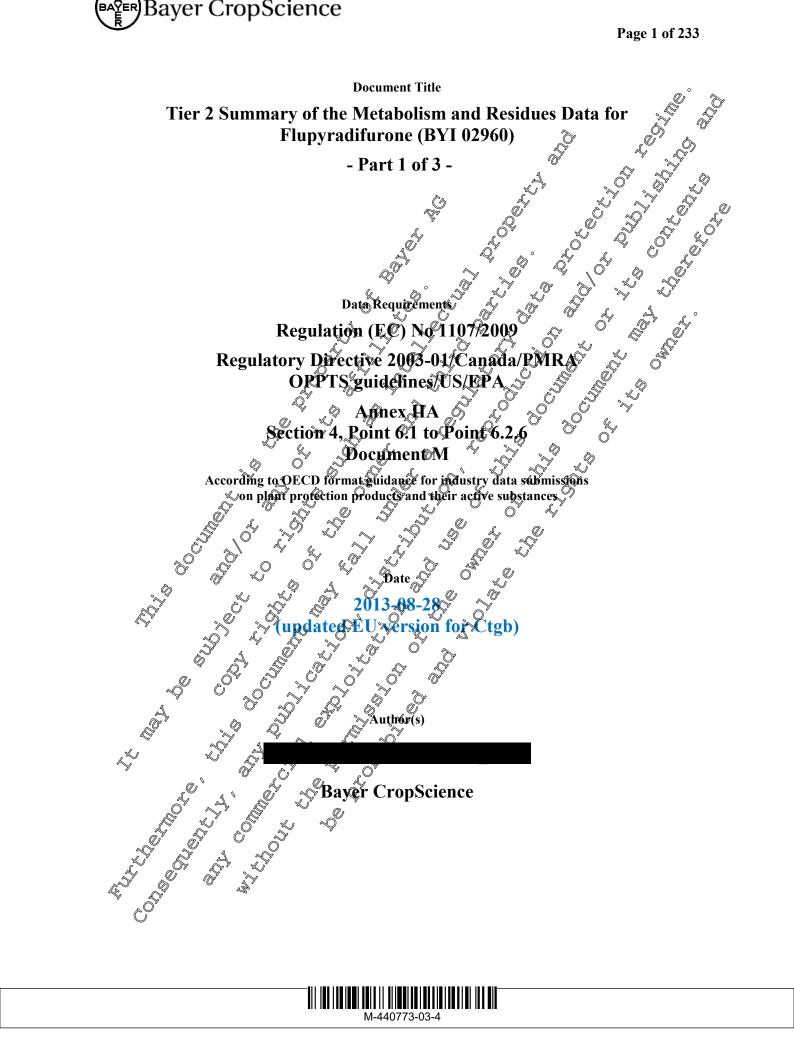


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Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Bayer CropScience

IIA 6 Metabons... 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 routined, stored frozen for home periods of time prior to their analysis, the effects of frozen storage on the residue levels were the frozen for the prior to the effects of frozen storage on the residue levels were the frozen for the prior to the effects of frozen storage on the residue levels were the frozen for the prior to the effects of frozen storage on the residue levels were the frozen for the prior to the effects of frozen storage on the residue levels were the frozen for the prior to the effects of frozen storage on the residue levels were the frozen for the prior to the effects of frozen storage on the residue levels were the frozen for the prior to the effects of frozen storage on the residue levels were the frozen for the prior to the effects of frozen storage on the residue levels were the frozen for the prior to the effects of frozen storage on the residue levels were the frozen for the prior for the prio

In this summary section (KIIA 6.1.1), the name by EAF will be used for the metabolite BXF 02966 difluoroethyl-amino-furanone, which is relevant to the tested residue definition: <u>Name</u> <u>Metab. No.</u> <u>Standardy "dosvier name"</u> DFEAF <u>M34</u> BYI 02960-diffuoroethyl-amino-furanone

Name	
DFEAF	

Ietab. No.	
M34	

► 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 below. Table 6.1 1 a suprimarizes the storage period of matrices obtained in European field hials, whereas table 6.1.1-16 refer to uses outside Europe (uses of the Global Jont Review Submission partner countries

Table 6.1.1-1a: EV uses Periods of frozen storage (Approx \$18° Of of plant-based samples (between Sampling and analysis) for matrices relevant to this dossier

Sample material & A	Longest storage	St St	udy
Erop Watrix	duration (t)	Report no.	Annex point KIIA
Lettuce Chead Contraction State	y 300 A	10-2503	6.6.3.1.1/01
inner leaves washed inner leaves		10-3223	6.5.4.1/01
withing water	225	10-3223	6.5.4.1/01
hops dried cone y	219	10-3407	6.5.4.2/01
dried cone 2 2	223	10-2225	6.3.1.2/01
apple	220	10-3407	6.5.4.2/01
apple	131	11-2077	6.3.1.3/02
apple fruit peeled fruit peeled fruit peeled fruit	159	10-3171	6.5.4.4/01
		Са	ontinued on next

Continued on next page...

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

Table 6.1.1-1a (cont'd):EU uses: Periods of frozen storage (5pprox.. -18°C) of plant-based samples
(between sampling and analysis) for matrices relevant to this dossier

S	Sample material		Stu	dy Annex point KIIA 6.5.49/02 6.5.49/02 6.5.49/02 6.5.49/02 6.5.49/02 6.5.49/02 6.5.49/02 6.5.49/02	Ŭ 🏠
Crop	Crop Matrix duration Report no. An		Annex point	Or .	
	TVIAU IX	(d)	<u> </u>	KIIA	Ô
apple	washed whole fruit		Õ	6.5.49/02 6 ⁴ 6	d a a a a a a a a a a a a a a a a a a a
(con'd)	raw juice		4	. 64 24	<i>Q</i>
	juice	ČĄ	Å.		Å.
	raw sauce	- The second sec			
	sauce	264 A		6549402	K ^O '
	washings	A. ~~	6° 4	L 0	s O í
	wet pomace				D.
	dried pomace 🔬	6 5		di se	
	retentate			6 A	e °
	stain rest			<u> </u>	
grape	bunch of grape	273		6.5.4.6/01	
	berry				
	berry raisin			\$ 49	
	must wine at botteling				
	must wine at botteling pomace	2730 2730 A	10-0406	6.5,4.6/01	
	pomace 2	19 09 ⁴ 9	× 0	N N N N N N N N N N N N N N N N N N N	
	pomace			Ş	
				2	
	jetty 5 0 Spice, pasteurised	260 O	\$0-340¢	6.5.4.6/01	
	wine of first taste test	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	10-32406		
blueberry	i fisult 4 & A	495 54 295 54	₩ ² 4PR	6.3.2.5/01	
blueberry		195 5 2123	NO.10637		
tomato	Ofruit Q A	323	\mathbb{N} $11-2007$	6.3.1.5/02	
	washed whole fruit				
	-peeled front				
	Traw Juffee				
	Q JUICA A A A	4 4			
	in purse of o		10.0107	6.5.4.0/01	
v A	puree of the former of the for	320	10-3186	6.5.4.8/01	
J.	preserve of or or	, K			
		~9 [×]			
L.	stain rest	y y			
, ,	wasaongs & V				
L. L	Arial fort				
	fruit washed whole fruit raw julice julice raw purce purce preserve peel stain rest washings peeling water dried fruit pasto	259	10-3186	6.5.4.8/01	
nepper a	fruit	368	11-2081	6.3.1.6/03	
N N		500			
	A A A		Con	ntinued on next pag	ge
	45				
Ô					

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

Table 6.1.1-1a (cont'd):EU uses: Periods of frozen storage (6pprox.. -18°C) of plant-based samples
(between sampling and analysis) for matrices relevant to this dossier

\$	Sample material	Longest storage	Study Study
Crop	Matrix	duration (d)	Report no. Annex point 7
cucumber	fruit	226	11-206
	washed whole fruit		
	fermented fruit	ČA	
	preserve	231	0-3184 65.4.9(b) 0 - 3184 65.4.9(b) 0 - 3186
	brine	4	
	washings	A Q	
watermelon	whole fruit	353 🥎	0-3184 05.4.9/01 0-3184 05.4.9/01 0 0 0 0 0 0 1.1.8/04 0 0 0 0 0 0 0 0 0 0 0 0 0
	pulp 👔	\$ 386.5	6.3.1.8/04
	peel	386 ~~	
barley	grain 🔬		A10-2508 6.6.3.1.1891
	straw		A10-250 6.0.3.1.1001
	green material	540	10,2503 6.6.2,1.1/01
carrot	root O ^V	·S 29 .0	19-2503 6 5.1.1/01
turnip	root (body)	م 180 م	010-2503 0.6.3.1 01
	leaf dia a construction of the construction of	\$ 177 \$	10-2503 0 6.6.2.1.1/01
potato	tuber	200	6. CB. 1. 2/01
leek	Whole plant without roots	Q 45	6.3.1.3/01
cucumber	fruit⁄y 🐧 🖉	× 117 × ×	11-2552 6.6.3.1.4/01
onion	bull of o	2° 29 ×	<u>(11-2553</u> 6.6.3.1.5/01
bean	Pod 2 Q	\$¥14	Q1-255 6.6.3.1.6/01
pea	S dry seed in the second	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	11-2556 6.6.3.1.7/01
winter rape	O seed & k, Y	117 A	12,2554 6.6.3.1.8/01
Č,			<u> </u>

Table 6 4 1-1b: Uses of the GIR partner countries: Reriods of frozen storage of plant-based samples (between sampling and analysis) for matrices retevant to this dossier

	Soviale material		St	udy
Crop	Sample material		Report no.	Annex point KIIA
NAFTA countries		? . Ø		
orange	fruite Q Q	351	RARVY012	6.3.2.1/01
stration − strategy −	pulp y y Q	352	-	
	dried pomace 0	280		
^A	Aashings a	279		
	marpalade , ~	276		
Ű,	washed per	272	RARVY035	6.5.4.3/02
	peel without oil	269	KAK V 1055	0.3.4.3/02
	Swet pomace	268		
	stain rest	267		
Ô	raw juice	266		

Continued on next page ...

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

Table 6.1.1-1b (cont'd):Uses of the GJR partner countries: Periods of frozen storage of plant-based
samples (between sampling and analysis) for matrices relevant to this dossier

Sample material		Longest storage	Study
Crop	Matrix	duration	Report no. Annex point
•		(d)	KIIA
NAFTA countries			
orange	unwashed ripe peel		
(cont'd)	oil	20%	
	washed fruit	T	RARVY035 \$5.4.30
	juice	£ 259	
	dried pomace	A 246 Q	
grapefruit	fruit	230 ~	RARVY012 6.3.2.1/01
lemon	fruit 🔬	<u>8</u> 235	
mandarin	fruit O	2 198	RAR P064 6.3.2.1/02.
almond	nutmeat without shell	<u></u> 491 Q	RARVY 016 6.3.2.2/01
pecan	nutmeat without shelk	× × 364 O	
apple	fruit	<u>لي 170 مح</u>	RARVY018 6.22.3/01
pear	fruit C X	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
grape	bunch of grap	271 Š	BARV 907 6.3.2 4 01
blueberry	fruit O L O	8 179 S	IR OPR 6 52.5/01
prickly pear cactus	fruit	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	1R-4PR 5.3.2.6/01
	pad y	× 101 × ×	VNO.10722 50.3.2.6/01
potato	tuber 🖓 🖗 Ö	318 5	RARVY019 6.3.2.7/01
	Grisps (chips)	\$ \$46	O Y
	Swet peel 🔊 🔊 🥿	<u>\$</u> 214	ý "Ø
Ő	cooked tuber with peel	A 212 A	
	Cooking Water O &		\mathbb{Q}
EG EG		ý ² 211	RARVY038 6.5.4.16/01
	steamed, mashed tuber		• KARV 1058 0.5.4.10/01
li v V	starch s	م م م م	
م م	Stlakes Star		
Ę	peeted tuber	2020	
Ŵ	Washed wher . O	<u>Ö</u> [®] 1999	
tomato	fruit of of o	266	
bell pepper	fruit J J	يش 247	RARVY022 6.3.2.8/01
chili 🖉	fruit V	~ ⁹ 189	0.5.2.0/01
<u></u>	Aried trait 0 0) 197	
celery	stalk C	238	RARVY005 6.3.2.9/01
bean 0 4	Arv see	243	
pea 🖧 🖄	dry fed 5	259	RARVY028 6.3.2.11/01
pea 4 Gy		1	Continued on next page
\cup			

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

Table 6.1.1-1b (cont'd):Uses of the GJR partner countries: Periods of frozen storage of plant-based
samples (between sampling and analysis) for matrices relevant to this dossier

Sa	ample material	Longest storage	Stu	ıdy
Сгор	Matrix	duration	Report no.	Annex point
-		(d)	<u> </u>	KIIA
NAFTA countries		1	- O ^y	
peanut	seed	217	RARV\$010	6.3.2 2/01
	peanut butter	395	, A	
	meal	390	Q	
	nut without shell		RÅRVY032	6.5.4 5 /01 5 ¢
	refined oil	389 - Q		6.3.2 2/01 2 4 6.3.2 2/01 2 4 6.5.4 65/01 5 4 5 6.5.4 65/01 5 5 6.5.4 5 5 6.5
	roasted peanut	465 m		
soybean	meal			Ŷ`ŶĬ
	defatted flour O	× 26 0	6 °° °°	6.9.2.13 (°
	dry seed		RARVY011	6.9.2.13 Q9
	refined oil	262		
	milk 2			
cotton	undelinted seed	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	RARVYOLI	6.9.2.13 (9) ^v ^v ^v ^v ^v ^v ^v ^v
	gin byproduct Q			Ö 😽
	meal Q V O	4540		
	hull	450 03	RARVY@3	6.5.4.14/01
	preclarified oil	445		6.5.4.14/01
	neutratised crude oil	445.cs		ÿ
	sour extracted of a			
1 1		<u>چَکْ مَحْطًا</u>		(2 2 15/01
barley	keynel and čob, húsked	× 401 ¢ × 325 ¢	RARV 7001	6.3.2.15/01
corn	keynel and cob, husked	2 325 <u>23</u> 2 469	RARVY002	6.3.2.16/01
		\$69 5 ¹⁰	RARVY004	(2 2 17/01
sorghum 🖉	grain S		KAKVY004	6.3.2.17/01
wheat	graun (C)	\$390 (694) ¹		
, , , , , , , , , , , , , , , , , , ,	Stresh pasta	بَ ^م (426 مُ		
C				
() I	Cooked dreed pasta	5 ⁵⁷ 45 ⁷		
~\$	whotomeal Q			
A	white flour	408	RARVY003	6.3.2.18/01
	White bread ~		KAR V 1005	0.3.2.10/01
, KJ	Wholemeal bread	387		
~	starch	412		
, O	ooking water S	412	1	
, O ^Y	shorts			
		408		
coffee 5 5	green bear	115		
	- Toasted bean	121	RARVP074	6.3.2.19/01
	instant coffee	119		
sugarça	stalk	178	RARVX001	6.5.4.18/01
hops	kiln-dried cone	226	RARVY008	6.3.2.20/01
1000		220	101111111000	0.3.2.20/01

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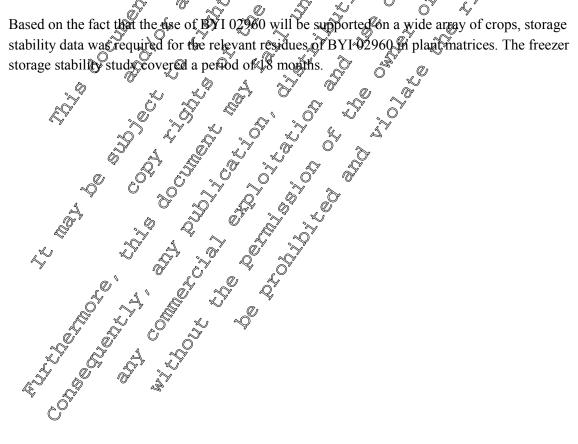
Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

Table 6.1.1-1b (cont'd): Uses of the GJR partner countries: Periods of frozen storage of plant-based samples (between sampling and analysis) for matrices relevant to this dossier

Sample material		Longest storage	Stu	udy
Сгор	Matrix	duration (d)	Report no.	Annex point 7
Brazil			Ô	
coffee	bean	152	I11 -0 08	6.3.2 0/02
orange	fruit	127	110-006	6,2,2.1/02
Guatemala,		Ţ		
coffee	green bean	×113	RARVP075	\$~6.3.2\$\$P/01 \$ \$
Chile		A Q	b d	
blueberry	fruit	242 ×	* IR-4PR NO.10537	6.3.2 09/02
Australia	0			A A A
blueberry	fruit		AIR-4PK NO.10637	6.3.2.5/01
potato	tuber tuber	2107 X	BCS-0358	6.32.7/03 C
sweet potato	tuber	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	BCS-0358	63.2.7/03
tomato	fruit 🔏 🙆 🦉	b 6250 X	BCS-048	©6.3.2.8/02
New Zealand				
blueberry	fruit to the second sec		NO.10637	¢6.3.2.5/01

1 Ő Ŝ

Based on the fact that the use of BYI 02960 will be supported on a wide array of crops, storage



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

Report:	KIIA 6.1.1/01, ; , B.C.; , A.M. 2012
Title:	Storage stability of BYI 02960, difluoroacetic acid, and difluoroethyl-amino-furanone in plant matrices (18-month data)
Report No. & Document No.:	RARVP046-1, dated November 1, 2012
	(updated version of M-428412-01-1, dated April 3, 2012
Guidelines:	 EPA Ref. OPPTS 860.1380 Storage Stability Data OECD Guideline for the Testing of Cherocals No. 506 Stability of Pesticide Residues of Stored Commodities PMRA Residue Chemistry Guidelines, Reg. Dir. 98-92, 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), spinact/leaves and tomato fruit (high water content), wheat grain (high statch content), bean seed (high protein content), cattlee bean and soybean seed (high oil content), and sugar cane were separately spiked with 50 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 three exceptions of very brief intervals at 6.5°C on April 14 at 4.2°C on August 24 and at 0.0°C on August 25, too short for the samples to thaw as documented by average temperatures and greater than-19°C on those days) for later ase. For day 0 analysis, three treated samples of each material were chosen, as well as one control sample of each. Samples were then also malysed after frominal intervals of 1, 2½, 5-6, 12 and 18 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 recovere. Samples used for concurrent recoverees 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, **1** & **1** (cf. report residue analysis of the samples) The bOQ was 0.0 kmg/kg for parent and DFEAF and 0.05 mg/kg for DFA, expressed in BX 02960 equivalents



The mean values of the concurrent recovery rates per compound, sample material, and spiking level were in the range of 81-007%, with relative standard deviations in the range of 3-14%. Details of recovery data are shown in table 6.1.1-5.

At day 0 overage 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 12 months of frozen storage (362-372 days), storage stability recoveries, corrected to day 0, ranged from 81-114% for BYI 02960, 76-90% for DFEAF, and 74-104% for DFA.

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In samples analysed after approximately 18 months of frozen storage (556-560 days), storage stability recoveries, corrected to day 0, ranged from 84-110% for BYI 02960, 81-102% for DFEAF, and 103-146% for DFA. At all sampling dates and in all sample materials, the relevant components of the \mathcal{Q} residue of BYI 02960 were above 70%. Even in the case of the lower values in the given ranges there was no evidence of any continued degradation of any of the analytes in any of the sample materials. Thus, all analytes can be considered stable in all relevant plant matrix types for a period of at least 18 months (556 to 560 days).

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

III. Conclusions residues of BYI 02960 (including parent compound DFEAF, and DFA) Were stable in orange fruit 4 spinach leaves and tomato fruit, wheat grain, bean seed coffee bean and soyoean seed, and sugarcane, representing a wide array of plant-based sample materials. These results validate the residue volues reported in all supervised field trials and processing studies with respect to storage stability of samples frozen prior to analysis.

(The storage stability study as reported and summarized in this section will be continued until a storage period of 2 years has been covered. The presented interim report submitted covers approx. 18 months of storage.) (The storage stability study as reported and summarized in this section will be continued until a

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

	Storage Concurrent Recovery in stored samples (%)				es (%)
Matrix	period	recovery		normalized	corrected
	(d)	(%)	apparent	to day 0	for recovery
sugar cane	0	(100)	100	100 🔊	100
C	29	101	92	92 🔏	S91 S
	77	91	85 🔊	85	93 ~
	149	98	94 💎	Ø4	
	372	87	93C	⁶ %93 ه	2 AP07 2
	559	89	SZ	92° °C	1040
coffee bean	0	(100)	0 ⁰ 94	<u>, 190</u> ~	0 ¹ 00
(green)	33	97	93 ° 50 0 80 ×5	× × 99 0 · ·	≥ <u></u>
	81	81		2 890 m	99
	152	85 🐊	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	Q 190	0 [×] 120 [×]
	370	93 🖉	83 2	y 788, O ^v	k) <u>89</u> (S
	560	91 0	<u>in 2</u>	0 100 0	104
orange fruit	0	(100)	\$ \$96 \$	<u>0</u> 190 S	109
	28	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	84		2° • • • •
	77	86 4	N X O	2 980 pc) <u>(</u> 111
	148	104	84	0 [×] 87	© [∞] 81
	365	02	× 80 %	83	log 79
	556°~y	80 🖗		^{الر} 94 ک	113
soybean seed	<u> </u>	(10 0)	<u> </u>	& <u>100</u>	100
	28		\$ ⁷ 93 \$	QI01 K	100
	\$ 75 F	\$93		£ 104@	104
× C		× 94	× 197 ~~	105	106
Ő) 9 6 (%)	98 0	^{ي 106}	103
	558	\$ 95 A	890	- <u> </u>	93
navy bean				<u>~ 100</u>	100
« ¥		<u>91</u>		96	117
	149			99	117
	\$ 148 70 148			111	123
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	304 558 ~~			114 84	112 94
	Q^r	J (100)	940 940 295	100	100
tomato fruit	0		≈ <u>~</u> * <del>30</del> ≈ ~92	97	100
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	276 A	<u> </u>	89	94	103
A ST	1480		0 ¹⁰⁵	111	98
	37.0 /	<u>6</u> 107	95	101	107
²	370 558	102 @	93	99	92
spinach		(100) (100)	91	100	100
	26	Q 96	97	106	100
ji G	ক্রীন্ট	90	85	93	94
	147	94	104	114	110
spinach	364	119	97	106	81
õ	557	98	100	110	103

Continued on next page ...

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

Summary of stability data for deep-frozen samples fortified with BYI 02960 Table 6.1.1-2 (cont'd): – samples fortified at 1 mg/kg

	Storage	Concurrent	Reco	very in stored sampl	es (%)
Matrix	period (d)	recovery (%)	apparent	normalized to day 0 🔊	es (%) corrected for recovery
wheat grain	0	(100)	94	100	100 . 5
	27	93	90	95 🔬	5 ⁹⁹⁷ 5
	76	89	82	87	2 93° A
	186	97	109 🕎	2 16	
	362	92	77	۵۶۶۵ ی	
	557	97	J.	<u>\$ 97.</u>	94 C C
Table 6.1.1-3:	Summary of – samples for	stability data for rtified at 1 mg/k	or deep-frozen same	~ (U^*	

	sumpres for	4			
	Storage	Concurrent		very in stored sample	es (%) & V
Matrix	period	recovery	apparent of	normalized to day 0	> corrected
	(d)	(%)		🔬 to day 0 🖉	for recovery
sugar cane	0	(100)		y 300 2	10 9
	29	97 0	\$ 960 G	⁰ 99 0	Č [°] ¥00
	77	97		Q 94 o	<u>چ</u> 94
	149 🕷	<u>لَّةُ 86° كَلَّ 286° كَلَّةُ 286° كَلَّةُ 2005</u> 097 مَنْ 2005	100	گر ×102 ⊘	116
	372 🏹		99	\$101 ×	102
	559 ^{°¥}	A 93 ·	3 187 6 ⁹	<u>4</u>	° 147
coffee bean	Å,	(100)	90	0 \$400 Y	100
(green)	³³	- 5 ⁹⁰ ~	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	98	99
	N 81 0'	× 77 ×			99
ð			2 94 2 79 2 79 2 (1) 2 (1) 2 (1) 3 (1)	sv 104	118
	A70 🔊	73	<u>,</u>	© 87	108
	56QC	ير» 85 ⁽¹⁾		120	128
orange front		× (100)	× 6 ⁹⁵ 2 ⁵	$\bigcirc^{\mathbb{Y}}$ 100	100
- 1		×98 0 ⁵	× 100 /	× 106	102
	N 77 1	Ø [*] 89 1 [*]	<u>_</u> ~ 99 ~	105	112
<i>.</i>	148	87 87 °	× \$93 \$	98	107
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	(365 °	×94 ~	×y 98	103	104
1	556 O*	~~ ⁹ 92 /		126	131
soybean seod		Q (100)	19 19	100	100
	~ ²⁸ 1	× ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	° ~ ° 71	89	79
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	75	مَنْ 93 مَنْ	× 82	103	88
(× 140	L 7 <u>5</u>	s 98	124	131
Å	371		≶ 79	99	107
	558	79	87	110	111
navy bean	× ¹ 558 × 00	(100) آھي.	96	100	100
S 2	<u>\$</u> 6 ~	» 96	99	104	104
	75	98	108	113	110
the gr	148	79	100	104	127
navy bean	364	80	78	82	98
	558	99	128	134	130

Continued on next page...

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

Summary of stability data for deep-frozen samples fortified with DFA Table 6.1.1-3 (cont'd): - samples fortified at 1 mg/kg

	Storage	Concurrent	Reco	very in stored sample	es (%)
Matrix	period (d)	recovery (%)	apparent	normalized to day 0 🔊	corrected for recovery
tomato fruit	0	(100)	101	100	100 . 9
	28	99	96	95 🔬	\$ ⁹⁹⁷ \$\$
	76	94	94 🚴	92° ″	َمَ [*] 100 م
	148	84	98 💎	Ø 6	100 107 107 107 107 107 107 107
	370	98	10,5		
	558	102	134	Q 133 C	
spinach	0	(100)		× 000 ~	<u> </u>
	26	98	<u> </u>	y 97 00	×99 5
	75	90		20 N	97
	147	85 🚄		Q 99	
	364	70 💭	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	۲ <u>۲</u> 74 °	106
	557			<u>0</u> 146	138
wheat grain	0	(100)	y . 992 J	190 S	
	27	.097	Í 08 .		× 102
	76	88	6 9 9	102 A	107
	186	78 ~	98 4	0 ⁹ 197	0 126
	362	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	2 71 W	78 2	<i>©</i> 100
	557	4 88 Q	\$ 1 46	× 126 × ~	2 132

: Summar of stability data for deep-frozen samples fortified with BYI 02960-tifluor oethylaminofuranone – samples fortified at 1 mg/kg Table 6.1.1-4:

	Storage	Concurrent	Reco	Very in stored sample	es (%)
Matrix	period	Concurrent Tecovory (%)	apparent	onormalized to day 0	corrected for recovery
sugar cane		(1400) 🔊		100	100
	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		× 0° 8° ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	74	91
		5° 98° 2	× 8P 7 × 588 5 × 588 5	80	95
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	049	/ _1M7 ~~	°≥ 112	102	96
4	372 0	\$9 [°] 87 <i>4</i> 54		85	108
	5 <i>5</i> 9	2 87 4 4 83 0 4	× × 94	85	112
coffee bean	~~ ⁰ A	(100)	× 101	100	100
(green)	33	<u>مَ</u> 100 م	9 4	93	94
l l	× 81	L 890	88	87	99
Å	452	<u>4</u>)78	\$ 100	99	85
	√370 5		86	85	95
Û	560	a 00	89	88	101
		ý		Сог	ntinued on next page.

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

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

Age Concurrent recovery (%) (100) 100 98 119 97 (100) 97 (100) 97 (100) 99 103 84 97 (100) 99 103 84 97 (100) 99 103 8117 84 97 009 107 93 116 93 114 96 96 96	apparent 104 109 94 110 ~ 92 0 22 0 24 0 107 0 24 0 24 0 102 0 25 0 24 0 102 0 25 0 24 0 102 0 25 0 24 0 25 0 2	2 × 101 × 86 × 2 × 102 × 2 × 100 × 2	corrected for recovery 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 100 101 100 100 100 100 100 100 100 100 100 100 100 101 100 102 100 103 100 121 121
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$ \begin{array}{c} 104 \\ 109 \\ 94 \\ 92 \\ 92 \\ 92 \\ 92 \\ 92 \\ 92 \\ 92 \\ 92$	to day 0 100 105 90 005 88 81. 90 910 92 93 94 95 96 97 99 99 97 98 99 99 99 99 90 910	for recovery 100
100 98 3 119 5 97 6 97 6 97 7 (100) 99 103 8 117 84 (100) 97 099 103 117 84 (100) 099 099 107 099 0107 099 0107 0107 3 116 93 114 94 96	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	105 4 96 005 088 099 0 299 0 299 0 299 0 299 0 299 0 299 0 299 0 2910 2910 2910 2910 2910 2910 2910 291	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
98 98 119 97 97 (100) 99 103 84 97 (100) 99 103 84 97 (100) 99 107 99 107 107 99 107 107 99 107 99 103 117 107 99 103 117 116 97 106 99 103 117 117 117 116 97 117 117 117 117 117 117 117	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	90 05 088 081. 099 000 099 099 0 099 0 0 0 0 0 0 0 0 0 0 0 0 0	96° 96° 87° 91° 90° 100° 100° 90° 100° 90° 100° 90° 100° 90° 100° 90° 100° 90° 100° 90° 100° 90° 100° 90° 10° 90° 10° $10^{$
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$ \begin{array}{c} 110 & & \\ 92c & & $	005 088 090 090 100 108 108 108 100 100 100 100 100 100 100 100 101 102 102 101 102 101 102 105	96° 96° 87° 91° 90° 100° 100° 90° 100° 90° 100° 90° 100° 90° 100° 90° 100° 90° 100° 90° 100° 90° 100° 90° 10° 90° 10° $10^{$
97 97 97 (100) 99 103 8 117 84 97 (100) 99 103 91 103 91 103 91 100 00 <td>$\begin{array}{c ccccccccccccccccccccccccccccccccccc$</td> <td>88 81. 999 999 870 870 999 999 999 999 999 999 999 9</td> <td>0 100 107 107 91 0 0 100 101 100 101 100 100 100</td>	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	88 81. 999 999 870 870 999 999 999 999 999 999 999 9	0 100 107 107 91 0 0 100 101 100 101 100 100 100
97 (100) 99 103 3 117 84 97 (100) 97 (100) 09 107 107 107 107 107 107 116 93 114 114 114 114 114 100 110	0107 106 ° 106 ° 106 ° 107 106 ° 107 106 ° 107 107 107 107 107 107 107 107	81. 81. 990 999 870 870 870 999 90 90 90 90 90 90 90 90	0 100 107 107 91 0 0 100 101 100 101 100 100 100
(100) 99 103 8 117 84 97 (100) 99 (100) 97 97 97 97 97 97 97 116 97 97 97 114 97 11 11 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	0107 106 ° 106 ° 106 ° 107 106 ° 107 106 ° 107 107 107 107 107 107 107 107	00 99 99 0 108 79 0 814 0 91 0 91 0 0 102 0 102 0 102 0 102 0 102 0 102 0 103 0 0 0 0 0 0 0 0 0 0 0 0 0	0 100 107 107 91 0 0 100 101 100 101 100 100 100
99 103 117 84 97 (100) 09 009 009 009 009 009 009 0	$ \begin{array}{c} 106 \circ \\ 0 & 04 \\ 0 & 0117 \\ 0 & 85 \\ 0 & 5 \\ 0$	y y y y y y y y y y y y y y	91 91 91 0 100 89 0 100 89 0 89 0 89 0 89 0 89 0 89 0 89 0 94
103 117 84 97 (100) 107 09 09 107 107 107 107 107 107 107 107	0 0	370 370 2 108 3 79 3 81/2 7 90 3 910 4 910 3 86 3 102 3 102 3 100 3 102	91 91 0 ⁷ 100 89 109 109 87 87 0 89 6 87 0 89 6 94 90 100
3 117 84 3 97 100 069 107 116 93 114 93 114 93 114 93 114 96	85 6 7 85 7 85 7 102 7 102 7 102 7 103 7 103 7 104 7 104 7 104 7 104 7 105 105 105 105 105 105 105 105	370 370 2 108 3 79 3 81/2 7 90 3 910 4 910 3 86 3 102 3 102 3 100 3 102	0 100 0 4 101 5 4 89 5 3 5 3 6 87 0 89 6 94 90 100
84 97 (100) 09 09 09 09 07 09 09 09 09 09 09 09 09 09 09	85 6 7 85 7 85 7 102 7 102 7 102 7 103 7 103 7 104 7 104 7 104 7 104 7 105 105 105 105 105 105 105 105	79.0 81.0 910 910 910 910 910 910 910 91	101 89 109 93 87 89 94 90 100
97 0 (100) 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	85 6 7 85 7 85 7 102 7 102 7 102 7 103 7 103 7 104 7 104 7 104 7 104 7 105 105 105 105 105 105 105 105	8 k 9 k 9 k 9 k 9 k 100 c 102 c 102 c 100 c 102 c 100 c	89 89 109 109 2 33 4 87 5 89 6 94 90 100
(100) 009 0107 0107 0107 0107 0107 0114	√	Ø Ø0 Ø 100 100 0 910 0 0 86 0 0 102 0 0 102 0 0	109 3 4 87 89 94 90 100
009 0, 107 107 107 107 107 107 107 107	$ \begin{array}{c} 102 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0$	0100 ℃ 91℃ €91℃ €91 ***********************************	109 3 4 87 89 94 90 100
009 0, 107 107 107 107 107 107 107 107	⁶ 103 √ 88 0 5 164 √ 10 √ 116 √	86 × 102 × 102 × 100 × 1	87 89 94 90 100
$3 - 116^{\circ}$ 4 - 93 3° 3° 114° 7° 1000 7° 1000	⁶ 103 √ 88 0 5 164 √ 10 √ 116 √	86 × 102 × 102 × 100 × 1	0 89 Ø 94 Ø 90 100 100
$3 - 116^{\circ}$ 4 - 93 3° 3° 114° 7° 1000 7° 1000	$ \begin{array}{c} & 103 \\ & 88 \\ & 88 \\ & 5$	86 × 102 × 102 × 100 × 1	© 94 90 100
	→ 164 → 10 → 10 → 116 →		90 100
, (10 0) , (10 0)	0 810 0 116 9		100
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	× \$116×	0 005 4	
	2 5 ² 1165	© 0105 √	121
~ 103 J	1.00	7	
		<u> </u>	98
N 128			86
) 0 97 %	▲ ▲ ▲ ▲	³ 276	87
	890	81	80
		- A'	100
		u v	102
			96
			95
			91
			93
	× × × × × × × × × × × × × × × × × × ×		100
			105
	91		94
	, , , , , , , , , , , , , , , , , , ,		95 98
			98 87
	87	84	87
		$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$

## Tier 2, IIA, Sec. 4, Point 6: Flupyradhurone (BY102960)

Study No.	Cron			Spike		Recov	very (%)		v° >>
GLP Year	Crop, Matrix	a.s./metabolite	n	level (mg/kg)	Individual recoveries	Min	Max	Mean	RSP
<i>RARVP046</i> GLP: yes	spinach leaf	BYI 02960	13	1.0	84, 90, 99, 93, 99, 92, 89, 95,95, 115, 124, 00, 00	845 ⁵ 7	124	98 7 7	\$ 11 \$
2011		difluoroacetic acid	13	1.0	124, 99, 96 101, 99, 101 97, 99, 90, 89, 87, 84, 69, 70, 106, 107	69 °			
		BYI 02960- difluoroethyl- aminofuranone	13 ©		102 12, 104 106 108, 99 102, 118, 110, 012, 98 0105, 5 99	98 5 6	₩ <b>92</b>	196 x	
	orange fruit	BYI 02960	13^ (%) *		101093,95088, 89/104,0 67,111,97,0 107,97,75,85			, 93 5 S	13
		difluoroaceric acid	13¢ ©		94,93,96,98, 99,102,56,87, 86,94,94,90, 93			∀ 92	7
		BYI 02960- difluordethyl- aminetaranon	130 0		104, 103, 105, 102, 98, 115, 82, 120, 119, 101, 92, 99, 05		پ [*] 120	103	10
	soybean seea	BYI 02960			97, 90, 91, 95, 92, 92, 92, 88, 94, 96, 95, 98, 93	©88	98	93	3
		difluoroacetic actu	187 *		66, 95, 76, 88, 90, 90, 96, 75, 75, 74, 73, 80, 77	66	96	81	12
6	¥()) (//	BYI 02960-	13°	$\sim$ $\sim$	<ul> <li>2, 109, 101,</li> <li>2, 106, 104,</li> <li>102, 116, 118,</li> <li>84, 85, 104, 90</li> </ul>	84	118	102	11
	navy bean	BYI 02960 difluoroacette acid BYU 02960- difluoroacette acid BYU 02960- difluoroethyl- aminofuranone			105, 104, 125, 93, 90, 96, 93,104, 97, 106, 121, 100, 101	90	125	103	10
		diflueroaceth acid	Q13	1.0	96, 91, 100, 95, 96, 100, 96,79, 78, 82, 78, 99, 98	78	100	91	10
		BY 02960- diffuoroethyl- aminofuranone	13	1.0	109, 94, 103, 121, 98, 103, 111, 118, 115, 98, 88, 116, 113	94	121	107	10

Table 0.1.1-5. Recovery data for the relevant residues of D 11 02900 in various plant matrices	Table 6.1.1-5:	Recovery data for the relevant residues of BYI 02960 in various plant matrices
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Continued on next page...

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

Study No.	Creat			Spike		Recov	very (%)		@° ≫
GLP Year	Crop, Matrix	a.s./metabolite	n	level (mg/kg)	Individual	Min	Max	Mean	RSD
		<b>DJH</b> 0 <b>0</b> 60	1.0	1.0	recoveries	O ^v	10-	<u> </u>	Ô
RARVP046	sugarcane	BYI 02960	13	1.0	97, 105, 99, 107, 96, 92, 89,	83	107	95 . S	~~~7 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
GLP: yes 2011				(	98, 99, 83, 90, 5 388, 89	Ç*	چر چر		
		difluoroacetic acid	13	1.0 🕅	*100, 97, 97, 95, 98, 98, 97, 85,	85		S\$5 *	یہ ج میں
					86, 96, 97, 92, 95	° Q			
		BYI 02960- difluoroethyl-	13	≪1.0 ©	116,114,100, 88,990,101,84,	84 V		K)97 ~~	> 14
		aminofuranone	O'		#16, 117, 87, 86, 86, 81	¢ ~		To,	Ś
	coffee bean,	BYI 02960	13~ ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	× 1.0	92, 97, 95, 100, 94, 83, 86, 88,			91 (91) 0	≥ 7
	green	<u> </u>			83, 100 86, 87 95			<u>_</u> ?	
		difluoroacetic acid	130		89,89,94,02, 87,77,73,80,	of a	^م 94 کې	≫ [™] 83	9
			Ş	Å 0	49, 72, <b>3</b> 8, 79 90 4 5	.0	0 - Ân		
	*	B 21 02960- difluorgethyl- amin@uranon@			103, 103, 9%, 198, 92, 92, 86,≋ 20, 1,5%, 92,⊈	6 ⁸⁴	ر 120 *	98	12
	tomate	BYI 02960	12		89, 84, 94 95, 408, 81, 88,	0° 50 49 Ø81	109	95	10
	fruit				109, 90, 81, 103, 001		109	95	10
		difluoroacetic actid	13	1.00	99, 105, 100, 99, 99, 99, 96, 91,	82	105	97	7
Ŕ					82, 85, 101, 94, 102≜102				
		BYI 02960-	120	1.0	182, 108, 98, 103, 102,	94	120	106	8
4		aminofuranone	Ĵ.		[®] 120, 116, 94, 99, 105, 117				
A A	wheat grain	BYI 02960	•138 ©		97, 87, 99, 96, 90, 96, 82, 90,	82	104	94	8
N.	s s s s s s s s s s s s s s s s s s s			y y	104, 103, 82, 95, 98				
COT		diflue for action of the second	43 Q	1.0	85, 98, 92, 95, 99, 97, 79, 81, 75, 70, 73, 85,	70	99	86	12
		BYI (3960- difteoroethyl-	13	1.0	91 97, 104, 107, 102, 00, 100	84	109	100	7
		aminofuranone			103, 99, 109, 84, 104, 104, 92, 92, 97, 102				

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

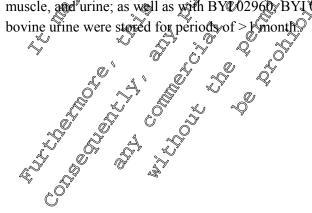
## ► ANIMAL MATRICES

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

Table 6.1.1-6:	Periods of frozen storage (approx15 to -20°C) of animal-based sample	es (betw	veen
	sampling and analysis) for matrices relevant to this dossier	. O'	Ś

	· -			$\checkmark$	
Sam	ple material	Analyte	Longest storage	Report no.	udy Amæx point KIIA. Q Ø 6.4.201
Animal	Matrix	group*	duration	Report no.	Annex points
		0 1	Â		$\mathbb{K}$ IIAQ. $\mathbb{O}^{\mathbb{V}}$ $\mathbb{K}$
ruminant	milk	A	<u> </u>	RARVP950	6.4.201
		В	Q 25		
	cream	A & B	Ser Ser		
	whey	A		RARVE950	KIIAQ 0 0 0 0 0 0 0 0 0 0 0 0 0
		В		~ 1	
	fat	A S			
		R u	× 40 × 22 × 22 × 22 × 22 × 22 × 22 × 22	* 0	
	kidney	A O	× 22 × 2		
		₿¢ B¢	40 K		
	liver				
		₿ Ŝ	× 37 × ×		ð .
	muscle				
		В	A A S		
	urine S	A&B	$\frac{3}{\sqrt{33}}$		
poultry	egg	A & B	~ ³⁷ 125 @		6.4.1/01
	fat O	A & B	$\begin{array}{ccc} & 125 \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & &$		
	diver ô	A & B	× × 11 ~		
Č	musele «	A & B	<u></u>		
	excreta 🏑 🤞	A			
* analyte grou	up A compares BY1029	60, B 902960-a	cetyl-AmCP, and BYI 02	9@-ОН	
analyte grou	ip B comprises DPA	* \$		Ň	

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



Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (E	BYI 02960)
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Report:	KIIA 6.1.1/02, , S.M., & , A.M.	.; 2012	
Title:	BYI 02960 – Magnitude of the Residue in Dairy Cov	WS	0
Report No. & Edition No.	RARVP050 M-428416-02-1		
Guidelines:	<ul> <li>OPPTS 860.1480 – Meat/milk/poultry/eggs</li> <li>OECD Guideline 505</li> <li>APVMA Residue Guideline No. 23</li> <li>DACO 7.5 – Meat/milk/poultry/eggs</li> </ul>		
GLP:	yes (certified laboratory)	Ŵ	

I. Materials and Methods

To determine the freezer storage stability of the relevant residues of BV1 02960 in animal materials, individual control samples of bovine fat, kidney, liver, and nuscle 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 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 tonger than one month, it is only very marginally so – an exceedance of only 16%. As the primary purpose of the drine 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-rick 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, & & 2012, KIIA 3.3/03), which was validated prior to and parallel to the residue analysis of the samples. The OQ of the method was 0.02 mg/kg for DFA, expressed in BYI 02960 equivalents.



The mean values of the concurrent recovery rates per compound, sample material, and spiking level were in the range of  $60_{88\%}$  with standard deviations < 10%. Details of recovery data are shown in table 6.1.1-800

At day 6 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 100-10%. 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.

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

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 for kidney, liver, and muscle. These results validate the residue values reported in the cattle feeding study L. with respect to storage stability of samples frozen prior to analysis. In addition, data from the pant storage stability study (KIIA 6.1.1/01) in watery matrices odicate that the crucial components of the Provide a state of the state of residue in urine, BYI 02960 parent compound and DFA, are stable in gorage, thus wiidating the residue values reported in the cattle feeding study. 

Table 6.1.1-7:	Summary of stability data for - samples fortified at 0.20 m	or deep-frøzen	samples #	ortified with D	₽ AFA
	- samples fortified at 0.20 m	ng/kg		· ~ · · · · ·	ř

Matrix	Storage period (d)	Concurrent recovery	apparent	Very in stored sample normalized to the store of the sto	for regovery
bovine fat	0		85.8	y 900 Č	J00
	43	87.8	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	× 100 × ~	98.0
bovine kidney	0		68.7 🗇	100	O [*] 100
	43	72.5	° 72.5°	¥ \$05.5 ×	<i>©</i> 100
bovine muscle	0 %	4 - Q	653	~100 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	100
	48,	69.00	0° 399.0 0°	ky 105.6 S	100
bovine liver	<u>í</u> ð		\$ 60.3	000 ×	100
	43 43	°∼68.0√	~ 6 <b>5</b> \$	L 109.10	96.7
	Č al	~~ «.	N LY N		

~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	~	s an	and the second s	Oř	9	Ø1	107	
Table 6,407-8:	C ÂN	\sim	1000		·	~~ ·	\wedge .	
1 able 6,40¥-8:	Concurrent re	covery (tata for	D ΓA	in variou	s anin	nal matrices	3
R V	(7/ n		*	0	() [.] %	.//	O	

Study No.	Matrix			Spiße	A ^Y	Reco	very (%)		
GLP Year	Matrix	A a.s. Abetabolite		level (mg/kg)/	S Individual recoveries	Min	Max	Mean	RSD
RARVP050	bovine fat	denuoreacetic acto	5	0.20	80, 87, 91, 88, 88	80	91	87	4.7
GLP: yes	kidney€」			~00.20	70, 72, 65, 73, 72	65	73	70	4.6
	bovine muscle	Difluor Gacetic acid	50°	0.20	63, 66, 68, 68, 71	63	71	67	4.4
	Dovine liver	ditteroroacetic acid,	5	0.20	58, 61, 63 71, 65	58	71	64	7.7

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

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.

interruption during the work process, it must be ensured that the stability during possible storage of samples in extracts is always guaranteed. Additionally when construct interruption during the work process, it must be ensured that the stability during possible sprage of samples in extracts is always guaranteed. Additionally, when conductions could analysis on egular, samples, the entire analytical procedure is routinely monitored by performing concurrent recoveries with each sample set. samples in extracts is always guaranteed. Additionally, when conductions residue analysis on regular

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 fluoroethyl)amino]foran-2(5H)-one IUPAC name: 4-[[(6-chloropyridin-3-54)meth ifluoroethyl)amino]-CAS name: 2(5H)-furanone, 4-[/(6-chlore-3 pyridinyl)methyl](9,2 951659-40-8 CAS #: Empirical formula: C12 H11 Cl F2 N2 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 (for a 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 BYI02960 has also been investigated in livestock (lactating goats and laying hens) in addition to the

tems, two different radiolabets were used in all plant and shown below. As BYI 02960 contains separate ring systems, animal studies. These labo

С Ś * denotes position of radiolabel selled the methyl * denotes position of radiolabel, labelled in the 4group of the paridiny methyle noiety position of the furanone ring incrmethyl-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.

also conducted with [ethyl-1- ¹⁴ C]BY102960.
Additionally, residue levels of difluoroacetic acid wire estimated by LC MS/MS according to the conditions of residue analytical method 01304 in selected samples conditional crops included in the BY102960 (furanome 4-1°C)BY102960. The live live is an elevel of the result of the selected samples conditions of residue analytical method 01304 in selected samples conditions (IBY 02960 r) [furanome 4-1°C)BY102960. The live live is an elevel of the result of the res
* denotes position of radiotabel, labelled in the ϕ hyle Q ϕ
group of the diffuoroethyl aminomolety of the diffuoroethyl aminomolety
lethy 1-14CARVI 07960 A A
Additionally residue levels of diffuor accetic and were estimated by I CANS/Meaccording to the
anditions of residue analytical method 01004 in Master Frankley of Perturbitions at the next and confined
rotational arap matchalian studios and atad with sitler investigation and CODV 0206 ar
[furanone-4- C]BY102960. Addus, DFA levels nave been determined in all crops included in the
BY 102960 metabolism program. For livestock tissues, applioroacetic acid levels were estimated on
basis of the rat data. Additionally, high resolution LCCMS analyses (non-GPP) were performed for
selected samples and confirmed the estimations.
Numerous metabolites were identified in the metabolism studies. The chertical 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. C
All residue values given in make 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.
All residue values given in marker refer to parent compound equivalents if not indicated otherwise. The overall summary of the metabolism in plants and fivestock 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, roge 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.

				~
T-11- () 1 1.	Crops and application	4 1	A	
	t rons and annucation	techniques used '	in nne	metanousmasminies
10010 0.2.1 1.	crops and approaction	teeningues used	ш	moutomanes

				(C)		
Crop group	Crop		Application technique			. Ó
fruits	apple		Coliar application	0		
fruits	tomato	ĺ	soil drench			,
root crops	potato		to leave two at one first / so			
oilseeds	cotton		foliar application			0
cereals	rice		foliar application / 1 granular application			
					\odot 0	_

In Europe, soil drench applications will no be developed however, soft applications are important in other regions which will apply for import tolerances in tutures g.g. NAFTA OUSA Canada and South America (Brazil). Therefore, the metabolism studies covering soil grench 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 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, C]-, [pyridinylmethyl-14C]- and $[\text{ethyl-1-}^{14}\text{C}]BYI 02960:$

Report	KIIA 6.2. 101,
Title:	Metabolism of furanone 4-14 CIBYI 02960 in tomatoes
	MEF_11/016
Edition No	M-ATH 352-QF-3 & X & X
Guidelines:	OPCD 50 Metabolism in Crops
	US EP Residue Chemistry Test Guideline OPPTS 860.1300: Nature of the Residue –
A	Plants, Livestock
	PMRA Regulatory Directive Dir28/02: Residue Chemistry Guidelines Section 2: Nature of
	the Residue – Plants, Livestock
	Japanese MARF, 12 Nousan SP47
<u> </u>	European Parliament and Council Regulation (EC) No 1107/2009
GLP	Yes a for the

[%]Executive Summarv

The metabolism of [furanons4-14C]BYI 02960 in tomatoes was investigated according to the maximum envisager use pattern globally. Four tomato plants were treated by soil drench application with [furgeone-4-14C]BYI 02960 formulated as an SL 200. The first application was performed at BBCH 5 (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 TR values are shown in the following table:

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

Matrix	Timing and Application	PHI (days)*	ppn (ang a.s. equiv./kg)
tomato fruits	two drench applications, at BBCH 15 and 14	69	Q096 5 K
tomato flowers	days later; 2 x 300 g a.s./ ha	°n.a. ∱	€ 0.721 ^C

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. vater mixture 84.8% and 93.6% of the TRR were released, respectively

Parent compound and metabolites of the extracts of tomato flowers and fruit were analysed by HPLC. Identification was performed by HPLQ and/or FLC corchromatography 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 dentified

Parent compound was the man component in both matrices and epresented 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 02960difluoroethyl-appino-figanone represented 10.3% of the FRR. Infomato flowers, no radioactive glucose (or isomeric carbohydrates) was detected. BY \$2960-difluor bethyl-amino-furanone was detected as a minor metabolite and represented 9.2% of the PRR. 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-4-14] BYJ92960 was moderately metabolised in tomatoes. The following metabolic routes ð Ô Ò were observed: Ô

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

a metabolic pathway of [furanone-4-14C]BYI 02960 in tomatoes can be On the ba proposed

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

I. Materials and Methods

A. Materials	
1. Test Material:	
Chemical structure	Cl = N + F + Cl + F
Radiolabelled test material	[furanone-4-14C]BYI 02960 27 0 2 2
Specific radioactivity (before radiodilution) (after radiodilution)	$3.94 \text{ MBq/mg/th} 6.46 \mu \text{Ci/mg} $ $1.31 \text{ MBq/mg/(35.50 \mu \text{Ci/mg})} $ $2.999\% (HPV C) $
Chemical Purity	
Radiochemical purity	
	299% (HPLC and TEC) or 5 5 5 6

The supplied radiolabelled test compound [furnione $4^{2/4}$ C]BY1 02960 was dissalled 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 air adequate amount of \$L 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 μ Ci/mgQ

- 2. Soil: "Einheitserde", pH CaCl = 5.8, 85% white moor pear and 15% clay, enriched with water soluble nutrient salt mixture (ENS)
- 3. Plant Tomate variety "Philona", representative for fruiting crops

K)

B. Study Design

Experimental condițions:

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

The tomato plants were treated with SEQ00 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 future of the residues in flowers. The flowers were collected with their receptacles and were stored in a freezer ($\leq -18^{\circ}$ 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, but in pieces and stored in a freezer ($\leq -18^{\circ}$ C) for optional metabolite investigations. The frozen flowers were homogenized with a high speed blender in liquid nitrogen (Polytron). The bomogenised flower sample was stored in a freezer ($\leq -18^{\circ}$ C) until extraction.

On the day when the three remaining to mato 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°C). Harvesting of newly ripe fruits was continued 2 - 3 times oweek over a total period of approx. three weeks until the plants produced no new fruits (at BBCH 89: fully ppe). 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 effreezer (\leq -18°C) until extraction.

C. Analytical Proceedines 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 acetomtrile/water (10, 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 blonder (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 traction (percolate) was collected and the cartridge was finsed with acetonitrife/water (8:2, w) and methanol/dichloromethane (1:1, v/v). The percolate and the acetonitrife/water fraction were combined and concentrated by rotary evaporation in vacuo. The concentrated extracts of fruits and flowers were analysed by HPLC.

Quantification

Parent compared 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.

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

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 \mathcal{Q} chromatographic systems (reversed phase HPLC and normal phase TLC). Minor metabolites (residue levels $\leq 0.01 \text{ mg/kg}$) were identified by HPLC co-chromatography, only.

