Milk and cream,	0.5
11.5	PT General population	10.3	CEREALS	0.3	Potatoes	0.3
10.5	FR all population	6.6	CEREALS	1.7	Other lettuce and other salad plants	0.8
8.4	NL general	5.3	CEREALS	1.1	Lettuce	0.6
7.3	UK vegetarian	4.9	CEREALS	1.3	Lettuce	0.3
7.1	LT adult	5.2	CEREALS	0.6	Lettuce	0.4
6.8	DK adult	5.6	CEREALS	0.5	Milk and cream,	0.2
6.5	FR infant	2.3	Milk and cream,	1.8	CEREALS	1.2
6.1	UK Adult	4.0	CEREALS	1.1	Lettuce	0.3
5.3	FI adult	3.7	CEREALS	0.7	Lettuce	0.5
0.8	PL general population	0.2	Other root and tuber vegetables	0.2	Potatoes	0.1

Conclusion:	
The estimated Theoretical Maximum Daily Intakes (TMDI), based on pTMRLs were below the ADI. A long-term intake of residues of BYI 02960 is unlikely to present a public health concern.	



IIA 6.9.2 NEDI/IEDI calculations

The TMDI calculation for BYI 02960 shows that residues at the level of the proposed MRLs do not result in a chronic risk to the consumer. Therefore, a refined risk assessment using processing factors and STMR values instead of MRLs is not necessary and was not conducted.

IIA 6.9.3 NESTI/IESTI calculations

The short-term dietary risk assessment for BYI 02960 was calculated based on the uses presented above (sections 6.3, 6.4, and 6.6.3) and on the Acute Reference Dose (ARfD) of 0.35 mg/kg body weight, which was derived from the acute neurotoxicity study in rats (cf. section 3, point 5.11).

The intake estimation was calculated using the EFSA PRIMo model (Version 2a) including diets from various countries in Europe. In a first, very conservative approach, the MRL values derived from supervised residues trials (total residues; as summarized in table 6.7.2-21) 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.

Table 6.9.3-1: 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 in table 6.9.3-1 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-leaved 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

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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.

IIA 6.10 Other/special studies

None.

IIA 6.11 Summary and evaluation of residue behaviour and reasonable grounds**IIA 6.11.1 Summary and evaluation of residue behaviour****Summary of plant metabolism:**

The metabolism of BYI 02960 was investigated in five target crops (tomato, potato, apple, cotton and rice) following application of [furanone-4-¹⁴C]BYI 02960 and [pyridinylmethyl-¹⁴C]BYI 02960.

Additionally, [ethyl-1-¹⁴C]BYI 02960 was applied in one tomato metabolism study. In all other plant metabolism studies, the fate of the difluoroethane moiety of the active substance was tracked by analysing extracts of all RACs for non-radiolabelled difluoroacetic acid, the most plausible metabolite which can be formed from the moiety after cleavage of the parent compound. The analysis of difluoroacetic acid was initiated after detecting difluoroacetic acid as a major soil metabolite in the aerobic soil degradation studies.

The active substance was applied either as an SL formulation for drench and foliar application, an FS formulation for seed/tuber treatment or as a granule to cover the special application technique in rice in Japan.

One to two spray applications were conducted in apples, cotton and rice with a maximum seasonal application rate of 400 g a.s./ha. In rice, additionally a granule application was conducted at the same maximum seasonal application rate. Soil treatments were performed in tomato (drench application) and in potato (in-furrow application) at a maximum seasonal application rate of 600 g a.s./ha. Tuber treatment of potatoes was also conducted with a dressing rate of 10 g a.s./dt (corresponding to approx. 250 g a.s./ha).

An overview of the use pattern from the metabolism studies in target plants is summarized in Table 6.11.1-1



Table 6.11.1-1 Use patterns in the target plant metabolism studies

Crop	Radiolabel	Appl. technique	No. of appl.	Actual single application rate	Appl. interval [days]	BBCH growth stage	PHI [days]
Tomato	[furanone-4- ¹⁴ C], [pyridylmethyl- ¹⁴ C], [ethyl-1- ¹⁴ C]	drench appl.	2	300 g a.s./ha 300 g a.s./ha 300 g a.s./ha	14	14-15 51-59	56-86 ¹
Potato	[furanone-4- ¹⁴ C], [pyridylmethyl- ¹⁴ C]	tuber treatment	1	10 g a.s./dt = 245 g a.s./ha or = 270 g a.s./ha	-	03	97
Potato	[furanone-4- ¹⁴ C], [pyridylmethyl- ¹⁴ C]	in-furrow appl.	1	626 g a.s./ha 626 g a.s./ha	-	03	97
Apple	[furanone-4- ¹⁴ C], [pyridylmethyl- ¹⁴ C]	spray appl.	1	86 g/(ha x m CH) 87 g/(ha x m CH)	-	69	98
Apple	[furanone-4- ¹⁴ C], [pyridylmethyl- ¹⁴ C]	spray appl.	2	86 & 86 g/(ha x m CH) 85 & 87 g/(ha x m CH)	14	69 85	14
Cotton	[furanone-4- ¹⁴ C], [pyridylmethyl- ¹⁴ C]	spray appl.	1	209 g a.s./ha	-	16	169
Cotton	[furanone-4- ¹⁴ C], [pyridylmethyl- ¹⁴ C]	spray appl.	2	209 & 176 g a.s./ha 206 & 177 g a.s./ha	155	15-16 95-97	14
Rice	[furanone-4- ¹⁴ C], [pyridylmethyl- ¹⁴ C]	granular appl.	1	409 g a.s./ha 434 g a.s./ha	-	13-15	127
Rice	[furanone-4- ¹⁴ C], [pyridylmethyl- ¹⁴ C]	spray appl.	2	175 & 240 g a.s./ha 178 & 236 g a.s./ha	97	13-15 87-89	29

¹ harvest period of approx. 4 weeks since tomato fruits ripen continuously

The total radioactive residues (TRR) of the raw agricultural commodities (RACs) of all target crops are summarized in Table 6.11.1-2. The TRR values were low in all edible matrices (max. 1.286 mg/kg in apple fruits in the double spray experiment).

Table 6.11.1-2 Total radioactive residues (TRR) in plant matrices under investigation

RAC		TRR (mg/kg) [furanone-4-UL- ¹⁴ C]	TRR (mg/kg) [pyridinylmethyl- ¹⁴ C]	TRR (mg/kg) [ethyl-1- ¹⁴ C]
Tomato fruits	drench appl.	0.096	0.130	0.201
Tomato flowers	drench appl.	0.721	1.254	2.230
Potato tubers	tuber treatment	0.078	0.076	(0.441) ³
Potato tubers	in-furrow	0.171	0.115	(0.618) ³
Apple fruits	single spray	0.280	0.079	(0.760) ³
Apple leaves	single spray	38.957	56.715	n.c.
Apple fruits ¹	double spray	1.133	1.868	n.c.
Apple fruits ²	double spray	1.286	0.545	(1.221) ³
Apple leaves	double spray	102.919	134.841	(99.846) ³
Cotton intermediate	single spray	12.391	14.153	n.c.
Cotton gin trash	single spray	0.191	0.310	(0.367) ³
Cotton lint	single spray	0.009	0.007	n.c.
Cotton seeds	single spray	0.013	0.045	(0.135) ⁴
Cotton gin trash	double spray	2.767	2.344	(2.351) ³
Cotton lint	double spray	4.993	8.846	n.c.
Cotton seeds	double spray	0.016	0.068	(0.125) ³
Rice kernels	granule appl.	0.140	0.050	(0.288) ³
Rice husks	granule appl.	1.404	1.602	(2.973) ³
Rice straw	granule appl.	2.879	3.280	(4.325) ³
Rice kernels	spray appl.	0.659	0.620	(0.661) ³
Rice husks	spray appl.	24.098	23.957	(24.450) ³
Rice straw	spray appl.	19.891	24.731	(24.790) ³

¹ with surface wash² without surface wash³ estimated TRR in hypothetical metabolism study with [ethyl-1-¹⁴C]BYI 02960 (for explanation see below)⁴ worst case TRR in hypothetical metabolism study with [ethyl-1-¹⁴C]BYI 02960 since no analysis of the seed extract was performed and therefore no information on label-specific metabolites is available; DFA residue was added to TTR determined in metabolism study.

n.c. not calculated since intermediate extract was not analysed for DFA residues

Example for estimation of a hypothetical TRR (TRR of apple fruit in the single application experiment):

