Document Title

Summary of the residues in or on treated products, food and feed for Fluopyram

Data Requirement(s)

Regulation (EC) No 1107/2009 & Regulation (EC) No 283/2013

Document MCA

Section 6: Residues in or on treated products, food and feed

According to the Guidance Document SANCO/10181/2013 for applicants on preparing dossiers for the approval of a chemical active substance

Date

2021-02-26

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Version history

Date [yyyy-mm-dd]	Data points containing amendments or additions[1] and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4, 'How to revise an Assessment Report'.

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CA 6 RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED

Fluopyram (AE C656948) was included in Annex I to Council Directive 91/414/EEC in 2013 (Regulation (EU) No 802/2013, Entry into Force on August 22, 2013). This Supplementary Dossier contains only data which were not submitted at the time of the Annex I inclusion of fluopyram under Council Directive 91/414/EEC and which were therefore not evaluated during the first EU review. All data which were already submitted by Bayer AG (former Bayer CropScience) for the Annex I inclusion under Council Directive 91/414/EEC are contained in the Draft Assessment Report (DAR) and its Addenda and are included in the Baseline Dossier provided by Bayer AG.

CA 6.1 Storage stability of residues

Data on the storage stability of fluopyram in plant matrices was submitted and reviewed for the first approval of fluopyram according to Reg (EC) 1107/2009. The studies described in the DAR and the addendum to the DAR are still considered adequate.

New storage stability studies, including all analytical targets, have been conducted in various plant matrices. These studies have been conducted to cover all commodity groups. The available data are summarised within the following tables.

Overall, the stability of residues of fluopyram and its metabolites is well demonstrated:

- Fluopyram : 3 years in all commodity category, 6 months in honey
- Fluopyram-benzamide : 3 years in all commodity category, 6 months in honey
- Fluopyram-pyridyl-acetic-acid : 6 months in acidic matrix, 6 months in honey and 3 years in all other commodity category
- Fluopyram-pyridyl-carboxilic-acid : 3 years in all commodity category, 6 months in honey
- Fluopyram-7-hydroxy : 3 years in all commodity category except acidic matrices (2 years), 6 months in honey
- Fluopyram-methyl-sulfoxide : 2 years in all commodity category, 6 months in honey

A new storage stability study of fluopyram-pyridyl-acetic-acid in acidic matrix (strawberry) is ongoing and will be submitted by January 2022.

No storage stability study on residues of fluopyram and its metabolites in animal matrices was conducted. Thus, all samples from the poultry and cattle feeding studies were analysed within 30 days.

CA 6.1.1 Storage of residue samples

CA 6.1.2 Storage stability in plant and animal matrices

Table 6.1.2- 1: Overview of stability data for fluopyram residues in samples stored under deep-frozen conditions (< -18°C)

Commodity	Category	Analytes	Maximum storage period covered	Reference
Already EU-reviewed data (Baseline dossier)				
orange (fruit)	acid	FLU FLU-benzamide FLU-PAA FLU-PCA	6 months (interim)	M-298687-01-1
Cereal (green material	water	FLU-methyl-sulfoxide	3 months	M-274729-02-1

Commodity	Category	Analytes	Maximum storage period covered	Reference
Cereal grain Cereal straw	starch dry matrix		24 months 18 months	
orange (fruit)	acid	FLU FLU-benzamide FLU-PAA FLU-PCA	36 months 36 months 6 months 36 months	M-356046-02-1
lettuce (head) rape (seed) dry pea (seed) wheat (grain)	water oil protein starch	FLU FLU-benzamide FLU-PAA FLU-PCA FLU-7-OH	36 months	M-299461-03-2
rape (seed) dry pea (seed) orange (fruit)	oil protein acid	FLU-7-OH FLU-methyl-sulfoxide	24 months	M-389465-01-1
cabbage (head) wheat grain potato tuber grape (bunches)	water starch acid	FLU-PCA	30 months	M-237350-01-1
New data				
Tomato (fruit) wheat (green material) rape (seed) dry pea (seed) wheat (grain) potato (tuber) grapes (bunches)	water oil protein starch acid	FLU FLU-benzamide FLU-7-OH	8h (at -1°C) then 7d (at -7°C)	M-480441-06-1
cucumber (fruit) sunflower (seed) dry bean (seed) barley (grain) strawberry (fruit)	water oil protein starch acid	FLU-methyl-sulfoxide	24 months	M-754395-01-1
honey	sugar	FLU FLU-benzamide FLU-PAA FLU-PCA FLU-7-OH FLU-methyl-sulfoxide	6 months	M-681002-02-2

Table 6.1.2- 2: Summary of stability data for fluopyram residues in plant products (< -18°C)

Category	Commodity	Maximum storage period covered	document	Dossier Reference
Fluopyram				
water	lettuce head	36 months	M-299461-03-2	KCA 6.1.2/03
oil	rape seed	36 months	M-299461-03-2	KCA 6.1.2/03
protein	dry pea	36 months	M-299461-03-2	KCA 6.1.2/03
starch	wheat grain	36 months	M-299461-03-2	KCA 6.1.2/03
acid	orange fruit	36 months	M-356046-02-1	KCA 6.1.2/02
miscellaneous	honey	6 months	M-681002-02-2	KCA 6.1.2/08
Fluopyram-benzamide				
water	lettuce head	36 months	M-299461-03-2	KCA 6.1.2/03
oil	rape seed	36 months	M-299461-03-2	KCA 6.1.2/03
protein	dry pea	36 months	M-299461-03-2	KCA 6.1.2/03
starch	wheat grain	36 months	M-299461-03-2	KCA 6.1.2/03
acid	orange fruit	36 months	M-356046-02-1	KCA 6.1.2/02
miscellaneous	honey	6 months	M-681002-02-2	KCA 6.1.2/08
Fluopyram-pyridyl-acetic-acid				
water	lettuce head	36 months	M-299461-03-2	KCA 6.1.2/03
oil	rape seed	36 months	M-299461-03-2	KCA 6.1.2/03
protein	dry pea	36 months	M-299461-03-2	KCA 6.1.2/03
starch	wheat grain	36 months	M-299461-03-2	KCA 6.1.2/03
acid	orange fruit	6 months	M-356046-02-1	KCA 6.1.2/02
miscellaneous	honey	6 months	M-681002-02-2	KCA 6.1.2/08
Fluopyram-pyridyl-carboxylic-acid				
water	lettuce head	36 months	M-299461-03-2	KCA 6.1.2/03
oil	rape seed	36 months	M-299461-03-2	KCA 6.1.2/03
protein	dry pea	36 months	M-299461-03-2	KCA 6.1.2/03
starch	wheat grain	36 months	M-299461-03-2	KCA 6.1.2/03
acid	orange fruit	36 months	M-356046-02-1	KCA 6.1.2/02
miscellaneous	honey	6 months	M-681002-02-2	KCA 6.1.2/08
Fluopyram-7-hydroxy				
water	lettuce head	36 months	M-299461-03-2	KCA 6.1.2/03
oil	rape seed rape seed	36 months 24 months	M-299461-03-2 M-389465-01-1	KCA 6.1.2/03 KCA 6.1.2/04

Category	Commodity	Maximum storage period covered	document	Dossier Reference
protein	dry pea dry pea	36 months 24 months	M-299461-03-2 M-389465-01-1	KCA 6.1.2/03 KCA 6.1.2/04
starch	wheat grain	36 months	M-299461-03-2	KCA 6.1.2/03
acid	orange fruit	24 months	M-389465-01-1	KCA 6.1.2/04
miscellaneous	honey	6 months	M-681002-02-2	KCA 6.1.2/08
Fluopyram-methyl-sulfoxide				
water	cereal green material cucumber fruit	3 months 24 months	M-274729-02-1 M-754395-01-1	KCA 6.1.2/01 KCA 6.1.2/07
oil	rape seed sunflower seed	24 months 24 months	M-389465-01-1 M-754395-01-1	KCA 6.1.2/04 KCA 6.1.2/07
protein	dry pea dry bean	24 months 24 months	M-389465-01-1 M-754395-01-1	KCA 6.1.2/04 KCA 6.1.2/07
starch	cereal grain barley grain	24 months 24 months	M-274729-02-1 M-754395-01-1	KCA 6.1.2/01 KCA 6.1.2/07
acid	orange fruit strawberry fruit	24 months 24 months	M-389465-01-1 M-754395-01-1	KCA 6.1.2/04 KCA 6.1.2/07
miscellaneous	cereal straw honey	18 months 6 months	M-274729-02-1 M-681002-02-2	KCA 6.1.2/01 KCA 6.1.2/08

Table 6.1.2- 3: Summary of the deep-frozen storage stability periods for each commodity type, for each analytical target

Analytes	Commodity Categories	Acceptable Maximum Storage duration
Fluopyram	Plant - high water content	36 months
	Plant - high oil content	36 months
	Plant - high protein content	36 months
	Plant - high starch content	36 months
	Plant -high acid content	36 months
	High sugar content	6 months
FLU-benzamide	Plant - high water content	36 months
	Plant - high oil content	36 months
	Plant - high protein content	36 months
	Plant - high starch content	36 months
	Plant -high acid content	36 months
	High sugar content	6 months

Analytes	Commodity Categories	Acceptable Maximum Storage duration
FLU-pyridyl-acetic-acid	Plant - high water content	36 months
	Plant - high oil content	36 months
	Plant - high protein content	36 months
	Plant - high starch content	36 months
	Plant -high acid content	36 months
	High sugar content	6 months
FLU-pyridyl-carboxylic-acid	Plant - high water content	36 months
	Plant - high oil content	36 months
	Plant - high protein content	36 months
	Plant - high starch content	36 months
	Plant -high acid content	36 months
	High sugar content	6 months
FLU-7-hydroxy	Plant - high water content	36 months
	Plant - high oil content	36 months
	Plant - high protein content	36 months
	Plant - high starch content	36 months
	Plant -high acid content	24 months
	High sugar content	6 months
FLU-methyl-sulfoxide	Plant - high water content	18 months
	Plant - high starch content	24 months
	Plant - high oil content	24 months
	Plant - high protein content	24 months
	Plant - high acid content	24 months
	High sugar content	6 months

Data already evaluated during the first EU review process for inclusion on Annex I.

Data Point:	KCA 6.1/01
Report Author:	[redacted]
Report Year:	2008
Report Title:	Letter granting BayerCropScience permission to cite exclusive use fluopicolide data submitted by Valent U.S.A. Corporation
Report No:	M-300103-01-1
Document No:	M-300103-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	--
Previous evaluation:	
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	

Data Point:	KCA 5.2/02
Report Author:	[redacted]
Report Year:	2008
Report Title:	Letter of Access granting BayerCropScience permission to cite exclusive use data submitted by Valent U.S.A. Corporation for fluopicolide technical fungicide, sub. No. 2007-4477
Report No:	M-300426-01-1
Document No:	M-300426-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	
Previous evaluation:	submitted for status of evaluation unclear
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	

Data Point:	KCA 6.1/03
Report Author:	[redacted]
Report Year:	2008
Report Title:	Phase report: 6 months stability in orange of study 07-02 - Storage stability of residues of AE C656948 and its metabolites (AE F148815, AE C657188 and BCS-AA10139) in orange during deep freeze storage for up to 24 months
Report No:	MR-08/036
Document No:	M-298687-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC amended by the Commission directive 2032/VI/95 rev. (1997); US EPA Residue Chemistry Test Guideline OPPTS 860.1380: Storage Stability Data
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted rev. 1 to Vol.3 of DAR B7 August 2012 (references relied on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

This 6 months interim study report was finally included in a full final report which is summarised in the present dossier under reference M-356046-02-1 (KCA 6.1.2/02).

Data Point:	KCA 6.1/04
Report Author:	[redacted]
Report Year:	2007
Report Title:	Storage stability of AE C638206 and its metabolites AE C657378 (3-OH-BAM), AE C653711 (BAM) and AE 1344122 (P1x) in/on cereals (rest of plant, grain, straw) for 25 months
Report No:	MR-178/04
Document No:	M-274729-02-1
Guideline(s) followed in study:	not specified
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

This study was conducted in the frame of the fluopicolide (AE C638206) project. It is considered to be relevant for this fluopyram EU renewal as AE 1344122 (Alias Fluopyram-methyl-sulfoxide) is a common metabolite between these two fungicides.

The purpose of this study was to determine the storage stability of residues of fluopicolide and its metabolites AE C657378, AEC653711 (BAM) and AE 1344122 (alias Fluopyram-methyl-sulfoxide) in fortified control samples of plant origin (wheat straw, grain, green material) during freezer storage at ≤- 18 °C for 25 months.

For the purpose of this dossier, the following summary will give results only for FLU-methyl-sulfoxide (AE 1344122) which is a common metabolite of fluopicolide and fluopyram.

I. Materials and Methods

To determine the freezer storage stability of a common metabolite of FLU-methyl-sulfoxide in cereals (wheat straw, grain, and green material), 5 g aliquots of the homogenised control materials were weighed into the bottles and were fortified with the spiking solutions containing the four analytes, resulting in a fortification level of 0.10 mg/kg in all matrices. After fortification, the solvent was allowed to evaporate for about 15 min. In addition, Untreated samples of each sample material were prepared for control and recovery experiments. Subsequently, the bottles were closed and deep-frozen until analysis (at ≤ -18 °C), except for the day-0 samples (five spiked samples, two control samples and two control samples for method validation recoveries).

The boxes containing the sample material for control samples were also stored in frozen conditions and were analysed at the nominal storage intervals of 0, 30, 90, 180, 360, 540, and 760 days.

On day 0 (zero-time analyses) five spiked samples and two control samples were analyzed. Since these samples are recovery samples, it was not necessary to include concurrent recoveries.

At each sampling interval at least three fortified and five control samples were removed from the deep-freezer and allowed to reach room temperature (nominal day 30 and 90 only three fortified and three control

samples were removed from the deep-freezer). Subsequently, two of the control samples of each sample material were fortified with the test items to determine the concurrent recoveries (fortification levels were at the same magnitude as the spiked storage samples). The samples were extracted and analysed concurrently with the third control sample and the spiked storage samples.

In order to determine residues of FLU-methyl-sulfoxide in cereals matrices the analytical method 00782/M002 was used (Schoning, R. and [redacted] 01/09/2003, M-226098-02-2, see MCA section 4.1.2). Despite not being fully validated according to SANCO/3029/99 rev.4, the method is considered fit for purpose

Briefly, residues of FLU-methyl-sulfoxide were extracted with a mixture of acetone/water adjusted with sulphuric acid to pH 2. After addition of L-cysteine hydrochloride (250 mg/L) and filtration, the extract is filled up to volume. An aliquot is concentrated to the aqueous remainder. The solution is again adjusted to pH 2, NaCl is added and partitioned twice against MTBE. For the determination of FLU-methyl-sulfoxide, an aliquot is concentrated to dryness and dissolved in a mixture of acetonitrile/water/ammonium acetate. The residues are quantified by reversed phase HPLC and MS/MS-detection.

The limit of quantitation (LOQ) is 0.01 mg/kg in all tested sample materials.

For quantitation, the matrix-matched standards were used.

II. Findings

In control samples, residues of FLU-methyl-sulfoxide were below 30 % LOQ.

Summaries of concurrent recoveries conducted as a part of this study are presented in the Table 6.1.2- 4. The obtained mean concurrent recoveries per fortification level of FLU-methyl-sulfoxide ranged between 63 % – 80 % for wheat straw, 71 % – 86 % for wheat grain, and 66 % – 117 % for wheat green material.

Summaries of the residue behaviour of FLU-methyl-sulfoxide in stored samples are presented in the Table 6.1.2- 5.

- Uncorrected mean recoveries in wheat straw show a stability period of 18 month for deep frozen residues (mean recoveries ranged between 73 % and 89 %).
- Uncorrected mean recoveries in wheat grain show a stability period of 24 month for deep frozen residues (mean recoveries ranged between 70 % – 98 %).
- Uncorrected mean recoveries in wheat green material show a stability period of 90 days for deep frozen residues (mean recoveries ranged between 70 % – 85 %). As the fresh concurrent recovery means in green materials are quite low (below 70%), the apparent instability of the residues after storage can actually be attributed to a low analytical method performance.

III. Conclusions

Residues of FLU-methyl-sulfoxide fortified to control samples of wheat grain at 0.1 mg/kg were stable for at least 760 days. In wheat straw, the residues were stable for at least 540 days. In wheat green material a stability of 90 days only could be demonstrated. This result can be attributed to the low performance of the analytical method as the concurrent recoveries are also very low. A new storage stability study (M-754395-01-1, see CA 6.1.2.7) show better results.

Assessment and conclusion by applicant:

Residues FLU-methyl-sulfoxide in wheat grain at 0.1 mg/kg were stable for at least 760 days. In wheat straw, the residues were stable for at least 540 days. In wheat green material a stability of 90 days only could be demonstrated.

Table 6.1.2- 4: Concurrent recovery data for FLU-methyl-sulfoxide

Plant material	Fortification Level [mg/kg]	Nominal Storage Interval (days)	FLU-methyl-sulfoxide Single Recoveries [%]*	Mean [%]	RSD [%]	Standard deviation
Wheat (straw)	0.10	0	73; 76; 83; 93; 76	80	10.0	8.0
		30	75 ; 46**	75	-	-
		90	79 ; 79	79	-	-
		180	68 ; 72	70	-	-
		360	69 ; 73	71	-	-
		540	81 ; 80	80	-	-
		760	61 ; 65	63	-	-
	Overall Mean, RSD and standard deviation [%]			**75**	**10.2**	**7.7**
Wheat (grain)	0.10	0	61;79; 82; 64; 83	74	14.3	10.5
		30	74; 77	76	-	-
		90	85; 76	81	-	-
		180	88; 83	86	-	-
		360	82; 85	84	-	-
		540	75; 71; 68	71	4.6	3.5
		760	89; 64	77	-	-
	Overall Mean, RSD and standard deviation [%]			**77**	**11.1**	**8.6**
Wheat (green material)	0.10	0	74; 79; 79; 82; 73	78	**4.2**	**3.3**
		30	76; 78	77	-	-
		90	95; 87	91	-	-
		180	65; 67	66	-	-
		360	83; 86	84		-
		540	117; 118	117		-
		760	67; 65	66	-	-
	Overall Mean, RSD and standard deviation [%]			**82**	**19.2**	**15.7**

RSD: relative standard deviation
If sample size (n) < 2, then standard deviation is not a consideration; only the mean is reported
Fortification as: **FLU-methyl-sulfoxide ;** determination as: **FLU-methyl-sulfoxide ;** calculated as: **Fluopyram**
*On day 0 (zero time analyses) five spiked samples and two control samples were analyzed. Since these samples are recovery samples, it was not necessary to include concurrent recoveries at 0-time.
**this recovery was excluded from the data set and is not included in the mean calculation

Table 6.1.2- 5: Storage stability data and concurrent recovery data for FLU-methyl-sulfoxide (fortification at 0.10 mg/kg)

Commodity	Nomial Storage Period (days)	FLU-methyl-sulfoxide Residue Level in Stored Samples			Day 0 Normalized % Recovery*	Average % of Fresh Concurrent Recoveries	Mean Corrected % Recovery*
		mg/kg	% of nominal spiking level	Mean % recovery			
Wheat (straw)	0	0.073 0.076 0.083 0.093 0.076	73 76 83 93 76	80	100	80	80
	30	0.095 0.079 0.091	95 79 91	88	110	75*	117
	90	0.077 0.096 0.093	77 96 93	89	111	79	113
	180	0.072 0.074 0.074	72 74 74	73	92	70	104
	360	0.077 0.077 0.082	77 77 82	79	98	71	111
	540	0.079 0.077 0.080	79 77 80	79	98	80	99
	700	0.059 0.060 0.056	59 60 56	58	73	63	92
Wheat (grain)	0	0.061 0.079 0.082 0.064 0.083	61 79 82 64 83	74	100	74	100
	30	0.095 0.100 0.100	95 100 100	98	133	76	129
	90	0.094 0.080 0.108	94 80 108	94	127	81	116
	180	0.061 0.077 0.071	61 77 71	70	94	86	81

Commodity	Nomial Storage Period (days)	FLU-methyl-sulfoxide Residue Level in Stored Samples			Day 0 Normalized % Recovery[a]	Average % of Fresh Concurrent Recoveries	Mean Corrected % Recovery[b]
		mg/kg	% of nominal spiking level	Mean % recovery			
	360	0.080 0.090 0.089	80 90 89	86	117	84	102
	540	0.074 0.067	74 67 *	71	95	71	100
	760	0.074 0.077 0.082	74 77 82	78	105	77	101
Wheat (green material)	0	0.074 0.079 0.079 0.082 0.075	74 79 79 82 75	78	100	78	100
	30	0.071 0.071 0.069	71 71 69	70	90	77	91
	90	0.078 0.085 0.092	78 85 92	85	109	91	93
	180	0.069 0.068 0.064	69 68 64	67	86	66	102
	360	0.082 0.080 0.081	82 80 81	81	104	84	96
	540	0.091 0.114 0.117	91 114 117	107	138	117	91
	760	0.056 0.062 0.065	56 62 65	61	78	66	92

a Day-0 Normalized Recovery = (Average recovery / average recovery at day 0) × 100%
b Mean Corrected percent recovery = (Mean % recovery (stored) / Average of fresh concurrent recoveries) × 100%
*One recovery sample was identified as outlier, therefore was removed from the data set

Data Point:	KCA 6.1/05
Report Author:	[redacted]
Report Year:	2010
Report Title:	Storage stability of residues of AE C656948 and its metabolites (AE F148815, AE C657188 and BCS-AA10139) in orange during deep freeze storage for up to 36 months
Report No:	07-02
Document No:	M-356046-02-1
Guideline(s) followed in study:	EU Directive 91/414/EEC amended by the Commission directive 7032/VI/95 rev.5 (1997) US EPA Residue Chemistry Test Guideline, OPPTS 860.1380: Storage Stability Data OECD Test Guideline 506, adopted 16 October 2007
Deviations from current test guideline:	Deviation to OECD 506 : samples spiked at 20xLOQ instead of 10X. Nevertheless, stability of residues is still assessable at this level.
Previous evaluation:	yes, evaluated and accepted rev. 2 to Vol.3 of DAR of 17 November 2017 (reference cited as)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/officially recognised testing facilities
Acceptability/Reliability:	Yes

The study was initiated to evaluate the stability of fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl-carboxylic acid (AE C657188), fluopyram-pyridyl-acetic acid (BCS-AA10139) in matrices of plant origin (orange fruits) during freezer storage period of 36 months (at ≤ -18 °C).

I. Materials and Methods

To determine the freezer storage stability of the relevant residues of fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl-acetic acid (BCS-AA10139, alias FLU-PAA), and fluopyram-pyridyl-carboxylic acid (alias AE C657188, alias FLU-PCA) in matrices of plant origin (orange fruits), 10-g aliquots specified as ''spiked samples'' individual samples were fortified with 100 µL of the spiking solution at 20 mg/L, resulting in a concentration of 0.20 mg/kg of fluopyram or FLU-benzamide or FLU-PCA or FLU-PAA. The plastic bottles were sealed and stored in frozen conditions immediately after the fortification. The boxes containing the sample material for control samples were also stored in frozen conditions (at ≤ -18 °C) and were analysed at the nominal storage intervals of 0, 3 6, 12, 18, 24, and 36 months.

Concurrent recovery experiments were performed at all storage intervals by spiking control samples with a mixture of fluopyram, FLU-benzamide, FLU-PAA or FLU-PCA or at a level of 0.01 and 0.20 mg/kg.

On day 0 (zero time analysis) three spiked samples per test item and one control sample were analysed. In parallel, four concurrent recoveries were conducted: one at the level of 0.01 mg/kg and three at 0.20 mg/kg.

All samples were analysed according to the analytical method 00984 ([redacted], 05/02/2007, M-283301-01-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Briefly, residues were extracted from 10 g of sample material (5g for straw) by two successive extractions using a high speed blender with a mixture of acetonitrile:water (80:20; v:v). After centrifugation the extract volume was adjusted. Then, the extracts were diluted ten times by adding the internal standards:

- dilution performed under acidic conditions and measured in negative electrospray ionization for the determination of FLU-PCA.
- dilution performed under basic conditions and measured in positive electrospray ionization for the determination of fluopyram, FLU-benzamide and FLU-PAA.

*Due to its instability, the analytical standard of fluopyram-pyridyl-acetic acid (BCS-AA10139) was made available under its sodium salt form (BCS-AA10189) which was used as reference item.

The limit of quantitation (LOQ) (expressed as parent fluopyram equivalents) for each single analyte is 0.01 mg/kg in all tested sample materials.

The quantification was carried out using internal stable labelled standards.

II. Findings

In control samples, residues of fluopyram, FLU-benzamide, FLU-PAA and FLU-PCA were below the Limit of Quantification (< 0.01 mg/kg for each test item expressed as fluopyram). Summaries of concurrent recoveries conducted as a part of this study are presented in the Table 6.1.2- 6 to Table 6.1.2- 9. The obtained individual and overall mean concurrent recoveries were satisfactory and were in the range of 70 -110 %, with the RSD < 20% for all tested analytes at two fortification levels.

- For fluopyram, uncorrected mean recoveries in orange show a stability period of 36 months for deep frozen residues (mean recoveries ranged between 93 % and 107 %).
- For FLU-benzamide, uncorrected mean recoveries in orange show a stability period of 36 months for deep frozen residues (mean recoveries ranged between 86 % – 102 %).
- For FLU-PCA, uncorrected mean recoveries in orange show a stability period of 36 months for deep frozen residues (mean recoveries ranged between 92 % – 102 %).
- For FLU-PAA, uncorrected mean recoveries in orange show a stability period of 6 months for deep frozen residues (mean recoveries ranged between 78 % – 91 %).

III. Conclusions

Residues of fluopyram and its metabolites (fluopyram-benzamide, fluopyram-pyridyl-carboxylic-acid) fortified at 0.2 mg/kg to control samples of orange (fruit) were stable during deep-frozen storage for at least 36 months. Residues of FLU-pyridyl-acetic acid were stable in orange fruit only over 6 months of storage

Assessment and conclusion by applicant:

The study is acceptable.
Residues FLU, FLU-benzamide, FLU-PCA in orange (fruit) at 0.2 mg/kg were shown to be stable for 36 months.
Residues of FLU-pyridyl-acetic-acid were stable in orange (fruit) only over 6 months of storage.