In tomato flowers (additional plant matrix), parent compound was also identified by HPIO and 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-chromato and The conjugate BYI 02960-OH-glyc was additionally cleaved by alkalinghydrobysis and its corresponding aglycon was identified by HPLC co-chromatography

Storage stability:

ĺ

Detailed evidence was provided in the report to show that the quantified pattern of parent composited 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 analotical work up fincluding identification and characterization of metabolites) which did not exceed a period of 6 months in total. In accordance with the QE@D Gudance for the festing of Chemical 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.

11. Results and Discussion

The metabodism of [furanone-4-14] BYA 02966 was investigated in formato fruits and flowers following two drench applications. The total radioactive residue (JRR) in fruits and flowers accounted for 0.096 mg/kg and 721 mg/kg respectively. The major portion of radioactivity was extracted conventionally by acetonitrile/water mixtures (84.8% 193.6% 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 cochromatography (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 glurose (or isomeric carbohydrates) and BYI 02960difluoroethyl-argino-furanone. The identity of the carbohydrate was elucidated by HPLC and TLC cochromatography 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 pethods were not considered selective enough to discriminate the configuration of the sugar moiety. Therefore, the isolated polar fraction was assigned more generally as aucose carbohydrates. The minor metabolites BYI 02960-difluoroethyl-amino-furanone and BYI 02990-OH-glyc were identified by HPLC co-chromatography only, since their residue levels were rather low ($\leq 0.01 \text{ mg/kg}$). The configuration of the hexose in BYI 02960-OH-glyc was identified

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

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 fruit and tomato flowers). For BYI 02960-difluoroethyl-amino-furanone, the assignment was additionally confirmed by HPLC co-chromatography. The conjugate BYI 02960-OH-gHc was cleaved by abaline hydrolysis and the resulting aglycon BYI 02960-OH was additionally identified by HPLC cochromatography.

The distribution of the parent compound and metabolites is shown in Table 6.2.4.4. In total, 79.2 and 93.6% of the TRR were identified in tomato fluits and flowers, respectively.

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

	tomate	o fruits	🗸 📎 toppato	flowers
TRR [mg/kg] =	Q \$0.096		Č 20.721	
	, ^O ‰ of ∂RR ∾	v″≪ong/kg	Nof BRR	¢ng/kg
Conventionally extracted	& <u>84.8</u>	~ 0.084°	O _ O ^{3.6} ∑	°م~ 0.675
Extract for analysis	<u>₹</u> 83.5 °C	\$ 0, 08 0 Q	[©] 93.6	۵.675 🖌
Losses (not analysed)		0.001		
Total extracted	o ⁷ \$ 4.8 0 ⁷	0.081	9 3. 6	0.675
Unextractable (PES*)	15.2	0.605	£ 6.4 S	0.046
Accountability	100.0 ×	° `^% 096 Õ [™]	الإيم 10 0	0.721
*			0 4	

* post extraction solid

Table 6.2.1-4: TRR alues and distribution of parent compound and metabolites in tomatoes (fruit and flowers) after drench application of [furanone-4¹⁴C]B/1 02960

	tomate	ofruits a a	tomato	flowers
TRR [mg/kg] = 2			0.721	
Compound (BYI 02969-)	× %TRR	mg/kg	% TRR	mg/kg
Conventional extra@ion A				
BYI 02960 (parent compound)	<u>,</u> 309, 0	Ø .034	77.9	0.561
glucose/carbohydrates	× 27.5 g	0.026		
difluoroeth Tamino-furanone	10.3	0.010	9.2	0.066
OH-glyc V		0.005	6.6	0.048
Total Adentified	Ø Ø9.2	0.076	93.6	0.675
unknown 1	4.30	0.004		
Total characterised	402°	0.004	<0.1	<0.001
Analysed extract(s)	\$3.5	0.080	93.6	0.675
Extract(s) not analyzed	1.3	0.001		
Total extracted Unextractable (PES*)	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

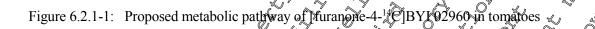
* post extraction solids

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 BY 2960difluoroethylamino-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 turanone-4-¹⁴C]BYB 02960 in tomatoes is well of understood and the following metabolic pathway is proposed.



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

Report:	KIIA 6.2.1/02, .; 2011
Title:	Metabolism of [pyridinylmethyl-14C]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 0107/2009
GLP	yes A Q Q Q Q Q Q

Execotive Summary

The metabolism of [pyridinylmethyl-¹⁴C]BXI 02960 in contatoes was investigated according to the maximum envisaged use pattern globally. Four tomato plants were treated by soil thenchapplication with [pyridinylmethyl-¹⁴C]BYI 02960 formulated as an SL 200. The first application was performed at BBCH 15 (5th leaf on main shoot upfolded and a second application application application application application application application rate corresponded to 300 g a.s./ba, the total application rate was 600 g a.s./ba.

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

Table 6.2.1-5: FRR values in tomato fruits and flowers after drepch application of [pyridinylmethyl-

Matrix 2			Application		, ON	PHI (days)*	ppm (mg a.s. equiv./kg)
tomato fruits		Ytwo difench a	applications, at x 300 ga.s./ha	BBCH 15	and 14	73	0.130
tomato flowers	<u>, , , , , , , , , , , , , , , , , , , </u>	day Hater; 2	🗴 300 g a.s./ha	0 [′]		n.a.	1.254

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

Parent compound was a major component in both matrices and represented 24.2% of the TRR in tomato fuits 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

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 of the oxidation of the methylene group to a carboxofic 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 [pyridin 0 methy] 4^{4} CJBY 102960 in tomators can be proposed.

. K.Materials and Methods
KMaterials and Methods
1. Test Material:
Chemical structure
1. Test Material:
Radiolabelled testonaterist [pyrieinylmethyl-1%]BYF02960
Specific radioadivity 4 4 37 MBq/mg 18.08 μ Ci/mg) (before radiodilution) 437 MBq/mg 18.08 μ Ci/mg) (after radiodilution) 4.46 MBq/mg $(3937 \ \mu$ Ci/mg)
Chamical Durity $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$
Radio Gremical purity 2 > 98% (FIPLC) and TLC)

The supplied radiolabelled test compound [pyrddinylmethyl-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 uci/mg).

Bayer CropScience 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 granhous of the test facility (controlled temperature, humidity and light conditions). The tomato plantowere 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 on the soil around each tomato plant. At each application, a total volgine of 200 mL vas applied, corresponding to 14507 MBq or to 100.0 mg a.s. (= 25 mg a 9/planty. Based on a planting density of 12,000 tomato plants/ha in agricultural practice, the application rate corresponds to 300 g a.s. tha, which was anticipated to be the maximum single drench application rate in agricultural fractice

Sampling:

When the plants reached the growth stage BBCH 61 (first forwer of the first inforescence 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 (< -18°C) on the same day Sampling of newly opened flowers was continued 2 - 3 times a week over a total period of approx. Ove weeks unit the ord of towering (at BBCH 69: 9 or more inflorescences with open flowers? The tomate plant from which the Howers were cut was sampled, cut in pieces and storedon a freezer (\$18°C) for optional pretabolite investigations. The frozen flowers were homogenized with a high speed blender in liquid hitrogen (Polytron). The homogenised flower sample was stored in a freezer (2>18°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 coordin, the ripe tomate fruits were harvested and were stored in a freezer $(\leq -18^{\circ}C)$ Harvesting of newlo ripe fruits was continued 2 - 3 times a week over a total period of approx, three weeks within the plants produced no new fruits (at BBCH 89: fully ripe). The frozen fruits were cut in pieces and were honogenised with a high speed blender (Polytron). The homogenised tomato fruit samples were stored in portion on a freezer (\leq -18°C) until extraction.

C. Analytical Procedures Extraction:

The homogenised tomato flowers were extracted three times with a mixture of acetonitrile/water (8:2, $v/\sqrt{2}$ and one time with a mixture of acetonitrile/water (1:1, v/v) and a portion of the homogenised tomato Puits 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

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

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 SPO 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/dichlotomethane (101, v/v). The percolate and the acetonitrile/water fraction were combined and concentrated by rotary evaporation is vacuo. The concentrated extracts of fruits and flowers were analysed to HPLC.

Quantification:

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

Identification and characterisation:

Parent compound was identified in tonato fruits and flowers by reversed phase HPLC cochromatography using a radiolabelled reference compound. Confirmation of the assignment by a second chromatographic method was shown in the tanato study performed with fluranene-4-¹⁴C]BYI 02960. The main metabolite in tonato fruits was identified by CC-MS/MS analysis after isolation of the compound by semperparative 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 cochromatography 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 13 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 spapprose 6 to 7 months in total) and that the chromatograms represented the metabolic pattern in the samples at barvest. Thus, no additional storage stability data have to be provided according to QECD Guidance for the Testing of Chemicals 501 (2007).

II. Results and Discussion

The metabolism of pyrion ylmethyl-¹⁴ BYI 02960 was investigated in tomato fruits and flowers following two dench 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 0.254 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.

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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 carbolig drates 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 HPLO 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 affective to from BYI 02960-acetic acid, a metabolite which was dentified e.g. in the apple metabolism and confined rotational crops studies and which co-eluted with BYI 02960-QH-glyousing the neutral profiling method.

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

The distribution of the parent compound and metabolites is shown in Table 6.2. 8. In total, 86.3% and 96.5% of the TRR were identified in tornato fruits and flowers, respectiv

	Distribution of tradioactivity in the extracts of the tomato matrices fruits and flowers
Table 6 2 1 6.	Distribution of a dispativity in the art state of the terrete instring Quite and flowers
1a0100.2.1-0.	Distribution of a dioactivity the the expracts particle to mathematices in units and nowers
	after diench application of [pyridin fimethyl-14C]BYI 02960
	The second s

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	tomato	fraits		flowers
$TRR [mg/kg] = \bigotimes^{\gamma} \bigvee^{\gamma}$	×0.130		1.254	
	[™] of PRR [™]	mg/kg_	% of TRR	mg/kg
Conventionally extracted	98.5	a 0.128 x	96.5	1.209
Extract for analysis	98.5 ⁰	Q. Q. 28 ~	96.5	1.209
Losses (not analysed)				
Total extracted 🔊 🖧	≫ .98.5 ∞	≶ 0.128 [°]	96.5	1.209
Unextractable (PESS) A	L.5.	0,0092	3.5	0.044
Accountability	<u></u> <u></u> <u></u> <u></u>	× % .130	100.0	1.254

Bayer CropScience 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	o fruits	tomato	flowers
TRR [mg/kg] =	0.130		1.254	
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	p@g/kg
Conventional extraction			0	
BYI 02960 (parent compound)	24.2	0.031	<u></u> 66.2	0.8 29 0
6-CNA	13.2	0,017	7.0	0:987
CHMP-di-glyc	37.1	1 .048	8.0	~0.100 J
CHMP-glyc	5.1	ي 0.007	D [♥] 9.5 √	0.119 6
CHMP	3.3	1 0.004 Q	° 4	
OH-glyc	3.4	0.004	0° 5.9 \C	1.209 1.209
Total identified	86.3 🔬	° 0.112 🗸	<u>ک</u> 96.5 کړ	××××××××××××××××××××××××××××××××××××××
unknown 1	5.50	Ø 0,007 🖉	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	A °
unknown 2	348 0	Ø.005 Q		
unknown 3	3.0	0.00€ →		
Total characterised	12.2	x 0.2016 x	≪<0.10 [°]	ي ۲۰۰۵ (000 م)
Analysed extract(s)	0 ⁹ 98 5	× 0.128 0	~~ 96 5	ي 209.
Extract(s) not analysed				°∼,
Total extracted	່ ູ 🔊 98.5 ຕັ້	\$ 0.128	© 96.5_	1.209
Unextractable (PES*)	10	© 0.002 c [™]	<u></u> 2.5 (0.044
Accountability	1 69 .0	©0.130 ×	1400.0	1.254

* post extraction solids

* post extraction solids

- @leavage of the pyridinylmethylamine bond followed by conjugation with carbohydrates or followed by ox dation of the methylene group to a carboxylic group, which were the major metabolic routes, and Š
- major metabolic routes, and hydroxylation of the metabolic routes and hy

with glucose, which was the minor metabolic coute. Thus, [pyridinylmethyl-¹⁴GBYI 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

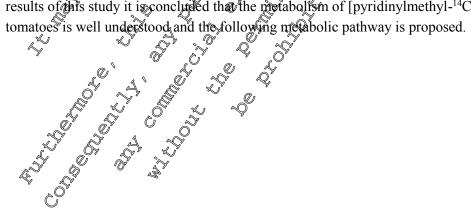
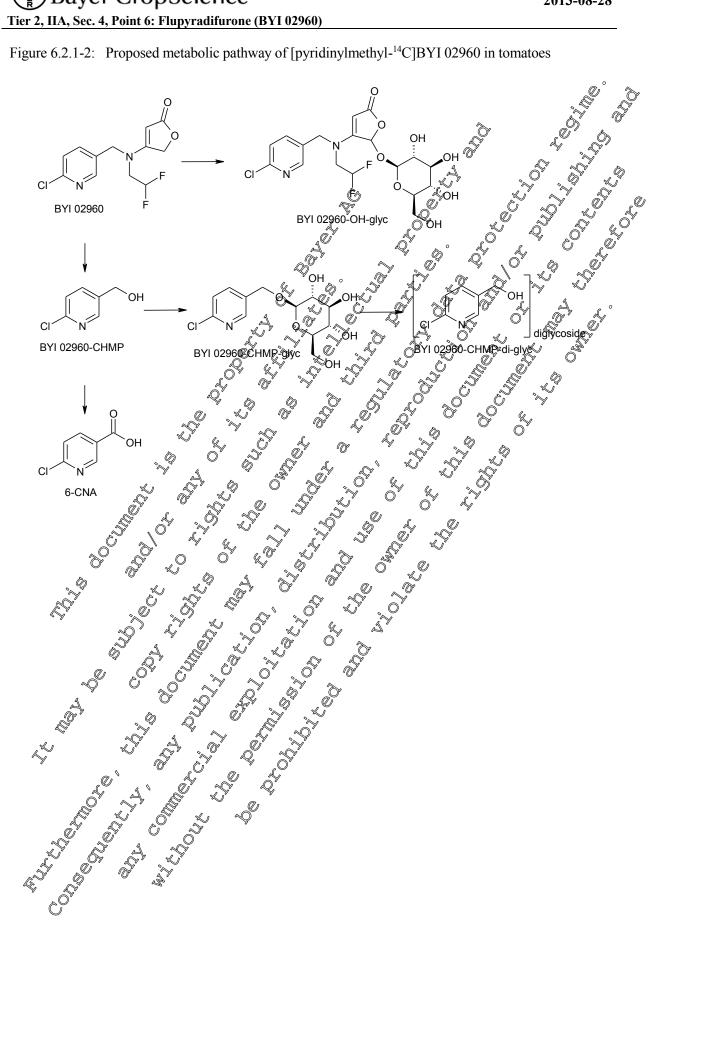


Figure 6.2.1-2: Proposed metabolic pathway of [pyridinylmethyl-14C]BYI 02960 in tomatoes



Bayer CropScience

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

Report:	KIIA 6.2.1/03,,, 2011
Title:	Metabolism of [ethyl-1-14C]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 107/2009
GLP	yes A Q o d o

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 BBCIF 14-15 (4th to 5th leaf on main shoot unfolded) and a second application at BBCH 5139 (first to 9th) inflore scence visible and first bud erect). The single application rate corresponded to 300 g a.s./ba, the total application rate was 600 g a.s./ba.

The flowers were sampled from one tornato plant at 14to 36 days after the fast application and the fruits were harvested from the remaining two tornato plants at 56 to 86 days after the last application. The TRR values are from in the following tables

Table 6.2.1-8: FRR values in tomato fruits and flowers after dreach application of [ethyl-1-

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

* PHI: preharvest interval (corresponds to days after fast 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 925% and 98.3% of the TRR from fruits and flowers, respectively.

Parent compound and metabolites in the expacts 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

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 of fruits and flowers.

[Ethyl-1-¹⁴C]BYI 02960 was metabolised to a significant extent in tomatoes. The following metabolise 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 B\$4 02960-difluoroethylaminofuranone and
- hydroxylation of the methylene group of the curanone mojety followed by conjugation with glucose

On the basis of these results, a metabolic pathway of ethyl 1-14C1BYI 02960 in comatoes can be proposed.

Northericals and Mathads
I. Materials and Methods
A. Materials
The second secon
Radiolabelled test material @ [ethyl-1-14@]BYI 02960
(before radiodilution) $3.93 \text{MBq/mgs}(106.28 \mu\text{Ci/mg})$
Chemical Purity 2 2 > 99% (CPLC)
Radiochemical purity $A = 99\%$ (HPEC and TLC)

The supplied radiolabelled test compound [effyl-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 temainder 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 μ Ci/mg).

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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 cultivated in the greenhouse of the test facility (controlled temperature humidity and light onditions).

The tomato plants were treated with SL 200 formulated [ethyl-1-14] BYD02966 by drench application. Two applications were performed, each at an application rate of 300 gas. /ha. The first application was conducted at a growth stage of BBCH 14-15 (4th to 5th leaf on man shoot unfolded) and the second at a growth stage of BBCH \$1-59 (Jet to 9) inflorescence visible and first budgerect) The interval between the two applications was 14 days. The agreeous application solution was ported into a circular furrow prepared in the sol around the prate plant. At each application, atotal olume of 150 mL was applied, corresponding to 98,50 MBq or to 75.1 mg a.s. i= 25 sa.s./plant). Based on a planting density of 12,000 tomato plants/ha in agricultaral 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 forwer of the first inforescence open), the open flowers were sampled from one of the three tomato plants a 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°C) on the same day Sampling of newly opened flowers was continued 2 - 3 times a week over a total period of approx. Our weeks until end of flowering (at BBCH 69: 9 or more inflorescences with open flowers? The tomate plant from which the flowers were cut was sampled, cut in pieces and stored in a freezer (218°C) for optional pretabolite investigations. The frozen flowers were homogenized with a high speed blender in liquid tritrogen (Polytron). The homogenised flower sample was stored in a freezer (2>18°C) until extraction.

On the day when the two repaining tomato plants reached the growth stage BBCH 81 (10% of fruits show typical fully ripe coordin, the ripe tomate fruits were harvested and were stored in a freezer $(\leq -18^{\circ}$ C. Con the same day. Howesting of newly rips 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 crashed and homogenised with a high speed blender (Polytron). The homogenised to finite samples was stored in portions in a freezer (\leq -18°C) until extraction.

C. Analytical Procedures Extraction:

The homogenised tomato flowers and tomato fruits were extracted three times with a mixture of acconitrile water (8:2, v) using a high speed blender (Polytron). After each extraction step, extracts and solid 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 labely specify main metabolite, three different stationary phases were used for TLC co-chromatography. Thus, dissinguar TLC systems confirmed the assignment un ambiguously.

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 barvest 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 IPLC chromatogram 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 [pyriduy]metayl-¹⁴C]BY102960 and [furanone-4-¹⁴C]BY102960, it was shown that the profiles of tomato truits 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.

Il Results and Discussion

The metabolism of [ethyl-1-¹/₂]BVL 2960 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 0.20 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.

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 thromatographic technique. The main metabolite difluoroacetic acid was identified after semi-preparative isolation by TLC co-chromatography with an authentic reference compound. Two dissimilar systems formation phase and reversed phase TLC) and one modified normal phase system were applied. TLC cochromatography with three different stationary phases showed chromatographic correspondence of radioactivity in the isolated fraction with the radiolabelled reference compound diffuoroacetic acid. tomato flowers this metabolite was identified by comparison of the HPL@ profiles of that and flowers.

The minor metabolite BYI 02960-difluoroethyl-amino-furnone as 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 tomate fruits and flowers by comparison of the HPLC profile with a corresponding profile obtained in the tomato metaboliser stud performed with [furgione-4-¹⁴C]BYI 02960. In the latter study, the metabolite BXF 02960-OH give had been dentified in tomato fruits by reversed phase HPLO 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 faits and flowers, respectively.

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		<u>) x x 0 (</u>		
	tomato	fruits	tomato f	lowers
	🖌 i 🛇 🗛 🖉 01		2.230	
	of TRR	y ung /kg	% of TRR	mg/kg
Conventionally Extracted C	205 299.5 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	0.200	98.3	2.192
Extract for analysis	9.5	0.200	98.3	2.192
Losses (not analysed)	n.q.y	🌾 n.q.	n.q.	n.q.
	~ 99,5 ,0	0.200	98.3	2.192
Unextractable (PES*)		0.001	1.7	0.037
Accountability 0	100.0	0.201	100.0	2.230
* post extraction solids n.q. not quantified			· · · · · ·	
n.q. not quantified				
n.q. not quantified				
le la la				
n.q. not quantified				
V				

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$T_{ab} = (2 + 1) + (2 + $	Dis Churtism of motion stights in the outro to of the town to matrice of further and flowers
I able $0.2.1-\infty$	Distribution of radioactivity instructs of the tornato matrices fruits and howers
	after drively and a stick of [atten] 1 14CIDVIPOOCO V
**	aller drench abencallow of relinvi-i-sults kast2900s
~~ "	Distribution of radioactivity in the extracts of the torrato matrices fruits and flowers after drepch application of [ethyl-1- $\frac{1}{2}$ C]BXL92960

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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

	tomate	o fruits	tomato	flowers
TRR [mg/kg] =	0.201		2.230	
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	p&g/kg
Conventional extraction			- Or	
BYI 02960 (parent compound)	10.0	0.020	33.0	0.7 36 0
difluoroacetic acid	86.6	Ø _{\$} 174	59.8	1334 N
difluoroethyl-amino-furanone	2.2	1 .004	3.1	~0.068 J
OH-glyc	0.6	ي 0.001	Q [♥] 2.4 ≪	0.054 6
Total identified	99.5	ے 0.200 £	· 98.3	20192 0
Total characterized	@	~>	lo ^o → ∖c	\$ Ø
Analysed extract(s)	99.5	° 0.200	98.3	2.192
Extract(s) not analysed	0*		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	L 0
Total extracted	9925 0	Ø.200 - Q	<u> </u>	2 .192
Unextractable (PES*)	0.5	~~0.00 [°]	A . 0.7 x	0.03
Accountability	(100.0)	x 0:201 x	×100.0	S 2.230
* post extraction solids	0× × ×	Y ZY NO		<u>, </u>

st extraction solids

HI. Conclusions

Based on the metabolites identified the following metabolic routes were defined;

- oxidative deavage of the difluoroethy anine bond and formation of difluoroacetic acid,
- cleavageof the pyridio lamite bond and formation of BY 02960-difluoroethyl aminofuranone, and
- hydroxylation of the methylene group of the furanone moiety followed by conjugation with glucose. Õ Š

[Ethyl-1, 2]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 to concluded that the metabolism of [ethyl-1-

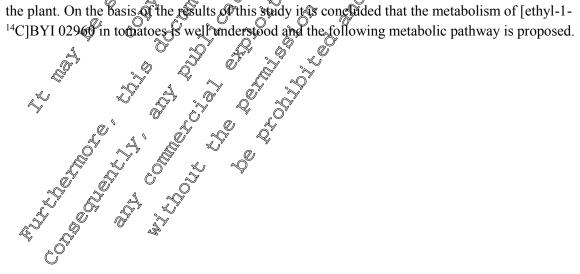
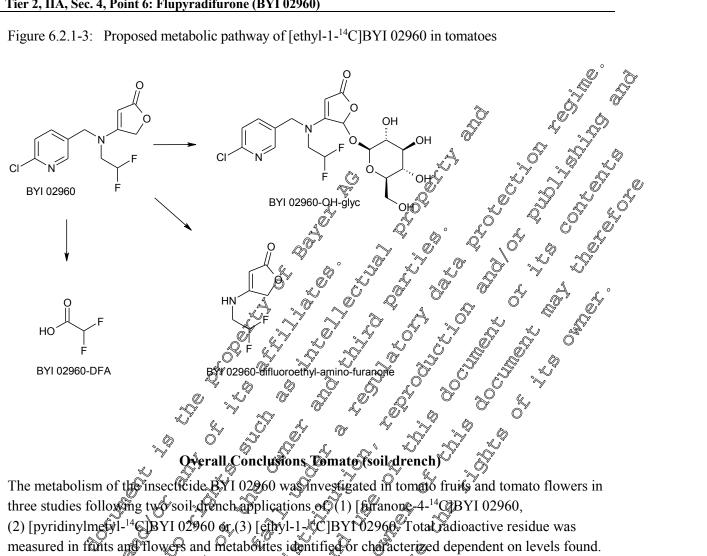


Figure 6.2.1-3: Proposed metabolic pathway of [ethyl-1-¹⁴C]BYI 02960 in tomatoes



The metabolism of the insecticide BYI 02960 was investigated in tomato fruits and tomato flowers in three studies following two soil-drench applications of (1) [furanone-4-14 CaBYI 02960, (2) [pyridinylmetyl-14G]BYI 02960 or (3) [ethyl-1-4C]BY1 02960 Total adioactive residue was measured in finits and flowers and metabolites identified or characterized dependent on levels found.

C]BYI 02960 (1) Label T: [furanone= 4^{14}

At harvest, the total tadioactive residue (FRR) 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 gucose for isomeric carbohydrates) and BYI 02960difluoroethylamino auranone. 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 metabolities were detected, the proportions differed significantly. Most significant was that parefit compound was by far the main constituent in flowers and represented nearly 80% of the TRRs

On basis of the netabolices 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 [pyridinylmetyl-14C]BYI 02960, 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 invery 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, the presenting the edible raw of 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-glyc, the label-specific procursor metabolites of BYI 02960-QHMP-th-glyc, and BYI 02960-OH-glyc, a metabolite common to all three radioabels bested. In tomato flowers, the same metabolites were identified as in the fruits, with the exception of BYI 02960-CHMP. Of However, the proportions of the compound's different 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-4-145] - and [ethyl-1-14C]BYI 02960. A minor metabolic route was hydroxylation of the methylene group of the furanone moiety followed by conjugation with gloose. This metabolic route resulted in the non-label specific metabolite BYI 02960-OH-glys, which was identified in corresponding concentrations in the tomato studies performed with [furanone-40⁴C]- and [ethyl-1-1⁴O]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 radiotabels.

(3) Label 3: $[ethy]_{2}^{14}C]_{14}BYI 02960$

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 pyridia ylmethyl-¹⁴ (JBYI 02960 and [furanone-4-¹⁴C]BYI 02960. Since it is known that parent degrades to diffuoroacetic acid in soil, the diffuoroacetic acid residue is probably the result of both uptake of this metaboline from the soil and degradation of the parent compound BYI 02960 in the plant, Diffuoroacetic 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 aftifuorethyl-amino-furanone and BYI 02960-OH-glyc.

Parent compared 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

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 monlabel 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 rad@labels.

When considering the results from all metabolism studies conducted on tomato, it can be concluded that BY102960 is rather extensively metabolised infinites considered on total of omajorand Gaminor metabolites were found, and all major and 3 minor have been identified. The distribution of parent compound and metabolites in the edible commodity genator during is summarized for Table 6.2.1-11.

TRR values and distribution of parent compound and metabolites in tomato fruits after drench application of radiolabelled BYI 02960	r
	0

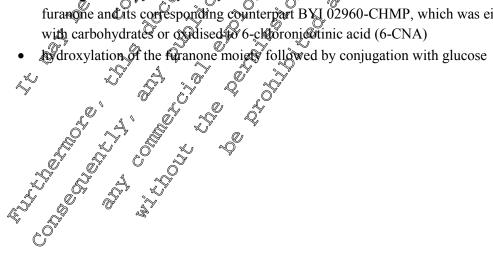
	tomato fruits					
Radiolabel	[furanon	$e-4-^{14}C$]	[pyridinylr	nethyl-14C]	[ethyl-	-1- ¹⁴ C) o
TRR [mg/kg] =	0.096		0.1	30	0.2	200° 5
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg 🕜	% TRR	mg kg
BYI 02960	35.9	0.034	24.2	0.031	10.0	9 .020 Ø
difluoroacetic acid			Ĉa	Ž,	86.6	°∼ 0.17
glucose/carbohydrates	27.5	0.026	- T	, di		
6-CNA			L 13.2	JOO.017	× 0	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
CHMP-di-glyc		1	37.1	0.048		o _s or
CHMP-glyc			5.1 ~	00 07 ⁴		
СНМР		\$. *	° 3.3°	0.0040		
difluoroethyl-amino- furanone	10.3	0.010				Â, 0.004°
OH-glyc	5.5	0.005	3.40	0.004 O	🗞 0.6	0001
Total identified	79.2	0,076	Ø 86.3	0 0.112	§ 99.5	് 0.200
Total characterised	4.3 0	9 ,004, \$	2 2.2 0	0.016		b
Analysed extract(s)	83,5	0.080	98.5	9.128 0	<u>م</u> 99.5 م	0.200
Extract(s) not analysed	@1.3 «	0.001	ç P	<u>~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~</u>	<u> </u>	
Total extracted	84.8	0.081	98. 5 Ø	0,128	° 995	0.200
Unextractable (PES*)	15.2%	0.015	© 1.5 [™]	0.002 ×	ه0.5	0.001
Accountability 🗞		0.096	<} 100₀0 [∿]	∞ 0.130	100.0	0.201

* post extraction solids 🔌

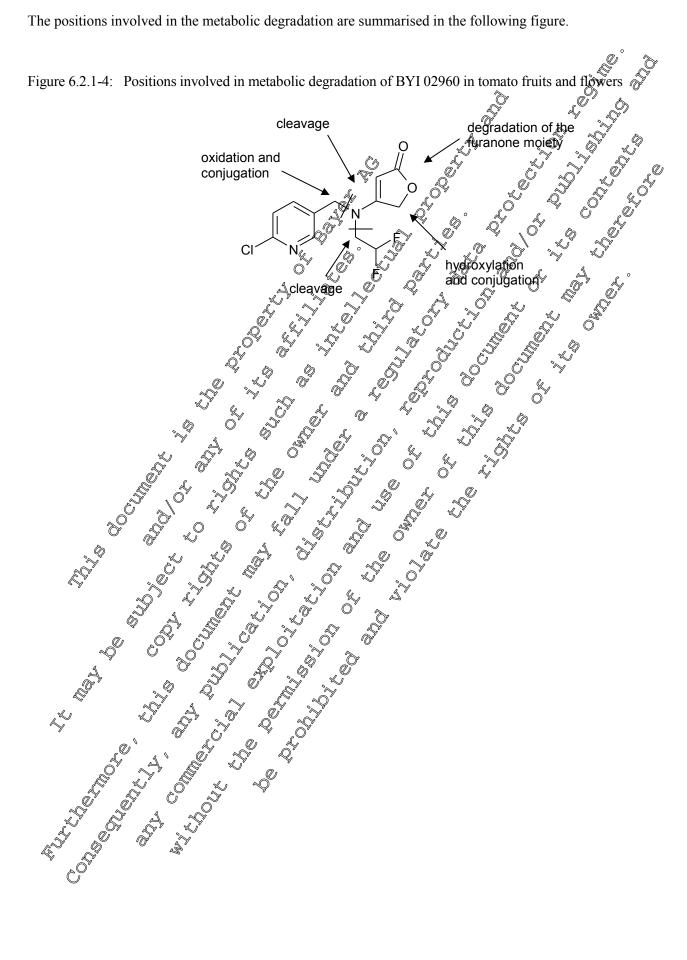
Label specific metabol fes are printed mitalics

On basis of the metabolites identified, biotransformation of BYL 02960 in tomato proceeds by the following pathways a strange of the strange ng pathways a start of the diffeoroethylamine bond and formation of diffuoroacetic acid

- complete degradation of the furancine moviety and incorporation of carbon atoms into the natural compound port, e.g. into glocose/Carbohydrates
- cleavage of the pyriding lmethy lamine bond and formation of BYI 02960-difluoroethylaminofuranone and its conceptoring counterpart BXL 02960-CHMP, which was either conjugated



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



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-

Report:	KIIA 6.2.1/04, .; 2011 A 6 2 4
Title:	Metabolism of [furanone-4- ¹⁴ C]BYI 02960 in potatoes
Report No &	MEF-10/769
Edition No	M-415234-01-2 L O ^V L O ^V L
Guidelines:	OECD 501 Metabolism in Crops US EPA Residue Chemistry Test Guideline OPPTS 860 1900: 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 81470 European Parliament and Council Regulation (BC) Not 1707/2009
GLP	yes a ki ki wi y w a a a

Lexective Summar

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 ap FS 480 at an application rate of 10.0 g a.s./at (= 254 g a.s./ha, seed density 25 dt/ha). In the other experiment, [furanone-4-¹⁴C]BYF 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 porato tubers and the remaining part of the plant after tuber treatment or im-furrow application of [furatione-4,14C]B&1 02960
$1a0100.2.1^{-1}2.$	The values in parato thoses and the regnanting part of the plant after tuber treatment of
	16/2 turrow ann to at 1 on at 1 turatione_1/2 ¹⁴ CIBAST (12960)

Matrix O C Timing and Application	PHI (days)*	ppm (mg a.s. equiv./kg)
potato tubers to tuber treatment at planting (BBCH 03),	97	0.078
potato leaves and roots, 100 g a.s. (0)	97	6.97
remainders of the seed potatoes	97	36.21
potato tubers	97	0.171
potato leaves and roots in-fur two application at planting remainders of the seed potatoes (BBCH 03), 626 g a.s./ha	97	7.01
remainders of the seed potatoes	97	3.43

* PHI2 preharsest integral (corresponds to days after last treatment (DAT) at the start of harvest/sampling)

The potato tobers (The potato tobers)) 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.

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-aminofuranone

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

- hydroxylation of the methylene group of the mranone moiety followed boconjugation with glucose, and
- cleavage of the pyridinylmethylamine bond

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

A. Materials 1. Test Material: Chemical structure
A. Materials 1 Test Material:
1. Test Material: $\sqrt[3]{2}$ $\sqrt[3]{2$
$\frac{\partial}{\partial t} = \frac{\partial}{\partial t} = \frac{\partial}$
Radiolabened test material (furanone de ¹⁴ C]BYI 02960
Specific radioactivity 394 MBq/mg (106.46 aCi/mg)
Chemical Purity
Radiochemical purity $\langle \mathcal{Q} \rangle > 99\%$ (HPLC and TLC).

The supplied adiolabelled test compound furarione-4¹⁴CJBYI 02960 was dissolved in acetonitrile. Formulation of the test compound was performed prior to the application. For both formulations (FS 480 and \$L 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. Solv "**Final d**" (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 mfurrow 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 \mathbb{Z}^{2} 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 of the S 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. Fo purpose, three seed potatoes were treated with a defined volume of the aqueous application suspension. The potatoes were placed in the furtows before application to preven any loss of the application suspension. To ensure a regular treatment, the seed potatoes were treated of the upper side, allowed to dry, then turned and treated on the other side. The forrow was closed when the application suspension was dried on the seed potatoes. A total volume of 763,29L was applied, corresponding to 50.2 MBq or to 12.7 mg a.s.. The actual seed treatment rate was 10.0 g as ./dt sorresponding to 254 g a.s./ha. The seed density was 25 dena.

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 pupp sprover was rinsed with mL water, 09 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 Demaining 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 petatoes, corresponding to apactual application rate of 626 g a.s./ha. The seed density was 22 dt/ha. À

Sampling:

Potato tubers were sampled as raw agricultural commodity (RAC) from the tuber treatment experiment and from the m-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. K,

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°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°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 adioactivity ju the extracts was determined by LSC and in the solids by combustion followed by **ESC**. 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 experiments were combined and subjected to a cleanstep using an SPE RP 18 cartridge (Phenomenex, Strata C18-E, 20 g), which was conditioned with acetonitrile beforehand. The flow-through fraction (percolate) was sollected and the cartfidge 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 everporation in vacuo for HPLC analysis.

The remaining solids after conventional extraction of the potato tubers in the m-furrow treatment experiment were subjected two times to extraction with aceton trile/water (10, v/v) onder 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 HPKC analysis. In the tuber treatment experiment, the solids remaining after conventional extraction and unted to 0.026 mg/kg, only. Moreover, shice the distribution of the radioactivity in the extracts and solids after conventional extraction was nearly identical within the infurrow 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 metabolities 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 mentioed by co-chromatography with reference compounds and/or by Comparison with the HPLC profiles obtained in the potato metabolism study with [pyridinylmethyl-14CPBYI 02960.

Storage stability:

Storage stability: The conventional extractions and the first HPLC analyses of the potato tuber extracts were performed not later then 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 as //dt (254 gs a.s./ha). In the other experiment [furanone-4-14G]BYI 02960, formulated as an SL 200, was spraved in the furrow onto the soil prior to planting of the seed potatoes at an application rate of 620 g a.s. Tha...

At harvest, the total radioactive residues (TRR) in potato tubers were now after tuber treatment and after in-furrow application and secounted for 0.078 mg/kg and 0.101 mg/kg, respectively. The TRR values in the remaining plant parts (leaves, poots 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 adioactivity in the porato tubers was extracted conventionally by acetonitrile/water mixtures (67.0% to 69.0% of the TRR) as shown in Table 62.1-12 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-diffuoroethyl-amino-furanone and BYI 02960-OHglyc. The assignment opparent 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]BYL(2960, 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 to chromatography on the present study, the identification of parent compound (mail constituent in the profile) of as 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 purior, label-specific metabolite BYI 02960-difluoroethyl-amino-furanone was identified by HREC corchromatography with an authentic reference compound in the tuber extract obtained after infurrow application. In the profile of the tuber treatment experiment, the metabolite was assigned by comparison of the two profiles.

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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.

	r i i i j	0
Table 6 2 1 12.	Distribution of radioactivity in the extracts of the potato tubers after tu	har traatmont or
1a0100.2.1-15.	Distribution of radioactivity in the extracts of the polato tubers after tu	
	in-furrow application of [furanone-4- ¹⁴ C]BYI 02960	
	in-runow application of [runanone-+- C]D1102900	

11	L	-	<u>R</u>	
	tuber trea	atment	in-furrow	application 5 tubers 5
	potato ti	ubers	potato	tubers of
TRR [mg/kg] =	0.078	Č,	0.17	
	% of TRR	mg/kg	ℚ % of TRR	Smg/kg
Conventionally extracted	67.0	م 0.052 L	69.0	Q 0.168 S
Extract for analysis	63.1) 0.049	©° 65.3√	Ç 0.411
Losses (not analysed)	3.9	0.003 ×	3.8	\$0.006 °
Microwave extraction	&		6.3	ັ∻> 0.01¥
Extract for analysis	0	, <u>č</u> F	6.20	C 04011 °
Losses (not analysed)		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	A SQM	S0.001
Total extracted	\$ 7.0 . Y	× 0.052	5.3	0.129
Unextractable (PES*)	Q 33,0 ×	U_0.026 KJ	24.7	0.042
Accountability	يري 1000 کې	40.078	~ 1000 S	9.171
* post extraction solids	R & B	8 8		°~y

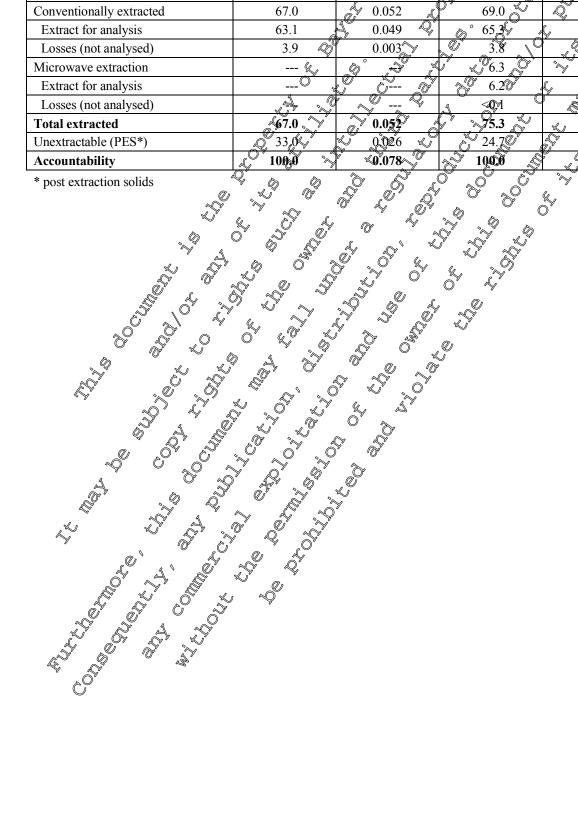


Table 6.2.1-14:TRR values and distribution of parent compound and metabolites in potato tubers after
tuber treatment and in-furrow application of [furanone-4-14C]BYI 02960

	tuber tr	eatment	in-furrow	application	
	potato	tubers	potato tubers 🔊 🔗		
TRR [mg/kg] =	0.078		0.171	e s	
Compound (BYI 02960-)	% TRR	mg/kg	% TØRR	mg/kg	
Conventional extraction			4		
BYI 02960 (parent compound)	40.0	0,031	50.6	× 0:986	
difluoroethyl-amino-furanone	4.2	\$0.003	2.9	~0.005 (
OH-glyc	6.6	ي 0.005	0 [°] 4.4 x	0.00	
Subtotal identified	50.8	0.039 Q	∘ 57 .9 ℃	[©] 9(0)99 ک	
unknown 1	7.2	0.006	<u>0</u> 5.9 \C	Ø.009 Ø	
unknown 2	1.7 🔬	° 0.00	y <u>v</u> »		
unknown 3	2.40*	Ø 0,002	<u> </u>	0.003	
unknown 4		Ø .001 		× & ~	
Subtotal characterised	£12.4 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	~0.010	C . 01.4 x	0.01	
Conventional extracts not analysed	3.9	√ 0:003 √C	3.8	ې 0. 0 06	
Total conventional extraction	67.0 67.0	v <u>0</u> .052_v	~~~ 6 29	Ø.118	
Microwave extraction	<u>Ŷ</u> à à	$\rightarrow 2'$		×9	
BYI 02960 (parent compound)	, S O	S P Ó	° 6.2	0.011	
Subtotal identified		······································	<u>6.2</u>	0.011	
Microwave extracts not analysed	6× 5- 0×	Ø ¥	A AN.1 Q	< 0.001	
Total microwave extraction	^Q ^{S³}	Å 5 *	· .3.	0.011	
Total identified 🔬 🔗	ୁହ 50. ଚ ୁର	× • Q,03 9	& 64.2 ⁹	0.110	
Total characterised	Q 12.4 S	0.010	O″ %. 4	0.013	
Analysed extract(s)	\$63.1~	<u> </u>	2 71.5	0.122	
Extracts not analysed	& 3.2×	0.003	3.8	0.006	
Total extracted 2	67.0	0.052	75.3	0.129	
Unextractable (PES*)	\$33.0 ~~	° 0.026	24.7	0.042	
Accountability	100.0	C 0 ,078 🔿	100.0	0.171	

* post extraction solids

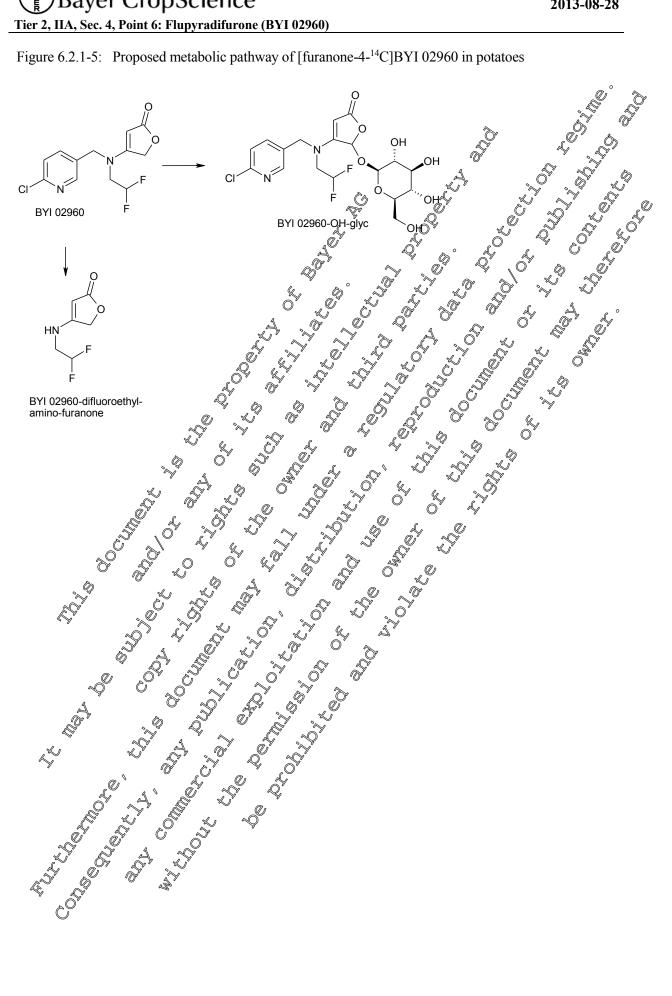
ATI. Conclusions

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

hydroxylation of the methylene group of the furanone moiety followed by conjugation with glusose, and

• cleavage of the pyridinylmethylam he bond

Thus, [furanone-4-¹⁴]BYL 2960 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.



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Report:	KIIA 6.2.1/05,
Title:	Metabolism of [pyridinylmethyl-14C]BYI 02960 in potatoes
Report No & Edition No	MEF-10/710 M-415078-01-2
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 107/2009
GLP	yes

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

Executive Sommary

The metabolism of [pyridinylmethyl-¹⁴C]BXI 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 heated with [pyridinylmethyl,⁴⁴C]BYI 02960 formulated as an FS 480 at an application rate of 10.0 g a.s./dt (= 276 g a.s./ha, seed density 27 d/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 potators were sampled. The TRR values determined are shown in the following table:

Table 6.2.1-15: TKR values in polato tubers and the remaining part of the plant after tuber treatment or in-furrow application of [pyfteinylmethyl-140]BYL02960

Matrix Timing and Application C	PHI (days)*	ppm (mg a.s. equiv./kg)
notato tubers	97	0.076
potato leaves and roots 10.0 g a.s./dc	97	8.40
remainders of the second potatoes	97	33.33
potato tubers	97	0.115
potato leaves ap@roots 0 (BBCHI 03),@26 g a@/ha 0	97	12.44
remainders of the seed potatoes	97	6.91

* PHropreharvest interval (corresponder to days after last treatment (DAT) at the start of harvest/sampling)

The potato tubers (edible faw agricultural compodity) were extracted conventionally. Four extraction steps with acetonitrile/water mixture@eleased 93.4% of the TRR in the tuber treatment experiment and 90.4% of the TRA in the in-furtow 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.

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 carboxytic group.

On the basis of these results, a metabolic pathway of [pyridinyhethyl \mathcal{C}]BYP02960 in potatoes and be proposed.

	I. Materialscand Methods
A. Materials	I. Materials and Methods
1. Test Material:	
Chemical structure	* position of the N S S S S S S S S S S S S S S S S S S S
Radiolabelled test material 4	pyridinglinethy[^{2/4} C]BYI 02960
Specific radioactivity O	$2.37 \text{ MBq/mg} (118 (28 \mu \text{Ci/mg}))$ > 99% (HPC) 2
Chemical Burity	> 992% (HPC) 0 4
Chemical Purity Radiochemical purity	

The supplied radio abelled test compound [pyrdinylmethyl_¹⁴C]BYI 02960 was dissolved in acetonitrile. Formulation of the test compound was performed prior to the application. For both formulations (FS 480 and SC 200); adequate parts of this stock solution were transferred into special glass vials and evaporated to drypess. 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 swifting. For the preparation of the SL 200 formulation, the liquid blank formulation was added to the test tem using a magnetic stirrer. The mixture was diluted with water to get the aqueous application solution.

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

3. Want Kotato, variety Cilena", representative for root crops

B. Study Design

Experimental conditions:

Two experiments were performed representing the intended application types (tuber treatment and *m*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 \mathbb{Z}^{2} 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 of 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. F 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 preven any loss of the application suspension. To ensure a regular treatment, the seed potatoes were treated of the upper side, allowed to dry, then turned and treated on the other side. The forrow was closed when the application suspension was dried on the seed potatoes. A total volume of 787,29L was applied, corresponding to 59.1 MBq or to 13.5 mg a.s.. The actual seco treatment rate was 10.0 g ars./dt corresponding to 270 g a.s./ha. The seed density was 27 dena.

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 vas sprayed onto the soil in the furrow. The empty pupp sprover was rinsed with mL water, 09 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 Demaining 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 petatoes, corresponding to apactual application rate of 626 g a.s./ha. The seed density was 28 dt/ha. À

Sampling:

Potato tubers were sampled as raw agricultural commodity (RAC) from the tuber treatment experiment and from the m-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. K,

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°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°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 adioactivity if 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 SPF 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 methanial/dichloromethane (C1, v/o). The percolate and the acetonitrile/water fraction were combined and concentrated by obtary evaporation in vacue for HPLCC analysis.

Quantification:

Parent compound and metabolites in the extracts were analysed by reversed phase HPLC coupled to a radioactivity detector with a glass schritillator cell. The HPCC chromatograms (Finetabolite 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 HPEC and normal phase TLC). Minor metabolites (<10% of the TRR and representing <0001 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 Festing 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 deeped 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.

Bayer CropScience

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

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./dg 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 few after tuber treatment and after in-furrow application and accounted for 0.076 mg/kg and 0.155 mg/kg, respectively. The PRR 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 potito tubers was extracted conventionally by acetonitrile/vater 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 >001 mg/kg) were parent compound and the metabolite BYI 02960-6-CNA. Both components were identified by co-chromatography with two difference thromatographic systems. They were identified in the tuber extract of the in-fuffic metabolites by 102960-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 1% fortation of the confined rotational crops study and to that of tomato fruits of the tomato metabolites had been identified with spectroscopic methods in these studies.

The minor metabolite BY1 02960-OH glyc was identified by HPLC co-chromatography after semipreparative isolation. Co-chromatography was performed using an acidic reversed phase HPLC method to ensure separation from BYL 02960-acetic acid, a metabolite co-eluting with BYI 02960-OH-glyc using the protifing method, as shown in the apple metabolism and the confined rotational crop studies. The configuration of the conjugated nexose 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 opparent 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 n-furrow application, respectively.

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 tr	eatment	in-furrow application			
	potato	tubers	potato tubers 🔊 🔗			
TRR [mg/kg] =	0.076	0.076				
	% of TRR	mg/kg	% of RR	mg/kg		
Conventionally extracted	93.4	0.071	<u>م</u> 90.4	0.1 0 4 0		
Extract for analysis	89.8	0,068	86.7	0.100		
Losses (not analysed)	3.7	\$0.003	3.6	~0 ^{0.004}		
Total extracted	93.4	چ 0.071	୦ ^୭ 90.4 🖑	0.104 ×		
Unextractable (PES*)	6.6	چ 0.005 Q	° 9.60			
Accountability	100.0	0.076	0 ² 100.0 \C	Ø.115 Ø		
* post extraction solids	K,					
	o* ,		` ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	d A o		
	A . 0		1 5			

Table 6.2.1-17: TRR values and distribution of parent compound and metabolites in potate tubers after tuber treatment and in purrow application of pyridinylmethyl-¹⁴ (BYI 02960

		y y y - Oy -		
		eatment	🗘 👸-furrøð i	application
<i>Q n</i>	s parato	tubers 🔊 🔬	potato	tubers
TRR [mg/kg] =	^م ر 0.076	<u>0</u> 4 64	0.115	\sim
Compound (BYI 02960-)	& % TRR	⊘mg/kg →	× % FRR	mg/kg
Conventional extraction			j ^v "S ^v "S	
BYI 02960 (parent compound)	\$ 40.2 °	© <u>0</u> 0031 &	44.0	0.051
	2015 N	×0.016 [©]	0 4 <u>8</u> .4	0.021
6-CNA-glycerol-glu	Ď "S ^o _~ .	0: 0 03	@ 23	0.003
CHMP-di-glyc		× 0.003	5.3	0.006
CHMP-al-glyc C C C CHMP-glyc C C C C C C C C C C C C C C C C C C C	0 & &	0.003 0.005 0.005	2.4	0.003
CHMP	$\begin{array}{c} O^{*} & \underline{3.9} \\ \hline & \underline{3.9} \\ \hline & 67 \end{array}$	\$ 0.003 ×	J 3.9	0.004
OH-glyc	0.7		4.7	0.005
Total identified	80.5 °C	9.061	80.9	0.093
	<u>, 0</u> 1.9 <u>, 1</u> .9	[∞] 0.001		
unknown 2 & A	2.3	0 0092	2.8	0.003
unknown 2 2 4 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2		× 0.004	3.0	0.003
Total characterised	9.3 gr	ۍ 0.007	5.8	0.007
Analysed extract(s)	89.8	0.068	86.7	0.100
Analysed extract(s)	38 .	0.003	3.6	0.004
Total extracted	93.4	0.071	90.4	0.104
Unextractable (PES*)	6.6 V	0.005	9.6	0.011
Accountability	100.0	0.076	100.0	0.115
* post autractic solid	w _ v			

* 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 carbohy thates • 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 to lowed by con ٠ with glucose, which was the minor metabolic oute.

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

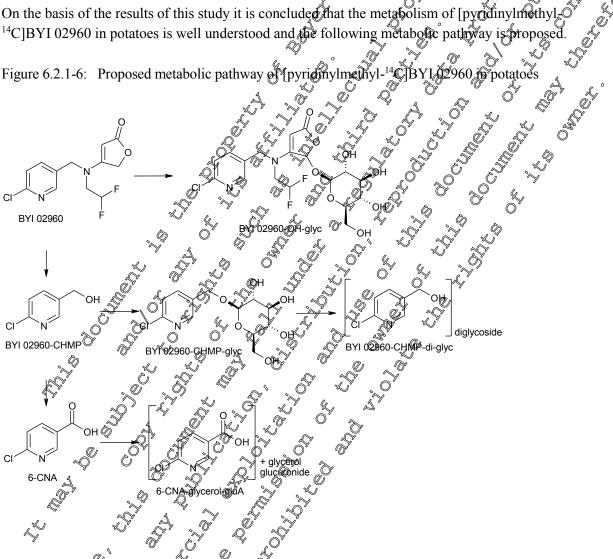


Figure 6.2.1-6: Proposed metabolic pathway

Overall Conclusions Potato (Tuber treatment and in-furrow application)

The metabolism of the insecticide BYF02960 was investigated in potatoes in two studies following tuber or sell appreation with (f) [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 metaboling 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 nonradiolabelled metabolite difluoroacetic acid - which cannot be detected with the radiolabels used revealed even higher concentrations of this metabolite compared to the parent compound.