1. DFA was analysed in the apple extract obtained in the metabolism study with [furanone-4-¹⁴C]BYI 0260
 $\Rightarrow 0.23 \text{ mg DFA/kg, corresponding to } 0.69 \text{ mg a.s. equiv./kg (see KIIA 6.2.1/12)}$
2. TRR determined in apple fruit extract: 0.280 mg a.s. equiv./kg (see KIIA 6.2.1/06)
 \Rightarrow all label-specific metabolites detected are subtracted from the TRR to get a TRR that is based on parent compound and metabolites common to all three radiolabels only (= corrected TRR):
 $0.280 \text{ mg/kg} - 0.201 \text{ mg/kg (glucose)} - 0.009 \text{ mg/kg (BYI 02960-difluoroethyl-amino-furanone)} = 0.070 \text{ mg/kg}$
3. A hypothetical TRR is calculated by adding the DFA residue to the corrected TRR:
 $0.070 \text{ mg/kg} + 0.69 \text{ mg/kg} = 0.76 \text{ mg/kg}$

Corresponding TRR values of sample matrices were very well comparable when considering the studies performed with [furanone-4-¹⁴C]BYI 02960 and [pyridinylmethyl-¹⁴C]BYI 0296. Only for the apple and some of the cotton matrices significant differing TTR values were determined. These differences can be traced back to some label-specific metabolites. These metabolites show a different uptake and/or translocation behaviour compared to the parent compound and concentrate in varying



compartments of the crop. For example, radiolabelled glucose, a natural product formed after complete cleavage of the furanone moiety of the parent compound, can be found in high concentrations in the repository organ of apples – the fruits. Thus, the TRR in fruits is significant higher in the study performed with [furanone-4-¹⁴C]BYI 02960, which allows the detection of the natural compound by incorporated radioactivity. Another example is metabolite 6-CNA, a weak acid that shows a pronounced phloem mobility and is therefore transported selectively into the cotton seeds as phloem sink. This behaviour seems even more distinct for the metabolite difluoroacetic acid. Based on the “cold analysis” of the metabolite in representative extracts of all plant metabolism studies, high concentrations of difluoroacetic acid are present in potato tubers, all fruits and in seeds/kernels. Thus the hypothetical TRR values estimated for these matrices are generally higher compared to the measured TRR values in the studies performed with [furanone-4-¹⁴C]BYI 02960 and [pyridinylmethyl-¹⁴C]BYI 0296. The tomato metabolism study with [ethyl-1-¹⁴C]BYI 02960 confirmed these results. It showed also very high concentrations of difluoroacetic acid in fruits (> 80% of the TRR) and a TRR that was significant higher (by a factor of approx. 2) compared to the studies performed with the other radiolabels.

Thus metabolite difluoroacetic acid was the main compound detected in tomato fruits and flowers, in potato tubers, in apple fruits after early spray application of the a.s., in rice kernels after granule application and in cotton seeds in both application scenarios and in cotton gin trash after early spray application. The difluoroacetic acid proportion was generally higher if the crop was treated at an early growth stage, only. Certainly, a second late application with the active substance shifted the proportion in favour of the parent compound. Thus, parent compound was the main constituent in apple fruits, in rice kernels and as well in cotton gin trash after a second late spray treatment. However, parent compound was always an prominent constituent of the residue. In rice husks and straw, as well as in the cotton intermediate, parent compound was the main compound even after one early application. In tomato fruits and potato tubers, parent compound was the second most common constituent considering all application scenarios. Overall, major compounds detected in the studies were the metabolites 6-CNA, BYI 02960-CHMP-glyc and -CHMP-di-glyc, glucose, BYI 02960-difluoroethyl-amino-furanone and BYI 02960-OH-glyc besides difluoroacetic acid and parent compound. The amounts of parent compound and these metabolites in edible matrices of target crops are shown in Table 6.11.1-3 (single application) and Table 6.11.1-4 (double application).

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Table 6.11.1-3 Residues [mg a.s. equiv./kg] of BYI 02960 and major metabolites in edible matrices after one application

Compound (BYI 02960-)	potato tubers				apple fruits		rice kernels		cotton seeds	
	tuber treatment		in-furrow appl.		foliar appl.		granule appl.		foliar appl.	
Label	F	P	F	P	F	P	F	P	F	P
TRR	0.078	0.076	0.171	0.115	0.280	0.079	0.140	0.050	0.013²	0.045²
BYI 02960	0.031	0.031	0.097	0.051	0.021	0.034	0.032	0.035		
DFA		0.39 ¹		0.54 ¹	0.69 ¹			0.06 ¹		0.09 ¹
glucose/carbohydr.	---		---		0.201		0.038			
6-CNA		0.016		0.021		0.004		0.002		
CHPM-di-glyc		0.003		0.006		---		---		
CHMP-glyc		0.003		0.003		0.004		---		
difluoroethyl-amino-furanone	0.003		0.005		0.009		---			
OH-glyc	0.005	0.005	0.007	0.005	0.001	0.004	---	---		

¹ LC-MS/MS analysis of non-radiolabelled DFA in the extract obtained in the metabolism study² analysis of the extracts was not feasible due low extraction efficiency and high matrix load in the extracts**Table 6.11.1-4 Residues [mg a.s. equiv./kg] of BYI 02960 and major metabolites in edible matrices after two applications**

Compound (BYI 02960-)	tomato fruits			apple fruits - with surface wash		apple fruits - w/o surface wash		rice kernels		cotton seeds	
	drench application			spray application				spray appl.		spray appl.	
Label	F	P	E	F	P	F	P	F	P	F	P
TRR	0.096	0.130	0.201	1.133	1.868	1.286	0.545	0.659	0.620	0.016²	0.068²
BYI 02960	0.034	0.031	0.020	0.809	1.652	0.946	0.467	0.373	0.467		
DFA			0.174				0.12 ¹		0.24 ¹		0.06 ¹
glucose/carbohydr.	0.026			0.193		0.182		0.023			
6-CNA		0.017			0.009		0.008		0.019		
CHPM-di-glyc		0.048			---		---		---		
CHMP-glyc		0.007			0.010		0.005		---		
difluoroethyl-amino-furanone	0.010		0.004	0.007		0.003		---			
OH-glyc	0.005	0.004	0.001	0.014	0.024	0.014	0.009	---	---		

¹ LC-MS/MS analysis of non-radiolabelled DFA in the extract obtained in the metabolism study² analysis of the extracts was not feasible due low extraction efficiency and high matrix load in the extracts

Except for the metabolite difluoroacetic acid, the amounts of the single metabolites in edible matrices were very low. Only the natural compound glucose was found at levels > 0.1 mg/kg in one crop matrix, namely apple fruits. Thus parent compound, difluoroacetic acid and glucose are the only compounds which accounted for residue concentrations > 0.05 mg/kg in edible crops. Since glucose is a natural compound with no toxicological concern, parent compound BYI 02960 and metabolite difluoroacetic acid were the two compounds analysed for in the plant residue trials in the EU. Additionally, metabolite BYI 02960-difluoroethyl-amino-furanone was included in the residue definition for data collection since it was expected as a major metabolite in leafy crops after soil application as indicated by the confined rotational crop studies.



Metabolism studies conducted in five crops representing four diverse crop groups (fruiting crops, root crops, oilseeds and cereals) showed similar profiles, with the unchanged parent compound and metabolite difluoroacetic acid representing the major part of the residue. Analysis of a variety of crops from European field trials shows that the residues are, in fact, dominated by parent compound and difluoroacetic acid. Of course, short pre-harvest intervals in the field trials increased the proportion of parent in the total residue.

The proposed metabolic pathway in plants (primary and rotational crops) is shown in Figure 6.11.1-1. The metabolism in all plants was very similar. The main reactions involved were:

- Oxidative cleavage of the difluoroethylamine bond and formation of difluoroacetic acid,
- complete degradation of the furanone moiety and incorporation of carbon atoms into the natural compound pool, e.g. into glucose/carbohydrates,
- cleavage of the pyridinylmethylamine bond and formation of BYI 02960-difluoroethylamino-furanone and BYI 02960-CHMP / BYI 02960-6-CNA,
- formation of BYI 02960-CHMP was followed by conjugation with carbohydrates and sulphate or by oxidation of the methylene group to a carboxylic group and subsequent conjugation with glycerol and glucuronic acid,
- hydroxylation of the furanone or the difluoroethyl moiety followed by conjugation with carbohydrates and sulphate, and
- oxidative degradation of the furanone moiety to an acetic acid group followed either by conjugation with a carbohydrate or by further oxidation or degradation of the moiety.
- Halogenation (bromination/chlorination) of the furanone moiety of the parent compound was most probably a process which took place in the soil.

Thus, oxidative cleavage of the molecule, oxidation and hydroxylation were the primary transformation steps followed by conjugation reactions with glycosides, sulphate and glucuronic acid.

Generally, these main metabolic reactions were also observed in the rat studies on absorption, distribution, metabolism and elimination (ADME) of BYI 02960 (refer to KIIA 5.1). Parent compound and all major plant metabolites, or at least subsequent metabolites which imply the presence of the plant metabolites as transient metabolites were detected, although BYI 02960 shows a more moderate degradation behaviour in rats compared to plants.