Table 6.1.2- 6: Concurrent recovery data for fluopyram (AE C656948)

Plant material	Fortification Level [mg/kg]	Storage Interval (days)	Fluopyram Single Recoveries [%]	Mean [%]	RSD [%]	Standard deviation
Orange (fruits)	0.01	0	97	97	-	-
		3	85	85	-	-
		6	87	87	-	-
		12	86	86	-	-
		19	90	90	-	-
		24	95	95	-	-
		36	101	101	-	-
	0.2	0	105; 102; 103	103	1.5	1.5
		3	95; 95	95	-	-
		6	98; 101	100	-	-
		12	99; 96	98	-	-
		19	96; 95 ; 90	93	3.1	2.9
		24	93 ; 93 ; 93	94	1.2	1.2
		36	105 ; 104 ; 113	107	4.6	4.9
	Overall Mean, RSD and standard deviation [%]			**97**	**6.8**	**6.6**

RSD: relative standard deviation
If sample size (n) ≤ 2, then standard deviation is not a consideration; only the mean is reported
Fortification as: **fluopyram;** determination as: **fluopyram;** calculated as: **fluopyram**

Table 6.1.2- 7: Concurrent recovery data for fluopyram-benzamide

Plant material	Fortification Level [mg/kg]	Storage Interval (days)	FLU-benzamide Single Recoveries [%]	Mean [%]	RSD [%]	Standard deviation
Orange (fruits)	0.01	0	104	104	-	
		3	98	98	-	
		6	104	104	-	-
		12	106	106	-	-
		19	103	103	-	
		24	83	83		-
		36	96	96		-
	0.2	0	102; 102; 102	102	0.0	0.0
		3	92; 93	93	-	
		6	93; 93	93	-	-
		12	96 ; 91	94	-	-
		19	93 ; 88 ; 90	90	2.8	2.5
		24	91 ; 89 ; 90	90	1.1	1.0
		36	94 ; 97 ; 100	97	3	3.0
	Overall Mean, RSD and standard deviation [%]			**96**	**6.3**	**6.0**

RSD: relative standard deviation
If sample size (n) ≤ 2, then standard deviation is not a consideration; only the mean is reported
Fortification as: **FLU-benzamide;** determination as: **FLU-benzamide;** calculated as: **Fluopyram**

Table 6.1.2- 8: Concurrent recovery data for fluopyram-pyridyl-acetic-acid

Plant material	Fortification Level [mg/kg]	Storage Interval (days)	FLU-PAA Single Recoveries [%]	Mean [%]	RSD [%]	Standard deviation
Orange (fruits)	0.01	0	88	88	-	-
		3	82	82	-	-
		6	77	77	-	-
		12	79	79	-	-
		19	78	78	-	-
		24	70	70	-	-
		36	81	81	-	-
	0.2	0	88; 89; 88	88	0.7	0.6
		3	91; 91	91	-	-
		6	82; 79	81	-	-
		12	72 ; 72	72	-	-
		19	83 ; 87 ; 84	85	2.5	2.1
		24	83 ; 86 ; 81	83	3.0	2.5
		36	78 ; 76 ; 82	79	3.9	3.1
	Overall Mean, RSD and standard deviation [%]			**82**	**7.2**	**5.9**

RSD: relative standard deviation
If sample size (n) ≤ 2, then standard deviation is not a consideration; only the mean is reported
Fortification as: **FLU-PAA;** determination as: **FLU-PAA;** calculated as: **Fluopyram**

Table 6.1.2- 9: Concurrent recovery data for fluopyram-pyridyl-carboxilic-acid

Plant material	Fortification Level [mg/kg]	Storage Interval (days)	FLU-PCA Single Recoveries [%]	Mean [%]	RSD [%]	Standard deviation
Orange (fruits)	0.01	0	96	96	-	-
		3	101	101	-	-
		6	95	95	-	-
		12	103	103	-	-
		19	84	84	-	-
		24	77	77	-	-
		36	79	79	-	-
	0.2	0	98; 99; 99	99	0.6	0.6
		3	98; 100	99	-	-
		6	92; 98	95	-	-
		12	96; 106	101	-	-
		19	94; 92 ; 94	93	1.2	1.2
		24	94 ; 97 ; 98	96	2.2	2.1
		36	96 ; 97 ; 94	96	1.6	1.5
	Overall Mean, RSD and standard deviation [%]			**95**	**6.9**	**6.6**

RSD: relative standard deviation
If sample size (n) ≤ 2, then standard deviation is not a consideration; only the mean is reported
Fortification as: **FLU-PCA;** determination as: **FLU-PCA;** calculated as: **Fluopyram**

Table 6.1.2- 10: Storage stability data for fluopyram (fortification at 0.200 mg/kg)

Commodity	Storage Period (days)	Fluopyram Residue Level in Stored Samples			Day 0 Normalized % Recovery[a]	Average % of Fresh Concurrent Recoveries	Mean Corrected % Recovery[b]
		mg/kg	% of nominal spiking level	Mean % recovery			
Orange (fruit)	0	0.220 0.204 0.220	110 102 110	107	100	103	104
	3	0.200 0.197 0.201	100 99 101	100	94	97	103
	6	0.206 0.210 0.207	103 105 103	104	97	100	104
	12	0.208 0.193 0.205	104 97 102	101	94	98	103
	19	0.204 0.197 0.182	102 98 91	97	91	93	104
	24	0.189 0.185 0.177	95 95 89	93	87	94	99
	36	0.204 0.220 0.220	102 110 110	107	100	107	100

a Day-0 Normalized Recovery = (Average recovery / average recovery at day 0) × 100%
b Mean Corrected percent recovery = (Mean % recovery (stored) / Average of fresh concurrent recoveries) × 100%

Table 6.1.2- 11: Storage stability data for fluopyram-benzamide (fortification at 0.200 mg/kg)

Commodity	Storage Period (days)	FLU-benzamide Residue Level in Stored Samples			Day 0 Normalized % Recovery[a]	Average % of Fresh Concurrent Recoveries	Mean Corrected % Recovery[b]
		mg/kg	% of nominal spiking level	Mean % recovery			
Orange (fruit)	0	0.207 0.205 0.203	104 102 101	102	100	102	100
	3	0.174 0.169 0.172	87 84 86	86	84	93	92

Commodity	Storage Period (days)	FLU-benzamide Residue Level in Stored Samples			Day 0 Normalized % Recovery[a]	Average % of Fresh Concurrent Recoveries	Mean Corrected % Recovery[b]
		mg/kg	% of nominal spiking level	Mean % recovery			
	6	0.173 0.169 0.178	86 85 89	87	85	93	93
	12	0.189 0.202 0.200	94 101 100	98	96	94	105
	19	0.175 0.191 0.181	88 96 91	92	90	90	102
	24	0.182 0.180 0.185	91 90 92	91	89	90	101
	36	0.207 0.193 0.201	103 96 101	100	98	97	103

a Day-0 Normalized Recovery = (Average recovery / average recovery at day 0) ×100%
b Mean Corrected percent recovery = (Mean % recovery (stored) / Average of fresh concurrent recoveries) × 100%

Table 6.1.2- 12: Storage stability data for fluopyram-pyridyl-acetic-acid (fortification at 0.200 mg/kg)

Commodity	Storage Period (days)	FLU-PAA Residue Level in Stored Samples			Day 0 Normalized % Recovery[a]	Average % of Fresh Concurrent Recoveries	Mean Corrected % Recovery[b]
		mg/kg	% of nominal spiking level	Mean % recovery			
Orange (fruit)	0	0.184 0.182 0.180	92 91 90	91	100	88	103
	3	0.158 0.166 0.172	79 83 86	83	91	91	91
	6	0.159 0.154 0.152	80 77 76	78	86	81	96
	12	0.120 0.111 0.116	60 55 58	58	64	72	80
	19	0.125 0.129 0.119	62 64 59	62	68	85	73
	24	0.115 0.116 0.114	57 58 57	57	63	83	69
	36	0.0947 0.0957 0.0969	47 48 48	48	53	79	60

a Day-0 Normalized Recovery = (Average recovery / average recovery at day 0) × 100%
b Mean Corrected percent recovery = (Mean % recovery (stored) / Average of fresh concurrent recoveries) × 100%

Table 6.1.2- 13: Storage stability data for fluopyram-pyridyl-carboxilic-acid (fortification at 0.200 mg/kg)

Commodity	Storage Period (days)	FLU-PCA Residue Level in Stored Samples			Day 0 Normalized % Recovery[a]	Average % of Fresh Concurrent Recoveries	Mean Corrected % Recovery[b]
		mg/kg	% of nominal spiking level	Mean % recovery			
Orange (fruit)	0	0.208 0.205 0.199	104 103 100	102	100	99	103

Commodity	Storage Period (days)	FLU-PCA Residue Level in Stored Samples			Day 0 Normalized % Recovery[a]	Average % of Fresh Concurrent Recoveries	Mean Corrected % Recovery[b]
		mg/kg	% of nominal spiking level	Mean % recovery			
	3	0.190 0.193 0.191	95 97 95	96	94	99	97
	6	0.191 0.188 0.196	96 94 98	96	94	95	101
	12	0.186 0.196 0.185	93 98 93	95	93	101	94
	19	0.185 0.185	92 93	93	91	93	99
	24	0.183 0.183	92 91	92	90	96	95
	36	0.204 0.187	102 94	98	96	96	102

a Day-0 Normalized Recovery = (Average recovery / average recovery at day 0) × 100%
b Mean Corrected percent recovery = (Mean % recovery (stored) / Average of fresh concurrent recoveries) × 100%

Data Point:	KCA 6.1/06
Report Author:	[redacted]
Report Year:	2010
Report Title:	Storage stability of residues of AE C656948 and its metabolites (AE F148815, AE C657188, BCS-AA10139 and BCS-AA10065) in plants during deep freeze storage for up to 36 months
Report No:	06-06
Document No:	M-299461-03-2
Guideline(s) followed in study:	EU Directive 91/414/EEC amended by the Commission directive 7032/VI/95 rev.5 (1997); US EPA Residue Chemistry Test Guideline, OPPTS 860.1380: Storage Stability Data; OECD Test Guideline 506, adopted 16 October 2007
Deviations from current test guideline:	Deviation to OECD 506 : samples spiked at 2xLOQ instead of 10X. Nevertheless, stability of residues is still assessable at this level.
Previous evaluation:	yes, evaluated and accepted rev. 1 to Vol.3 of DAR of 17 August 2012 (references relied on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/officially recognised testing facilities
Acceptability/Reliability:	Yes

The study was initiated to evaluate the stability of fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl-carboxylic acid (AE C657188), fluopyram-pyridyl-acetic acid (BCS-AA10139) and fluopyram-7-hydroxy (BCS-AA10065) in matrices of plant origin (lettuce head, wheat grain, dry pea seed, and rape seed) during freezer storage (at ≤ -18 °C).

6. Materials and methods

To determine the freezer storage stability of the relevant residues of fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl-carboxylic acid (AE C657188, alias FLU-PCA), fluopyram-pyridyl-acetic acid (BCS-AA10139, alias FLU-PAA) and fluopyram-7-hydroxy (BCS-AA10065) in matrices of plant origin (lettuce head, wheat grain, dry pea seed, and rape seed), individual samples (in form of 10 g-aliquots specified as ''spiked samples'') were fortified with fluopyram or fluopyram-benzamide or fluopyram-pyridyl-carboxylic acid or fluopyram-pyridyl-acetic acid (BCS-AA10139) (sodium salt of BCS-AA10139) or fluopyram-7-hydroxy (depending on the test system) at the level of 0.20 mg/kg. Afterwards, the samples were stored in plastic containers at ≤-18 °C and were analysed at the nominal storage intervals of 0, 3, 6, 13, 18, 24, and 36 months.

In lettuce head (high water content matrix) and wheat grain (high starch content matrix) the following analytes were tested: fluopyram, fluopyram-benzamide, fluopyram-pyridyl-acetic acid and fluopyram-7-hydroxy.

In dry pea seed (protein content matrix) and rape seed (oil content matrix) the following analytes were tested: fluopyram, FLU-benzamide, FLU-PAA and FLU-PCA.

To demonstrate the accuracy of the fluopyram and its metabolites determination during this study, recovery experiments were performed prior to sample storage (initial method validation) and at each storage interval by fortifying stored control samples with all the test items.

A set of three recoveries at 0.01 mg/kg and at 0.20 mg/kg for each matrix were conducted before the nominal storage interval of Day 0. For this purpose, control samples were freshly fortified with fluopyram, FLU-benzamide, FLU-PAA, FLU-PCA and FLU-7-hydroxy at 0.01 mg/kg and at 0.20 mg/kg and then analysed.

Concurrent recoveries were conducted at the nominal storage intervals of 0, 3, 6, 13, 18, 24 and 36 months. For this purpose, stored control samples were freshly fortified with a mixture of fluopyram, FLU-benzamide, FLU-PAA, FLU-PCA and FLU-7-hydroxy at 0.01 and 0.20 mg/kg. The freshly fortified samples were then extracted and analysed concurrently with the control and spiked samples of these nominal storage intervals.

In order to identify any degradations into FLU-methyl-sulfoxide (AE 1344122), this compound was quantified during each storage interval analysis. Therefore, procedural recoveries on this analyte were carried out in order to assure the validation of the results. No degrations into FLU-methyl-sulfoxide were observed during the study.

On day 0 (zero-time analysis) three spiked samples per test item and one control sample were analysed. In parallel, four concurrent recoveries were conducted: one at the level of 0.01 mg/kg and three at 0.20 mg/kg.

The stored control samples were either analysed directly as control samples (one) or freshly fortified for recoveries (four: one at 0.01 mg/kg and three at 0.20 mg/kg). The freshly fortified samples were analysed concurrently with the remaining control samples and the stored spiked samples.

All samples were analysed according to the analytical method 00984 ([redacted], 05/02/2007, M-283301-01-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Briefly, residues were extracted from 10 g of sample material (5g for straw) by two successive extractions using a high speed blender with a mixture of acetonitrile:water (80:20; v:v). After centrifugation the extract volume was adjusted. Then, the extracts were diluted ten times by adding the internal standards:

- dilution performed under acidic conditions and measured in negative electrospray ionization for the determination of FLU-PCA.
- dilution performed under basic conditions and measured in positive electrospray ionization for the determination of fluopyram, FLU-benzamide and FLU-PAA.

*Due to its instability, the analytical standard of fluopyram-pyridyl-acetic acid (BCS-AA10139) was made available under its sodium salt form (BCS-AA10189) which was used as reference item.

II. Findings

No interferences greater than the LOQ were observed in any of the control extracts from any matrix. Summaries of concurrent recoveries conducted as a part of this study are presented in the Table 6.1.2- 14 to Table 6.1.2- 19. The obtained mean concurrent recovery data were between 70 and 110% at each fortification level except for :

- FLU-benzamide in rape seed at 3 months : 124 % (20xLOQ level)
- FLU-PCA in rape seed at 24 & 36 months 64 & 66 % (20xLOQ level)

This results show the accuracy of the fluopyram, FLU-benzamide, FLU-PAA, FLU-PCA and FLU-7-hydroxy determination during the conduct of the study. The low method performance for FLU-PCA at 24 and 36 explains the low residue measured in stored samples at these timepoints.

Moreover, the overall mean concurrent recoveries are in the acceptable range of 70-110%, with the RSD < 20 % for fluopyram, FLU-benzamide, FLU-PAA, FLU-PCA, FLU-7-hydroxy, and FLU-methyl-sulfoxide (AE 1344122) in all tested matrices (lettuce head, wheat grain, dry pee seed, and rape seed).

Summaries of the residue behaviour in stored samples are presented in the Table 6.1.2- 20 to Table 6.1.2-24.

- For fluopyram, uncorrected mean recoveries in all tested matrices show a stability period of 36 months for deep frozen residues (mean recoveries ranged between 93 – 106 %, 95 – 108 %, 95 – 103 %, and 95 – 115 % in lettuce (head), wheat (grain), dry pea (seed), and rape (seed), respectively)
- For fluopyam-benzamide, uncorrected mean recoveries in all tested matrices show a stability period of 36 months for deep frozen residues (mean recoveries ranged between 84 – 96 %, 86 – 101 %, 83 – 96 %, and 85 – 124 %, in lettuce (head), wheat (grain), dry pea (seed), and rape (seed), respectively.
- For fluopyram-pyridyl-acetic acid, uncorrected mean recoveries in all tested matrices show a stability period of 36 months for deep frozen residues (mean recoveries ranged between 73 – 95 %, 82 – 95 %, 77 – 90 %, and 80 – 96 %, in lettuce (head), wheat (grain), dry pea (seed), rape (seed), respectively.
- For fluopyram-7-hydroxy uncorrected mean recoveries in lettuce (head) and wheat (grain) show a stability period of 36 months for deep frozen residues (mean recoveries ranged between 95 – 104 % and 94 – 104 %, respectively). The stability of residues of fluopyram-7-hydroxy were not tested in dry pea (seed) and rape (seed).
- For fluopyram-PCA, uncorrected mean recoveries in dry pea seed show a stability period of 36 months for deep frozen residues (mean recoveries ranged between 87 – 101 %. In rape (seed) the residues were stable over 18 months (uncorrected mean recoveries ranged between 87 – 101 %). At 24 and 36 months the uncorrected mean recovery were 67 % and 70%. As the concurrent recovery mean at 24 and 37 months is quite low (64 and 66% respectively), the low value measured in the stored sample are due to the poor efficiency of the method the day of analyses. The stability of the fluopyram-PCA residues was not tested neither in lettuce head nor in wheat (grain).

III. Conclusions

Residues of fluopyram and its metabolites (fluopyram-benzamide and fluopyram-pyridyl-acetic-acid) at 0.2 mg/kg were stable over 36 months at T ≤ -18°C in all tested matrices, i.e. in lettuce (head), wheat (grain), dry pea (seed), and rape (seed 37 months).

Residues of fluopyram-7-hydroxy were stable in lettuce (head) and wheat grain over 36 months.

Residues of fluopyram-pyridyl-carboxylic-acid were stable in dry pea (seed) over 36 months. In rape (seed), fluopyram-pyridyl-carboxylic acid showed a 18 months stability. The results at 24 and 37 months at 67% and 70% respectively can be explained by the poor efficiency of the analytical method the days of analyses as the concurrent recoveries were quite low (64% and 66%).

Assessment and conclusion by applicant:

The study is acceptable.
Residues FLU, FLU-benzamide, FLU-PAA in lettuce (head), wheat (grain), dry pea (seed) and rape (seed) at 0.2 mg/kg were shown to be stable for 36 months.
Residues of FLU-7-OH were stable in lettuce (head) and wheat (grain) over 36 months of storage.
Residues of FLU-PCA were stable in dry pea over 36 months of storage and over 18 months in rape (seed).

Table 6.1.2- 14: Concurrent recovery data for fluopyram

Plant material	Fortification Level [mg/kg]	Storage Interval (days)	Fluopyram Single Recoveries [%]	Mean [%]	RSD [%]	Standard deviation
Lettuce (head)	0.01	0	98	98	-	-
		3	94	94	-	-
		6	86	86	-	-
		13	77	77	-	-
		18	93	93	-	-
		24	91	91	-	-
		36	80	80	-	-
	0.2	0	103; 108; 105	105	2.4	2.5
		3	111; 107; 102	107	4.2	4.5
		6	101; 99; 98	99	1.5	1.5
		13	114; 111; 118	114	3.1	3.5
		18	95; 96; 96	96	0.6	0.6
		24	87; 97; 96	93	5.9	5.5
		36	96; 98; 100	98	2.0	2.0
	Overall Mean, RSD and standard deviation [%]			**98**	**9.6**	**9.5**
Wheat (grain)	0.01	0	88	88	-	-
		3	93	93	-	-
		6	94	94	-	-
		13	88	88	-	-
		18	84	84	-	-
		24	94	94	-	-
		36	99	99	-	-
	0.2	0	97; 94; 96	96	1.6	1.5
		3	105; 106; 101	104	2.5	2.6
		6	99; 99; 99	99	0.1	0.0
		13	113; 116; 114	114	1.4	1.5
		18	106; 102; 103	104	2.0	2.1
		24	97; 106; 100	101	4.5	4.6
		36	100; 95; 99	98	2.7	2.6
	Overall Mean, RSD and standard deviation [%]			**100**	**7.6**	**7.5**
Dry pea (seed)	0.01	0	103	103	-	-
		3	96	96	-	-
		6	86	86	-	-
		13	86	86	-	-
		18	93	93	-	-
		24	89	89	-	-
		36	104	104	-	-
	0.2	0	91; 91; 94	92	1.9	1.7
		3	107; 100; 106	104	3.8	3.8

Plant material	Fortification Level [mg/kg]	Storage Interval (days)	Fluopyram Single Recoveries [%]	Mean [%]	RSD [%]	Standard deviation
		6	99; 98; 97	98	1.0	1.0
		13	109; 112; 113	111	1.9	2.1
		18	96; 101; 102	100	3.2	3.2
		24	101; 96; 93	97	4.2	4.0
		36	88; 93; 93	91	3.2	2.9
	Overall Mean, RSD and standard deviation [%]			**98**	**7.6**	**7.4**
Rape (seed)	**0.01**	0	94	94	-	-
		3	99	99	-	-
		6	90	90	-	-
		13	87	87	-	-
		18	85	85	-	-
		24	85	85	-	-
		36	90	90	-	-
	0.2	0	109; 104; 99	104	4.8	5.0
		3	113; 106; 108	109	3.3	3.6
		6	97; 101; 103	100	2.8	3.1
		13	119; 98; 108	108	9.7	10.5
		18	91; 96; 91	93	3.1	2.9
		24	93; 88; 97	93	4.9	4.5
		36	94; 91; 94	93	1.9	1.7
	Overall Mean, RSD and standard deviation [%]			**98**	**8.9**	**8.7**

RSD: relative standard deviation
If sample size (n) ≤ 2, then standard deviation is not a consideration; only the mean is reported
Fortification as: **fluopyram;** determination as: **fluopyram;** calculated as: **fluopyram**

Table 6.1.2- 15: Concurrent recovery data for fluopyram-benzamide

Plant material	Fortification Level [mg/kg]	Storage Interval (days)	FLU-benzamide Single Recoveries [%]	Mean [%]	RSD [%]	Standard deviation
Lettuce (head)	0.01	0	91	91	-	
		3	102	102	-	
		6	105	105	-	-
		13	93	93	-	-
		18	101	101	-	
		24	77	77		-
		36	105	105		-
	0.2	0	101; 105; 102	103	2.0	2.1
		3	103; 102; 103	103	0.5	0.6
		6	98; 101; 93	97	4.2	4.0
		13	112; 106; 109	109	2.8	3.0
		18	89; 92; 92	91	1.9	1.7
		24	90; 91; 94	92	2.3	2.1
		36	97; 94; 95	95	1.6	1.5
	Overall Mean, RSD and standard deviation [%]			**98**	**8**	**7.5**
Wheat (grain)	0.01	0	91	91		
		3	102	102		
		6	104	104		
		13	99	99		
		18	80	80		
		24	99	99		
		36	102	102		
	0.2	0	91; 91; 94	92	1.9	1.7
		3	107; 106; 103	105	2.0	2.1
		6	97; 96; 94	96	1.6	1.5
		13	109; 111; 110	110	0.9	1.0
		18	102; 98; 95	98	3.6	3.5
		24	100; 95; 89	95	5.8	5.5
		36	92; 91; 96	93	2.8	2.6
	Overall Mean, RSD and standard deviation [%]			**98**	**7**	**7.2**
Dry pea (seed)	0.01	0	99	99	-	-
		3	102	102	-	-
		6	94	94	-	-
		13	88	88	-	-
		18	89	89	-	-
		24	80	80	-	-
		36	99	99	-	-
	0.2	0	90; 88; 93	90	2.8	2.5
		3	100; 98; 97	98	1.6	1.5
		6	95; 95; 93	94	1.5	1.2
		13	109; 111; 104	108	3.3	3.6
		18	93; 91; 91	92	1.3	1.2
		24	94; 89; 93	92	2.9	2.6
		36	97; 90; 92	93	3.9	3.6
	Overall Mean, RSD and standard deviation [%]			**95**	**7**	**6.6**
Rape (seed)	**0.01**	0	87	87	-	-
		3	109	109	-	-
		6	91	91	-	-

Plant material	Fortification Level [mg/kg]	Storage Interval (days)	FLU-benzamide Single Recoveries [%]	Mean [%]	RSD [%]	Standard deviation
		13	115	115	-	-
		18	114	114	-	-
		24	90	90	-	-
		36	101	101	-	-
	0.2	0	86; 99; 98	94	7.7	7.2
		3	124; 126; 123	124	1.2	1.5
		6	96; 89; 93	93	3.7	3.5
		13	116; 98; 109	107	8.0	8.6
		18	94; 90; 90	91	2.5	2.3
		24	88; 80; 83	84	4.8	4.0
		36	100; 90; 93	94	5.4	5.1
	Overall Mean, RSD and standard deviation [%]			**99**	**13**	**12.9**

Fortification as: **FLU-benzamide ;** determination as: **FLU-benzamide;** calculated as: **Fluopyram**

Table 6.1.2- 16: Concurrent recovery data for fluopyram-pyridyl-carboxilic-acid

Plant material	Fortification Level [mg/kg]	Storage Interval (days)	FLU-PCA Single Recoveries [%]	Mean [%]	RSD [%]	Standard deviation
Lettuce (head)	0.01	0	71	71	-	
		3	106	106	-	
		6	100	100	-	-
		13	83	83	-	-
		18	96	96	-	
		24	103	103	-	-
		36	85	85	-	-
	0.2	0	90; 97; 95	94	3.8	3.6
		3	101; 101; 97	100	2.3	2.3
		6	96; 96; 94	94	1.8	1.5
		13	94; 96; 99	96	2.6	2.5
		18	90; 101; 101	97	6.5	6.4
		24	91; 97; 104	97	6.7	6.5
		36	89; 97; 98	95	5	4.9
	Overall Mean, RSD and standard deviation [%]			**95**	**8**	**7.2**
Wheat (grain)	0.01	0	85	85	-	-
		3	85	85	-	-
		6	101	101	-	-
		13	87	87	-	-
		18	80	80	-	-
		24	80	80	-	-
		36	87	87	-	-
	0.2	0	92; 101; 98	97	4.7	4.6
		3	82; 88; 92	87	5.8	5.0
		6	88; 90; 88	89	1.3	1.2
		13	91; 90; 91	91	0.6	0.6
		18	92; 96; 90	93	3.3	3.1
		24	96; 91; 96	94	3.1	2.9
		36	89; 87; 95	90	4.6	4.2
	Overall Mean, RSD and standard deviation [%]			**90**	**6**	**5.5**
Dry pea (seed)	0.01	0	107	107	-	-
		3	108	108	-	-
		6	109	109	-	-
		13	86	86	-	-
		18	82	82	-	-
		24	80	80	-	-
		36	106	106	-	-
	0.2	0	88; 89; 89	89	0.7	0.6
		3	101; 101; 99	100	1.2	1.2
		6	85; 89; 90	88	3.1	2.6
		13	86; 83; 85	85	1.8	1.5
		18	83; 90; 94	89	6.3	5.6
		24	84; 88; 88	87	2.7	2.3
		36	88; 94; 89	90	3.6	3.2
	Overall Mean, RSD and standard deviation [%]			**91**	**9**	**8.4**
Rape (seed)	**0.01**	0	95	95	-	-
		3	94	94	-	-
		6	107	107	-	-

Plant material	Fortification Level [mg/kg]	Storage Interval (days)	FLU-PCA Single Recoveries [%]	Mean [%]	RSD [%]	Standard deviation
		13	116	116	-	-
		18	101	101	-	-
		24	85	85	-	-
		36	78	78	-	-
	0.2	0	97; 96; 103	99	3.8	3.8
		3	93; 89; 94	92	2.9	2.6
		6	90; 92; 90	91	1.5	1.3
		13	106; 87; 97	97	9.8	9.5
		18	105; 113; 87	102	13.1	13.3
		24	65; 61; 66	64	4.1	2.6
		36	69; 67; 62	66	5.5	3.6
	Overall Mean, RSD and standard deviation [%]			**89**	**17**	**15.4**

Fortification as: **FLU-PCA ;** determination as: **FLU-PCA ;** calculated as: **Fluopyram**

Table 6.1.2- 17: Concurrent recovery data for fluopyram-pyridyl-acetic-acid

Plant material	Fortification Level [mg/kg]	Storage Interval (days)	FLU-PAA Single Recoveries [%]	Mean [%]	RSD [%]	Standard deviation
Lettuce (head)	0.01	0	93	93	-	
		3	92	92	-	
		6	108	108	-	-
		13	91	91	-	-
		18	97	97	-	
		24	90	90		-
		36	90	90		-
	0.2	0	93; 90; 99	94	4.9	4.6
		3	104; 94; 99	99	5.1	5.0
		6	97; 96; 96	96	0.6	0.6
		13	107; 106; 111	108	2.4	2.6
		18	100; 106; 88	98	9.4	9.2
		24	91; 94; 91	92	1.9	1.7
		36	95; 86; 95	92	5.6	5.2
	Overall Mean, RSD and standard deviation [%]			**96**	**7**	**6.6**
Wheat (grain)	0.01	0	91	91	-	-
		3	90	90		-
		6	108	108	-	-
		13	85	85	-	-
		18	88	88		-
		24	89	89		-
		36	82	82	-	-
	0.2	0	84; 82; 86	84	2.4	2.0
		3	90; 89; 93	91	2.3	2.1
		6	92; 85; 85	87	4.6	4.0
		13	104; 102; 103	103	1.0	1.0
		18	94; 96; 95	95	1.1	1.0
		24	92; 85; 84	87	5.0	4.4
		36	85; 81; 77	81	4.9	4.0
	Overall Mean, RSD and standard deviation [%]			**90**	**8**	**7.5**
Dry pea (seed)	0.01	0	80	80	-	-
		3	89	89	-	-
		6	95	95	-	-
		13	88	88	-	-
		18	90	90	-	-
		24	73	73	-	-
		36	76	76	-	-
	0.2	0	83; 80; 77	80	3.8	3.0
		3	88; 90; 86	88	2.3	2.0
		6	85; 85; 83	85	1.5	1.2
		13	101; 105; 101	102	2.3	2.3
		18	87; 80; 88	85	5.1	4.4
		24	79; 74; 82	78	5.2	4.0
		36	85; 87; 83	85	2.4	2.0
	Overall Mean, RSD and standard deviation [%]			**86**	**9**	**7.8**
Rape (seed)	**0.01**	0	94	94	-	-
		3	96	96	-	-
		6	97	97	-	-

Plant material	Fortification Level [mg/kg]	Storage Interval (days)	FLU-PAA Single Recoveries [%]	Mean [%]	RSD [%]	Standard deviation
		13	98	98	-	-
		18	82	82	-	-
		24	87	87	-	-
		36	92	92	-	-
	0.2	0	94; 92; 88	91	3.3	3.1
		3	91; 91; 92	91	0.6	0.6
		6	90; 88; 88	89	1.1	1.2
		13	115; 96; 104	105	9.1	9.5
		18	88; 86; 82	85	3.6	3.1
		24	79; 82; 78	80	2.6	2.1
		36	86; 90; 81	86	5.3	4.5
	Overall Mean, RSD and standard deviation [%]			**90**	**9**	**7.8**