(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 BYD02960-difluoroethy aminofuranone, a metabolite specific to the furanone-label. The metabolites identified work also detected in tomato fruits after soil application and in the confined rotational crop studies.

BYI 02960 was metabolised moderately in potatoes: bydroxylation of the methylone 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 polato study performed with [pyridinylmethyl-¹⁴C]BYI 02900 illustrating that the results of the metabolism studies were in good conformity.

(2) Label 2: [pyridinylmethyl-¹⁴C]BYL @296@

(2) Label 2: [pyridinylmethyl-¹⁴C]BY [92966, 7 C O Only one major metabolite (6-CNA) was detected besides parent compound. Additionally, eight minor metabolites were detected, five there of were identified BYI 02960-001-glye, a metabolite common to both radiolabels tested, and the gabel-specific metabolites BVI 02960-CHMP, BXP 02960-CHMPglyc, BYI 02960-CHMP-di-glyc and 6-CNA-glycerol-gluA. These metabolites or at least the aglycons were also detected in tomato fruit 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 metholene 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 hydroxydation of the methylene group of the furanone moiety followed by conjugation with glucose. This metabolic route resulted in the non-label specific metabolite BYI 02966-OH-glyc, which was identified in nearly identical concentrations in the potato study performed with [furanone 244C]BYI 02960. Thus, the results of the present metabolism study in potatoes are in good coofformits 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 Yound, and all majorand 6 minor have been identified. The distribution of parent compound and metabolites in the edible composity potato deber is summarized in Table 6.2.1-18.

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

	Potato tuber							
Radiolabel	[furanone-4- ¹⁴ C]			[pyridinylmethyl- ¹² C]				
	tuber tr	eatment	in-furro	w appl.	tuber treatment		in Furrow appl.	
TRR [mg/kg] =	0.0)78	0.1	71	0.0	7 6	0,1 0	¥5
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	ÓW TRAC	mĝ∕kg
BYI 02960	40.0	0.031	56.9	المجم 0.097	40.2	0.031	Å4M	0.051
6-CNA			Ŕ	4	21.5	0.016	~Q ⁷ 8.4 _×	
6-CNA-glycerol-gluA			~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	ألم	.0 [%]	%	2.3	04093
CHMP-di-glyc			Ą	, Q	4:4	Q.003	¢ Ç	J. Ø.006
CHMP-glyc			- AD	~	<u></u> 3.7 '	©0.0Q₽"		©0.003
СНМР		Ŵ	, b°	- S ⁰ - 1	J 3.L	0.003	3.9	0.004
difluoroethyl-amino- furanone	4.2	0.003		Č0.005				ý.
OH-glyc	6.6	0.005	4.A	02007	₼ 6,70	0.005	4.7	0.005
Total identified	50.8	Ø.039	64,2	0.11	8,96,5	9 ,061	800	0.093
Total characterised	12.4 🦿	0.01	<u> </u>	90.01 <i>3</i> 0	9.3	T.007	<i>©</i> 5.8	0.007
Analysed extract(s)	63.10	0.049	71.5	0,122	89.8	0.068	s≪ ⁸ 86.7	0.100
Extract(s) not analysed	<i>д</i> ,9 [°]	× 0.003 @		Ø.006	× 30F	_00003 _{(/}	3.6	0.004
Total extracted	\$ 7.0	∞ 0.052	75.3	∜0.12¢€	<i>8</i> 3.4	0.0710	90.4	0.104
Unextractable (PES*)	33.0	0.026	24.7 0	0.042	6.6	0.005	9.6	0.011
Accountability		0 .078	100.0	0. 171 ×	10 0.0 ×	0.076	100.0	0.115

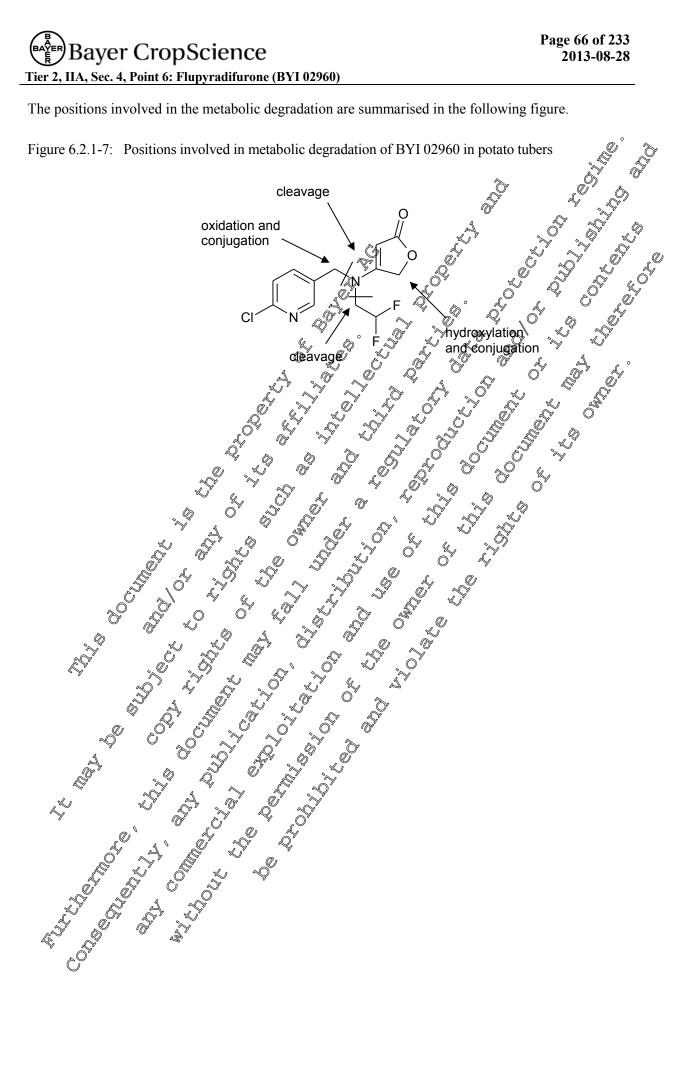
* post extraction solids 🔬

Label specific metabol

In order to gain information on the fate of the difluoroethane morety of BYI 02960, the tuber extracts obtained in the potato metabolism study with [pyridinytmethyl^AC]BYI 02960 were additionally analysed for non-radiolabelled difluoroacetic acid by LC-MS/MS according to residue method 01304 (see KIKA 6.2./12). High levels of this metabolite were found. After tuber treatment and in-furrow application, difluoroacetic acid accounted for 039 mg as. 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 diffuoroacetic acid is the main residue in potato tubers. Since it is known that BYI 02960 degrades to diffuoroacetic acid m soil, the high concentration of diffuoroacetic 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
- creavage of the pyridinylmethylamine bond and formation of BYI 02960-difluoroethylamino-
- furance 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



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

Report:	KIIA 6.2.1/06, .; 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.1 (97/2009
GLP	yes $(\mathcal{G}^{\circ} \mathcal{J}^{\circ} \mathcal{J}$

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 caropy height (86 g a.s./(ha x m CH) or also referred to 86 g g.s./ha/m CH) at the end of flowering (BBCH 69). In another experiment, one apple tree was treated twice with 86 g ags./(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 have stell Concirrently, the leaves were sampled to support metabolism investigation. The FKR values are shown in the following table?

Table 6.2.1-19.	TRE values in appl	e fruits a	nd leaves	after	folia applicatio	on of furanone-4-
	¹⁴ C]BYL02960	A	S.	O,		

Matrix	Timing and Application of the second	PHI (days)*	ppm (mg a.s. equiv./kg)
apple fruits	Mie foliar spray application at BBCH 69,	98	0.280
apple leaves	$\sqrt{2}$ $\sqrt{86}$ g $\frac{2}{3}$ (ha x m CH) $\sqrt{2}$	98	38.957
apple fruits ¹		14	1.133
apple fruits ²	and 14 days PHL 2 x 86 g a.s./(hay x m CHJ)	14	1.286
apple leaves	Z x 86@/a.s./(ha/x m (CHJ)	14	102.919

* PHI: preharvest therval (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 fronts 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: Flupyraditurone (BY1 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 (200% of the GRR) 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 7.3% of the TRR. All other metabolites were minor. Four basic metabolic routes were detected.

- hydroxylation of the furanone or the difluoracthyl molety followed by conjugation,
- complete degradation of the furance moiory and incorporation of carbon atoms into the natural compound pool, i.e. into glucose carbon drates
- cleavage of the pyridinylmethylamine bond and
- oxidative degradation of the furance morely to an acetic acid group followed by conjugation with a carbohydrate.

On the basis of these results, a metabolic pathway of [furanone-4-4]BYJ 02960 m apples can be proposed.

The second Mathematical Second Mathematical Second							
The second secon							
y I. Waterian and stethods							
A. Materials	9						
I. Lest Material: $\mathcal{A} = \mathcal{A}^{*} \mathcal{A}^{*0} \mathcal{A}^{*} \mathcal{A}^{*0} \mathcal{A}^{*0}$							
Chemical structure							
	* position of the						
	radiolabel						
Radiolabelled test material Specific radioactive A C C C C C C C C C C C C C							
Radiolabelled test material [furanone ²¹⁴ C]BY1 02960							
Specific radioactivity							
Specific radioactively (before radiodilution) 3.94 MBcrag (106.46 µCi/mg)							
(after radiodilution) 107 MBq/mg (53.24 μ Ci/mg)							
Specific radioactively 3.94 MBα/mg (106.46 μCi/mg) (after radiodilution) 1.97 MBα/mg (53.24 μCi/mg) Chemical Putity 99% (HPLC) Radiocheroical putity > 99% (HPLC)							
Radiochemical purity 2 > 99% (HPLC and TLC)							

The test compound was formulated as an SL 200. In order to prepare an appropriate stock solution, the supplied radiolabelted 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).

4" (sandy loam soil from Germany), pH (CaCl₂) = 6.8, 6% sand, 18% (ilt and anic carbon, cation exchange capacity (CEC) of 8.3 meq/100 g, riety "James Grieve", representative for fruiting crops 2. Soil: 13% clay, 1.2% organic carbon, cation exchange capacity (CEC) of 8.3 meg/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 0,2960 ip orchards. In one experiment (single foliar spray application), the appletrees were treated at a targed application rate of 75 g a.s./(ha x m CH) (canopy height) at the end of Nowering (BBCH 69) In the other experiment (double foliar spray application), one apple the 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 large fate corresponds to the anticipated maximum application rate. The apple trees used in the present study bad a canopy height (CH) of 0.5 m and were cultivated in the sandy loam soil," 45 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 00 mL of the aqueous application solution using a hand pump spray Prior to the application, each apple tree was covered with a projective plastic wrap of 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 muse solutions was determined and subtracted from the amount in the application solution. A fotal 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 carbon hoght of 0.5 m was treated for the first time with 10 mL of the aqueous application solution using a lond purp 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°C) on the dage \hat{f} harvest until extraction.

All apple fruits of the single application experiment were cut in pieces. The process were mixed and divided into aliquots. The apple aliquots were stored in a freezer ($\leq -18^{\circ}$ C) until needed for extraction, The apple fruits of the double application experiment were separated into two subsamples. Approx.

Extraction: Single application experiment (apple fruits): The apple pieces were extracted three times with a maxture of acetomitrile/water to the extracts was determined by LSC, by the solide be of the RAC was determined by CSC, by the solide be remaining solids. All extracts were combined and subjected to a clean up step using an SPE RP 18 cartridge (Phenomenex, Strata C19-E, 209), which was conditioned with accionitate beforehand. The flow-through fraction (percolate) was collected and the cartfridge 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 rotated evaporation in vacuo. The pH value of the concentrate was adjusted to pH 7 with animonium carbonate before chromatographic analysis by HPLC. The solids remaining after the conventional expaction steps were sigmitted two times to a microwave-assisted extraction step with acetonitrile/water (1, v/x) After each extraction step, extracts and solids were separated by centrifugation. The extracts were combined and concentrated by rotary evaporation in vacuo. Prior to HPL 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 rotaty 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 topH 7, with any nonjugar carbonate for HPLC analysis.

Double application experiment (apple fruits):

je S

The subsample of the whole apple fruits which was stored at room temperature was subjected to a surface wash with dichloromethane. An align of the dichloromethane surface wash was dispersed with 5 mL of vater 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°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 carbonates 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-op procedure and the concentration step were also identical with the concentration of the single application experiment. 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

Ouantification:

...OC coupled to so(= metabolite profiles) Parent compound and metabolites in the extracts were analysed by reversed phase HP6C coupled to a radioactivity detector with a glass scintillator cell The HPLC chromatogram (= 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 pyriding lmethyl-14CBYI 02960. In this study parent compound and an interabolites common to both radio abels were identified by two independent chromatographic methods (reversed phase HPEC and normal phase FLC) 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 metabolic profiles.

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

Storage stability:

All extraction and surface was experiments and the first to LC 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 month 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 coronatograme recorded at different times during the study showed that the profiles of the extracts dignot change significantly during the analytical work up, which did not exceed approx mine 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 ord of flowering (BBCH 69). In the other experiment, one apple tree was treated two tones 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 vas 172 g a.s./(ha x m CH). The actual single application rate were slightly above the antiepated 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 metabolities. The apple truit 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 RAG was 0.280 mg/kg in the single application experiment. In the double application experiment, the apple fruits had a TRR of 9.1.133 mg/kg (extraction including surface wash) and 0.286 mg/kg (extraction without surface wash). The TRR values of the apple leaves were high. 38.957 mg/kg were found in the traves of the single application experiment and 102.910 mg/kg in the teaves of the double application experiment. Apple leaves were only sampled to support the dentification of metabolites

The radioactive residues were efficiently extracted with acetonitrile/wher mixtures after conventional and exhaustive extraction procedures (86.5% to 96.5% of the FRR) as shown in Table 6.2.1-20 and Table 6.2.1-21. The apple fruits of the double application experiment were additionally surfacewashed with dichloromethane and then extracted conventionally with acetonitrile/water mixtures. A portion of 75% 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 wask 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 [pyridinyImetby]-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 isometic 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.

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-cheomatography.

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 tester as B 1 02960acetic acid-glyc, BYI 02960-OH-glyc, BYI 02960-acetic acid, BYI 02960-difluoroethyl-OH-gl 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-diffuoroethyl-amino-furanone was identified in the extract of apple fruits of the double application experiment by HPLC coefficiency with a nonradiolabelled 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 6.2.7-23.

Single application experiment						
	apple 🖓	fruits 🖉	apple leaves			
TRR [mg/kg] = 0 0 0	°~~ ~ 0.289		38.957	7		
	୬ %®fTRR©	mg/kg	% of TRR	mg/kg		
Conventionally extracted	<u>ر</u> 59.	K 0.168	94.3	36.725		
Extract for analysis	~ 587 ~	0.164	94.0	36.604		
Losses (not analysed)		0.003	0.3	0.121		
Microwave extraction	9.5 ⁰	0.027				
Extract for analysis	~~~ 9 <u>9</u>	0.026				
Losses (not analysed)	CC0 .1	< 0.001				
Cellulase expract S &	17.2	0.048				
Total extracted 3 1	86.5	0.242	94.3	36.725		
Unextractable PES*)	13.5	0.038	5.7	2.232		
Accountability 3	100.0	0.280	100.0	38.957		

Table 6.2 1 Distribution of radioactivity in the extracts of apple fruits and leaves after a single foliar application of furgion -4 4 C BY 0 2960 -2

* post extraction solids

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

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

		d	ouble applicat	<u>ion expe</u> rimer	nt		
	apple fr		apple fr		apple le	aves	ð
	incl. surfac	e wash	without surf	ace wash	ð		
TRR $[mg/kg] =$	1.13	3	1.28	36 a	§ 102.9	19 . 5	
	% of TRR	mg/kg	% of TRR	mg/kg 🔬			Ô
Surface wash with DCM	7.5	0.085	<i>~</i>	Å.	25	×	1
Conventionally extracted	81.9	0.928	~9 .1.9	1.082	96,5	999.2 80	
Extract for analysis	81.6	0.925	ي 91.2	Ø.173	∢ 96.0 _^	986843	,þ
Losses (not analysed)	0.3	0.003	ر گ ^{ور} 0.7	0.009°	0.4	උ0.436 @	,¥
Cellulase extract	3.9	0.044	» «	,	Ŷ, Ŷ	<u>0.436</u>	
Total extracted	93.3	1.057	<u></u> ∘91.1 <i>©</i>	<u></u>]%182	~96.5 "*	99.280	
Unextractable (PES*)	6.7	0.056	Ø 84	× 0.104	\$ 3.5	3.639	
Accountability	100.0	1.133	, 1 09 .0	0, 1.286	1000	3.639 (102.919)	
Surface wash with DCM Conventionally extracted Extract for analysis Losses (not analysed) Cellulase extract Total extracted Unextractable (PES*) Accountability * post extraction solids						×	
		Ŷ					

 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

leaves after a single foliar treatment of [furanone-4- ¹⁺ C]BY102960					
	annle	fruits	apple	leaves	
TRR [mg/kg] =	0.280		36.715		
Compound (BYI 02960-)	% TRR	mg/kg	% TORR	mg/kg S	
Conventional extraction	/01111		1		
BYI 02960 (parent compound)	7.4	0,021	26.0	× 10.438	
glucose/carbohydrates	50.3	1 1 1 1 1	2.5	~00.991	
difluoroethyl amino furanone	3.2	2.009			
acetic acid-glyc	0.3	<u>3</u> 0.001 Q	° 6.4	r 2@486 @	
OH-glyc	0.4	0.001	<u>36.</u>	₫4.062 Ø	
acetic acid	0.2 (° 0.004	2.5	0.956	
difluoroethyl-OH-glyc	0		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	2264	
ОН	A		20 0.40 C	0 .244	
Subtotal identified	587	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	A 69.9 K	31.14	
unknown 1			× 030	~ 0.00	
unknown 3		× × ×		0.020 09.646	
unknown 4				×,	
unknown 5	, <u> </u>		2	×>	
unknown 6			© 2.000 © 1.6	0.609	
unknown 0	y N A	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	× × 1.5 0	0.578	
unknown 7 view of the second s			4.9	1.895	
Subtotal characterised &			<u> </u>	5.463	
Conventional extracts for analysed		× 0.003	O %.3	0.121	
Total conventional extraction	59.8~	0.168	© 0.3	36.725	
Microwave extraction	<i>(</i>		\$94.3 \$	30.725	
MICTOWAVE EXITAL POIL	<u></u>	0.018	~		
glucose/carboh@tates	\$.4 \$.4 1.0	0.003	<i>Ø</i>		
Subtotal identified	7.5	C 19.921			
unknown ² Subtotal characterised		0.005			
Subtotul enalueterised		<0.003			
Microwave extracts not analysed S Total microwave extraction	0, <0,1 ² , 0,1 0,0 0,0 0,0 0,0 0,0 0,0 0,0 0,0 0,	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~			
		Ø.02 7			
Cellulase digestion		<u> </u>			
glucose/carbohydrates	15.0 ×	≪ <u> </u>			
difluoroethyl-amino-furanone		0.006			
Subtotal characterised	0 47.2 V	0.048			
Total cellulase extraction	~~ 17Q ⁷	0.048			
Total identified	83.4	0.234	79.9	31.141	
Total characterised	2.0	0.005	14.0	5.463	
Analysedextracter	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

 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

Surface wash Sufface wash<	<u> </u>
surface wash surface wash surface wash surface wash $(12.91)^\circ$ TRR [mg/kg] = 1.133 1.286 102.919 Compound (BY102960-) % TRR mg/kg % TRR mg/kg glucose/carbohydrates 0.2 0.002 $(12.91)^\circ$ $(12.91)^\circ$ glucose/carbohydrates 0.2 0.002 $(12.91)^\circ$ $(12.91)^\circ$ $(12.91)^\circ$ octic acid 0.1 0.001/° $(12.91)^\circ$ $(12.91)^\circ$ $(12.91)^\circ$ $(12.91)^\circ$ OH 0.1 0.001/° $(12.91)^\circ$ $(12.91)^\circ$ $(12.91)^\circ$ $(12.91)^\circ$ $(12.91)^\circ$ unknown 8 0.1 $(0.001)^\circ$ $(12.91)^\circ$	ý ô
Compound (BY1 02960-) % TRR mg/kg % TRR<	
Surface wash Surface wash parent compound 6.7 0.076 0.002 OH-glyc 0.2 0.002 0.002 0.002 Carcia caid 0.11 0.000 0.002 0.002 0.002 OH-glyc 0.22 0.002 0.002 0.002 0.002 0.002 0.002 0.003	<u> </u>
parent compound 6.7 0.076 0 glucose/carbohydrates 0.2 0.002 0 0 OH-glyc 0.2 0.002 0 0 0 OH 0.1 0.001 0 0 0 0 Subtotal identified 7.2 0.002 0 0 0 0 unknown 8 0.1 0.001 0 0 0 0 0 subtotal identified 7.2 0.003 0	©∕kg ⊘
glucose/carbohydrates 0.2 0.002 0.002 OH-glyc 0.2 0.002 0.002 0.002 acetic acid 0.1 0.000 0.002 0.002 0.002 OH 0.1 0.000 0.002 0.002 0.001 0.001 Subtotal identified 7.2 0.0852 0.002 0.002 0.001 unknown 8 0.1 0.000 0.002 0.001 0.001 0.001 Subtotal identified 7.2 0.00852 0.001 0.002 0.002 0.002 0.003 0.002 0.003 0.012 0.003 0.012 0.003 0.01 0.003 0.01 0.003 0.01 0.003 0.01 0.003 0.01 0.003 0.01 0.000	
OH-glyc 0.2 0.003 0.003 0.07 0.003 0.07 0.003 0.07 0.003 0.07 0.003 0.07 0.003 0.07 0.003 0.07 0.003 0.07 0.003 0.07 0.003 0.07 0.003 0.07 0.003 0.07 0.003 0.07 0.003 0.07 0.003	Ű
acetic acid 0.1 0.0012 0.0012 0.0012 Subtotal identified 7.2 0.082 0.0012 0.0012 0.0012 unknown 8 0.1 0.0012	S v
OH 0.1 0.001 0.7 7 7 Subtotal identified 7.2 0.082 7 7 7 unknown 8 0.1 <0.000	
OH 0.1 0.001 0.7 7 7 Subtotal identified 7.2 0.082 7 7 7 unknown 8 0.1 <0.000	Ő,
unknown 8 <0.1 <0.001/m Subtotal characterised 0.3 0.003 0.017 0.003 0.017 0.003 0.01 0.003 0.01 0.003 0.01 0.003 0.01 0.003 0.01 0.003 0.01 0.003 0.01 0.003 0.01 0.003 0.01 0.003 0.01 0.003 0.01 0.003 0.01 0.003 0.01 0.003 0.01 0.003 0.01 0.003 0.01 0.003 0.01 0.003 0.01 0.003 0.01 0.0003 0.01 0.003 <t< td=""><td>Ş</td></t<>	Ş
unknown 8 <0.1 <0.001/2 0 0 0 Subtotal characterised 0.3 0.003 0<	~~
unknown 9 0.3 0.003 0.3 0.003 0.3 0.003 0.3 0.003 0.3 0.003 0.3 0.003 0.3 0.003 0.3 0.003 0.3 0.003 0.3 0.003 0.3 0.003 0.3 0.003 0.01 0.01 0.01 0.01 0.01 0.01 0.01 0.012 0.012 0.003 0.07 0.001 0.003 0.001 </td <td>Å</td>	Å
Total surface wash 7.5 0.085 0 0.945 0.946 57.9 5 glucose/carbohydrates 13.6 0.154 44.2 0.82 36 5 glucose/carbohydrates 13.6 0.154 44.2 0.82 36 5 difluoroethyl-amino-furanone 0 0.007 4.2 0.007 4.2 1 OH-glyc 0.8 0.009 0.5 0.007 4.2 1 acetic acid 0.6 0.007 0.11 0.014 1.7.3 1 acetic acid 0.6 0.007 0.7 0.009 1.2 1 OH 0.9 0.010 0.8 0.000 0.6 1 Subtotal identified 81.6 0.925 91.1 1.171 87.5 9 unknown 1 0.5 1 unknown 5 0.5 1 unknown 6 <td< td=""><td><u> </u></td></td<>	<u> </u>
Total surface wash 7.5 0.0855 0 0.946 57.9 5 glucose/carbohydrates 13.6 0.154 44.2 0.946 57.9 5 glucose/carbohydrates 13.6 0.154 44.2 0.946 57.9 5 glucose/carbohydrates 13.6 0.154 44.2 0.948 36 6 difluoroethyl-amino-furanone 0 0.002 0.007 4.2 1 0003 0.07 4.2 1 OH-glyc 0.8 0.002 0.7 0.009 4.2 1 1 2 0.007 4.2 1 2 1 2 0 1.2 1 3 2 0.007 4.2 1 2 1 2 1 2 0 0.007 4.2 1 2 1 2 1 2 1 2 1 2 1 2 1 2 1 2 1 2 1 2 1	je v v v v v v v v v v v v v v v v v v v
Conventional extraction A <tha< th=""> A A A</tha<>	
parent compound 64.7. 633 766 0.946 57.9 5 glucose/carbohydrates 13.6 0.154 14.2 0482 36 36 difluoroethyl-amino-furanone 0.8 0009 0.5 0.007 4.2 0482 36 acetic acid-glyc 0.8 0009 0.5 0.007 4.2 04 17.3 1 acetic acid 0.6 0.007 0.7 0.009 1.2 01 1.3 1 1.3 1.4 0.112 0.11 0.014 0.7 0.009 1.2 01 0.009 0.12 0.12 0.11 0.014 0.7 0.009 0.6 0.000 0.6 0.000 0.6 0.000 0.6 0.000 0.6 0.000 0.6 0.000 0.6 0.000 0.6 0.000 0.6 0.000 0.6 0.000 0.6 0.000 0.000 0.6 0.000 0.000 0.000 0.000 0.000 0.000 0.0	
glucose/carbohydrates 13.6 0.154 14.2 0.82 16 difluoroethyl-amino-furanone 0.8 0.009 0.5 0.007 4.2 0.11 0.007 4.2 0.11 0.007 4.2 0.1 0.007 4.2 0.007 4.2 0.2 0.1 0.1	9.547
difluoroethyl-amino-furanone 0 0 0.2 0003 0.7 acetic acid_glyc 0.8 0009 0.5 0.00% 4.2 OH-glyc 0.1 0014 17.3 1 acetic acid 0.6 0.00% 0.7 0009 1.2 difluoroethyl-OH-glyc 0.7 0009 0.7 0009 1.2 OH 0.00 0.00% 0.7 0009 0.6 Subtotal identified 81.6 0.925 91.1 1.171 87.5 9 unknown 1 0.7 0.00% 0.6 0.6 0.00% 0.6 0.6 unknown 2 0.6 0.6 0.00% 0.6 0.6 unknown 3 0.5 0.00% 0.1 0.002 0.5 0.00% unknown 4 0.5 0.00% 0.1 0.002 1.2 unknown 5 0.5 0.00% 0.1 0.002 8.5 <td>3.687</td>	3.687
acetic acid-glyc 0.8 0009 0.5 0.00% 4.2 OH-glyc 1.0 00.012 0.1 0.014 17.3 1 acetic acid 0.6 0.000 0.7 0009 1.2 difluoroethyl-OH-glyc 2.1 1 OH 0.0 0.010 0.8 0.000 0.6 1 Subtotal identified 81.6 0.925 91.1 1.171 87.5 9 unknown 1 0.6 1 unknown 2 0.6 1 unknown 3 0.5 1 unknown 4 0.5 1 unknown 5 0.5 1 unknown 7 0.5 1 unknown 8 2.5 1 unknown 7 0.1 0.002 <td< td=""><td>0.736</td></td<>	0.736
OH-glyc 1.0 0.012 0.11 0.014 17.3 1 acetic acid 0.6 0.000 0.7 0.009 1.2 difluoroethyl-OH-glyc 2.1 1 OH 0.0 0.010 0.8 0.000 0.6 1 Subtotal identified 8106 0.925 91.1 1.171 87.5 9 unknown 1 0.6 1 unknown 2 0.5 1 unknown 3 0.5 1 unknown 4 0.5 1 unknown 5 0.5 1 unknown 6 0.5 1 unknown 4 0.5 1 unknown 7 0.5 1 unknown 8 0.5 <td>4.274</td>	4.274
acctic acid 0.6 0.000 0.7 0.009 1.2 difluoroethyl-OH-gtx 2.1 0.000 0.8 0.000 0.6 Subtotal identified 816 0.925 91.12 1.171 87.5 9 unknown 1 0.6 0.000 unknown 2 0.5 0.000 0.6 unknown 3 0.5 0.000 0.6 unknown 4 0.5 0.000 0.000 0.000 unknown 5 0.5 0.000 0.1 0.002 1.2 unknown 6 0.5 0.000 0.1 0.002 1.2 unknown 7 0.1 0.002 1.2 0.000 0.1 0.002 8.5 0.0003 0.7 0.009 0.4 0.000 0.4 0.000 0.4 0.000	7.856
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Subtotal characterised 0.3 0.003 0.1 0.002 8.5 Conv. extracts not analysed 0.3 0.003 0.7 0.009 0.4 Total conv. extraction 84.9 0.928 91.9 1.182 96.5 9 Cellulase dige 0 on 3.3 0.037 0.007 0.007 0.007 0.007 glucose/carlsonydrates 3.3 0.037 0.007 0.007 0.007 0.004 Subtotal characterised Total cellulase extraction 3.9 0.044	1.210
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Cellulase dige@on 3.3 0.037 glucose/carbohydrates 3.3 0.037 difluoroeth/l-amike-furanone 0.6 0.007 Subtotal identified 3.9 0.044 Subtotal characterised Total cellulase extraction 3.9 0.044	9.280
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Subtotal characterised Total cellulase extraction 3.9 0.044	
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	0.063
	8.781

m 11

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

		double application experiment						
	apple fruits incl.apple fruits withoutsurface washsurface wash			apple le	eaves 0°			
Analysed extract(s)	93.0	1.054	91.2	1.173	96.0	Ø8 .843		
Extracts not analysed	0.3	0.003	0.7	0.009	0.4	ر 0.430		
Total extracted	93.3	1.057	91.9	1.182	96.5 🔊	99 280		
Unextractable (PES*)	6.7	0.076	8.1	0.1.07	3.50"	<u></u> , Ø.639√		
Accountability	100.0	1.133		1,286	100.0	102.919		

* post extraction solids

OF SOL , Î

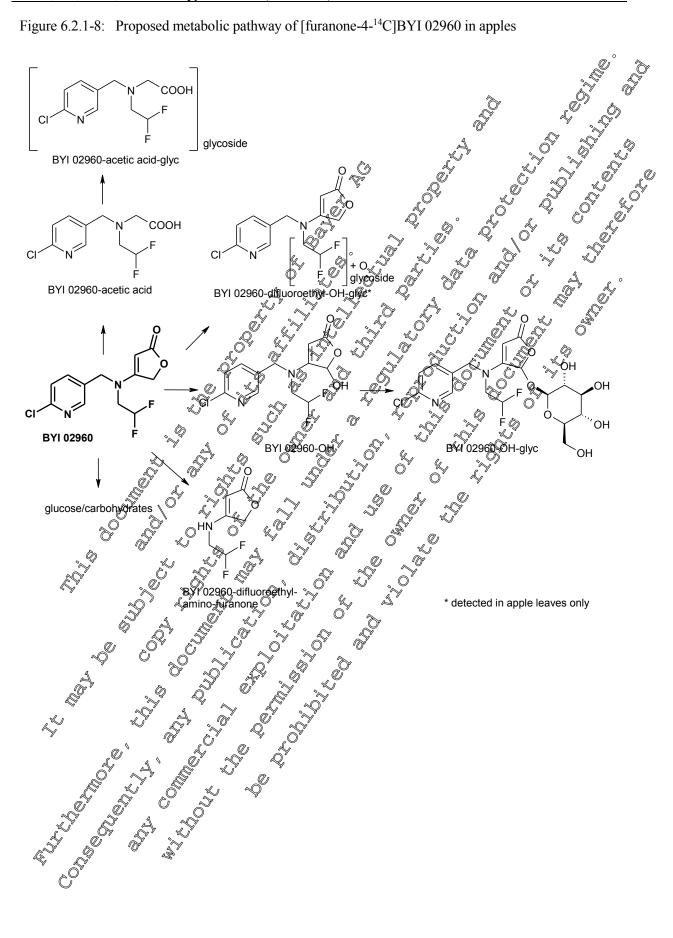
Four major metabolic routes of [furanone-4-14 BY] 2960 were observed in apples:

- hydroxylation of the furation of the diffuoreethyl more by conjugation with • carbohydrates, complete degradation of the furanone mojety and incomporation of carbon atoms into the
- natural compound pool, i.e. into glucose/carbohydrates,
- oxidative degradation of the furance moiety to an acetic acid group followed by • conjugation with a carbohydrate, and
- cleavage of the pyridiny methylamine bond. • \bigcirc

On the basis of the study it is concluded that the metabolism of [furanone-4-

On the basis of the sesults of this study to is cancillated that the metabolism of [furanone-4-¹⁴C]BY102960 br apples is well understood and the following metabolic pathway is proposed.

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



Bayer CropScience

Report:	KIIA 6.2.1/07,
Title:	Metabolism of [pyridinylmethyl-14C]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 107/2009
GLP	yes de la companya de

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

Executive Sommary

The metabolism of [pyridinylmethyl-¹⁴C]B\$I 02960 formalated as an SL 200 was investigated in apples after foliar spray application. In one experiment (single application experiment), two apples trees were treated at an actual application rate of 87 g a.s./ha/per meter canopy height (\$7 g a.s./ha x m CH)) at the end of flowering (BBCH 69) in another experiment, one apple free 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) in another experiment, one apple free 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 as./(ha x m CH) at 14 days before barvest. 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 TRE values are shown in the following table: 4

	`	~ _ ¥			
Table 6.2 1-24. $\square R = 1$	n Innleafrui	its and leave	le after Miar	monlication o	f [nvridinvlmethvl_
Table 0.2.1-24. FILL Fulles	n approxyui	ns que reav		application	pyriantynneuryi-
Table 6.2.1-24: PRR values i	àn O		Ô° 4	S. S.	
\bigcirc C \square	90 °	°∀ Ø	$\sim \sim \sim$		

Ø

Matrix 3	Timing and Application	PHI (days)*	ppm (mg a.s. equiv./kg)
apple fruits	one foliar spray application at BBCH 69,	98	0.079
apple leaves	Sy g a.s. (tra x m CH)	98	56.715
apple fruits ¹	Two formar spray applications, ap BBCH 69	14	1.868
apple fruits ²	\mathbb{O}^{\vee} \mathbb{O}^{\vee} and \mathbb{M} days \mathbb{O}^{\vee} \mathbb{O}^{\vee}	14	0.545
apple leaves	87 and 85 g a.s./(bax m @H)	14	134.841

PH/Opreharvest interval (corresponds to days after last treatment (DAT) at the start of harvest/sampling)
 determined from the extraction experiment with prior surface wash

 2 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 & subsample of the apple fruits of the double application experiment was surface-washed with dichloromethane (10.0% 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

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. Devertheless, warged 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 detected: 5% of the TRR, all other metabo

- hydroxylation of the furanone or the difluoroethyl moiety followed by conjugation with carbohydrates,
- cleavage of the pyridinylmethylamine bood 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 of by further degradation of the moiety

On the basis of these results, a metabolic pathway of byriding meth 4¹⁴C BYI 02960 in apples was proposed.

	Š	🦉 I, Materia	ls and Meth	øds "		Ý
A. Materials	Ĵ,			londs (
1. Test Material:	\$ \$		ĵ, o			
Chemical structure	× A			^y O	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
				Lo ^r §		
			° *		$\mathscr{O}_{\mathscr{I}}^*$ position of t	he
l ô ^r	10°		N S	~6 [%] , %	v position of t	iic
	Ş ^a ₄ O	o ko /		.5 0		
			<u>~~~~</u> ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~			
Radiolabelled test r	materiars st	Invrianavime	thyl-14C]BY	1.02960		
Specific radioactori	ty a m			9		
(before radiodilatio	ng S	437 MBg/m	g (#18.08gr(Ci/mg)		
(after radiod aution)		2.18 MBq/m	g (58.92 µCi	i/mg)		
Chemical Purity		′ > 9 9% (HP€	Č)			
Radiochemical puri	ty o	>@8%(ÈPPL	C and TLC)			
			Ŷ.			

The test compound was formulated as an SL 200. Therefore the supplied radiolabelled test compound [pyridinylmethy], 44 C]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-tadiolabelled 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 μ Ci/mg).

Bayer CropScience

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

2. Soil: 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 meg/100 g,

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 02900 for the experiment (single foliar spray application), the apple trees were total of the variety of BYI 02900 for the experiment (single foliar spray application), the apple trees were total of the variety of BYI 02900 for the experiment (single foliar spray application), the apple trees were total of the variety of BYI 02900 for the experiment (single foliar spray application), the apple trees were total of the variety of BYI 02900 for the experiment (single foliar spray application) for the 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 taget rate correspond to the anticipated maximum application rate. The apple trees had a conopy height (CH) of 0.5 m and were 4," in a planting container with a surface area of 0.091 m². cultivated in the sandy loam soil " The plants were grown in the glass toofed regetation area of the test facility which allows curtivation 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 on were freate Deach with 10 mL of the aqueous application solution using a hand permp sprayer. Prior to the application, each apple tree was covered with a protective pastic whap to prevent radioactivity spreading into the vegetation area. After application, the hand pump sprayer was rinsed with water and acconitrite and the plastic wrap was rinsed with methanol. The radioactivity in the ringe 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 s.s. From a planting achisity of 3000 trees/ba an actual application rate of 87 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 hard pump sprayer and the protective plastic wrap were rinsed to determine the losses of radioactivity Que 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./(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 stored in a freezer (\leq -18°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°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°C) on the day of harvest. The remaining whole fruits were stored overnight at room temperature. On the next day the apple 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 pixture of acetonitrile water (8:2, 4v) and one time with time with a mixture of acetonitrile/water (1:1, v/v) using a high speed blender. The adioactivity in the extracts was determined by LSC, in the solids by compustion 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 648-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, v, v/v) The percolate and the acetonitrile/water fraction were combined and conceptrated by rotary evaporation in vacuo. The pH value of the concentrate was adjusted to part with ammonium carbonate before chromatographic analysis by HPLC.

Double application experiment (apple fruits):

 \bigcirc The subsample of the whole applorruits which was stored at foom temperature was subjected to a surface wash with dichloromethane. An aliquet of the dichloromethane surface wash was dispersed with 1 mL of water by ultrasonic treatment. The dehlor onethane was removed by a nitrogen stream and the remaining concentrate was analysed by MPLC The washed apples were cut into pieces. The apples pieces were mixed, divided into aliquots and stored in a freezer (\leq -18°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 puble pplic on 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 abquot 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 an@ysis.∧ Ô

Aliquots of the spple leaves of the single and double application experiment were extracted as described for the freux 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 chromatopraphic methods (reversed, 9) phase HPLC and normal phase TLC) or by LC-MS/MS after semi-preparative isolation of the semi-preparative iso 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 solated 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 chromatogram's recorded at different times during the study showed that the profiles of the extracts of 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

IF Results and Discussion

The metabolispoof [pytidiny] wethy C]B& 02960 was investigated in apple fruits and leaves following twodifferent spray scenarios. In one experiment (single application experiment), two apple trees were reated at an actual application rate of 87 g a.s./(ha x m CH) (CH = meter canopy height) at the end of flowering (BBCH O). 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 havested 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 acconitrite/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 6.21-26).

HPLC analysis of the surface wash and the conventional stracts of apple fruits and leaves after both spray scenarios revealed that all notabolite 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 sprace wash) by comparison.

The main compound in apple traits and leaves of the single and the double application experiment was parent compound. It is identified in the extracts of apple fruits of both experiments and in the extract of apple leaves of the single application experiment bo HPLC co-chromatography with a radiolabelled reference compound. For contribution, 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 anothe fractions were subjected to LC-MS/MS analysis. Thus, metabolites B\$7 02960-CHMP-glyc, BYI 02960-difluoroethyl-OH-glyc, BYI 02960-difluoroethyl-OH-glyc, BYI 02960-difluoroethyl-OH-glyc and the corresponding aglycons BYI 02960-acetic acid and BYI 02960-OH were identified. The isolated metabolites were used for HPL 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-

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 give and BYI 02960acetic 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 subquantification 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. Reanalyses 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 subuantification of 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 Confided rotational crops soldy.

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 ß-glucosidase cleaved the conjugate BYI 02960-OH-glyc. HPLC co-chromatography of the cleavage product with the toference compound BYI 02960-OH located the conjugating hydroxy group at the 5-position of the furance moiety. Concurrently, the successful enzymatic deavage revealed D-glucose as the configuration for the conjugated herose. For the metabolite BYI 02960-difluoroethyl-OH-glyc, the position of the hydroxy group being the Brik for the conjugation could not be exactly determined by LC-MS/MS. However, the fragmentation pattern indicated hydroxylation and conjugation in the diffuoroethyl side chain.

The minor metabolites BYI 02960-6-CNA and BYL02960 CHMP were identified in apple fruits of the single application experiment by HPLC co-chromatography with authentic reference compounds. Following identification of metabolites by LCAIS/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% (998.5% of the TRR were identified in the apple fruit after foliar application as summarized in Table 6.2.2-27 and Table 6.2. 928.

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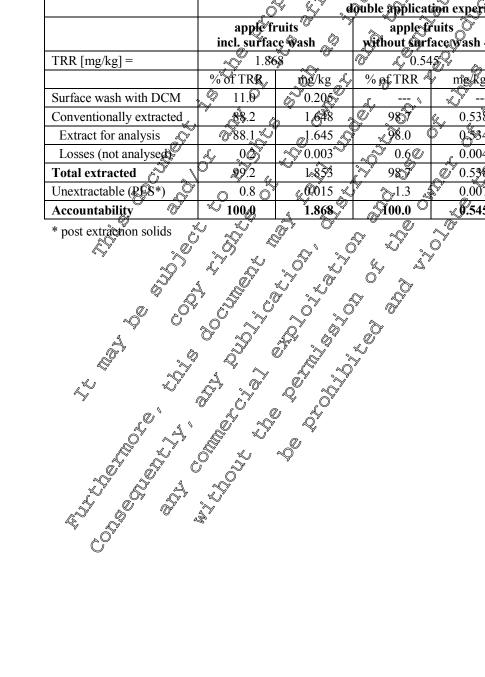
Bayer CropScience Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

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

		<u> </u>		
	apple	fruits	apple	leaves
TRR [mg/kg] =	0.079)	\$6.71	5 0 5
	% of TRR	mg/kg	% of RR	mg/kg
Conventionally extracted	94.2	0.074	A96.7	6 [×] 54.859 [×] ,0
Extract for analysis	94.2	0,074	96.5	\$ 54.727
Losses (not analysed)		☞	0.2	~0.132 ×
Total extracted	94.2	ي 0.074	0ళ 96.7 🖑	54.859° «
Unextractable (PES*)	5.8	ے 0.005 D	° 3.3	1855
Accountability	100.0	0.079	0 ² 100.0 \C	5 6.715
* post extraction solids	×	o° 5° ×		

Table 6.2.1-26: Distribution of radioactivity in the extracts of apple fruits and baves after a double foliar application of [pyrfdinylmethyl-40]BY1 02960 Ĩ

	.Ô	double application experiments						
	apple fi incl. surfac	uits 🦉 🕺	apple fruits apple léaves					
TRR $[mg/kg] =$	1.86		V L 0.54		Õ L ¥4.8	341		
	% of TRR	ŵg/kg 🗸	% 🔊 TRR 🖗	mes kg 、	🛇 % of TRR	mg/kg		
Surface wash with DCM	11.0	0.205	<u> </u>		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~			
Conventionally extracted	88 .2	1648	Ø 987	۵.538	98.4	132.635		
Extract for analysis	88.1	1.645	% .0 [©]	0,534 ,	97.8	131.847		
Losses (not analysed)		~~0.003 [~] ~	0.6C	0.004	0.6	0.788		
Total extracted	0 9 9.2	1.853	^م ري کې	0.538	98.4	132.635		
Unextractable (ROS*)	0.8	ي 1000∫ي (00015	∑ <u></u> 1.3 <u>4</u>	0.007	1.6	2.206		
Accountability 0	∞ 100,9	1.868	A00.0	\$.545	100.0	134.841		



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

 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		36.71				
Compound (BYI 02960-)	% TRR	mg/kg	% TORR	mg/kg S			
Conventional extraction		00		5 5 0			
BYI 02960 (parent compound)	43.1	0.034	24.5	7 13.882			
6-CNA	5.0	1 .004	<u> </u>	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~			
CHMP-di-glyc		L 0	D 0.6 K	0.342 6			
CHMP-glyc	4.7	<u>.</u> 0.004	• 14.40	r 8041 @			
СНМР	4.0	0.003		¢.727_Û			
acetic acid-glyc	3.5 🌾	° 0.00	Ø5.1 O	°∼ 2.891 ×			
OH-glyc	4.90	Ø 0,004	<u>م</u> ر 19.9	11,278			
acetic acid	330 0	Ø .002 Q	10 14 C	9 .767			
difluoroethyl-OH-glyc	J.4 ~	~ 0.00°	\$ \$ \$ \$ \$ \$ \$	3.63			
AMCP-difluoroethanamine	8.4	x 0:997 x	× 0.4	Q 0.255			
ОН		0.001	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	Ø.484			
Total identified	Q	~ 0.06 2	4.8	× 42.399			
unknown 1	, 🛴 🕰 2.7 🕉	\$ 0.002 S	ð <u>~</u> 0	«,			
unknown 2	66	0.005 °		⊃ [∞]			
unknown 3	5 5 O	©0.003 ×	A 1.8 Q	1.000			
unknown 4 🏾 🔊	Q 2.0 5	~ 0.002 ×	3.6	2.052			
unknown 5 🔬 🖉		× ·~ ×	k, z				
unknown 6	19 .Q 5	×	0 ¥.1	0.634			
unknown 7	P 2~	<u>, 9 </u>	0.6	0.322			
unknown 8	& -× ,	6 <u> </u>	<u>سم الم الم الم الم الم الم الم الم الم ال</u>				
unknown 9 🔊 🖉 🖉	0 <i>4</i> §	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	2 .6	1.477			
unknown 10	9 A 87	8 6,- 6	1.3	0.762			
unknown H	\$	g <u>,</u> g , y	0.5	0.295			
unknown 12 5	\$ \$ · · >	u 25					
unknown 13 unknown 14	\$ `~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	0 <u>~</u>					
unknown 14 🖗 👌 🕵			0.6	0.353			
unknown 15 O C		°	0.6	0.315			
unknown 16	Ý <u>Q</u> ' Q'	<i>`</i> `	1.2	0.682			
unknown		×	0.7	0.403			
unknown 8 2 4			2.4	1.386			
unknown 19 🗸 🖓 🦕			1.9	1.088			
unknown 20 Of C	0.6°	< 0.001	2.5	1.445			
unknown 21 🖉 🐴 🛇 🖉			0.2	0.114			
Total characterised	@ 5.3	0.012	21.7	12.328			
Analysed extract(s)	94.2	0.074	96.5	54.727			
Extracts for analysed			0.2	0.132			
Total extracted 5	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

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

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 frui			pple fruits without apple leaves				
	surface		surface		۰۰ (۱) ۱۹			
TRR [mg/kg] =		1.868		0.545	Ô.	134.841		
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg 🦼	% TRR	S mg⊀Qg		
Conventional extraction			<i>≈</i> ∧	×,				
parent compound	88.4	1.652	~\$5 .6	0.467	48	64.981 Ø		
6-CNA	0.5	0.009	1.5	6008	<u>, 9.3</u>	0.456		
CHMP-di-glyc			₄©″	Ô ⁴	0.2	¢.\$27 ¢		
CHMP-glyc	0.5	0.010	v 0.9 🔊	0.063	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	§.842		
CHMP	0.7	0.013	· 0.8 0	_0,004 _ 7	r ≈0.6 .	0.779		
acetic acid-glyc	0.6	0.012	0 0.8	×0.004	\$ 5.0	6.799		
OH-glyc	1.3	0.024	0.7	Q 0.009	15.€	20.729		
acetic acid	0.8	×0.015×	× 1.1 %	β .0 06 (×1,0	1.4,10		
difluoroethyl-OH-glyc)	<u>v</u> j		0 [°]	\$4.9	6,674		
AMCP-difluoroethanamine	4.5_0	0,085	A.1	0.025	0.8	م1.015		
ОН	1,6	0.020	<u>م</u> 1.0 م	0,005	Ĵ, OŤ,	0.944		
Total identified	98.Š	\$ 1.8 39	§ 97.6		84.5	113.933		
unknown 1	<u></u>	~~~	V 4-	Û ^y A	⁰			
unknown 2	<u> </u>	<u> </u>	Ø	× × · · ·	g -			
unknown 3 🗞	0.2	\$ 0.0 6 \$	الأسر- مريد ليك	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	0.8	1.070		
unknown 4 🔬	Ø \$	Ő ?	<u> </u>	× (<u> </u>	2.652		
unknown 5		0\$		P	§ 0.2	0.222		
unknown 6		N	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	£, Ø	0.3	0.406		
unknown 7 🖉 🔊	Š G	<u> </u>			0.4	0.601		
unknown 8	00	<u> </u>	ð 4	·	0.4	0.504		
unknown 9 🔊	¢-	1 2	⁰	×	1.6	2.150		
unknown	~~~~ , j			~~	0.5	0.721		
unknown 1	\$ <u>9</u> *	~~~~ ⁰	× ~~ ~	Ç	0.5	0.654		
unknown 12	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		× ×		0.2	0.323		
unknown 12 unknown 13	©0.3 Å	<u> </u>			0.4	0.518		
unknown 14 🕡	~~~~ Č	<u> </u>	ř – – – – – – – – – – – – – – – – – – –					
unknown 15 🔷 👌	0 st	<u>~~~</u>	لا					
unknown 16	\$ <i>^</i>	F* <u>, 9</u> _ ,	«) —		1.0	1.380		
unknown 7 ~	Ø 		×		0.5	0.669		
unknown 18	A.P	<u>Ø</u> Y			1.7	2.291		
unknown 19	× ~~~	<u>~~~~</u>			0.8	1.091		
unknown 20 C	~ 0.L~	0.002	0.5	0.002	1.7	2.248		
unknown 21 0 v		¥			0.3	0.413		
Total characterised	×9.6	0.011	0.5	0.002	13.3	17.914		
Analysed extract (S	0 99.0	1.850	98.0	0.534	97.8	131.847		
Extracts not an avsed	0.2	0.003	0.6	0.004	0.6	0.788		
Totalextracted	× 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		

* post extraction solids

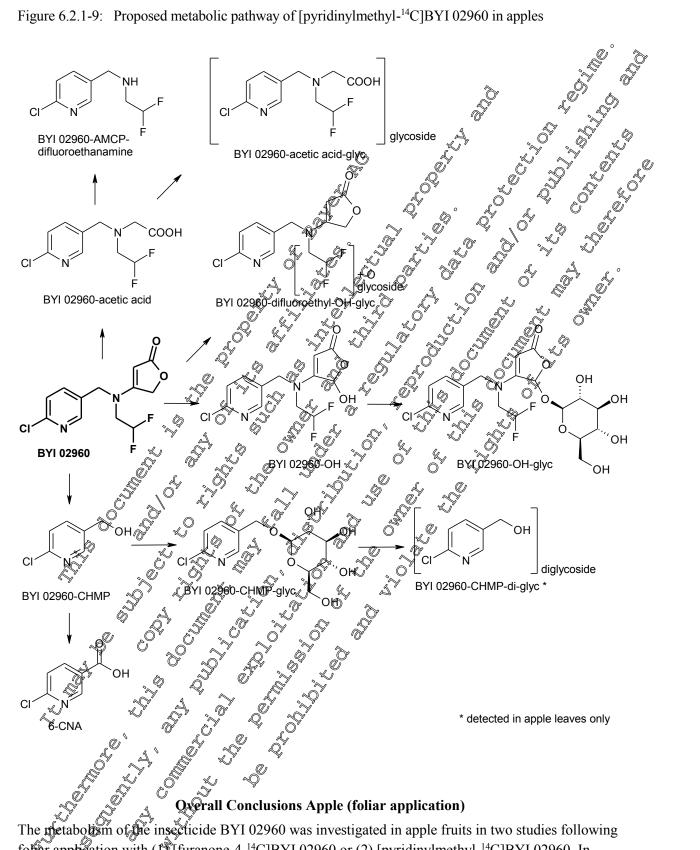
III. Conclusions

As expected, the residues were dominated by parent compound in the double application experiment, whereas the metabolite pattern was the same in both same in bot Three major metabolic routes of [pyridinylmethyl-¹⁴C]BYI 02960 were observed in apples:
hydroxylation followed by conjugation

- hydroxylation followed by conjugation with carbohydrates,

the of the and the state the of the and the state of the On the basis of the results of this study it is concluded that the metabolismon (pypdimylmethyl-"CJBY102960 in apples is well understood and the following metabolic pathway is proposed."