In the ADME studies, parent compound was highly bioavailable and rather moderately metabolized. 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 at 40.9% to 77.7% of the dose. Plant metabolite 6-CNA and its conjugates are covered by the rat metabolites 6-CNA and BYI 02960-hippuric acid, which were detected in sum at a maximum of 16.8% of the dose rate. Both metabolites were considered together since hippuric acid is the subsequent metabolite of 6-CNA and can only be formed via 6-CNA. The hydroxylated parent compound BYI 02960-OH accounted for 10.8% to 28.9% of the dose and even additional conjugates of this metabolite were detected in the rat. Thus toxicological coverage is given. Metabolite BYI 02960-difluoroethyl-amino-furanone (DFEAF) was detected at lower levels. It represented up to 3.5% of the dose, only. Therefore additional toxicity testings were initiated and it was shown that the metabolite was neither acutely



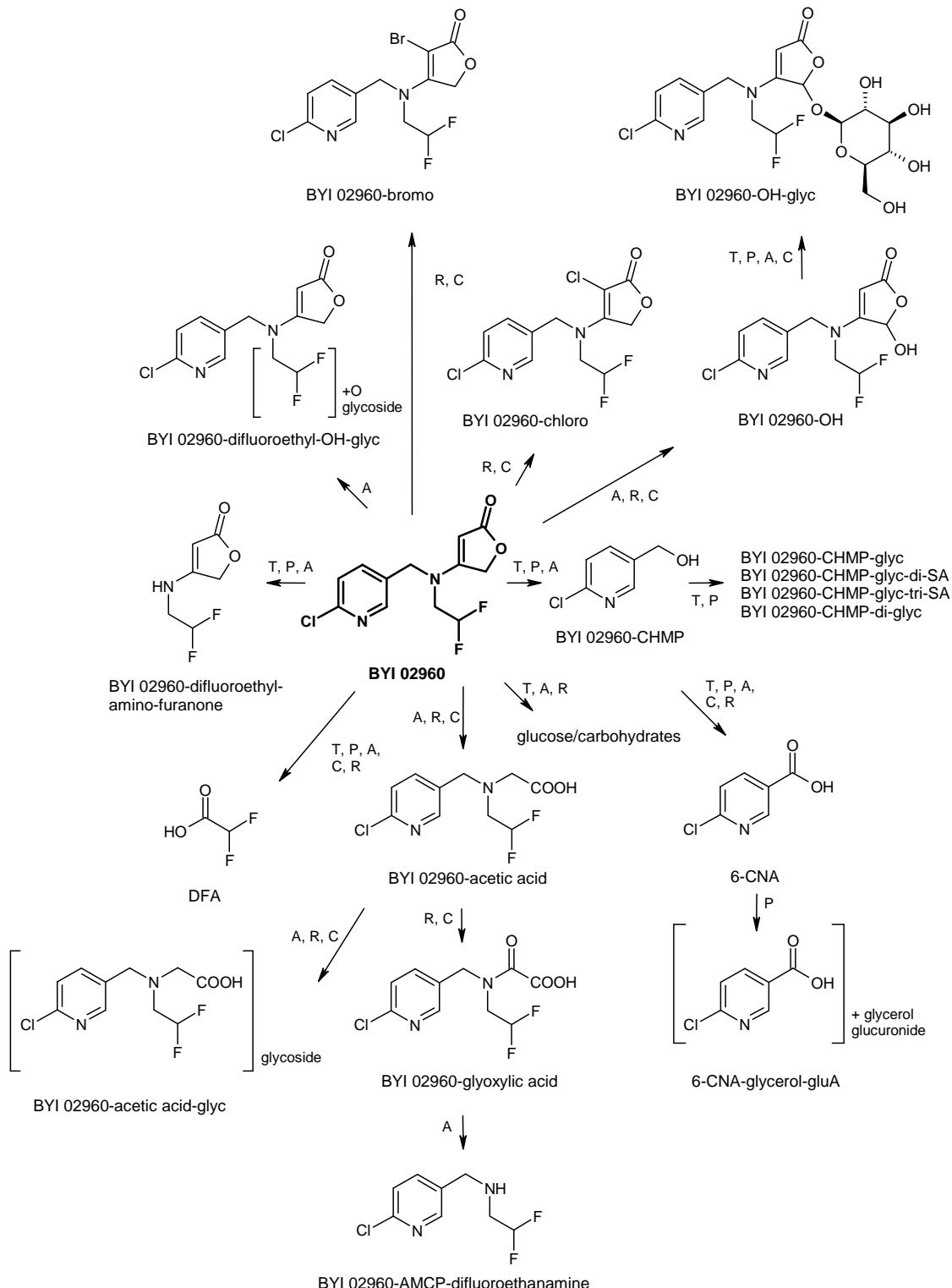
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toxic nor exhibits a genotoxic potential. Subacute administration of BYI 02960-difluoroethyl-amino-furanone to rats revealed that it is less toxic than parent and thus covered by the endpoints derived for the parent compound BYI 02960.

A detailed view on the toxicological coverage of all plant metabolites is summarized in Section 3 KIIA 5.10.



Figure 6.11.1-1 Proposed metabolic pathway of BYI 02960 in target plants



T Tomato

R Rice

P Potato

C Cotton

A Apple

glyc

glyc-di-SA

glyc-tri-SA

glycoside

glycoside-disulphate

glycoside-trisulphate

di-glyc

gluA

diglycoside

glucuronic acid

Summary of livestock metabolism:

The metabolism of Flupyradifurone (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-¹⁴C]BYI 02960 and [furanone-4-¹⁴C]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-¹⁴C]BYI 02960. However, following the dosage of [furanone-4-¹⁴C]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 ¹⁴CO₂, an observation which was also made in rat studies with the [furanone-4-¹⁴C]-labelled test compound (KIIA 5.1). The total radioactive residues in the eggs and edible tissues as well as the concentrations of the identified metabolites are summarised in Table 6.11.1-5.

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-4-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 Table 6.11.1-6.



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Table 6.11.1-5 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-4-¹⁴C] 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



Table 6.11.1-6: 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-¹⁴C]- or [furanone-4-¹⁴C] 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										
TRR (mg/kg)	Milk		Muscle		Fat		Kidney		Liver	
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 natural compounds like fatty acids in all poultry tissues and lactose in the milk of goats.

Notwithstanding these observations, the unchanged parent compound is a significant, if not the dominating constituent of the residue in milk, eggs and edible tissues of both species. Other metabolites determined in comparable concentrations are the natural compound lactose in the milk of goats after administration of [furanone-4-¹⁴C]BYI 02960, and BYI 02960-acetyl-AMPC in eggs and tissues of poultry after administration of [pyridinylmethyl-¹⁴C] BYI 02960.

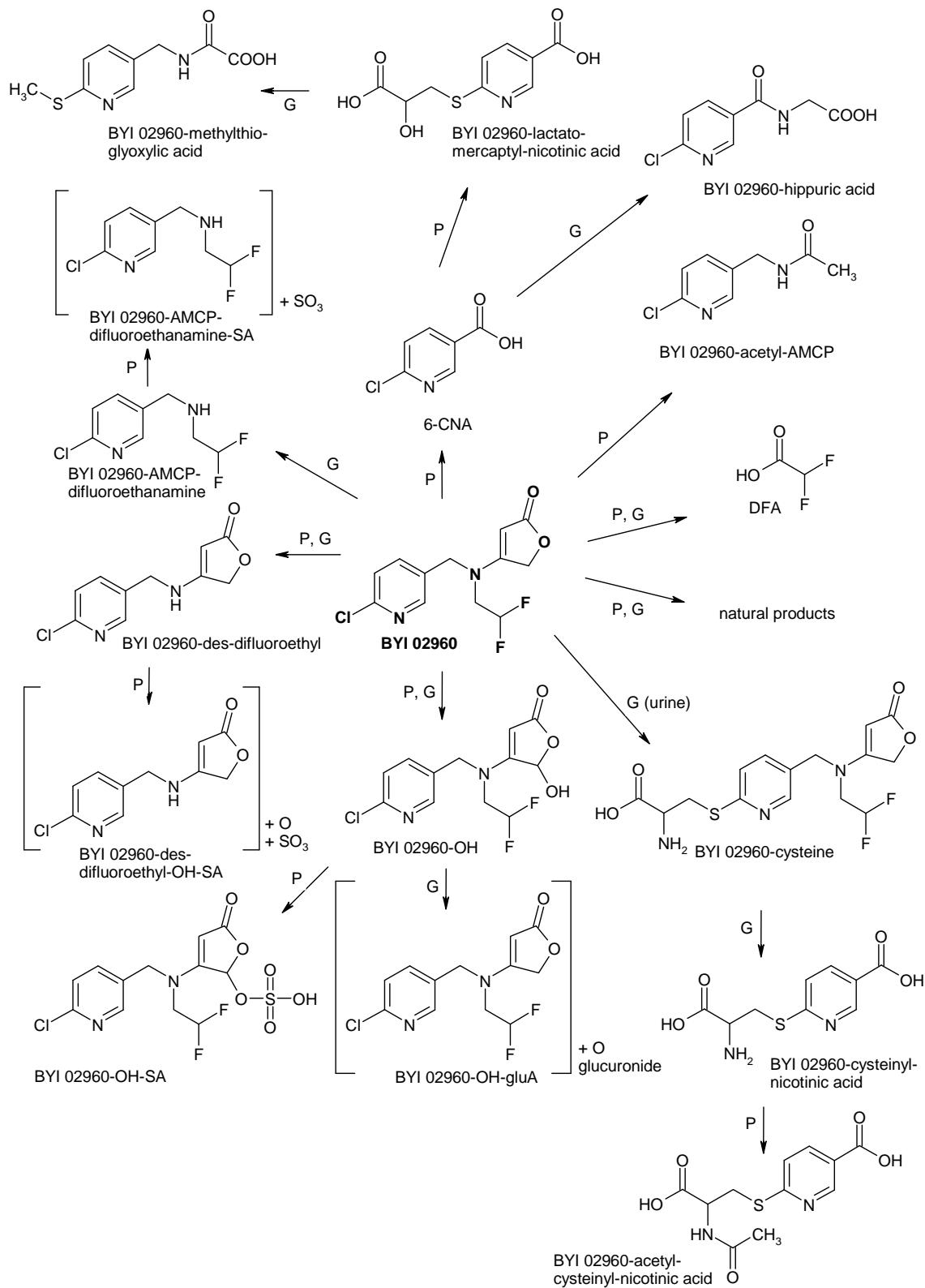
The proposed metabolic pathway of BYI 02960 in edible tissues of livestock, milk and eggs is shown in Figure 6.11.1-2. The main metabolic reactions involved were:

- 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 or lactose
- Substitution of the chloro group of BYI 02960-6-CNA with glutathione followed by degradation resulting in the conjugates BYI 02960-acetyl-cysteinyl-nicotinic acid and BYI 02960-lactato-mercaptyl-nicotinic acid
- Cleavage of the difluoroethyl group forming BYI 02960-des-difluoroethyl followed by hydroxylation and conjugation with sulfuric acid to BYI 02960-des-difluoroethyl-OH-SA
- Cleavage of the furanone ring and conjugation with sulfonic acid forming BYI 02960-AMCP-difluoroethanamine-SA
- Cleavage of the furanone ring and the difluoroethyl group forming an amine followed by acetylation to BYI 02960-acetyl-AMCP and BYI 02960-AMCP-difluoroethanamine
- Cleavage of the difluoroethyl group forming BYI 02960-des-difluoroethyl and difluoroacetic acid

Difluoroacetic acid was determined in selected livestock samples by high resolution LC-MS subsequently to the metabolism studies since rat studies conducted with [ethyl-1-¹⁴C]BYI 02960 showed major amounts of this metabolite in organs and tissues. Extrapolation of rat data suggested high difluoroacetic acid levels in livestock tissues as well which was confirmed by the non-radioactive LC-MS analyses. Based on these findings, it was concluded that difluoroacetic acid is a major livestock metabolite and should be - besides parent compound - part of the residue definition for data collection, risk assessment and enforcement.



Figure 6.11.1-2 Metabolic pathway of BYI 02960 in milk, eggs and edible organs and tissues of lactating goats and laying hens following oral administration of [pyridinylmethyl-¹⁴C]- or [furanone-4-¹⁴C] BYI02960 (G: Goat, P: Poultry)





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These main metabolic reactions were also observed in the rat studies on absorption, distribution, metabolism and elimination (ADME) of BYI 02960 (refer to KIIA 5.1) and therefore the toxicological coverage of the livestock metabolites is given. Parent compound and all major plant metabolites, or at least subsequent metabolites which imply the presence of the livestock metabolites as transient metabolites were detected.

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 including 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 (see also 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 (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.

**Summary of confined rotational crop metabolism:**

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

The total radioactive residues (TRR) of the raw agricultural commodities (RACs) of the rotational crops in the three subsequent rotations are summarized in Table 6.11.1-7. The TRR values of all matrices declined significantly from the 1st to the 3rd rotation.

Table 6.11.1-7 TRR values in the different RACs of the three rotations after soil application of BYI 02960

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

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

The levels of parent compound decreased with increasing plant back intervals but nevertheless parent formed a main portion of the residues in all of the RACs in all rotations, except for wheat grains.

Other major metabolites (accounting for >10% of the TRR in at least one RAC in one rotation) were

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



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- BYI 02960-bromo-amino-furanone, which was only major in wheat hay of the 3rd rotation
- BYI 02960-OH, which was major in wheat grains of the 1st rotation, only
- Glucose/carbohydrates, which were identified as the main portion in wheat grains after exhaustive extraction and as a major metabolite in turnip roots after conventional extraction.

The amounts of parent compound and these major metabolites in the matrices of confined rotational crops are shown in Table 6.11.1-8 to Table 6.11.1-10 for all rotations.

Table 6.11.1-8 Residues [mg a.s. equiv./kg] of BYI 02960 and major metabolites in the matrices of rotational crops (1st rotation)

Compound (BYI 02960-)	wheat forage		wheat hay		wheat straw		wheat grain	
Label	F	P	F	P	F	P	F	P
TRR	0.783	1.407	2.003	2.409	6.290	9.015	0.478	0.177
BYI 02960	0.365	0.640	0.672	0.676	2.459	3.261	0.002	0.015
DFA	0.27 ¹		0.96 ¹		0.60 ¹		3.45 ¹	
glucose/carbohydr.	---		---		---		0.338	
bromo-amino-furanone	0.016		0.033		0.172		---	
difluoroethyl-amino-furanone	0.077		0.205		0.374		---	
glyoxylic acid	0.124	0.172	0.227	0.176	0.965	0.615	0.024	0.011
OH-glyc	0.028	0.048	0.067	0.135	0.242	0.296	0.007	0.009
6-CNA-glycerol-gluA (2 + 3)		0.199		0.569		1.900		0.036
OH	0.010	0.019	0.038	0.052	0.161	0.239	0.011	0.019

(2 + 3) isomer 2 and/or isomer 3

F = [furanone-4-¹⁴C]-label

P = [pyridinylmethyl-¹⁴C]-label

Compound (BYI 02960-)	Swiss chard intermediate		Swiss chard mature		turnip leaves		turnip roots	
Label	F	P	F	P	F	P	F	P
TRR	0.848	1.358	0.871	1.483	0.679	0.815	0.074	0.072
BYI 02960	0.460	0.779	0.371	0.687	0.437	0.508	0.041	0.042
DFA	0.24 ¹		0.48 ¹		0.24 ¹		0.06 ¹	
glucose/carbohydr.	---		---		---		0.003	
bromo-amino-furanone	---		---		---		---	
difluoroethyl-amino-furanone	---		0.010		---		---	
glyoxylic acid	0.031	0.021	0.041	0.039	0.045	0.021	0.009	0.006
OH-glyc	0.072	0.101	0.119	0.162	0.076	0.076	<0.001	0.002
6-CNA-glycerol-gluA (2 + 3)		---		---		---		---
OH	0.017	0.024	0.017	0.023	0.012	0.011	<0.001	<0.001

¹ LC-MS/MS analysis of non-radiolabelled DFA in the extract obtained in the confined rotation crop study performed with [furanone-4-¹⁴C]BYI 02960

(2 + 3) isomer 2 and/or isomer 3

F = [furanone-4-¹⁴C]-label

P = [pyridinylmethyl-¹⁴C]-label

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 Table 6.11.1-9 Residues [mg/kg] of BYI 02960 and major metabolites in the matrices of rotational crops (2nd rotation)

Compound (BYI 02960-)	wheat forage		wheat hay		wheat straw		wheat grain	
Label	F	P	F	P	F	P	F	P
TRR	0.193	0.308	1.081	1.009	1.519	2.148	0.103	0.057
BYI 02960	0.124	0.183	0.314	0.283	0.538	0.804	0.001	0.001
DFA	0.06 ¹		0.42 ¹		0.18 ¹		0.78 ¹	
glucose/carbohydr.	---		---		---		---	
bromo-amino-furanone	0.006		0.107		0.093		---	
difluoroethyl-amino-furanone	0.016		0.075		0.090		---	
glyoxylic acid	<0.001	---	0.020	0.010	0.018	0.036	0.001	0.001
OH-glyc	0.007	0.008	0.037	0.045	0.08	0.112	0.002	0.003
6-CNA-glycerol-gluA (2 + 3)	---	0.044	---	0.242	---	0.472	---	0.007
OH	0.003	0.005	0.021	0.019	0.047	0.079	0.003	0.004