Fortification as: **FLU-PAA ;** determination as: **FLU-PAA ;** calculated as: **Fluopyram**

Table 6.1.2- 18: Concurrent recovery data for fluopyram-7-hydroxy

Plant material	Fortification Level [mg/kg]	Storage Interval (days)	FLU-7-OH Single Recoveries [%]	Mean [%]	RSD [%]	Standard deviation
Lettuce (head)	0.01	0	86	86	-	
		3	96	96	-	
		6	95	95	-	-
		13	87	87	-	-
		18	92	92	-	
		24	92	92		-
		36	97	97	-	-
	0.2	0	105; 111; 107	108	2.8	3.1
		3	104; 105; 102	104	1.5	1.5
		6	102; 99; 103	101	2.0	2.1
		13	108; 109; 115	111	3.4	3.8
		18	100; 96; 96	97	2.4	2.3
		24	98; 97; 96	97	1.0	1.0
		36	96; 95; 99	97	2.2	2.1
	Overall Mean, RSD and standard deviation [%]			**100**	**7**	**6.8**
Wheat (grain)	0.01	0	86	86	-	-
		3	92	92	-	-
		6	98	98	-	-
		13	87	87	-	-
		18	83	83	-	-
		24	90	90	-	-
		36	93	93	-	-
	0.2	0	98; 100; 100	99	1.2	1.2
		3	107; 106; 107	107	0.5	0.6
		6	99; 101; 100	100	1.0	1.0
		13	115; 117; 117	116	1.0	1.2
		18	97; 94; 93	95	2.2	2.1
		24	99; 101; 95	98	3.1	3.1
		36	98; 97; 97	97	0.6	0.6
	Overall Mean, RSD and standard deviation [%]			**99**	**9**	**8.4**
Dry pea (seed)	0.01	0	89	89	-	-
		3	100	100	-	-
		6	92	92	-	-
		13	86	86	-	-
		18	84	84	-	-
		24	90	90	-	-
		36	96	96	-	-
	0.2	0	94; 98; 97	96	2.2	2.1
		3	111; 108; 106	108	2.3	2.5
		6	100; 96; 100	99	2.2	2.3
		13	108; 109; 108	108	0.5	0.6
		18	93; 95; 90	93	2.7	2.5
		24	92; 94; 94	93	1.2	1.2
		36	98; 98; 101	99	1.7	1.7
	Overall Mean, RSD and standard deviation [%]			**97**	**7**	**7.1**
Rape (seed)	**0.01**	0	103	103	-	-
		3	105	105	-	-

Plant material	Fortification Level [mg/kg]	Storage Interval (days)	FLU-7-OH Single Recoveries [%]	Mean [%]	RSD [%]	Standard deviation
		6	97	97	-	-
		13	90	90	-	-
		18	95	95	-	-
		24	84	84	-	-
		36	97	97	-	-
	0.2	0	106; 102; 102	103	2.2	2.3
		3	109; 106; 103	106	2.8	3.0
		6	93; 95; 96	95	1.6	1.5
		13	120; 99; 113	111	9.7	10.7
		18	90; 88; 87	88	1.7	1.5
		24	97; 96; 98	97	1.0	1.0
		36	105; 107; 99	104	4.0	4.2
	Overall Mean, RSD and standard deviation [%]			**99**	**8**	**8.1**

Fortification as: **FLU-7-OH ;** determination as: **FLU-7-OH ;** calculated as: **Fluopyram**

Table 6.1.2- 19: Concurrent recovery data for fluopyram-methyl-sulfoxide (AE 1344122)

Plant material	Fortification Level [mg/kg]	Storage Interval (days)	FLU-methyl-sulfoxide Single Recoveries [%]	Mean [%]	RSD [%]	Standard deviation
Lettuce (head)	0.01	0	82	82	-	
		3	94	94	-	
		6	109	109	-	-
		13	101	101	-	-
		18	104	104	-	
		24	103	103		-
		36	86	86	-	-
	0.2	0	93; 103; 103	100	5.8	5.8
		3	117; 111; 115	114	2.8	3.2
		6	109; 108; 112	110	1.8	2.1
		13	103; 102; 106	104	2.0	2.1
		18	108; 107; 109	108	0.9	1.0
		24	108; 108; 107	108	0.5	0.6
		36	104; 116; 112	111	5.5	6.1
	Overall Mean, RSD and standard deviation [%]			**105**	**8**	**8.0**
Wheat (grain)	0.01	0	94	94	-	-
		3	83	83	-	-
		6	106	106	-	-
		13	92	92	-	-
		18	106	106	-	-
		24	91	91	-	-
		36	93	93	-	-
	0.2	0	101; 111; 102	105	5.3	5.5
		3	93; 96; 95	95	1.6	1.5
		6	101; 101; 97	100	2.3	2.3
		13	90; 89; 94	91	2.9	2.6
		18	108; 100; 106	105	4.0	4.2
		24	104; 107; 104	105	1.6	1.7
		36	100; 101; 103	101	1.5	1.5
	Overall Mean, RSD and standard deviation [%]			**99**	**7**	**6.7**
Dry pea (seed)	0.01	0	83	83	-	-
		3	94	94	-	-
		6	80	80	-	-
		13	107	107	-	-
		18	80	80	-	-
		24	84	84	-	-
		36	99	99	-	-
	0.2	0	90; 94; 94	93	2.5	2.3
		3	100; 102; 99	100	1.5	1.5
		6	90; 92; 94	92	1.8	2.0
		13	94; 95; 77	89	11.4	10.1
		18	99; 100; 96	98	2.1	2.1
		24	103; 109; 94	102	7.4	7.5
		36	106; 109; 104	106	2.4	2.5
	Overall Mean, RSD and standard deviation [%]			**95**	**9**	**8.7**
Rape (seed)	**0.01**	0	102	102	-	-
		3	118	118	-	-

Plant material	Fortification Level [mg/kg]	Storage Interval (days)	FLU-methyl-sulfoxide Single Recoveries [%]	Mean [%]	RSD [%]	Standard deviation
		6	78	78	-	-
		13	94	94	-	-
		18	101	101	-	-
		24	116	116	-	-
		36	104	104	-	-
	0.2	0	99; 99; 101	100	1.2	1.2
		3	107; 106; 103	105	2.0	2.1
		6	95; 96; 99	97	1.9	2.1
		13	102; 84; 97	94	9.8	9.3
		18	113; 113; 108	111	2.6	2.9
		24	110; 110; 109	110	0.5	0.6
		36	111; 108; 103	107	4.3	4.6
	Overall Mean, RSD and standard deviation [%]			**103**	**9**	**8.9**

Fortification as: **FLU-methyl-sulfoxide ;** determination as: **FLU-methyl-sulfoxide ;** calculated as: **Fluopyram**
* : this value was corrected by the suppression of the interferent present in the control sample (0.0028 mg/kg); the initial value was 132%.

In order to identify any degradations into FLU-methyl-sulfoxide (AE 1344122), this compound was quantified during each storage interval analysis. Therefore, procedural recoveries on this analyte were carried out in order to assure the validation of the results. No degradation into FLU-methyl-sulfoxide were observed during the study.

Table 6.1.2- 20: Storage stability data and concurrent recovery data for fluopyram (fortification at 0.200 mg/kg)

Commodity	Storage Period (days)	Fluopyram Residue Level in Stored Samples			Day 0 Normalized % Recovery[a]	Average % of Fresh Concurrent Recoveries	Mean Corrected % Recovery[b]
		mg/kg	% of nominal spiking level	Mean % recovery			
Lettuce (head)	0	0.208 0.193 0.207	104 97 103	101	100	105	96
	3	0.207 0.198 0.206	104 99 103	102	101	107	95
	6	0.207 0.209 0.217	104 105 109	106	105	99	107
	13	0.177 0.200 0.219	89 100 110	100	99	114	87
	18	0.212 0.191 0.203	106 96 102	101	100	96	106
	24	0.188 0.180 0.191	94 90 96	93	92	93	100
	36	0.205 0.207 0.215	103 103 107	104	103	98	106
Wheat (grain)	0	0.206 0.198 0.211	103 99 105	102	100	96	106
	3	0.197 0.199 0.202	99 99 101	100	98	104	96
	6	0.200 0.190 0.182	100 95 91	95	93	99	96
	13	0.210 0.207 0.205	105 104 103	104	102	114	91
	18	0.206 0.211 0.206	103 105 103	104	102	104	100
	24	0.208 0.193 0.209	104 97 104	102	100	101	101

Commodity	Storage Period (days)	Fluopyram Residue Level in Stored Samples			Day 0 Normalized % Recovery[a]	Average % of Fresh Concurrent Recoveries	Mean Corrected % Recovery[b]
		mg/kg	% of nominal spiking level	Mean % recovery			
	36	0.211 0.215 0.221	106 108 110	108	106	98	110
Dry pea (seed)	0	0.185 0.194 0.187	93 97 94	95	100	92	103
	3	0.202 0.199 0.202	101 99 101	100	105	104	96
	6	0.192 0.193 0.191	96 96 96	96	101	98	98
	13	0.207 0.202 0.207	104 101 103	103	108	111	93
	18	0.205 0.206 0.198	103 103 99	102	107	100	102
	24	0.201 0.199 0.183	100 100 91	97	102	97	100
	36	0.198 0.197 0.200	99 98 100	99	104	91	109
Rape (seed)	0	0.242 0.217 0.233	121 109 116	115	100	104	111
	3	0.207 0.213 0.218	103 107 109	106	92	109	97
	6	0.198 0.195 0.192	99 98 96	98	85	100	98
	13	0.197 0.199 0.193	99 100 98	99	86	108	92
	18	0.192 0.189 0.202	96 95 101	97	84	93	105
	24	0.190 0.188 0.193	95 94 96	95	83	93	102

Commodity	Storage Period (days)	Fluopyram Residue Level in Stored Samples			Day 0 Normalized % Recovery[a]	Average % of Fresh Concurrent Recoveries	Mean Corrected % Recovery[b]
		mg/kg	% of nominal spiking level	Mean % recovery			
	37	0.199 0.211 0.183	99 105 92	99	86	93	106

a Day-0 Normalized Recovery = (Average recovery / average recovery at day 0) × 100%
b Mean Corrected percent recovery = (Mean % recovery (stored) / Average of fresh concurrent recoveries) × 100%

Table 6.1.2- 21: Storage stability data and concurrent recovery data for fluopyram-benzamide (fortification at 0.200 mg/kg)

Commodity	Storage Period (days)	FLU-benzamide Residue Level in Stored Samples			Day 0 Normalized % Recovery[a]	Average % of Fresh Concurrent Recoveries	Mean Corrected % Recovery[b]
		mg/kg	% of nominal spiking level	Mean % recovery			
Lettuce (head)	0	0.183	92	93	100	103	90
		0.177	89				
		0.193	97				
	3	0.199	100	96	103	103	93
		0.192	96				
		0.185	93				
	6	0.190	95	95	102	97	98
		0.192	96				
		0.189	94				
	13	0.163	82	84	90	109	77
		0.169	85				
		0.172	86				
	18	0.183	91	96	103	91	105
		0.192	96				
		0.201	100				
	24	0.177	88	89	96	92	96
		0.176	88				
		0.179	90				
	36	0.182	91	91	98	95	96
		0.184	92				
		0.180	90				
Wheat (grain)	0	0.180	90	92	100	92	100
		0.186	93				
		0.186	93				
	3	0.203	101	101	110	105	96
		0.202	101				
		0.205	102				
	6	0.176	88	93	101	96	97
		0.191	96				
		0.190	95				
	13	0.175	88	86	94	110	78
		0.171	86				
		0.168	84				
	18	0.189	95	99	108	98	101
		0.203	101				
		0.202	101				
	24	0.183	91	90	98	95	95
		0.178	89				
		0.179	90				

Commodity	Storage Period (days)	FLU-benzamide Residue Level in Stored Samples			Day 0 Normalized % Recovery[a]	Average % of Fresh Concurrent Recoveries	Mean Corrected % Recovery[b]
		mg/kg	% of nominal spiking level	Mean % recovery			
	36	0.187 0.180 0.185	94 90 92	92	100	93	99
Dry pea (seed)	0	0.182 0.182 0.175	91 91 87	90	100	90	100
	3	0.197 0.188 0.192	98 94 96	96	107	98	98
	6	0.185 0.177 0.183	93 89 91	91	101	94	97
	13	0.164 0.163 0.170	82 82 85	83	92	108	77
	18	0.179 0.181 0.190	90 91 95	92	102	92	100
	24	0.179 0.176 0.169	90 88 85	88	98	92	95
	36	0.168 0.177 0.176	84 88 88	87	97	93	93
Rape (seed)	0	0.174 0.168 0.171	87 84 85	85	100	94	91
	3	0.252 0.244 0.248	126 122 124	124	146	124	100
	6	0.195 0.198 0.189	97 99 95	97	114	93	104
	13	0.167 0.171 0.177	84 86 89	86	101	107	81
	18	0.195 0.196 0.187	97 98 94	96	113	91	106
	24	0.168 0.175 0.171	84 87 86	86	101	84	102

Commodity	Storage Period (days)	FLU-benzamide Residue Level in Stored Samples			Day 0 Normalized % Recovery[a]	Average % of Fresh Concurrent Recoveries	Mean Corrected % Recovery[b]
		mg/kg	% of nominal spiking level	Mean % recovery			
	37	0.186 0.183 0.179	93 92 90	92	108	94	98

a Day-0 Normalized Recovery = (Average recovery / average recovery at day 0) × 100%
b Mean Corrected percent recovery = (Mean % recovery (stored) / Average of fresh concurrent recoveries) × 100%

Table 6.1.2- 22: Storage stability data and concurrent recovery data for fluopyram-pyridyl-acetic-acid (fortification at 0.200 mg/kg)

Commodity	Storage Period (days)	FLU-PAA Residue Level in Stored Samples			Day 0 Normalized % Recovery[a]	Average % of Fresh Concurrent Recoveries	Mean Corrected % Recovery[b]
		mg/kg	% of nominal spiking level	Mean % recovery			
Lettuce (head)	0	0.185 0.186 0.197	92 93 99	95	100	94	101
	3	0.190 0.193 0.178	95 97 89	94	99	99	95
	6	0.186 0.187 0.184	93 94 92	93	98	96	94
	13	0.169 0.165 0.163	85 83 82	83	87	108	77
	18	0.164 0.153 0.168	82 76 84	81	85	98	82
	24	0.157 0.163 0.159	79 81 79	80	84	92	87
	36	0.153 0.140 0.144	77 70 72	73	77	92	79
Wheat (grain)	0	0.166 0.162 0.166	83 81 83	82	100	84	98
	3	0.173 0.176 0.170	87 88 85	87	106	91	95
	6	0.168 0.173 0.172	84 87 86	86	105	87	99
	13	0.185 0.190 0.185	93 95 93	93	113	103	91
	18	0.180 0.192 0.197	90 96 99	95	116	95	100
	24	0.173 0.182 0.172	86 91 86	88	107	87	101

Commodity	Storage Period (days)	FLU-PAA Residue Level in Stored Samples			Day 0 Normalized % Recovery[a]	Average % of Fresh Concurrent Recoveries	Mean Corrected % Recovery[b]
		mg/kg	% of nominal spiking level	Mean % recovery			
	36	0.162 0.169 0.164	81 84 82	82	100	81	102
Dry pea (seed)	0	0.164 0.158 0.170	82 79 85	82	100	80	103
	3	0.174 0.173 0.180	87 87 90	88	107	88	100
	6	0.171 0.164 0.163	86 82 81	83	101	85	98
	13	0.178 0.167 0.175	89 84 87	87	106	102	85
	18	0.173 0.187 0.179	87 93 90	90	110	85	106
	24	0.156 0.154 0.150	78 77 75	77	94	78	98
	36	0.151 0.157 0.172	76 79 86	80	98	85	95
Rape (seed)	0	0.181 0.180 0.181	91 90 91	91	100	91	100
	3	0.174 0.174 0.173	87 87 86	87	96	91	96
	6	0.161 0.184 0.167	80 92 84	85	93	89	96
	13	0.192 0.192 0.190	96 96 95	96	106	105	91
	18	0.183 0.171 0.180	92 86 90	89	98	85	105
	24	0.160 0.171 0.158	80 86 79	82	90	80	102

Commodity	Storage Period (days)	FLU-PAA Residue Level in Stored Samples			Day 0 Normalized % Recovery[a]	Average % of Fresh Concurrent Recoveries	Mean Corrected % Recovery[b]
		mg/kg	% of nominal spiking level	Mean % recovery			
	37	0.153 0.168 0.157	76 84 79	80	88	86	93

a Day-0 Normalized Recovery = (Average recovery / average recovery at day 0) × 100%
b Mean Corrected percent recovery = (Mean % recovery (stored) / Average of fresh concurrent recoveries) × 100%

Table 6.1.2- 23: Storage stability data and concurrent recovery data for fluopyram-7-hydroxy (fortification at 0.200 mg/kg)

Commodity	Storage Period (days)	FLU-7-OH Residue Level in Stored Samples			Day 0 Normalized % Recovery[a]	Average % of Fresh Concurrent Recoveries	Mean Corrected % Recovery[b]
		mg/kg	% of nominal spiking level	Mean % recovery			
Lettuce (head)	0	0.209 0.197 0.218	104 98 109	104	100	108	96
	3	0.192 0.192 0.202	96 96 101	98	94	104	94
	6	0.193 0.195 0.199	97 98 100	98	94	101	97
	13	0.195 0.200 0.193	98 100 97	98	94	117	88
	18	0.186 0.184 0.205	93 92 102	96	92	97	99
	24	0.188 0.188 0.194	94 94 97	95	91	97	98
	36	0.198 0.204 0.203	99 102 101	101	97	97	104
Wheat (grain)	0	0.210 0.203 0.209	105 102 104	104	100	99	105
	3	0.200 0.203 0.203	100 102 102	101	97	107	94
	6	0.188 0.187 0.191	94 93 96	94	90	100	94
	13	0.210 0.204 0.208	105 102 104	104	100	116	90
	18	0.195 0.196 0.190	97 98 95	97	93	95	102
	24	0.199 0.193 0.189	99 96 95	97	93	98	99

Commodity	Storage Period (days)	FLU-7-OH Residue Level in Stored Samples			Day 0 Normalized % Recovery[a]	Average % of Fresh Concurrent Recoveries	Mean Corrected % Recovery[b]
		mg/kg	% of nominal spiking level	Mean % recovery			
	36	0.202 0.195 0.199	101 97 100	99	95	97	102
Dry pea (seed)	Not determined						
Rape (seed)	Not determined						

a Day-0 Normalized Recovery = (Average recovery / average recovery at day 0) × 100%
b Mean Corrected percent recovery = (Mean % recovery (stored) / Average of fresh concurrent recoveries) × 100%

Table 6.1.2- 24: Storage stability data and concurrent recovery data for fluopyram-pyridyl-carboxilic-acid (fortification at 0.200 mg/kg)

Commodity	Storage Period (days)	FLU-PCA Residue Level in Stored Samples			Day 0 Normalized % Recovery[a]	Average % of Fresh Concurrent Recoveries	Mean Corrected % Recovery[b]
		mg/kg	% of nominal spiking level	Mean % recovery			
Lettuce (head)	Not determined						
Wheat (grain)	Not determined						
Dry pea (seed)	0	0.182 0.188 0.179	91 94 89	91	100	89	103
	3	0.187 0.209 0.212	93 104 106	101	111	100	101
	6	0.193 0.188 0.189	97 94 94	95	104	88	108
	13	0.172 0.174	86 87	87	96	85	102
	18	0.173 0.174	86 87	87	96	89	98
	24	0.191 0.186 0.177	96 93 89	93	102	87	107
	36	0.164 0.180 0.181	82 90 91	88	97	90	97
Rape (seed)	0	0.183 0.167 0.178	92 83 89	88	100	99	89
	3	0.172 0.172 0.177	86 86 89	87	99	92	95
	6	0.187 0.184 0.186	94 92 93	93	106	91	102
	13	0.187 0.193 0.186	94 97 93	95	108	97	98
	18	0.194 0.207	97 104	101	115	102	99

Commodity	Storage Period (days)	FLU-PCA Residue Level in Stored Samples			Day 0 Normalized % Recovery[a]	Average % of Fresh Concurrent Recoveries	Mean Corrected % Recovery[b]
		mg/kg	% of nominal spiking level	Mean % recovery			
	24	0.135 0.134 0.133	67 67 66	67	76	64	104
	37	0.130 0.133 0.155	65 66 78	70	80	66	106

a Day-0 Normalized Recovery = (Average recovery / average recovery at day 0) × 100%
b Mean Corrected percent recovery = (Mean % recovery (stored) / Average of fresh concurrent recoveries) × 100%

Data Point:	KCA 6.1/07
Report Author:	[redacted]
Report Year:	2010
Report Title:	Storage stability of residues of AE C656948 metabolites (AE 1344122 and BCS-AA10065) in dry pea, rape and orange during deep freezer storage for up to 24 months
Report No:	MR-10/045
Document No:	M-389465-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC amended by the Commission directive 7032/VI/95 rev.5 (1997) US EPA Residue Chemistry Test Guidelines OPPTS 860.1380: Storage Stability Data OECD Test Guideline 506, adopted 16 October 2007
Deviations from current test guideline:	Deviation to OECD 506 : samples spiked at 2xLOQ instead of 10X. Nevertheless, stability of residues is still assessable at this level.
Previous evaluation:	yes, evaluated and accepted rev. 2 to Vol.3 of DAR of 17 November 2017 (reference cited in)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/officially recognised testing facilities
Acceptability/Reliability:	Yes

The purpose of this study was to determine the storage stability of residues of the fluopyram metabolites fluopyram-methyl-sulfoxide (AE 1344122) and fluopyram-7-hydroxy (BCS-AA10065) in dry pea and orange (for 25 months), and rape (for 24 months) under freezer conditions at about ≤-18 °C.

I. Materials and Methods

To determine the freezer storage stability of the relevant residues of fluopyram metabolites fluopyram-methyl-sulfoxide and fluopyram-7-hydroxy in dry pea (seed), rape (seed), and orange (fruit), 10- g aliquots specified as ''spiked samples'' individual samples were fortified with 100 µL of the spiking solution at 20 mg/L, resulting in a concentration of 0.20 mg/kg of FLU-methyl-sulfoxide or FLU-7-hydroxy. The plastic bottles were sealed and stored in frozen conditions immediately after the fortification. The boxes containing the sample material for control samples were also stored in frozen conditions (at ≤ -18 °C) and were analysed at the nominal storage intervals of 0, 3, 7, 12, 18 and 25 months for orange, at 0, 4, 7, 13, 19 and 25 months for dry pea and at 0, 3, 6, 12, 18 and 24 months for rape seed.

Concurrent recovery experiments were performed at all storage intervals by spiking control samples with a mixture of FLU-methyl-sulfoxide and FLU-7-hydroxy at a level of 0.01 and 0.20 mg/kg.

On day 0 (zero time analysis) three spiked samples per test item and one control sample were analysed. In parallel, four concurrent recoveries were conducted: one at the level of 0.01 mg/kg and three at 0.20 mg/kg.

All samples were analysed according to the analytical method 00984 ([redacted], 05/02/2007, M-283301-01-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA-4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Briefly, residues were extracted from 10 g of sample material (5g for straw) by two successive extractions using a high speed blender with a mixture of acetonitrile:water (80:20; v:v). After centrifugation the extract volume was adjusted. Then, the extracts were diluted ten times by adding the internal standards:

- dilution performed under acidic conditions and measured in negative electrospray ionization for the determination of FLU-PCA.
- dilution performed under basic conditions and measured in positive electrospray ionization for the determination of fluopyram, FLU-benzamide and FLU-PAA.

*Due to its instability, the analytical standard of fluopyram-pyridyl-acetic acid (BCS-AA10139) was made available under its sodium salt form (BCS-AA10189) which was used as reference item.

II. Findings

In control samples, residues of FLU-methyl-sulfoxide and FLU-7-hydroxy were below LOQ. Summaries of concurrent recoveries conducted as a part of this study are presented in the Table 6.1.2- 25 and Table 6.1.2- 26. The overall mean concurrent recoveries of FLU-methyl-sulfoxide and FLU-7-hydroxy were satisfactory and were in the range of 70 -110 %, with the RSD < 20% for all tested analytes at two fortification levels.

Summaries of the residue behaviour of FLU-methyl-sulfoxide and FLU-7-hydroxy in stored samples are presented in the Table 6.1.2- 27 to Table 6.1.2- 28.

- For FLU-methyl-sulfoxide, uncorrected mean recoveries in orange show a stability period of 24-25 months for deep frozen residues (mean recoveries ranged between 85 – 99%, 91 – 96%, 91 – 103%, for dry pea (seed), rape (seed), and orange (fruit), respectively)
- For FLU-7-hydroxy, uncorrected mean recoveries in orange show a stability period of 24-25 months for deep frozen residues (mean recoveries ranged between 85 – 99 %, 91 – 96 %, 91 – 103 %, for dry pea (seed), rape (seed), and orange (fruit), respectively)

III. Conclusions

Residues of FLU-methyl-sulfoxide and FLU-7-hydroxy fortified at 0.2 mg/kg to control samples were stable in dry pea (seed) and orange (fruit) for 25 months and for 24 months in rape (seed) in frozen conditions (T ≤ -18°C).

Assessment and conclusion by applicant:

The study is acceptable.
Residues FLU, FLU-methyl-sulfoxide and FLU-7-hydroxy in dry pea (seed) and orange (fruit) at 0.2 mg/kg were shown to be stable for 25 months.
Residues FLU, FLU-methyl-sulfoxide and FLU-7-hydroxy in rape (seed) at 0.2 mg/kg were shown to be stable for 24 months.