Figure 6.2.1-9: Proposed metabolic pathway of [pyridinylmethyl-14C]BYI 02960 in apples



fohar application with (f) [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 nonradiolabelled difluoroacetic acid by LC-MS/MS according to residue method 01304 (see KIIA

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 4 were dominated by parent compound.

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

In the single application experiment a natural compound (gucose/carbohydrates) was by far the main compound in apple fruits, whereas parent compound was predominan on 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 froms 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 eaves BYI 02960-04-gly was identified as major metabolite, each of the other metabolites (6 to 7) represented lesothan 7% of the TRR Overall, four major metabolic routes were detected: (1) Kydroxylation of the parent compoind (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 sonjugation 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 pooloi.e. into glucose/carbohydrates, and (4) cleavage of the pyridwylmethylamine 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 metabolisty study with pyridiny methol-14 CTBYI 02960. Molecule cleavage led to the label-specific metabolite BYI 2960-difluore thyl-minorfuranone and degradation of the furanone moiety finally to gluebse/carbohydrotes. The corresponding counterparts BYI 02960-CHMP and BYI 02966-AMCP-difloroethanamine 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 corresponding study performed with [pyridinylmethyl-¹⁴C]BYP02960. S

(2) Label 2: [pyrhinylmethyl-SC]BY502960

Parent compound was the main compound detected in both experiments in apple fruits at harvest. All metabolites represented less than 10% of the T&R. Nine metabolites were identified in fruits from which BX7 02960-AMCP-difluoroethanamine was the most prominent one, followed by 6-CNA (single application experiment) or BX1 02960-Off-glyc (double application experiment). Overall, three major metabolic routes were detected: (f) 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 twooroutes ted to the non label-specific metabolites BYI 02960-acetic acid and BY1 02960-OH, their corresponding glycosides and to BY1 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-

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 at 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 opparent compound and metabolites in the set of the set o

		.00 (/	, A		LIN JO	<u> </u>		
		Ő	, 0°	📈 apple	fruits~	Â.	.1	0
Radiolabel		[furanon	e-47*C] (ΰ Q		[pyridin]In	neth@ ⁴ C]	ý.
	single	× , ×	🖉 doubl	e apply ace wash _ (single	appt	douldt w/o stirfa	appl. ace wash
TRR [mg/kg] =	0.2		6 ¥ ./	1966° ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	0.0	TO D	0.5	45
Compound (BYI 02960-)	% TRR	mg/kg	[™] TRR [™]	mgAkg	O TRR Ô	mgAg	ू‰∕TRR	mg/kg
BYI 02960	7.4	\$0.021	? <u>7</u> 3,6	9 .946	489	0.034	85.6	0.467
glucose/carbohydrates	~\$71.7 °	°≫ 0.2Q1	Q4.2	~ 0.1 8	l la		>	
6-CNA		<i>S</i>	Å. 0	4	5.00	0,004	1.5	0.008
CHMP-glyc		6 6		~~ ×		Q.004	0.9	0.005
CHMP		n Ö		Ô ^{\$} &	4.0	6 0.003	0.8	0.004
difluoroethyl-amino-		, Ø009	\$ 0.2	0.003	0 4	7		
acetic acid-glyc	£ 0.3	0.001	°≫0.5	S 0.00	\$.5	0.003	0.8	0.004
OH-glyc	0.40	∀ 0,0001	0.5	0.004	× 4.9	0.004	1.7	0.009
acetic acid		. 0 001 %		0.009	3.0	0.002	1.1	0.006
AMCP-diftuoroethanamine	Z.				8.4	0.007	4.1	0.023
difluoroethyl-OH-glyc	S A		Ô ^y ×	Ĵ` <u></u> 0'	1.4	0.001		
OH NO		<u> </u>	J 048	ي 20.01	0.8	0.001	1.0	0.005
Total identified	\$3.4	× 0.234	° 99.1	⊘1.171	78.9	0.062	97.6	0.532
Total characterised Analysed extra0(s)	్ల్లో 2.0	0 0.005	0.1	0.002	15.3	0.012	0.5	0.002
Analysed extraçõe(s)	0 85.3	0,239	°√ 91-2 Ø.7	1.173	94.2	0.074	98.0	0.534
Extract(s) not analysed \square	° 29.2	£0.003¢		0.009			0.6	0.004
Total extracted	Q 86.5	0.242	<u>م</u> ي 91.9	1.182	94.2	0.074	98.7	0.538
Unextractable (PES*)	13.5	Ø,Ø38	8.1	0.104	5.8	0.005	1.3	0.007
Accountability	1/200	-Q.2805	100.0	1.286	100.0	0.079	100.0	0.545

 Table 6.2.1-29
 TRR values and distribution of parent compound and metabolites in apple fruit after folds

 application of BYI 02960
 Image: Compound and metabolites in apple fruit after folds

* post extraction whiles Label specific metabolities are wrinted in italics

Analysis of apper 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, diffuoroacetic 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 diffuoroacetic 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

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:

- •
- •
- complete degradation of the furanone moiety and incorporation of carbon atoms into the natural compound pool, e.g. into gluese/carbohydrates • with carbohydrates or oxidised to 6-chloronicothac acid (6-CNA)
- hydroxylation of the furanone of difluor bethave moisty follo by conjugation with glacose • 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 faits and leaves

cleavadé oxidative degradation The second secon of the furanone moiety oxidation and conjugation hydroxylation and conjugation and conjugation

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

¹⁴ C]BYI 02960:	dies in cotton were conducted with [furanone-4- ¹⁴ C]-, and [pyridinylmethyl-
Report:	KIIA 6.2.1/08, ; 2011
Title:	Metabolism of [furanone-4-14C]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 Guideline Section 2. Nature of the Residue – Plants, Livestock Japanese MAFF, 12 Nousan 8147 European Parliament and Council Regulation (EC) No (107/2009
GLP	yes in a first start was

Æxecutive Summart

The metabolism of [furanone 4⁻⁴C]BVI 02960 formulated as an SP 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. Ana 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 209 g a.s./ha at BBCH 15 and a second time at an 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 there than 90% of the bolls were open and approx 50% to 70% of the leaves were discoloured or callen; BBCH 95 - 97). The total application rate in the double application experiment was 385 ga.s./ha?

Samples were taken from both experiments. Cotton seeds and gintrash were the raw agricultural commodities (RACs) harvested at maturing 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 BYT 02960. The TRR values of all plant matrices determined are shown in the following table:

snown in the following table:

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. equivAkg)
intermediate	one spray application,	28 2	12.391
gin trash	209 g a.s./ha (at BBCH 16)	169	0.194
lint		169	6 009 5 0
seeds	Ča.	×169	\$0.013
gin trash	two spray applications,	14	E 2.767 0 0
lint	209 g a.s./ha (at BBCH 15)	<u> </u>	x 4993 x 0
seeds	176 g a.s./ha (at BBCH 95 – 5)	.14	0.016

* PHI: preharvest interval (corresponds to days after ast treatment (DAT) of the stark of hardest/sampling)

The radioactive residues were extracted with actonifile/water mixtures on the ase 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 966% for kint (double application experiment). The profiles of the extracts comprising dreasonable amount of radioactivity were recorder by HPLC and all major and several minor components were identified. Identification of parent compound and metabolites common to both regiolabels was based on the metabolite profiles obtained in the cotton metabolism study with [pyridinylmethyl-10]BY102960 Additionally all assignments made in the present study were confirmed by HPLCoo-chromatography using authentic reference compounds. The identification rates ranged from 70.3% of the TRR for gin wash (single application experiment) to 86.0% for lint (double application experiment). [Garanone-4-14C]BYI 02960 was metabolised moderately in cotton. Parent compound was the most promition t component and represented approx. 40 - 70% of the PRR in all matrices analysed. The sum of metabolites BYI 02960-OH-glyc and BYI 02960-acetic acid represented another major fraction in alomatrices 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 trage components

The following metabolic router of [toranone 4-14 CBYI 02960 were observed in cotton:

- hydroxylation of the methylene group of the formanone moiety followed by conjugation,
- oxidative opening of the furantine multiple and acetic acid group followed by further oxidation of conjugation with a carbohydrate (e.g. abycosylation), and

halogenation (mostly brothination, to a minor extent chlorination) of the furanone moiety

Halogenation of the furanone moiet of the active substance probably occurred in the soil which was supported by the fact that small amount of halogenated parent compound were identified in the aerobic sol 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.

(1) n

I. Materials and Methods

A. Materials

1. Test Material:	
Chemical structure	CI N + F C + Position of the radiolabel
Radiolabelled test material	[furanone-4- $\frac{14}{2}$]BYI 02960 $\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$
Specific radioactivity	3.94 MBq/mg (106,46 μG/mg) (106,46 μG/mg)
Chemical Purity	>99% (HPLC) = 20% (HPLC) = 20
Radiochemical purity	$> 98\%$ (HPLC and TC) \sim \sim \sim \sim \sim

The supplied radiolabelled test compound [finanone4-14C] PYI 02960 was disserved in action trile. 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 of get the aqueous application solution.

"Einheitserde T" (standardæoil comaining 2 kg/m of awater soluble alt mixture), pH 2. Soil: $(CaCl_2) = 5.8, 85\%$ white moor peak, 15% elay, containing NQ₃, NH₄ OP_2O_5 , K₂O, Fe, Mn, B, Cu, "Carmen", Gossypium Rirsutton, representative for oil seeds Mo, Zn; distributer Germany

3. Plant Cotton, værety

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 opplication 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 cottor 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 gas./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. 0.078 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.

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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 in small piece. The gin trash chaff was combined with the empty capsules which had contained the lint and the seeds. trash and seed samples were further processed using a Polytron homogenizer and liquid more the generation of the second s homogenized samples were stored in a freezer ($\leq -18^{\circ}$ C) with analysis. Fint samples of the double application experiment were not submitted to any sample preparation geps and were stored in a freezer (\leq -18°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 BBCk122 – 24) to support the number of instabilities. The plant was cut off just above the soft surface and was cut in small pieces prior to extraction and analysis. C. Analytical Procedures Extraction: stage (two to four vegetative side shoots were visible BBCK 22 - 24) to support the identification of

Aliquots of all sample matrices were extracted three times with a mixture of aceronitrile/water (8:2, v/v) using a high speed blender. The cotton seed samples were additionally extracted with heptane prior to the first acetonitril@water@xtraction step. The radioactivity in the extracts was determined by LSC and in the solids by combustion follower 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 fint extracts were directly analysed by HPLC analysis after concentration The clean-up was performed using an SPCRP 18 cartridge (Phenomenex, Strata C18-E, 20 g), which was conditioned with methanol, water and an acetonit the water mixture beforehand. The flow-through fraction (percolate) was collected and the cartridge was rinsed with acetonitrile/water (8:2, v/v) and methanol/dichoromethane (2:1, 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 sobmitted 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 otary 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 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 metabolise profiles obtained in the cotton metabolism study with [pyridinylmethyl-¹⁴C]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 cochromatography 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 (2) 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 500 (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 frue lear 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 har vested 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, tesidues in seeds were not affected by the late second application. The TRR level of 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 0% 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

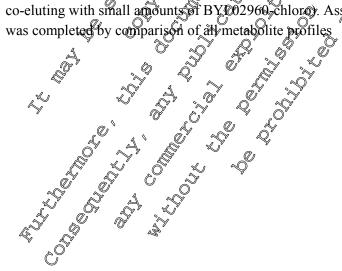
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assistance. Extraction efficiencies ranged from 23.4% of the TRR for seeds (single application experiment) to 96.6% for lint (double application experiment) as shown in Table 6.2.1-31 and Table 6.2.1-32. Due to the low radioactivity level in seeds, 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 alk 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 colchromatography 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 BXL02960-OH-gtvc an OBYL02960-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 acidie method and identification of both compounds became feasible by HPLC co-chromatography with authentic reference coropounds. Separation of the compounds revealed that BY402960-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 @2960 OH-glyc was identified as D-glucose in the corresponding apple metabolism study by the specific enzymation entropy with B-glucosidase.

Besides parent compound and the fraction comprising BYI 0260-0H-gly Sand BYI 02960-acetic acid, one additional major compound was detected only in girdrash, it was identified as BYI 02960-OH. All other metabolites detected in gin trash, line and as well in the intermediate were minor metabolites. They did not exceed 10% of the TRR in the single and the Gouble application experiment. Three of the minor metabolites were identified in gin trash by HPLC co-chromatography using authentic reference compounds: BYI 02960-glyoxy acid BYI 02960-acetic acid-glyc and BYI 02960-bromo (probably co-eluting with small another BY 202960, chloroy. Assignment of the metabolites in other matrices



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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						
	intern	intermediate		gin trash		eds		
TRR $[mg/kg] =$	12.3	91	0.19		0.0	130 0		
	% of TRR	mg/kg	% of TRR	mg/k@	% of TRR	mg/kg		
Conventional extraction	90.3	11.194	69.4	0.133	23.4	2 9 2003 Ø		
Extract for analysis	89.9	11.141	66.9	0,128	×¥			
Losses (not analysed)	0.4	0.053 🚿	2.6	0.005	Q3.4 [#] ~(0.003#		
Microwave extraction		{	10.9	0.021 ♥	×2	\$ ⁷ &		
Extract for analysis		A-	10.9Q	0.021		<u> 0</u> 0		
Losses (not analysed)		Q0	n.g.	🖉 n.q. 🖓	<u> </u>			
Total extracted	90.3	ر 11.194°° %	\$0.3	× 0.159	23.4	0.0 03		
Unextractable (PES*)	9.7	O 1.190	19.7		76.6	∡ 0.011 。		
Accountability	100.0	12391	© 100 .9	0.191 ₀	190.0	0.013		
* post extraction solids	<u>v</u> *			A O	×.			

post extraction solids the conventional extract was not further analyzed by HPLC due to the low radioactivity fevel is not quantified (< LOQ) # n.q. not quantified (< LOQ)

					(gip Pash, (int and seeds)	
Table 6 2 1 22.	Distribution of Podi	and with in the	avtracte ale ott	on matrice ((nin trach (lint and coode)	
1 a 0 10 0.2.1 - 52.	Distribution of gau	Uque vity memo	cautacis yeacon	Utinati ives (ging frash, gint and socus)	
	a		Or alla area			
	after two foliar app	licetions at I tim	ranone_//`_\#(`'IR'	MAMAAAAAA	- (())	
	and two ional app	nearions or pru	$anonc C \mu$	¥1 02 JQD		
	•	, <u>av</u>	· · · · · · · · · · · · · · · · · · ·	/ °^ `	(Co)	

		<u> </u>	y . V	<u>~</u>	A	
	o si	do	uble applicat	ion experim	ent	
	gin t			nt 🔊 👌	See see	ds
TRR [mg/kg] =	ي 2.76	7	°∑ °¥.99)3 × ×	0.01	6
	% of TRR	∼mg/kg♡	% of TRR	mg/kg	% of TRR	mg/kg
Conventional extraction	90.2	> 2.496	~ ^{96.6}	4.822	57.8	0.009
Extract for analogis	89.7 0	2 : 3 *82 ~	966	4.822		
Losses (not analysed)	0.5	• 9.014 0.155	@.q. 🖌	🧶 n.q.	57.8#	0.009#
Microwave extraction	<u> </u>	0.155	0 0			
Extract of analysis	₹ ^{3.8} \	0.504	Q.			
Losses (not analysed)	K 16	× Ø.051				
Total extracted	. 95.8	@ 2.65 P	≫,96.6	4.822	57.8	0.009
Total extracted 3 0 Unextractable (PES*) 3 5 Accountability 0 5	<u>ک</u> 4.2 ک	0,4\$76	3.4	0.170	42.2	0.007
Accountability	`∽ 100:0	^ 3.767	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

The TRR and distribution of parent and metabolitesin 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. As summary of the results is given in Table 6.2.1-35.

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

	ar treatment of [furanone-4- ¹⁴ C]BY102960					
	interm	ediate	gin t	rash		
TRR [mg/kg] =	12.391		0.191			
Compound (BYI 02960-)	% TRR mg/kg		% TORR	mg/kg		
Conventional extraction	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,		1			
BYI 02960 (parent compound)	42.3	\$,237	37.5	× 0.972		
glyoxylic acid			0.9	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		
acetic acid-glyc	8.6	L 1.068		2 4 0		
OH-glyc / acetic acid	24.9	3.082	• 12.8	r 0.0024 , ©		
OH	00			Ø.023_		
bromo/chloro	0.7 🖉	° 0.0890	200.6 D	°∼ 0.001		
Subtotal identified	76.50	, © 9,476	<u>∧</u> ♥ 63.7	0.422		
unknown 1	222	. 0.268	26 C	Q.005 A		
unknown 2			A 0 x,	-49		
unknown 3		× 0×22 ×	× · · · · · · · · · · · · · · · · · · ·			
unknown 4		× × ···· · · · · · · · · · · · · · · ·		Ø9.001		
unknown 5	Q . 6.6 s	0.823		×		
unknown 6						
unknown 7	25	0.310	. Ø (
unknown 8		Ø %	× × 0			
unknown 9	Q S					
unknown 10 🔬 🖓	<u> </u>					
unknown 11	Q.1 \$	×0.141	0 [°] 4 <u>··</u>			
unknown 12		<u>~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~</u>	<i></i>			
Subtotal characterised	& 13.4 ^y ,*	1.665 A	3.2	0.006		
Total conventional extraction	89.9	A 1.141	<i>Q</i> , 66.9	0.128		
BVI 02966 (parent compoind)			2.5	0.005		
acetic acid-glyc						
OH-glvc / acetic acid			2.9	0.006		
OH Q A			1.2	0.002		
Subtotal identified 5		r Ö	6.6	0.013		
unknown 1		ð,	4.2	0.008		
unknown 2 S		×				
unknown		1				
unknown 7						
Subtotal characterised			4.2	0.008		
Total microwaye extraction			10.9	0.021		
Total identified	26.5	9.476	70.3	0.134		
Total chargeterised	13.4	1.665	7.4	0.014		
Analysedextracts	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		
Unextractoble (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

 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 t	rash	lint			
TRR [mg/kg] =	2.767		04.993			
Compound (BYI 02960-)	% TRR	mg/kg	% TARŘ	mg/kg 🛇		
Conventional extraction		00				
BYI 02960 (parent compound)	53.3	1,476	70.3	3.512		
glyoxylic acid	1.6	1 .044	Ø 0.2 Č	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		
acetic acid-glyc	2.2	a 0.062 a				
OH-glyc / acetic acid	20.0	0.553 O	· 13.9	0,694 _0		
OH	0.6	0.016		· ··· ···		
bromo/chloro	2.3	° 0.0630° √	× 01.6 ~	0.078		
Subtotal identified	80.0	0 2,214	86.0	4,292		
unknown 1	49	Ø 135 Q	3.8	Ør.190 /		
unknown 2	×0.5 ×	~ ⁹ 0.01 ²	A . 00.4 × /	0.01		
unknown 3	0.4, ~	0.012 0	× 0.9 ×	0.033		
unknown 4		¢ _ 0,056 ~		Ç		
unknown 5	Å <u>0.8</u>	0.023	Č <u>(0</u> .3 Š	्र≪0.014		
unknown 6	× 0.5	~ 0.0 B ~	^~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		
unknown 7	°≫ 0,2,	Ø Ø Ø05 Ø	0.6	0.028		
unknown 8	& Q- 4	~ [~]	X . 09.5	0.024		
unknown 9	6 ₆ 9	L <u>~</u> ×	2.7 2.7	0.135		
unknown 10	\$ 0.2 °	<u>0009</u> %	1.20	0.062		
unknown 10 v v v v		× 0	0 4 <u>7</u>			
unknown 12	S <u>_</u> S		0.3	0.016		
Subtotal characterised	9.7	N 0.268 V	\$ 10.6	0.530		
Total conventional extraction	0 89,7 ×	2.482	96.6	4.822		
Microwave extraction 🐨 🐇 🖉						
BYI 02960 (parent compound)	1.1 °	~ Q.Q29 ~				
acetic actor glyc	S ALP C	[∞] ≪0.001. [©]				
OH-glyc / acetic acid		<i>&</i> 0.02 <i>≸</i>				
OH & A	<u></u>	0 <u>0</u> -				
Subtotal identified Strategy	č k ő	× 0.053				
unknown 1 🛷 🖉 🖉	× ×1.7 ×	0.046				
unknown 3 🔬 💛 🖓	0.1 0	0.001				
unknown and a construction of the construction		0.002				
unknown 7	⇒ Ø.1 ×	0.002				
Subtotal characterised	\$ 1.8 \$	0.051				
Total microwave extraction	Q 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 Aracts C 2	93.4	2.586	96.6	4.822		
Not analysed/losses# A	2.3	0.065	<0.1	< 0.001		
Totakextracted	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

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		7
TRR [mg/kg] =	12.391		0.191		2.767		4.998 6		
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR_j	Ømg/kg	% Z R R	ing/kg	D
BYI 02960 (parent compound)	42.3	5.237	40.0	0.076	5 4 .4	1.505	≈ 70.3	3.512	
glyoxylic acid			0.9	0.002	@1.6	0.044		00009	Å
acetic acid-glyc	8.6	1.068			Ó [♥] 2.3	0.065	2	\$~ \$	Ď
OH-glyc / acetic acid	24.9	3.082	J\$ 5.7	0.03	20,8	9 977	^۲ 13.9 ک	0.694	
ОН			@ [*] 13.1	0,025	0.6	Q.016C		Q ⁴	
bromo/chloro	0.7	0.089	0.6	00001	2.30	,	.s.¥.6	9 .078	
Total identified	76.5	9.476	Ø0.3	×0.134	8139	2 268	86.0	4.292	
unknown 1	2.2	0.268	6.8	0.005	6.5	0.181 (₽	0,190	
unknown 2	,			 ک	€ 0,50	0.014	0.4	0.018	
unknown 3	1.00	0,122	"Q [°]	(D Q5	0.014	0.9	0.043	
unknown 4		Ş ,	\$ 0.6°	0.00	<u>\$</u> 2.0	Ø.056			
unknown 5	Ø [%] 6.6	0.823	×			0.02	₽ 9.3	0.014	
unknown 6	6, - .			g) 6	v 18	QO13	G		
unknown 7	2.8	A 310	^{10°} ^	v "	0.3	0.007	0.6	0.028	
unknown 8	×	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	× 4	<u>~</u>	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	ĝ-òj	0.5	0.024	
unknown 9 🥎	,Q		sy	~~ · · · ·			2.7	0.135	
unknown 10 🔬 🖧	\$ \$	10	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	Ô [°] &	0.3	\$ 009	1.2	0.062	
unknown 11	~~1.1	@0.141		<u> </u>	Ő «	√			
unknown 12	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~			S 2	, <u>-</u> @		0.3	0.016	
Total characteris 🖓 🕺	13.4	1.665	7.4 🖉	^{©*} 0.014	<u>1</u>]?.5	0.319	10.6	0.530	
Analysed extracts	®9.9	&1.141	V 77.0°	0.448	@ ₁ 93.4	2.586	96.6	4.822	
Not analysed losses [#]	Ø 0.4 <u>1</u>		Å .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	_Q1.197₅	° 19.7≈	0,038	4.2	0.116	3.4	0.170	
Accountability	~9100.0~	0 12.39€	100%	0.191	100.0	2.767	100.0	4.993	

* post extraction solids

#. losses during clean up, concentration, etc O

. Conclusions

Metabolism of BY102966 was inderate 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 (e. glycosylation),

• Soxidative 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.

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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-¹⁴C]BYI 02960 in cotton is well understood and the following metabolic pathway is prop

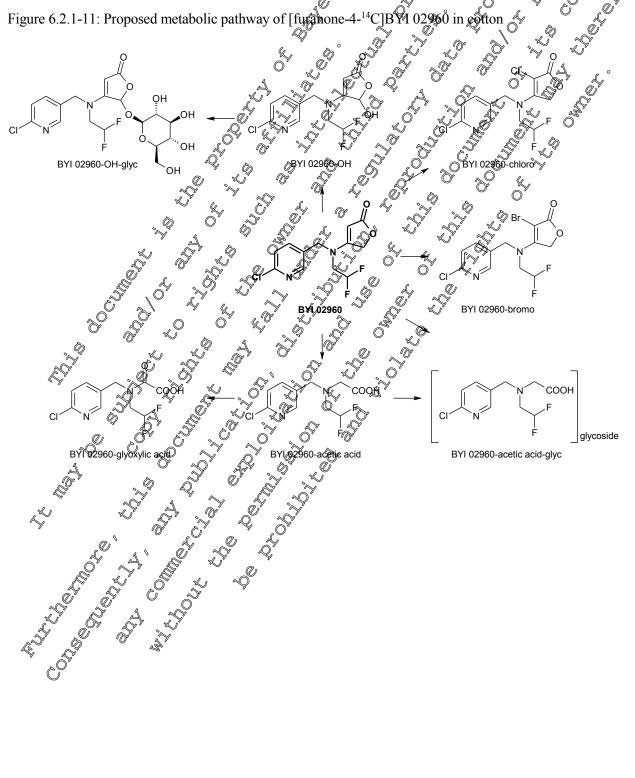


Figure 6.2.1-11: Proposed metabolic pathway of [furanone-4-1

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Report:	KIIA 6.2.1/09,,, 2011
Title:	Metabolism of [pyridinylmethyl-14C]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 0107/2009
GLP	yes <u>A</u> Q ° A C Q

Execotive Summary

The metabolism of [pyridinylmethyl-¹⁴C]BXI 02960 formulated as an SL 2000vas 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 when the sixth true leaf was unfolded application rate of 177 g a.s./ha at 15 days before halvest of the conton bolls (when more than 90% of the bolls were open and approx. 50% to 70% of the leaves were discoloured of faller; 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 trastowere the raw agricultural commodities (RA(s)) hardested 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 metabolite degradation behaviour of BYI 02960. The TRR value of all plant matrices determined are shown in the following table.

8			18		
T11 (010(TDD		1 1 1 1	1 1 1	1) 0 01	1
Table 6.2.1-36: TRR	Regimes the cotton (1)	termediate gin	trash lint se	eeds) after tollar a	application of
14010 0.2.1 200/114		selline analy 5m	up on, mu, o	cous, and roman	appinoution of
		A DAACON T			
\approx $n_{\rm m}$	ØInvimethvi₄™Ø`IRN	∕№029603% &			
Ş [Dy⊓			8		
~⊊ [руп	dinylmethyl 14C]BY	1302900 // 🏷	7		

Matrix Tining and Application	PHI (days)*	ppm (mg a.s. equiv./kg)
intermediate	28	14.153
gin trash @ 206 g a.s./ha (at BBGF1 16)	169	0.310
	169	0.007
seeds O A G	169	0.045
gin trash of two spray applications,	15	2.344
lint 206 g a.s./ha (at BBCH 16)	15	8.846
seeds 57 g a.s./ha (at BBCH 95 – 97)	15	0.068

* PHI: oreharvest 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 cochromatography (two different chromatographic systems) using authentic reference compounds or by LC-MS/MS. Minar compounds were assigned based on the metabolite profiles obtained in the eotton metabolism study with [furanone-4-14C]BYI 02960. The identification rates anged 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 poderately in cotton Parent compound was the most prominent component in all matrices, except for seeds of the side most prominent component in all matrices, except for seeds of the single application experiment, and accounted for approx. 23% to 73% of the TRR Addinonal major metabolites detected in cotton matrices were BYI 02960-acetic acid, BYI 02960 OH and the label-specific metabolity 6-CNA. other metabolites detected represented minor or trace components.

The following metabolic routes of [pyriding)methy 14C] BY I 02960 were observed inconton

- hydroxylation of the metholene group of the foranone moiet of ollowed by conjugation,
- cleavage of the pyriding methylamine bond followed by addation of the methylene group to •

, subwerdby further oxic , subwerdby further oxic , at chlorinaugh) of the furanone moiety , stive substance probably ocutired in the soil which was , so of have reacted the soil and been subjected to soil metabolism processes. , and have reacted the soil and been subjected to soil metabolism processes. , so of these results, a metabolic pathway of (Pyridmylmethyl-¹⁴C)BYI 02960 in cotton can be , posed.

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

I. Materials and Methods

A. Materials	
1. Test Material:	
Chemical structure	$ \begin{array}{c c c c c c c c c c c c c c c c c c c $
Radiolabelled test material	[pyridinylmethyl-bC]BYb02960
Specific radioactivity	4.37 MBq/mg (1) 8.08 (1) Ci/mg)
Chemical Purity	>99% THPLO
Radiochemical purity	> 98% (HPLC and TLC)

The supplied radiolabelled test compound pyridiny lmethyl-14 (18 YI) 14 (18 YI) 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 drynes? The fiquid blank formulation was added to the test item using a magnetic stirrer. The mixture was diluted with water to get the aqueous application solution.

"Einheitsende T" (standard soil containing 2 kg/m³ of a water soluble salt mixture), pH 2. Soil: $(CaCl_2) = 5.8, 85\%$ white moor peat, 15% clays containing NO₃, NH4⁺, PrO5, K₂O, Fe, Mn, B, Cu, Mo, Zn; distributer:

A Contraction cossypium hirsutum representative for oil seeds 3. Plant Jotton, variet armen

 \bigcirc

B. Study Design

Experimental conditions:

M

Two experiments were performed with a total of so cotton plants of the variety "Carmen". The experiments represented the intended foldar 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 frue 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 currivated in the standard soil "Einheitserde T" in planting pots with a surface area of approx. 2076 m² and avolume 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:

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

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 6 gin trash chaff was combined with the empty capsules which had contained the Ont 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 ($\leq -18^{\circ}$ 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°C) until analysis.

stage (two to four vegetative side shoots were visible; BBCH 23-24) 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.

Extraction:

Aliquots of all sample matrices were extracted three times with a mixture of acetometrile/water (8:2, v/v) using a high speed blender The cotton seed samples were additionall 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 mitermediate gin trash and seed extracts were subjected to a clean-up step, whereas the line extract was directly analysed by HPLC analysis after concentration. The cleanup was performed using an SPE/RP 18 cartridge (Phenomenex, Steata C18-E, 20 g), which was conditioned with methanol, water and an acctonitible/water mixture beforehand. The flow-through fraction (percolate) was collected and the cartrage was rinsed with acetonitrile/water (8:2, v/v). Elution of retained compounds was performed with methanel/dichloromethane (1:1, v/v). The percolate and the acetonitrile/water fraction (= rinse) were combined and concentrated by rotary evaporation in vactor prior to chromatographic malyso by HPLC. The solids remaining after the conventional extraction steps of the gor trash samples were additionally submitted two times to microwave-assisted extraction with acetonutrile/water mixtures (8:2, v/v and 1:1, v/v). After each extraction step, extracts and solids were separated by coltration. The extracts were combined and concentrated by rotary evaporation in Vacuo Prior to HPLC analysis.

Ouantification:

Parent compound and metabolites in the extracts were analysed by reversed phase HPLC coupled to a radioactivity detector with 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-chronatography 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-4-14C]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 (20 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 protonged storage period of the extract. Additionally, stability of parent composed, 6 CNA, BYI 02960-acetic acid, BYI/02960-OH-gloc and BYI 02960-OH was proven in a grain extract for at least 27 months in the confined relationatorop study with [pyridinylmethyl-¹⁴C]BYI 02960. Therefore, analysis of the extracts of seeds and quantification of the resulting netabolite profiles was deemed acceptable despite the protonged storage period.

Since the first metabolic analysis of the RACs as well as the last experiments with extracts (HPLC cochromatography) took place within 5.5 months, no turther stability investigations were necessary according to OECD Guidance for the Testing of Chemicals 504 (2007).

JIL Results and Discussion

The metabolism of [pyrtriny]methyl- C]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), ia. 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 hardest 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, give trash and line 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 space (BBCH 22-24) of support the elucidation of metabolites.

TRE 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

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

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 (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 line was < 0.01 pg/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 tash (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 28.7% 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 vere well a 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 metabolities study conducted with [furanone-4-¹⁴C]BYI 02960.

Parent compound and major metabolities 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 [furatione-44/C]B\$402960.

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 there fore 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 BYL12960 OH-gipe 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 are imbedded in the Int. 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 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 jothe 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 opprox 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, BY 102960-OH was identified as an additional major metabolite. All other metabolites detected in gin and the second seco 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 other the single or the double application. experiment. In a first step they were identified in gur trash by HPDC comparison. The metabolite profiles of the gin trash extracts were compared with the corresponding profiles obtained in the corresponding profiles obtained metabolism study with [furanone-4-14 GBYI 2960. Assignment of the metabolites in all other matrices was completed by comparison of the gin trash profiles with all other metabolic profiles.

Table 6.2.1-37:	Distribution of fadioactivity in the expracts of cottoo matrices (intermediate, gin	trash
	and seeds) after a single forther application of [pyndinylnicthyl-14C]BY102960	

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		sir	gle applicati	ion experime	ent	
	intern	rediate 🖤	l ∩° gin t	rash 🐐 🧪	see see	ds
TRR [mg/kg] =	intern 4.1 % of CRR	53 🖧 🤘		105 27	0.04	5
Conventional extraction	b‰ of≪CRR	53 5 ×	% ØTRR	mg/kg	% of TRR	mg/kg
Conventional extragion	950 .	1 4 4 4 5 1		192 53	28.3	0.013
Extract for analogis	° [≫] 94.2°°	13(3)36	8056	0.250	21.9	0.010
Extract for analogis	07	13.336 0.105	9.9 🐇	0.003	6.5	0.003
Microwave extraction	~~~-	°'	10.4	0.032		
Microwave extraction	^`	<u> </u>	(^N 10 [®]	0.032		
Microwave extraction	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	An.q.	n.q.		
Total extracted 🔊 🔬 🖉	.25 .0	[©] 13.440 [°]	% 92.0	0.285	28.3	0.013
Unextractable (PES [*])	چې 5.0 ^ک ې	0,543	8.0	0.025	71.7	0.032
Accountability	7 100.0	1¥.153	100.0	0.310	100.0	0.045
Extracted Losses (not analysed) Total extracted Unextractable (PES*) Accountability * post extraction solids n.q. not quantified (< LO(9)						

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	Li	nt	Seeds	
TRR $[mg/kg] =$	2.34	14	8.84	6	0.00	580° 55
	% of TRR	mg/kg	% of TRR	mg/k	% of TRR	mg/kg
Conventional extraction	89.3	2.094	99.2	8.724	66.6	29045
Extract for analysis	88.7	2.079	م 99.2	8,774	38,8	∿>0.02€
Losses (not analysed)	0.6	0.014 <	n.q.	n.q.	Q7.6 ~	0.0
Microwave extraction	7.9	0.185C	"()¥	×2	\$ ⁷ \$
Extract for analysis	4.7	0.140	Q [¥]	° 4	<u> </u>	00
Losses (not analysed)	3.2	\$ 975	~	, ذ V	<u> </u>	
Total extracted	97.2	ر 2.279°°	99.2	× 8.774	66.1 ×	0.04 5
Unextractable (PES*)	2.8	0.065	0.8	.00 73 (33.9	<u>0.023</u>
Accountability	100.0	27344	C 100.Q	8.846	100.0	0.068
* post extraction solids						No. Contraction of the second se

The TRRs and the distribution of parent and metabolites in the extracts is shown in Table 6.2.1-40. In total, 16.2% to 89.7% of the BRR were identified in the matrices of colton after foliar application. A summary of the robults in the colling is the colling of the tract of the colling of the colling of the tract of the colling of the tract of the colling of the tract of the colling o

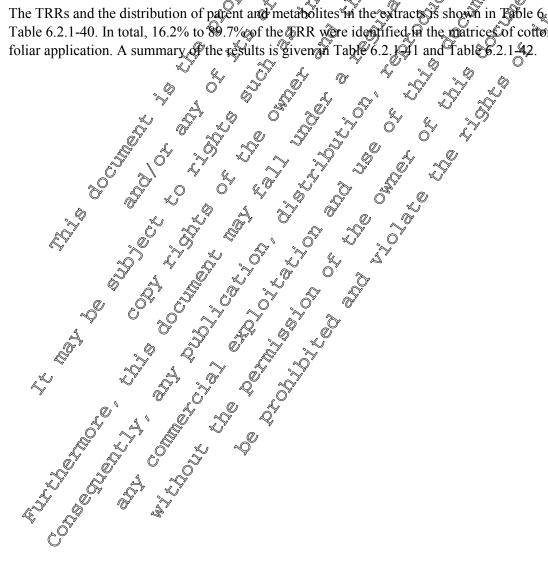


Table 6.2.1-39: TRR values and distribution of parent compound and metabolites in cotton matrices after a single foliar treatment of [pyridinylmethyl-14C]BYI 02960

		sir	igle applicat	ion experime	ent	<u> </u>
	intern	nediate	ediate gin trash			eds 🔊 🕺
TRR [mg/kg] =	14.1	53	0.3	10	0.04	
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Conventional extraction	1		1	A	<u> </u>	\$\$ Ø
BYI 02960 (parent compound)	36.9	5.221	م 24.7	0,077	_^≯	×~
6-CNA	2.1	0.298 🚿	18.5	0.057	Q16.2 ~	0.067
glyoxylic acid	1.5	0.209	2.1	0.007	×2	\$~ \$
acetic acid-glyc	6.4	0.899	Q,	° 4		00
OH-glyc / acetic acid	25.1	3,959	12,0	©0.037 [©]	<u> </u>	
ОН	1.2	0.168°°	A3.4 🔬	0.042	ð×	
bromo/chloro	0.5	0 0.064	×	~O (Ş	A 0
Subtotal identified	73.6	102+19	V 70.9	0.219	0 6.2	0.007
unknown 1	t i i i i i i i i i i i i i i i i i i i	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	2 .5	A 0.00	لاي 5.7	0,903
unknown 2	Č. (~~~~ 0.6 ^O	0 % 0 02 (0
unknown 3	0 1.4	0,196	S0	s \$	<u> </u>	ð
unknown 4	0. 2.8	0.403	<u>3</u> .4	0.010	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
unknown 5	2.8 ×J.1	0° 0.159	0, 1.0	0.0003 🥎	0 6	
unknown 6	°76.6	0.935	× 0,6×	Ø.002) 0	
unknown 7	3.9	Ø.551 0	· ··· »		Ôj	
unknown 10	<u> </u>	\$ 0.27 6 /	~~~ ×	×. 	Č	
unknown 11 🔬 🔅	Q 1.9 C	0.273	12	¢ 0.004	Š	
unknown 12		3 .130 ×	_ 0.6	0 00%		
Subtotal characterise	20.6	2.91%	9.9 K	0,021	5.7	0.003
Total conventional extraction ¹	% ,94.2	13.336	^{~)*} 80,6 [°]	0.250	21.9	0.010
Microwave extraction S						
Microwave extraction S	D A.	N O	1.6	0.005		
6-CNA		C	N 1.2	0.005		
OH-glyc Vacetic acid			€ .1\/7	0.005		
OH S			1.1	0.003		
Subtotal identified			6.1	0.019		
unknown 1 @ O C			3.8	0.012		
unknown 11		0 0	0.5	0.002		
Subtotal characterised			4.3	0.013		
Total microwavee extraction	~	~~~~~	10.4	0.032		
Total dentified	Ø 73 Ø	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~Q	13.336	91.0	0.282	21.9	0.010
Not analysed/Usses ²	00	0.105	0.9	0.003	6.5	0.010
Total extracted	95.0	13.440	92.0	0.285	28.3	0.003
Unextractable (PES*)	5.0	0.713	8.0	0.025	71.7	0.013
Accountability	100.0	14.153	100.0	0.025	100.0	0.032
* post extraction solid	100.0	17.133	100.0	0.510	100.0	0.040

* post extraction solid
 ¹ analysed extracts only
 ² losses during clean-up, concentration, etc

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

		do	uble applicat	tion experim	ent	<u></u>
	gin	gin trash lint				eds 🔊 🕷
TRR [mg/kg] =	2.34		8.84	46 🕻	» 0.0 0	580 A
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR 4	mgAQg
Conventional extraction		00	1	4	Ś	
BYI 02960 (parent compound)	51.3	1.204	73.0	6435	23	.0.016√√
6-CNA	2.0	0.047	0.4	0.031	\$5.0	× 0.005
glyoxylic acid	1.5	0.035	0.2	Q 0.015		
acetic acid-glyc	3.6	0.085			<u>~ - </u>	.0 \$
OH-glyc / acetic acid	20.6	0,484	14.6	Ø.295	4/.9	0.005
ОН	1.3	\$ 030	<u> </u>			
bromo/chloro	2.1	\$ 0.049°	_≫1.6 ×	0.1440	<u> </u>	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
Subtotal identified	82.6	1.935	89.70	\$936	33,3	▲ 0.023 ∘
unknown 1	0.4	<u>~0</u> ,010 ∧				0.02p
unknown 2	0.5 y	0.005			↓ ⁽	,
unknown 2 unknown 3	0.1	0.003				 0
unknown 4	0 2 2 %	0.0 .0 0.052 ×				»
unknown 5		0.031	<u></u>	0.02		
unknown 6			0.6	00051	0 2	
unknown 7				20.064	<u> </u>	
unknown 8		 		0.432		
unknown 9			~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	0,129	×	
unknown 10	× © 0.4 (0:009	0 0.\$	0.023 Č	~ }	
unknown 11		0:009 0033 ×	0.8	0.023	5.2	0.003
unknown 12		04033 x	0.5 م ©0.5 م	0.040	<u> </u>	0.005
Subtotal characterised		0.1,44		0.0 4 0 10 8 37	5.2	0.003
Total conventional extraction ¹	88.7 0	1	00	8.774	38.5	0.003
Microwave extraction	0.00.64		9952	0.//4	30.5	0.020
BYI 02960 (parent compound)	9. E	0.044	0.0	, <u> </u>		
6-CNA		0.044				
		\$ 07.002				
acetic acid-glyc	<u>05</u> 78		Ľ,			
OH-glyc / acetic acid		@ 0.0420 [°]	<u>~</u>			
	[*] ~ 3.9 · *	0.092				
unknown I		 0.9004 ∅ ∅ 0.002 				
unknown 2		\$0.002°				
unknown 5		0.003				
unknown of the second s	0.2					
unknown 11		0.006				
Subtotal characterised	0.8	0 0.018				
Total microwaveextraction	4.7					
Total identified	× 86,5, ×	2.028	89.7	7.936	33.3	0.023
Total characterised	«Q.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 Total	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 solid
 ¹ analysed extracts only

² losses during clean-up, concentration, etc

			- ¹⁴ C]BYI 02			<u> </u>
	single application experime					
	intern		0	rash	see	ds 🔿
TRR [mg/kg] =	14.1		0.31	Å	0.04	<u>5 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~</u>
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg∕kg	% TRO	
BYI 02960 (parent compound)	36.9	5.221	<u>گ</u> 26.3	\$ 9.082	<u>k</u> - 1	-
6-CNA	2.1	0.298	20.2	Q 0.063	J6.2	0:007
glyoxylic acid	1.5	0.209	2.1	0.007	õÇ	X
acetic acid-glyc	6.4	0.899	&		í <u>5</u>	
OH-glyc / acetic acid	25.1	<i>Q</i> 0559	1377	©0.043 [™]		
ОН	1.2	🌜 0.168)°	×ي 4.5	0.045		≪ ^v
bromo/chloro	0.5	0.064	0	~~~~ (× 4-	A °
Total identified	73.6	10,4 19 ~	76.8	<u>0.238</u>	16.2 🔬	0.007
unknown 1	4	<u>`</u>	6.4	0.020	5.7	Q 003
unknown 2	Q 1,4	v 🖉	0.6	ð,002		0
unknown 3	L 1.40	0,196 ×		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	Ş ,	Q
unknown 4	\$ 2,8	©0.403	3.4	0.000	<u></u>	
unknown 5	.ô.1	0.159	0 1.0Q	0.003 🔌		
unknown 6 🔊	6.6	0.935	¢ αβ [*]	\$0.002	<u> </u>	
unknown 7 🧔 🖉	<u>) 39</u>	0.551	,			
	ľ.9 4	§ 0.270	S'	× 2	S	
unknown 10	× 1.9	0.973	× 10 ⁴	&0.005°~		
unknown 12	S Q.S	×.130 ×	0.6	0'0.002		
Total characterised	29.6	> 2.917	\$14.2	Q:044	5.7	0.003
Analysed extract	×94.2 @	13.336	91,0	×0.282	21.9	0.010
Not analysed/losses# 🖉 🖉	0.7	9 .105	Ø.9	@ 0.003	6.5	0.003
Total extracted	25,0	0°13.440°	@92.0	0.285	28.3	0.013
Unextractable (PES*)	\$ 5.0	0,513	<u>80</u>	0.025	71.7	0.032
Accountability	≪ 100 € ^{©°°}		1,00.0	0.310	100.0	0.045
* post extraction solids						

Table 6.2.1-41: Summary of characterized and identified radioactive residues in cotton matrices after

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

		do	uble applicat	tion experim	ent	
	gin t	rash	li	nt	seeds	
TRR [mg/kg] =	2.34	14	8.84	46	0.00	580° 65
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	29 016 ¢
6-CNA	2.2	0.053	م 0.4	0 031	5,0	>>0.003
glyoxylic acid	1.5	0.035 <	0.2	0.015	Ö «	<u>v "¢"</u>
acetic acid-glyc	3.7	0.087.	()¥	×~	\$ ² &
OH-glyc / acetic acid	22.4	0.526	14.6Q	1.295	4.9	℃0.003©
ОН	1.3	\$9 30	~~	. Ø ^Q	<u> </u>	e e
bromo/chloro	2.1	0.049°°	¶.6 ≰	× 0.140	ð×	
Total identified	86.5) 2.028°	89.7	7036	33.3 O	<u>م</u> 0.023 م
unknown 1	0.6	07013	Ø Q	4	0 [*] "	
unknown 2	03	0.007	, ð ,		&	<u> </u>
unknown 3	Ø.1 (× 0.004	×~~	<i>y</i> (Û Ş	Õ
unknown 4	°2.2	0,052 🐇	S ~-9	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		6
unknown 5	Q 1.5	0.034	D .3	0.02	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
unknown 6	ູ≪0.2	° 0.004	0.6	0.0051	Q &	
unknown 7	, [×]	· · · · ·		\$0.064	<u> </u>	
unknown 8		_0 [×] ⁰	¥ 4.9 ≈	0.432	jûg	
unknown 9 🔊 🕺	Q.,	0,009	\$1.5 ×	0,129	õ	
unknown 10 🔬 🖓	_ © 0.4 C	0.0099		& 0.023s	D	
unknown 11		ي 039.039	0.8	0 0.070 €	5.2	0.003
unknown 12 5			0.5 K	0,0240		
Total characterised	🍇 6.9 🔊	0.462	~ 9,5	40.83 7	5.2	0.003
Analysed extractor	^O 93.4√	<u>3</u> .190	98.2	8.774	38.5	0.026
Not analysed bosses#	2 38	×0.0890°	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	J <0.001	27.6	0.019
Total extracted	97.2	2.279	S 99.2 V	8.774	66.1	0.045
Unextractable (PES*)	2.8	20,065	Å0×8	0.073	33.9	0.023
Accountability	106,0	2.346	100.0	8.846	100.0	0.068
Accountability * post extraction solids #. losses during clean up, concentration	tion, etc.		J.			

MI. Conclusions:

[Pyridinylmethyl-¹⁴C]BY 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 Stal, seven different metabolites were identified in gin trash and the intermediate, which showed the post pronounced metabolite patterns.

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

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

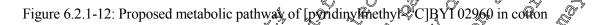
Bayer CropScience

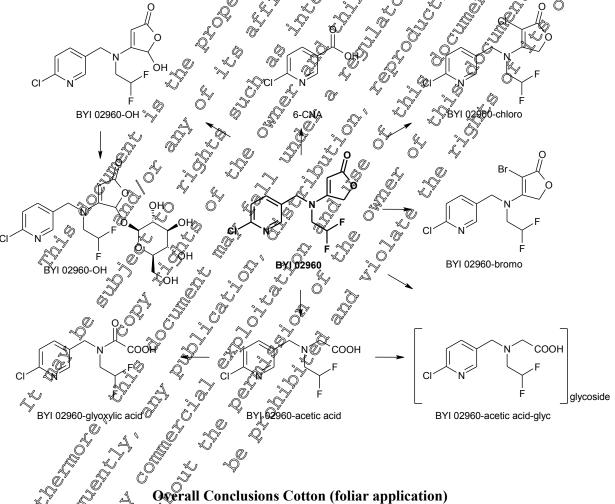
Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

- 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 supported by the fact that small amounts of halogenated parent compound were identified in the c 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 processe

On the basis of the results of this study it is conclude \mathcal{C} that the metabolism of [pypdiny] methy $\mathbb{P}_{p}^{\mathcal{C}}$ ¹⁴C]BYI 02960 in cotton is well understood and the following metabolic pathway is proposed





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) [fu⁴/₄ hone-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

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 Gere 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 (earble commodity) were very low in both

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 bit extract of the double application experiment. As expected, the residues of the double spray application experiments were dominated by parent compound. However, even after the single application, parent compound was the main residue in all matrices an alysed. One major metabolit fraction was detected in al matrices under investigation, comprising the co-eluring metabolites BXD02966 acetic acid and BYI 02960-OH-glyc. BYI 02960-acetic acid represented the main part of the fraction in the double application experiment, whereag the dominance was less pronounced in the single application experiment. In gin trash, BYK02960-OH was detected as additional major metabolite (12% of the TRR). All other metabolites represented less that 10% of the TRR in all matrices with BYI 02960acetic acid-glyc showing the highest portion (%) 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 furthone moiety to an acetic acid group followed either by conjugation with a carbohydrate of by further of dation, and (3) halogenation (bromination/sploringtion) of the furnious moiety. This gretabely sation step probably occurred in the soil and halogenated parent compound was then taken op by the plants. No label-specific metabolites were identified. All metabolic coutes were also detected in the cotton metabolism study with [pyridiny]methyl-14CHSYI 02960. Š

(2) Label 2: [pyridinylnethyl C]By 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-agetic agid, 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 furthone moiety to an acetic acid group followed by conjugation with a carbohydrate or by further ox dation, (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 faranone moiety. Only the second route led to a label-specific metabolite, all other routes were also detected in the cotton metabolism study with [furnaone-4-14C]BYI 02960 which showed no label-

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. 18 minor metabolites were found, and all major and 3 minor have been identified. The edible commodity couron seed showed only very low radioactive residues and analysis of the extract was only feasible in the study conducted with the pyridinylmethyl-label. The distribution of parefy compound and metabolites in the edible commodity seeds is shown in Table 6.2.1-43, the distribution in the feed commodity sin trash is summarised in Table 6.2.1-44.

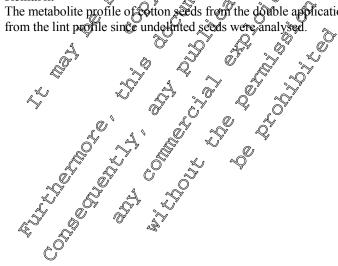
Table 6.2.1-43:	TRR values and distribution	of parent	compound	anding	tabolites	incott	on seeds	after
	foliar application of BYI 029	96Ø√		ž	K,	Â,	°≫ ,	۳.