(2 + 3) isomer 2 and/or isomer 3

 F = [furanone-4-¹⁴C]-label P = [pyridinylmethyl-¹⁴C]-label

Compound (BYI 02960-)	Swiss chard intermediate		Swiss chard mature		turnip leaves		turnip roots	
Label	F	P	F	P	F	P	F	P
TRR	0.311	0.332	0.263	0.438	0.158	0.230	0.014	0.022
BYI 02960	0.171	0.170	0.072	0.108	0.108	0.153	0.004	0.011
DFA/carbohydr.	0.12 ¹		0.15 ¹		0.09 ¹		<0.03 ¹	
glucose	---		---		---		0.002	
bromo-amino-furanone	---		---		---		---	
difluoroethyl-amino-furanone	0.032		0.046		0.002		---	
glyoxylic acid	---	---	---	---	0.002	---	<0.001	---
OH-glyc	0.036	0.058	0.047	0.111	0.020	0.025	<0.001	0.001
6-CNA-glycerol-gluA (2 + 3)		---		---		---		---
OH	0.006	0.013	0.006	0.016	0.002	0.004	---	---

¹ LC-MS/MS analysis of non-radiolabelled DFA in the extract obtained in the confined rotation crop study performed with [furanone-4-¹⁴C]BYI 02960

(2 + 3) isomer 2 and/or isomer 3

 F = [furanone-4-¹⁴C]-label P = [pyridinylmethyl-¹⁴C]-label



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Table 6.11.1-10 Residues [mg/kg] of BYI 02960 and major metabolites in the matrices of rotational crops (3rd rotation)

Compound (BYI 02960-)	wheat forage		wheat hay		wheat straw		wheat grain	
Label	F	P	F	P	F	P	F	P
TRR	0.111	0.117	0.254	0.321	0.462	0.491	0.047	0.017
BYI 02960	0.048	0.053	0.047	0.063	0.096	0.129	0.001	0.002
DFA	<0.03 ¹		0.03 ¹		0.06 ¹		0.15 ¹	
glucose/carbohydr.	---		---		---		---	
bromo-amino-furanone	0.006		0.027		0.034		---	
difluoroethyl-amino-furanone	0.013		0.021		0.024		---	
glyoxylic acid	---	0.001	0.001	---	---	0.013	---	---
OH-glyc	0.003	0.004	0.006	0.010	0.016	0.014	0.001	0.001
6-CNA-glycerol-gluA (2 + 3)		0.022		0.095		0.140		0.002
OH	0.001	0.001	0.004	0.005	0.010	0.010	0.001	0.001

(2 + 3) isomer 2 and/or isomer 3

F = [furanone-4-¹⁴C]-label P = [pyridinylmethyl-¹⁴C]-label

Compound (BYI 02960-)	Swiss chard intermediate		Swiss chard mature		turnip leaves		turnip roots	
Label	F	P	F	P	F	P	F	P
TRR	0.180	0.135	0.152	0.130	0.090	0.083	0.008	0.008
BYI 02960	0.066	0.042	0.051	0.036	0.065	0.058	0.006	0.005
DFA	<0.03 ¹		0.03 ¹		<0.03 ¹		<0.03 ¹	
glucose/carbohydr.	---		---		---		<0.001	
bromo-amino-furanone	---		---		---		---	
difluoroethyl-amino-furanone	0.025		0.024		0.001		---	
glyoxylic acid	<0.001	0.003	---	0.001	0.002	---	---	---
OH-glyc	0.040	0.033	0.033	0.036	0.009	0.008	<0.001	<0.001
6-CNA-glycerol-gluA (2 + 3)		---		---		---	---	---
OH	0.007	0.005	0.005	0.004	0.001	---	---	---

¹ LC-MS/MS analysis of non-radiolabelled DFA in the extract obtained in the confined rotation crop study performed with [furanone-4-¹⁴C]BYI 02960

(2 + 3) isomer 2 and/or isomer 3

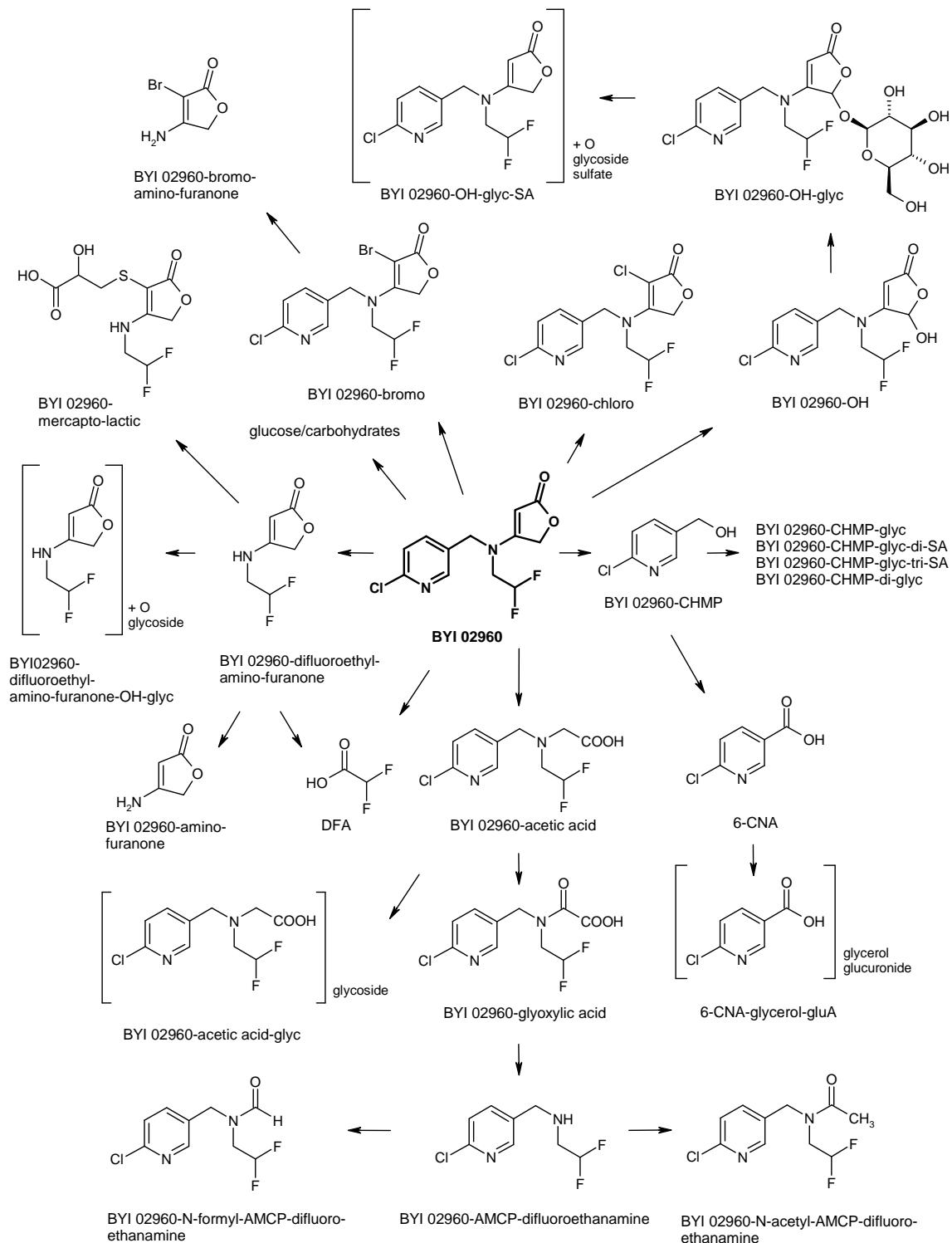
F = [furanone-4-¹⁴C]-label P = [pyridinylmethyl-¹⁴C]-label

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

A pathway for succeeding crops is proposed in Figure 6.11.1-3.

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Figure 6.11.1-3 Proposed metabolic pathway of BYI 02960 in confined rotational crops





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As field rotational crop studies became necessary, residue data have been collected on parent BYI 02960, difluoroacetic acid and BYI 02960-difluoroethyl-amino-furanone. These three compounds are adequate to assess the dietary risk of BYI 02960. Parent compound and difluoroacetic acid are major components detected in all RACs of all rotations. Since BYI 02960-difluoroethyl-amino-furanone occurred in high levels in Swiss chard matrices (representative for leafy crops) and first analysis on difluoroacetic acid indicated only marginal levels of this metabolite in these matrices, BYI 02960-difluoroethyl-amino-furanone was chosen as additional component to represent the relevant **residue definition for data gathering** and **risk assessment** in plants. All three compounds are specific to the use of BYI 02960 and suitable marker compounds for estimating residue levels of other metabolites, if necessary. All other metabolites, not included in the residue definition, show no toxicity concerns nor are they generally observed across most crop matrices.