Table 6.1.2- 25: Concurrent recovery data for fluopyram-methyl-sulfoxide (AE 1344122)

Plant materials	Fortification Level [mg/kg]	Nominal Storage Interval (days)	FLU-methyl-sulfoxide Single Recoveries [%]	Mean [%]	RSD [%]	Standard deviation
	0.01	0	92	92	-	-

Plant material	Fortification Level [mg/kg]	Nominal Storage Interval (days)	FLU-methyl-sulfoxide Single Recoveries [%]	Mean [%]	RSD [%]	Standard deviation
Dry pea (seed)		4	87	87	-	-
		7	89	89	-	-
		13	73	73	-	-
		19	83	83	-	-
		25	84	84	-	-
	0.20	0	82 ; 90 ; 100	91	9.9	9.0
		4	80 ; 84	82	3.4	2.8
		7	98 ; 98	99	0.7	0.7
		13	104 ; 111	108	4.6	4.9
		19	91 ; 92	92	0.8	0.7
		25	106 ; 106 ; 100	104	3.3	3.3
	Overall Mean, RSD and standard deviation [%]			**93**	**10.9**	**10.1**
Rape (seed)	0.01	0	94	94	-	-
		3	107	107	-	-
		6	122	122	-	-
		12	93	93	-	-
		18	122	122	-	-
		24	99	99	-	-
	0.20	0	95; 104; 101	100	4.6	4.6
		3	107; 104	106	2.0	2.1
		6	104; 112	108	5.2	5.7
		12	107; 111	109	2.6	2.8
		18	102; 104	103	1.4	1.4
		24	109; 114; 115	113	2.9	3.2
	Overall Mean, RSD and standard deviation [%]			**106**	**7.7**	**8.2**
Orange (fruit)	0.01	0	100	100	-	-
		3	101	101	-	-
		7	91	91	-	-
		12	90	90	-	-
		18	103	103	-	-
		25	97	100	-	-
	0.20	0	100; 100; 100	100	0.0	0.0
		3	97; 108	103	7.6	7.8
		7	109; 109	109	0.0	0.0
		12	118; 122	120	2.4	2.8
		18	100; 102	101	1.4	1.4
		25	125; 120; 116	120	3.7	4.5
	Overall Mean, RSD and standard deviation [%]			**105**	**9.6**	**10.1**

RSD: relative standard deviation
If sample size (n) < 2, then standard deviation is not a consideration; only the mean is reported
Fortification as: **FLU-methyl-sulfoxide ;** determination as: **FLU-methyl-sulfoxide ;** calculated as: **Fluopyram**

Table 6.1.2- 26: Concurrent recovery data for Fluopyram-7-hydroxy

Plant material	Fortification Level [mg/kg]	Nominal Storage Interval (days)	FLU-7-OH Single Recoveries [%]	Mean [%]	RSD [%]	Standard deviation
Dry pea (seed)	0.01	0	90	90	-	
		4	88	88	-	
		7	87	87	-	-
		13	81	81	-	-
		19	96	96	-	
		25	88	88	-	-
	0.2	0	94; 99; 100	98	3.3	3.2
		4	98; 89	94	6.8	6.4
		7	93; 95	94	1.5	1.4
		13	96; 98	97	1.3	1.4
		19	97; 100	99	2.2	2.1
		25	91; 92; 95	93	2.2	2.1
	Overall Mean, RSD and standard deviation [%]			**93**	**5.4**	**5.1**
Rape (seed)	0.01	0	95	95	-	-
		3	85	85	-	-
		6	80	80	-	-
		12	73	73	-	-
		18	85	85	-	-
		24	87	87	-	-
	0.2	0	99; 96; 99	98	1.8	1.7
		3	97; 95	96	1.5	1.4
		6	96; 90	93	4.6	4.2
		12	94; 93	94	0.8	0.7
		18	94; 95	95	0.7	0.7
		24	101; 96; 96	98	3.0	2.9
	Overall Mean, RSD and standard deviation [%]			**92**	**7.6**	**7.0**
Orange (fruit)	0.01	0	90	90	-	-
		3	75	75	-	-
		7	99	99	-	-
		12	83	83	-	-
		18	102	102	-	-
		25	101	101	-	-
	0.2	0	101; 99; 101	100	1.2	1.2
		3	95; 94	95	0.7	0.7
		7	94; 94	94	0.0	0.0
		12	97; 100	99	2.2	2.1
		18	103; 99	101	2.8	2.8
		25	99; 107; 106	104	4.2	4.4
	Overall Mean, RSD and standard deviation [%]			**97**	**7.8**	**7.5**

RSD: relative standard deviation
If sample size (n) < 2, then standard deviation is not a consideration; only the mean is reported
Fortification as: **FLU-7-OH ;** determination as: **FLU-7-OH ;** calculated as: **Fluopyram**

Table 6.1.2- 27: Storage stability data for fluopyram-methyl-sulfoxide (AE 1344122) (fortification at 0.20 mg/kg)

Commodity	Nominal Storage Period (days)	FLU-methyl-sulfoxide Residue Level in Stored Samples			Day 0 Normalized % Recovery[a]	Average % of Fresh Concurrent Recoveries	Mean Corrected % Recovery[b]
		mg/kg	% of nominal spiking level	Mean % recovery			
Dry pea (seed)	0	0.195 0.201 0.196	98 101 98	99	100	91	109
	4	0.158 0.162 0.165	79 81 82	81	82	82	98
	7	0.206 0.203 0.193	103 102 96	100	101	99	101
	13	0.218 0.199 0.202	109 99 101	103	104	108	95
	19	0.186 0.178 0.181	93 89 91	91	92	92	99
	25	0.192 0.195 0.188	96 98 94	96	97	104	92
Rape (seed)	0	0.191 0.206 0.211	94 101 104	100	100	100	100
	3	0.209 0.216 0.211	105 108 106	106	106	106	100
	6	0.212 0.206 0.208	106 103 104	104	104	108	97
	12	0.217 0.209 0.226	109 105 113	109	109	109	100
	18	0.196 0.211 0.190	98 105 95	99	99	103	96
	24	0.196 0.209 0.218	98 104 109	104	104	113	92
Orange (fruit)	0	0.199 0.202 0.202	99 101 101	100	100	100	100
	3	0.216 0.206 0.209	108 103 104	105	105	103	102
	7	0.227 0.204 0.210	113 102 105	107	107	109	98
	12	0.239 0.229 0.223	119 115 111	115	115	120	96

Commodity	Nominal Storage Period (days)	FLU-methyl-sulfoxide Residue Level in Stored Samples			Day 0 Normalized % Recovery[a]	Average % of Fresh Concurrent Recoveries	Mean Corrected % Recovery[b]
		mg/kg	% of nominal spiking level	Mean % recovery			
	18	0.201 0.207 0.187	100 103 93	99	99	101	98
	25	0.221 0.225 0.225	110 113 113	112	112	120	93

a Day-0 Normalized Recovery = (Average recovery / average recovery at day 0) ×100%
b Mean Corrected percent recovery = (Mean % recovery (stored) / Average of fresh concurrent recoveries) × 100%

Table 6.1.2- 28: Storage stability data for fluopyram-7-hydroxy (BCS-AA10065) (fortification at 0.20 mg/kg)

Commodity	Nominal Storage Period (days)	FLU-7-OH Residue Level in Stored Samples			Day 0 Normalized % Recovery[a]	Average % of Fresh Concurrent Recoveries	Mean Corrected % Recovery[b]
		mg/kg	% of nominal spiking level	Mean % recovery			
Dry pea (seed)	0	0.196 0.201 0.198	98 101 99	99	100	98	101
	4	0.173 0.175 0.162	86 88 81	85	86	94	90
	7	0.191 0.195 0.193	95 97 97	96	97	94	102
	13	0.197 0.188 0.184	99 94 92	95	96	97	98
	19	0.183 0.184 0.192	92 92 96	93	94	99	94
	25	0.190 0.192 0.187	95 96 94	95	96	93	102
Rape (seed)	0	0.195 0.188 0.197	97 94 98	96	100	98	98

Commodity	Nominal Storage Period (days)	FLU-7-OH Residue Level in Stored Samples			Day 0 Normalized % Recovery[a]	Average % of Fresh Concurrent Recoveries	Mean Corrected % Recovery[b]
		mg/kg	% of nominal spiking level	Mean % recovery			
	3	0.182 0.175 0.189	91 88 94	91	95	96	95
	6	0.180 0.183 0.181	90 91 91	91	95	93	97
	12	0.182 0.187 0.183	91 94 91	92	96	94	98
	18	0.185 0.184 0.186	93 92 93	93	97	95	98
	24	0.191 0.190 0.189	95 95 95	95	99	98	97
Orange (fruit)	0	0.198 0.200 0.200	99 100 100	100	100	100	100
	7	0.184 0.180 0.182	92 90 91	91	91	95	96
	7	0.199 0.189 0.193	99 95 96	97	97	94	103
	12	0.202 0.201 0.205	101 100 102	101	101	99	102
	18	0.201 0.202 0.193	101 101 97	100	100	101	99
	25	0.217 0.202 0.202	108 101 101	103	103	104	99

a Day-0 Normalized Recovery = (Average recovery / average recovery at day 0) × 100%
b Mean Corrected percent recovery = (Mean % recovery (stored) / Average of fresh concurrent recoveries) × 100%

Data Point:	KCA 6.1/09
Report Author:	[redacted]
Report Year:	2004
Report Title:	Final report - Determination of the storage stability of AE C638206 and the metabolites AE C653711 (BAM) and AE C657188 (PCA) in grape, potato, cabbage, and wheat grain
Report No:	C045739
Document No:	M-237350-01-1
Guideline(s) followed in study:	OPPTS 860.1380
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and Methods

This storage stability study was conducted for the fluopicolide project. As fluopyram-pyridyl-carboxylic-acid (FLU-PCA alias AE C657188) is a common metabolite between fluopyram and fluopicolide this study is presented in this EU renewal dossier.

The fluopicolide renewal is currently ongoing and the present study was submitted to the RMS Austria.

For a sake of clarity, only the results for fluopyram-pyridyl-carboxylic-acid are reported below.

Executive summary

The study investigated the stability of fluopicolide (AE C638206) and its metabolites, M-01 (BAM, AEC653711) and M-02 (AE C657188, alias FLU-pyridyl-carboxylic-acid) in grapes, potatoes, cabbage, and wheat grain under storage conditions at < -18 °C for a period of 30 months and residues were considered to be sufficiently stable over this period.

Portions of untreated substrate were fortified with the analytes. Separate batches were prepared for the fluopicolide and its metabolites. The substrates were wheat grain, grapes, potato tubers and cabbage leaves (head).

The fortification level was at a nominal concentration (fresh weight basis) of 0.1 mg/kg. The fortified specimens were stored in a freezer at T < -18 °C.

Fluopicolide and its metabolites were analysed according to the method 00782 ([redacted] E., 13/09/2002, M-217563-02-1 see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues were extracted with acidified water and acetone. After filtration and concentration, the solution is partitioned with MTBE and an aliquot of the MTBE phase is evaporated to dryness. The residues are

dissolved in methanol and methylated under acidic conditions. Then the mixture is cleaned up on a cartridge and diluted to be injected in the LC-MS/MS system.

FLU-PCA was analysed after derivatization as methyl ester using FLU-PCA-methyl-ester (AE 0815899) as reference item. However, the residue concentrations were calculated with respect to FLU-PCA.

The residue concentrations of the analytes in the specimen extracts were calculated from the detector signal (peak area) of each peak by means of a second order calibration curve.

The limit of quantification was 0.01 mg/kg for each analyte.

Findings

At each storage interval, fluopicolide and its metabolites were determined in the stored control samples and in the stored spiked samples according to the following method:

Freshly fortified specimens with a nominal concentration of 0.1 mg/kg were analysed concurrently with each set of stored specimens. The results of these concurrent recoveries are presented in the Table 6.1.2-29 for each of the tested matrices. The concurrent recoveries meet the acceptability criteria, the mean recoveries are within the range 70 - 110% for each analyte / matrix combination and the %RSD values are <20% (where applicable). These results demonstrate acceptable method performance and support the analytical results obtained for the fortified deep-frozen samples.

Summaries of the residue behaviour of FLU-PCA in stored samples are presented in the

Table 6.1.2- 30.

- For FLU-PCA, uncorrected mean recoveries in wheat (grain) and grapes show a stability period of 30 months for deep frozen residues (mean recoveries ranged between 74 – 97% (wheat), 79 – 91 (grapes), respectively)
- For FLU-PCA, uncorrected mean recoveries in potato (tuber) show a stability period of 12 months for deep frozen residues (mean recoveries ranged between 77 and 82 %. The low recovery level in the stored samples at 18 months can be attributed to a poor performance of the analytical method the day of analyses (concurrent recovery mean at 70% only). It is confirmed by the good results at the storage intervals of 24 and 30 months were the stored samples show residue levels at 77 and 78 %. It can be considered that fluopyram-PCA is stable 30 months in potato at T=-18°C.
- For FLU-PCA, uncorrected mean recoveries in cabbage show a stability period of 12 months for deep frozen residues (mean recoveries ranged between 73 and 90%)

III. CONCLUSION

Residues of FLU-pyridyl-carboxylic acid fortified at 0.1 mg/kg to control samples were stable in wheat (grain), grapes and potato for 30 months and for 12 months in cabbage) in frozen conditions (T ≤ -18°C).

Assessment and conclusion by applicant:

The study is acceptable.
Residues FLU-PCA in grape, potato, wheat grain and 0.1 mg/kg were shown to be stable for 30 months at T ≤ -18°C.
Residues FLU-PCA in cabbage at 0.1 mg/kg were shown to be stable for 12 months at T ≤ -18°C.

Table 6.1.2- 29: Concurrent recovery data for fluopyram-PCA (AE C657188)

Plant material	Fortification Level [mg/kg]	Nominal Storage Interval (months)	FLU-PCA Single Recoveries [%]	Mean [%]	RSD [%]
Wheat grain	0.1	0	99, 94, 93, 85, 89	90	4.0
		3	81, 78	80	-
		6	73, 80	77	-
		12	86, 83	85	-
		18	75, 77	76	-
		24	76, 74	75	-
		30	78, 75	77	
	Overall Mean, RSD and standard deviation [%]			**82**	**9.2**
Grapes	0.1	0	90, 82, 93, 96, 91	90	5.84.0
		3	78, 80	79	-
		6	68, 77	73	-
		12	87, 89	88	-
		18	75, 76	76	-
		24	75, 75	75	-
		30	92, 84	88	
	Overall Mean, RSD and standard deviation [%]			**83**	**9.5**
Potato tuber	0.1	0	71, 83, 87, 90, 81	83	8.8
		3	72, 72	72	-

Plant material	Fortification Level [mg/kg]	Nominal Storage Interval (months)	FLU-PCA Single Recoveries [%]	Mean [%]	RSD [%]
		6	79, 87	83	-
		12	96, 83	90	-
		18	71, 81	76	-
		24	73, 68	71	-
		30	86, 89	88	
			Overall Mean, RSD and	**81**	**9.9**
Cabbage leaves (head)	0.1	0	85, 84, 93, 98, 93	91	6.6
		3	74, 70	72	-
		6	75, 74	75	-
		12	77, 73	75	-
		18	71, 73	72	-
		24	72, 77	75	
		30	78, 81	80	
	Overall Mean, RSD and standard deviation [%]			**79**	**10.4**

RSD: relative standard deviation
If sample size (n) ≤ 2, then standard deviation is not a consideration; only the mean is reported
Fortification as: **FLU-PCA ;** determination as: **FLU-PCA-methylester ;** calculated as: **Fluopyram**

Table 6.1.2- 30: Storage stability data for fluopyram-PCA (AE C657188) following storage at T=-18°C

Commodity	Nominal Storage Period (days)	Spike level (mg/kg)	FLU-PCA Residue Level in Stored Samples			Day 0 Normalized % Recovery[a]	Average % of Fresh Concurrent Recoveries	Mean Corrected % Recovery[b]
			mg/kg	% of nominal spiking level	Mean % recovery			
Wheat grain	0	0.104	0.093 0.098 0.097 0.088 0.093	85 89 89 93 94	90	-	90	100
	3		0.075 0.083	72 80	76	84	80	95
	6		0.080 0.082	77 79	78	87	77	101
	12		0.089 0.114	86 110	97	108	85	114
	18		0.080 0.084	77 81	79	88	76	104
	24		0.082 0.082	79 80	80	89	75	107
	30		0.074 0.079	71 76	74	82	77	96
Grape		0.104	0.085 0.094 0.095 0.097 0.100	82 90 91 93 96	91		90	101
			0.085 0.090	82 87	84	92	79	106
			0.089 0.091	86 88	87	96	73	119
			0.087 0.091	84 88	86	95	88	98

Commodity	Nominal Storage Period (days)	Spike level (mg/kg)	FLU-PCA Residue Level in Stored Samples			Day 0 Normalized % Recovery[a]	Average % of Fresh Concurrent Recoveries	Mean Corrected % Recovery[b]
			mg/kg	% of nominal spiking level	Mean % recovery			
Potato	0	0.104	0.077	74	75	82	76	99
			0.079	76				
			0.090	87	89	98	75	119
			0.093	91				
			10*	991*	77	85	88	88
			0.080	77				
			0.074	71	82		83	99
			0.084	81				
			0.086	83				
			0.090	87				
			0.094	90				
	3		0.081	78	80	98	72	111
			0.086	83				
	6		0.078	75	77	94	83	93
			0.081	78				
	12		0.083	80	81	99	90	90
			0.086	82				
	18		0.058	56	57	70	76	75
			0.062	59				
	24		0.078	75	77	94	71	108
			0.082	79				
	30		0.081	79	78	95	88	89
			0.082	77				
Cabbage	0	0.104	0.087	84	90		91	99
			0.088	85				
			0.096	93				
			0.097	93				
			0.102	98				
	3		0.072	69	73	81	72	101
			0.080	77				

Commodity	Nominal Storage Period (days)	Spike level (mg/kg)	FLU-PCA Residue Level in Stored Samples			Day 0 Normalized % Recovery[a]	Average % of Fresh Concurrent Recoveries	Mean Corrected % Recovery[b]
			mg/kg	% of nominal spiking level	Mean % recovery			
	6		0.080 0.084	77 81	79	88	75	105
	12		0.079 0.080	76 76	76	84	75	101
	18		0.071 0.072	69 69	69	77	72	96
	24		0.085 0.087	82 83	83	92	75	111
	30		0.062 0.071	60 68	64	71	80	80

a Day-0 Normalized Recovery = (Average recovery / average recovery at day 0) × 100%

b Mean Corrected percent recovery = (Mean % recovery (stored) / Average of fresh concurrent recoveries) × 100%

* The analysed concentration was unrealistic. The result was considered as an anomaly and was therefore excluded from the formal stability data.

New studies

Data Point:	KCA 6.1/10
Report Author:	[redacted]
Report Year:	2017
Report Title:	Amendment no. 3 to final report - 7 days freezer storage stability study with different combinations of a total of 61 analytes (parent and metabolite molecules) and five matrix types (high water / acidic / starch / protein / oil)
Report No:	S13-03307
Document No:	M-480441-06-1
Guideline(s) followed in study:	Commission Regulation (EU) No 544/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the data requirements for active substances US EPA Residue Chemistry Test Guideline OPPTS 860.1380: Storage Stability Data OECD Test Guideline 506, adopted 16 October 2007
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted Study was not found in DAR/RAR and the Addenda
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and methods

A study was completed to determine the stability of residues of fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl-carboxylic acid (alias AE C657188, alias FLU-PCA) and fluopyram-7-hydroxy (BCS AA10065) in fortified control samples of material of plant origin (tomato (fruit), wheat (green material), grape (bunches), wheat (grain), potato (tuber), peas (dry peas) and oilseed rape (seeds) during storage for a period of 8 hours at +1°C followed by 7 days of storage at -7°C.

This study was conducted in order to cover frozen temperature exceedance during the sample shipment of residue trials.

The spiking solutions were prepared in acetonitrile for fluopyram, in acetonitrile/water (1/9) pH8+ammonia for Fluopyram-benzamide and for fluopyram-7-hydroxy and in acetonitrile/0.5% acetic acid (1/9) for fluopyram-pyridyl carboxylic acid.

The sample materials were homogenized in a cutter with dry ice. Individual aliquots were prepared by filling 50 mL Sarstedt tubes with 5 g of specimen material. Samples were fortified with 1.0 mg/kg of fluopyram and its metabolites. Some other aliquots were not spiked to be used as control sample and procedural recoveries. The storage containers were labelled with a unique sample number, the study number, sample material, storage interval and the compound analysed and then sealed and placed in a freezer at ±1°C immediately after the fortification. After 8 hours the storage containers were placed in a freezer at -7°C for 7 days.

On day 0 (zero-time analysis) five spiked samples per test item and one control sample were analysed to confirm the fortification level and performance of the method.

At each storage interval, one stored control sample and procedural recoveries were analysed concurrently with the stored spiked samples.

Residues of fluopyram in/on plant material were determined by HPLC-MS/MS according to BCS method 01207 based on the QuEChERS multi-residue method ([redacted] S., 11/12/2013, M-424756-02-1, see section MCA 4.1.2). Extraction of residues was done with acetonitrile/water (4/1, v/v) by shaking. An aliquot of the extract was taken and, when relevant, internal stable labelled standards were added. The solution was subjected to LC-MS/MS.

Quantification was performed with matrix matched calibration solutions and double injection of each sample and calibration solution. The Limit of Quantification (LOQ) of BCS Method 01207 is defined as 0.01 mg/kg as validated in the original report S10-00279.

After the day 7 analysis, a decrease of the recoveries in the stored samples was observed for tomato (fruit), wheat (green material) and peas (dry peas); this was not the case of the fresh fortifications. Following this observation, an extended extraction time was applied to the stored samples which led to better recoveries. Therefore, longer extraction times were applied for additional re-analysis and confirmation after 22 days storage at -7°C for wheat (green material) and after 30 days of storage at -7°C for tomato (fruit) and peas (dry peas).

Residues of the metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl-carboxylic acid (alias AE C657188, alias FLU-PCA) and fluopyram-7-hydroxy (BCS AA10065) in/on plant material were determined by HPLC-MS/MS according to analytical method 00984 ([redacted], 05/02/2007, M-283301-01-1, see MCA section 4.1.2). Extraction of residues was done using two successive extractions using a blender with acetonitrile / water (8/2, v/v). After centrifugation the extract volume was adjusted. Then, the extracts were diluted ten times by adding the internal standards. One first dilution was done under acidic conditions for the determination of fluopyram-pyridyl carboxylic acid. Another dilution was performed under basic conditions for the determination of fluopyram-benzamide and fluopyram-7-hydroxy. These extracts was analysed by HPLC-MS/MS.

Quantification was performed using calibration solutions in acetonitrile/ water (1/9, v/v) pH 8 or acetonitrile/ 0.5% acetic acid (1/9, v/v) and the internal standards in the same concentration as in the sample ex-tracts (4 ng/mL).

The Limit of Quantification (LOQ) of the method 00984 is defined as 0.01 mg/kg as validated in the original report.

Prior to the storage stability tests a method validation was performed using BCS methods 01207 and 00984.

Results and discussions

The performance of the analytical method was good during the conduct of the whole study. Indeed, mean concurrent recoveries were deemed acceptable (between 70 and 110%) and RSD was below 20%, as shown in Table 6.1.2- 32.

For the untreated samples as well for the treated samples, a sufficient number of samples has been tested for each storage period. In the control samples, the apparent residues were below 30% of the LOQ. The results of the spiked stored samples are summarised in the Table 6.1.2- 33.

Fluopyram and its metabolites were shown to be stable in all tested matrices during storage after 8 hours at +1°C followed by at least 7 days at -7°C.

Table 6.1.2- 31: Residue relative decrease (%) compared to initial analysis.

Commodity category	Sample material	FLU	FLU-benzamide	FLU-PCA	FLU-7-OH
High water	tomato green material	3* 10*	0 1	-5 -3	-3 1
High acid	grape	4	-6	-1	2
High starch	wheat grain potato	19 15	2 12	- -	0 -2
High protein	peas	-7*	4	-7	-4
High oil	rape seed	7	0	-	-10

*: The extracts were shaken for 15 minutes on day 30 compared to 2 minutes on day 7, which led to better recoveries. This longer extraction procedure was applied for all samples thereafter.

Conclusion

The storage stability demonstrated that the parent fluopyram as well as its metabolite fluopyram-benzamide, FLU-PCA and FLU-7-OH are stable for fluctuations of the temperature between +1 and -7 °C for at least one week in all commodity categories. Therefore, no degradation of residues is expected when fluctuation of temperature occurs due to defrost cycles or other reasons during the storage or the sample shipment.

Assessment and conclusion by applicant:

The study is acceptable.
Residues FLU-benzamide, FLU-PCA and FLU-7-OH are shown to be stable at a temperature between +1°C and -7°C during one week.

Table 6.1.2- 32: Concurrent recoveries of fluopyram and its metabolites from different matrices.

Matrix	Spike level (mg/kg)	Storage Interval (days)	Sample size (n)	Individual procedural recoveries (%)	Mean ±RSD (%)
fluopyram (AE C656948)					
tomato (fruit)	1.0	0	5	93,83,93,97,96	92 ±6.0
	1.0	7*	2	99,97	98
	1.0	30*	2	93,92	93
	Overall Mean, RSD and standard deviation [%] (n=9)				94 ±5.0
Wheat (green material)	1.0	0	5	81, 87, 82, 88, 100	88 ±8.6
	1.0	7*	2	86,91	89
	1.0	22*	2	97,93	95
	Overall Mean, RSD and standard deviation [%] (n=9)				89 ±3.3
Grape (bunches)	1.0	0	5	97,95,96,97,93	96 ±1.8
	1.0	7	2	95,91	93
	Overall Mean, RSD and standard deviation [%] (n=7)				95 ±2.3
Wheat (grain)	1.0	0	5	96,97,95,98,98	97 ±1.3
	1.0	7	2	85,88	87
	Overall Mean, RSD and standard deviation [%] (n=7)				94 ±5.6
Potato (tuber)	1.0	0	5	90,93,92,93,94	92 ±1.6
	1.0	7	2	96,96	96
	Overall Mean, RSD and standard deviation [%] (n=7)				93 ±2.3
Peas (dry peas)	1.0	0	5	88,72,80,89,81	82 ±8.4
	1.0	7*	2	81,85	83
	1.0	30*	2	99,92	96
	Overall Mean, RSD and standard deviation [%] (n=9)				85 ±9.2
Oilseed rape (seeds)	1.0	0	5	95,93,89,86,89	90 ±4.0
	1.0	7	2	90,86	88
	Overall Mean, RSD and standard deviation [%] (n=7)				90 ±3.7
Fluopyram-benzamide (AE F148815)					
tomato (fruit)	1.0	0	5	106,104,103,104,105	104 ±1.1
	1.0	7	2	106,107	107
	Overall Mean, RSD and standard deviation [%] (n=7)				105 ±1.3
Wheat (green material)	1.0	0	5	97,95,81,79,84	87 ±9.5
	1.0	7	2	78,87	83
	Overall Mean, RSD and standard deviation [%] (n=7)				86 ±8.8
	1.0	0	5	98,97,93,88,102	96 ±5.6

Matrix	Spike level (mg/kg)	Storage Interval (days)	Sample size (n)	Individual procedural recoveries (%)	Mean ±RSD (%)
Grape (bunches)	1.0	7	2	98,96	97
	Overall Mean, RSD and standard deviation [%] (n=7)				96 ±4.6
Wheat (grain)	1.0	0	5	98,96,98,89,102	97 ±4.9
	1.0	7	2	94,100	97
	Overall Mean, RSD and standard deviation [%] (n=7)				97 ±4.4
Potato (tuber)	1.0	0	5	100,117,94,117,104	106 ±9.7
	1.0	7	2	93,105	99
	Overall Mean, RSD and standard deviation [%] (n=7)				104 ±9.4
Peas (dry peas)	1.0	0	5	110,91,97,107,95	100 ±8.1
	1.0	7	2	94,90	92
	Overall Mean, RSD and standard deviation [%] (n=7)				98 ±8.0
Oilseed rape (seeds)	1.0	0	5	80,77,71,75,77	76 ±4.4
	1.0	7	2	83,71	77
	Overall Mean, RSD and standard deviation [%] (n=7)				76 ±5.8
AE C656948-pyridyl carboxylic acid (AE C657188)					
tomato (fruit)	1.0	0	5	99,100,91,87,88	93± 6.6
	1.0	7	2	93,108	101
	Overall Mean, RSD and standard deviation [%] (n=7)				95 ±7.9
Wheat (green material)	1.0	0	5	91,83,87,89,82	86 ±4.5
	1.0	7	2	81,89	85
	Overall Mean, RSD and standard deviation [%] (n=7)				86 ±4.6
Grape (bunches)	1.0	0	5	100,100,100,102,95	99 ±2.6
	1.0	7	2	96,99	98
	Overall Mean, RSD and standard deviation [%] (n=7)				99 ±2.5
Peas (dry peas)	1.0	0	5	93,87,84,89,90	89 ±3.8
	1.0	7	2	94,92	93
	Overall Mean, RSD and standard deviation [%] (n=7)				90 ±3.9
AE C656948-7-hydroxy (BCS-AA10065)					
tomato (fruit)	1.0	0	5	104,108,106,107,94	104 ±5.5
	1.0	7	2	110,110	110
	Overall Mean, RSD and standard deviation [%] (n=7)				106 ±5.2
Wheat (green material)	1.0	0	5	98,96,92,95,95	95 ±2.3
	1.0	7	2	94,101	98
	Overall Mean, RSD and standard deviation [%] (n=7)				96 ±3.0
Grape (bunches)	1.0	0	5	109,109,110,105,106	108 ±2.0
	1.0	7	2	94,108	101

Matrix	Spike level (mg/kg)	Storage Interval (days)	Sample size (n)	Individual procedural recoveries (%)	Mean ±RSD (%)
	Overall Mean, RSD and standard deviation [%] (n=7)				106 ±5.2
Wheat (grain)	1.0	0	5	109,111,110,104,108	108 ±2.5
	1.0	7	2	106,111	109
	Overall Mean, RSD and standard deviation [%] (n=7)				108 ±2.4
Potato (tuber)	1.0	0	5	109,99,105,105,107	105 ±3.6
	1.0	7	2	110,110	110
	Overall Mean, RSD and standard deviation [%] (n=7)				106 ±3.7
Peas (dry peas)	1.0	0	5	101,100,107,97,106	102 ±4.1
	1.0	7	2	106,101	104
	Overall Mean, RSD and standard deviation [%] (n=7)				103 ±3.7
Oilseed rape (seeds)	1.0	0	5	104,96,99,103,90	98 ±5.8
	1.0	7	2	108,109	109
	Overall Mean, RSD and standard deviation [%] (n=7)				101 ±6.7

*The extracts were shaken for 15 minutes on day 30 compared to 2 minutes on day 7, which led to better recoveries. This longer extraction procedure was applied for all samples thereafter.