11	
	cotton segul a Q Q O' Q' A
Radiolabel	[pyridinylmethyl- ¹⁴ C] O C
	$\begin{array}{c} \hline c \hat{o}(tton segd \\ \hline [pyridiny]methyl-4C] \\ \hline single appl. \\ \hline 0.0245 \\ \hline \% TR8 \\ \hline mg/kg \\ \hline $
TRR [mg/kg] =	
Compound (BYI 02960-)	0,023 2 2 0,068 0 2 2 0,068 0 2
BYI 02960	<u>0</u> 1 3 3 4 0 0 0 0 0 0
6-CNA	5.0×0.007
OH-glyc/ acetic acid	
Total identified	416.2 0.007 3\$.3 0.023 v v v
Total characterised 🔬	
Analysed extract(s)	
Extract(s) not analyse	
Total extracted	28.3 (0.0) (66.1 0 0.0) (66.1
Total extracted Unextractable (OS*)	
Accountability	
* post extraction solids	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$

Label specific metabolites are printed initalics

Remark:

The metabolite profile of otton seeds from the double application experiment shows most probably an interference



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

Table 6.2.1-44:	FRR values and distribution of parent compound and metabolites in gin trash after fol	iar
8	application of BYI 02960	
		0

	gin trash								
Radiolabel		[furanon	e-4- ¹⁴ C]		[pyridinylmethyl- ¹⁴ 0]				
	single	appl.	double	e appl.	single	appl.	Cuble	e æppl.	
TRR $[mg/kg] =$	0.1	91	2.7	67	0.2	N 0	2,3,44		
Compound (BYI 02960-)	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	Ô [%] TR R €	mĝ∕gkg	
BYI 02960	40.0	0.076	54.4	م 1.505	26.3	0.082^{2}	53.2	£1.247	
6-CNA			Ŷ) ??	20.2	0,063	~ [°] 2.2 ₄	0.053	
glyoxylic acid	0.9	0.002	K6	0.044	0 ♥ 2.1	\$ 2007	0° 15°	¢0,035	
acetic acid-glyc			2.3	0.06Q	°	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	©.7	©0.087	
OH-glyc/ acetic acid	15.7	0.030	20.8	0.577	ر 13.7 [،]	0:093	گ ^{22.4}	D 0.526	
ОН	13.1	0.025 _{&}	. 0,6	0.016	J 14.9	0045	13	0.030	
chloro/ bromo	0.6	0.00Ø	Q2.3	0.062	<u> </u>	\$	2.1	0.049	
Total identified	70.3	0 A 34	<u>,</u> @ 81.2 (2.268	76.8	> 0.298	86.5	2.028	
Total characterised	7.4	.0 .014	× 11.5	@ 319	J 14.2	6.044	6.0	0.162	
Analysed extract(s)	77.7	0.148	.93.4	2.586	\$ ¥.0	Ø)0.282C	× 90.4	2.190	
Extract(s) not analysed	2.6	0,005	2.3	0.065	م می 0.9	0.005	ي 3.8	0.089	
Total extracted	80Q	0.153	95.8	2 651	o 92€	Ø.285	۶≈ 97.2	2.279	
Unextractable (PES*)	¢9.7	. 🔊 0.0380	¥.2	_©0.116		~0.02 \$	2.8	0.065	
Accountability	3100.0	[≫] 0 <u>,1</u> 91	100.0	2.7 6 7	\$00.0	0.319	100.0	2.344	

* post extraction solids

Label specific metabolites are printed in italic

Analysis of cotton ared and gin trash extracts on the non-radioabelled metabolite difluoroacetic acid revealed that this metabolite represents a significant proportion of the restrices in the single and double application experiments. Diffuoroacetic 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 BYF02960 concentration. In the double application experiment, parent compound and several metabolites showed figher 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 diffuoroeffylamine bond and formation of difluoroacetic acid
- oxidative degradation of the further moiety to an acetic acid group followed either by conjugation with a carbohydrate or by further oxidation to BYI 02960-glyoxylic acid
- $\sqrt{2}$ xidative cleavage of the pyridinylmethylamine bond and formation of 6-chloronicotinic acid $\sqrt{2}$ (6- $\sqrt{2}$ NA) $\sqrt{2}$
- prodroxylation of the furanone moiety followed by conjugation with glucose
- Chalogenation (bromination and most probably chlorination, as well) of the furanone moiety

ð

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 de la companya de la comp 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

halogenation

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The positions involved in the metabolic degradation are summarised in the forewing figure

Figure 6.2.1-13: Positions involved in metabolic degradation of BYI 02960 in cotton of or the fur anone moiety

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Contraction of the part of the

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-

Report:	KIIA 6.2.1/10, ; 2011
Title:	Metabolism of [furanone-4-**C]BY102960 in paddy rice
Report No & Edition No	MEF-11/058
Guidelines:	OECD 501 Metabolism in Crops US EPA Residue Chemistry Test foundeline OPPTS 860.1000: 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 81470 European Parliament and Council Regulation (CC) NotTr07/2009
GLP	yes of the second secon

Executive Sommary

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 as./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 surfay application took place directly after transplanting of the rice seedlings at a fate of 475 g as./ha and the second approx. one month before harvest at a rate of 240 g as./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:

Matrix Timing and Application	PHI (days)*	ppm (mg a.s. equiv./kg)
kernéls fone granule application at transplanting of the	127	0.140
husks rice seedings (BBCH 13-15),	127	1.404
straw	127	2.879
kernels two spray appetations at transplanting of the rice seedlings (BBCH 13-15) and approx. 30	29	0.659
husks of rice seedlings (BBCH 13-15) and approx. 30	29	24.098
straw 2 A des before harvest (BBCH 87-89)	29	19.891

Table 6.2.1-45 TRR values in paddy rice (Rernels, husks and straw) after granular or spray treatment of [furanone4-14C] BYI 02960

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

The ratioactive 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

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 afterors. 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. 41% of the TRR. Halogenation of the furanone moiety of the active substance probably accurred in the paddy soil. Soil contact with parent compound resulted in both experiments. Either the active substance was aptiled 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 optrace components.

The following metabolic routes of [Faranone-4-14C/BYI.02960 vere observed in pader rice

- halogenation (mostly bromanation, to againor extent aforination) of the furanone moiety,
- hydroxylation of the methylene group of the furanche mojey,
- 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 furanon moies and incorporation of the carbon atoms into the natural compound pool, i.e. into glucose carbon drates

On the basis of these results, a metabolic pathway of [furanone-4, C]BXF02960 in paddy rice can be proposed.

A. Materials 1. Test Material:		
		* position of the radiolabel
Radiolabelled test material Specific radioactivity Chemical Purity	[furanone-4-14C]BYI 02960	
Specific radioactivity	3.94 MBq/mg (106.46 µCi/mg)	
Chemical Eurity	> 99% (HPLC)	
Radiockemical purity	> 99% (HPLC and TLC)	
Ô		

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.

4" (sandy loam soil from Germany), pH (CaCl 9= 6.8, 69% sand, 18% silt and ter kvel was maintained for als 2. Soil: 13% clay, 1.2% organic carbon, cation exchange capacity (CEC) of 8.3 meq/100 The soil was submerged and kept under paddy conditions. An adequate water level optimal growing conditions.

vat., representative for cereals 3. Plant Rice, variety "Nihonbare", Oryza sativa B. Study Design

Experimental conditions:

Experimental conditions: application and spray treatment Each experiment was conducted ander 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 files diffectly 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 manting container was flooded with water to simplate 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 prayer 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 mount of 34% MB for 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°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/x) 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 solide All extracts were combined per matrix. The extracts of the sample materials busks and straw were? subjected to a clean-up step using an SPE RP 18 cartridge (Phenomenex, Strata C182E, 2039), which was conditioned with acetonitrile/water (8:2: who have a strate of the s was conditioned with acetonitrile/water (8:2; v/v) beforehand. The Aow-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 emuls for and concentrated by rotary evaporation in vacue for HPLC analysis. Non-polar contaminations on the cartridge were eluted by rinsing with methanol/dightoromethane (1:1; v/). Volume and radioactivity of this fraction was also determined. Extracts of kernels were concentrated and analysed by HPI without any additional purification step.

The post-extraction solids (PES) Rkernels and straw of both experiments were subjected in an exhaustive extraction procedure. The solids were extracted for a fire step with acconitrate/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 extractionstep, extracts and solids were separated by filtration. The radioactivity in the extracts was determined by ASC, in the solids by combostion followed by LSC. The two extracts were combined per matrix, porified by SPE and concentrated by Potary 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 dicheromethane under addition of Celite. The corresponding exhaustive extract of rice kernels from the spray breatment was subjected to a diastase treatment (pH & 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: radioactivity detector with a gass scintillator cell. The HPLC chromatograms (= metabolite profiles) were integrated for quantification of compounds K,

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 theses 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

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

II. Results and Discussion

The metabolism of [furanone-4-14C]BYI 02960 was investigated in vice kernels, shaw and husks following two different application scenarios. In the grange 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 article of the seedling of the seedlings (approx. 3 to 5) leaves unfolded BBCH 13-15) at an article of the seedling of leaves unfolded, BBCH 13-15) at an application rate of 409 g a.s./ha. In the sprag application experiment, rice was treated two times, one time at an actual application rate of 175 ga.s./hadirectly 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 harves? The total application rate if 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 as that $\frac{1}{2}$

The raw agricultural commodities fice kernels and stray were collected in both experiments at maturity (BBCH \$9-92), 127 and 29 days after the last application Rice hasks were sampled during the preparation of rice kernel.

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 TRK in edible RAC rice kernels accounted for 0.140 mg/kg after granule application and for 659 ng/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 spragexperiment.

The radioactive residues were afficiently expected 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 6.27-46 and Table 6.2.1-47. Exhaustive extraction comprised two extractions steps with actionitrile/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.

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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 J. 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 grapule 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 glugese 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 Celiteshowed that by far the majority of the radioactivity remained in the aqueous phase and indicated the presence of ratural compounds. HPLC analysis of the aqueous phase confirmed the assumption. Nearly 90% of the extract was represented by glucose/carbohydrates. In all other manices, glucose/carbohydrates were assigned by profile comparison.

In rice straw, BYI 02960-bromo was dentified as the only major metabolite present the co-eletted with BYI 02960-chloro, which accounted for significant lower concentrations. Both metabolites were identified by LC-MS/MS analysis in rice strawin the spray application experiment following isolation of the fraction containing both compounds. Most probably, halogenation of the furanone moiety of the active substance occurred dready in the paddy spil and the metabolite was taken up by the rice plants. All other metabolites detected in the sample matrice 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-agetic acid and BYI 02960-QH was confirmed by HPLC co-chromatography and the metabolities BX102960 glyoxylic acid and BYI 02960-aceffc acid glyc were identified by comparing the HPLC profiles of the extracts with the consponding ones obtained in the rice study with the other radiolabel. Co-elution of metabolite BY 02960-OH-give with metabolite BYI 02960acetic acid was not expected since HPC-MS/MS analysis of the volated peak in the corresponding rice metabolism study with pyridiny lmethyl-14 CIBYI 02960 showed only BYI 02960-acetic acid. rice metabolism study with pyridinylmethol.¹⁴CHBYI 02960 showed only BYI 02960-acetic acid. Compounds in the actracts of hust's and in the achaustive extracts were also assigned by comparison of profiles.

Table 6.2.1-46: Distr	ribution of radioactivity in the extracts of rice matrices after one granule application
of [f	uranone-4- ¹⁴ C]BYI 02960

	granule application experiment					
	kernels		hu	sks	straw 🎢 🧃	
TRR $[mg/kg] =$		0.140		1.404	<i>S</i>	^{2.87} 8
	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	[™] mg/kg
Conventionally extracted	20.5	0.029	75.6	1.062	79.7	\$\$.294_Q
Extract for analysis	19.2	0.027	75.6 <i>چ</i>	1,062	79.0	2.275×
Losses (not analysed)	1.2	0.002	🕅 n.q.	n.q.	Ç0.7 🔹	0,020
Exhaustive solvent extr.	6.5	0.009	L	0 [×]	ي 11.5 ¢	@:331 g
Extract for analysis	5.6	0.008 🚄		Q	10.9	© 0.31 <i>3</i> ©
Losses (not analysed)	0.8	0.00	~>	<u> </u>	𝒫 \ @ .6	© 0.0017
Exhaustive NaCl extraction	41.8	0,058	é à	× -70	ð ×	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
aqueous phase	28.6	00040	Ő ×		ð t	A
organic phase	0.8	0.001°	<	· ···	· 0	Q* #
Losses (not analysed)	12.3	0.647	\sim $\overline{\diamond}$	<u>,, 0</u>	<u>ي</u>	~
Total extracted	68.7	D (0.0 96 s	75.6	1.062	Ø 91.2	02.625
Unextractable (PES*)	31,30%	0.044	24.4	\$	§ .8	ي 0.254
Accountability	100.0	0.140	A 100,00°	0 ¹ .404	مَ ^ح 100.0%	2.879
* post extraction solids	0, 4	j W	5 0)	Å 8	~~~ <u>«</u>	

post extraction solids

n.q. not quantifiable (residues $< \hat{L} \otimes Q$)

n.q. not quantifiable (residues < DØQ) Table 6.2.1-47: Distribution of radioactivity in the extracts of fice matrices after two spray applications of [furanone-4, ⁴C]B YI 02960

				<u> </u>	4	
	ς.δ [°] .	∧S S		ion_experimen	t	
	ker	nels 🧳 🥈	🕅 🔊 hu	sky 24 098	stra	IW
TRR $[mg/kg] = 0$	0 0	ر @0.659		24.098		19.891
	‱% of J RR	ر 00.659¢¢ mg/kg	Sof TRR	√nng/kg	% of TRR	mg/kg
Conventionally extracted	65.8	0.433	, 26 .9	21.917	83.7	16.643
Extraction analysis	65.8 65.8	0.4330	×€90.4 الم	21.790	83.3	16.561
Losses (not analysed)	l piq.	$O' n_{Q'}$	炎 0.A	0.127	0.4	0.081
Exhaustive solvent extr.	© 5.6 ×	Q.9 37	°		9.9	1.977
Extract for analysis	<u>ې څخې 5.2</u>	ð Ø.034	÷ ~~		9.9	1.964
Losses (not analysed)	0 Å3	× 0.00x	~~		0.1	0.013
Exhaustive MaCl/	3 ^{95.8}		, Ø			
diastase extraction ¹	Q.					
aqueous phase	<u>13.9</u>	~ 0.090°				
organic phase	∑″ <u></u> ~0.9	Q 0 290 6				
Losses (not analysed)	L 1.20	.008				
Total extracted A	87.2	0.574	90.9	21.917	93.6	18.620
Unextractable PES*	Ş‴ _≪ 1,2.8 _≈	0.085	9.1	2.182	6.4	1.271
Accountability	À 100.0	0.659	100.0	24.098	100.0	19.891

notifither APLC analysis for to high matrix load of the extract
 post extraction solids
 n.qcnot quadrifiable (residues < LOQ)

۵Ô

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

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 agricultural commodities of rice after granular and foliar application. A summary of the compound 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 metabolities in rice matrices after a single granule application of [furanone-4-¹⁴C]BYI 02960

	**	-	anulaanniiaa	tion experime	nt 💦		
	ker		Nini	sks _Q			
TRR [mg/kg] =	0.14		<i>P</i>	04,()		1 <u>4</u> % . (1)	
Compound (BYI 02960-)	% of TRR		% of TRR	or ♀ mg∂sg°	% of TAR	mg/kg	
Conventional extraction	70 01 1 KK		<u> </u>		\sim \sim \sim \sim \sim		
BYI 02960 (parent comp.)	17.5	0%024	<u>6</u> 723	1.016	57.6~	¥.658	
glucose/carbohydrates	17.3	0.002 «		07894	0 3U	0.088°	
glyoxylic acid		0.002 x			× 9,7	0.000	
acetic acid			N QP	\$ 0.006		× 0.051	
OH						0.024	
bromo/chloro			0.6	~0.008 ×	\$ \$0.6	0.306	
Subtotal identified	192	© 0.02)7	~ 0.0 y		م ² 75.6 ک	2.176	
unknown 1					0 /3.0 /	0.023	
unknown 3			1.3 2		1.1	0.023	
unknown 5	\$ \$ \$		<u> </u>		2 1.1 2 1.1	0.031	
unknown 7	 		× ~		× 0.4	0.033	
Subtotal characterised			<u>)</u>	8 ,019	→ 0.4 → 3.4	0.012	
Total conventional exer.		0.029	2 75.6	1.062	79.0	2.275	
Microwave extraction					19.0	2.275	
BYI 02960 (pare@ comp@	5.6	2. 00.008 /			6.4	0.183	
glucose/carbohydrates				<u> </u>	2.3	0.066	
glyoxylic açig	×7	4 8	- O ^Y a.	1 m	0.2	0.007	
acetic actor		, Â	× S [×]	<u>y</u>	0.2	0.007	
OH OH		\$ <u>,</u> \$	U AY	¢	0.2	0.005	
bromo/chloro	¥/	× .@	0 .		0.8	0.022	
Subtatal identified	S 5.60	<u>`~9.008</u> ~	<u> </u>		10.1	0.290	
unknown 3			~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		0.4	0.011	
unknown 4		<u>8</u>	,		0.3	0.007	
unknown 💭 🔍 🖓	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	2 ° ×			0.2	0.005	
Subtotal characterized	 ∢				0.8	0.023	
Total microwave extr.	5.6	Q 02008			10.9	0.313	
Exhaustive NaCl extraction				1			
organic phase 🖉 🔬	Q Q Y	² 0.001					
aqueous phases	28.6 ×	0.040					
glucose/carbohydrates	25.2	0.035					
Subtotal Mentified	~ 25.2	0.035					
Subtotal dentified	3.4	0.005					
Subtotal characterized	4.2	0.006					

Table continued on next page...

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

Total identified	granule application experiment							
	kernels		husks		straw			
	50.1	0.070	74.3	1.043	85.7	2.466		
Total characterised	4.2	0.006	1.3	0.019	4.2	\$.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	√ Q.Q\$7		
Total extracted	68.7	0.096	75.6	1.062	91.2	~Qx625 ¿		
Unextractable (PES*)	31.3	0.044	24.4	0.342	8:8	°~0.254		
Accountability	100.0	0.140	~100.0	E A04	109.0	2.879		

Accountability	100.0	0.140	A 100.0	£ ,404	109.0	
 post extraction solids 			Å,	,Ô¥		
n.q. not quantifiable (residues	s < LOQ)	/=	s [©]	Q, so		e e
			,*	,	q. "ó"	o Ĵ
Table 6.2.1-49: TRR value two spray	es and distribut	ution of parer	it compound	and metaboli	es in rice mat	pices after a
two spray	application of	f [furanone-4	- @C]BY19029		S.	.1
		1	pray applicat	ion experimen		D' A
	ker	nels 🔨	dru	sks 🗍 🚬 Õ	🔬 stra	aw S
TRR [mg/kg] =	0.6	89 67 ,	24.0) <u>980 x x</u>	\$ A9.8	91 0
Compound (BYI 02960-)	% of TRIO	mg/kg	% of TRR /	mg kg	S‰ of T \$€R	⊘ mg/kg
Conventional extraction	Q.					, ^y
BYI 02960 (parent comp.)	@,53.3 ≪	\$ 03351	\$ 74,8	17.90	⁰ 52,9	10.527
glucose/carbohydrates		0.012	° ~√2.1 "	0× 0¢497	OF QU	0.401
glyoxylic acid	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		Ø ^{\$}	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	ي 2.0	0.393
acetic acid-glyc	0.4	04003	L 0,2	[∞] 0.05	× 1.7	0.329
acetic acid 🔬	5.90	@ .039 ~	~7.0	J.693 «	9 7.2	1.432
OH		0 -2	Å	0 4	0.4	0.081
bromo/chloro	v _≈ 9.7 _₹	Q 011	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	L 0.380	10.1	2.016
Subtotal identified	≪ 63.k	% 416 (85.5	20,605	76.3	15.179
unknown 1 🔗 🔗	<u> </u>	<u> </u>	0.4	Ø.092	0.6	0.126
unknown 2 👸	Q	1 Å	8	×	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	ý <u>s</u> '				0.9	0.173
unknown 6 🖗 🖂	0.9	\$0006	9.4	0.103	1.2	0.229
unknown 7 🖉 🔊	Q.\$	0 0.0050	® 2.5	0.608	0.2	0.044
unknown 9	Ş	Q, <u>\$</u> -	0.9	0.211	3.1	0.609
Subtotal characterised	స్త్రా 2.7 ్లో	\$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
Microwave extraction	\$. O					
BYI 02960 (parent comp.)	رگ 3.3	Ø 0 21			3.6	0.720
glucose/carbohydrates		Q0.011			1.4	0.287
glyoxylic acid		Ø			0.1	0.023
acetic acid-glyc	× ^	Ş			0.7	0.132
acetic acid	0.3	0.002			0.8	0.149
OH L OF L	÷				0.1	0.025
OH Cr Cr	×				0.6	0.116
Subtotal identified	5.2	0.034			7.3	1.451
<u>0</u> -	•	-	•	T		

Table continued on next page...

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

	ker	nels	hu	sks	str	aw
unknown 1					0.3	0.062
unknown 3					0.1	0. Ø å
unknown 5					0.2	\$0,033
unknown 8					∞ 0.2	0.031
unknown 9				0	1.8	~ 0. 3 59
Subtotal characterised				4	2.6	Q\$13
Total microwave extr.	5.2	0.034		, the second sec	9,20	\$1.964
Exhaustive NaCl/diastase ex	ctraction			a s	Å.	
organic phase	0.9	0.006	e	- Q		
aqueous phase	13.7	0.090	Ô,	Å		
Total exhaustive extr.	14.6	0.096 🎽	·	~~		<u> </u>
Total identified	68.4	0.450	85.5	×20.605	××3.6 _<	16:030
Total characterised	17.2	0413	() () () () () () () () () () () () () (1.185	<u>9.5</u>	¥.896
Analysed extract(s)	85.6	0.564 🛠	90.4	O ⁷ 21 39 0	°0° 9301	,18.526°
Extracts not analysed	1.6	0.010	~ 0.5	0.127	0.5	0.094
Total extracted	87.2	<i>∲</i> 0,574	90.9	A21.917	93.6 _€	18.620
Unextractable (PES*)	12.8	6 085	Ø.1	× 2.882	S 64	©1.271
Accountability	100,0	~0.659 ~	×100.0	24.098	100 .0	19.891
* post extraction solids	- Q'	Q Q	ð S			/

Table 6.2.1-50: Summary of characterization and identification of radioactive residues in rice matrices after one granular application of fluranone-4-¹⁴C]BY102960

Š`_			Y Q		<u>~</u> 0 [×]	
K.				tion experime	Ω) [*]	
	🖉 🚕 ker	nels 🔊		sks 🔊 🦂	∛ str	aw
TRR [mg/kg] =		40 × ×	0 ² 0 ² 1.40		2.87	79
Compound (BYI 02960-)	% of TRR	mg/kg	% of TRR	Ų″ mg∕kg	% of TRR	mg/kg
Conventional expaction) Ö	LO X		<u>.</u>		
BYI 02960 (parent comp.)	رم 23.1 م	0:032	F 72.3	1.016	64.0	1.841
glucose/carbohydrates	<u> </u>	9.038		0.014	5.3	0.153
glyoxylic acid 🚬 🖉 🔬	6		~		2.0	0.056
acetic acid 🔬	~~~. ~~~.	0° 45	& 0Å	0.006	2.0	0.058
OH 🔊 🔬	J'K	<u> </u>	۲ <u>م</u>		1.0	0.029
bromo/chloro	N G	× -5	0.6	0.008	11.4	0.328
Total identified	50. 1	0,070	74.3	1.043	85.7	2.466
unknown 1 🔬 🔗		<u> </u>	й		0.8	0.023
unknown 🖉 👘 🔬	Q. 3.4	0.005				
unknown 3	× ~		1.3	0.019	1.5	0.043
unknown 4	N Q				0.3	0.007
unknown 5	K t	X			1.3	0.037
unknown 7 🔏 🔬		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~			0.4	0.012
Total characterised	4.2	0.006	1.3	0.019	4.2	0.122
Total characterised	ని 54.3 [*]	0.076	75.6	1.062	89.9	2.588
Extracts hot analysed A	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
Total extracted States 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)

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					
	ker	nels	hu	sks	str	aw X 🖓	
TRR [mg/kg] =	0.63	59	24.0)98	<u>ک</u> 19.8	910 6	
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	17.92	56.5	ĵ\$.247_¢	
glucose/carbohydrates	3.6	0.023	2.1	0,497	3,3	^م م 0.68	
glyoxylic acid			V	<i></i>	Č2.1 ×	Q 0,496	
acetic acid-glyc	0.4	0.003	6 .2	0 ^{\$} 0.054	ي 2.30° ي	@.461 & O	
acetic acid	6.1	0.040	7.0 4	Q 1,693	2.9 ×	© 1.581@	
ОН			-	, O ⁴ ⁴	00.5	0.1006	
bromo/chloro	1.7	0.011	° 1	0.3890	ð 10.7 ×	2,132	
Total identified	68.4	() .450		20,605	83.6	16.630	
unknown 1		A	0.4Q	0.092	, Q).9	0.188	
unknown 2	"			~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	≪ 0.3	\$ 053	
unknown 3	0.9	_ & 0.006 _≪	× 0.6	0,138	Ø 08	00.151	
unknown 4			0.10		9 .1	Ø 0.025	
unknown 5	Q ,	· ·			~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	0.206	
unknown 6	0,96	Ø .006	Ø.4	6 0. Î O 3	~~ k2	0.229	
unknown 7	0.8	0.005 [℃]	‴∕ 2.5 ©	[™] _02608	Q .2	0.044	
unknown 8	Š 🖇	6 <u>6</u> -	Ø		يڭ 0.2	0.031	
unknown 9	Q	\$ ⁷ ^	. 6.9	0.214°	<u>م</u> 4.9	0.969	
Subtotal characterised	£ 2.9	00.01	× 4.9 %	¢	6.9	1.382	
Total characterised ¹	A 7.2	Ø 0.193	4.9	©ĭ.185∜	9.5	1.896	
Analysed extract(s)	°∼y 85.6√	×,564	9.4	<i>≨</i> y 21.7 £0	93.1	18.526	
Extracts not analysed	× 146	20.010	~~~0.5 C	ØU27	0.5	0.094	
Total extracted 炎 🖌	89.2	0.574	2 90 8	21.917	93.6	18.620	
Unextractable (PES*)	\$12.8 Å	20,085	° 9.1	2.182	6.4	1.271	
Accountability	S 1000	0.659	200.0 ^	∑ 24.098	100.0	19.891	

including characterization by partitioning

post extraction solids

n.q. not quantifiable (residues

Conglusions

[Furanone-4-14C]BX192960 was moderately metabolised in rice. Residues were dominated by parent compound in the foldar apprication 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/carbonydrates was observed.

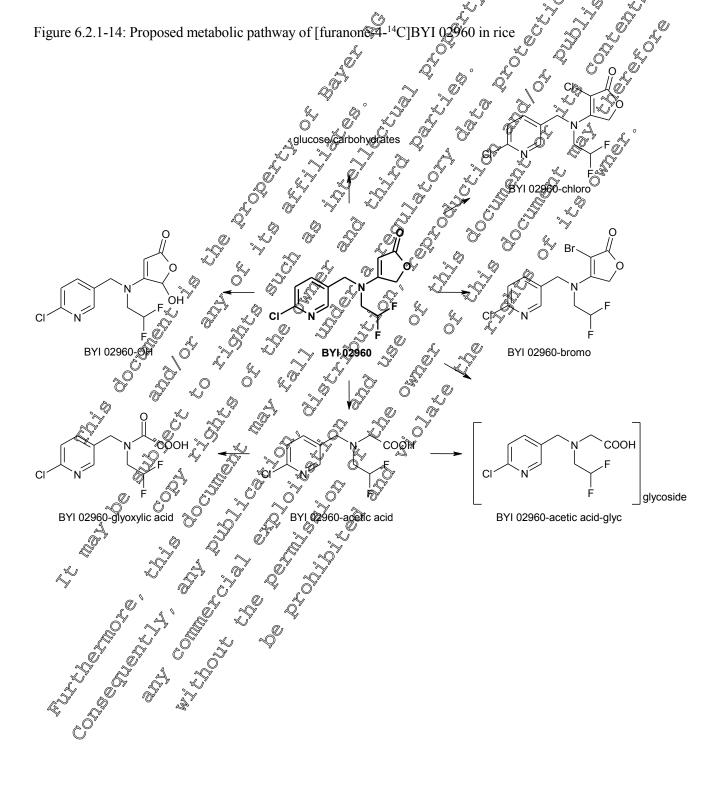
Generall four major metabolic routes of [furanone-4-14C]BYI 02960 were observed in rice:

- by drox pation 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.

¹⁴C]BYI 02960 in rice is well understood and the following metabolic pathway is proposed.

Figure 6.2.1-14: Proposed metabolic pathway of [furanone4-14C]BYI 02960 in rice



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

Report:	KIIA 6.2.1/11, , , , , , , , , , , , , , , , , ,
Title:	Metabolism of [pyridinylmethyl-14C]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 0107/2009
GLP	yes A Q O O

Executive Summary

The metabolism of [pyridinylmethyl-¹⁴C]BXI 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 in the second approx. one pronth before harvest at a rate of 236 g a.s./ha.

At maturity the rice plants were hat vested and separated this straw, kernels and husks. The TRR values of all plant platrices determined are shown in the following table:

Table 6.2.1-52	TRR values in page	dy rice (kernel	s, husks and s	s@aw) after	granular or	spray treatment of
, Q	[pyridinylmethyl-	²⁴ C]BAI 02960	/ ° _0		-	

Matrix	Timing and Application	PHI (days)*	ppm (mg a.s. equiv./kg)
kernels	one granule application at transplanting of the	127	0.050
husks	riceSeedlings (BBCH 13-5),	127	1.602
straw		127	3.280
kernels 🔬	two pray applications at transplanting of the	29	0.620
husks	rice seedlings (BBCH 13-15) and approx. 30	29	23.957
straw	days before harvest (BBCH 87-89)	29	24.731

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

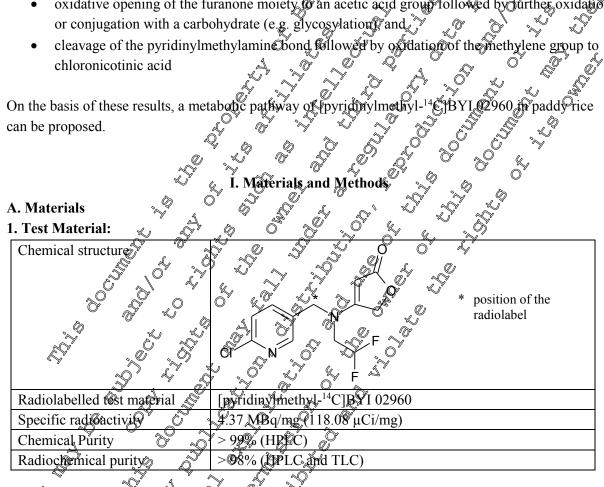
The radioactive residues were efficiently extracted with acetonitrile/water mixtures. When necessary, additional exhaustive extraction steps were applied to the solids of the first extraction. Extraction efficiencies ranged from 74.3% of the TRR for kernels (GR) to 97.3% for kernel (SP) after exhaustive extraction. The profites 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 $\geq 60\%$ 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 we

- halogenation (mostly bromination, to a minor extent chlorington) of the further more
- hydroxylation of the methylene group of the diranone moiety, •
- oxidative opening of the furanone moiety to an acetic acid group followed by further oxida or conjugation with a carbohydrate (e.g. glycosylation) and
- cleavage of the pyridinylmethylamin and followed by ordation of the methylene group to • chloronicotinic acid

On the basis of these results, a metabolic can be proposed.



The supplied radio abelle Crest compound [pytoinylmethyl-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 concentrate and index constant flow of nitrogen. The concentrate was then mixed with Sepiolite 30/60 & carrier grande, 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 a lass vial and evaporated to dryness. The liquid blank formulation was added to the texpitem and the mixture was homogenized by ultrasonication. Thereafter, the mixture was diluted with water to get the aqueous application solution.

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2. Soil: 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 the (granule of the second application and spray treatment). Each experiment as conducted under paddy conditions in a planting container with a surface area of 0.5 m². The plants were cultived in a climate chamber in a greenhouse under controlled environmental conditions.

Granule application:

The granules were distributed equally if the planting holes directly Before the second ing BBCID13-15) were transplanted into the holes. The target application rate was 400 g a.s. ba. After transplanting of the seedlings, the planting container was flooded with water to sipulate paddy conditions. An actual amount of 94.9 MBq or \$4.7 mg a.s. was applied, corresponding to 434 gas./ha Spray application: (M)

For the spray application, addefined 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 days before havest, ar actual amount of 250 g as ha was applied (515 MBq or 11.8 g).

Sampling:

In both experiments, the rice was barvested at maturity (BBCH 89-92). The panicles were cut off from the plants and the oce kernels were separated from papicles 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 température 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 (2 18°C) until malysig

C. Analytical Procedures

Extraction: 💭

Prior to extraction, the bomogenized sample materials were soaked in a solvent mixture of acetoniffile/water (8:2; v/v) for one night. Three extraction steps with acetonitrile/water (8:2; v/v) followed using an Lyraturax 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

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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 was of 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 for an exhaustive extraction procedure. The solids were extracted in a first step with acetonitrile water a v/v) and in a second step with acetonitrile/water (1/T, v/v) under microwave assistance (120 °C for 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 otary 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/o)? 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 treament to characterize the radioactivity incorporated into starch composents. Therefore, the extract was buffered with sectium 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 pixture was acquisted to pH.7.0 and the supernatant was separated from the solids by centrifugation. Partitioning with acconitrile followed after addition of sodium chloride. A second partitioning step with acetophrile followed after acjusting 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 metaboliter 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 of

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 cochromatography 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

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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 hole 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 as the structure of 200 g a.s./ha, the second application was slightly overdosed to achieve the intended maximum rate of 200 g a.s./ha, the second application was slightly overdosed to achieve the intended maximum seasonal application rate of 400 g a.s./ha.

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 for edible RAC rice kernels accounted for 0.050 mg/kg after granule application and for 0.620 mg/kg after pray 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 vater 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 6.2.1-33 and Gable 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 [furatione-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 thentification. 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.

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 self. 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 TRIC 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 HPCC cochromatography were BYI 02960-acetic acid-glyc, BYI 02960-gb oxylic acid, BYI 02960-OF and the label-specific metabolite 6-CNA

Table 6.2.1-53:	Distribution of radi	opertivity in	n the ext	ractsof	ricematrice	s after one	granule application	
10010 0.211 001	of [pyridinylmethy	⁴ CIBVI	12960		N N			
	of [pyridinyiniculu		02,000	8	Ŝ, Õ	~Õ (Õ N	

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			anule applica	tion experime	nto 🥾	
	ker ker	nels 🖓 🎣		sks 👡 🤅	stra	aw
TRR $[mg/kg] =$	¢ک 🖉 🖉	5,00	1.6		3.28	30
~	% of TRR	mgakg	% of FRR	mg/kg	of TRR	mg/kg
Conventionally extracted	\$ 62.8 [°]	0.032	_^%79.6 C	َ` \$4,275 °	≫ 81.6	2.677
Extract for analysis	L 628	0.032	S 796,	9.275	80.7	2.648
Losses (not analysed)	n.q.	1 1 1 1 1 1	J. ARQ.	A ACA	0.9	0.030
Exhaustive solven extr.	11.5	O.006	· · ś	× ~	7.7	0.252
Extract for analysis	L 11.5	0.006	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	_@	7.2	0.237
Losses (not analysed) 🔬	, 🔬 n.q.	🖓 🕅 🤄 🦓	· · · ·	~	0.5	0.016
Total exported	74.3	0.037	79.6	1.275	89.3	2.930
Unextractable (PES*)	257	6 ⁵ 0.013	<u>کې</u> 20.4	0.327	10.7	0.350
Accountability	1 000.0 K	× 0.050	O 160,0	1.602	100.0	3.280

Table 6.2.1-54:	Distribution of radioactivity in the extracts of rice matrices after two spray applications
	of [pyridinylmethyl-14C]BYI 02960

		S	pray applicat	ion experimen	ıt	
	ker	kernels		sks	str	aw 🏹 🖓
TRR [mg/kg] =	0.6	0.620		957	24.7	7310° 5
	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	[™] mg/kg
Conventionally extracted	88.4	0.548	90.3	21.625	84.0	2 9.764 ¢
Extract for analysis	88.4	0.548	89.8ھ	21,514	83.7	°∽20.708
Losses (not analysed)	n.q.	n.q.	T 0.5	A .111	CO .2	0,056
Exhaustive solvent extr.	5.6	0.035	L		ي 9.7 ⁽¹⁾	\$.393 ¢ P
Extract for analysis	4.9	0.031 🚄		Q ~	A 9.6	© 2.374
Losses (not analysed)	0.7	0.004	~~	<u> </u>	Ø.1	© 0.©20
Exhaustive NaCl/	3.3	0,021	ŝ	\$ 7	ð~»	~~~~~
diastase extraction ¹		Ő ^v	Ø "V	Å sø	à .	Â.
aqueous phase	1.8	0.011			<u> </u>	
organic phase	1.2	× 0.008	\sim \rightarrow	, , , 0	× , '	\$`{\$\$
Losses (not analysed)	0.3	D Q:003				
Total extracted	97.30	0.604	\$90.3	21,625	S 25.6	ال 23.157
Unextractable (PES*)	£,₹	0.017	9.2	Q.332 Ô	6.4	J [∞] 1.573
Accountability	400. 0 🖌	° 0 ≈5 20	109,0	∱ 23.95F	<u> </u>	24.731

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

* post extraction solids

* post extraction solids The TRR values and the distribution of parent compound and metabolities in the extracts is shown in 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

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

Table 6.2.1-55:	TRR values and distribution of parent compound and metabolites in rice matrices af	ter a
	single granule application of [pyridinylmethyl- ¹⁴ C]BYI 02960	
		0

		gr	anule applica	tion experime	ent	, e
	ker	nels	hu	sks	str	aw 🏹 👘
TRR [mg/kg] =	0.0	50	1.6	1.602		800 0
Compound (BYI 02960-)	% of TRR	mg/kg	% of TRR	mg/kg 🖉	% of TRR	[∞] mg/kg
Conventional extraction	•				A	25 0
BYI 02960 (parent comp.)	62.8	0.032	77.7	1,244	56.2	1.842
6-CNA			0.5	Ø .009	ČŽ.9 🔬	0.093
glyoxylic acid			al	.64	0.2	.0.007 / (
acetic acid		40]	<u></u>	0 1.8	0.058
ОН				Q A	Q 1	0.037
bromo/chloro		<u>. 2</u>	。 076 ⁻	°≫0.010	≈,11.7 ≪	\$ \$ \$ \$\$85
Subtotal identified	62.8	0.032	2 J8.8	1.263	\$ 73.9 [°]	2.423
unknown 3		4 - <u>-</u> %	<u> </u>	r 8	Ø.7	~ 0.0 2 3°
unknown 4	·	× · · · ·	~ 0.8	A0.012	0.7 4	0,022
unknown 6		· · · · · ·			\$ 0.5U	30 .016
unknown 7	2	(~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	<u>У "Õ</u>	ê a	0.047
unknown 9	<u> </u>	<u>r</u>		Å	\$0.3 ×	0.011
unknown 11	Q [*] ∅	Q	Ô Â-		0.4	0.015
unknown 12		<u> </u>			8 8	0.027
unknown 13	V 6	A A	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	× ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	. 0.9	0.031
unknown 14	0 x	<u>y</u>			× 1.0	0.033
Subtotal characterised	A	<u> </u>	<u>60.8</u>	0.6¥2	S 6.9	0.225
Total conventional extr	\$ ⁷ 62,8	0.032	^≫ 79.60	¥.275 Å	80.7	2.648
Microwave extraction			\$7 <i>a</i> ,	0******		21010
BYI 02960 (parent comp.)	6.8	. ^0.003 %			3.7	0.122
6-CNA	AN 18-7	0.002			1.0	0.031
acetic acid	<u>p </u>			Ø	0.2	0.006
OH Q V	29 A	· ~~···	Ö ₍₎	<i>w</i>	0.1	0.004
bromo/clatoro				*0 7	0.5	0.018
Subtotal identified	× 1,1.5	\$ 0.000			5.5	0.181
unknown 2					0.2	0.006
unknown 3		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	ř "Ô		0.2	0.003
unknown 5		0^{2} 0^{2}	Ő		0.1	0.005
unknown 6		<u>y</u> xy			0.2	0.003
unknown 1	3 -49				0.1	0.005
unknown 3	R				0.1	0.005
unknown 14					0.3	0.010
Subtotal characterised					1.7	0.010
Total microwaye extraction	0 ' 0 405	Q, 0.006			7.2	0.030
Total identific	74.3	0.037	78.8	1.263	79.4	2.603
Total characterised	×9		0.8	0.012	8.6	0.281
Analysed extract	0 74.3	0.037	79.6	1.275	87.9	2.884
Losses extracts of analysed &	C .				1.4	0.046
Totalextracted	n.q. 74.3	n.q. 0.037	n.q. 79.6	n.q. 1.275	89.3	2.930
Unextractable (PES*)			20.4		10.7	0.350
	25.7	0.013		0.327		
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 6.2.1-56:
 TRR values and distribution of parent compound and metabolites in rice matrices after two spray applications of [pyridinylmethyl-14C]BYI 02960

	spray application experiment							
	kor	nels		sks	str	aw N	S	
TRR [mg/kg] =	0.6		23.9		24.7	<u> </u>	1	
Compound (BYI 02960-)	% of TRR	mg/kg	% of TRR	mg/kg 🔞	% of TRR	mg/kg	-	
Conventional extraction	70011100	iiig/kg	70 01 1100	111g/Kg *0			-	
BYI 02960 (parent comp.)	72.0	0.447	₹77.3	18526	56,9	<u>^</u> ~14.07Å	3	
6-CNA	2.1	0.013	∞ 0.4	0.107	0,9 00.9 ~	0.297	- "(
glyoxylic acid	0.4	0.003	т.0. Т	0.107	2.0	\$.505 ¢	64	
acetic acid-glyc	0.6	0.003	0.2 4	0,048	1.2	0.30	1	
acetic acid	7.8	0.04	6.5	Ø.548		© 1.001	-	
OH	0.4	Ø.002			ð 0.6 ×	<u>0</u> 146	-	
bromo/chloro	1.5	0.009		0,2095	£.9	1.955	-	
Subtotal identified	84.8	A 0.526	∑ Ø 85.7-Q	20.524	06.2	Ø 18.844	-	
unknown 1					≪ 0.1	£2033	-	
unknown 2	Q	<u> </u>		Ő X	6 0 Q	00.031	1	
unknown 3	,0 ^y	<u> </u>	× × 0	× ÷ · · · · ·	§ .3	<u>ک</u> 0.079	1	
unknown 4			<u> </u>	0.0850	0.3	0.068	1	
unknown 5		~~~_^	<u>, </u>	<u> </u>	~ Q2	0.047	1	
unknown 6		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	0.2	Ø 2044	0 .2	0.049	1	
unknown 7	×		Ø	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	ي 0.1	0.022	1	
unknown 8 🏻 🔊	Ŷ		× ~	K Z	~ 0.2	0.042	1	
unknown 9 🔬	\$.0 <u>9</u>	0.00	×0 [*] 0.4	¢ (0.090 %	0.8	0.188	-	
unknown 10	2.2	0.008	1.7	©0.401∜	0.3	0.068	1	
unknown 11	°~ Ŵ	×^	Ş. 8	L U	0.3	0.070	1	
unknown 12	× &,	~	[∞] 0.2 €	6.0 44	0.7	0.171	1	
unknown 13 🔗 🖧 🦼	φ <u>φ.1</u>	S 0.0007	0 de 1	@0.106	1.1	0.279		
unknown 140	0.8	2005	0.9	0.221	2.9	0.718		
Subtotal characterised	2 3 3 6	0.022	× 4.1	¥ 0.990	7.5	1.864		
Total conventional extr	88.4	S` 0.548	89,8%	21.514	83.7	20.708]	
Microwave extraction]	
BYI 02960 (parent comp.)	\$ 3,2	<u></u> \$0.020			3.9	0.958		
6-CNA	ç <u> </u>	0.000	°		0.3	0.073		
glyoxylic acid		× , Ø	Ö,		0.2	0.052		
acetic acid aryc	2 -0				0.7	0.166		
acetic act	~~				0.7	0.183		
OH L L					0.2	0.043		
bromo/chloro	<u> </u>	<u> </u>			0.6	0.139		
Subtotal identified		Q 0.026			6.5	1.615		
unknown 1	×	e			<0.1	0.011		
unknown 2 2	\$~¥				0.1	0.036		
unknown 3	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 mi owave extraction	4.9	0.031			9.6	2.374		

Table continued on next page...

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	ker	rnels husks		straw		
Exhaustive NaCl / diastase ext	traction					
organic phase	1.8	0.011				Q° '
aqueous phase	1.2	0.007				N A
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	<u></u> 2.622
Analysed extract(s)	96.4	0.597	89.8	26,514	Ø¥.3	23.082
Extracts not analysed	0.9	0.007	0.5	\$ 0.111	ر © 0.3 ر	0 075
Total extracted	97.3	0.604 🦼	90.3	∱ 21.625	0 93.6	23.157
Unextractable (PES*)	2.7	0.01	9.7	× 20332 k	ð <i>6</i> /4	1.579
Accountability	100.0	0.620	。100 -0 ^{->}	^ ≈ 23.957	1 00.0 <	240731
* post extraction solids						
		A		1 5	* O.	