Toxicological coverage of metabolites was either shown in the rat ADME studies, in additional toxicological tests or by estimating the toxicological concern of the metabolite for the consumer by applying the TTC (Threshold of Toxicological Concern) concept (see Section 3 KIIA 5.10).

Studies were conducted to assess the **storage stability** of frozen samples. During a storage period of 12 months under deep-freezer conditions, the components of the relevant residues of BYI 02960 (including parent compound, BYI 02960-difluoroethyl-amino-furanone, and DFA) were stable in orange fruit, spinach leaves and tomato fruit, wheat grain, bean seed, coffee bean and soybean seed, and sugar cane, representing a wide array of plant-based sample materials. (This study will be continued until a final storage period of 2 years.) In addition, over a storage period of 43 days under deep-freezer conditions, DFA was shown to be stable in bovine fat, kidney, liver, and muscle. (Animal matrices were not stored for longer periods in the studies presented in this dossier.) These results validate the residue values reported in supervised field trials, processing studies, and feeding studies with respect to storage stability of samples frozen prior to analysis.

Full sets of **field residue trials** were presented, allowing the evaluation of the residue behaviour of BYI 02960 in the "safe use" crops lettuce and hops. In *lettuce*, trials were conducted in both the northern and southern European "residue" regions as well as in the greenhouse, to support both an "agricultural" use and a "home & garden" use. The most critical "region" proved to be the greenhouse; total residues of BYI 02960 dissipated rapidly in lettuce heads, to levels of 0.80-6.0 mg/kg on day 3 (envisaged PHI). The median value (STMR) in these trials was 2.2 mg/kg.

In *hops*, a full set of trials was conducted in the northern "residue" region; total residues of BYI 02960 dissipated rapidly in green cones, to values of <0.40-0.87 mg/kg on day 21 (envisaged PHI). In dried cones, relevant residue levels on day 21 (day 28 in 3 trials) ranged from 0.61-2.4 mg/kg. The respective median values were 0.47 and 1.2 mg/kg.

Based on these trials, in which residues of BYI 02960 itself and its metabolite make up by far the major proportion of the residue in crops, the proposed **enforcement residue definition** for BYI 02960 in *plant* commodities is parent compound + DFA, expressed in parent equivalents.

Processing trials were also conducted to support the "safe uses". In a radioactive hydrolysis study simulating processing conditions, no breakdown products of BYI 02960 >0.5% were determined, thus



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confirming the residue definition's validity for use in processing "field trials". In *lettuce*, simulated household "salad preparation" revealed that the largest proportion of the residues is on the outer leaves, which are usually removed in a first step in the kitchen. For *hops*, beer processing was conducted using simulated industrial practices. The processing factor (PF) for the relevant residues of BYI 02960 from hop cones to beer was 0.01.

Field rotational crop trials were also conducted to evaluate the effect of previous application of BYI 02960 on following crops. In large-scale trials, using multiple rotations (simulating crop failure, re-use of the field in the same season, and re-use in the following season) and multiple crop groups (a root crop, a leafy crop, and a cereal), it was evident that total residues in all relevant food and feed matrices were generally highest in the first rotation. Residues in *root*, *leafy*, and *cereal* crops due to field re-use ranged from 0.06-0.14 mg/kg, <0.04-0.16 mg/kg, and 0.11-0.65 mg/kg, respectively, in the primary food commodities. In straw and fodder, they were <0.07-0.39 mg/kg and 0.05-0.41 mg/kg, respectively.

(The crops and trials presented in this dossier represent the "safe uses" only. Further EU field residue and field rotational crop trials as well processing trials will be presented later, in a separate document. In addition, trials relevant to import tolerances will also be presented separately.)

Animal feeding studies were conducted in both dairy cattle and laying hens. The studies were conducted in a manner that allows their use in multiple registration regions (EU, NAFTA, Australia). Feeding studies should allow the evaluation of the effects of feeding a representative residue to livestock. As the primary residues of BYI 02960 in both plants and in livestock chiefly comprise parent BYI 02960 and its metabolite DFA, the feeding studies were also designed to allow "material balancing" in order to evaluate levels and calculate transfer factors for both the total residues and DFA, despite feeding parent compound only. This study design is specific to BYI 02960. Both studies showed that the primary components of the residue are, in fact, parent and DFA. Plateau residue levels were achieved for milk and eggs; residues were evaluated for all dose groups (4 per study). Transfer factors were calculated for all relevant matrices for both the total residue of BYI 02960 + DFA as well as for DFA alone. The basic study data as well as the evaluation of DFA transfer factors allowed MRL calculations for realistic worst-case animal diet conditions.

Based on these studies, in which residues of BYI 02960 itself and its metabolite make up by far the major proportion of the residue in all relevant tissues, the proposed **enforcement residue definition** for BYI 02960 in *animal* matrices is parent compound + DFA, expressed in parent equivalents.

MRLs for the uses of BYI 02960 described in this dossier (including primary and rotational crops as well as residues in edible animal commodities) were proposed, as follows:



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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

On the basis of the MRLs (as well as other relevant residue data), **dietary risk assessments** were conducted. In an evaluation of the *chronic* dietary risk, the TMDI, calculated according to the EFSA PRIMo model, amounted to between 0.8% (PL general population) and 29.4% (WHO Cluster Diet B) of the ADI. All ADI usage values in these evaluations are well below 100%; thus, a further, more refined risk assessment is not required.

As for the *acute* dietary risk, IESTI calculations (using proposed MRLs instead of HRs) indicated that the maximum contribution to the ARfD is approx. 54% for lettuce (children, IESTI 1 calculation), and thus far below 100%. Despite using the most conservative approach for the assessment of the acute risk, it is evident that no acute risk for consumers will arise from the uses of BYI 02960 as presented in this dossier.

IIA 6.11.2 Reasonable grounds in support of the petition

Bayer CropScience is requesting registration of BYI 02960 as an insecticide for several uses. In this Annex II dossier, only the so-called "safe uses" are described (lettuce and hops). Further data on other crops will be submitted in a separate document later in 2012.

To support this registration Bayer CropScience has evaluated the risk associated with registration on these crops. The hazard of the compound was assessed by the conduct of toxicology studies with BYI 02960 (acute, short-term and chronic) as well as respective toxicology studies with selected metabolites. Exposure to BYI 02960 residues was evaluated by the conduct of plant (primary and confined rotational crops) and animal metabolism studies to define the residues of concern followed by the conduct of field residue studies on primary and succeeding crops and feeding studies to define the magnitude of residue in food and feed items. Acute and chronic dietary exposure assessments according to the EFSA PRIMo model (revision 2) have shown that total human dietary exposure to BYI 02960 represents only a small portion of the acute and chronic reference doses even when calculating with the most conservative approaches. Occupational exposure assessments have shown acceptable Margins of Exposure for all use practices.



Bayer CropScience

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Therefore, there is reasonable certainty that no harm will result from the use of BYI 02960 when it is used according to the label.

Adequate MRLs have been proposed for all food and feed items and Bayer CropScience requests establishment of these MRLs.



List of metabolites

In the original study reports on BYI 02960 the metabolites are sometimes named by different synonyms. In order to present a common basis for the evaluation of the active substance and its degradation products a complete list of metabolites is presented.

The following abbreviations were used:

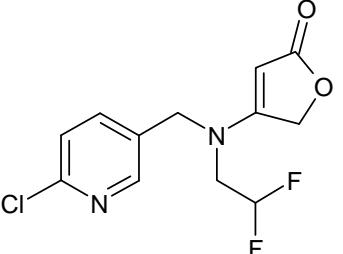
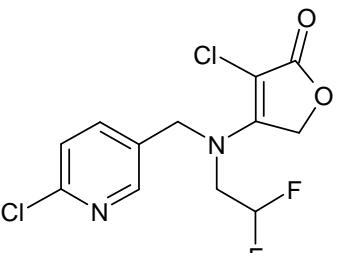
gluc = glucoside (conjugation with glucose)

glyc = glycoside (conjugation with a hexose)

gluA = glucuronide (conjugation with glucuronic acid)

OH = hydroxy

SA = sulfate

	Report name, Structure IUPAC name CAS name, [CAS number]	Molecular formula molar mass Other names / codes	Occurrence
a.s.	BYF 02960 (parent compound)  4-[[[6-chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino]furan-2(5H)-one (IUPAC) 2(5H)-furanone, 4-[[[6-chloro-3-pyridinyl)methyl](2,2-difluoroethyl)amino]- (CAS) CAS-No.: 951659-40-8	C ₁₂ H ₁₁ ClF ₂ N ₂ O ₂ 288.68 g/mol flupyradifurone	all matrices
M01	BYI 02960-chloro  3-chloro-4-[(6-chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino}furan-2(5H)-one	C ₁₂ H ₁₀ Cl ₂ F ₂ N ₂ O ₂ 323.13 g/mol BCS-CD27046	Plant: Swiss chard (CRC) (co-elution with BYI 02960-bromo) Environment: aerobic soil (minor)