Table 6.1.2- 33: Stability of residues of fluopyram and its metabolites in different matrices following storage at -7°C

Matrix	Spike level (mg/kg)	Storage interval (days)	Individual recovered residues (mg/kg)	Mean	Individual recoveries (%)	Mean	Relative decrease %**
		fluopyram					
Tomato (fruit)	1.0	0	0.934, 0.833, 0.927, 0.973, 0.963	0.926	93, 83, 93, 97, 96	92	-
	1.0	7*	0.791, 0.803, 0.838, 0.818, 0.847	0.819	79, 80, 84, 82, 85	82	**11**
	1.0	30*	0.871, 0.920, 0.933, 0.852, 0.857	0.887	87, 92, 93, 85, 86	89	**3**
Wheat (green material)	1.0	0	0.807, 0.867, 0.822, 0.877, 0.996	0.874	81, 87, 82, 88, 100	88	-
	1.0	7*	0.661, 0.708, 0.738, 0.706, 0.653	0.693	66, 71, 74, 71, 65	69	**22**
	1.0	22*	0.796, 0.792, 0.794	0.794	80, 79, 79	79	**10**
Grape (bunches)	1.0	0	0.973, 0.954, 0.959, 0.971, 0.925	0.956	97, 95, 96, 97, 93	96	-
	1.0	7	0.949, 0.893, 0.914, 0.941, 0.929	0.925	95, 89, 91, 94, 93	92	**4**
Wheat (grain)	1.0	0	0.965, 0.973, 0.946, 0.980, 0.984	0.970	96, 97, 95, 98, 98	97	-
	1.0	7	0.793, 0.779, 0.768, 0.810, 0.801	0.790	79, 78, 77, 81, 80	79	**19**
	1.0	0	0.903, 0.934, 0.915, 0.926, 0.940	0.924	90, 93, 92, 93, 94	92	-

Matrix	Spike level (mg/kg)	Storage interval (days)	Individual recovered residues (mg/kg)	Mean	Individual recoveries (%)	Mean	Relative decrease %**
Potato (tuber	1.0	7	0.774, 0.790, 0.774, 0.774, 0.814	0.785	77, 79, 77, 77, 81	78	**15**
Peas (dry peas)	1.0	0	0.879, 0.723, 0.796, 0.888, 0.812	0.820	88, 72, 80, 89, 81	82	**-**
	1.0	7*	0.789, 0.798, 0.819, 0.791, 0.674	0.774	79, 80, 82, 79, 67	77	**6**
	1.0	30*	0.907, 0.817, 0.945, 0.840, 0.895	0.881	91, 82, 94, 84, 89	88	**-7**
Oilseed rape (seeds)	1.0	0	0.946, 0.928, 0.894, 0.858, 0.887	0.903	95, 93, 89, 86, 89	90	**-**
	1.0	7	0.834, 0.814, 0.859, 0.828, 0.867	0.840	83, 81, 86, 83, 87	84	**7**
		fluopyram-benzamide					
Tomato (fruit)	1.0	0	1.061, 1.044, 1.033, 1.044, 1.045	1.045	106,104,103,104,105	104	**-**
	1.0	7	1.054, 0.911, 1.049, 1.123, 1.063	1.040	105, 91, 105, 112, 106	104	**0**
Wheat (green material)	1.0	0	0.974, 0.955, 0.808, 0.788, 0.843	0.874	97, 95, 81, 79, 84	87	**-**
	1.0	7	0.847, 0.834, 0.811, 0.867, 0.922	0.836	85, 83, 81, 87, 92	86	**1**
Grape (bunches)	1.0	0	0.978, 0.972, 0.927, 0.880, 1.020	0.955	98, 97, 93, 88, 102	96	**-**
	1.0	7	1.009, 1.062, 1.084, 1.009, 0.961	1.025	101, 106, 108, 101, 96	102	**-6**
Wheat (grain)	1.0	0	0.979, 0.956, 0.983, 0.895, 1.019	0.966	98, 96, 98, 89, 102	97	**-**
	1.0	7	0.911, 0.877, 0.909, 0.879, 1.046	0.944	91, 98, 91, 88, 105	95	**2**
Potato (tuber	1.0	0	1.000, 1.170, 0.939, 1.170, 1.042	1.064	100, 117, 94, 117, 104	106	**-**
	1.0	7	0.887, 0.923, 0.981, 0.996, 0.858	0.929	89, 92, 98, 100, 86	93	**12**
Peas (dry peas)	1.0	0	1.102, 0.905, 0.970, 1.074, 0.954	1.001	110, 91, 97, 107, 95	100	**-**
	1.0	7	0.873, 1.028, 0.942, 0.964, 0.989	0.959	87, 103, 94, 96, 99	96	**4**
Oilseed rape (seeds)	1.0	0	0.803, 0.771, 0.714, 0.754, 0.775	0.763	80, 77, 71, 75, 77	76	**-**
	1.0	7	0.709, 0.781, 0.849, 0.734, 0.738	0.756	71, 75, 85, 73, 74	76	**0**
		AE C657188 (fluopyram-pyridyl carboxylic acid)					
Tomato (fruit)	1.0	0	0.989, 0.997, 0.906, 0.872, 0.881	0.929	99, 100, 91, 87, 88	93	**-**
	1.0	7	0.967, 0.955, 0.987, 0.999, 0.981	0.978	97, 95, 99, 100, 98	98	**-5**
Wheat (green material)	1.0	0	0.907, 0.833, 0.871, 0.886, 0.816	0.863	91, 83, 87, 89, 82	86	**-**
	1.0	7	0.883, 0.941, 0.871, 0.864, 0.880	0.888	88, 94, 87, 86, 88	89	**-3**
Grape (bunches)	1.0	0	1.000, 1.003, 0.998, 1.016, 0.948	0.993	100, 100, 100, 102, 95	99	**-**

Matrix	Spike level (mg/kg)	Storage interval (days)	Individual recovered residues (mg/kg)	Mean	Individual recoveries (%)	Mean	Relative decrease %**
	1.0	7	0.983, 1.005, 1.038, 1.012, 0.979	1.003	98, 101, 104, 101, 98	100	-1
Peas (dry peas)	1.0	0	0.929, 0.872, 0.842, 0.890, 0.899	0.886	93, 87, 84, 89, 90	89	-
	1.0	7	0.927, 0.939, 0.947, 1.000, 0.932	0.949	93, 94, 95, 100, 93	95	-7
		fluopyram-7-hydroxy					
Tomato (fruit)	1.0	0	1.037, 1.083, 1.056, 1.070, 0.938	1.037	104, 108, 106, 107, 94	104	-
	1.0	7	1.054, 1.071, 1.063, 1.076, 1.111	1.075	105, 107, 106, 108, 111	107	-3
Wheat (green material)	1.0	0	0.976, 0.964, 0.922, 0.949, 0.949	0.952	98, 96, 92, 95, 95	95	-
	1.0	7	0.923, 0.943, 0.924, 1.003, 0.924	0.943	92, 94, 92, 100, 92	94	1
Grape (bunches)	1.0	0	1.091, 1.093, 1.101, 1.054, 1.061	1.080	109, 109, 110, 105, 106	108	-
	1.0	7	1.029, 1.057, 1.065, 1.085, 1.042	1.056	103, 106, 106, 109, 104	106	2
Wheat (grain)	1.0	0	1.092, 1.115, 1.101, 1.044, 1.085	1.087	109, 111, 110, 104, 108	108	-
	1.0	7	1.038, 1.095, 1.111, 1.090, 1.083	1.083	104, 109, 111, 109, 108	108	0
Potato (tuber	1.0	0	1.089, 0.988, 1.047, 1.051, 1.066	1.048	109, 99, 105, 105, 107	105	-
	1.0	7	1.084, 1.067, 1.065, 1.099, 1.049	1.073	108, 107, 107, 110, 105	107	-2
Peas (dry peas)	1.0	0	1.012, 1.003, 1.073, 0.972, 1.060	1.024	101, 100, 107, 97, 106	102	-
	1.0	7	1.032, 1.063, 1.029, 1.091, 1.085	1.060	103, 106, 103, 109, 108	106	-4
Oilseed rape (seeds)	1.0	0	1.041, 0.959, 0.985, 1.029, 0.895	0.982	104, 96, 99, 103, 90	98	-
	1.0	7	1.057, 1.090, 1.081, 1.095, 1.079	1.080	106, 109, 108, 109, 108	108	-10

*The extracts were shaken for 15 minutes on day 30 compared to 2 minutes on day 7, which led to better recoveries. This longer extraction procedure was applied for all samples thereafter.

**: (1 - final recovery mean / initial recovery mean) x100

Data Point:	KCA 6.1/11
Report Author:	[redacted]
Report Year:	2020
Report Title:	Storage stability of fluopicolide metabolites AE C657378 and AE C656948-methyl-sulfoxide (AE 1344122) in/on sunflower, dry bean, cucumber, strawberry and barley and AE C643890 in/on barley during freezer storage for up to 24 months - Final report
Report No:	M-754395-01-1
Document No:	M-754395-01-1
Guideline(s) followed in study:	OECD Guideline for the Testing of Chemicals. Stability of Pesticide Residues in Stored Commodities 506, 2007-10-16; US EPA OPPTS 860.1380, Storage Stability Data; Coordenação Geral de Acreditação do Inmetro-NIT-DICLA 035.
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted.
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities.
Acceptability/Reliability:	Yes

This study was conducted in the frame of the fluopicolide (AE C638206) project. It is considered to be relevant for this fluopyram EU renewal as AE 1344122 (Alias Fluopyram-methyl-sulfoxide) is a common metabolite between these two fungicides.

The purpose of this study was to determine the storage stability of residues of fluopicolide metabolites AE C657378, AE 1344122 (alias Fluopyram-methyl-sulfoxide) and AE C643890 in fortified control samples of plant origin (Sunflower (seed), Dry bean, Cucumber (fruit), Strawberry (fruit) and Barley (grain)) during freezer storage at ≤- 18 °C for 25 months.

For the purpose of this dossier, the following summary will give results only for FLU-methyl-sulfoxide (AE 1344122).

Materials and Methods

For the purpose of this dossier, the following summary will give results only for FLU-methyl-sulfoxide (AE 1344122) which is a common metabolite of fluopicolide and fluopyram.

Samples of sunflower seed (high oil matrix), dry beans seed (high protein matrix), cucumbers (high water matrix), strawberry (high acid matrix), barley grain (high starch matrix) were fortified with fluopicolide M05 (alias Fluopyram-methyl-sulfoxide, AE 1344122) at the level of 0.1 mg/kg and stored deep frozen (≤ -18°C) for a period of 24 months.

Portions of untreated substrate were fortified with individual analytes The fortification level was at a nominal concentration (fresh weight basis) of 0.1 mg/kg. The fortified specimens were stored in a freezer at < - 18 °C. After (nominal) 30, 90, 180, 360 and 540 days, three fortified per analyte and one control samples of each tested plant material were removed from the deep-freezer and analysed using the HPLC-MS/MS method 00783/M006 ([redacted], 2017, M-610859-01-1, see section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues are extracted with a mixture of acetone/water adjusted with sulfuric acid to pH 2. After addition of L-cysteine hydrochloride (250 mg/L), the extract is made to volume and an aliquot is concentrated to the aqueous remainder. The solution is again adjusted to pH 2. NaCl is added and partitioned twice with MTBE. An aliquot is concentrated to dryness and dissolved in methanol/water before LC-MS/MS analysis.

The limit of quantification (LOQ) for FLU-methyl-sulfoxide was 0.01 mg/kg for all matrices. The metabolite was not calculated as parent equivalents but the concentration is expressed as FLU-methyl-sulfoxide itself. The limit of detection (LOD) was estimated to be ≤ 30% of the LOQ.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels. Correlation coefficients were about 0.99..

The quantification was done by external standardization using matrix matched standards.

Analysis was performed within 24 hours after extraction.

Findings

In order to assess the accuracy of the residue analyses, recoveries were determined by analysing freshly fortified samples alongside the stored fortified samples. At all storage intervals except for day 0, two concurrent recoveries were determined at a level of 0.10 mg/kg. No additional concurrent recoveries were determined on day 0, since there were five freshly fortified storage samples.. The procedural recoveries meet the acceptability criteria; the mean recoveries are within the range 70 - 110% for each analyte / matrix combination and the %RSD values are ≤20% (where applicable). These procedural results demonstrate acceptable method performance and support the analytical results obtained for the fortified deep-frozen samples. Summaries of the residue behaviour of FLU-methyl-sulfoxide in stored samples are presented in the Table 6.1.2- 35.

- For FLU-methol-sulfoxide, uncorrected mean recoveries in orange show a stability period of 24-25 months for deep frozen residues (mean recoveries ranged between 85 – 97%, 87 – 101%, 96 – 109%, 90 – 103%, 84 – 104%, for sunflower seed, dry pea (seed), cucumber (fruit), strawberry (fruit) and barley (grain), respectively).

Conclusion

Representative plant commodities (sunflower seed, dry bean seed, cucumber, strawberry, and barley grain) were fortified at 0.1 mg/kg with -methyl-sulfoxide. The fortified samples were stored at low temperatures at -18°C and analysed at set intervals. The recoveries show that residues of FLU-methyl-sulfoxide are stable for up to 24 months in the tested commodities.

Assessment and conclusion by applicant:

The study is acceptable.
Residues FLU-methyl-sulfoxide in sunflower seed, dry bean seed, cucumber, strawberry, and barley grain at 0.1 mg/kg were shown to be stable for 24 months at T = -18°C.

Table 6.1.2- 34: Summary of concurrent recoveries of FLU-methyl-sulfoxide from various plant matrices

Matrix	Spike level (mg/kg)	Storage Interval (days)	Sample size (n)	Individual procedural recoveries (%)	Mean
Fluopyram-methyl-sulfoxide					
Sunflower seed	0.1	0	1	88	88
		30	2	83, 75	79
		97	2	80, 85	83
		183	2	85, 86	86
		387	2	79, 88	84
		568	2	81, 84	83
		756	2	76, 77	77
Dry bean seed	0.1	0	1	81	81
		32	1	84	84
		96	1	87	87
		182	1	81	81
		392	2	75, 77	76
		573	2	82, 82	82
		761	2	79, 82	81
Cucumber	0.1	0	1	96	96
		30	1	88	88
		93	2	96, 95	96
		178	2	89, 83	86
		385	2	97, 94	96
		567	2	87, 85	86
		752	2	84, 74	79
Strawberry	0.1	0	1	104	104
		30	2	84, 83	84
		98	2	100, 101	101
		182	2	88, 80	84
		386	2	77, 76	77
		567	2	88, 86	87
		755	2	99, 98	99
Barley grain	0.1	0	2	82, 92	87
		31	2	93, 92	93
		91	2	83, 85	84

Matrix	Spike level (mg/kg)	Storage Interval (days)	Sample size (n)	Individual procedural recoveries (%)	Mean
		183	2	83, 83	83
		360	1	77	77
		540	2	88, 93	91
		758	2	82, 83	83

Table 6.1.2- 35: Stability of fluopicolide M-05 (FLU-methyl-sulfoxide) residues in various matrices following storage at -18°C

Sample Material	Actual Storage Period [days]	Residue Level in Stored Samples				Day-0 Normalized Recovery [%][a]	Average of Fresh Concurrent Recoveries [%]	Average Corrected Recovery [%][b]
		mg/kg	% of nominal spiking level	Average recovery [%]	RSD [%]			
	AE C656948-methyl-sulfoxide							
Sunflower (seed)	0	0.0931 0.0957 0.0957 0.0960 0.0976	93 96 96 96 98	96	1.9	100	88	109
	30	0.0878 0.0833 0.0876	88 83 88	86	3.3	90	79	109
	97	0.0841 0.0833 0.0875	84 83 87	85	2.5	89	83	102
	183	0.0916 0.0921 0.0930	92 92 93	92	0.6	96	86	107
	387	0.0992 0.101 0.0902	99 101 90	97	6.1	101	84	115
	568	0.0883 0.0995 0.102	88 99 102	96	7.7	100	83	116
	756	0.0891 0.0884 0.0944	89 88 94	90	3.6	94	77	117
Dry bean (seed)	0	0.0934 0.0921 0.0913 0.0974	93 92 91 97	93	2.8	100	81	115
	32	0.0935 0.0899 0.0904	93 90 90	91	1.9	98	84	108

	96	0.0947 0.0948 0.0948	95 95 95	95	0.0	102	87	109
	182	0.0922 0.0866 0.0851	92 87 85	88	4.1	95	81	109
	392	0.105 0.102 0.0964	105 102 96	101	4.5	109	76	133
	573	0.0953 0.0901 0.0761	95 90 76	87	11.3	94	80	109
	761	0.0861 0.0864 0.0887	86 86 89	87	2.0	94	81	107
Cucumber (fruit)	0	0.0913 0.105 0.0998 0.0982	91 105 100 98	99	5.9	100	96	103
	30	0.0970 0.0928 0.107	97 93 107	99	7.3	100	88	113
	93	0.0995 0.0990 0.102	99 99 102	100	1.7	101	96	104
	178	0.0969 0.0955 0.0958	97 95 96	96	1.0	97	86	112
	385	0.108 0.110 0.109	108 110 109	109	0.9	110	96	114
	567	0.0974 0.0988 0.0958	97 99 96	97	1.6	98	86	113
	752	0.0889 0.0951 0.0937	89 93 94	92	2.9	93	79	116
Strawberry (fruit)	0	0.110 0.104 0.0947 0.104 0.0876	110 104 95 104 88	100	8.7	100	104	96
	30	0.106 0.102 0.102	106 102 102	103	2.2	103	84	123
	98	0.109 0.101 0.100	109 101 100	103	4.8	103	101	102
	182	0.0955 0.0943 0.0863	96 94 86	92	5.8	92	84	110

	386	0.105 0.106 0.0728	105 106 73	95	19.8	95	77	123
	567	0.0869 0.0928 0.0914	87 93 91	90	3.4	90	87	103
	755	0.089 0.0918 0.1045	89 92 105	95	8.9	95	99	96
Barley (grain)	0	0.0844 0.0854 0.0877 0.0854 0.0844	84 85 88 85 84	85	1.9	100	82	104
	31	0.0916 0.0805 0.0847	92 80 85	86	7.0	101	90	96
	91	0.102 0.102 0.108	102 102 108	104	3.3	122	84	124
	183	0.0960 0.0883 0.0878	96 88 88	91	5.1	107	83	110
	391	0.0856 0.0815 0.0845	86 81 85	84	3.1	99	77	109
	573	0.0966 0.0735 0.0965	100 73 96	90	16.3	106	91	99
	758	0.0875 0.0902 0.0903	88 90 90	89	1.3	1.5	83	107

Mean values were calculated with unrounded values. Therefore minor deviations may occur when the values given in the table are used.

[a] Day-0 Normalized Recovery [%] = (Average Recovery [%] / Average recovery at Day-0 [%]) X 100

[b] Average Corrected Recovery [%] = (Average Recovery [%] / Average of Concurrent Recoveries [%]) X 100

Data Point:	KCA 6.1/12
Report Author:	[redacted]
Report Year:	2020
Report Title:	Report amendment no. 1 to final report - Residue analytical method 01594 and short term storage stability of fluopyram (AE C656948) and its metabolites AE F148815, AE C657188, BCS-AA10189, BCS.AA10065 and AE 1344122 in/on honey by HPLC-MS/MS
Report No:	M-681002-02-2
Document No:	M-681002-02-2
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC Guidance document on residue analytical methods, SANCO/825/00/rev. 8.1, European Commission, Directorate General Health and Consumer Protection 16/11/2010 European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration Data Requirements for Annex II (Part A, Section 4) and Annex III (Part A, Section 5) of Directive 91/414, SANCO/3029/99 rev. 4, 11/07/00 OECD 506, 2007; OECD Guideline for the Testing of Chemicals – Stability of Pesticide Residues in Stored Commodities SANTE/11956/2016 rev.9
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Report: Residue analytical method 01594 and short-term storage stability of fluopyram (AE C656948) and its metabolites AE F148815, AE C657188, BCS-AA10189, BCS AA10065 and AE 1344122 in/on honey by HPLC-MS/MS. [redacted]; 2020; report No. S19-00990, document No. M-681002-02-2

Guideline(s): Yes
Regulation (EC) No 1107/2009
SANCO/825/00/rev. 8.1, 16/11/2010
SANCO/3029/99 rev. 4, 11/07/00
OECD Test Guideline 506, adopted 16 October 2007
SANTE/11956/2016 rev.9

Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The storage stability of residues of fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815, FLU-benzamide), fluopyram-pyridyl-carboxylic-acid (AE C657188, FLU-PCA), fluopyram-pyridyl-acetic-acid (BCS-AA 10139, FLU-PAA), fluopyram-7-hydroxy (BCS-AA10065, FLU-7-OH), fluopyram-methyl-sulfoxide (AE 1344122, FLU-methyl-sulfoxide) was investigated in honey. The conduct of this study was integrated in the analytical method 01594 (M-681002-02-2, see section 04) and reported in the same document.

Honey control samples (1 g) were separately fortified with each analyte at 0.1 mg/kg (10 x LOQ) and stored frozen at T≤-18°C for 1 month (28 days), 3 months (89 days) and 6 months (181 days). Five fortified samples and two control samples were analysed on the day of storage initiation (day zero). Subsequently, three fortified samples per analyte and one control sample were analysed after storage at each time point, with two freshly fortified concurrent recovery samples. Recovery samples were fortified with a mixture of the analytes, each at 0.1 mg/kg.

Samples were analysed using the validated analytical method 01594 ([redacted], 2020, M-681002-02-2, see MCA section 4.1.2). Honey samples were extracted by full dilution with the extraction solvent, shaking with acetonitrile/water (1:1, v/v).

- An aliquot was diluted with water (adjusted to pH 8 with ammonia) and analysed for fluopyram , FLU-benzamide, FLU-PAA and FLU-7-OH by HPLC-MS/MS in positive ion mode.
- A separate aliquot was diluted with 0.5% acetic acid and analysed for FLU-PCA and FLU-methylsulfoxide by HPLC-MS/MS in negative ion mode.

The Limit of Quantification (LOQ) in honey was 0.01 mg/kg for fluopyram and its metabolites, expressed as fluopyram.

The quantification is done against matrix matched standards.

The linearity was demonstrated in each analytical batch with a 1/x weighting calibration curve established with at least 5 concentration levels. Correlation coefficients were above 0.99.

The extracts were analysed within 2 days after extraction. The storage stability of honey final extract was demonstrated in this study for 8 days at 1 to 10°C in the dark.

Results and discussions

No residues of fluopyram or its metabolites were detected in the honey control samples used for the storage stability study.

In order to assess the accuracy of the residue analyses, recoveries were determined by analysing freshly fortified samples alongside the stored fortified samples. At all storage intervals except for day 0, two concurrent recoveries were determined at a level of 0.10 mg/kg as presented in Table 6.1.2- 36. No additional concurrent recoveries were determined on day 0, since there were five freshly fortified storage samples. The concurrent recoveries meet the acceptability criteria; the mean recoveries are within the range 70 - 110% for each analyte / matrix combination and the %RSD values are <20% (where applicable). These procedural results demonstrate acceptable method performance and support the analytical results obtained

for the fortified deep-frozen samples. Summaries of the residue behaviour of fluopyram and its metabolites in stored honey samples are presented in the

Table 6.1.2- 37.

- For fluopyram, uncorrected mean recoveries in honey show a stability period of 6 months for deep frozen residues (mean recoveries ranged between 103 % and 110 %).
- For FLU-benzamide, uncorrected mean recoveries in honey show a stability period of 6 months for deep frozen residues (mean recoveries ranged between 89 % – 108 %).
- For FLU-PCA, uncorrected mean recoveries in honey show a stability period of 6 months for deep frozen residues (mean recoveries ranged between 76 % – 98 %).
- For FLU-PAA, uncorrected mean recoveries in honey show a stability period of 6 months for deep frozen residues (mean recoveries ranged between 84 % – 92 %).
- For FLU-7OH, uncorrected mean recoveries in honey show a stability period of 6 months for deep frozen residues (mean recoveries ranged between 99 % – 110 %).
- For FLU-methylsulfoxide, uncorrected mean recoveries in honey show a stability period of 6 months for deep frozen residues (mean recoveries ranged between 88 % – 100 %).

The data indicate that fluopyram and its metabolites are stable in honey for at least 6 months when stored frozen at -18°C or below.

Conclusion

The storage stability study demonstrated that residues of parent fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815, FLU-benzamide), fluopyram-pyridyl-carboxylic-acid (AE C657188, FLU-PCA), fluopyram-pyridyl-acetic-acid (BCS-AA 10139, FLU-PAA), fluopyram-7-hydroxy (BCS-AA10065, FLU-7-OH), fluopyram-methyl-sulfoxide (AE 1344122, FLU-methyl-sulfoxide) are stable in honey for 6 months when stored frozen at ≤-18°C.

Assessment and conclusion by applicant:

The study is acceptable.
residues of parent fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815, FLU-benzamide), fluopyram-pyridyl-carboxylic-acid (AE C657188, FLU-PCA), fluopyram-pyridyl-acetic-acid (BCS-AA 10139, FLU-PAA), fluopyram-7-hydroxy (BCS-AA10065, FLU-7-OH), fluopyram-methyl-sulfoxide (AE 1344122, FLU-methyl-sulfoxide) are stable in honey for 6 months when stored frozen at ≤-18°C

Table 6.1.2- 36: Concurrent recoveries in honey for fluopyram and its metabolites

Analyte	actual storage interval [d]	Concurrent Recoveries [%]		
		0.10 mg/kg* Single Values		Mean
Fluopyram m/z 396 → 173	0	-		-
	28	108	112	110
	89	108	111	110
	181	105	110	108
FLU-benzamide m/z 190 → 170	0	-		-
	28	102	106	104
	89	107	104	106
	181	108	107	108

FLU-PCA m/z 224 → 180	0	-		-
	28	108	95	102
	89	87	90	89
	181	94	87	91
FLU-PAA m/z 240 → 194	0	-		
	28	91	96	94
	89	88	92	90
	181	94	96	95
FLU-7-OH m/z 413 → 173	0			-
	28	108	111	110
	89	104	107	106
	181	110	110	110
FLU-methylsulfoxide m/z 252 → 61	0	-		-
	28	109	103	106
	89	79	86	83
	181	91	80	86

Table 6.1.2- 37: Storage stability data for fluopyram and its metabolites in honey at T ≤ -18ºC

Commodity	Fortification level (mg/kg)	Storage period (days)	stored residue (mg/kg)	Mean stored residue (mg/kg)	stored recovery (%)	Mean stored recovery (%)	Mean concurrent recovery (%)	Day-0 normalized recovery [a] (%)	Mean corrected stored recovery [b] (%)
Fluopyram (AE C656948)									
Honey	0.1	0	0.109, 0.110, 0.105, 0.115, 0.113	0.110	109, 111, 105, 115, 113	110	-	100	-
	0.1	28	0.109, 0.107, 0.111	0.109	109, 107, 111	109	110	99	99
	0.1	89	0.0996, 0.105, 0.103	0.103	100, 105, 103	103	110	93	94
	0.1	181	0.110, 0.108, 0.107	0.108	110, 108, 107	108	108	98	101
Fluopyram-benzamide (AE F148815)									
Honey	0.1	0	0.105, 0.108, 0.112, 0.107, 0.107	0.108	105, 108, 112, 107, 107	108	-	100	-
	0.1	28	0.0984, 0.0977, 0.0989	0.0983	98, 98, 99	98	104	91	94
	0.1	89	0.0904, 0.0873, 0.0887	0.0888	90, 87, 89	89	106	82	84
	0.1	181	0.0953, 0.0100, 0.0977	0.0977	95, 100, 98	98	108	91	91
Fluopyram-pyridyl-carboxilic-acid (AE C657188)									
Honey	0.1	0	0.0887, 0.0779, 0.0877, 0.0894, 0.0927	0.0873	89, 78, 88, 89, 93	87	-	100	-
	0.1	28	0.0927, 0.0955, 0.0904	0.092	93, 96, 90	93	102	106	91
	0.1	89	0.0732, 0.0758, 0.0784	0.0758	73, 76, 78	76	89	87	85
	0.1	181	0.0934, 0.100, 0.101	0.0981	93, 100, 101	98	91	112	108
Fliopyram- pyridyl -acetic-acid (BCS AA10189)									
Honey	0.1	0	0.0883, 0.0957, 0.0916, 0.0918, 0.0942	0.0923	88, 96, 92, 92, 94	92	-	100	-
	0.1	28	0.0907, 0.0933, 0.0882	0.0907	91, 93, 88	91	94	98	97
	0.1	89	0.0859, 0.0849, 0.0811	0.0840	86, 85, 81	84	90	91	93

Commodity	Fortification level (mg/kg)	Storage period (days)	stored residue (mg/kg)	Mean stored residue (mg/kg)	stored recovery (%)	Mean stored recovery (%)	Mean concurrent recovery (%)	Day-0 normalized recovery [a] (%)	Mean corrected stored recovery [b] (%)
	0.1	181	0.0939, 0.0933, 0.0939	0.0937	94, 93, 94	94	95	101	99
Fluopyram-7-hydroxy (BCS AA10065)									
Honey	0.1	0	0.374[c], 0.111, 0.109, 0.110, 0.111	0.110	374[c], 111, 109, 110, 111	110	-	100	-
	0.1	28	0.114, 0.102, 0.103	0.106	114, 102, 103	106	110	96	96
	0.1	89	0.0996, 0.0951, 0.102	0.0989	100, 95, 102	99	106	90	94
	0.1	181	0.100, 0.0994, 0.100	0.0998	100, 99, 100	100	110	90	91
Fluopyram-methyl-sulfoxide (AE 1344122)									
Honey	0.1	0	0.0972, 0.0811, 0.102, 0.102, 0.0992	0.0963	97, 81, 102, 102, 99	96	-	100	-
	0.1	28	0.117, 0.0917, 0.0914	0.100	117, 92, 91	100	106	104	94
	0.1	89	0.0808, 0.0850, 0.103	0.0896	81, 85, 103	90	83	93	109
	0.1	181	0.0920, 0.0845, 0.0858	0.0874	92, 85, 86	88	86	91	103

[a] Normalized Recovery = (Average % recovery / Average % recovery at day 0) X 100%
[b] Corrected percent recovery = (Average % recovery / Average % of fresh concurrent recoveries) X 100%
[c] Result excluded as an outlier

CA 6.1.3 Storage stability in sample extracts

In most of the studies, the time between the beginning of the sample preparation and the sample analysis did not exceed 24 hours. If not the case, the maximum storage period of extracts is covered by stability experiments conducted in the course of the analytical methods validations presented in section CA 4.1.2 :

- 00984/M003 (M-467323-03-1) for plant materials
- 01594 (M-681002-02-2) for honey.