Table 6.2.1-57: Summary of characterization and identification of radioactive residues in rice matrices after one granular application of [pyrdiny]methyl-4C]BY 02960

	~~~~	The state of the s	anule annliva	tionexperime	nt 🔊 🗸	<u>Ş</u>
	kernels & husks & s					aw
TRR [mg/kg] =	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	50 °	<u>م</u> ر ا.ھ	<u>ny</u> or	×3.2	
Compound (BYI 02960-)	% of TRR	Ömg/kg	‰ of TR¶√	'nşğ/kg 🌾	% of TRR	mg/kg
BYI 02960 (parent comp.)	69.6		r. 77∿7	× 1.240	\$ 59.9	1.964
6-CNA	47	ð.002 Ø	0 [°] 0.5 «	0.009	3.8	0.125
glyoxylic acid	8 X-		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	Å L	0.2	0.007
acetic acid	<u> </u>	× ~ ~	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	л. <u>П</u>	1.9	0.063
OH CO NO		~~~~^^	° ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	v , , , , , , , , , , , , , , , , , , ,	1.2	0.040
bromo/chloro		¢° ±	~ 0.6°	0.010	12.3	0.403
	©74.3	0.037	<b>78.8</b>	ي 1.263	79.4	2.603
unknown 2 2		<u> </u>	~ ~	Ø	0.2	0.006
unknow 🕄 🔍 🖉	<u>67 - 57 - 57 - 57 - 57 - 57 - 57 - 57 - </u>	<u></u>	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		0.8	0.026
unknown 4		0° <u>kj</u>	Ky 0. <b>48</b>	0.012	0.7	0.022
unknown 5	<u> </u>		<u>}</u>		0.2	0.005
unknown 6			~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		0.6	0.019
unknown 7 🖓 🗘 🔊		<u>× ,×-</u>	<u>⊳</u>		1.4	0.047
unknown 9	 	<u> </u>	Ŭ		0.3	0.011
unknown	Q				0.4	0.015
unknown 12	, ³ (	v ×			1.0	0.032
unknown 13	<u>~</u> ?	, ⁶ ·			1.7	0.056
unknown 14					1.3	0.044
Total characterised 🖂 🔬		×	0.8	0.012	8.6	0.281
Analysed extract(s)	_ <b>₹</b> _ 74.3-Q	0.037	79.6	1.275	87.9	2.884
Losses/extracts nor 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)

after two spray applications of [pyridinylmethyl- ¹⁴ C]BYI 02960						
	spray application experiment					N O
	kernels		husks		Ş str	ay o
TRR [mg/kg] =	0.6	20	23.9	057 0	24.7	31 .
Compound (BYI 02960-)	% of TRR	mg/kg	% of TRR	mg/kg	% of TR	jing/kg
BYI 02960 (parent comp.)	75.2	0.467	<u>م</u> 77.3	18(526	60,8	^م ري 15.020
6-CNA	3.1	0.019	☞ 0.4	0.107	<u>0</u> 1.2 ~	<b>0,39</b> 1
glyoxylic acid	0.4	0.003	~		× 2.30	£9.557 &
acetic acid-glyc	0.6	0.003	0.2 4	Q 0.048	L.9	Õ 0.475
acetic acid	7.8	0.04	6.5	َرِي @¥.548	Ø7.3	\$ 1. <b>8</b> /4
ОН	0.4	Q.002	s° <del>s</del> -		0.8	<b>0</b> .189
bromo/chloro	1.5	@.009 @		0,2015	8.5	<u>2.09</u> 4°
Total identified	88.9	A 0.5 <b>52</b>	[©] 85.7 [®]	20.524 _C	° <b>82</b> .7	20.460
unknown 1	"	, ~ ,			ي 0.2	° 043
unknown 2	0	_& L	~~~~ ×		Q 99	$\tilde{O0.068}$
unknown 3					<b>9</b> .3	l 0.079
unknown 4	Q* _@	~ھ	87 (ST	0.085	^م ري 0.3 م	0.068
unknown 5		Ø Å	, <u>0</u> ,	ô ^y ô <u>-</u>	~~~ <b>%</b> 2	0.047
unknown 6	$\mathcal{O}$ ,		[∞] 0.2	^{عر} في 10044 م	<b>9</b> .2	0.049
unknown 7	Š~ "	g ig-	Ø <u></u> *		<b>Q</b> 0.1	0.022
unknown 8	Q	\$ [*]	× _~	× ÷	<u> </u>	0.042
unknown 9 🔬	S 2,3	0.000	<u>~</u> 0.4	¢0,090 🌫	0.8	0.188
unknown 10	<u>\$91.2</u>	Ø 0. <b>00</b> 8	5 17	©ó.401∜	0.3	0.068
unknown 11	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	<u> </u>	<u> </u>		0.3	0.070
unknown 12	~ ~	<u>~</u>	0.2	6.044	0.7	0.171
unknown 13 Or 🖉 🧹	P 9.9	§ 0.0052	0 0 C	<i>©</i> 0.106	2.2	0.555
unknown 140	× 0.8 A	@005	° ₀ 9.9	0.221	4.7	1.154
Total characterised	2 7 <b>3</b> 7	0.045	<b>4.1</b>	0.990	10.6	2.622
Analysed extract(s)	\$ \$26.4	\$ 0.597	89,87	21.514	93.3	23.082
Losses/extracts not analysed	\$ 0.9 ×	<b>6</b> 007 (	0.5	0.111	0.3	0.075
Total extracted	S 97.0	× 0.604	. 90.3	21.625	93.6	23.157
Unextractable (PPS*)	° ~2.7 ,	0.0.9	9.7	2.332	6.4	1.573
Accountability	<b>100.0</b>	0.6620	) ⁽⁾ 100.0	23.957	100.0	24.731

 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

### AIII. Conclusions

[Pyridinylmethyl- 14 C]BYI 02960 is metabolized moderately in rice. Residues were dominated by parent compound in the folia capplication experiment, and parent compound was also the main compound in all matrices of rice after granule application. In total, seven different metabolites were identified by rice array, 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,

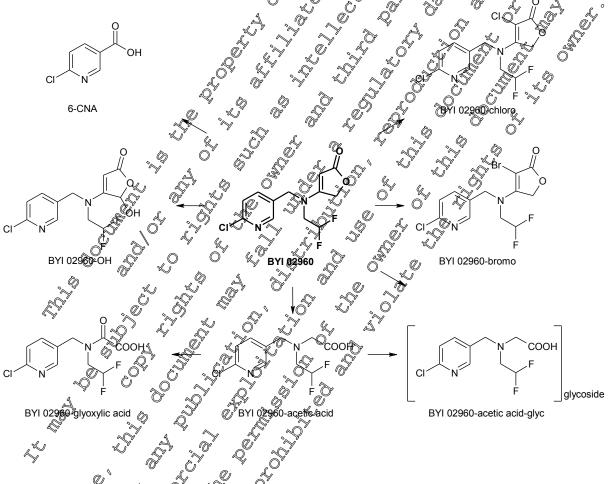
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- Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)
  - oxidative degradation of the furanone moiety to an acetic acid group followed by either conjugation with a carbohydrate or further oxidation, and
  - halogenation (mostly bromination, to a minor extent chlorination) of the furanone moievy,

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 metabolisph of [pyridinyhpethy].⁴C]BYI 02960 in rice is well understood and the following metabolic pathway is proposed.

Figure 6.2.1-15: Proposed metabolic pathway of pridinglmethyl, ¹⁴ClBar 0296 in Rice



### Averal Conditions Rice (foliar and granule application)

The metabolism of the insecticide BYF02960 was investigated in rice kernels, straw and husks following two different application scenarios of (1) [furanone-4-¹⁴C]BYI 02960 or (2) [pyridinylmetayl-¹⁴CBYI 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 appraval additionally 30 days before harvest. The total target application rate in both experiments was 400 g a.s./ha. Regardless of the 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 scenaro. After granule application, the main residue in rice kernels was represented by a natural compound (glucose/carbohydrates), indicating a quite intense degradation of the faranone 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 of compound or glucose, an additional major metabolite (>40% 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 exidation 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) hydroxytation of the furanone moiety of the parent compound and (4) halogenation (bronunation, 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 cansformation path if the plant.

Only the second route led to a tabel-specific metabolite. A corresponding counterpart was detected in the rice study performed with [pyridinylmethyl¹⁴C]BYI 02960. The concentrations of the metabolites common to both rabiolabels 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: [pyridiny]methyl C]BY 02960

As for the study with the futanone 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 metabolite 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)

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 [furnaone-4-¹⁴C]BYI 02960. The concentrations of the metabolism study marice of the present metabolism study marice of the present metabolism study marice of the present metabolism study marice of the corresponding study performed with [furnaone-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 pa	rent compound and	metabolités in rice	kerness after
	granule and foliar opplication of E	¥۲1 02960 کې		°∼y

	·						/		
		× 0 × 0	s.	rice®	ernels &				
Radiolabel	w k	[furanon		, Ý	~~~~ 0	pyridinyln	nethyl-14C]		
0.	🏟 granu@ a	pplication	🖉 foliar ap	plication	. granule aj	oplication	foliar ap	plication	
TRR [mg/kg] =		40 3	0.6 ^م رکز ک	558 ⁶⁷ U	° ≪°0.0	50	0.6	20	
Compound (BYI 02960	% TRR«	/ mg/kg	% TRR	∀mg/l@	%TRR	≫mg/kg	% TRR	mg/kg	
BYI 02960	23	~ 0.032	² 560	Ø ₂ 373	69.6	0.035	75.2	0.467	
glucose/carbohydrates	26.9	0.038	°∼\$.6	~0.023					
6-CNA					^{4.7}	0.002	3.1	0.019	
glyoxylic acid	K	× 4.40°		Õ	Ø		0.4	0.003	
acetic acid-gfyc		2 C	0.4	@0.003			0.6	0.003	
acetic acto	. 6 '	\$~_ <del>`</del> +-	°° 6.1 ≰				7.8	0.048	
OH NO	X ×	. 0 ,		A			0.4	0.002	
Chloro/ bromo	Ç` <u>~~</u>	J ?	P.7	≫0.011			1.5	0.009	
Total identified	50.1 C	0.0750	\$68.4	<b>0.450</b>	74.3	0.037	88.9	0.552	
Total characterised 🖉	A.2	<b>^0,006</b>	ُمَ 17,2	0.113			7.4	0.045	
Analysed extract(s)	<b>Q</b> .3	<b>0.076</b>	85.6	0.564	74.3	0.037	96.4	0.597	
Extract(s) for analysed 🔪 🖗	a ^{14.4}	0.020	≈≈1.6	0.010			0.9	0.007	
Total extracted	<u>∢</u> 68 71	<b>9</b> ,096	\$ 87.2	0.574	74.3	0.037	97.3	0.604	
Unextractable (PES*)	₹ 3¥.3	Q0.044	12.8	0.085	25.7	0.013	2.7	0.017	
Accountability	<b>400.0</b>	0.149	100.0	0.659	100.0	0.050	100.0	0.620	
* past avtraction halida		, <del>R</del>							

* post extraction solids Label specific metal of the apprint of in italie

Analysis of the extracts of rice kernels and the feed items husk and straw on the non-radiolabelled metabolite affluoroacetic acid revealed that this metabolite represents a significant proportion of the residues on 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 the proceeds of the following pathways:

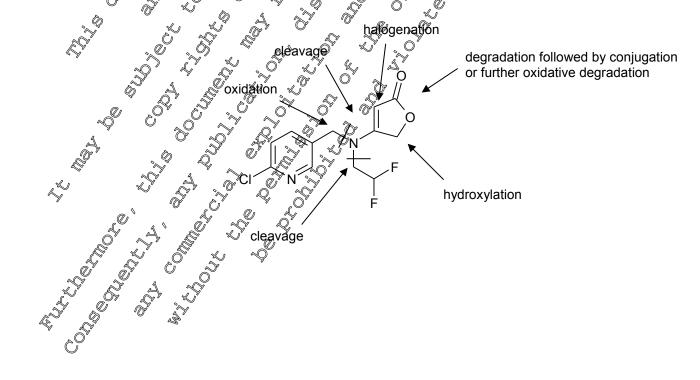
- oxidative cleavage of the difluoroethylamine bond and formation of difluer acetic acid
- oxidative degradation of the furanone moiety to an acetic acid group followed either by conjugation with a carbohydrate or by further oxidation to BY 202960-glyox fic acid
- complete degradation of the furanone moiety and incorporation of carbon from into the natural compound pool, e.g. into glucose carbonydrates
- oxidative cleavage of the pyridiny methylamine bond and formation of 6-chloronicotinic and (6-CNA)
- hydroxylation of the furanone moiety
- halogenation (bromination and most probably chloringtion, a well) of the fur anone moiety

It is considered likely that hat be so in the furnione morety of the active substance occurred in the paddy soil/sediment. Uptake of the soil metabolize results in plant residues.

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

Ŵ

Figure 6.2.1-16 Positions involved in metabolic degradation of B&I 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 stracts originated from the metabolism studies conducted with either [furanone-4-14Cf or [pyridiny]methy ¹⁴C]BYI 02960:

æ.

Report:	KIIA 6.2.1/12, , , , , , , , , , , , , , , , , , ,
Title:	Determination of residues of difluoroactic acid in extracts of samples from plant
	incluoonsin and commed rotational rops studies and appendition of D 11 (2)00 0
Report No &	$MR-11/050 \qquad $
Edition No	MR-11/050 M-422550-01-1
<b>Guidelines:</b>	OECD 501 Metabolism in Coops , O , S , O , O , O , O
	US EPA Residue Chemistry Test Guidelice OPPTS 860.1500: Nature of the Residue – 4 Plants, Livestock
	PMRA Regulatory Diffective Dir98-02 Residue Chemistry Guidelines Section 2: Nature of
	the Residue – Plants Livestock
	Japanese MAFF 2 Nousan 8147
	European Parliagent and Council Regulation (PC) NoO107/2009
GLP	yes white the second seco

Executive Sommacy

Due to the fact that only one material of the study was performed with [ethyl-1-14C]BYI 02960, additional information on the fate of the driluoroethane moiet of BYI 02960 was provided in the present report. Residues of nontradiolabelled offluoroacetic acid, the most plausible metabolite that can be formed from the diffuoroethme majery due to cleavage a BYI 02960, were determined in extracts of samples of apples, potatoes, gotton, tice, wheat, Swiss chard and turnips originating from plant metabolism and confined votational crops studies after application of [pyridinylmethyl-¹⁴C]BY102960 or [furanone 4²¹⁴C]BYI 02960. To estimate the residue levels of difluoroacetic acid in these crop samples non-radiolabelied diffuoroacetic and was analyzed according to the provisions of residue analytical method 01304. As expected from the results of the tomato metabolism study performed with [ethy] -14 COBYI 02960, diffuoroacetic acid was detected in all crops as a major metabolite accounting for significant proportion of the BYI 02960 residue.

### Material and Methods

Samples of apples, potatoes, cotton, fee, wheat, Swiss chard and turnips were harvested and extracted in different point metabolists and confined rotational crop studies following application of either [furanone-474C]BYI 02260 or [pyridin9]methyl-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 diffution an isotopically labelled internal standard solution was added and the extract was analysed boLC-MS/MS according to residue analytical method 01304. No further sample work up was performed.

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:	F F C C C C C C C C C C C C C	Ĵ,
Chemical structure	OH A R A R	Å
	F	
		<i>v</i>
	$F = \frac{1}{2} $	
Name of Compound	Difluoroacetic acid (BES-AA56716 OFA) of A	0
Certificate of Analysis	AZ 16523, dated 2010-03-31	
Chemical name	Difletoroacetric acid	
Purity	980% (WW) 2 2 2 2 4 4 4	
2. Internal Standard:	F $F$ $F$ $F$ $F$ $F$ $F$ $F$ $F$ $F$	
Chemical structure	F Diflustoacetic acid C ₂ (BCS-AB60481-ISTD, DFA-ISTD)	
N C P	$F_{13}$ $C$ $V$	
Name of Compound	Difluoroacetic acid C ₂ (BCS-AB60481-ISTD, DFA-ISTD)	
Certificate of Analysis	KATH 15199-1-4, dated 2010 6-14	
Chemical narge	Sødium@tifluerø(13C)acetat	
Purity of the of	3 > 99%	
	F   Diflustoacetic acid   C   KATH 15199-14   dated   2010   64	

#### 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 pray application of BYI 02960

Matahalian standa	Crea	Samela	Use notton ()	Desider %- "	Defenere
Metabolism study	Crop	Sample material	Use pattern	Residues [mg/kg]	Reference n Apssier 2 KIIA62.1/06
Metabolism of [furanone-4- ¹⁴ C]-	apple	fruits	one foliar spray application at BBCH269,	09.23	KIIA 2.1/06
BYI 02960 in apples		leaves	96 all a line of CII)	Q 0.63	
		fruits	two foliar spray applications, at .	Ň	
		leaves	2 x 86 g a.s./(Set x m (P1) 0	@ 0.45.C	A &
Metabolism of [pyridinylmethyl- ¹⁴ C]- BYI 02960 in potatoes	potato	tuber 2	BBCH 97), BBCH 97), C	\$ 0.13 \$ 0.13	KMA 6,24/05
			in-factow spiraly application at planting (BBCH 99),		Ŝ
			(BBCH 03), 10.0 gazs./dt in-fat/ow spiraly application at planting (BBCH 03), 26 g as ha		2 2
Metabolism of	cotton	gin trash 🔊	one spray apprication	0.04	KIIA 6.2.1/09
[pyridinylmethyl- ¹⁴ C]- BYI 02960 in Cotton		seeds >	one spray application 206 g a.s./ha (at BBCH 16)	0,003	
after Spray Application		gin trash	wo spoy applications, 20 206 Qu.s./hayat BBCH 16)	0.02	
Ű		sceds	175 g a.s./ba (at BBCH 950 97)	0.02	
Metabolism of [pyridinylmethyl- ¹ C]-	rice >	straw	two spray applications dato the plants at different growth stages,	0.39	KIIA 6.2.1/11
BYI 02960 in paddy		hust,	178 g a.s./hc (at BBCH 13 - 15)	0.46	
rice	*)		236 g a.s. ba (at BBCH 87-89 one granular application at the	0.08	
Ê,		husk ~	time of transplanting	0.12	
29 °		grains O	434 g a.s./ha (at BBCH 13 - 15)	0.02	
[pyridinylmethyl-1 BYI 02960 in packy rice					
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		$\sim$			
			Š [*] . D		
	× ~~	~ ~			
	Ê,	, ~ ~			
Le la			, ,		
	Č Õ	× ×			
	A A A A A A A A A A A A A A A A A A A				
õ					

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

Metabolism study	Rotation	Сгор	Sample material	Residues	Reference in
·			-	[mg/kg]	dossier
Metabolism of	1 st rotation	wheat	forage	0.09 🖒	KIBA 6.6.2401
[furanone-4-			hay	0.32	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
¹⁴ C]BYI 02960 in Confined Rotational			straw	0.20	\$ \$\$ 6
Crops			grain	1.15 🥎	dossiev 37 KIP 6.6.201
cropo		Swiss	intermediate Ø	0.08	
		chard	mature 0	.0,16	
		turnip	intermediate mature leaves	0.08	
			forots ~ @	$Q^{\prime} 0.00^{\prime}$	o û
	2 nd rotation	wheat	forage of	1 3 0 2 .	
		O V	nav N N		
		A	Watting of the second s	0.06	
			Ygrain S A		
		Seviss x	intermediate	0.04 0.04	
		6 ^{cs} hard & "	nature of o	S 0.05	L.
	Q.	turnip	leaves N O	0.03 .	
	, Qi	sti ni	roots	0.01	v
	3 rd rotation	wheat	forage 🔧 🖉	0.00	
		Wheat		t≪ 0æ91	
			straty	9.02	
		ĝ Õ	grain O	<u>م</u> لام کې م	
		Swiss	Sintermediate 0 /	√ < 0.01	
		chard	matore 2 0 leaves ~ 2	0.01	
Č,		turnip 🥎		< 0.01	
		chard turnip	Toots O S	< 0.01	
en EG		à ô	netisions 0		
			netwions .		

Significant levels of diffuorbacetic acid (DFA) were detected in most of the primary and confined rotational crops under investigation intespective of the appleation technique. High diffuorbacetic acid concentrations after to har smay application indicate that this metabolite is also formed in plants and not only in soll.

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 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 matrice	softe
	application	Ô	s l		×,

u	upphonition						a,	Č,	່ 🚿	seeds	
Compound		potato	tubers		apple			ernel®			
(BYI 02960-)	tuber tr	eatment	in-furro	w appl.	Ø ⁹ foliar	appl.	granul	e appl.	Sfoliar	@ppl.	
Label	F	Р	F	P 🎢	۶F	P 📎		Ô, Ő	∀F ू	P	
TRR	0.078	0.076	0.171	0.115	0.280	0 79	0.140	0.050	0.013 ²	40,945	
BYI 02960	0.031	0.031	0.097	0.951	Ø.021 🔬	0.034°	0.032	0.035	× ,		
DFA		0.39 ¹	4	0.54 ¹ %	0.69		ð	0.061		0.02^{1} °	
glucose			2		0.291		0.038		- Q		
6-CNA		0.016	Ĩ	0.021	Ø . 1	€⁄0.0046		0.002	Z (9.007	
CHPM-di-glyc		0.003		Ø.006		~~	Ő.				
CHMP-glyc		0.003	y 0	0.003		Q.904	ð é) <u> </u>			
difluoroethyl- amino-furanone	0.003		0:005	Ű.	0 .009	á V	8				
OH-glyc	0.005	6.0 05 候	0.007	0.005	0.001	0.004		b			

LC-MS/MS analysis of non-radiolabeller DFAOn the extract obtained in the restabolism study

analysis of the extracts was not feasible due low extraction efficiency and high matrix load in the extracts [furanone-4-¹⁴C]-label \sim $R = [pyridinylmethyl-¹⁴C]/label <math>\sim$ 2 $F = [furanone-4-{}^{14}C]-label$

of BYI 02960 and major metabolites in enible matrices after two Table 6.2.1-63: Residues mg/kg _ Oxpplic Oxions_ Ø G

Compound (2) (BYI 02960-)		mato frui	<i>U</i>	with s		NO SI	fruit	rice k	ernels	cotton	seeds
	dren	ch applica	ntion	Ô ^Ŷ «	spray ap	plication	Y	spray	appl.	spray	appl.
Label	E	_δ Ρ΄ _α (È 🔬	F °	P O	F ≫	Р	F	Р	F	Р
TRR	0.096	0.130	0.209	1.133	1.868	1,286	0.545	0.659	0.620	0.016 ²	0.068
BYI 02960 🔊	0.034	0.091	ð.020	~0,809	⊳.652	0.946	0.467	0.373	0.467		0.016
DFA 🔬		Ŭ a	0.174		, Ø		0.121		0.241		0.061
glucose	0.026	\hat{Q}	Ű.	0.193	²	0.182		0.023			
6-CNA		0.017	\sim	á s	0.009		0.008		0.019		0.003
CHPM-di-glyc	<i>S</i>	0048			⁹						
CHMP-glyc	Ø) ^{\$}	0.007	<u></u>	- Q	0.010		0.005				
difluoroethyl-			×S [°]	~~							
difluoroethyl-	0,000		0.004	% .007		0.003					
Turanone	~~~ (\sim	2	r							
OH-gly¢	0.005	0.00	0.001	0.014	0.024	0.014	0.009				0.003

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

Canalysis of the extracts was not feasible due low extraction efficiency and high matrix load in the extracts $F = [fura@ne-4-^{14}C]-label$ $E = [ethyl-1-{}^{14}C]$ $P = [pyridinylmethyl-{}^{14}C]-label$

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

Bayer CropScience

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

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. Ngh 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 radio abelled BYL 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 grops revealed the same picture. For the edible crops wheater ains, Swiss chard and turnin roots, difference acid accounted also for a high proportion of the residue, especially in the early rotations. In when grains, difluoroacetic acid represented by far the main proportion of a hypothetical TRR (for i please refer to KIIA 6.11, to in all three rotations as shown in the following tables. difluoroacetic acid represented by far the main proportion of a by pothetical TBR (for more details

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	whea	at hay	wheat	straw	wheat	t grain	
Label	F	Р	F	Р	F	Р	F	ð	
TRR	0.783	1.407	2.003	2.409	6.290	9.015	0.478	0.177	4.9
BYI 02960	0.365	0.640	0.672	0.676	2.459	3.261	0.002	0.015	\$ \$ \$ 6
DFA	0.271		0.96 ¹		0.601		3.45		
glucose							Ø∕338	Ô	
bromo-amino- furanone	0.016		0.033		\$ /172	Å			
difluoroethyl- amino-furanone	0.077		0.205	Ŵ	0	2 ,	Q U-	Ŷ,Ő	
glyoxylic acid	0.124	0.172	0.227	0.∜76	Ø.965 (*	0.615	0.0 24 0 0 07	0.011	
OH-glyc	0.028	0.048	0.067	0.135≪	0.242	0.296	0007	0.009	
6-CNA-glycerol- gluA (2 + 3)		0.199		0.569		ð.900 j		0.036	
ОН	0.010	0.019	0038	\$0.052 ×	0.161 ×	0.239	0.871	Q 019	Ğ, Ö

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

 $P = [pyridin@methyl2]^4C]-label$ $F = [furanone-4-^{14}C]-label$

	1		y j		Ø	4) Ø
Compound (BYI 02960-)	Swiss interm	ediate	Swiss Smat	Swiss chard matore		turnip leaves		roots
Label	F & @:848 &	Ø «	SF a,	Р	F	P O	ð 4	, P
TRR	@: 848 /	/ 1.358	0.879	P 1.483	6 679	Ø.815	0.07	0.072
BYI 02960 රි	0.460	0.709	0.371	0.687	0.437		0.041	0.042
DFA 🔊	0,2	.0	0.48^{1}		0.2		0.06^{1}	
glucose	<u>-0</u> *) Ø	{		TOS I		0.003	
bromo-amino- furanon		922	R.		z "S			
difluoroethyl- amino-furanone	\$°_^		0.010		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	\$ 7		
glyoxylic acid	0.030	0.021	00041	<u>6</u> .039 🔍		0.021	0.009	0.006
OH-glyc	0072		0.119	0.162	0.076	0.076	< 0.001	0.002
6-CNA-glycerol- gluA (2 + 2)	ð				j v			
OH S	Q.Q.Y7	₄ 0.024 ≈	0.017	°0.029	0.012	0.011	< 0.001	< 0.001

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

Compound	wheat	forage	whea	it hay	wheat	straw	wheat	grain
(BYI 02960-)		P					- micat	grann A
Label	F	-	F	P	F	P	F	67 (2) (2) (2) (2) (2) (2) (2) (2) (2) (2)
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	0.004	0.001
DFA	0.061		0.421		0.18		0.781	~
glucose							Ű	Ô
bromo-amino- furanone	0.006		0.107	4	6 2093			
difluoroethyl- amino-furanone	0.016		0.075		0.090			¢× ,ć
glyoxylic acid	< 0.001		0.020	0.010	Q.018	0.036	0.004	0.001
OH-glyc	0.007	0.008	0.037	0.045%	0.08	0.142	0002	0.003
6-CNA-glycerol- gluA (2 + 3)		0.044	2	0.242		@ .472	\$ <u>`</u>	grain 0.001 0.001 0.001 0.001 0.001 0.007 0.007
ОН	0.003	0.005	00021	%0 .019 ×	0.047	0.079	0,003	Ø 004
$\mathbf{E} = \mathbf{f}_{11} \mathbf{r}_{12} \mathbf{r}_{13} \mathbf{r}_{14} \mathbf{r}_{1$	1 lobel	Ю	, midime la	nother 1 140	1 And I	S'	y _o	Ň
$F = [furanone-4-^{14}C]$		ŝ		nethyl- ¹⁴ C	C]-Cabel			
$F = [furanone-4-^{14}C]$ Compound (BYI 02960-)	Swiss	ŝ	Swi ś s	hethyl- ¹⁴ C	[]-abel	0.079 0.079 2 2 4 1 eaves ~		roots
Compound	Swiss	chard &	Swi ś s	chard ture	turnip	leaves a	y turnig F	0.007 0004
Compound (BYI 02960-)	Swiss intern F	chard & ediate	Swiss anat	chard ture	turnip	leaves «		
Compound (BYI 02960-) Label	Swiss	chard & ediate P 0 332	Swiss Anat F (0.263	chardy ture	turnip	leaves « P (0.230 0.153	F 🖉	B
Compound (BYI 02960-) Label TRR	Swiss intern F 0.311 0.171 0 123 0	chard & ediate P 0 332	Swiss E	Chard ture 0.438	turnip # 6 0.158 @ 108	leaves ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	F 🔊	8 0.022
Compound (BYI 02960-) Label TRR BYI 02960 DFA	Swiss intern F 0.311 0.171 0 123 0	chard & ediate P 0,332	Swiss Anat E 0.263 0.072	chard ture 0.438 0.108	turnip <i>F</i> 0.158 0.09 ¹ 0.09 ¹	leaves ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	F 0.004	8 0.022
Compound (BYI 02960-) Label TRR BYI 02960 DFA glucose	Swiss intern F 0.311 0.121 0.121 0	chard & ediate P 0 332	Swiss Anat E 0.263 0.072	chardy urc 0.438 0.108	turnip <i>F</i> 0.158 0.09 ¹ 0.09 ¹	leaves « P (0.230 0.153	F 0.004 0.004 <003 ¹	8 0.022
Compound (BYI 02960-) Label TRR BYI 02960 DFA glucose	Swiss interm F 0.311 0.121 	chard & ediate P 0 332	Swiss Swiss 0.070 0.15 ¹	Chardy ture 0.438 0.108	tiffnip 2F 0.158 0.09 ¹ 	leaves ~ P & 0.230 Ø.153	F 0.004 0.004 <003 ¹	8 0.022
Compound (BYI 02960-) Label TRR BYI 02960 DFA glucose	Swiss interm F 0.311 0.121 	chard & ediate P 0 332	Swiss Swiss 0.070 0.15 ¹	Chardy ture 0.438 0.108	tiffnip 2F 0.158 0.09 ¹ 	leaves ~ P & 0.230 Ø.153	F 0.004 0.004 <003 ¹	8 0.022
Compound (BYI 02960-) Label TRR BYI 02960 DFA glucose bromo-amino- furanone difluorochyl-	Swiss intern F 0.311 0.12 0.12 0.032	chard & ediate • P 4 0.332 0.1700	Swiss Swiss 0.070 0.15 ¹ 0.046 0.046	Chardy ure 0.4387 0.108	turnip <i>F</i> 0.158 0.09 ¹ 0.09 ¹	leaves ~ P & 0.230 Ø.153	F 0.004 0.004 0.002 0.002	8 0.022
Compound (BYI 02960-) Label TRR BYI 02960 DFA glucose bromo-amino- furanone difluoroctivil- amino-furanone	Swiss intern F 0.311 0.12 0.12 0.032	chard & ediate • P 4 0.332 0.1700	Swiss Swiss 0.070 0.15 ¹ 0.046 0.046	Chardy ture 0.438 0.108	tiffnip 2F 0.158 0.09 ¹ 0.09 ¹ 0.00 ² 0.00 ²	leaves ~ P & 0.230 Ø.153	F 0.004 0.004 0.002 7	0.011
Compound (BYI 02960-) Label TRR BYI 02960 DFA glucose bromo-amino- furanone difluorochyl- amino-furanone glyoxylic acid	Swiss interm F 0.311 0.121 	chard & ediate P 0.1705 0.1705	Swiss Swiss 0.070 0.15 ¹	Chardy ture 0.438 0.108	tiffnip 0.158 0.108 0.009 ¹ 0.0062 0.0062	leaves ~ P & 0.230 Ø.153 Ø V V V V V V V V V V V V V V V V V V	F 5.014 0.004 6003 ¹ 0.002 7 <0.001	0.022 0.011

¹ LC-MS/MS analysis of non-radiolabelled DFA in the extract obtained in the confined rotation crop study performed with [furanone 4+4C]B/I 02969 (2 + 3) isomer 2 and/or isomer 3 0 J-label P = [peridinylinethy,

$$(2+3)$$
 isomer 2 and/or isomer 3 \bigcirc

$$F = [furanone-4-rac{1}{2}C] - label P = [pyridinylphethylowC] - label$$

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

Compound (BYI 02960-)	wheat	forage	whea	it hay	wheat	straw	wheat	grain
Label	F	Р	F	Р	F	Р	F	B
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	0.004	0.002
DFA	< 0.031		0.031		0.061		0,154	
glucose					~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		Ű	Č
bromo-amino- furanone	0.006		0.027	4	§ 2034			
difluoroethyl- amino-furanone	0.013		0.021	Ŵ				grain 0.002 0.002 0.002 0.001 0.001 0.001
glyoxylic acid		0.001	0.001	-\$		0.013	🖉	
OH-glyc	0.003	0.004	0.006	0.010	0.01	0.0404	0001	0.001
6-CNA-glycerol- gluA (2 + 3)		0.022		0.095		@.140		
ОН	0.001	0.001	0004	0.005	0.010	0.040	0.001	Q 001
(2+3) isomer 2 ar	nd/or isom	er 3 🧳	5 O	ř N	×Q	\sim	S A	y "Ş
$F = [furanone-4-{}^{14}C]$]-label P =	= [pyridin	vlmethyl-	¹⁴ C Jabel				
(2 + 3) isomer 2 ar F = [furanone-4- ¹⁴ C] Compound (BYI 02960-)]-label P =	= [pyridin	slmethyl-	¹⁴ Clelabel	tugenip		turnif	
$\mathbf{F} = [\text{furanone-}4^{-14}\text{C}]$ Compound]-label P =	= [pyridin chard ediate	slmethyl-	¹⁴ Clelabel whard ure P	tugsnip			Proots R
$\mathbf{F} = [\text{furanone-}4^{-14}\text{C}]$ Compound (BYI 02960-)]-label P = Swiss (intermy F * 0.180/	= [pyridin Chard ediate P 0:435	Swiss F Control Swiss F Swiss F Control F Swiss F Swis Swiss F Swiss F Swiss F Swiss F Swiss F Swiss F Swiss F Swiss F Swiss F Swiss F Swiss F Swiss F Swiss F Swiss F Swiss F Swiss F Swiss F Swiss F Swiss F Swiss Swiss F Swis Swiss F Swis F Swiss F Swiss F Swis F Swis F Swis Swis F Swis F SwiS	¹⁴ C blabel whard ure P 9.130	tuasnip	2 1eáves 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	5 5 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	Proots R 0.008
$F = [furanone-4-^{14}C]$ Compound (BYI 02960-) Label TRR BYI 02960]-label P = Swiss of interm F [∞] 0.180 0.0066	= [pyriding chard gdiate P 0:135 0.042	Swiss 5wiss 10at F 152 0.051 0.	¹⁴ Clelabel whard ure P	tugenip 2 0.090 0.055	leáves 0.083 0.058	x turnif F 0.008 Q.006	Proots R 0.008
$F = [furanone-4-^{14}C]$ Compound (BYI 02960-) Label TRR]-label P = Swiss (intermy F * 0.180/	= [pyriding chard gdiate P 0:135 0.042	Swiss F Control Swiss F Swiss F Control F Swiss F Swis Swiss F Swiss F Swiss F Swiss F Swiss F Swiss F Swiss F Swiss F Swiss F Swiss F Swiss F Swiss F Swiss F Swiss F Swiss F Swiss F Swiss F Swiss F Swiss F Swiss Swiss F Swis Swiss F Swis F Swiss F Swiss F Swis F Swis F Swis Swis F Swis F SwiS	¹⁴ C blabel whard ure P 9.130	tuasnip	leaves 0.083 0.058	x turnif F x 0.006 0 .006	Proots R 0.008
$F = [furanone-4-^{14}C]$ Compound (BYI 02960-) Label TRR BYI 02960 DFA glucose]-label P = Swiss of interm F [∞] 0.180 0.0066	= [pyriding chard gdiate P 0:135 0.042	Swiss 5wiss 10at F 152 0.051 0.	¹⁴ C blabel whard ure 0.130 0.036 0.0	tuşnip 0.090 0.055	Ieaves P 0.058	x turnif F 0.008 Q.006	Proots R 0.008
$F = [furanone-4-^{14}C]$ Compound (BYI 02960-) Label TRR BYI 02960 DFA glucose	-label P = Swiss interm F 0.180 0.0066 \$0.03 ¹ 0 	= [pyridin chard ediate P 0:035 0.042	Vimetayi- Swiss post F 2 62152 20.0510 0.63 4 	¹⁴ C blabel whard ure P 9.130	tuşnip 0.090 0.055	Ieaves P 0.058 2 4	x turnif F x 0.006 0 .006	Proots R 0.008
$F = [furanone-4-^{14}C]$ Compound (BYI 02960-) Label TRR BYI 02960 DFA glucose]-label P = Swiss of interm F [∞] 0.180 0.0066	= [pyridifk chárd & gdiate P 0:035 0.042 0.042	Swiss F 6/152 0.051 0.03	¹⁴ C stabel whard, ure 9.130 0.036 0.	tugnip 0.090 0.005 0.001 0.005	leaves v P 0.083 0.058 2 4 4 4 4 4 4 4 4 4 4 4 4 4		Proots R 0.008
$F = [furanone-4-^{14}C]$ Compound (BYI 02960-) Label TRR BYI 02960 DFA glucose bromo-amino- furanone difluoroeth M- amino-furanone glyoxylic acid	-label P = Swiss of interm F ~~ 0.180 0.0066 ~~ 0.0066 ~~ 0.026 ~~ 0.026 ~~ 0.026 ~~ 0.001 ~~	= [pyridiff chárd gdiate P 0.435 0.042	Swiss F 6/152 70.051 0.63 5 6/252 7 0.051 7 6/252 7 7 6/252 7 7 7 6/252 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	¹⁴ C blabel whard, ure 9.130 0.035 0.035 0.04	tugnip 0.090 0.055 0.031 0.055 0.031 0.001 70.001 70.001 70.001	2 leáves 0.0588 0.0588 0.0588 0.0588 0.0588 0.0588 0.0588 0.058		Proots Proots Proots 0.005
$F = [furanone-4-^{14}C]$ $Compound$ $(BYI 02960-)$ Label TRR $BYI 02960$ DFA $glucose$ bromo-amino- furanone $glucose$ $difluoroeth - amino-for anone$ $glyoxylic acid$ $OH-glyc$	$\begin{array}{c} -label P = \\ \hline Swiss of \\ interm \\ \hline F & & \\ \hline 0.180 \\ 0.0066 \\ \hline 0.03^{1} \\ \hline 0.026 \\ \hline 0.026 \\ \hline 0.026 \\ \hline 0.001 \\ \hline$	= [pyridift chárd gdiate P 0.935 0.042 0.042	Vimetayi- Swiss post F 2 62152 20.0510 0.63 4 	¹⁴ C stabel whard, ure 9.130 0.035 0.035 0.055 0.001 0.001 0.0036	tugnip 0.090 0.055 0.031 (70.031 (70.001) 70.001 70.001 70.002 0.009 0	leaves v P 0.083 0.058 2 4 4 4 4 4 4 4 4 4 4 4 4 4		Proots P.008 0.005
$F = [furanone-4-^{14}C]$ Compound (BYI 02960-) Label TRR BYI 02960 DFA glucose bromo-amino- furanone difluoroeth 4- amino-foranone glyoxylic acid	-label P = Swiss of interm F ~~ 0.180 0.0066 ~~ 0.0066 ~~ 0.026 ~~ 0.026 ~~ 0.026 ~~ 0.001 ~~	= [pyridiff chárd gdiate P 0.435 0.042	Swiss F 6/152 70.051 0.63 5 6/252 7 0.051 0.63 7 6/252 7 0.051 7 7 6/252 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	¹⁴ C blabel whard, ure 9.130 0.035 0.035 0.04	tugnip 0.090 0.055 0.031 (70.031 (70.001) 70.001 70.001 70.002 0.009 0	2 leáves 0.0588 0.0588 0.0588 0.0588 0.0588 0.0588 0.0588 0.058		Proots Proots Proots 0.005

¹ LC-MSMS analysis of non-radiolabelled DFN in the extract obtained in the confined rotation crop study performed with [furapione-4-4C]BX10296@7

(2+3) isomer 2 and/or isoprier 3 F = [furanone-4-14C]-label P = [pprtdinylopethyl-4C]-label Q

High difluoroacetic acid concentrations fere 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).

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 a difluoroacetic acid is expected to have a pronounced phloen mobility and will therefore be transported in these repository parts of plants known as phloem sinks? significant residue levels/proportions in fruits, tubers, roots and in seeds. On basis of the results

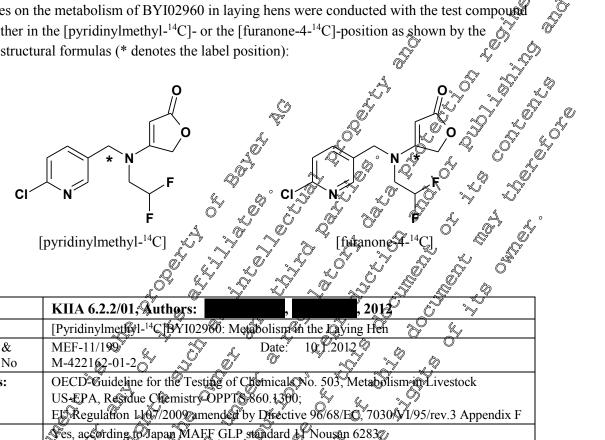
Additional non-GLP studies -initiated to gain information on the system with of DFA fron firmed this assumption. In one study BYI 02960 was applied on assumption of the system of the s residue levels of BYI 02960, difluoroacetic acid, BYJ 02960-difluotoethyl aming Juranone and 6-CNA were determined in mature fruits of lower and higher plant parts at different time point 3, 7, 5° 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 phloen mobility of this metabolite. Highest DFA levels (approx. 0, 5 mg/kg) were Yound in cucumbers sampled 14 and 21 days after application. BYI 02960-diflueroethyl-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 phloent mobile and can be transported within the plant.

With this information an additional experiment was conducted: Dethyler-14C BYI 02960 was applied on cucumber leaves scenario 1; two single droplets on either de 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 plan 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 radiolaber chosen, it is very likely that metabolite difluoroacetic acid is the phloem mobile compound detected.

The report of these non OLP spidies to summarized in chapter 6.10.

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, Suthers: , , , , , , , , , , , , , , , , , , ,
Title:	[Pyridinylmet Byl-14C] BY 102960: Metabolismen the Laying Hen
Report No &	MEF-11/199 Date: 10: 2012 Meter 10: 2012 Meter 10: 2012 Date: 10: 2012 Meter 10: 2012 Date: 10: 2012 Meter 10: 2012 Date: 10:
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Guidelines:	OECD-Guideline for the Testing of Chemical No. 503, Metabolism in Livestock
	US-EPA, Residue Chemistry OPPT 5860.1390; &
	EL Regulation 110//2009 amended by Directive 96/68/EC, 7030/V1/95/rev.3 Appendix F
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Testing Facility	
and Dates	Germany
Ê,	Germany Germany C Experimentation work: 5.3.2010 – 11.02011

### **Executive Summary**

The metabolism and excretion of pyridiny Imetay 1-14 CBYI 02960 (common name: flupyradifurone) were investigated in laying hensias a model to poultry. Six hens were orally dosed once daily in the morning for 14 consecutive days with an advieous 0.5% Tragacanth® suspension of 1.02 mg/kg body weight which corresponded to 16.98 mg d.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, fiver and excreta were extracted and analysed for parent compound and metabolities.

### 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 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 argnificance of these organs for excretion and metabolism. These values corresponded to 0.05% and 0.08% of the total alose, 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 size 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 ax animals. Eggs, muscle and liver as well as excreta were extracted with mixtures of acetonitrile/water and pure acetonitrile. Ear 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 exciteta was similar to those in the eggs, organs and tissues. Therefore, parent compound and metabolities were isolated from excreta and identified by LC-MS/MS or NMR spectroscopy. The identified metabolities 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 far (approx. 10 - 20%) and amounted to less than 0.017 mg/kg. Major netabolites 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.

The metabolite pattern of the extracts of the current hen study and the hen study with the ¹⁴C-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:

		Ğ			- 5
	Eggs	- An	Q		
	(day 3 to	Muscle	$\mathcal{L}^{O'}$ Fat	Lover	
	13.25)		<u> </u>		L.
TRR [mg/kg]	0.084	0.070	<u>`</u> @ð.021 [™]	0.435	
Sample/Report name	% of %	% of S/kg	So of Spaller	% of marks	ř
BYI 02960-	TRR Dg/kg	TRR	TRR mg/kg	TRR mg/kg	e °
Conventional extraction	96,₽, 0.08₽	92,6 0.064∕	79.7 0.017	74.6 \$324	Ŵ.
lactato-mercaptyl-nicotinic acid	4.0 0.003	3.6 0.002	Å `>> k	15.5 0.068	*
acetyl-cysteinyl-nicotinic acid	چ کی ک	V × ×	۲ ^۲	0.00	
6-CNA	7.2~0.006	8.8 0.00	1 \$ <0.001	<b>4</b> 0,028	
des-difluoroethyl-OH-SA	_&&	≫>1 0£19ľ01	\$.6 <b>0</b> .001	€3.1 ∞0.014	
acetyl-AMCP	23.1 0.0194	40.2 0.028		6.5 0.027	
des-difluoroethyl	8.9 0.007	9,2 0.007	5.0 0.001	1 8 0.008	
AMCP-difluoroethanamin@SA	5.1 20.004	····	29- ~ ~ · ·	<b>3</b> 0.3 0.001	
OH-SA	5.1 0.004	Ž1.8 ŠØ.004	5.0 + 0.001 16.2 + 0.003 5.5 + 0.003	22.5 0.098	
OH S O	18.0 0.015	8.1 0.000	55 0,001	1.5 0.007	
parent compound & &	19.8 0.017	<b>9</b> .8 <b>02</b> 007	15.3 0.003	0.9 0.004	
Identified in conventional extract	86.2 0.072	84.2 0.052	77.9 0.016	58.6 0.255	
Characterised on convention extra	9.9 0.008	8. 0.006	1.8 <0.001	16.0 0.070	
Exhaustive extraction	n.a	or n.a.	🖉 n.a.	19.8 0.086	Ţ
Total characterised	86.2 0.072		77.9 0.016	58.6 0.255	
Total characterised 5 5	9.9 0.008	8.4 0.006	1.8 <0.001	35.8 0.156	
Total extracted	96.1 6,081	Ø2.6 <u>0.064</u>	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 0 2	100,0 0.084	100.0 0.070	100.0 0.021	100.0 0.435	

The main metabolic reactions of [pyridinylmethyl-14] BYI 02960 in the laying hen are:

Hydroxylation in position 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 choro group of BYI 02960-6-CNA with glutathione followed by degradation resulting in the conjugate BYI 02960-acetyl-cysteinyl-nicotinic acid and BYI 02960-lactatomercaptyDnicotinic acid.
- Eleavage of the diflueroethyl 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

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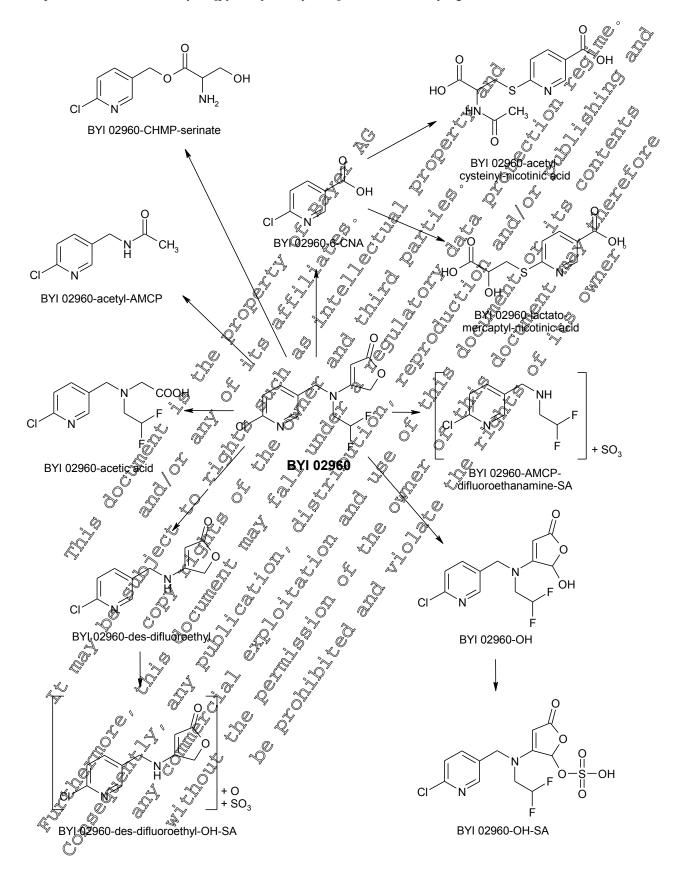
### Tier 2, IIA, Sec. 4, Point 6: Flupyradifurone (BYI 02960)

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- Oxidative degradation of the furanone ring forming BYI 02960-acetic acid •
- Cleavage of the pyridinylmethyl moiety forming an alcohol conjugated with serine (BYI 02960-•

Cleavage of the furance ring and the difluoroethyl group forming an amine followed by the second seco

### Proposed metabolic Pathway of [pyridinylmethyl-¹⁴C]BYI 02960 in laying hens:



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

#### I. Materials and Methods

### A. Materials

A. Materials	ý s					
1. Test Material						
IUPAC Name	4-{[(6-chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino, 4uran-6) 2(5H)-one					
Code name	BY102960					
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	$[pyridinylmethy]_{\mathcal{A}}^{\mathcal{A}}C]$					
Specific radioactivity	4.37 MBq/mg $\approx$ 118.08 μCi/mg (delivered sample before radiodilution) 3.50 MBq/mg = 2.10 x 10° dpm/mg = 94.59 μCi/mg $\approx$ 27.34 Ci/mol° (sample after radiodilution)					
Radiochemical purity	> 99 % by radio-HPLC and 98 % by radio-TLC &					
Nonradioactive test substance	Baten BY 02960 PU-02 2 2 2 2 2					
Chemical purity	99×4% & ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~					
Dose level	14 oral doses of 1.02 mg/kg bw/day by gavage					
Vehicle	0.5 % aqueous Tragacama suspension					
2. Test Animals						

### ) Tost Ani

2. Test Animals 🔬 🖧	
Species	Kaying Ken (Gallus gallus domesticus)
Strain	White Leghorn" N & L
Sex and numbers involved O Age	6 Out of 48 here were selected by maximum egg production
	$(\mathfrak{g} - 8 \mathfrak{m}onths)$ $(\mathfrak{g}' + \mathfrak{g}')$
Body weight Acclimatization Identification	1.63 kg at first administration, 1.60 kg at sacrifice
Acclimatization	35 days 0 2 2 0
Identification	Cage Japelling and whog tags
Housing	Individually in stainless steel metabolism cages for laying hens
	allowing atmost quantitative collection of eggs and excreta (supplier:
Housing A A	proom temperature $2^{\circ}$ - 30°C, relative humidity 30 - 57%. 16 Wight 28 h dark cycle, air change 10 – 15 times per hour
A ° ä	Oroom temperature 22 - 30°C, relative humidity 30 - 57%.
Feed and water	16 Plight Sh dark cycle, air change 10 – 15 times per hour
Feed and water	Hons wefe 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
L'AL	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/nen), tap water,
	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
Feed and water	
× sov	
$\bigcirc$	

### **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 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 copp. The hens received a mean dose of 1.67 mg (3.51 x 10⁸ 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 adioactivity of the actually administered amounts served as reference for the calcalation of total radioactivity in the biological samples. The administration volume was 10 mL 40g body weight. The cose 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 on the phorning before administration) and the number of eggs was recorded for all hers. After sampling, the eggshells were discarded, and the egg white and yolk were weighed and then thoroughly mixed An alrouot sample of each egg mix was taken for the determination of the total radioactivity in triplicate by ESC. The residual amount of the eggonix from all mimals was stored at ca. - 18°C until metabolite analysis.

The excreta of each hen were collected once daily from the collecting this as quantitatively as possible. The individual samples over homogenised after the addition of water, before recording the total weights. Arcaliquot of each fragtion was processed for adioactivity measurement by combustion/LSC. The remaining samples were stored in freezer until metabolite analysis.

### Sacrifice and sampling of organs and ssues

The treated hens were weighed and sacrificed care hours after the last (14th) dosage. The animals were anaesthetised using carbon dioxide, sacrificed by decapitation and exsanguinated. The organs and tissues were prepared in indiately after sacrifice. Lover without the gall bladder, kidneys, leg and breast muscle skin without subcutaneous tat, subcutaneous fat and eggs from the ovary and oviduct were sampled immediately after sacrifice and their from weights were recorded. The gall bladders were purctured for the collection of the bile fuid 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 subchaneous 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.

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/JESC (solid san is a directly by LSC (e.g. combined eggs and extracts). Eggs, muscle, liver and excreta weter extracted 3 times with acetonitrile/water 80:20 (v/v) and finally with pure acetonic le. For eggs@muscleand liver the combined conventional extracts were subjected too clean-up step using an SPE-cartridge. Post extraction solids of liver were exhaustively extracted twice with acetonigale/water (1:5/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 meneptane phase. All stracts were used for quantification of parent compound and metabolites by HPLC

### Radioactivity measurement

<u>Kadioactivity measurement</u> Solid samples were combusted prior to radioactivity determination and the formed  14   14   12   12  absorbed in an alkaline trapping solvent. The determination of radioactivity of liquid samples was conducted by liquid scintillation counting (LSQ) using sub-samples (1 - 3 Geplicates). 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 (LCD) was established at approximately 10 dpm per aliquot after instrument background correction. The limit of quantification (LOQ) was established as 2 times the background adioactivity (dpm) of each instrument/method

### Metabolite analysis

The prepared extracts were subjected to HPL Spring Preversed phase column (C18) and the eluting solvents water/formic acid 99 (v/v) and acetonitrite/wate@formic/acid 97:2:1 (v/v/v) in the gradient mode. Detection was performed by a UV- (234 mm) and a radiosotope 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 radioassaged 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-chromatograph for identification of metabolites.

Metabolites were isplated from the excrete identified by LC-MS/MS or NMR-spectroscopy and used as reference compounds for the identification of metabolites in eggs and liver by HPLC cochromatograph@Other_reference compounds were taken from the goat metabolism study also using the pyridiny wethy C - Levelled 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

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### **II. Results and Discussion**

### A. Recovery and Elimination of Radioactivity

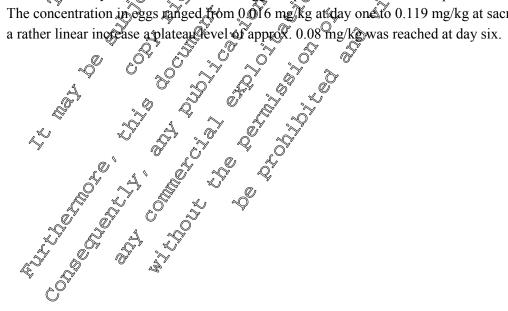
The recovery of radioactivity in laying hens after administration of an average daily dose of 1.02 mg [pyridinylmethyl-¹⁴C]BYI 02960 per kg bw/day on 14 consecutive days is presented in Table 2.2-1. The overall recovery accounted for 96.11% of the totally administered dose. The remaining amount of radioactivity (approx. 4%) was expected to still be present in the gastro-intestinal tract at sacrifice, to the short period between the last administration and sacrifice.

Table 6.2.2-1:	Distribution of residues in eggs oral administration of 14 daily	, muscle, fat, l	iver and kidney	of laying here	following 🐇
	oral administration of 14 daily	doses of [pyric	linylroethyl-14C	BX102960 at	a dose rate
	C1 00 /I				. Š

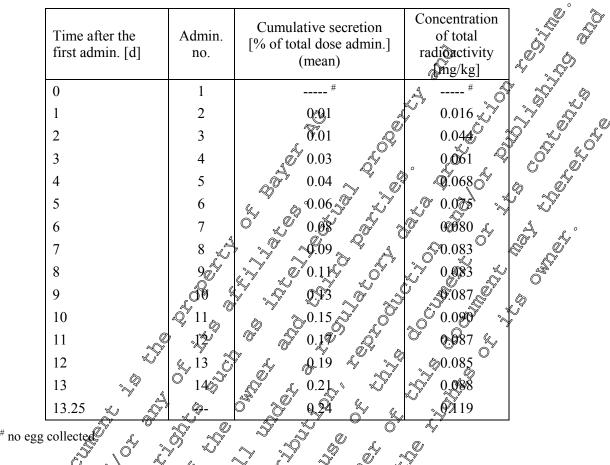
of 1.02 mg/kg			~~~~~	
Sample	Percent of to dose administer	al Control	fadioactivity	
Liver			∞Q435 ≪	
Kidney	<b>0.05</b>	× × č	1.072	ð O
Eggs from ovary/oviduct	0.05 0.05 0.01		0.090	
Muscle, total	0 0 0 9 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	S O	0.070 Č	×
Liver Kidney Eggs from ovary/ovidact Muscle, total	<b>0</b> .02		0435 1.072 0.127 0.070 0.090 0.090	*
Fat, total 🛸 🕵	S & 0.02		0.021	
Organs/tissues, total	0 0.04 0 0.37 0 0.24		, Q, L'	
Foos	0 <u>0</u>		0.080	
LACICIA, IOIUI	0 50.24 © 5 95.59		-Ay	
Excreta total	í 👡 🥵 1 .		Q	