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	Report name, Structure IUPAC name CAS name, [CAS number]	Molecular formula molar mass Other names / codes	Occurrence
M02	BYI 02960-bromo 3-bromo-4-[(6-chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino)furan-2(5H)-one	C ₁₂ H ₁₀ BrClF ₂ N ₂ O ₂ 367.58 g/mol BCS-CD27042	Plant: Swiss chard (CRC) (co-elution with BYI 02960-chloro)
M03	BYI 02960-OH 4-[(6-chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino]-5-hydroxyfuran-2(5H)-one	C ₁₂ H ₁₁ ClF ₂ N ₂ O ₃ 304.68 g/mol BYI 02960-hydroxy BCS-CQ74364	<u>Animal:</u> rat lactating goat laying hen <u>Plant:</u> tomato potato apple cotton rice Swiss chard (CRC) turnips (CRC) wheat (CRC)
M04	BYI 02960-OH-gluA (isomer 1) + O glucuronide	C ₁₈ H ₁₉ ClF ₂ N ₂ O ₉ 480.81	Animal: rat lactating goat



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	Report name, Structure IUPAC name CAS name, [CAS number]	Molecular formula molar mass Other names / codes	Occurrence
M05	BYI 02960-OH-gluA (isomer 2) 3-{{(6-chloropyridin-3-yl) methyl}(2,2-difluoroethyl) amino}-5-oxo-2,5-dihydrofuran-2-yl beta-D-glucopyranosiduronate	C ₁₈ H ₁₉ ClF ₂ N ₂ O ₉ 480.81	Animal: lactating goat
M06	BYI 02960-OH-gluA (isomer 3) 3-{{(6-chloropyridin-3-yl) methyl}(2,2-difluoroethyl) amino}-5-oxo-2,5-dihydrofuran-2-yl beta-D-glucopyranosiduronate	C ₁₈ H ₁₉ ClF ₂ N ₂ O ₉ 480.81	Animal: rat lactating goat
M07	BYI 02960-OH-gluA (isomer 4) 3-{{(6-chloropyridin-3-yl) methyl}(2,2-difluoroethyl) amino}-5-oxo-2,5-dihydrofuran-2-yl beta-D-glucopyranoside + O-glucuronide	C ₁₈ H ₁₉ ClF ₂ N ₂ O ₉ 480.81	Animal: lactating goat
M08	BYI 02960-OH-glyc 3-{{(6-chloropyridin-3-yl)methyl}(2,2-difluoroethyl)amino}-5-oxo-2,5-dihydrofuran-2-yl beta-D-glucopyranoside	C ₁₈ H ₂₁ ClF ₂ N ₂ O ₈ 466.83 g/mol BYI 02960-OH-gluC BCS-CR46036	Plant: tomato potato apple cotton (co-elution with BYI 02960-acetic acid) Swiss chard (CRC) turnips (CRC) wheat (CRC)

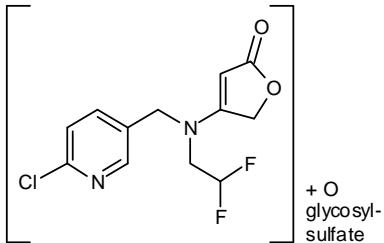
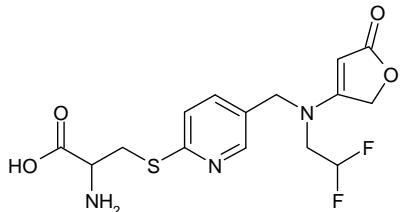
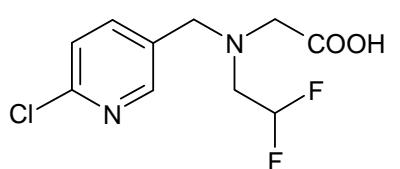
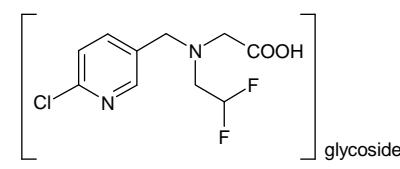


Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

	Report name, Structure IUPAC name CAS name, [CAS number]	Molecular formula molar mass Other names / codes	Occurrence
M09	BYI 02960-OH-SA 	C ₁₂ H ₁₁ ClF ₂ N ₂ O ₆ S 384.75 g/mol	Animal: rat laying hen
M10	BYI 02960-iso-OH 	C ₁₂ H ₁₁ ClF ₂ N ₂ O ₃ 304.68 g/mol	Animal: rat lactating goat
M11	BYI 02960-difluoroethyl-OH-glyc 	C ₁₈ H ₂₁ ClF ₂ N ₂ O ₈ 466.83 g/mol	Plant: apple
M12	BYI 02960-OH-glyc-SA (isomer 1) 	C ₁₈ H ₂₁ ClF ₂ N ₂ O ₁₁ S 546.89 g/mol	Plant: Swiss chard (CRC) turnips (CRC)



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

	Report name, Structure IUPAC name CAS name, [CAS number]	Molecular formula molar mass Other names / codes	Occurrence
M13	BYI 02960-OH-glyc-SA (isomer 2)  + O glycosyl-sulfate	C ₁₈ H ₂₁ ClF ₂ N ₂ O ₁₁ S 546.89 g/mol	Plant: Swiss chard (CRC) turnips (CRC) wheat (CRC)
M14	BYI 02960-cysteine  S-(5-{[(2,2-difluoroethyl)(5-oxo-2,5-dihydrofuran-3-yl)amino]methyl}pyridin-2-yl)cysteine	C ₁₅ H ₁₇ F ₂ N ₃ O ₄ S 373.38 g/mol	Animal: lacating goat
M15	BYI 02960-acetic acid  N-[(6-chloropyridin-3-yl)methyl]-N-(2,2-difluoroethyl)glycine	C ₁₀ H ₁₁ ClF ₂ N ₂ O ₂ 264.66 g/mol BCS-CQ74373	Plant: apple rice cotton (co-elution with BYI 02960-OH-glyc) Swiss chard (CRC) turnips (CRC) wheat (CRC) Animal: laying hen
M16	BYI 02960-acetic acid-glyc 	C ₁₆ H ₂₁ ClF ₂ N ₂ O ₇ 426.8 g/mol	Plant: apple rice cotton Swiss chard (CRC) turnips (CRC) wheat (CRC)

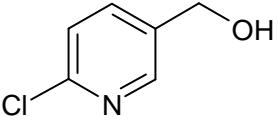
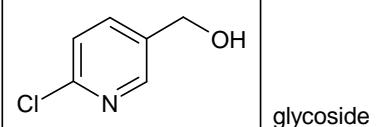
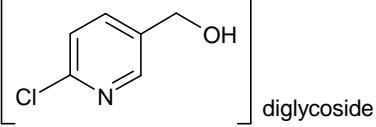
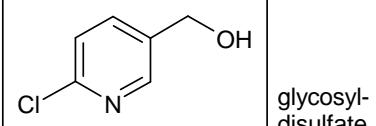
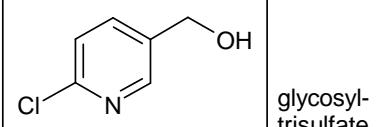


Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

	Report name, Structure IUPAC name CAS name, [CAS number]	Molecular formula molar mass Other names / codes	Occurrence
M17	BYI 02960-glyoxylic acid 	C ₁₀ H ₉ ClF ₂ N ₂ O ₃ 278.64 g/mol BCS-CQ74372	Plant: rice cotton Swiss chard (CRC) turnips (CRC) wheat (CRC)
M18	BYI 02960-methylthio-glyoxylic acid 	C ₉ H ₁₀ N ₂ O ₃ S 278.64 g/mol	Animal: lactating goat
M19	BYI 02960-des-difluoroethyl 	C ₁₀ H ₉ ClN ₂ O ₂ 224.65 g/mol BCS-AB49019	Animal: rat lactating goat laying hen Environment: Aerobic soil (minor)
M20	BYI 02960-des-difluoroethyl-OH-SA 	C ₁₀ H ₉ ClN ₂ O ₆ S 320.71 g/mol	Animal: laying hen