Fluopyram and fluopyram-benzamide were found to be stable in extracts of matrices of plant origin for at least four weeks at 4 ± 3 °C which was tested within the validation of method 00984/M003.

For honey samples, the extracts were analysed within 2 days after extraction. The storage stability of honey final extract was demonstrated in this study for 8 days at 1 to 10°C in the dark.

Data Point:	KCA 4.1.2/25
Report Author:	[redacted].
Report Year:	2015
Report Title:	Modification M003 of the residue analytical method 00984 for the determination of AE C656948, its metabolite AE F148815 and tebuconazole in an orange (fruit), wheat (grain), wheat (straw), bean (seed), lettuce (head), rape (seed) and hop (dry cone) by HPLC-MS/MS and a cross validation of the analytical methods 00984 and 00984/M003
Report No:	00984/M003
Document No:	M-467323-03-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 rev. 4, 11/07/00 Guidance document on residue analytical methods, SANCO/825/00/rev. 8.1, European Commission, Directorate General Health and Consumer Protection 16/11/2010 US EPA Residue Chemistry Test Guideline OCSPP 860.1340: Residue Analytical Method
Deviations from current test guideline:	-
Previous evaluation:	
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	

This residue analytical method is fully summarized in section MCA 4.1.2 but additionally the part dealing with the stability of residues in sample extracts is presented here.

The stability of residues of fluopyram and fluopyram-benzamide in plant final extracts was checked for the tested sample materials over a period of 4 weeks. The following tables show the recoveries comparing initial day of analysis and analysis after storage of the final samples at 4 ± 3°C under dark conditions over the given periods.

All analytes were found to be stable in plant final extracts for at least 4 weeks (with the exception of hop cone dried).

In hops extracts, the results show that fluopyram-benzamide were stable after 4 weeks. Only the results for fluopyram show a deviation of 24% compared to the Day 0 values. It is suggested to analyse samples of hops within 24h after extraction and so will be recommended using modification M003 of method 00984.

Table 6.1.3- 1: Storage of fluopyram in plant extracts (Mass Transition 397 > 208)

Sample Material	FL* [mg/kg]		Recovery Rates [%]					Mean	Mean deviation [%] **
Lettuce head	0.10	initial analysis	93	98	95	96	94	95	n.a.
		4 week reanalysis	96	97	94	93	92	94	1%
Orange fruit	0.10	initial analysis	90	90	89	91	88	90	n.a.
		4 week reanalysis	88	91	93	91	88	90	1%
Wheat grain	0.10	initial analysis	87	95	94	96	93	93	n.a.
		4 week reanalysis	89	92	95	96	92	93	0%
Wheat straw	0.10	initial analysis	84	87	89	88	88	87	n.a.
		4 week reanalysis	89	90	89	89	89	89	2%
Rape seed	0.10	initial analysis	92	92	90	92	92	92	n.a.
		4 week reanalysis	91	91	92	91	91	91	0%
Bean dry seed	0.10	initial analysis	93	94	95	93	95	94	n.a.
		4 week reanalysis	94	89	95	96	94	94	0%
Hop cone dried	0.10	initial analysis	63 (75[a])	70 (82[a])	69 (81[a])	64 (76[a])	66 (78[a])	66	n.a.
		4 week reanalysis[b]	87 (120[a])	64 (97[a])	88 (121[a])	89 (122[a])	80 (113[a])	82	24% (46%)

* FL = Fortification Level

** Mean deviation [%] between initial analysis and days of reanalysis. For the calculation of the mean deviation as it appears in the table above, unrounded values were used. Therefore, minor deviations may occur between the mean deviation shown above and when the values given for the mean values in the table are used for calculation.

a Values before correction. The control sample used yielded residue levels of more than 30% (0.0036 mg/kg) of the LOQ and therefore the recovery was background-corrected for this signal

b The recovery values are deviating by 24% compared to the day 0 values, therefore it is suggested to analyse samples of hops within 24h after extraction.

Table 6.1.3- 2: Stability of fluopyram-benzamide in plant extracts (Mass Transition 190 > 170)

Sample Material	FL* [mg/kg]		Recovery Rates [%]					Mean	Mean deviation [%] **
Lettuce head	0.10	initial analysis	97	97	96	94	98	96	n.a.
		4 week reanalysis	92	91	96	97	91	93	3%
Orange fruit	0.10	initial analysis	96	96	97	97	95	96	n.a.
		4 week reanalysis	98	92	96	94	91	94	2%
Wheat grain	0.10	initial analysis	85	96	95	94	94	93	n.a.
		4 week reanalysis	95	102	94	97	99	97	5%
Wheat straw	0.10	initial analysis	92	90	91	93	90	91	n.a.
		4 week reanalysis	101	95	98	96	99	98	7%
Rape seed	0.10	initial analysis	91	94	89	93	90	91	n.a.
		4 week reanalysis	93	91	95	90	88	91	0%
Bean dry seed	0.10	initial analysis	96	93	93	96	94	94	n.a.
		4 week reanalysis	95	98	95	92	96	95	1%
Hop cone dried	0.10	initial analysis	93	82	95	92	91	91	n.a.
		4 week reanalysis	95	93	95	93	92	94	3%

* FL = Fortification Level

** Mean deviation [%] between initial analysis and days of reanalysis. For the calculation of the mean deviation as it appears in the table above, unrounded values were used. Therefore, minor deviations may occur between the mean deviation shown above and when the values given for the mean values in the table are used for calculation.

Assessment and conclusion by applicant:

The study is acceptable.
Plants extracts are found to be stable for four weeks.

Storage stability in standards solutions

Data Point:	KCA 6.1/13
Report Author:	[redacted]
Report Year:	2019
Report Title:	Determination of the standard stability for prothioconazole, JAU 6476-desthio, fluopyram, fluopyram-benzamide, JAU 6476-alpha-hydroxy-desthio, JAU 6476-3-hydroxy-desthio, JAU 6476-4-hydroxy-desthio, JAU 6476-5-hydroxy-desthio, JAU 6476-6-hydroxy-desthio, 1,2,4-triazole, triazole alanine, triazole acetic acid and triazole lactic acid
Report No:	P602176032
Document No:	M-654837-01-1
Guideline(s) followed in study:	European Commission Guidance Document for Generating and Reporting Methods of Analysis inSupport of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 Guidance document on residue analytical methods; SANCO/825/00 rev. 8.1, European Commission, Directorate General Health and Consumer Protection, 2010-11-16 US EPA Residue Chemistry Test Guideline OCSPP 860.1340: Residue Analytical Method OECD (2007). Guidance Document on Pesticide Residue Analytical Methods, Enviroment No. 72 and Series on Pesticides No. 39
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted Study was not found in DAR/RAR and the Addenda
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The stability of fluopyram (AE C656948), fluopyram-benzamide (AE C656948-benzamide), and other compouds in standard solutions was analyzed over a storage period of 184 to 404 days. For sake of clarity only the results concerning fluopyram and fluopyram-benzamide are reported here.

Table 6.1.3- 3: Stability in standard solution

active substance	analyte	investigated storage period at < 6 °C [days]	devation [%]
fluopyram	fluopyram	205	3.9
	fluopyram-benzamide	205	4.3

deviation [%] = 100 - ((counts per µg/L)$_{fresh}$ / (counts per µg/L)$_{aged}$ x100)

Different secondary standard solutions were stored for 184 to 404 days at T< 6 °C until analysis. The standards were diluted and analyzed by HPLC-MS/MS for fluopyram and fluopyram-benzamide (transitions m/z 397>208 for FLU and 190>170 for FLU-benzamide). The standards solutions were found to be stable for the investigated storage period.

For the determination of the standard stability, primary standard solutions at 500 mg/L in acetonitrile were diluted and secondary standard solutions in acetonitrile/water (1/9) were generated. Internal standard was added to the secondary standard solutions, as this is the general procedure for the analysis in the laboratory.

Since the internal standard is not part of the analysis of the standard stability in this study and the results of the internal standards were not reported, information regarding the internal standard solutions is not given in this report.

The secondary standard solutions were stored at < 6 °C for 184 to 404 days. For the determination of standard stability the aged secondary standards were compared to freshly prepared secondary standards, diluted from fresh primary standards.

Table 6.1.3- 4: Stability of fluopyram and fluopyram-benzamide in standard solution

Analyte	date of prepara-tion	storage time [days]	Peak area [counts]			Mean [counts]	RSD [%]	Concentra-tion of analyte [µg/L]	Counts per µg/L
fluopyram	2017-06-26	205	1161107	1141608	1221848	1154531	3.1	10.005	115395
			1141652	1140029	1120941				
	2018-01-17		1259884	1202839	1097841	1199109	6.0	10.000	119911
			1213374	1284982	1135793				
	deviation [%]								3.9
fluopyram-benzamide	2017-06-26	205	221488	219422	218482	220897	1.1	4.7716	46294
			221023	219562	226405				
	2018-01-17		236044	234430	223066	230205	2.8	4.7691	48270
			226914	237235	223540				
	deviation [%]								4.3

Counts per µg/L = mean (counts) / concentration of the analyte (µg/L)
deviation [%] = 100 - ((counts per µg/L)$_{fresh}$ / (counts per µg/L)$_{aged}$ x100)

The standard solutions were found to be stable for the investigated storage period.

Assessment and conclusion by applicant:

The study is acceptable.
Standards solutions are found to be stable for 205 days at T<6°C..

CA 6.2 Metabolism, distribution and expression of residues

CA 6.2.1 Metabolism, distribution and expression of residues in plants

Data to address this point were presented in the dossier submitted for first inclusion in Annex and were deemed acceptable following evaluation and peer review at EU level (2013).

Additionally metabolism studies using [phenyl-UL-^{14}C]- and [2,6-pyridinyl-^{14}C]-labelled fluopyram were conducted in seed treatment (wheat) and rice.

For details of data submitted previously please refer also to the Baseline dossier CA 6.2. For completeness, a summary of these previously submitted studies are included below.

Data already evaluated during the first EU review process for inclusion on Annex I (no new studies)

Metabolism in grape (foliar spray application)

Data Point:	KCA 6.2.1/01
Report Author:	[redacted]
Report Year:	2006
Report Title:	Metabolism of [phenyl-UL-^{14}C]-AE C656948 in grapes after spray application
Report No:	MEF-06/088
Document No:	M-282177-01-1
Guideline(s) followed in study:	US EPA OPPTS 860.1300; Canadian PMRA DACO 8.3; EU 91/414/EEC amended by 96/68/EC
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted revised to Vol.3 of DAR B7 August 2012 (references relied on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The metabolism of [phenyl-UL-^{14}C]fluopyram, formulated as a SC 500 (Suspension Concentrate), was investigated in grape vines following three foliar spray applications corresponding to a total application rate of 500 g a.s./ha. The applications were performed at growth stages BBCH 17, 71 and 81. Approximately 100 g a.s./ha were applied for the first treatment, 200 g a.s./ha for the second, and again 200 g a.s./ha for the last application.

Leaves of the summer cut (BBCH 71) were analysed as an intermediate sample on the same day of the second application. Furthermore, grapes and leaves were harvested at maturity of grape berries with a pre-harvest interval (PHI) of 18 and 19 days, respectively.

The TRR of the edible RAC (grapes) was 1.86 mg eq/kg and was considerably lower than those of summer cut leaves and leaves at harvest (28.55 mg eq/kg and 48.06 mg eq/kg, respectively). The major amount of radioactivity (93.9–98.1% of the TRR) was effectively extracted with acetonitrile/water from all RACs leaving only minor amounts < 6.1% remaining in the solids (PES). Parent compound and metabolites in the extracts were quantified by HPLC.

A total of 98.2% (28.03 mg eq/kg) of the TRR was identified in summer cut leaves, 98.7% (1.83 mg eq/kg of the TRR) in grapes, and 93.9% (45.10 mg eq/kg) in the leaves at harvest.

The unchanged fluopyram was the major compound (>90% TRR) detected in all matrices. Apart from parent, the residues consisted of fluopyram-benzamide (M25) and fluopyram-7-hydroxy in grape (both <.1% TRR). In the leaves at harvest, the 7- and 8-hydroxy metabolites, and the glucoside conjugate of the 7-hydroxy were also detected (all <1% TRR).

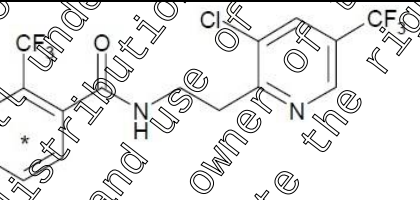
The metabolic reactions involved:

- Hydroxylation of the ethyl linking group of the a.s. forming 7- or 8- hydroxy metabolites
- Conjugation of the fluopyram-7-hydroxy with glucose
- Hydrolytic cleavage and a subsequent oxidation lead to fluopyram-benzamide

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	 * position of the radiolabel
Compound	AE C656948
IUPAC name	N-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]ethyl}-2-(trifluoromethyl)benzamide
CAS name	Benzamide, N-[2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-(trifluoromethyl)-(9Cl)
CAS #	658066-35-4
Radiolabel position	Phenyl-UL-^{14}C
Specific radioactivity	3.85 MBq/mg (104.2 µCi/mg)
Purity Chemical Purity	99% (HPLC) > 99% (TLC) > 99% (HPLC)

2. Soil: "Monheim 2" (sandy loam from Germany), pH ($CaCl_2$) = 6.4, 60.0% sand, 28.2% silt and 11.8% clay, 1.3% organic carbon, cation exchange capacity (CEC) of 10 meq/100 g

3. Plant: Grapevine (*Vitis vinifera*), variety: "Mueller Thurgau"

B. Study Design

1. Experimental conditions:

The grapevine plant was grown in the vegetation area (building 6682) of Bayer CropScience AG, Metabolism / Environmental Fate, Monheim, Germany, which allows plant growth under natural sunlight and temperatures, with a glass roof that can automatically close at the beginning of a rainfall. The plant was cultivated in a 35 L bucket with a surface area of 0.125 m^2, filled with a sandy loam soil. The plants were sprayed with [phenyl-UL-^{14}C]fluopyram formulated as a SC 500 (Suspension concentrate) and the spraying was performed using a graphic spray pistol.

The metabolism study simulated the envisaged use pattern and was based on a targeted application rate of 500 g a.s./ha as three sprayings. To compensate losses during applications up to 20% excess of a.s. was applied. Three applications were performed at growth stage BBCH 17–19, BBCH 71 and BBCH 81, at application rates of approximately 100 g a.s./ha, 200 g a.s./ha and 200 g a.s./ha, respectively. This resulted in a total application rate of about 504 g a.s./ha after subtraction of the losses in the rinses. The time interval between the first and the second application was 42 days and 49 days between the second and third application.

2. Sampling

Summer cut: Directly after drying of the spray of the second application at growth stage fruit set (BBCH 71), the grapevine plant was pruned according to agricultural practice. The leaves and stalks of this summer prune were cut into small pieces, and were homogenised with liquid nitrogen using an Ultra-Turrax homogeniser. The complete homogenised plant material was used for extraction.

Grapes: At maturity (BBCH code 89 and PHI of 18 days), whole bunches of grapes were cut from vines, and the single grapes were cut from the stems. A representative aliquot of the grapes was surface washed and used for extraction. The remaining grapes were stored in aliquots at *ca.* -20°C.

Leaves at harvest: One day after the grapes had been sampled the leaves (at BBCH 89, PHI of 19 days) were cut from the vines. A representative aliquot of these leaves was cut into small pieces, and was homogenised with liquid nitrogen using an Ultra-Turrax homogeniser and was used for extraction. The rest of the leaves were stored at *ca.* -20°C.

C. Analytical Procedures

The grape RACs were extracted and the extracts were analysed by reversed phase HPLC and TLC. The identification of parent compound and metabolites was based on co-chromatography experiments.

1. Extraction

In general, samples of the grape RACs were extracted 3 times with a mixture of acetonitrile/water (8/2, v/v) using an Ultra-Turrax. The extracts were separated from the solids by centrifugation. The radioactivity of

the extracts was determined by volume measurement and LSC. The solids (PES) were air-dried, homogenised and weighed. Aliquots thereof were combusted and measured for radioactivity by LSC.

Grape berries were additionally surface washed prior to homogenization. After extraction, due to high sugar content, a phase separation acetonitrile : water occurred. Hence, the phases (organic and aqueous sugar phase) were separated using a separation funnel resulting in the combined acetonitrile phases and the combined water phases after all three extraction steps. Prior to HPLC analysis, an aliquot of the combined acetonitrile phases was concentrated using a rotary evaporator.

The ^{14}C-radioactivity of liquid samples was determined by liquid scintillation counting (LSC) using Quicksafe A containing 5% of water. The radioactivity in solid samples was measured by combustion. The released $^{14}CO_2$ was absorbed in an alkaline scintillation cocktail and radio assayed by LSC.

The total radioactive residues (TRR) in the RACs of grapes were determined by summation of the radioactivity in the combined acetonitrile/water extracts and in the PES. The residue levels are expressed as parent compound equivalents per weight. The combined extracts were analysed by HPLC for quantification of metabolites.

2. Identification and characterisation:

Identification of metabolites was done by co-chromatography using HPLC and TLC methods. Each metabolite was co-chromatographed either with its authentic reference compound and/or radiolabelled metabolites, which were obtained in the plant study applying [pyridyl-2,6-^{14}C]fluopyram to beans.

3. Storage stability:

The RACs were extracted and analysed within a few days after sampling. All samples were stored at temperatures ≤ -18 °C. A few days after extraction, the earliest metabolite profiles of the combined extracts were obtained using a preliminary HPLC method until the final profiling method for the use in all plant metabolism studies was available. The extracts were analysed again using HPLC approximately eight to ten months after the initial analysis. The profiles from the initial and the late analysis showed no significant differences in the compound pattern. Therefore it was concluded that the results of this study were not negatively influenced by storage effects.

II. Results and Discussion

The metabolism of [phenyl-UL-^{14}C]fluopyram, formulated as a SC 500, was investigated in grape following three foliar applications at application rates of approximately 100 g a.s./ha, 200 g a.s./ha and 200 g a.s./ha, respectively.

The TRR amounted to 28.55 mg eq/kg in summer cut leaves, 1.86 mg eq/kg in grapes and 48.06 mg eq/kg in leaves at harvest (Table 6.2.1-1). The TRR of the edible RAC (grapes) was significantly lower than those of the leaves cut in summer or at harvest.

Table 6.2.1-1: TRR values in grape matrices after application of [phenyl-UL-^{14}C]fluopyram

Matrix	Timing and Applic. No.	PHI (days)	TRR (ppm, mg a.s. equiv./kg)
Summer cut leaves	Two applications: at growth stages BBCH 17-19 and 71 1 x 100 g a.s./ha and 1 x 200 g a.s./ha	0	28.55
Grapes	Three applications: at growth stages BBCH 17-19, 71 and 81 1 x 100 g a.s./ha and 2 x 200 g a.s./ha, total 504 g/ha	18	1.86
Leaves at harvest	Three applications: at growth stages BBCH 17-19, 71 and 81 1 x 100 g a.s./ha and 2 x 200 g a.s./ha, total 504 g/ha	19	48.06

The grape matrices were extracted with acetonitrile/water (8/2; v/v), the extracts were analysed by HPLC and, where necessary, TLC and parent compound, and metabolites were identified.

Nearly the complete radioactive residues in summer cut leaves, 98.2% (28.03 mg eq/kg) of the TRR, was extracted by acetonitrile/water (

Table 6.2.1-2), whereas 1.8% (0.52 mg eq/kg) of the TRR remained in the solids (PES). For grapes, 80.0% (1.49 mg eq/kg) of the TRR, was found in the surface wash, while 18.6% (0.34 mg eq/kg) of the TRR was extracted by acetonitrile/water (

Table 6.2.1-2), leaving 1.4% (0.03 mg eq/kg) of the TRR in the solids (PES). The majority of the radioactive residue in leaves at harvest, 93.9% (45.10 mg eq/kg) of the TRR, was extracted by acetonitrile/water (

Table 6.2.1-2), whereas 6.1% (2.96 mg eq/kg) of the TRR remained in the solids (PES). Due to the low radioactive residue, the solids (PES) from all matrices were not further investigated.

Table 6.2.1-2: Distribution of radioactivity in the extracts of the grape matrices after application of [phenyl-UL-^{14}C]fluopyram

	Summer cut		Grapes		Leaves at harvest	
TRR [mg eq/kg] =	28.55		1.86		48.06	
	% of TRR	mg eq/kg	% of TRR	mg eq/kg	% of TRR	mg eq/kg
Surface wash	n.a.	n.a.	80.0	1.49	n.a.	n.a.
Acetonitrile/water extract	98.2	28.03	18.6	0.34	93.9	45.10
Solids (PES)	1.8	0.52	1.4	0.03	6.1	2.96
Total extracted	98.2	28.03	98.7	1.83	93.9	45.10
Accountability	100.0	28.55	100.0	1.86	100.0	48.05

n.a. not applicable

For the elucidation of metabolites, the solvent extracts (acetonitrile/water) were analysed by HPLC or by TLC with radiodetection. Metabolites were either identified by co-chromatography (HPLC, TLC) with authentic reference compounds or with metabolites isolated and identified from beans.

Summer cut: In the extracts of summer cut leaves, only parent compound (98.2% of the TRR, 28.03 mg eq/kg) was detected (Table 6.2.1-3).

Grapes: In the surface wash of grapes, only parent compound (80.0% of the TRR, 1.49 mg eq/kg) was detected. Due to the viscosity (high sugar content), the acetonitrile/water extracts separated into two phases: The acetonitrile phase of the grapes (18.2% of the TRR, 0.34 mg eq/kg), contained parent compound (contributing to 17.3% of the TRR, 0.32 mg eq/kg). Hence, other than parent, metabolites detected in grapes were very minor. fluopyram-benzamide amounted to 0.7% (0.012 mg eq/kg) of the TRR. In addition, fluopyram-7-hydroxy (0.3% of the TRR, 0.005 mg eq/kg) was detected (Table 6.2.1-3).

The aqueous phase contained only 0.4% (0.01 mg eq/kg) of the TRR in total, and again fluopyram was the major compound (0.3%, 0.008 mg eq/kg of the TRR). Overall, parent compound accounted for 97.6% (1.82 mg eq/kg) of the TRR in grapes (Table 6.2.1-3). A very polar minor metabolite (0.1%, 0.002 mg eq/kg of the TRR) remained unassigned, but was characterised by its extraction and chromatographic behaviour.

Leaves at harvest: In the extracts of leaves at harvest, parent compound (91.8% of the TRR, 44.11 mg eq/kg) was again detected as the predominant part of the residues (.

Table 6.2.1-3). A minor metabolite in the extracts of leaves was the fluopyram-7-hydroxy-glc (0.7%, 0.35 mg eq/kg of the TRR). Furthermore, the metabolites fluopyram-7-hydroxy (0.7% of the TRR, 0.35 mg eq/kg) and fluopyram-8-hydroxy (0.6% of the TRR, 0.28 mg eq/kg) were identified.

Table 6.2.1-3: Summary of characterisation and identification of radioactive residues in grape matrices after spray application of [phenyl-UL-^{14}C]fluopyram

	Summer cut		Grapes		Leaves at harvest	
TRR [mg eq/kg] =	28.55		1.86		48.06	
Compound	% of the TRR	mg eq/kg	% of the TRR	mg eq/kg	% of the TRR	mg eq/kg
AE C656948, a.s., fluopyram	98.2	28.03	97.6	1.82	91.8	44.11
fluopyram-8-hydroxy (M18)	-	-	-	-	0.6	0.28
fluopyram-7-hydroxy (M08)	-	-	0.3	< 0.01	0.7	0.35
fluopyram-7-hydroxy-glc (M11)	-	-	-	-	0.7	0.35
fluopyram-benzamide (M25)	-	-	0.7	0.01	-	-
Total identified	98.2	28.03	98.6	1.83	93.8	45.09
Unknown 1 (GR0)	-	-	0.1	< 0.01	-	-
Total characterised	-	-	0.1	< 0.01	-	-
Solids (non-extractable residue)	1.8	0.52	1.4	0.03	6.1	2.96
Accountability	100.0	28.55	100.0	1.86	100.0	48.05

III. Conclusions

After foliar spray application of *ca.* 500 g/ha [phenyl-UL^{14}C]fluopyram, most of the recovered radioactivity was detected in leaves at harvest (48.06 mg eq/kg) and summer cut leaves (28.55 mg eq/kg), whereas significantly less residues were detected in grapes (1.86 mg eq/kg).

The radioactivity was easily extracted (minimum ~93% of the TRR) and identification of metabolites was performed by co-chromatography using HPLC and TLC methods.

Unchanged parent compound was the predominant portion of the TRR in all matrices, accounting for more than 90% of the TRR. Metabolism of fluopyram was rather limited in grapevine and none of the metabolites was detected in portions higher than 1.0% of the TRR. The label specific compound fluopyram-benzamide was detected in grapes only, and amounted to 0.7% of the TRR. Further metabolic reactions involved in the degradation of fluopyram were the hydroxylations of parent compound forming the fluopyram-7-hydroxy and 8-hydroxy. The latter as well as the glucoside conjugate of fluopyram-7-hydroxy were only detected in leaves.

Hence major metabolic reactions involved in the degradation of fluopyram were:

- Hydroxylation of the ethyl linking group of the a.s. forming 7- or 8- hydroxy metabolites
- Conjugation of the fluopyram-7-hydroxy with glucose
- Hydrolytic cleavage and oxidation leading to fluopyram-benzamide

From the results of this study, the metabolic pathway of [phenyl-UL-^{14}C]fluopyram in grapevines is proposed in Figure 6.2.1-1.