Total Recovery			9	-

### 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



### Table 6.2.2-2:Time course of total radioactivity in eggs following oral administration of 14 daily<br/>doses of [pyridinylmethyl-14C] BYI02960 at a dose rate of 1.02 mg/kg



### 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 detaily 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 digher diactor 1.2) than the levels of the land eggs collected at sacrifice (0.119 mg/kg). This indicated that the egg yolk was not a preferential, the 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 subcutateous 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 2% 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 Miciency 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/x) followed by pure acetonitrile. Additionally, solids of liver were exhaustically 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

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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 much a cost x = 1eggs (based on the 1st extraction), 0.005 mg/kg (7.4%) for muscle, 0.004 mg/kg (20.3%) for fat (based in the second se on the  $2^{nd}$  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 eg	gs, muscle at and l	liver samples f	following ora	N° ^{(°}
	administration of 14 daily	doses of pyridinyln	nethy	/102\$60 at &	dose rate of
	1.02 mg/kg				Â

	1			~~~ .	17
	Eggs (1 st extraction)	Muscle	Fat S (De extraction)		ver 。
TRR [mg/kg]	0%984	0.070	🖇 🕺 🔞 🖓 🕹	[©] 0.4	30
	Wer where	TRAC mg/kg	%of FRR \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	∽% of Ô ∕ TRR	∬ mg/kg
Conventional extraction	<u></u>	92.6 9.064	0.040 0.040	<u>.</u> 7¥4.6	0.324
- acetonitrile/water (8/2, v/v) 🥡	96 0 <del>0</del> 81	\$92.6 0.064	ð <u>,</u> ð	74.6	0.324
- acetonitrile phase			Ø9.7 0.017C		
- n-heptane phase 👸 👔	5 ,5 <del>-</del> , Q	Ø «	n.d. n.d		
Exhaustive extract:	· · 2		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	19.8	0.086
Total extracted	\$26.1 0.081 3.9< 0.003	92.6 0.664	\$ <b>79.7 _}0.017</b>	94.5	0.411
Post-extraction solids (PES)	3.9 0.003	D.4 @.005	20.3 0.004	5.5	0.024
Accountability	100.0 0.084	100.05 0.070	100.0 0.021	100.0	0.435

n.d.: not detected

To demonstrate storage stability, a second extraction and simple 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 Atentification of Parent Compound and Metabolites in Excreta

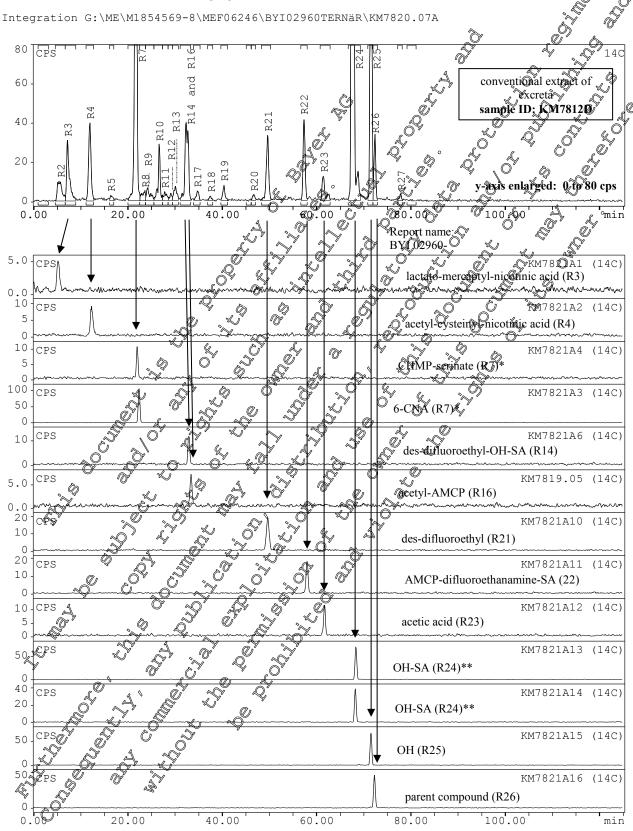
The extract of excreta was used isolate the metabolities using a ternary reversed phase HPLC system. The three eluents employed were:

- A: 1L water + 1.54 mL and nonium hydroxide solution (25%) + 0.8 mL formic acid, adjusted to pH 7
- B: acetonitrile / eluent  $A_{\rm c}(99:1; V/v)$
- C: methanol / tetrahydrofurane  $(1:1 \sqrt{v})$

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 putilication steps. In case of BYI 02960-OH, the position of the hydroxy group was identified by HPLC & 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.

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



* 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

#### 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 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 summaris Table 6.2.2-4. Of the total radioactivity, ca. 86% was identified in eggs \$4% in muscle 78% in fat and 59% in liver. All other residues were characterised by their extraction and chromatographic behaviour.

tissues of laying hens	followi	nooral	admini	stration	$\frac{1}{14}$	Milly dose		
[pyridinylmethyl- ¹⁴ C]							,s or	A
	E SE	70% V		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	A			
	@(day	~3x0 .	[©] Mu	scre,	Ő I	at S	ΓĎ	ver 🔊
	13	25)					Ű	È
TRR [mg/kg]	0.0	)84	<u></u> .0.0	)705	(( ))	02	S 0.4	<b>†</b> 35
Sample/Report name	20 of	mg/kg	So of	O Cha/ka	% of	mg/kg	% of	mg/kg
BYI 02960-	TRR	×		mg/kg	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		TRR	
Conventional extraction	96,₽	0.081	9206	0.064	79,7	0,017	<i>6</i> <b>3</b> 4.6	0.324
lactato-mercaptyl-nicotini acid	<b>\$</b> .0	0003	\$3.6	<b>@</b> .002	« <u>`</u> ،	J*\$	15.5	0.068
acetyl-cysteinyl-nicotinic acid	b (	) Ç	>	/&		- A	0.3	0.001
6-CNA* des-difluoroethyl-OH-SAO	7.9	0.006	858	0,006	198	< 0.001	6.4	0.028
des-difluoroethyl-OH-SAO	≪ <b>j</b> _∧	<b>~</b>	<b>2</b> .1	0.001		<b>@0.001</b>	3.1	0.014
acetyl-AMCP	/23.1 ₀	0.019	40,2	0.028	28. <b>S</b> ⁄		6.3	0.027
des-difluoroethyl	8.9	0.007	2.9	0.007	500	0.001	1.8	0.008
AMCP-duteroroethanamine-SA	~	°>	<u>"0"</u>	Ø ~	@		0.3	0.001
OH-SA	5.1	0.00		0.00 ⁰	16.2	0.003	22.5	0.098
OH ' O' C	18:00	0,005	8.1	0.006	5.5	0.001	1.5	0.007
parent compound	<b>19.8</b>	0017		0.007	15.3	0.003	0.9	0.004
identified in the conventional extract	86.2×	×0.073 مرجع	* 84. <b>2</b> 5	^{\$} 0.059	77.9	0.016	58.6	0.255
characterised in the conventional extract	.9 <u>.9</u>	<b>0</b> 5008	Ø8.4	0.006	1.8	< 0.001	16.0	0.070
Exhaustive extraction	'n	.a. 🔊	n.	a.	r	ı.a.	19.8	0.086
Total characterized	86.2	<b>Ø</b> 072	84.2	0.059	77.9	0.016	58.6	0.255
Total characterized	¢ 9.9_0	0.008	8.4	0.006	1.8	< 0.001	35.8	0.156
Total extract of the second se	96,1	0.081	92.6	0.064	79.7	0.017	94.5	0.411
Solids	~9.9	0.003	7.4	0.005	20.3	0.004	5.5	0.024
Accountability C	100.0	0.084	100.0	0.070	100.0	0.021	100.0	0.435

Table 6.2.2-4: Radioactive residues of parent compound and metabolities in eggs and edible organs 

### Metabolites in Eggs

Besides the parent compound (0.017 mg/kg; 19.8%), major metabolites in eggs were BYI 02960acetyl-AMCP (0.019 mg/kg; 23.1%) and BYI 02960-OH (0.015 mg/kg, 18.0%). Further metabolites

covelution with BY1 02960-CHMP-serinate was excluded for eggs, muscle and liver

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

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  $\leq$  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 BYI02960-OH-SA (0.003 mg/kg, 16.2%). All other metabolites were detected in amounts <0001 fmg/kg.

### Metabolites in Liver

The majority of the radioactive resulue (approx, 75%) was expected with actionitrie/watey. The main metabolites in this extract were BYI 02960-OH-SA (0.098 mg/kg, 02.5%), BYI 029604actatomercaptyl-nicotinic acid (0.068 mg/kg, 15.5%), BYI 02960-6-CNA (0.028 mg/kg, 6.4%) and BYI 02960-acetyl-AMCP (0.022 mg/kg, 6.3%). The parent compound was only detected in negligible amounts of 0.004 mg/kg. All other metabolites amounted to  $\leq 0.014$  mg/kg, except one unknown metabolite (0.027 mg/kg).

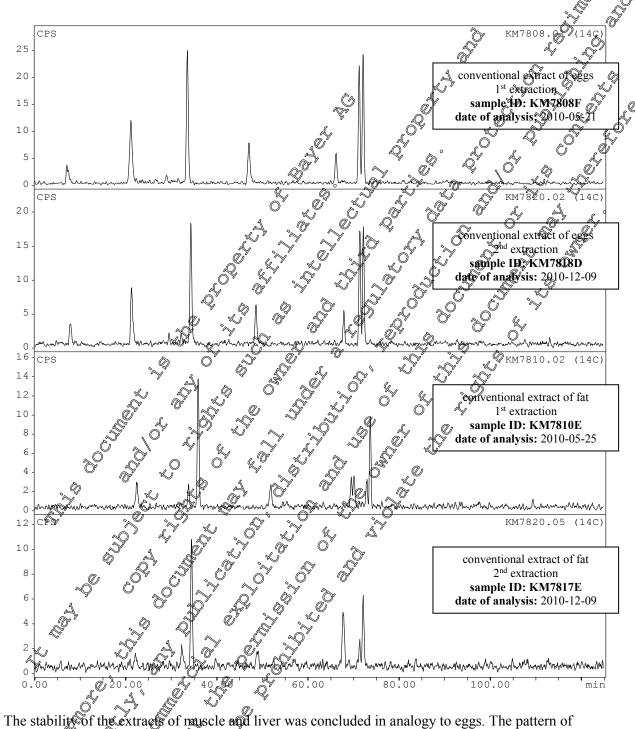
Metabolites in the exhaustive extract of liver ranged from 0.002 to 0.027 mg/kg. They were not identical with the metabolites in the accetorufrile/water extract. All unknown metabolites in the exhaustive extract of liver were not further investigated, due to their low amount and the high concentration 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).

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 metabolities (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.
- 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 BY102960 hydroxy, BX102960 OH-SA, BXF02960 desdifluoroethyl, BYI 02960 des-difluoroethyl OH-SA and BYI 02960 are tic active. They were detected in approximately the same low amounts as in the study with the furanone abel.
- The cleavage of the molecule was a significant reaction in the metabolism. A prominent portion of label specific metabolities was detected in the edible tissues. Major label specific metabolites were BYI 02960-6-CNA and BYT 02960-acetyl-AMOP.
- The main metabolic reactions of the bying hen are
  - Hydroxylation in position 5 of the furance ring forming BVF 92960-OH followed by conjugation with sulfur acid to BYL 2960 OH-SAS
  - Oxidative cleavage of the pyridiny wethy bridge forming BYI 02960-6-CNA
  - Substitution of the chloro group of BYI 02960 CNA with glutathione followed by
  - degradation resulting in two conjugates BYI 02960-acetyl-cysteinyl-nicotinic acid and BYI 02960-lactato-mercapty nicothnic acid
  - Cleavage of the diffuoroetbyl group forming BOI 02960-des-difluoroethyl followed by hydroxylation and conjugation with softuric acid to BYI 02960-des-difluoroethyl-OH-SA
  - Cleavage of the furatione ring and conjugation with sulfonic acid forming BYI 02960-AMCR difluctoethanamine SA
  - Oxidative degradation of the furanon oring forming BYI 02960-acetic acid
  - Cleavage of the pyridinylmenhyl-budge forming an alcohol conjugated with serine (BYI 02960-CHMP-seripate)
  - Cleavage of the furance 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 §?

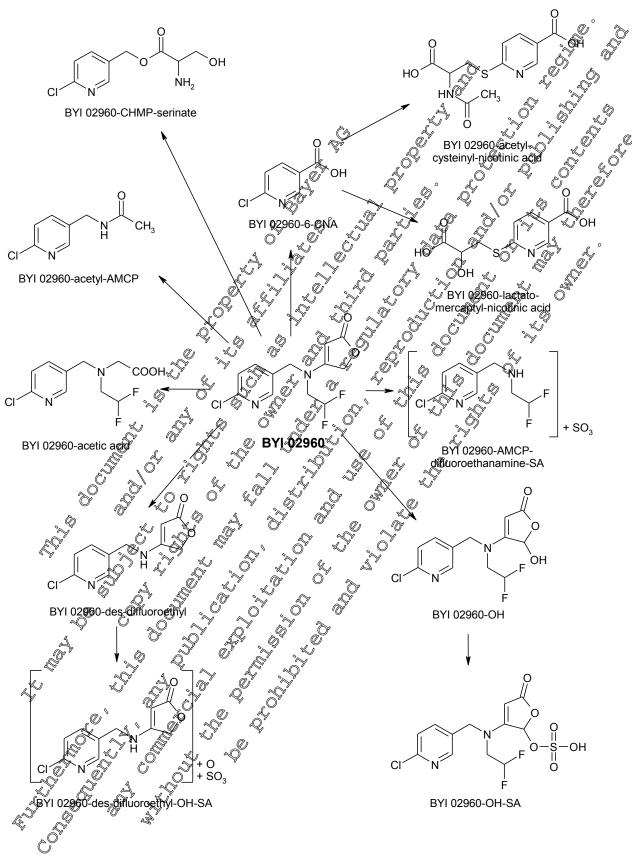


Figure 6.2.2-3: Proposed metabolic pathway of [pyridinylmethyl-14C] BYI02960 in laying hens

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

Report:	KIIA 6.2.2/02, Authors: , , , , , , , , , , , , , , , , , , ,
Title:	[Furanone-4- ¹⁴ C]BYI02960: Metabolism in the Laying Hen
Report No &	
Document No	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, 7930/VI/95/rev. Appendix F
GLP	Yes, according to Japan MAFF GLP standard 11 Nousan (283;
	US EPA – FIFRA GLP (40CFR Part 160);
	Principles of GLP, German Chemical Qaw, current version of Annex D
Testing Facility	
and Dates	Germany of the second s
	Experimental work: 12.2.2010 9.12.2000

### **Executive Summary**

The metabolism and excretion of [furgeone/4^{4/4}C]BYI 02960 (common name: fupyradifurone) were investigated in laying hens as a model for poultry. Six hens were orallo dosed once daily in the morning for 14 consecutive days with an aqueous 0.5% Tragscanth, suspension of 1.05 mg of the active substance per kg body weight which corresponded to 17.12 mg as /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 arsacrifice in the dissected organs and tissues (muscle, fat, liver, kidney, skin and eggs from ovary/oviduco). Eggs, muscle, fat liver and excreta were extracted and analysed for parent compound and netabolites.

### Recovery and Elimination of Radioactivity

The overall recover or ate 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. The 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 desidues 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 (05%). 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 kichey (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

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

(0.257 mg/kg) and muscle (0.183 mg/kg). The residue level of the total muscle corresponded to about 0.50% of the total dose assuming a value of 40% of the body weight for this tissue. Assuming values of 12% and 4% of the body weight for fat and skin, the residues in both tissues accounted for about 0.41% of the total dose.

#### 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 extracted with acetonitrile/water followed by acetonitrile/water/formic acid using microwave assistance. The extraction efficiencies were above 81% of the fotal racioctivity 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 accionitril 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 furatione label and were caused by the cleavage and subsequent total degradation of the furatione ring to smaller carbon units (C-1- or C-2-fragments), entering the carbon pool of endogenous compounds. Apart of these fragments was probably also converted to  ${}^{14}CO_2$  as can be suggested from the lower overall recovery if compared with the study using the metabolicarly stable pyridinylmethyl label.

Residues in the acetonit/ile/water- and exhapstive extracts were analysed by HPLC with radiometric detection. The metabolite patterns of the current ben study and the corresponding extracts of the hen study with the pyridinylmethyl-label were comparable, except the label specific metabolites. Parent compound, BY 202960-OH, BYI 02960-OH SA, BYI 02960-des-difluoroethyl, BYI 02960-des-difluoroethyl OH-SA were dentified 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 62.2/01]. 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.

sb The identification rate, including the parent compound, metabolites and results identified as fails acids after saponification, accounted for approx. 50% for fage day 2 to 7.63% for egaday A for sarefice, 17% for muscle, 96% for fat and 59% for liver. All other results as well as the fases during the concentration procedures were characterised by their extraction- or chromatographic behaviour. The identification rate, including the parent compound, metabolites and residues identified as faily acids after saponification, accounted for approx. 59% for cogs day 2 to 7.63% for the sacrifice, 17% for muscle 0.00% for the sacrifice of the

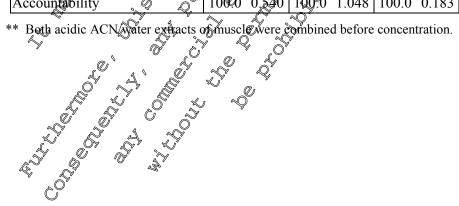
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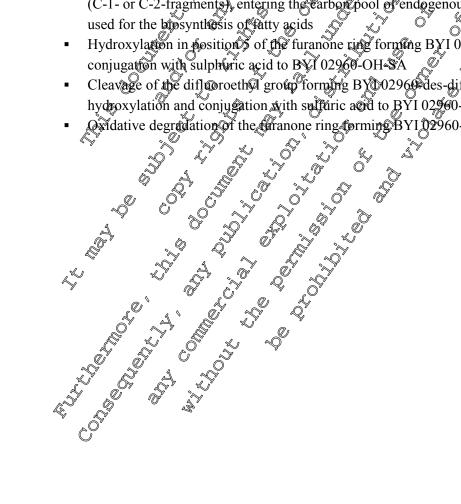
The concentration of the total radioactivity (expressed as mg a.s. equiv. /kg) as well as the distribution of the parent compound and metabolites and the identification rates in all edible samples are summarised in the following table: Ø

<b>C</b> .									
Eggs (day 2 to 7)		Eggs (day 8 to 13.25)		Muscle		Fat		Lived	
0.540		1.048		0.183		0.427 0		<i>्रेथ्र.</i> 1	78
% of TRR	mg/kg	% of TRR	ŵ nyg/kg	% of TRR	ung/kg	% of TRR	mg/kg	≪‰of ♀TRR≪	mg/kg
65.0	0.351	66.8	0.701	38.6	0.071	980	0.42	720	1.571
52.0	0.281	58.3	0.611	8.Í	<b>0</b> 015	\$5.9	<b>0</b> .410	51.5 ~	ź.121
0.1	0.00 🌾	n.d 🎗	) n.d.	0.5	0.000		, °~	0.2	0.004
1.2	0.006	0K.6		296	0005	107	Å	0.8	0.017
0.6	0,003	≫0.5 ू	0.005	NY R	y (	§ 4		5.1	0.112
		[♥] 1.6©		, S		Ş		0.8	0.018
23%	~ V	<u>1</u> 6	0.016			S.	Ű	0.5	0.010
\$8.5	0.316	62.5°			ð	. 0		58.9	1.282
6.4	0.035	4.3	0.045	22.0	\$ <b>0</b> \$ <b>0</b> 40	ي 2.6	0.011	13.2	0.288
		0				~~n.d.		n.d.	
	<i>(</i> )	S.	J.	0′	õ i	Ç,			
∲¥7.0 <u>~</u>	0.038	~ ¥	V,	8.L	0.015	n.	.a.	9.6	0.209
8,3	0.045	10.0		3 <b>9</b> 5		n.	.a.	12.1	0.264
Ø.1	Q.006	5 ³ .3	(> (	see 🖉	see ** n.a.		.a.	n.a.	
16.5	0.08	16.9	0.170	47.9	0.087	n.	.a.	21.7	0.473
58.5	<b>Q</b> 316	2.5	0.656	≫6.5	0.030	95.9	0.410	58.9	1.282
\$22.9≈	y0.124	21.2	0.223	[®] 69.7	0.128	2.6	0.011	34.9	0.761
815	0.440	83,8	0.898	86.2	0.158	98.5	0.421	93.8	2.044
×17.5	<u>0</u> 9094	<b>4</b> .7	0.155	10.3	0.019	n.d.	n.d.	4.5	0.098
1.1	0.00¢	1.5	0.016	3.5	0.006	1.5	0.006	1.7	0.036
1 AR	0.540	100.0							
	(day) 0.: % of TRR 65.0 52.0 0.1 1.2 0.6 2.30 2.30 58.5 7.0, 8,3 Ф.1 16.5 72.9 81.5 72.9 81.5 72.9	Eggs (day 2 to 7) 0.540 % of TRR mg/kg 65.0 0.351 52.0 0.281 0.1 0.00% 1.2 0.006 0.6 0.003 2.3 0.013 2.3 0.045 2.3 0.045 2.5 0.316	Eggs (day 2 to 7)Eggs (day 13. $0.540$ $1.0$ % of TRRmg/kg mg/kgTRR $65.0$ $0.351$ $66.8$ $52.0$ $0.281$ $58.3$ $0.1$ $0.004$ $n.d$ $1.2$ $0.006$ $9.6$ $0.6$ $0.003$ $8.5$ $2.3$ $0.013$ $1.6$ $2.3$ $0.013$ $1.6$ $2.3$ $0.013$ $1.6$ $2.3$ $0.013$ $1.6$ $2.3$ $0.013$ $1.6$ $2.3$ $0.013$ $1.6$ $2.3$ $0.013$ $1.6$ $2.3$ $0.013$ $1.6$ $2.3$ $0.013$ $1.6$ $2.3$ $0.013$ $1.6$ $2.3$ $0.013$ $1.6$ $2.3$ $0.013$ $1.6$ $2.3$ $0.013$ $1.6$ $2.3$ $0.013$ $1.6$ $2.3$ $0.013$ $1.6$ $2.3$ $0.013$ $1.6$ $2.3$ $0.013$ $1.6$ $2.3$ $0.013$ $1.6$ $2.3$ $0.013$ $1.6$ $2.3$ $0.038$ $3.6$ $3.3$ $0.045$ $10.9$ $7.0$ $0.038$ $3.6$ $8.3$ $0.045$ $10.9$ $5.8.5$ $0.316$ $62.5$ $22.9$ $0.124$ $21.2$ $815$ $0.440$ $82.8$ $4.7.5$ $0.994$ $4.7$	Eggs (day 2 to 7)Eggs (day 8 to 13.25) $0.540$ $1.048$ % of TRRmg/kg% of TRRmg/kg $65.0$ $0.351$ $66.8$ $0.701$ $52.0$ $0.281$ $52.0$ $0.281$ $52.0$ $0.281$ $52.0$ $0.281$ $52.0$ $0.281$ $52.0$ $0.281$ $52.0$ $0.281$ $52.0$ $0.281$ $52.0$ $0.281$ $52.0$ $0.281$ $52.0$ $0.281$ $52.0$ $0.281$ $52.0$ $0.281$ $52.0$ $0.281$ $52.0$ $0.281$ $52.0$ $0.281$ $52.0$ $0.281$ $52.0$ $0.281$ $52.0$ $0.281$ $52.0$ $0.281$ $52.0$ $0.281$ $52.0$ $0.281$ $52.0$ $0.281$ $52.0$ $0.281$ $52.0$ $0.005$ $6.4$ $0.003$ $62.5$ $0.656$ $6.4$ $0.035$ $4.3$ $0.045$ $10.7$ $0.038$ $3.6$ $0.038$ $8.3$ $0.045$ $10.7$ $0.038$ $3.6$ $0.038$ $8.3$ $0.440$ $8.3$ $0.656$ $22.9$ $0.124$ $21.2$ $0.223$ $81.5$ $0.916$ $52.5$ $0.656$ $52.9$ $0.124$ $52.5$ $0.656$ $52.9$ $0.124$ $52.9$ $0.235$ <td>Eggs (day 2 to 7)Eggs (day 8 to 13.25)Mu0.5401.0480.1% of TRRmg/kg% of TRR% of 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$52.0$ 0.281 $$8.3$ 0.611 $8.1$ $9015$ $$5.9$ $0.421$ $52.0$ 0.281 $$8.3$ 0.611 $8.1$ $9005$ $$6.0004$ $1.2$ 0.0060.60.0072.60.004 $$7.9$ $2.3$ 0.0131.60.0162.40.009 $2.3$ 0.0131.60.0162.90.905 $6.4$ 0.935 $4.3$ 0.04522.00.004 $2.3$ 0.0131.60.01522.00.009 $2.3$ 0.0131.60.0162.90.905 $6.4$ 0.935 $4.3$ 0.04522.00.004 $2.6$ 0.0131.60.11210.30.019 $7.0$ 0.0383.60.0388.10.015n.a. $8.3$ 0.04610.60.10747.70.087n.a. $58.5$ 0.31662.50.6566.50.03095.90.410 $2.9$ 0.12421.20.22369.70.1282.60.011 $8.6$ 0.44082.80.87886.20.15898.50.421<	Eggs (day 2 to 7)Eggs (13.25)MuscleFatLip (13.25) $0.540$ $1.048$ $0.183$ $0.427$ $2.1$ % of TRRmg/kg% of TRR% of mg/kg% of TRR% of mg/kg% of TRR $65.0$ $0.351$ $66.8$ $0.701$ $38.6$ $0.071$ $98.5$ $0.427$ $2.1$ $65.0$ $0.351$ $66.8$ $0.701$ $38.6$ $0.071$ $98.5$ $0.421$ $720^{\circ}$ $52.0$ $0.281$ $58.3$ $0.611$ $8.1$ $0.015$ $95.9$ $0.410$ $51.5$ $0.1$ $0.006$ $0.007$ $26$ $0.007$ $26$ $0.002$ $0.2^{\circ}$ $1.2$ $0.006$ $96.0065$ $0.007$ $26$ $0.007$ 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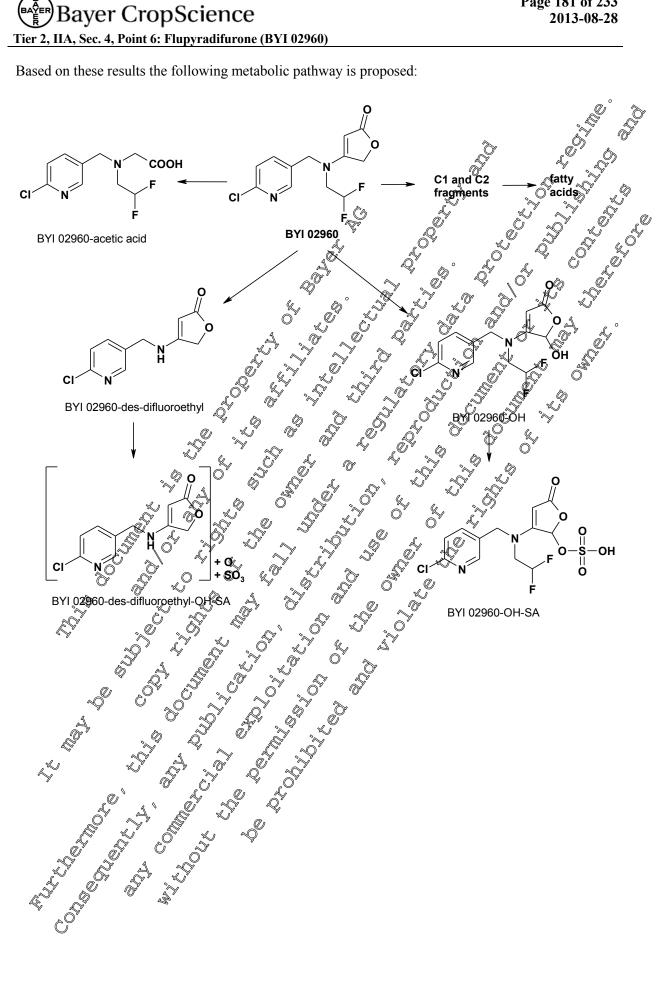


The metabolic and excretion behaviour of [furanone-4-¹⁴C]BYI 02960 in the laying hen can be characterised by the following observations:

- 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 mained 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-fragment 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
- The relatively high values in liver and kidney at sacrifice 6 hours after the fast 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 tissoes. Unextracted residues were quite low and amounted to  $\leq 0.036 \text{ mg/kg}$ .
- Parent compound and non label specific metabolifes, such as BOI 02960-OH BYI 02960-OH-SA, • BYI 02960-des-difluoroethyl and BYI 02960-des-difluoroethyl-OHSA were identified a similar amounts as in the study using the pyridinylmethyl abel. Additionally, By 02960-acetic acid was detected in excreta.
- The main metabolic reactions of [furapone-4-14C]BYI 02960 in the Paying hen are
  - Cleavage and subsequent total degradation of the furanone ting forming smaller carbon units (C-1- or C-2-fragments), entering the earbour pool of endogenous compounds and then being used for the bosynthesis of fatty acids
  - Hydroxylation in position of the furanone ring forming BYI 02960-OH followed by
  - Cleavage of the difloroetho group forming By 002960 des-difluoroethyl followed by hydroxylation and conjugation with suffaric and to BYI 02960-des-difluoroethyl-OH-SA
  - Qxidative degradation of the thranone ring torming BYI 02960-acetic acid



Bayer CropScience



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

### I. Materials and Methods

## A. Materials

A. Materials	L'and the second
1. Test Material	
IUPAC Name	4-{[(6-chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino}furan-
	4-{[(6-chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino}f@ran- 2(5H)-one
Code name	BY102960
Common name	flupyradifurone (proposed ISO)
Empirical formula	$C_{12}H_{11}CIF_2N_2O_2$
Molar mass	288.68 g/mol 2
Labelling position	[furanone-4- ¹⁴ C]
Specific radioactivity	4.24 MBq/mg = 114.50 $\mu$ Ci/mg (delivered sample) before
	radiodilution and a second s
	$3.50 \text{ MBq/mg} = 2.10 \text{ x} 40^8 \text{ dpm/mg} = 94.59 \text{ µCi/mg}^2 27.3 \text{ Ci/mg}^3$
	(sample after radiodilation) a fraction of the second seco
Radiochemical purity	> 98% by radio-HPLC and > 99% by radio-TLC
Nonradioactive test substance	Batch BY 02960-PU-02 ~ ~ ~ ~ ~
Chemical purity	99.4% & & & & & & & & & & & & & & & & & & &
Dose level	14 oral doses of 1.05 mg4g bw day by gavage
Vehicle	(\$5% accession) = %
29 4	
2. Test Animals 🔬 🖉	
Species	Laying Ben (Gallus gallus domesticus)

,	
Species	Raying Ben (Gallus gallus domesticus)
Strain	"White Leghorn" S
Breeding facility	
Sex and numbers in Wived	6 out of 18 hears were selected by maximum egg production
Age	6 – Semonths
Age Body weight Acclimatization	1.56 kg at first administration, 5.56 kg at sacrifice
Identification	Cage labeling and wing tags
Housing , O', O'	Individually in stainless steel metabolism cages for laying hens
	allowing almost quantitative collection of eggs and excreta (supplier:
	Individually in stainless steel metabolism cages for laying hens allowing almost quantitative collection of eggs and excreta (supplier:
× × A	goom tomperature 21 - 26°C, relative humidity 39 - 56%.
	<ul> <li>From temperature 21 - 26°C, relative humidity 39 - 56%.</li> <li>16 h light / Sr dark cycle, air change 10 – 15 times per hour</li> <li>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</li> <li>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</li> </ul>
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 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 dos

The oral administration was carried out with a knob cannula attached to avglass syringe Immediately after dosage, the swallowing was supported by a gentle massage of the throat towards the copp. The hens received a mean dose of 1.63 mg (3.42 x 10⁸ 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 adioactivity of the actually administered amounts served as reference for the calcalation of total radioactivity in the biological samples. The administration volume was 10 mL 40g 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 on the phorning before administration) and the number of eggs was recorded for all hers. After sampling, the eggshells were discarded, and the egg white and yolk were weighed and then thoroughly mixed An alrouot 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 anomals was stored at ca. - 18°C until metabolite analysis.

The excreta of each hen were collected once daily from the collecting this as quantitatively as possible. The individual samples were homogenised after the addition of water, before recording the total weights. Arcaliquot of each fragtion was processed for radioactivity measurement by combustion/LSC. The remaining samples were stored in freezer until metabolite analysis.

## Sacrifice and sampling of organs and ssues

The treated hens were weighed and sacrificed carb hours after the last (14th) dosage. The animals were anaesthetised using carbon dioxide, sacrificed by decapitation and exsanguinated. The organs and tissues were prepared in indiately after sacrifice. Lover 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 besh worghts 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 suboutaneous 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.

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 deg 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 A.SC (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;  $\langle N \rangle$ ). And aliquot of the excreta of day 13 was extracted with acetonitrile/water (8:2, v/v) follower by pure acetonitrile. All extracts were used for quantification of parent compound and metabolites by HPKC.

## Radioactivity measurement

Solid samples were combusted prior to radioactivity determination and the formed 4CO₂ absorbed in an alkaline trapping solvent. The determination of radioactivity of louid 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 ubtracted. For all samples, the limit of detection LOD kwas established at approximately 40 dpm per about 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, hauscle, fat and liver, were concentrated and analysed by thin layer chromatography on sitica geoplates and raciolumprography for setection 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 rarioactivity from the HPLCocolumn and interferences during detection due to chemiluminescence. Therefore a washing step of the HRC 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 (= 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 ppmvalue, The completeness of the chromatographic dution for the adapted HPLC profiling method (including the washing step) was shown for expracts 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 musele 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 exhausticely extracted twice with acetonitrile/water (1:1; v/v) followed by 2 extractions with acetoutirile/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-

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

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/wat 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 S comparison of the TLC profiles (KIIA 5.1.2/01; KIIA 6.2.3/02). Radioactive residues in the n-heptine phases were analysed by TLC and visualised in an iodine vapour chamber Residues in the n-heprane phase of fat were further identified by investigation of the partition behaviour after seponification and subsequent acidification. 

## **II. Results and Discussion**

## A. Recovery and Elimination of Radioactivity

The recovery of radioactivity in laying heng after administration of an average daily dose of 1005 mg/ [furanone-4-14C]BYI 02960 per kg bw/day on 14 consecutive days is presened 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 gastre-intestinal tractat sacrifice, due to the short period between the last administration and sacrifice. Another part is probably exhaled as *4CO2. The formation of carbon dioxide was also shown in a rat quantitative whole-body autoradiography study using the same labelling position (KIIA 5. 2/02).

#### Distribution of residues in eggs muscle fat, liver and kidney of laying hens following oral Table 6.2.2-5: administration of 14 daily doses of fur anone/4-14 CDBY102960 at a dose rate of 1.05 mg/kg

	- A
Sample Sample does administered	Concentration of total radioactivity [mg/kg]
$\overline{\mathcal{L}}$ iver $\overline{\mathcal{L}}$	2.178
	1.083
Eggs from ovary/ovidact	2.774
$\mathcal{O}$ uscle, total $\mathcal{O}$ $\mathcal{O}$ $\mathcal{O}$ $\mathcal{O}$ $\mathcal{O}$ $0.50$	0.183
Skin total 20 2007	0.257
Muscle, total Skin total Fat, total Orgentiation total	0.427
$\begin{array}{c c} & & & & & & \\ \hline & & & & & \\ \hline & & & & \\ \hline & & & &$	
Organs/tissues, total Eggs 2 2.35 Excreta total 7 78.01	0.757
Excreta total 78.01	

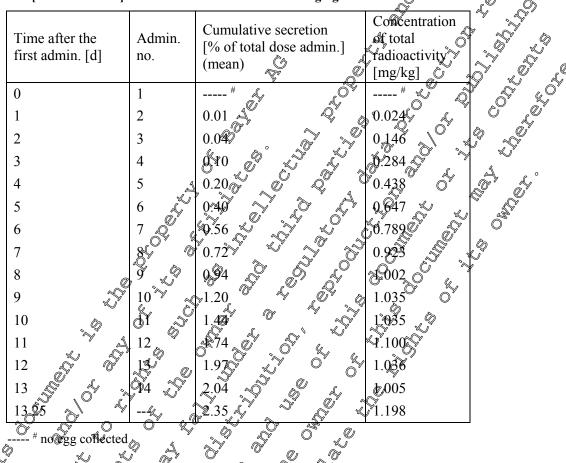
An average appoint of 2.35% of the total dose was measured in the eggs. At sacrifice, the residues in the organs and tissues were calculated mestimated to be about 1.8% of the total dose from which about 28% was detected in the skeletal muscle (0.5%). Until sacrifice, the excreted radioactivity accounted for \$8.01% of the total dose.

## B. Levels and Time Course of Total Radioactivity in Eggs

, A

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 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



## 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 aniministered, 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), thin (0.257 mg/kg) and musele (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

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

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 (1.6%) 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 (sa. 52 and 58%), fat (ca. 96%) and liver (ca. 52%). The residues in the n-heptane phase of muscle amounted to only a. 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 at 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 and 10% of the total radioactivity was detected in the neutral extracts and between and 40% in the acidic extracts of eggs, muscle and liver Cosses of radioactivity during the extraction procedure of eggs, muscle and liver were between 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 bomogenizer, filter etc.). A summary of the extraction efficiency of the samples is presented in Table 6.207.

probably, the losses of radioactivity were caused by adhesion of radioactive fat a mon-polar matrix or the equipment (e.g. Polytron to mogenizer, filter etc.). A summary of the extraction efficiency of the samples is presented in Table 6.207.

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

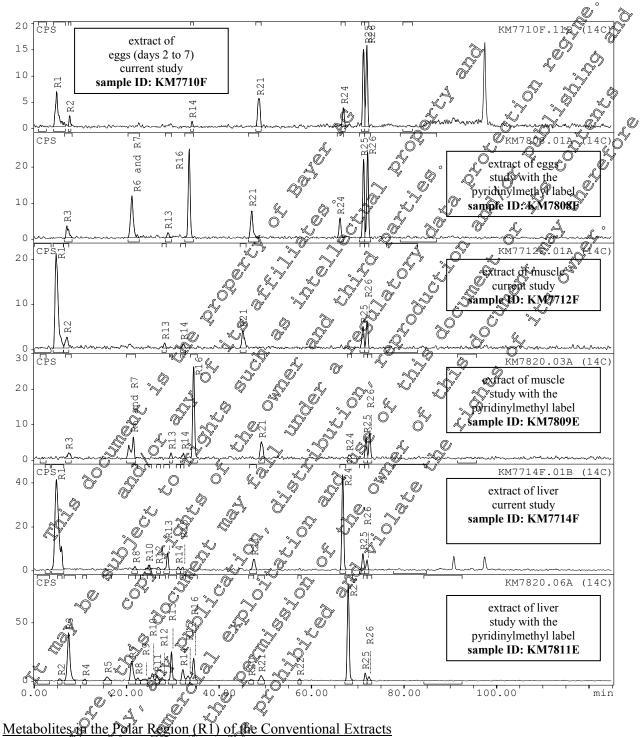
1.05 mg/kg	i uuiij e		JI		0] D	10290	o ur u u		01	v° >>
	Egg (days 2		(days	ggs s 8 to 25)	Mus	cle	°Fa	t	Pive .	
TRR [mg/kg]	0.54	0	1.0	)48	0.13	83 🔬	0.42	27 🔊	207	'8 Ø
	% of TRR ⁿ	ng/kg	% of TRR	m¢kg	% of TRR	mg⁄kg	% of TRR	ng kg	‱f JRR∡	mg∕k Øgℓ
Conventional extraction	65.0 0	0.351	66.85	0.701	38.6Ô	0.071	98.5	0.425	72.1	1.571 <b>0</b> ″
- n-heptane phase		0.281	583	0.611		0.01.5	95,9	0,410	5 ÊS	1,121
- ACN/water extract	13.0 0	0.070	8.5	0.089	<b>`3</b> 0.4 .	Ø.056		~ ~	20.7	¥.450
- subregion 1		0.046	64	° 0.05	27:4	0.050		.a. 📎	19:6	0.426
- subregion 2	2.7	0.015	3,6	0038	nod.	æd.	°б [°] п	.aly	A,1	0.024
- losses during the conventional extraction	10.7	ð.058	10.7°	~0.112 >	10.3	0.019	n.d.	n.d. 4	n.d.	n.d.
Exhaustive extraction	R	a s			×,	.Ö	Ű	ŝ	0	
- combined neutral	₹¢7.0 4	0.038	₹≫3.6	[%] Ø.038	8.1	0.015	n.a.	\$9.6×	0.209	
ACN/water extract	\$ _Q			) S		, ²	C)	, "Y	Ĩ	
- 1 st acidic ACN/water extract		0.045		**	39,5	0.072	ida.	2.1	0.264	
- 2 nd acidic ACN/water extract		9.006	<b>€</b> 3.3	0.035	See**	∼n.a.	©n.a.	<u> </u>		
- losses during the exhaustive extraction	6.8	0.03	4.1	0.043	n.đ.)	nat	n.z	4.5	0.098	
Total extracted	81.5	0.440	\$83.8	ר.878	\$6.2	0.158	98.5	0.421	93.8	2.044
Total losses	17,3	0.094	14	0.155	/ 10.3	0.019	n.d.	n.d.	4.5	0.098
Solids	01.1 <u>k</u>	9.006	×1.5	@.016	3.5	0.006	1.5	0.006	1.7	0.036
Accountability 0	100_0	0.540	100	1.048	100%0	0.183	100.0	0.427	100.0	2.178

** Both acidic ACN/water extracts of mascle were combined before concentration.

## E. Quantification Identification and Charagerisation 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 (KHA 6.22/01) using the HPLC system described there. Additionally, BYI 02960-des offluoroethyl was identified in the extract of muscle by HPLC co-chromatography with the ratiolabelled 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.

Figure 6.2.2-4: Comparison of HPLC profiles of the acetonitrile/water extracts from eggs, musle and liver of the current study and the study with the pyridinylmethyl ¹⁴C-label



Metabolites in the polar region **R**1 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

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

dig of 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  $n_{s}$ 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 tatty 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 furance laber and were canced by the cleavage and subsequent degradation of the furanone ring to smaller carbon mits

Distribution of Parent Compound and Metabolites in Eggs, Organs and Tissues The distribution of the parent compound and metabolites in Grace 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 The distribution of the parent compound and metabolites in toggs, argans and tisbles is commarised in Table 6.2.2-8. The identification rate was \$9% in eggs (days 2 to //), 63% in eggs (days 8 to sacrifice), 17% in muscle, 96% in fat and 59% in lister. All other residues were characterised by their extraction or chromatographic behaviour 4 and 50% in lister. All other residues were characterised by their extraction or chromatographic behaviour 4 and 50% in lister. All other residues were characterised by their extraction or chromatographic behaviour 4 and 50% in lister. All other residues were characterised by their extraction or chromatographic behaviour 4 and 50% in lister. All other residues were characterised by their extraction or chromatographic behaviour 4 and 50% in the first fir Table 6.2.2-8. The identification rate was 5% in eggs (days 2 to 7), 63% in eggs (days 8 to sacrifice),

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Table 6.2.2-8:Radioactive residues in eggs, muscle, fat and liver samples following oral<br/>administration of 14 daily doses of [furanone-4-14C] BYI02960 at a dose rate of<br/>1 05 mg/kg

1.05 mg/kg										V 🏷
		ggs 2 to 7)	(day	ggs 8 to 25)	Mu	scle	F F	at	Li	ça Vet
TRR [mg/kg]	0.5	540	1.0	)48	0.1	183 🔬	0.4	27 🖉	Į.	78 🔊
Sample/Report name BYI 02960-	% of TRR	mg/kg	% of TRR	mĝøkg	% of TRR	mg/kg		mág/kg	% of TRR	rog/kg
Conventional extraction	65.0	0.351	66.8 ₁	0.701	38.60	0.071	98.5	0.424	72.4	1.570
n-heptane phase (identified as fatty acids)	52.0	0.281	583	0.611	8.9	0æ915	\$ ^{5.9}	0410	5¥.5	121
des-difluoroethyl-OH-SA	0.1	0.001	n.d.	• n.d.	0.5	×0.001	r õ	, ×	0.2	0.004
des-difluoroethyl	1.2	0.000	n.a. 0,€	0.067	2,6/	0,005	Ĩ	al.	Q.8	0.017
OH-SA	0.6	0_003	Ø:5	02005	'n₽¢d.	n.d.	¢.	0 ^y	\$.1	9.112
ОН		0.013	° [%] 1.6 [™]	0.016		0.004	) ×	/ _% ,	0.8	0.018
Parent compound	2.2	0.043	1.6	0.016	20	0.005	Ő		0.5	0.010
identified in the conventional extract	5 ^{8.5}	09316	° 62.5	0.656	¥6.5	0.095	<u>م</u> 95.9 ک	§0.4104	\$ 58.9	1.282
characterised in the conventional extract	6.4	0.035	4.9	0.045	220	0,040	2.6	0.011	13.2	0.288
losses during the conventional extraction	010.7¢	0.058°	10.7	0.112	10.3	0.040	×10.	d.	n.	d.
Exhaustive extraction		0	ð	°∼ N	×	<b>W</b>				
- neutral ACN/wat@extract	7.0	Ø.038 ·	<u>)</u> 3.6	0.038	, 8.1	Ø.015 [*]	∛n.	a.	9.6	0.209
- 1st acidic ACN Sater Stract	8.3	0.045	10.0	0.105	39,51	0.072	n.	a.	12.1	0.264
- 2 nd acidic ACN/water extract	£.1	0,006	33	Q.035	See	***	n.	a.	n.	a.
characterised in the state of t	¢16.5,₄	0.089	\$16.9	\$0.177°	47.7	© 0.087	n.	a.	21.7	0.473
Total identified	585	0.316	62.5	0,656	6.5	0.030	95.9	0.410	58.9	1.282
Total identified	<b>%2</b> 2.9	Ø.124	¢\$1.2%	0.223	69.7	0.128	2.6	0.011	34.9	0.761
Total extracted &	81.5	⁹ 0.449	83.8	0.878	86.2	0.158	98.5	0.421	93.8	2.044
Total losses	180.5 ≫	0,094	164.7	0 <b>9</b> 755	10.3	0.019	n.d.	n.d.	4.5	0.098
Solids 🔬 🔴 🔬	1.14	0.006	1.50	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.

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## Metabolites in Legs

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.%) was detected in the n-heptane phase and identified as natural fats. The parent compound, BY5 02960-OH, FYI 02960-OH-SA, BYI 02960-des-difluoroethyl and BYI 02960-des-difluoroethyl OH-SA were identified in low amounts ( $\leq 0.016$  mg/kg). Metabolites in the polar region R K see Fig. 6.2.2-4) were not further quantified due to the low concentration ( $\leq 0.010$  mg/kg).

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## Bayer CropScience 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 ( $\leq 0.005$  mg/kg) of parent compound, BYI 02960-OH, BX 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 chromatograph? 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 uring viver and kidney of the goad (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 surface of the sample in the same set of th analysis could not be performed, due to the high mater burden in the extract.

## Metabolites in Fat

The dominating part of the radioactive fesidues in fat (0.410 mg/kg 95.9%) was dentified as forty acids after saponification. The fatty acids were formed from smaller carbon units (C-1) or CQfragments), which entered the carbon pool of epdogenous compounds after eleavage and subsequent degradation of the furanone ring A small part 0.01 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 (1.12,1 mg/kg, 51,5%) was identified as matural fats. Parent compound, BYI 02960-OH, BYI 02960-OH-SA, BYI 02960-des-difluoroethyl and BYI 02960-desdifluoroethyl-OIGSA 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 bow amounts between 0.004 and 0.008 mg/bg

Metabolites in the poter region R1 of the acetonityle/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 5.1.2/06) and in the urine, liver and kidney of the lactating goat, (KIIA 6.2.3/02) was demonstrated by comparison of the thip layer chromatograms.

## Metabolites in Excre

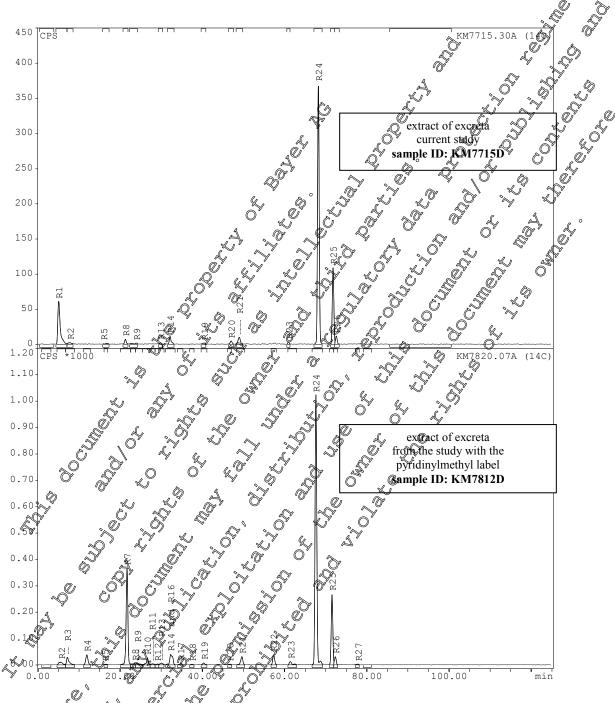
The metabolite profiles of excreta of the current study and the study using the pyridinylmethyl label were very similar, except for the label specific compounds - e.g. in the region of polar metabolites (R1). A comparison of the pointes from both studies is shown in Figure 6.2.2-5. The quantification of

metabolites is given in Table 6.2,2-9.

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	Q°

	D		0/ 00 *	
Report name	Rt	Area	% of RA	, Ű, Ď
BYI 02960-	[min]	[%]	in sample	
Polar metabolites	5.0	14.28	3.5	
des-difluoroethyl-OH-SA	32.C	2.05	چ 1.9	
des-difluoroethyl	48.9	2.27	2, 2.2	`\$`\$
acetic acid	61.0	0.46	0.4	Ŷ,Ô ^y
OH-SA	68.2	58.10	_@°55.15	5 . S
ОН	71.8	<b>j</b> ø.45 %	15.6	
Parent compound	2.6 x	1.78	×1.7 ~	*~~`\'
Total			© 94.8	
Sum identified (w/o polar metabolites)			769	
		¥ .0 ⁴		× ×
	Ğ Ş			Ũ,
	j N		5° 6° 5°	
		) A		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
	O L	, Or	à. °	Ď
		~ 2	S O .	
	' '			
	' "" (	, <i>S</i>		
des-difluoroethyl acetic acid OH-SA OH Parent compound Total Sum identified (w/o polar metabelites)				
Report name BYI 02960- Polar metabolites des-difluoroethyl-OH-SA des-difluoroethyl acetic acid OH-SA OH Parent compound Total Sum identified (w/o polar metabolites)				

## 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 ¹⁴C-label



The dominating metabolite was BYF 02960-OH-SA (55.1%) followed by the free aglycone BYI 02960-OH (15.6%). Only fraces 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 43.5% of the radioactivity in the excreta.

## F. Storage Stability of Residues

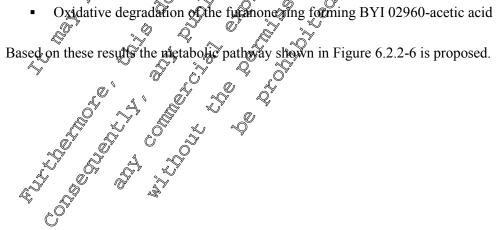
All samples of eggs, and edible organs and tissues were extracted within approx. 2 months after sample collection. The first metabolite profile was recorded latest within about 3 weeks after sample

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 wying 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 G-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 ocovery in comparison to the study with the metabolically stable pyridinylmethyl ladel.
- The relatively high values in liver and kigney 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 the after the first administration.
- The extraction rates were above 80% for eggs and edible tissues. Unextracted residues were quite low and amounted to  $\leq 0.036$  mg/kg. 1 al
- Parent compound and fron-label specific metabolites, such as BX 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, BY102960-acetic acid was detected in excerta.
- The main metabolic reactions of furanone-4-14 CBYI 02960 BY the laying hen are:
  - Cleavage and subsequent total degradation of the furanene ring forming smaller carbon units (C-1/2 or C-2-fragments), entering the sarbon pool of endogerous compounds and then being used for the biosynthesis of fairly acids
  - Aydroxylation in position 5, of the Turanove ring forming BYI 02960-OH followed by conjugation with sulphume acid to BY 029600 H-SA
  - Cleavage of the diflue wethy group forming BYI 2960-des-difluoroethyl followed by hydroxylation and conjugation with sulfaric acid to BYI 02960-des-difluoroethyl-OH-SA
  - Oxidative degradation of the furanonering forming BYI 02960-acetic acid



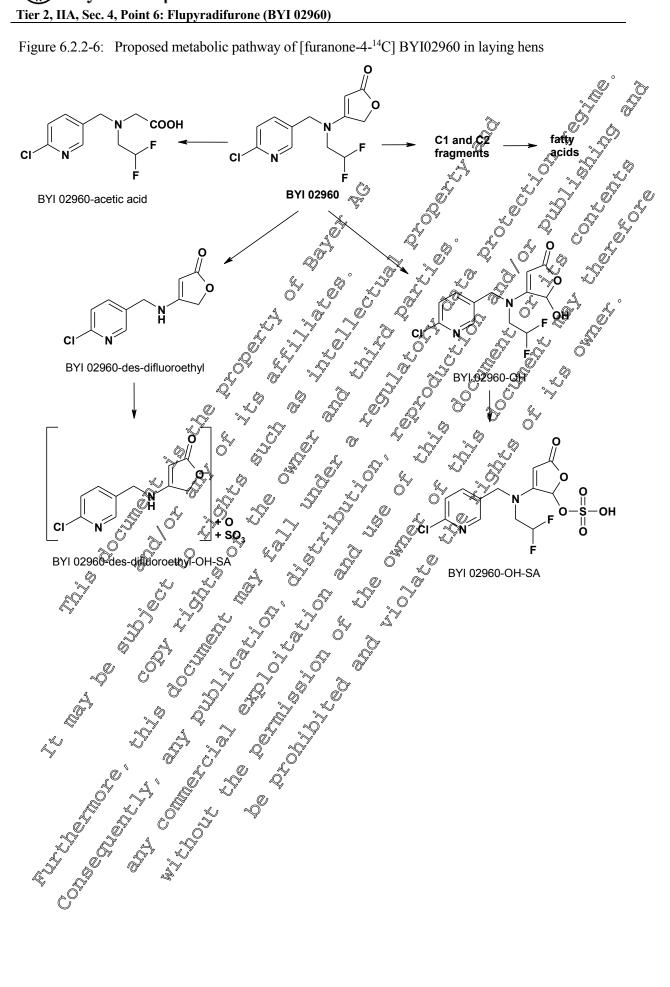
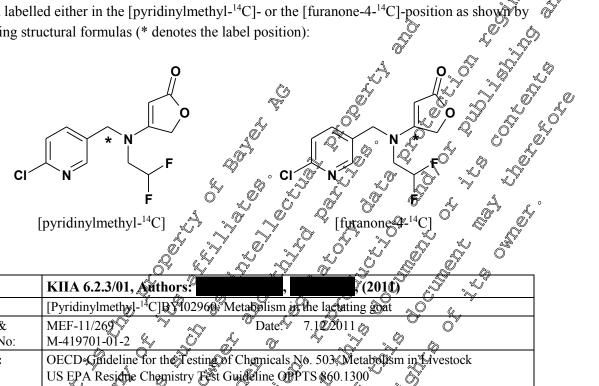


Figure 6.2.2-6: Proposed metabolic pathway of [furanone-4-14C] BYI02960 in laying hens

#### **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):



Report:	KIIA 6.2.3/01, Authors: (2010)
Title:	[Pyridinylmethy]-14C]BY102960 Metabolism in the lactating goat
Report No &	MEF-11/269 Date: 7.122011
Document No:	M-419701-01-2 & O & O & S
Guidelines:	OECD-Gnideline for the Testing of Chemicals No. 503 Metabolism in Livestock
	US EPA Residue Chemistry Fist Guideline OPTS \$60.1300
	Nate of the Residue – Plants, Live tock y O
	European Parliament and Council Regulation (EG) No 1107/2009
GLP:	Pes, according to Japan MAFF CILP standard P Nousan 6282
_ ()	TUS 28A - FIFRA GLP (40GFR Part ¥60):
ð	Principles of GLP, German Chemical Law Current Version of Annex 1
Testing Facility	
and Dates	Germany of a start of the
	Experimental work: $9.6.2000 - 6.52011$

## Executive Summary

The metabolism and excretion of pyridevine byl-14 BYI 02960 (common name: flupyradifurone) were investigated in the lactating goal as a nodel for ruminants. The goat was orally dosed once daily for five consecutive days in the morning after milling with 1.0 mg of the active substance per kg body weight which corresponded to 244 mg Qs. /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 ssues wellas 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%) wo 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 faeca excretion stated 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 P.345 mg/kg at sacrifice, 102 hours after the first administration. The time course in the vening and of morning milk pool samples showed a pronounced durnal 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 to 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 adioactivity 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 organs.

## Metabolism

For analysis of parent compound and metabolites, mills 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 furance tabel (KIIA 6.2.3/02) were comparable with the exception of the label specific metabolites.

All prominent metabolites of the extracts of mok, muscle, fat, kidney and liver were also detected in urine. Therefore, the isolation and identification of metabolites were performed using the urine samples. Isolated metabolites were identified by LC-MS/MS and used as radiolabelled reference compounds for the identification in milk, muscle, fat, kidney and liver by HPLC co-chromatography. The identification of the minor metabolite BYI 02960-AMCP-difluoroethanamine was performed in the liver by HPLC co-chromatography with the non-radiolabelled reference compound. Approximately 99% of the total radioactivity in milk, muscle, fat and kidney and ca. 93% in liver was identified. All other residues were characterised by their extraction behaviour. The unchanged parent compound was by far the major constituent of the residue in milk, organs and tissues and amounted to

0.165 mg/kg (88.8%) for milk, 0.349 mg/kg (98.0%) for muscle, 0.105 mg/kg (99.2%) for fat, 0.650 mg/kg (34.8%) for kidney and 1.028 mg/kg (84.6%) for liver.

Extensive metabolism was detected in the kidney, where the main portion of metabolites was found. The major metabolite in the kidney was BYI 02960-OH, with a concentration of 0.299 mg/kg (16.0%). Four prominent glucuronic acid conjugates of the hydroxylated parent compound were detected in the kidney as well as in the urine. The four isomers were named BYI 02960-OH-gluA (isomer 1 to isomer 4); their concentration ranged from 0.112 mg/kg (6.0%) to 0.175 mg/kg (9.5%). BYI 02960-OH gluA (isomer 2) and BYI 02960-OH-gluA (isomer 3) are diaster comers, 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) of an unknown position.

A further metabolite was BYI 02960-hippuric acid, which was detected in milk (0.0).7 mg/kg, 9.1%) and kidney (0.178 mg/kg, 9.5%). Traces of BY 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 BYk 02960-methythioglyoxylic acid in milk and muscle and BYI 02960-AMCP-diffuoro than an in Fidney and liver. The concentration of each was below 0.020 mg/kg.

The concentration of the total radioactivity (expressed as mg as equication /kg) as well as the distribution of the parent compound and metabolites and the identification rates in milk and while its sues are summarised in the following table (

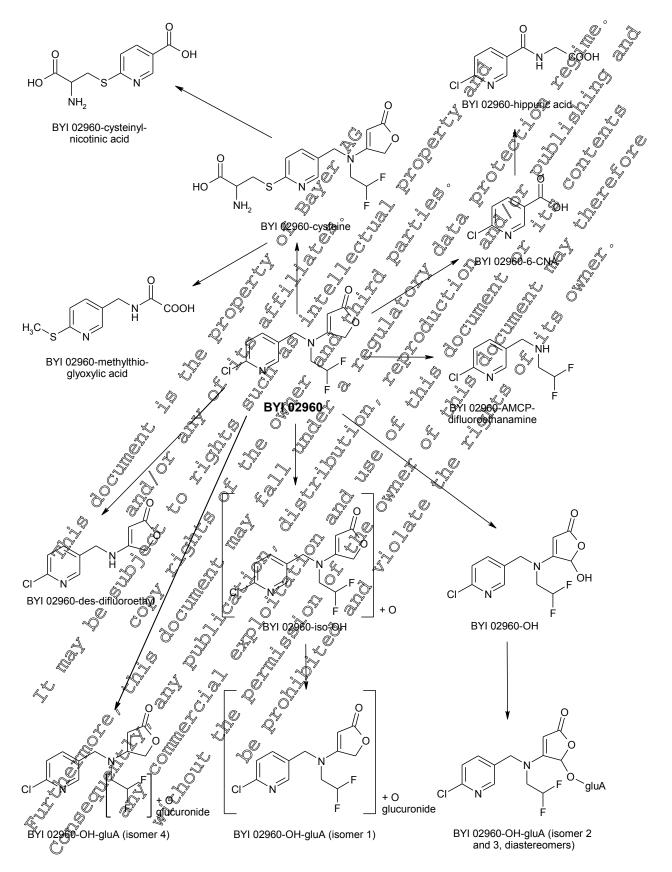
	•Ô	× _>		107	· · · ·	~~~	°∼¶"	, Ôj		
	M (24 to	ilk 🖗 1,062 h)	Mu	ð ».		at	Ka	mey	Li	ver
TRR [mg/kg]	<i>©</i> 0,1	,86 _ C	, ØS	56 🔊	0.	106 0	≪1.8	369	1.2	215
TRR [mg/kg]	% of TRR	¢ng/kg		, mg/kg	% of TRR	106 © ² mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
ACN/wateræxtract		0.1.84	99°,4		<u>9</u> 9.2	0,105	98.8	1.847	92.8	1.128
cysteinyl nicotinioacid hippuric acid methylthio-	Å.			\$* *		×	6.1	0.114	4.8	0.058
hippuric acid		0.04		0 ²	~		9.5	0.178	0.8	0.010
methylthio- glyoxylic acid OH-gluA (isomer 1) OH-gluA (isomer 2) OH-gluA (isomer 3)	155	0.04 0.003		0.005	Ş					