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

	Report name, Structure IUPAC name CAS name, [CAS number]	Molecular formula molar mass Other names / codes	Occurrence
M21	BYI 02960-CHMP  6-chloropyridin-3-ylmethanol (IUPAC) 3-pyridinemethanol, 6-chloro- (CAS) CAS-No.: 21543-49-7	C ₆ H ₆ ClN O 143.57 g/mol BCS-AA52175 6-CPA CPA (6-chloro-3-pyridyl)methanol BCS-AA52175 IM-0	Plant: tomato potato apple turnips (CRC) wheat (CRC)
M22	BYI 02960-CHMP-glyc 	C ₁₂ H ₁₆ ClN O ₆ 305.72 g/mol	Plant: tomato potato apple
M23	BYI 02960-CHMP-di-glyc 	C ₁₈ H ₂₆ ClNO ₁₁ 467.86 g/mol	Plant: tomato potato apple Swiss chard (CRC) turnips (CRC) wheat (CRC)
M24	BYI 02960-CHMP-glyc-di-SA 	C ₁₂ H ₁₆ ClN O ₁₂ S ₂ 465.84 g/mol	Plant: Swiss chard (CRC)
M25	BYI 02960-CHMP-glyc-tri-SA 	C ₁₂ H ₁₆ ClN O ₁₅ S ₃ 545.9 g/mol	Plant: Swiss chard (CRC)



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

	Report name, Structure IUPAC name CAS name, [CAS number]	Molecular formula molar mass Other names / codes	Occurrence
M26	BYI 02960-CHMP-serinate 	C ₉ H ₁₁ ClN ₂ O ₃ 230.65 g/mol	Animal: laying hen
M27	6-CNA 6-chloronicotinic acid (IUPAC) CAS-No.: 5326-23-8	C ₆ H ₄ ClN ₂ O ₂ 157.56 g/mol 6-chloronicotinic acid IC-0 BYI 02960-6-CNA BCS-AA35572	Animal: rat laying hen Plant: tomato potato apple cotton rice Swiss chard (CRC) turnips (CRC) wheat (CRC) Environment Aerobic soil (major)
M28	BYI 02960-6-CNA-glycerol-gluA (isomer 1) 	C ₁₅ H ₁₈ ClN ₂ O ₁₀ 407.76 g/mol	Plant: potato (isomer was not assigned) wheat (CRC)
M29	BYI 02960-6-CNA-glycerol-gluA (isomer 2 & 3) 	C ₁₅ H ₁₈ ClN ₂ O ₁₀ 407.76 g/mol	Plant: wheat (CRC)



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

	Report name, Structure IUPAC name CAS name, [CAS number]	Molecular formula molar mass Other names / codes	Occurrence
M30	BYI 02960-hippuric acid N-[(6-chloropyridin-3-yl)carbonyl]glycine	C8 H7 Cl N2 O3 214.61 g/mol	Animal: rat lactating goat
M31	BYI 02960-cysteinyl-nicotinic acid 6-[(2-amino-2-carboxyethyl)sulfanyl]nicotinic acid	C9 H10 N2 O4 S 242.26 g/mol	Animal: lactating goat
M32	BYI 02960-acetyl-cysteinyl-nicotinic acid 6-[(2-acetyl-2-carboxyethyl)sulfanyl]nicotinic acid	C11 H12 N2 O5 S 284.29 g/mol	Animal: laying hen
M33	BYI 02960-lactato-mercaptyl-nicotinic acid 6-[(2-hydroxy-2-carboxyethyl)sulfanyl]nicotinic acid	C9 H9 N O5 S 243.24 g/mol	Animal: laying hen

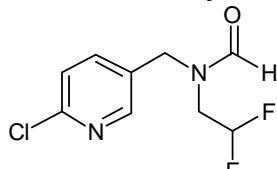
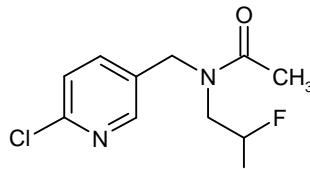
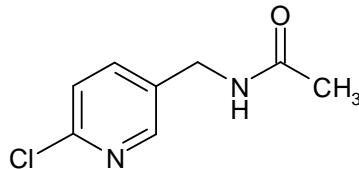
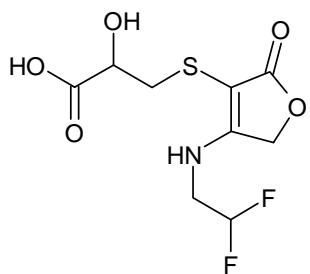


Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

	Report name, Structure IUPAC name CAS name, [CAS number]	Molecular formula molar mass Other names / codes	Occurrence
M34	BYI 02960-difluoroethyl-amino-furanone 	C ₆ H ₇ F ₂ N O ₂ 163.12 g/mol DFEAF BCS-CC98193	Plant: tomato potato apple Swiss chard (CRC) turnips (CRC) wheat (CRC) Animal: rat
M35	BYI 02960-difluoroethyl-amino-furanone-OH-glyc 	C ₁₂ H ₁₇ F ₂ N O ₈ 341.27 g/mol	Plant: Swiss chard (CRC) wheat (CRC)
M36	BYI 02960-AMCP-difluoroethanamine 	C ₈ H ₉ ClF ₂ N ₂ 206.62 g/mol BCS-AA10710	Plant: apple Animal: lactating goat
M37	BYI 02960-AMCP-difluoroethanamine-SA 	C ₈ H ₉ ClF ₂ N ₂ O ₃ S 286.69 g/mol	Animal: laying hen

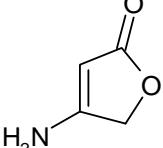
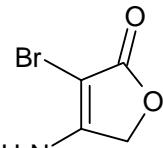
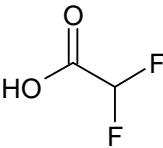
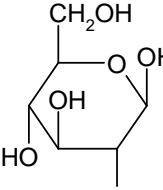


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	Report name, Structure IUPAC name CAS name, [CAS number]	Molecular formula molar mass Other names / codes	Occurrence
M38	BYI 02960-N-formyl-AMCP-difluoroethanamine  N-[(6-chloropyridin-3-yl)methyl]-N-(2,2-difluoroethyl)formamide	C ₉ H ₉ ClF ₂ N ₂ O 234.63 g/mol	Plant: Swiss chard (CRC) turnips (CRC)
M39	BYI 02960-N-acetyl-AMCP-difluoroethanamine  N-[(6-chloropyridin-3-yl)methyl]-N-(2,2-difluoroethyl)acetamide	C ₁₀ H ₁₁ ClF ₂ N ₂ O 248.66 g/mol	Plant: Swiss chard (CRC) turnips (CRC)
M40	BYI 02960-acetyl-AMCP 	C ₈ H ₉ ClN ₂ O 184.63 g/mol AMCP BYI 02960-AMCP	Animal: laying hen
M41	BYI 02960-mercapto-lactic acid  3-((4-[(2,2-difluoroethyl)amino]-2-oxo-2,5-dihydrofuran-3-yl)sulfanyl)-2-hydroxypropanoic acid	C ₉ H ₁₁ F ₂ NO ₅ S 283.25 g/mol	Plant: wheat (CRC)



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	Report name, Structure IUPAC name CAS name, [CAS number]	Molecular formula molar mass Other names / codes	Occurrence
M42	BYI 02960-amino-furanone  4-aminofuran-2(5H)-one	C ₄ H ₅ N O ₂ 99.09 g/mol	Plant: Swiss chard (CRC) turnips (CRC) wheat (CRC)
M43	BYI 02960-bromo-amino-furanone  4-amino-3-bromofuran-2(5H)-one	C ₄ H ₄ Br N O ₂ 177.99 g/mol	Plant: wheat (CRC)
M44	DFA  difluoroacetic acid (IUPAC) Acetic acid, 2,2-difluoro- (CAS) CAS-No.: 381-73-7	C ₂ H ₂ F ₂ O ₂ 96.03 g/mol difluoroacetic acid BYI 02960-DFA BYI 02960- difluoroacetic acid BYI 0260-Difluoro- acetic-acid BCS-AA56716	Animal: rat lactating goat laying hen Plant: tomato potato apple cotton rice Swiss chard (CRC) turnips (CRC) wheat (CRC) Environment aerobic soil (major) aerobic water/sediment (major)
M45	Glucose (hexose)  D-glucose assumed structure	C ₆ H ₁₂ O ₆ 180.16 g/mol glucose/carbohydrates D-glucose	Plant: tomato apple rice turnips (CRC) wheat (CRC)



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

	Report name, Structure IUPAC name CAS name, [CAS number]	Molecular formula molar mass Other names / codes	Occurrence
M46	Lactose 	C ₁₀ H ₁₈ O ₁₁ 314.25 g/mol	Animal: lactating goat
M47	BYI 02960-azabicyclosuccinamide 	C ₁₂ H ₁₄ F ₂ N ₂ O ₄ 288.25 g/mol BCS-CS64875	Environment: water – aquatic photolysis (major)
M48	BYI 02960-succinamide 	C ₁₂ H ₁₃ ClF ₂ N ₂ O ₃ 306.69 g/mol BCS-CR74729	Environment: water – aquatic photolysis (major)
M49	BYI 02960-deschlorohydroxysuccinamide 	C ₁₂ H ₁₄ F ₂ N ₂ O ₄ 288.25 g/mol DCHS	Environment: water – aquatic photolysis (minor)