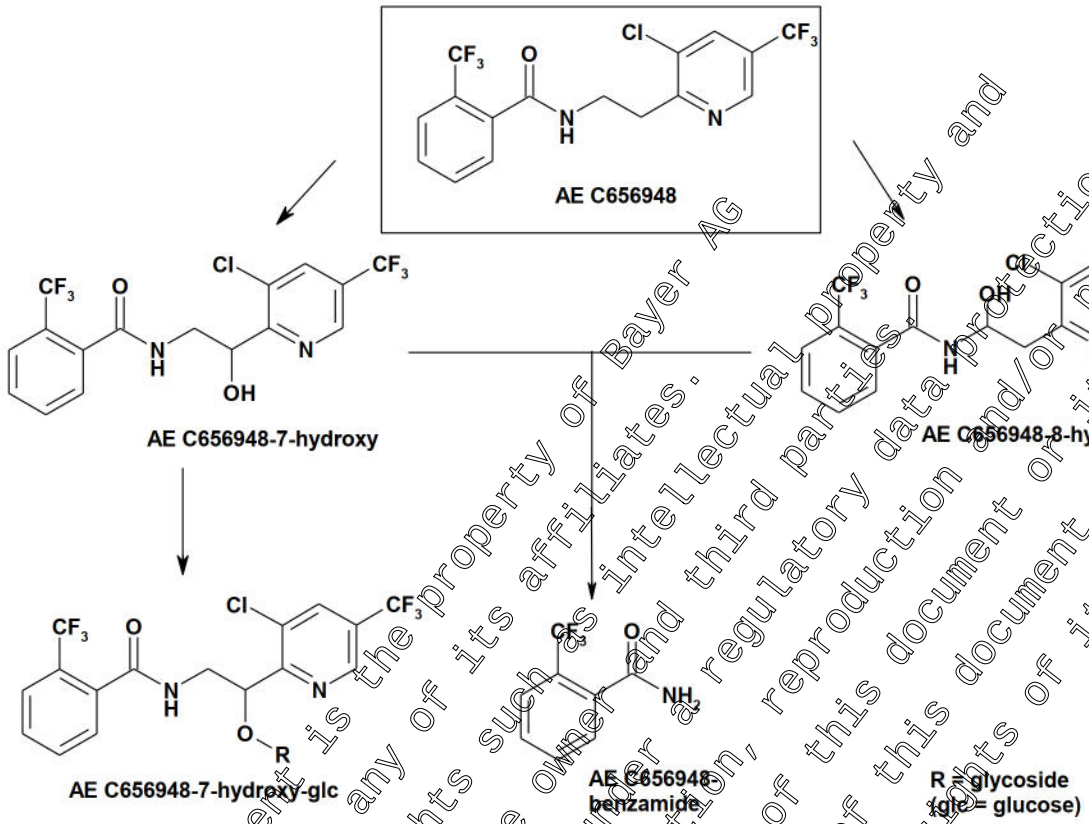


Figure 6.2.1-1: Proposed metabolic pathway of [phenyl-UL-^{14}C]AE C656948 (fluopyram) in grape

Assessment and conclusion by applicant:

The study is valid and acceptable.

Metabolism studies in grape were conducted with [pyridyl-2,6-^{14}C] fluopyram:

Data Point:	KCA 6.2.1/02
Report Author:	[redacted]
Report Year:	2006
Report Title:	Metabolism of [pyridyl-2,6-14C]AE C656948 in grapes after spray application
Report No:	MEF-06/086
Document No:	M-282460-01-1
Guideline(s) followed in study:	US EPA OPPTS 860.1300; Canadian PMRA DACO 8.3; EU 91/414/EEC amended by 96/68/EC
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted rev. 1 to Vol.3 of DAR, B.7 August 2012 (reference relied on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The metabolism of [pyridyl-2,6-^{14}C]fluopyram, formulated as a SC 500 (Suspension Concentrate), was investigated in grape vines following three foliar spray applications. The applications were performed at growth stages BBCH 17, 71 and 81. Approximately 100 g a.s./ha were applied for the first treatment, 200 g a.s./ha for the second, and again 200 g a.s./ha for the last application and the total application rate was approximately 500 g a.s./ha.

Leaves of the summer cut (BBCH 71) were analysed as an intermediate sample on the same day of the second application. Grapes and leaves were harvested at maturity with a pre-harvest interval (PHI) of 18 and 19 days, respectively.

The TRR of the edible RAC (grapes) was 1.70 mg eq/kg and was considerably lower than those of summer cut leaves and leaves at harvest (64.18 mg eq/kg and 42.66 mg eq/kg, respectively). The major amount of radioactivity (94.7–97.9% of the TRR) was effectively extracted with acetonitrile/water from all RACs leaving only minor amounts < 6.0% with the solids (PES). Parent compound and metabolites in the extracts were quantified by HPLC.

Parent compound and metabolites in the extracts were quantified by radio-HPLC. A total of 97.0% of the TRR was identified in grapes and 94.7% in leaves.

The unchanged fluopyram was the major compound detected in all extracts, comprising more than 95% of the TRR in both summer cut leaves and grapes, and approximately 91% of the TRR in leaves at harvest. Apart from parent, the residues consisted of fluopyram-pyridyl-carboxylic acid (PCA, M43), the 7- and 8-hydroxy metabolites, and the conjugate 7-hydroxy-glucoside. A total of 96.7% (62.05 mg eq/kg) of the

TRR was identified in summer cut leaves, 97.0% (1.65 mg eq/kg of the TRR) in grapes, and 94.7% (40.43 mg eq/kg) in the leaves at harvest.

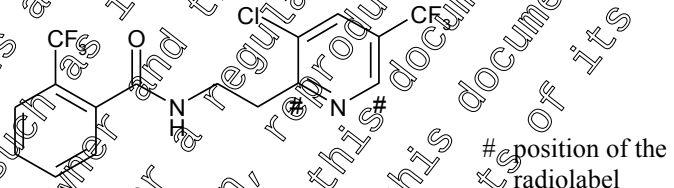
On the basis of the nature and amount of metabolites found in the extracts of grapes and leaves, the metabolic pathway of [pyridyl-2,6-14C]fluopyram in grapes was proposed and consisted of:

- Hydroxylation of the ethyl linking group of the a.s. forming 7- or 8- hydroxyl metabolites
- Conjugation of the AE C656948-7-hydroxy with glucose
- Hydrolytic cleavage and oxidation lead to fluopyram-pyridyl-carboxylic acid

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	 # position of the radiolabel
Compound	AE C656948
IUPAC name	*N*-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]ethyl}-2-(trifluoromethyl)benzamide
CAS name	Benzamide, *N*-[2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-(trifluoromethyl)-(9Cl)
CAS #	658066-35-4
Radiolabel position	Pyridyl-2,6-^{14}C
Specific radioactivity	3.85 MBq/mg (104.2 µCi/mg)
Purity Chemical Purity	> 98% (HPLC) > 98% (TLC) > 99% (HPLC)

2. Soil: "Monheim 2" (sandy loam from Germany), pH ($CaCl_2$) = 6.4, 60.0% sand, 28.2% silt and 11.8% clay, 1.3% organic carbon, cation exchange capacity (CEC) of 10 meq/100 g

3. Plant: Grapevine (*Vitis vinifera*), variety: "Mueller Thurgau"

B. Study Design

1. Experimental conditions:

The grapevine plant was grown in the vegetation area (building 6682) of Bayer CropScience AG, Metabolism / Environmental Fate, Monheim, Germany, which allowes plant growth under natural sunlight and temperatures, with a glass roof that can automatically close at the beginning of rainfall. The plant was cultivated in a 35 L bucket with a surface area of 0.125 m^2, filled with a sandy loam soil. The plant was sprayed with [pyridyl-2,6-^{14}C]fluopyram formulated as a SC 500 (Suspension Concentrate) using a graphic spray pistol.

The metabolism study simulated the envisaged use pattern and was based on a targeted application rate of 500 g a.s./ha as three sprayings. To compensate losses during applications, up to 20% excess of a.s. was applied. Three applications were performed at growth stage BBCH 17–19, BBCH 71 and BBCH 81, at actual application rates of approximately 100 g a.s./ha, 200 g a.s./ha and 200 g a.s./ha, respectively. This resulted in a total application rate of 498 g a.s./ha after subtraction of the losses in the rinses. The time interval between the first and the second application was 42 days and 49 days between the second and third application.

2. Sampling

Summer cut leaves: Directly after drying of the spray of the second application at growth stage fruit set (BBCH 71), the grapevine plant was pruned according to agricultural practice. The leaves and stalks of this summer prune were cut into small pieces, and were homogenised with liquid nitrogen using an Ultra-Turrax homogeniser. The complete homogenised plant material was used for extraction.

Grapes: At maturity (BBCH code 89 and PHI of 18 days), whole bunches of grapes were cut from vines, and the single grapes were cut from the stems. A representative aliquot of the grapes was surface washed and used for extraction. The remaining grapes were stored in aliquots at *ca.* -20°C.

Leaves at harvest: One day after the grapes had been sampled the leaves (at BBCH 89, PHI of 19 days) were cut from the vines. A representative aliquot of these leaves was cut into small pieces, and was homogenised with liquid nitrogen using an Ultra-Turrax homogeniser and was used for extraction. The rest of the leaves were stored at *ca.* -20°C.

C. Analytical Procedures

The grape RACs were extracted and the extracts were analysed by HPLC and TLC. The identification of parent compound and metabolites was based on co-chormatography experiments.

1. Extraction

In general, samples of the grape RACs were extracted 3 times with a mixture of acetonitrile/water (8/2, v/v) using an Ultra-Turrax. The extracts were separated from the solids by centrifugation. The radioactivity of the extracts was determined by volume measurement and LSC. The solids (PES) were air-dried, homogenised and weighed. Aliquots thereof were combusted and measured for radioactivity by LSC.

Grape berries were additionally surface washed prior to homogenization. After extraction, due to high sugar content, a phase separation acetonitrile : water occurred. Hence, the phases (organic and aqueous sugar phase) were separated using a separation funnel resulting in the combined acetonitrile phases and the combined water phases after all three extraction steps. An aliquot of the combined acetonitrile phases was concentrated prior to HPLC analysis using a rotary evaporator.

The ^{14}C-radioactivity of liquid samples was determined by liquid scintillation counting (LSC) using Quicksafe A containing 5% of water. The radioactivity in solid samples was measured by combustion. The released $^{14}CO_2$ was absorbed in an alkaline scintillation cocktail and radio assayed by LSC.

The total radioactive residues (TRR) in the RACs of grapes were determined by summation of the radioactivity in the combined acetonitrile/water extracts and in the PES. The residue levels are expressed as parent compound equivalents per weight. The combined extracts were analysed by HPLC for quantification of metabolites.

2. Identification and characterisation:
Identification of metabolites was done by co-chromatography using HPLC and TLC methods. Each metabolite was co-chromatographed either with its authentic reference compound and/or radiolabelled metabolites, which had been isolated and identified in a supplemental cell culture study and/or metabolism studies in beans and in rat.

3. Storage stability:

The RACs were extracted and analysed within a few days after sampling. All samples were stored at temperatures ≤ -18 °C. A few days after extraction, the earliest metabolite profiles of the combined extracts were obtained using a preliminary HPLC method until the final profiling method for the use in all plant metabolism studies was available. The extracts were analysed again using HPLC approximately eight to ten months after the initial analysis. The profiles from the initial and the late analysis showed no significant differences in the compound pattern. Therefore, it was concluded that the results of this study were not negatively influenced by storage effects.

II. Results and Discussion

The metabolism of [pyridyl-2,6-^{14}C]fluopyram, formulated as a SC 500, was investigated in grape following three foliar applications, at single application rates of approximately 100 g a.s./ha, 200 g a.s./ha and 200 g a.s./ha, respectively.

The TRR amounted to 64.18 mg eq/kg in summer cut leaves, 1.70 mg eq/kg in grapes and 42.66 mg eq/kg in leaves at harvest (Table 6.2.1-1). The TRR of the edible RAC (grapes) was significantly lower than those of the leaves cut in summer or leaves at harvest.

Table 6.2.1-4: TRR values in grape matrices after application of [pyridyl-2,6-^{14}C]fluopyram

Matrix	Timing and Applic. No.	PHI (days)	a.s.TRR (ppm, mg a.s. equiv./kg)
Summer cut leaves	two applications: at growth stages BBCH 17-19 and 71 1 x 100 g a.s./ha and 1 X 200 g a.s./ha	0	64.18
Grapes	three applications: at growth stages BBCH 17-19, 71 and 81 1 x 100 g a.s./ha and 2 X 200 g a.s./ha	18	1.70
Leaves at harvest	three applications: at growth stages BBCH 17-19, 71 and 81 1 x 100 g a.s./ha and 2 X 200 g a.s./ha	19	42.66

The grape matrices were extracted with acetonitrile/water (8:2, v/v), the extracts were analysed by HPLC and, where necessary, TLC, and parent compound and metabolites were identified.

Nearly the complete radioactive residue (97.3% of the TRR, 62.43 mg eq/kg) in summer cut leaves was extracted by acetonitrile/water (), leaving only 2.7% (1.75 mg eq/kg) in the solids (PES). Nearly the complete radioactive residues (97.1% of the TRR (1.65 mg eq/kg)) on or in grapes, were in the acetonitrile surface wash or extracted by acetonitrile/water (Table 6.2.1-5), leaving only 2.1% (0.04 mg eq/kg) in the solids (PES). The majority of the radioactive residues in leaves at harvest (94.7% of the TRR (40.43 mg eq/kg)) was extracted by acetonitrile/water (Table 6.2.1-5), whereas 5.2% (2.23 mg eq/kg) of the TRR remained in the solids (PES). Due to the low radioactive residue, the solids (PES) from all matrices were not further investigated.

Table 6.2.1-5: Distribution of radioactivity in the extracts of the grape matrices after application of [pyridyl-2,6-^{14}C]AE 656948

	Summer cut leaves		Grapes		Leaves at harvest	
TRR [mg eq/kg] =	64.18		1.70		42.66	
	% of TRR	mg eq/kg	% of TRR	mg eq/kg	% of TRR	mg eq/kg
Surface wash	n.a.	n.a.	76.9	1.31	n.a.	n.a.
Acetonitrile/water extract	97.3	62.43	20.2	0.34	94.7	40.43
Solids (PES)	2.7	1.75	2.1	0.04	5.2	2.23
Total extracted	97.3	62.43	97.1	1.65	94.7	40.43
Accountability	100.0	64.18	100.0	1.70	100.0	42.66

n.a. not applicable

For the elucidation of metabolites, the solvent extracts (acetonitrile/water) were analysed by HPLC or by TLC with radiodetection. Metabolites were either identified by co-chromatography (HPLC, TLC) with authentic reference compounds or with isolated metabolites in the course of other metabolism studies.

Summer cut leaves: Parent compound (95.7% of the TRR, 61.39 mg eq/kg) was detected as the predominant part of the residues in the extracts of summer cut leaves (Table 6.2.1-3). Several minor metabolites were detected in the extracts of the summer cut leaves. fluopyram-pyridyl-carboxylic acid (0.3% of the TRR, 0.21 mg eq/kg) was identified in the acetonitrile/water extract. The minor metabolites fluopyram-7-hydroxy (0.3% of the TRR, 0.20 mg eq/kg), fluopyram-8-hydroxy (0.2% of the TRR, 0.13 mg eq/kg), and fluopyram-7-hydroxy-glc (0.2% of the TRR, 0.12 mg eq/kg) were also assigned (Table 6.2.1-3). Additionally, one unknown compound (0.6% of the TRR, 0.39 mg eq/kg) was characterised by extraction and chromatographic behaviour.

Grapes: Parent compound (76.9% of the TRR, 1.31 mg eq/kg) was the only component detected in the surface wash of grapes. Due to the viscosity (high sugar content), the acetonitrile/water extracts separated into two phases. The acetonitrile phase of the grapes contained 19.5% (0.33 mg eq/kg) of the TRR of which parent compound (contributing 18.3% of the TRR, 0.31 mg eq/kg) was the predominant residue.

Hence, metabolites detected in grapes were minor, fluopyram-pyridyl-carboxylic acid, amounted to 0.9% (0.015 mg eq/kg) of the TRR. In addition, fluopyram-7-hydroxy (0.3% of the TRR, 0.005 mg eq/kg) was assigned (Table 6.2.1-3). The aqueous phase contained only 0.7% (0.01 mg eq/kg) of the TRR in total, and again fluopyram was the major compound (0.6% of the TRR, 0.008 mg eq/kg). Overall, the parent compound accounted in total for 95.8% (1.63 mg eq/kg) of the TRR in grapes (Table 6.2.1-3). A very polar minor metabolite (0.1% of the TRR, 0.002 mg eq/kg) remained unassigned, but was characterised by its extraction and chromatographic behaviour.

Leaves at harvest: In the extracts of leaves at harvest parent compound (91.3% of the TRR, 39.0 mg eq/kg) was again detected as the predominant part of the residues. Minor metabolites in the extracts of leaves were fluopyram-7-hydroxy (1.0% of the TRR, 0.43 mg eq/kg), fluopyram-7-hydroxy-glc (0.8% of the TRR, 0.34 mg eq/kg), fluopyram-8-hydroxy (0.8% of the TRR, 0.34 mg eq/kg) and fluopyram-pyridyl-carboxylic acid (0.8% of the TRR, 0.33 mg eq/kg).

Table 6.2.1-6: Summary of characterisation and identification of radioactive residues in grape matrices after application of [pyridyl-2,6-^{14}C]fluopyram

	Summer cut leaves		Grapes		Leaves at harvest	
TRR [mg eq/kg] =	64.18		1.70		42.66	
Compound	% of the TRR	mg eq/kg	% of the TRR	mg eq/kg	% of the TRR	mg eq/kg
AE C656948, a.s., fluopyram	95.7	61.39	95.8	1.63	91.3	39.00
fluopyram-8-hydroxy (M18)	0.2	0.13	n.d.	n.d.	0.8	0.34
fluopyram-7-hydroxy (M08)	0.3	0.20	0.3	0.01	1.0	0.43
fluopyram-7-hydroxy-glc (M11)	0.2	0.12	n.d.	n.d.	0.8	0.34
fluopyram-pyridyl-carboxylic acid (M43)	0.3	0.21	0.9	0.02	0.8	0.33
Total identified	96	62.05	97.0	1.65	94.7	40.43
Unknown 1 (GR0)	-	-	0.1	0.01	-	-
Unknown 2 (SC5)	0.6	0.39	-	-	-	-
Total characterised	0.6	0.39	0.1	< 0.01	-	-
Losses (phase separation)	-	-	0.8	0.01	-	-
Solids (non-extractable residue)	2.7	1.75	2.1	0.04	5.2	2.23
Accountability	100.0	64.18	100.0	1.70	100.0	42.66

III. Conclusions

After foliar spray application of [pyridyl-2,6-^{14}C]fluopyram, most of the recovered radioactivity was detected in summer cut leaves (64.19 mg eq/kg) and leaves at harvest (42.66 mg eq/kg), whereas significantly less residues were detected in grapes (1.70 mg eq/kg).

The radioactivity was easily extracted (minimum = 94% of the TRR) and identification of metabolites was done by co-chromatography using HPLC and TLC methods.

Unchanged parent compound was the predominant portion of the TRR in all matrices, accounting for more than 90% of the TRR. Metabolism of fluopyram was rather limited in grapevine and none of the metabolites were detected in portions of more than 1.0% of the TRR. The label specific compound fluopyram-pyridyl-carboxylic acid (PCA, M43) amounted to up to 0.9% of the TRR. Further metabolic reactions involved in the degradation of fluopyram were the hydroxylation of parent compound forming the fluopyram-7-hydroxy and 8-hydroxy metabolites. The latter as well as the glucoside conjugate of fluopyram-7-hydroxy were only detected in leaves.

Hence major metabolic reactions involved in the degradation of fluopyram were:

- Hydroxylation of the ethyl linking group of the a.s. forming 7- or 8- hydroxy metabolites
- Conjugation of the fluopyram-7-hydroxy with glucose
- Further oxidation and hydrolytic cleavage led to fluopyram-pyridyl-carboxylic acid

From the results of this study, the metabolic pathway of [pyridyl-2,6-^{14}C]fluopyram in grapevines is proposed in Figure 6.2.1-2.

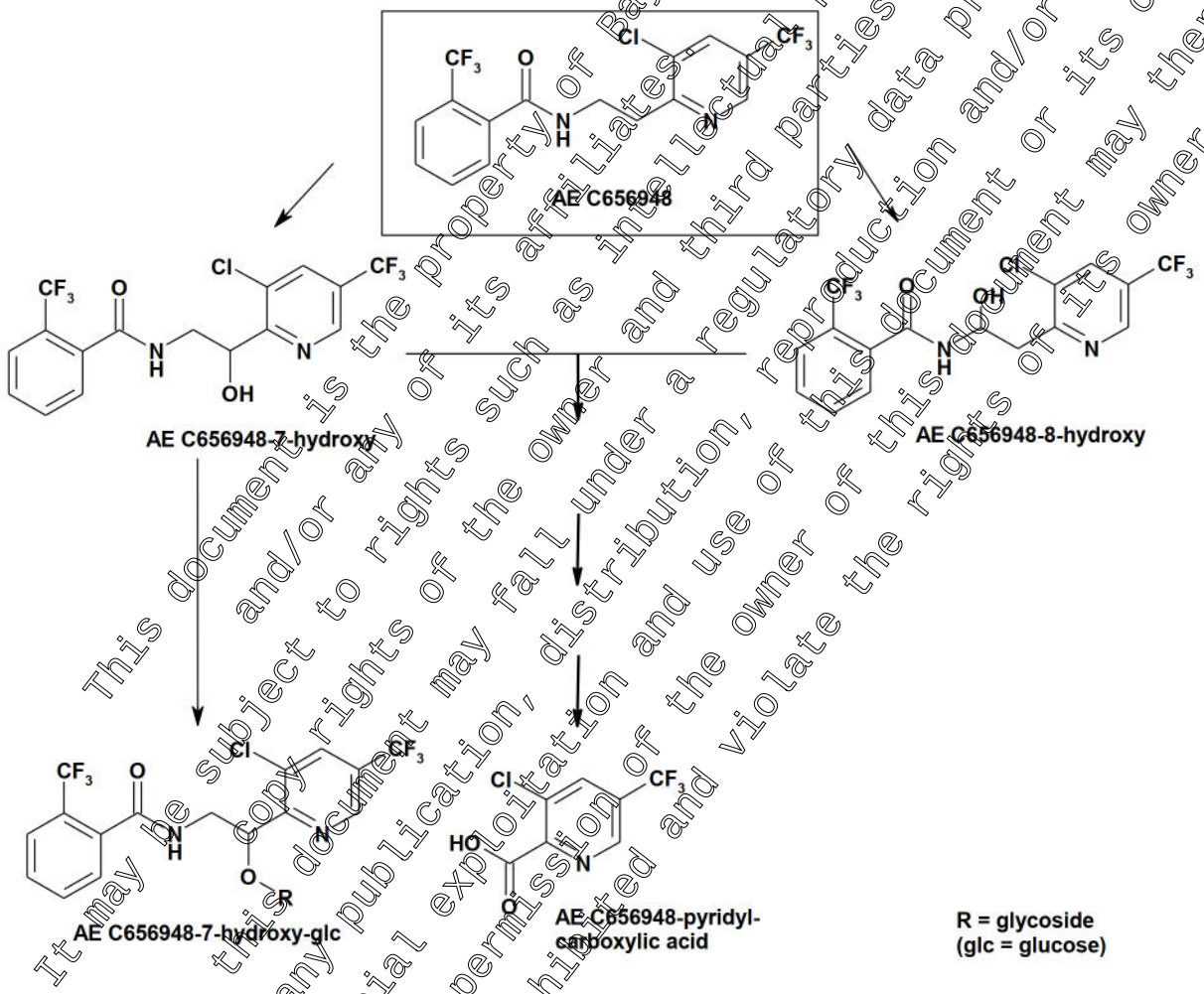


Figure 6.2.1-2: Proposed metabolic pathway of [pyridyl-2,6-^{14}C]AE C656948 (fluopyram) in grape

Assessment and conclusion by applicant:
The study is valid and acceptable.

Metabolism in potato (foliar spray application)

Data Point:	KCA 6.2.1/03
Report Author:	[redacted]
Report Year:	2007
Report Title:	Metabolism of [phenyl-UL-14C]AE C656948 in potatoes
Report No:	MEF-05/512
Document No:	M-286400-01-1
Guideline(s) followed in study:	US EPA OPPTS 860.1300, Canadian PMRA Ref.: DACO 6.7, EU 91/414/EEC amended by 96/68/EC
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted rev. 1 to Vol.3 of DAR, B.5 August 2012 (references relied on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

A metabolism study in potato were conducted with [phenyl-UL-^{14}C] fluopyram.

Executive Summary

The metabolism of [phenyl-UL-^{14}C]fluopyram, formulated as a SC 500 (Suspension Concentrate), was investigated in potato following three foliar spray applications. The applications were performed at growth stages BBCH 16, 55 and 71. Approximately 167 g a.s./ha were applied for each treatment and the total application rate was about 500 g a.s./ha.

Tubers and leaves were harvested at maturity with a pre-harvest interval (PHI) of 51 days.

The TRR of the edible RAC (tubers) was 0.008 mg eq/kg and was considerably lower than that of leaves (47.64 mg eq/kg). The major portion of radioactivity (96.7–99.4% of the TRR) was effectively extracted with acetonitrile/water from all RACs leaving only minor amounts ≤ 3.3% with the solids (PES). Parent compound and metabolites in the extracts were quantified by HPLC.

Unchanged fluopyram was the major compound detected in all extracts, comprising between 68.8% (0.006 mg eq/kg) of the TRR in tubers and 98.0% (46.69 mg eq/kg) of the TRR in leaves. fluopyram-benzamide was identified in the extract of tubers (0.001 mg eq/kg, 7.1% of the TRR) and also assigned in

the extract of leaves (0.23 mg eq/kg, 0.5% of the TRR). fluopyram-7-hydroxy was detected in low amounts in the extract of tubers and leaves.

A total of 77.1% (0.006 mg eq/kg) of the TRR was identified in tubers and 99.2% (47.28 mg eq/kg) of the TRR in leaves.

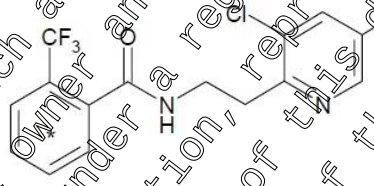
The metabolic reactions in potato involved were:

- Hydroxylation of parent compound leading to fluopyram-7-hydroxy
- Hydrolysis of fluopyram-7-hydroxy forming fluopyram-benzamide

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	 * position of the radiolabel
Compound	AE C656948
IUPAC name	*N*-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]ethyl}-2-(trifluoromethyl)benzamide
CAS name	Benzamide, *N*-[2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-(trifluoromethyl)-(9Cl)
CAS #	658066-35-4
Radiolabel position	Phenyl-UL-^{14}C
Specific radioactivity	3.85 MBq/mg (104.2 µCi/mg)
Purity Chemical Purity	> 98% (HPLC) > 99% (TLC) > 99% (HPLC)

2. Soil: "Monheim 3" (sandy loam from Germany), pH ($CaCl_2$) = 6.4, 60.7% sand, 26.3% silt and 13.0% clay, 1.36% organic carbon, cation exchange capacity (CEC) of 5.9 meq/100 g.

3. Plant: Potato, variety: "Cilena"

B. Study Design

1. Experimental conditions:

A total of six potato plants were grown in the vegetation area (building 6682) of Bayer CropScience AG, Metabolism / Environmental Fate, Monheim, Germany, which allows plant growth under natural sunlight and temperatures. The plants were cultivated in a planting container with a surface area of 1 m^2, filled with a sandy loam soil. The plants were sprayed with [phenyl-UL-^{14}C]fluopyram formulated as a SC 500 using a computer controlled track sprayer with a flat fan nozzle directed onto the plants.

The metabolism study simulated the envisaged use pattern and was based on a proposed total application rate of 500 g a.s./ha as three sprayings. To compensate losses during applications, 10% excess of a.s. was applied. Three applications were performed at growth stage BBCH 16, BBCH 55 and BBCH 71. The actual rates for each or the three applications were 167.3 g a.s./ha, 175.6 g a.s./ha and 176.0 g a.s./ha and resulted in a total actual application rate of 518.8 g a.s./ha (nominal 500 g a.s./ha). The intervals between the first and the second applications were 16 days and 11 days between the second and third applications.

2. Sampling

Leaves: At maturity (BBCH code 97 and PHI of 51 days), the leaves of the plants were cut above the soil surface. A representative portion of leave material was cut into small pieces and was homogenised with liquid nitrogen using a Polytron. An aliquot of the homogenised sample was used for extraction. Residual sample material was stored in aliquots at ca. -20 °C.

Tubers: At maturity (BBCH code 97 and PHI of 51 days) tubers were dug out of the soil. Tubers were left to dry and dry soil particles were removed by hand. Then the tubers were washed with water and the radioactivity of the wash solution was determined. The wash solution was not further investigated.

Half of the washed tubers were cut into slices and half of these slices were homogenised with liquid nitrogen using a Polytron. A suitable aliquot of the homogenised sample material was used for extraction. All other remaining sample material was stored in aliquots at ca. -20 °C.

C. Analytical Procedures

The potato RACs were extracted and the extracts were analysed by HPLC. The identification of parent compound and metabolites was based on LC-MS and co-chromatography experiments.

1. Extraction

In general, samples of the potato RACs were extracted 3 times with a mixture of acetonitrile/water (8/2, v/v) using a Polytron. The extracts were separated from the solids by suction through a filter and combined. The radioactivity of the extracts was determined by volume measurement and LSC. The solids (PES) were air-dried and weighed. Aliquots thereof were combusted and measured for radioactivity by LSC.

Prior to HPLC analysis combined extracts were mixed with the emulsifier HOT 5902 and concentrated. Tuber extracts were furthermore precipitated with acetonitrile and the precipitate was separated and dissolved in acetonitrile.