glyoxylic acid	õ 🔹		)C	0.005 ( 0.005 (	y.					
OH-gluA (isomer 1)	>		60	Ô			6.0	0.112		
On-gluA soller 2)		Ø-					9.3	0.175	1.4	0.016
	, <u>~</u> ~	y x	°, Q	)			8.4	0.158		
OH-ghtA (isomer 4)	×, 0	, 0.					7.5	0.141		
AMCP-	Ő	<i>a</i> , [*]	$\chi^{O'}$				1.1	0.020	1.2	0.015
difluoroethanamine	Ů,	\$ \$	Ç″							
OH S S	·						16.0	0.299		
Parent compound	* 0 5 5 5 8 8 8 8 8 8 8 8 8 8 8 8 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	⁹ 99.5	0.185	99.5	0.354	99.8	0.106	98.9	1.848	92.9	1.129
Softigds	0.5	0.001	0.5	0.002	0.2	< 0.001	1.1	0.021	7.1	0.086
Accountability	100.0	0.186	100.0	0.356	100.0	0.106	100.0	1.869	100.0	1.215

The metabolic reactions of [pyridinylmethyl-¹⁴C-]BYI 02960 in the lactating goat are:

- Hydroxylation followed by conjugation with glucuronic acid forming two diastereomeric conjugates of BYI 02960-OH (BYI 02960-OH-gluA, isomer 2 and 3), the hydroxylation and conjugation being in the 5-position of the furanone ring. One isomer (BYI 02960-OH-gluA isomer 4) with hydroxylation and conjugation in the difluoroethyl side chair and one isopher (BV) 02960-OH-gluA, isomer 1) with hydroxylation and conjugation in an unknown position
- Oxidative cleavage of the pyridinylmethyl bridge forming BYI 02960, the CNA followed by

A Fold A Fold Used by degrada A cysteme forming FY10 a cysteme forming FY1

#### Proposed metabolic pathway of [pyridinylmethyl-¹⁴C]BYI 02960 in the lactating goat:



## I. Materials and Methods

A. Materials	Q° &
1. Test Material	
IUPAC Name	$\begin{array}{c} 4-\{[(6-chloropyridin-3-yl)methyl](2,2-difluoroefhyl)amino\} for an-2(5H)-one & & & & & & & & & & & & & & & & & & &$
Code name	BY102960
Common name	Flupyradifurone (proposed ISO)
Empirical formula	C ₁₂ H ₁₁ ClF ₂ N ₂ O ₂
Molar mass	
Labelling	[pyridinylmethyl ⁴ C]
Specific radioactivity used for administration	$3.92 \text{ MBq/mg} = 405.93 \ \mu\text{C/mg}$ (delivered sample before radiodilution) $3.425 \text{ MBq/mg} = 2.06 \text{ x } 10^{\circ} \text{ dpm/mg} = 82.57 \ \mu\text{Ci/mg} \neq 26.72 \ \text{Ci/mol}^{\circ}$ (sample after radiodilution)
Radiochemical purity	>99 % (HPLC) ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
Dose level	5 datty oral doses of 1 mg/kg bw by gavage
Vehicle	Capsule 0 7 7 7 7 7 7 7 7 7 7
	<ul> <li>&gt; 99 % (HPL C)</li> <li>5 datty oral doses of 1 mg/kg bw/by gavage</li> <li>Capsule</li> <li>Capsule</li></ul>
2. Test Animals	
Species	Lactating goat (Gapra hircus) *
Strain 😽	
Breeding facility	Member of the Landesverband
Sex and numbers involved	1 female animale of the second s
Age 2 2 3	La. 36 months C Q P
Body weight	60 kg at first administration, 59 kg at sacrifice
Acclimatization	Zelays O LT & AT
Identification	Eskin 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 anymals (goat, sheep, and pig), supplied by
	device.
S A S	room temperature 17 - 24°C, relative humidity 48-93%
	12 h light / 12 h dark cycle, air change $10 - 15$ times per hour.
Accumulzation	The goat was fed <i>ad libitum</i> with hay, hay pellets, apples and supplementary ruminant feed ("Raiffeisen LammGold", supplied by
Č	During the test period, the average feed consumption was 2.48 kg/day, tap water was offered a <i>d 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 orag 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 MBo/mg. In total, five gelatine capsules containing the test compound were operated. They were stored the -18° C untit administration. The remaining test compound was stored in solid form ogether 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 60.42 mg. The totally administered amount and radioactivity accounted for 302.03 mg and 62,067,165,000 dpm, respectivel. The total amount of radioactivity administered to the animal served as reference value ( $R_0 = 100\%$ ) for the percentage calculation of the total radioactivity if the brological samples.

# Sampling of milk, urine and facees during the m-life phase

The goat was milked in the morning immediately prior to each administration, about 8 hours later in the afternoon, and directly before carrifice (8, 24, 32, 48, 56, 72, 80, 96 and 102 hours after the first administration). The milk weights were recorded.

cooling in intervals of 24 Kours 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 defonised water into the vessels 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 coffecting 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 dissues

The animal was sacrificed approx of 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 Gagular vein and finally terminated by intracardiac injection with approx. 10 mL of the veterinary drog "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 of

## 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 metabolite analysis. The remaining samples of each organ or tissue were stored at  $\leq$  -18 °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 antil extraction.

Aliquot samples of milk, muscle, kidney and liver were extracted with acetonitrile/water (8:2; 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 nelding 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 if 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 (COD) was established at approximately 10 dpm measured per aliquot after instrument background correction. The limit of quantification (LQC) 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 of mill and the tissues, and sample of urife were subjected to HPLC using a reversed phase column ( $C_{18}$ ) and the eluting solvents water/formic acid 99:1 (v/v) and acetonitrie water/formic acid 97:2:1 (v/v) in the gradient mode. Detection was performed by a UV-(254 nm) and a radiot sotope detector with a glass head 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 ecoveries were between 94.4 and 106.5% of the injected radioactivity Radiotabelled and ren-labelled reference compounds were used in co-chromatography for identification of metabolites.

As a second chromatographic method, thin fayer chromatography (TLC) was employed on silica gel plates and radioluminography for retection of radioactive spots. As a solvent system a mixture of acetonitrile/water/formic cid (70.25:5,  $\sqrt{v/v}$ ) was used.

The electrospray ionisation mass spectra (ESI) were obtained with an LTQ Orbitrap XL mass spectrometer Othermo, Sar 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. ¹H-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

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 & 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. Oring [pyridiny methy ¹⁴C]BYI 02960 per kg body weight on five consecutive days is presented in Table 9.2.3-1. The overall recovery accounted for 88.75% of the total dose. Much of the remaining adioactivity (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 moscle. Until sacrifice, the excretion of radioactivity accounter for \$202% of the related dose. A portion of 71.74% was found in the urine and 13.28% in the faeces. The urine ry and faecal excretion started immediately after the first administration

$T_{abl} \in (2, 2, 1)$	Distribution of residues in milk, muscle, fat, liver and kidney of actating goats
1 able 0.2.3 - 1.	Distribution of residues in mirk, indecie, in, inveging kidney of actating goals
	following oral administration of Sdaily boses of [pyridhylmethyl-14C] BYI02960 at a
	dase rate of 1.0 ppg/kg 2

			¢*
Å.	Sample ~ 5 40	Percent of that	Concentration of total radioactivity [mg/kg]
Ê, ^g '	Liver , St	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	1.215
		× 0.10 [×]	1.869
_	Muscle, total	2,40	0.356
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	Fatotal	م م 0.25	0.106
A B	Total of organistissues	2.94	
	Liver Kaney Musche totak Fat total Total of organytissues Milk 0 – 102 h Urine, 05 102 h	290 0.25 2.94 0.78 71.74	0.186*
	Urine, 05 102 h	71.74	
(13.28	
<u> </u>	Total exceed	85.02	
Ĵ,	Potal Recovery	88.75	
	Faeces, 0 – 102 h Total experied Fotal Recovery * : Mark 24 – 102 h	· · · · · · · · · · · · · · · · · · ·	

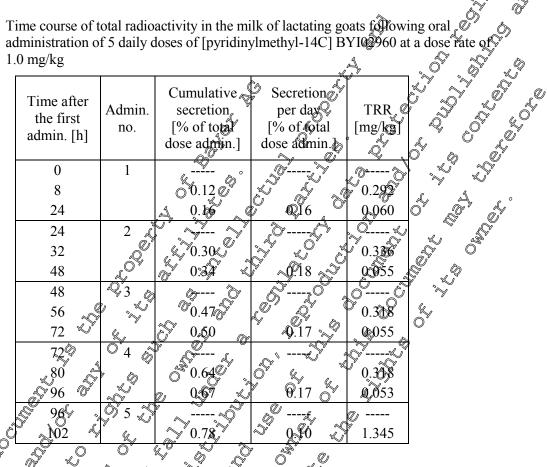
B. Levels and Time Course of Total Radioactive Residues in Milk

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

Bayer CropScience 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:



C. Total Radioactive Residues fa the Dissected Organs and Tisspes

The concentration of the total adioactivity 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 (215 mg/kg) mdicating 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 22% of the body weight for these tissues.

D. Extraction Efficience of Residues

Milk (24 to 102%), muscle, kit mey and live 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 acetonipile/water (8:2, v/v) and n-heptane followed by a solvent partition yielding an acetoortrile/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

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.

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. Summary of the quantification of parent compound and A Cart metabolites in milk, muscle, fat, kidney and liver is presented in table 62.3

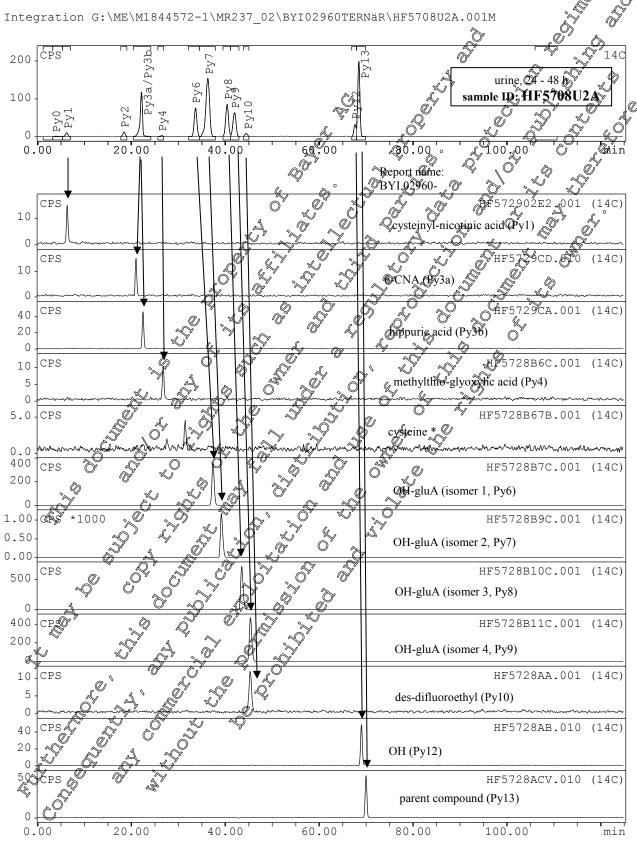
Isolation and Identification of Parent Compound and Metabolites in Uring

Isolation and Identification of Parent Compound and Metabolites in Uring O O Metabolites were isolated from uring (24 to 48 h) and identified by LC-MS/MS investigations. An assignment of all identified metabolites to the metabolic profile of under is presented in Figure 6.2.3-1. BYI 02960-OH-gluA (isomer 1@Py6) to BYI 02960 DH-gluA (isoprer 4, Py9) were identified as glucuronic acid conjugates of hydoxylated BYI 02960 by LC-MS/MS, The LG-MS/MS fragments of BYI 02960-OH-gluA (isomer 1) and BYP02960-OH-gluA (isomer 4) were different from each other and were different from BYI 02960-OH-glu (isomer 2) and BYI 02960-OH-gluA (isomer 3). Most probably BYI 02960-OH-glue (isomer 2) and BYO 02960-OH-gluA (isomer 3) are two diastereomers because of their identical KC-MSONS fragments. Therefore, jowas concluded that BYI 02960 was hydroxylated and conjugated with glucuronic acid in three different positions of the molecule. The hydroxylation of the two diaster comers (isopper 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 (2960-OH-SA was clearly identified by NMR-spectroscopy in the laying hen study with the pyridinylmet yl laber)KIIA 6.2.2/01). In the case of isomer 4, the position of the hydroxylation was assigned to the difloroethyl 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 somers of BYI 02960-OH-gluA to their corresponding

Further evidence for the assignment of the four isomers of BYI 02960-OH-gluA to their corresp aglycone and decomposition product is presented in the lactating goat study with the furanone label JKIIA 6.2.3/02

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

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.



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 pon-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 sumparised in Table 6.2.3-3. Approx. 99% of the total radioactivity of milk, muscle, fat and kidney and approx 93% in liver were identified.

Table 6.2.3-3:	Radioactive residues of parnt compound and metabolites in milk, arine and edib	le
	organs of lactating goats following oral administration of daily doses of	-
	[pyridinylmethyl- ¹⁴ C] BY402960 of a dose rate of 1.0 mg/kg &	ſ

		L.	~~	Ľ. M	_c O	al "		~	<u>s</u>	Y
	M (24 to	ilk 1002 h)	K Mu	şčte	k K	at č	Kat	iney of	Êi	ver
TRR [mg/kg]	0¢	86	0.3	56	20 .1	106	¢1.8	869 ^{°°} ·	1.2	215
Sample/ Report name BYI 02960-	‱of ¥¥RR≰	mg/kg	% of STRR(mg/kg	¢% of TR ≹ ∕	mg/kg	% of TBR	mg⁄Rg	% of TRR	mg/kg
- ACN/water extract	99.3	0.184	<u>9</u> 9.4	0.953	<i>Q</i> 9.2	0.105 ×	98.8	<u>1.847</u>	92.8	1.128
cysteinyl- nicotinic acid hippuric acid methylthio- glyoxylic acid OH-gluA (isomer 1) OH-gluA (isomer 2)	99,5 9:4 15	у д У	0 <u> </u>	, ¢	, % <i>a</i> .	×&	6:10 ~	0.114	4.8	0.058
nicotinic acid hippuric acid methylthio- glyoxylic acid OH-gluA (isomer 1) OH-gluA (isomer 2) OH-gluA (isomer 3)	9:1	0.007	~~~	, Ŷ-		Å »(@9.5	0.178	0.8	0.010
methylthio-	115	Q.003	₹¥.3	.005 .005	S C	,÷				
	()	0 %	, ĝ		Å.	. O				
OH-gluA (isomer 1)	Q		- A	101		~ ~	6.0	0.112		
OH-gluA (Isomer 2)	S.	<u>e</u>		§~ «	ç	×	9.3	0.175	1.4	0.016
OH-gluA (isomer 3)		, [*] 4	»°») %			8.4	0.158		
							7.5	0.141		
AMCP-		~	<u> </u>	~ <i>4</i>	Ş		1.1	0.020	1.2	0.015
difluoroethana@aine 💭	δ^{*}	Ů () ^v O	¥					
OH N N			@	Ô.			16.0	0.299		
Parent coppround \gg	88.8	00165	-40° 298.0	0 .349	99.2	0.105	34.8	0.650	84.6	1.028
Total identified	, 99.3 ∧	0.184	^{\$\$} 99,40	0.353	99.2	0.105	98.8	1.847	92.8	1.128
Total extracted	[*] 99,50	0.185	995	0.354	99.8	0.106	98.9	1.848	92.9	1.129
Solids	0,5	0,001	LO.5	0.002	0.2	< 0.001	1.1	0.021	7.1	0.086
Accountability	Ĵ0.0 (Ø.186 [°]	100.0	0.356	100.0	0.106	100.0	1.869	100.0	1.215
	×.	- N								

Parent compound was by far the dominating constituent of the residue in milk, organs and tissues. Its concentration mounted to 0.465 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 hydrox ation and conjugation in an unknown position.

A further major metabolite was BYI 02960-hippuric acid, which was detected in the mill 0.01 mg/kg, 9.1%) and kidney (0.178 mg/kg, 9.5%). Traces of BYI 02960-hippuric acid were also found for liver (0.010 mg/kg,). BYI 02960-cysteinyl-nicotinic acid was identified in kidney (0.114 mg/kg, 619 and in liver (0.058 mg/kg, 4.8%). Minor metabolites were BYI 02960-methylthio-glyoxylc acidin 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 ≤ -1 SC or for a short time in a refriger for. All samples of milk, and edible organs and tissues were extracted within approx ax weeks after sample collection. The first metabolite profile was recorded one day after the sart of the extraction and sample preparation. Hence, investigations on storage stability of the residues in the samples were not necessary and it can be conduded that the metabolic profiles represent the residues in the matrices at sacrifice.

III. Conclusion

The metabolic and excretion behaviour of [pyridinylmethyl-1@]BYI 02960 in the lactating goat can be characterised by the following biservations

- The concentration of radioactivity in milk and edibre tissues was rather low compared to the dose Ś level and the dosing period of five days.
- level and the dosing period of five days. The evaluation of these concentrations should more over consider the fact that an exaggerated dose level of 24.36 mg/kg feed/day was attministered. Durthermore, the significant amount of radioactivity detected in usine 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 sidue level in milk howed a propounced 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 way 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 hydrox lated BYI 02960, BYI 02960-hippuric acid and BYI 02960-cysteinylnicofinic acid were identified.

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- 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 and forming two • diastereomeric conjugates of BYI 02960-OH (BYI 02960-OH-gluA, Gomer 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 a unknown position
 - Oxidative cleavage of the pyridinylmethyl pridge forming BYI @2960-64 NAfollowed conjugation with glycine to BYI 02960-hippuric acid
 - Substitution of the chlorine atom of BY1 02960 with glutathione followed by degradation resulting in BYI 02960-cysteine
 - Oxidative cleavage of the pyridin methy bridge of BYI 02960 cys@ine førming B • cysteinyl-nicotinic acid
 - Degradation of BYI 02960-Osteine in the systeme group and furanones ring forming • BYI 02960-methylthio-gloxylic acid
 - Cleavage of the furance ring forming BYI 2960, AMCP ifluor ethansmine •

Cleavage of the diffusion o

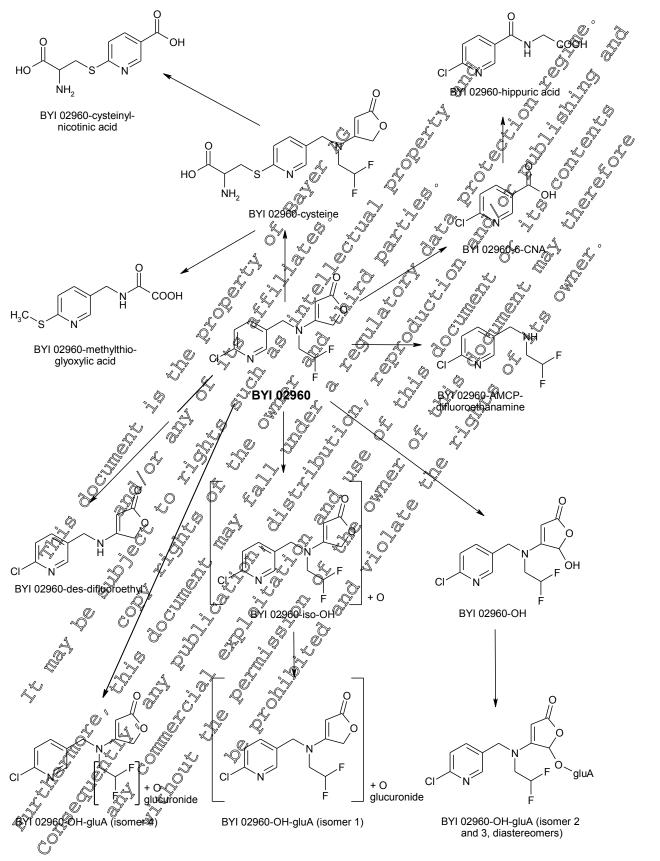


Figure 6.2.3-2: Proposed metabolic pathway of [pyridinylmethyl-14C] BYI02960 in the lactating goat

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Tier 2.	IIA. Sec.	4. Point 6:	Flupyradifurone	(BYI 02960)
1101 2, 1	m, scc.	- , i omt o.	rupyraunurone	(DII 02/00)

Report:	KIIA 6.2.3/02, Authors: , , , , , , , (2011)]
Title:	[Furanone-4- ¹⁴ C]BYI02960: Metabolism in the lactating goat	
Report No & Document No:	MEF-11/268 Date: 16.12.2011	
Guidelines:	OECD-Guideline for the Testing of Chemicals No. 503, Metabolism Livestock US EPA Residue Chemistry Test Guideline OPPTS 860.1300 Nature of the Residue – Plants, Livestock European Parliament and Council Regulation (EC) No 1107/200	
GLP:	Yes, according to Japan MAFF GLP standard 11 Nousan 6253; US EPA – FIFRA GLP (40CFR Part 160); Principles of GLP, German Chemical Law, current version of Annex 1	
Testing Facility		Ø .
and Dates:	Germany Experimental work: 19.4.2010 20.4.201 P	j

Executive Summary

The metabolism and excretion of [furmone_4,1⁴C]BVI 02960 (common name: fuppyradfurone) were investigated in the lactating goat as a model for runninants. 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 beed/day. The animal was sacrificed about six hours after the last administration. Total radioactive residues (TfAR) were determined in milk and excrete at various sampling intervals, and in muscle, fat, kidney and laver at sacrifice. Milk, edible organs and tissues and urine were analysed for the were hanged parent compound and metabolites.

Recovery and Eligenation of Radioactivity

The overall recovery amounted to 7%94% of the total dose. Much of the remaining radioactivity (ca. 21%) was expected to still be present in the gastro-intestinal tract at sacrifice, due to the short period between the last administration and sacrifice. A significant proportion is probably exhaled as $^{14}CO_2$ since the formation of carbon broxide 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 much.

Until sacrifice, the excretion of adioactivity accounted for 72.14% of the total dose. A portion of 69.15% was found in the urine and 3.0% in the faces. The urinary and faecal excretion started immediately after the first administration

Total Radioactive Residues in Mills, Orgons 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 esidue increased significantly during the eight hour period after the second and the fifth administration followed by a decrease measured prior to the delivery of the next dose. A plateau level of about 1.1 mg/kg was reached ca. 50 hours after the first administration.

In the **organs and tissues**, the highest concentrations were determined in liver (1.746 mg/kg) and kidney (1.472 mg/kg) indicating the significance of these organs for excretion and metabolism. These

values corresponded to 0.65% and 0.09% of the total dose, respectively. For muscle and fat 0.539 mg/kg and 0.265 mg/kg, respectively, were determined. The radioactivity concentration of the muscle corresponded to 2.91% and of fat to 0.57% of the total dose assuming a value of 30% and 20% of the body weight for these tissues.

Metabolism

For analysis of parent compound and metabolites, milk, muscle, kidney and liver were explacted 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 fatioactive residue. Solids of kidney and liver were exhaustively extracted with mixtures of acetonitrile/water extracts of 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 0990 mg/kg. The extraction residues of kidney and liver were completely solublised 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 goar study with the pytidinylmethyl label (KfrA 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 fiver 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 profile based on the comparison with the urine profiles of the current study and the study with the profile based on the comparison of BY102960. The glux were isolated and identified by comparison of the LC MS/MS data. Further identification of the BY1 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 pulk was identified after isolation and acetylation by HPLC co-chromatography with the radioabelled reference compound.

Approximately 91% otopic 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 way the main constituent of the residue in organs and tissues and amounted to 0.475 mg/kg (880%) 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 metabolites in kidney was BYI 02960-OH (hydroxylation in the 5-position of the furanone ring), which amounted to 0.215 mg/kg (14.6%). Four prominent glucuronic acid conjugates of hydroxylated parent compound were detected in the kidney and in the urine. The four isomers were named BYI 02960-OH-gluA (isomer 1 to isomer 4). Their concentrations were between 0.032 mg/kg (2.2%) and 0.069 mg/kg (4.7%) in the kidney. BYI 02960-OH-gluA (isomer 2) and BYI 02960-OH-

gluA (isomer 3) were diastereomers, which were hydroxylated and conjugated in 5-position of the furanone ring. BYI 02960-OH-gluA (isomer 4) was hydroxylated and conjugated in the difluoroethyl side chain and BYI 02960-OH-gluA (isomer 1) in an unknown position. A minor metabolite was all a 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 ($\leq 5 \text{ min}$) were specific for the furanone label. Their concentrations ranged from 0.021 to 0.037 mg/kg for kitney and from 0.019 kg 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 5.1.2/0) 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 furanche ring of the molecule obviously underwent extensive biotransformation to C-1- and C-2-tragments 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 4COr, which might explain the lower overall recovery as compared to the study with the metabolically stable pyridiny methyloabel. The concentration of the total radioactivity (expressed as tog a.s. equiv, arg) as well as the distribution of the parent compound and metabolites and the identification rates jomilk and edible tissues are summarised in the following table:

	~	<u>%</u>) A	' T	~		<u></u>	<i>î</i> a		
) N (24) to	filk 5 102 h) 046	Mu		Fa	at ×	Kid	pney	Li	ver
TRR [mg/kg]	Ĩ.	Q\$46	, 05	39			\sim	170	1.7	746
TRR [mg/kg] Sample/ Report name BYI 02960- ACN/water extract Lactose	%`of TRR	mg/kg	T RR	mg/kg	of TRRC	Źmg/kg	Ø% of TRR	mg/kg	% of TRR	mg/kg
	90.7	0.948	95.0 	0.592	88.4	Q.734	89.1	1.311	73.3	1.280
Lactose Polar merabolites OH-gluA (isomer 1) OH-gluA (isomer 2)	66,8 03	0,698	<u>O</u> *		<u> </u>	ŷ				
Polar metabolites	Ø 	, 4	\$^4.4 C	€ 0.024 0.024	5.0 ⁰	0.013	10.0	0.148	12.1	0.211
OH-gluA (isomer 😥 🎾 🦨			4.4 -4 -4 -4 -4 	0	<u> </u>		2.2	0.032		
OH-gluA (isomer 2)		s s s s s s s s s s s s s s s s s s s	. «	~ 4	Ö		2.2	0.032		
OH-gluA (isomer 3) OH-gluA (isomer 4)	<u> ~</u>	, Ö, (-9 -9) Ô			4.7	0.069		
OH-gluA (isomer 4)	Ď <u> </u>	· · · · · · · · · · · · · · · · · · ·		-0			3.5	0.052		
Des-diflueroethyl	Š	OF STREET	×2-	ж у			1.3	0.019		
ОН 🖓 🕺	<u></u>	~ 4	[©] 1.8 _C	M 010	2.9	0.008	14.6	0.215		
Des-difluctoethyl OH Parent-compound	23.9	0.25	88.1	0.475	80.5	0.213	50.5	0.744	59.8	1.045
Total identified	9¢7	0.948	89.9	0.484	83.4	0.221	79.0	1.163	59.8	1.045
10tul extracted	() 1.T	.956	Q95.2	0.513	94.1	0.249	100.0	1.472	100.0	1.746
Solids	8.6	×0.099,	^v 4.8	0.026	5.9	0.016	n.d.	n.d.	n.d.	n.d.
Accountability 🖉 🖉	1000	1.096	100.0	0.539	100.0	0.265	100.0	1.472	100.0	1.746
Solids	<u>1040</u>									

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

The metabolic and excretion behaviour of [furanone-4-¹⁴C]BYI 02960 in the lactating goat can be characterised by the following observations:

The concentration of radioactivity in milk and edible tissues were relatively high compared to the dose level and the dosing period of five days. This was mainly caused by the partial instability of the radiolabel used. For a small portion of the total dose the furanone ring obviously underwent extensive biotransformation to C1- and C2-fragments that resulted in an accumulation of radioactivity in biomolecules during dosing for five days.
 A part of these fragments was probably also converted to the terminal product ¹⁴CO₂ as can be done

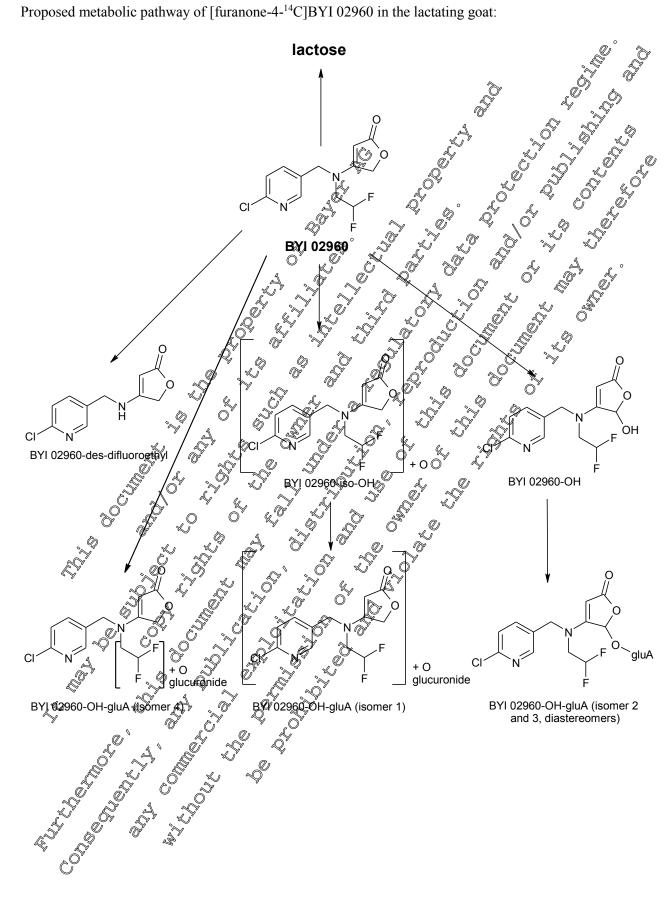
derived from the lower overall recovery compared to the study with the metabolically stable pyridinylmethyl label.

- 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 significant amount of radioactivity detected in urine and significant amount of radioactivity detected in urine and significant amount of radioactivity detected in the residues are further metabolised and finally eliminated
- The total radioactive residues in mill showed a dintrial 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 efficient 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 layer (ca. 60%) Approximately 24% of the total radioactivity in the milk was identified as parent compound.
- The main residue in the milk was radioactive factose (appro . 67%)
- Extensive metabolism was detected for the kidney BYI 02960-QH, four glucuronic acid conjugates of hydroxylated BYI 02960 and BYI 02960 des-diffuoroethyl were identified.
- The main metabolic reactions in the lactating goat are
 - Cleavage and subsequent degradation of the far anone ring forming small carbon units (C-1- or C2-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 BY1 02960-OH (BYL02960-OH-gluA, isomer 2 and 3) with hydroxylation and conjugation in the 5 position of the furatione ring, one isomer (BYI 02960-OH-gluA, isomer 4) with the hydroxylatico 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 alluoroothyl group forming BYI 02960-des-difluoroethyl

Based on the results the metabolism of [furanone-4-¹⁴C]BYI 02960 in the lactating goat can be described by the metabolic pathy ay shown on the next page.

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 BYI02960 Flupyradifurone (proposed ISO) $C_{12}H_{11}CIF_{2}N_{2}O_{2}$ 288.68 [furanone-4- ¹⁴ C]
Code name	BY102960
Common name	Flupyradifurone (proposed ISO)
Empirical formula	$C_{12}H_{11}CIF_2N_2O_2$
Molar mass	
Labelling	[furanone-4- ¹⁴ C]
Specific radioactivity used for administration	(derivered sample betwire radioality on) $3.50 \text{ MBq/mg} = 240 \text{ x} 10^{\circ} \text{ dpm/mg} = 9459 \mu\text{CPmg} = 27.31 \text{e}\text{i/mol} $
Radiochemical purity	>99% (HPLC) ~ ~ ~ ~ ~ ~ ~ ~ ~
Dose level	5 datty oral doses of 1 mg/kg by by gavage
Vehicle	Capsule O S S S S S S
2. Test Animals	 > 99 % (HPL C) 5 datty oral doses of 1 mg/kg bw/by gavage Capsule Capsule
Species	L'actating goat (Gapra hircus)
Strain N	"Weiße Deutsche Edelziege"
Breeding facility	
Sex and numbers involved	1 févnale animal
Age of the second secon	24 months 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
Body weight	4/kgat first administration 42 kg at sacrifice
Acclimatization 0, 6	7 days
Identification	strin matching of the second s
Housing	During accliniatization period: raised stall with a metal grid as base
	During the test period; electro-polished stainless steel metabolism cage
	for farmy animals (goat sheep and nig) supplied
	24 months A7 kg ar first administration 42 kg at sacrifice 7 days Sin matking 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 The cage was equipped with a variable-restraining device. room temperature 20 - 25°C, relative humidity 40-55%
	poom temperature 20 - 25°C, relative humidity 40-55%
	12 holight 42 h dark cycle, air change 10 – 15 times per hour.
Feed and water A	The goat was fed <i>ad libitum</i> with hay, hay pellets, apples and
	supplementary ruminant feed ("Raiffeisen LammGold", supplied by
J & A J	
	During the test period: electro-polished stainless steel metabolism cage for farp animals (goat, sheep, and pig), supplied The cage was equipped with a variable-restraining device. room temperature 20 - 25°C, relative humidity 40-55% 12 h/12 h dark cycle, air change 10 – 15 times per hour. The goat was fed <i>ad libitum</i> with hay, hay pellets, apples and supplementary ruminant feed ("Raiffeisen LammGold", supplied by During the test period, the average feed consumption was 1.63 kg/day, tap water was offered a <i>d libitum</i>
	tap water was offered aa libitum
Ũ	

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 0.0 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. It was five gelatine capsules containing the test compound were prepared. They were stored at \leq 98 °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 methode (2 L writer + 154 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 faece during the inflife phase

The goat was milked in the morning immediately pror to each administration, about 8 hours later in the afternoon, and directly before sacrifice (8, 24, 92, 48, 36, 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 of 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 faces, the collecting grid was cleaned prior to each administration. No samples of the rinong 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 combust of LSC. The remainder was stored at room temperature for metabolite analysis.

Sacrifice and dissection of organs and tissues

The animal was accrificed 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 jupular win and finally terminated by intracardiac injection with approx. 10 mL of the veterinaty drug T 61[®]". Following sacrifice, the following organs and tissues were sampled: liver without gall bradder kidneys, two different types of muscle (round and loin), and two different types of 4at (perifenal 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 frequer 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 muntil 102 h after the first administration and composite samples of muscle (loin and cound) 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 acetonitoffe/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 reference phase. Solids of kinney and liver were in addition exhaustively extracted with mixtures of acetonitrile/water, OI 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, gnantification 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 00 - 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 cas determined by LSC.

Metabolite analysis

The purified extracts and samples of trine were subjected to HPLC using a reversed phase column (C18) and a tennary elution gradient. Detection was performed by a UV- (254 nm) and a radioisotope detector with a glass bead scintillator. It 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 CSC. The recoveries were between 95.3 and 105.9% of the injected radioactivity. Radiolabelled and non-labelled inference 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 acetonit the water formic acid (70:25:5, v/v/v) was used:

The electrospray ion sation 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 muk, 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® carted ge 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 accontint water extracts of kidney and liver were quantified and further characterised by TLC (solyint system: accontint water) formic acid 70:255, v/v/0. Their commonality with polar metabolites in urine of rate of grades of succession of the metabolites 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 a cetylated radioactive factose as reference compound. Acetylated radioactive lactose was synthesised from adioactive lactose.

II. Results and Discussion

A. Recovery and Elimination of Radioactivity

The recovery of radioactivity after administration of a daily does of 1.0 mg [furanone-4-¹⁴C]BYI 02960 per kg body weight on five consecutive days is presented in Table 6.2.3-4. The overall recovery accounted for 08.94% 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 ⁴CO₂ since the formation of carbon dioxide had been clearly shown in a rad quantitative whole body autoradiography study using the test compound labelled at the same position (KIIA 5.1.202). Af sacrifice, the residues in the organs and tissues dissected from the body were calculated or estimated to be 222% of the total dose from which about 69% was detected in skeletal phasele.

Until sacrifice, the excretion of cadioactivity accounted for 72.14% of the total dose. A portion of 69.15% was found in the units and 3.0% in the faeces. The unitary and faecal excretion started immediately after the first administration.

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

Tate of 1.0 mg/kg		
Sample	Percent of total dose administered	Concentration of total Totalioactivity
Liver	0.65	A. 1.746 20 6
Kidney	Q_09	1.746° 5° 5° 1.473 5° 5°
Muscle, total	\$2.91	
Fat, total	مَنْ 0.57 ⁽⁰⁾	
Total of organs/tissues	4.22 Q	
Milk, 0 – 102 h	2.58	(9.961 ° ° ° °
Urine, 0 – 102 h		L Q Y V
Faeces, 0 – 102 h	1 x 300 7 7	
Total excreted	× × × × 2.14	
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 1213 mg/kg at 36 hours after the first administration. At sacrifice, the residue conceptration was 1005 mg/kg. The time course in the evening and morning rivilk poor samples showed a pronotinced durnal 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 dolivery of the pext dose. A plateau level of about 1.1 mg/kg was

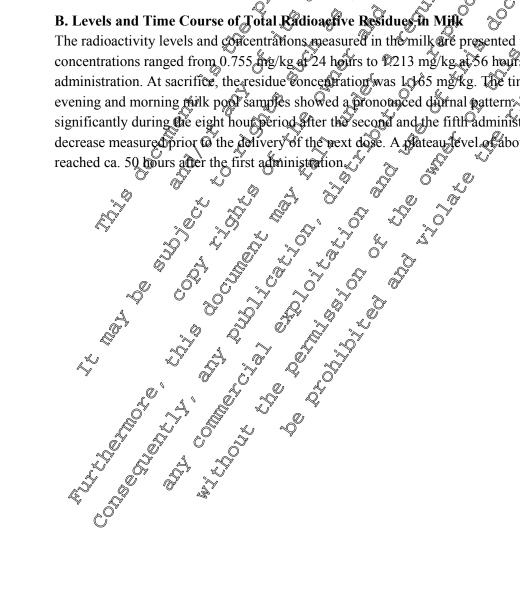
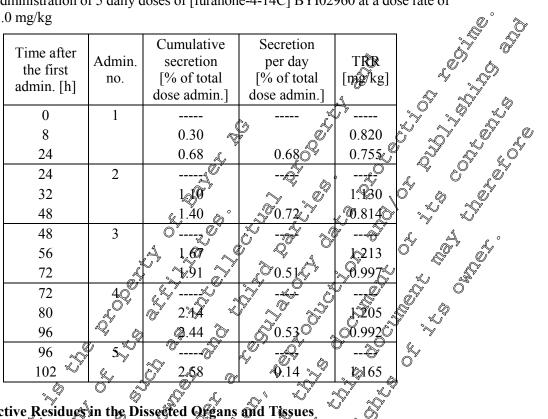


Table 6.2.3-5: Time course of total radioactivity in the milk of lactating goats following oral administration of 5 daily doses of [furanone-4-14C] BYI02960 at a dose rate of 1.0 mg/kg



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. (fast 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 metabolisor. In relation of the total dose administered, these values, corresponded to 0.65% and 0.09%, respectively. For muscle and fat 0.539 pg/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 vanie of 30% approximation 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 (\$2,v/v) and pcheptane followed by solvent partition yielding an acetonitrile/water phase and an n-heptane hase 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 mile (24 to 102 h), 95.2% for muscle, 94.1% for fat, 89.8% for kidney and 73.9% for liver. Postextraction residues of kidney and liver were extracted exhaustively with mixtures of acetonits e/water, 0.1 N HCL and 0.1 N NaOH using microwave assistance. The residues in the exhaustive extracts fanged 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

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

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 b) 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 B: acetonitrile / eluent A (99:1; v/v) C: methanol / tetrahydrofurane (1:1; v/v) Detailed information can be found in the report

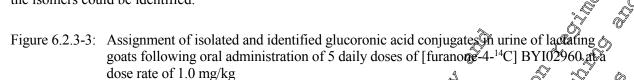
Detailed information can be found in the report.

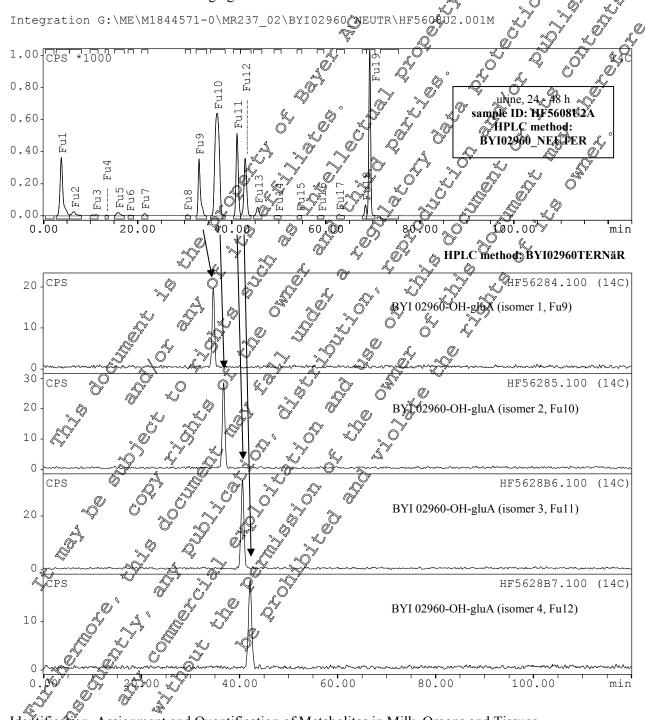
Metabolites in the extracts as well as in the urine (24 to 102 h) were assigned by comparison of the metabolite profiles and their retention times. A summary of the quantification of parent compound and A Cart metabolites in milk, muscle, fat, kidney and liver is presented in table 62.3-6

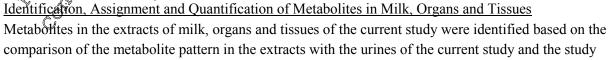
Isolation and Identification of Parent Compound and Metabolites in Urings

The identification of parent compound, BY, 02960 OH (hydrox vation in 5-position of the foranone ring) and BYI 02960-des-difluorochyl was performed in the prine of the goat study with the pyridinylmethyl label (KIIA 6,223/01) by spectroscopic methods. Four isomers of the glucuronic acid conjugate of hydroxylated B & 02960 were solated and identified in the urine of the current study and the study with the pyridin fmethy Dabel. 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 BY 02960-OH QuA isomers was achieved by comparison of the LC-MS/MS spectra and by comparison of the decomposition fates 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 DH-gluA (isomer 1) & BYL 02960 DH-gloA (isomer 4) were identified as glucuronic acid conjugates of hydroxylated BX 02960 by LC-MS/QIS. The LC-MS/MS fragments of BYI 02960-OHgluA (isomer 1) and BYI 02960-QH-gluA (isomer 4) were different from each other and were different from BY 12960-OH-gfrA (isomer 2) and Bol 02960-OH-gluA (isomer 3). Most probably BYI 02960-OH-gluA (in other a) and BYI 02960-OH-gluA (isomer 3) are two diastereomers, due to their identical C-MSMS spectra. Therefore, it was concluded that BYI 02960 was hydroxylated and conjugated with glucuron aciden 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 BYE 2960-OH-SA. The position of the hydroxylation of BY 102960-OH-SA was chearly identified by NMR-spectroscopy in the laying hen study with the pyridinylmethy@abel (KIIA \$2.2/Q1). For isomer 4, the position of the hydroxylation was assigned to the difluoroetbyl side chain based on the fragments 225 (ESI+) and 223 (ESI-) which prove the presence of the unchanged pyrithylmethyl and furanone moieties. For isomer 1, the position of the hydroxydation could not be derived from the mass spectra, therefore the position remains unknown. The assignment of the BY 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 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.







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 access of the sector of the total radioactivity in milk, ca. 90% in muscle, ca. 83% in fat access of the sector of the total radioactivity in milk, ca. 90% in muscle, ca. 83% in fat access of the sector of the total radioactivity in milk, ca. 90% in muscle, ca. 83% in fat access of the sector of the total radioactivity in milk, ca. 90% in muscle, ca. 83% in fat access of the sector of the total radioactivity in milk, ca. 90% in muscle, ca. 83% in fat access of the sector of the sector of the total radioactivity in milk, ca. 90% in muscle, ca. 83% in fat access of the sector o

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 kidnes and 1.645 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 patent compound were detected in the kidney. The four conjugates were named BYI 02960-OH gluA disomer 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 diastereometrs (BY) 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 diffuoroethyl side chain and one isomer (BYE02960-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

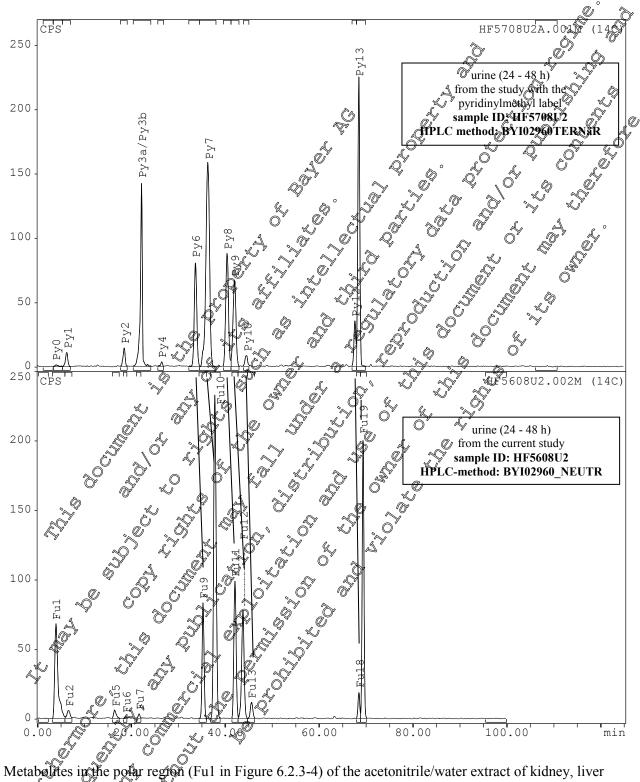
unknown position of the molecule. Metabolites in the exhaustive extracts of kidney and liver were characterised by their extraction behaviour. They were not further investigated due to the high matrix content.

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

Table 6.2.3-6: Radioactive residues of parnt compound and metabolites in milk, urine and edible organs of lactating goats following oral administration of 5 daily doses of [furanone-4-¹⁴C] BYI02960 at a dose rate of 1.0 mg/kg a°

		ose rat								Ľ
	Mill (24 to 10		Mus	scle	F	at	\sim	ļney	0) ~ 1.7	ver
TRR [mg/kg]	1.04	6	0.5	39	0.2	265	À.4	472	×1.7	46
Sample/ Report name BYI 02960-	% of TRR ⁿ	ng/kg	% of TRR	mg/kg	% of ØTRR	mg/kg	5% of TRR	mg/kg	₩ ₩ ₩	mgAk
- ACN/water extract	90.7 ().948	95.0	0.512	88.4	0234		J.311	73.3	1.28
Lactose Polar metabolites OH-gluA (isomer 1) OH-gluA (isomer 2) OH-gluA (isomer 3) OH-gluA (isomer 4) Des-difluoroethyl OH	66.8 (0.698 		2 0.024 2 			10.0 2.2 2.2 45 3.5 1.3 145	0.032 0.032 0.032 0.032 0.069 0.052 0.019 0.052 0.019 0.052 0.744 1.163 1.472 *n.d.		221 221 1.04 1.74 1.74 1.74
Parent compound Total identified Total extracted Solids Accountability Composition Composi										

Figure 6.2.3-4: Comparison of the HPLC profiles of urine of lactating goats with the pyridinylmethyl ¹⁴C- (top) and the furanone-4-¹⁴C-label (bottom)



Metabalites in the polar region (Fu1 in Figure 6.2.3-4) of the acetonitrile/water extract of kidney, liver and prine 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 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 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 \leq -18 °C or for a short time in a refrigerator. All samples of milk, and edible organs and tissues were extracted within approxiseven, weeks after sample collection. The first metabolite profile was pecorded not fater than two days after the start of the extraction and sample preparation. The first metabolite profiles were used for the quantification of metabolites, except for the profile of milk. The quantification of metabolites, except for the profile of milk. The quantification of metabolites, except for the profile of milk. 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 [for an one 4-14C] BYI 02960 in the lactating goat can be characterised by the following observations:

• The concentration of radioactivity in milk and edible tissues were relatively high compared to the dose level and the dosing period of five days. This was mainly caused by the partial instability of the radiolabel used. For a small portion of the total dose the turanone ring obviously underwent extensive biotransformation of 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 ${}^{14}CO_2$ as can be derived from the lower over of recovery compared to the study with the metabolically stable pyriomylmethyl label.

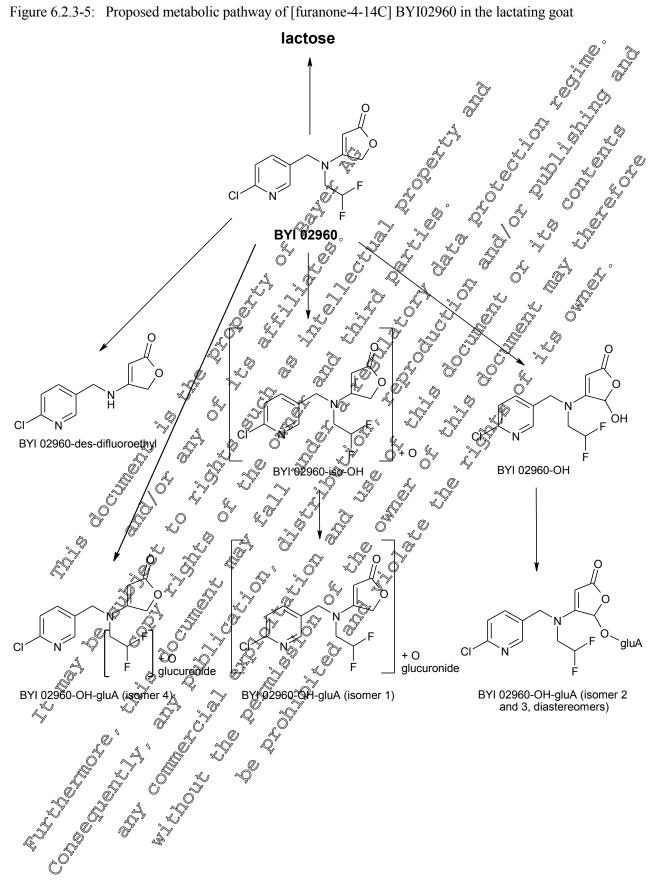
- However, the fact should be considered that an exaggerated dose level of 28.82 mg/kg feed/day was administered. Furthermore, the significant amount of radioactivity detected in urine and faeces and the relatively high radioactivity in liver and kidney at sacrifice indicate that the residues are further metabolised and finally eliminated.
- The total radioactive residues in milk showed a diurnal pattern after the second administration as they beclined 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%.

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- 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 glucaronic 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 furancene ring forming small carbon units it . 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 hydrox dation and conjugation in the 5-position of the furamonearing, one isomer (B) 02960 OH-gluA, isomer 4) with the hydroxylation and conjugation in the difluor withyl side chain and one isomer • Cleavage of the difluoroethy Ugroup forming BY 102960 des-diffuoroethy 1 (BYI 02960-OH-gluA, isomer 1) with the hydroxylation and conjugation in an unknow position

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 studiegon 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 603 (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-structur which are of known potential toxicological concern.

The dominating constituent of the residue in the milk and the edible organs and tissues of the got 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 lover were determined as glucuronide conjugates of BYI 02960-QF and as BYI 02960 pippuric 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 goar. 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 knidey and liver also at low concentrations ($< 0.020 \text{ mg/k} Q^2$)

The only major goat metabolite@vhich was not found in the cat is By 1 02960 cystemyl 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-chloronicothic acid with cysteine under loss of the chlorine atom. The compound is year polar and easily exercitable via urine which is supported by the fact that it was only found in kidney and fiver but not in the peripheral compartments of muscle or fat. In this context it hould also be noted that the lactating goat was dosed at an exaggerated dose level of ca. 24 mg/kg dry feed which is approximately of times higher as the 1X dose level in the cashe 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 toxic trogical concern became apparent. Thus a pig metabolism study was rendered annecessar

Nature of residue in fish IIA 6.2.5

The draft European data requirements (SANCO/17802/2010 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 feet 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." Ŀ

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 accumulation in fish as indicated by an octanol/water partition coefficient less than approximately