The ^{14}C-radioactivity of liquid samples was determined by liquid scintillation counting (LSC) using Quicksafe A containing 5% of water. The radioactivity in solid samples was measured by combustion. The released $^{14}CO_2$ was absorbed in an alkaline scintillation cocktail and radio assayed by LSC.

The total radioactive residues (TRR) in the RACs of grapes were determined by summation of the radioactivity in the combined acetonitrile/water extracts and in the PES. The residue levels are expressed as parent compound equivalents per weight. The combined extracts were analysed by reversed phase HPLC for quantification of metabolites.

2. Identification and characterisation:

Parent compound was identified by LC-MS-spectroscopy of an isolated HPLC-fraction of the extract of tubers. Metabolites were either identified by co-chromatography with reference compounds taken from a metabolism study in rat and/or in cell culture or characterized by their extraction and chromatographic behaviour.

3. Storage stability:

All samples were stored at temperature of -20°C. All samples were extracted within four days after sampling. The earliest metabolite profiles of the combined extracts were obtained by HPLC coupled to a radioactivity detector using a preliminary HPLC method until the final profiling method for the use in all plant metabolism studies was available. Within approximately one month, the extracts were analyzed using this final HPLC method. The profiles showed no significant differences in the compound pattern and in the amounts of metabolites compared to the very first quantifications. Therefore, it was concluded that the residues and the extracts were stable over the time of the analyses.

II. Results and Discussion

The metabolism of [phenyl-UL-^{14}C]fluopyram, formulated as a SC 500 (Suspension Concentrate), was investigated in potato following three foliar applications, at a total application rate of 518.8 g a.s./ha.

The TRR amounted to 0.008mg eq/kg in tubers and 47.64 mg eq/kg in leaves (Table 6.2.1-1). The TRR of the edible RAC (tuber) was significantly lower than those of the leaves.

Table 6.2.1-7: TRR values in potato matrices after application of [phenyl-UL-^{14}C]fluopyram

Matrix	Timing and Applic. No.	PHI (days)	TRR (ppm, mg eqiv./kg
Tubers	three applications: at growth stages BBCH 16, 55 and 71 3 x 167 g a.s./ha	51	0.008
Leaves	three applications: at growth stages BBCH 16, 55 and 71 3 x 167 g a.s./ha	51	47.64

The potato matrices were extracted with acetonitrile/water (8/2, v/v), the extracts were analysed by HPLC and parent compound and metabolites were identified.

The majority of the radioactive residue of tubers, 96.7% (0.008 mg eq/kg) of the TRR, was extracted using acetonitrile/water (8/2, v/v) and 3.3% (<0.001 mg eq/kg) of the TRR remained in the solids (Table 6.2.1-8). The major amount of radioactivity in leaves, 99.4% (47.35 mg eq/kg) of the TRR was extracted using acetonitrile/water (8/2, v/v) and 0.6% (0.29 mg eq/kg) of the TRR remained in solids (Table 6.2.1-8). Due to the low radioactive residue, the solids (PES) from all matrices were not further investigated.

Table 6.2.1-8: Distribution of radioactivity in the extracts of the potato matrices after foliar spray application of [phenyl-UL-^{14}C]AE 656948

	Tubers		Leaves	
TRR [mg eq/kg] =	0.008		47.64	
	% of TRR	mg eq/kg	% of TRR	mg eq/kg
Acetonitrile/water extract	96.7	0.008	99.4	47.35
Solids (PES)	3.3	<0.001	0.6	0.29
Total extracted	96.7	0.008	99.4	47.35
Accountability	100.0	0.008	100.0	47.64

For the elucidation of metabolism, the solvent extracts (acetonitrile/water) were analysed by HPLC. The parent compound was identified by LC-MS, metabolites were identified by co-chromatography (HPLC) with reference compounds.

Tubers: The parent compound was the major compound and was found in amounts of 68.8% (0.006 mg eq/kg) of the TRR. The only other compounds detected in tubers were metabolite fluopyram-benzamide (7.1% of the TRR, 0.001 mg eq/kg)and metabolite fluopyram-7-hydroxy (<0.001 mg eq/kg, 1.2% of the TRR).

Leaves: The parent compound was the major compound and was found in amounts of 98.0% (46.69 mg eq/kg) of the TRR. The only other metabolites detected were fluopyram-benzamide (0.5% of the TRR, 0.23 mg eq/kg). and fluopyram-7-hydroxy (0.36 mg eq/kg, 0.8% of the TRR).

All other minor unassigned metabolites were characterised by their extraction and chromatographic behaviour.

Table 6.2.1-9: Distribution of parent compound and metabolites in the extracts of potato matrices after foliar spray application of of [phenyl-UL-^{14}C]fluopyram

	Tubers		Leaves	
TRR [mg eq/kg] =	0.008		47.64	
Compound	% of the TRR	mg eq/kg	% of the TRR	mg eq/kg
AE C656948, a.s., fluopyram	68.8	0.006	98.0	46.69
fluopyram-7-hydroxy (M08)	1.2	<0.001	0.8	0.36
fluopyram-benzamide (M25)	7.1	0.001	0.5	0.23
Total identified	77.1	0.006	99.2	47.28
Unidentified compound MET 2	-	-	0.1	0.07
Unidentified compound MET 3	3.1	<0.001	-	-
Unidentified compounds in the solution of the precipitate (dissolved in acetonitrile)	12.4	0.001	-	-
Distillate of the combined extracts	4.1	<0.001	-	-
Total characterised	19.5	0.002	0.1	0.07
Solids (non-extractable residue)	3.3	<0.001	0.6	0.29
Accountability	100.0	0.008	100.0	47.64

III. Conclusions

After foliar spray application of [phenyl-UL^{14}C]fluopyram, most of the recovered radioactivity was detected in leaves (47.64 mg eq/kg), whereas only very little residues were detected in tubers (0.008 mg eq/kg).

The radioactivity was easily extracted (> 96% of the TRR) and identification of metabolites was done by co-chromatography using HPLC and by LC-MS.

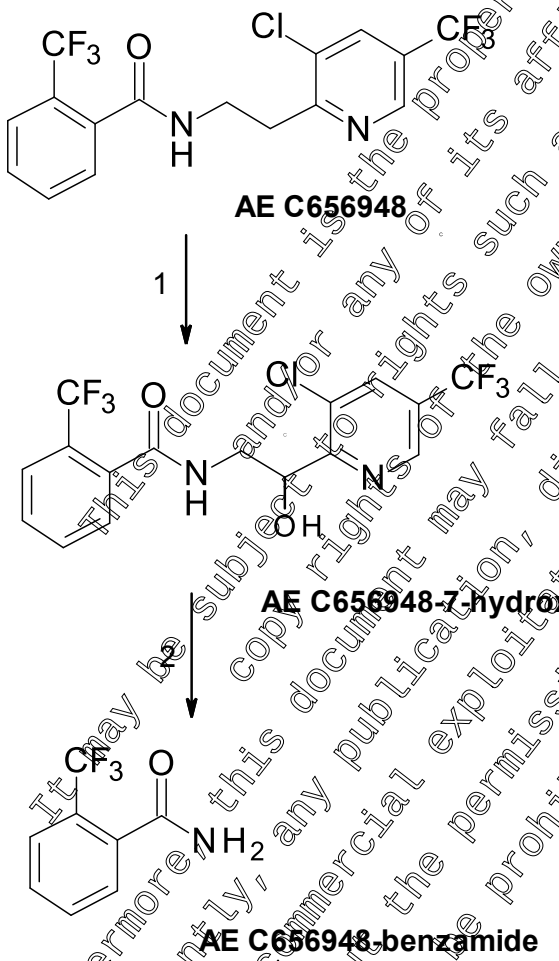
The major residue was parent compound and amounted to 68.8% of the TRR in tubers and 98.0% of the TRR in leaves. The label-specific compound fluopyram-benzamide was the main metabolite in tubers (7.1%

of the TRR), followed by fluopyram-7-hydroxy (1.2% of the TRR). Both metabolites were also detected as minor residues in leaves (each < 0.8% of the TRR).

The following metabolic routes were deduced:

- Hydroxylation of parent compound leading to fluopyram-7-hydroxy
- Hydrolysis of fluopyram-7-hydroxy forming fluopyram-benzamide

On the basis of the nature and amount of metabolites found in the extracts of tubers and leaves, the metabolic pathway of [phenyl-UL-^{14}C]fluopyram in potatoes is proposed below.



1–hydroxylation **2**–hydrolysis / **cleavage**

Figure 6.2.1-3: Proposed metabolic pathway of [phenyl-UL-^{14}C]AE C656948 (fluopyram) in potato

Assessment and conclusion by applicant:
The study is valid and acceptable.

A metabolism study in potato were conducted with [pyridyl-2,6-^{14}C] fluopyram:

Data Point:	KCA 6.2.1/04
Report Author:	[redacted]
Report Year:	2007
Report Title:	Metabolism of [pyridyl-2,6-^{14}C]AE C656945 in potatoes
Report No:	MEF-05/513
Document No:	M-286531-01-1
Guideline(s) followed in study:	US EPA OPPTS 860.1300; Canadian PMRA ref.: DACO 6.1; EU 91/414/EEC amended by 96/68/EC
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted rev. 1 to Vol.3 of CAR B7 August 2012 (reference relied on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The metabolism of [pyridyl-2,6-^{14}C]fluopyram, formulated as a SC 500 (Suspension Concentrate), was investigated in potato following three foliar spray applications performed at growth stages BBCH 16, 55 and 71. Approximately 167 g a.s./ha were applied for each treatment so the total application rate was about 500 g a.s./ha.

Tubers and leaves were harvested at maturity with a pre-harvest interval (PHI) of 51 days.

The TRR of the edible RAC (tubers) was 0.012 mg eq/kg and was considerably lower than that of leaves (21.67 mg eq/kg). The major portion of radioactivity (95.3–99.6% of the TRR) was effectively extracted with acetonitrile/water from all RACs leaving only minor amounts ≤ 4.7% with the solids (PES). Parent compound and metabolites in the extracts were quantified by HPLC.

Unchanged fluopyram was the major compound detected in potato leaves, representing 98.1% (21.26 mg eq/kg) of the TRR in leaves. It also represented 23.2% (0.003 mg eq/kg) of the TRR in tubers.

Label-specific fluopyram-pyridyl-carboxylic acid was the main metabolite in the extract of tubers (0.006 mg eq/kg, 49.8% of the TRR) and was also observed in the extract of leaves in low levels (0.11 mg eq/kg, 0.5% of the TRR). fluopyram-7-hydroxy (M08) was detected in low amounts in the extract of tubers (<0.001 mg eq/kg, 1.1% of the TRR) and of leaves (0.12 mg eq/kg, 0.6% of the TRR).

A total of 74.1% (0.009 mg eq/kg) of the TRR was identified in tubers and 99.2% (21.49 mg eq/kg) of the TRR in leaves.

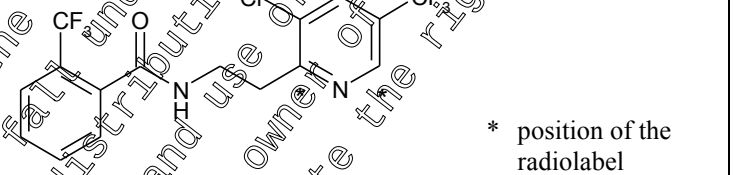
The metabolic reactions involved were:

- Hydroxylation of parent compound leading to fluopyram-7-hydroxy
- Hydrolysis of fluopyram-7-hydroxy and a subsequent oxidation forming fluopyram-pyridyl-carboxylic acid.

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	 * position of the radiolabel
Compound	AE C656948
IUPAC name	*N*-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]ethyl}-2-(trifluoromethyl)benzamide
CAS name	Benzamide, *N*-[2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-(trifluoromethyl)-(9Cl)
CAS #	658066-35-4
Radiolabel position	Pyridyl-2,6-^{14}C
Specific radioactivity	3.85 MBq/mg (104.2 µCi/mg)
Purity Chemical Purity	> 98% (HPLC) > 98% (TLC) 99% (HPLC)

2. Soil: "Monheim 3" (sandy loam from Germany), pH ($CaCl_2$) = 6.4, 60.7% sand, 26.3% silt and 13.0% clay, 1.36% organic carbon, cation exchange capacity (CEC) of 5.9 meq/100 g

3. Plant: Potato, variety: "Cilena"

B. Study Design

1. Experimental conditions:

A total of six potato plants were grown in the vegetation area (building 6682) of Bayer CropScience AG, Metabolism / Environmental Fate, Monheim, Germany, which allows plant growth under natural sunlight and temperatures. The plants were cultivated in a planting container with a surface area of 1 m^2, filled with a sandy loam soil. The plants were sprayed with [pyridyl-2,6-^{14}C]fluopyram formulated as a SC 500 using a computer controlled track sprayer.

The metabolism study simulated the envisaged use pattern and was based on a proposed total application rate of 500 g a.s./ha as three sprayings. To compensate losses during applications, 10% excess of a.s. was applied. Three applications were performed at growth stage BBCH 16, BBCH 55 and BBCH 71, at application rates of 170.1 g a.s./ha, 170.5 g a.s./ha and 165.1 g a.s./ha. This resulted in a total application rate of 505.7 g a.s./ha. The intervals between the first and the second applications were 16 days and 11 days between the second and third applications.

2. Sampling

Leaves: At maturity (BBCH code 97, PHI of 51 days) the leaves of the plants were cut above the soil surface. A representative portion of leave material was cut into small pieces and was homogenised with liquid nitrogen using a Polytron. An aliquot of the homogenised sample was used for extraction. Residual sample material was stored in aliquots at ca. -20 °C.

Tubers: At maturity (BBCH code 97, PHI of 51 days) tubers were dug out of the soil. Tubers were left to dry and dry soil particles were removed by hand. Then the tubers were washed with water and the radioactivity of the wash solution was determined. The wash solution was not further investigated.

Half of the washed tubers were cut into slices and half of these slices were homogenised with liquid nitrogen using a Polytron. A suitable aliquot of the homogenised sample material was used for extraction. All other remaining sample material was stored in aliquots at ca. -20 °C.

C. Analytical Procedures

The potato RACs were extracted and the extracts were analysed by reversed phase HPLC. The identification of parent compound and metabolites was based on LC-MS and co-chormatography experiments.

1. Extraction

In general, samples of the potato RACs were extracted 3 times with a mixture of acetonitrile/water (8/2, v/v) using a Polytron. The extracts were separated from the solids by suction through a filter. The radioactivity of the extracts was determined by volume measurement and LSC. The solids (PES) were air-dried and weighed. Aliquots thereof were combusted and measured for radioactivity by LSC.

Prior to HPLC analysis, combined extracts were mixed with the emulsifier HOT 5902 and concentrated. Tuber extracts were furthermore precipitated with acetonitrile and the precipitate was separated and dissolved in acetonitrile.

The ^{14}C-radioactivity of liquid samples was determined by liquid scintillation counting (LSC) using Quicksafe A containing 5% of water. The radioactivity in solid samples was measured by combustion. The released $^{14}CO_2$ was absorbed in an alkaline scintillation cocktail and radio assayed by LSC.

The total radioactive residues (TRR) in the RACs of grapes were determined by summation of the radioactivity in the combined acetonitrile/water extracts and in the PES. The residue levels are expressed as parent compound equivalents per weight. The combined extracts were analysed by HPLC for quantification of metabolites.

2. Identification and characterisation:

Parent compound and fluopyram-pyridyl-carboxylic acid were identified by LC-MS-spectroscopy of isolated HPLC-fractions of the extract of tubers, fluopyram-7-hydroxy was identified by co-chromatography with reference compound from a cell culture study.

3. Storage stability:

All samples were stored at temperature of ≤ -20 °C. All samples were extracted within four days after sampling. The earliest metabolite profiles of the combined extracts were obtained by HPLC coupled to a radioactivity detector using a preliminary HPLC method until the final profiling method for the use in all plant metabolism studies was available. Within approximately one month, all extracts were analyzed using this final HPLC method. The profiles showed no significant differences in the compound pattern and in the amounts of metabolites compared to the very first quantifications. Therefore, it was concluded that the residues and the extracts were stable over the time of the analyses.

II. Results and Discussion

The metabolism of [pyridyl-2,6-^{14}C]fluopyram, formulated as a SC 500, was investigated in potato following three foliar applications, at a total application rate of 505.7 g a.s./ha.

The TRR amounted to 0.012 mg eq/kg in tubers and 21.67 mg eq/kg in leaves.The TRR of the edible RAC (tuber) was significantly lower than that of the leaves.

Table 6.2.1-10: TRR values in potato matrices after application of [pyridyl-2,6-^{14}C]fluopyram

Matrix	Timing and Applic. No.	PHI (days)	TRR (ppm, mg eqiv./kg)
Tubers	three applications: at growth stages BBCH 16, 55 and 71 3 x 167 g a.s./ha	51	0.012
Leaves	three applications: at growth stages BBCH 16, 55 and 71 3 x 167 g a.s./ha	51	21.67

The potato matrices were extracted with acetonitrile/water (8/2, v/v) the extracts were analysed by HPLC and parent compound and metabolites were identified.

The majority of the radioactive residue of tubers, 95.3% (0.012 mg eq/kg) of the TRR, was extracted using acetonitrile/water (8/2, v/v) and 4.7% (0.001 mg eq/kg) of the TRR remained in the solids (Table 6.2.1-11). The major amount of radioactivity in leaves, 99.6% (21.57 mg eq/kg) of the TRR was extracted using acetonitrile/water (8/2, v/v) and 0.4% (0.10 mg eq/kg) of the TRR remained in solids (Table 6.2.1-11). Due to the low radioactive residue, the solids (PES) from all matrices were not further investigated.

Table 6.2.1-11: Distribution of radioactivity in the extracts of the potato matrices after foliar spray application of [pyridyl-2,6-^{14}C]AE 656948

	Tubers		Leaves	
TRR [mg eq/kg] =	0.012		21.67	
	% of TRR	mg eq/kg	% of TRR	mg eq/kg
Acetonitrile/water extract	95.3	0.012	99.6	21.57
Solids (PES)	4.7	0.001	0.4	0.10
Total extracted	95.3	0.012	99.6	21.57
Accountability	100.0	0.012	100.0	21.67

For the elucidation of the metabolites, the solvent extracts (acetonitrile/water) were analysed by HPLC. The parent compound and metabolites were identified by LC-MS/MS and co-chromatography (HPLC) with a radiolabelled reference compound.

Tubers: Parent compound was found in amounts of 23.2% (0.003 mg eq/kg) of the TRR in tubers. The main metabolite in tubers was fluopyram-pyridyl-carboxylic acid, which amounted to 49.8% (0.006 mg eq/kg) of the TRR. A minor metabolite, fluopyram-7-hydroxy was detected in the extract of tubers (<0.001 mg eq/kg, 1.1% of the TRR).

Leaves: Parent compound represented the major portion of the TRR in leaves with 98.1% (21.26 mg eq/kg) of the TRR in leaves. Two minor metabolites, fluopyram-7-hydroxy (0.12 mg eq/kg, 0.6% of the TRR) and fluopyram-pyridyl-carboxylic acid (0.11 mg eq/kg, 0.5% of the TRR) were

detected in the extract of leaves.

All other minor unassigned metabolites were characterised by their extraction and chromatographic behaviour.

Table 6.2.1-12: Summary of characterisation and identification of radioactive residues in potato matrices after foliar spray application of [pyridyl-2,6-^{14}C]fluopyram

	Tubers		Leaves	
TRR [mg eq/kg] =	0.012		21.67	
Compound	% of the TRR	mg eq/kg	% of the TRR	mg eq/kg
AE C656948, a.s., fluopyram	23.2	0.003	98.1	21.26
fluopyram-7-hydroxy (M08)	1.1	0.001	0.6	0.12
fluopyram-pyridyl-carboxylic acid (M43)	49.8	0.006	0.5	0.11
Total identified	74.1	0.009	99.2	21.49
Unidentified compound MET 1	3.6	<0.001	-	-
Unidentified compound MET 2	2.7	0.001	0.4	0.08
Unidentified compounds in the solution of the precipitate (dissolved in acetonitrile)	11.6	0.001	-	-
Distillate of the combined extracts	4.4	<0.001	-	-
Total characterised	21.2	0.003	0.4	0.08
Solids (non-extractable residue)	4.7	0.001	0.4	0.10
Accountability	100.0	0.012	100.0	21.67

III. Conclusions

After foliar spray application of [pyridyl-2,6-^{14}C]fluopyram, most of the recovered radioactivity was detected in leaves (21.67 mg eq/kg), whereas only very little residues were detected in tubers (0.012 mg eq/kg).

The radioactivity was easily extracted (> 95% of the TRR) and metabolites were identified by co-chromatography using HPLC and by LC-MS/MS.

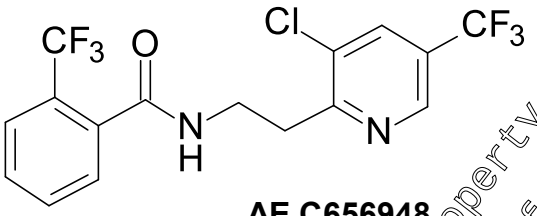
The label-specific compound fluopyram-pyridyl-carboxylic acid was the main compound in tubers (49.8% of the TRR), while the parent compound accounted for 23.2% of the TRR and fluopyram-7-hydroxy to 1.1% of the TRR. The major residue in leaves was the parent compound (98.1% of the TRR). fluopyram-7-hydroxy and fluopyram-pyridyl-carboxylic acid were both detected as minor metabolites in leaves (< 0.6% of the TRR).

The following metabolic routes were deduced:

- Hydroxylation of parent compound led to fluopyram-7-hydroxy

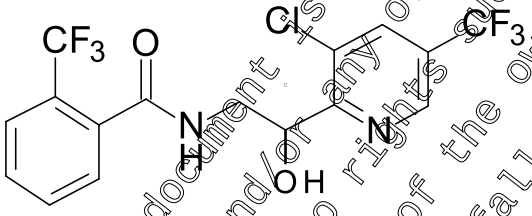
- Hydrolysis of fluopyram-7-hydroxy and a subsequent oxidation forming fluopyram-pyridyl-carboxylic acid

On the basis of the nature and amount of metabolites found in tubers and leaves, the metabolic pathway of [pyridyl-2,6-14C]fluopyram in potatoes is proposed below.



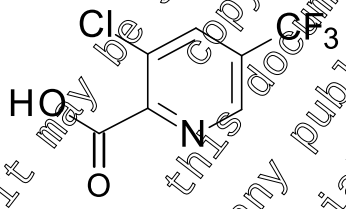
AE C656948

1



AE C656948-7-hydroxy

2



AE C656948-pyridyl-carboxylic acid

1–hydroxylation **2**–hydrolysis / **cleavage**

Figure 6.2.1-4: Proposed metabolic pathway of [pyridyl-2,6-^{14}C]AE C656948 (fluopyram) in potato

Assessment and conclusion by applicant:
The study is valid and acceptable.

Metabolism in beans (foliar spray application)

Metabolism studies in beans were conducted with [phenyl-UL-^{14}C]fluopyram:

Data Point:	KCA 6.2.1/05
Report Author:	[redacted]
Report Year:	2011
Report Title:	Metabolism of [phenyl-UL-14C]AE C656948 in beans after spray application
Report No:	MEF-06/085
Document No:	M-283061-02-1
Guideline(s) followed in study:	US-EPA OPPTS 860.1300; Canadian PMRA PACO 6.3; EC 91/414/EEC amended by 96/68/EC
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted rev. 1 to Vol.3 of DAR B.8 August 2012 (reference relied on)
GLP/Officially recognised testing facilities:	yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The metabolism of [phenyl-UL-^{14}C]fluopyram, formulated as a SC 500, was investigated in beans following two foliar spray applications. The applications were performed at growth stages BBCH 51 and BBCH 75. The single application rates were 268.8 g a.s./ha and 259.3 g a.s./ha for the first and the second application, respectively, and the total application rate was 528 g a.s./ha.
The immature RACs investigated green beans and foliage were taken four days after the second application. The mature RACs beans ("succulent beans") and straw were harvested 29 days after the last application. A portion of the mature beans were dried for 11 days and were analyzed as edible RAC "dry beans".

The TRR of the edible RACs (green, succulent and dry beans) ranged between 0.07 mg/kg and 1.40 mg/kg and was considerably lower than those of foliage and straw (36.66 mg/kg and 16.55 mg/kg, respectively). The major amount of radioactivity (93.9 – 98.1% of the TRR) was effectively extracted with

acetonitrile/water from all RACs leaving only minor amounts < 6.1% with the solids (PES). Parent compound and metabolites in the extracts were quantified by HPLC.

In green beans, the parent compound was the only compound detected in the extracts, amounting to 93.9% (1.31 mg/kg) of the TRR. The foliage TRR comprised of 93.8% parent compound. Other five metabolites were identified in foliage, none of them exceeding 2.2% of the TRR. The major part of the residues in succulent and dry beans consisted of fluopyram-benzamide, accounting for > 50% (0.04-0.08 mg eq/kg) of the TRR. The unchanged parent compound accounted for >10% of the TRR in both RACs (< 0.01 mg/kg in succulent bean and 0.02 mg/kg in dry bean). Five additional metabolites were identified in succulent and dry beans, each accounting for ≤ 0.01 mg eq/kg. In the straw, the residues again comprised of approximately 90% parent compound. None of the other five metabolites identified in straw exceeded 4.1% of the TRR.

A total of 93.9% of the TRR (1.31 mg/kg) was identified in green beans and 97.9% (35.90 mg eq/kg) in bean foliage, 83.6% (0.059 mg eq/kg) of the TRR was identified in succulent beans, 91.6% (0.11 mg eq/kg) of the TRR in dry beans, and 96.7% (16.0 mg eq/kg) of the TRR in bean straw. Only a few minor unknown components (each < 10% of the TRR and < 0.05 mg eq/kg) were characterised by their chromatographic behaviour in foliage and mature beans.

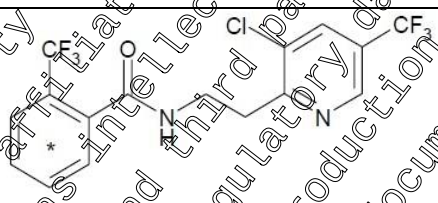
The metabolic reactions involved:

- Hydroxylation of the ethyl linking group of the a.s. forming 7- or 8- hydroxyl metabolites
- Cleavage leading to fluopyram-benzamide
- Conjugation of hydroxylated metabolites with hexoses and subsequent higher conjugations with malonic and glucuronic acid

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	 * position of the radiolabel
Compound	AE C656948
IUPAC name	*N*-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]ethyl}-2-(trifluoromethyl)benzamide
CAS name	Benzamide, *N*-[2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-(trifluoromethyl)-(9CI)
CAS #	658066-35-4
Radiolabel position	Phenyl-UL-^{14}C
Specific radioactivity	3.85 MBq/mg (104.2 µCi/mg)
Purity Chemical Purity	> 98% (HPLC) > 98% (TLC) > 99% (HPLC)

2. Soil: "Monheim 3" (sandy loam from Germany), pH ($CaCl_2$) = 6.4, 60.7% sand, 26.3% silt and 13.0% clay, 2.34% organic carbon, cation exchange capacity (CEC) of 5.9 meq/100 g

3. Plant: Bush beans, variety: "Dublette"

B. Study Design

1. Experimental conditions:

The bean plants were grown in the vegetation area (building 6682) of Bayer CropScience AG, Metabolism / Environmental Fate, Monheim, Germany, which allowes plant growth under natural sunlight and temperatures. Approximately 40 bush bean plants (variety: Dublette) were cultivated in a planting container with a surface area of 1.0 m^2, filled with a sandy loam soil. The plants were sprayed with [phenyl-UL-

[14C]fluopyram formulated as a SC 500 and the spraying was performed using a computer controlled track sprayer.

The metabolism study simulated the envisaged use pattern and was based on the maximum proposed application rate (500 g a.s./ha). To compensate losses during applications, 10% excess of a.s. was applied. Two applications were performed at growth stage BBCH 51 and BBCH 75, at actual application rates of 268.8 g a.s./ha and 259.3 g a.s./ha, respectively. This resulted in a total application rate of 528.1 g a.s./ha. The time interval between the first and the second application was 28 days.

2. Sampling

Green beans and foliage: As about 50% of the beans had a harvestable size (BBCH code 75; 4 days after treatment), these green beans (whole pods) were picked by hand. The green beans were cut into small pieces and were homogenised with liquid nitrogen using an Ultra-Turrax homogeniser. The complete homogenised plant material was used for extraction. At the same growth stage, a representative sample of the leaves (foliage) was cut from the bean plants. The leaf material was cut into small pieces and homogenised with liquid nitrogen using an Ultra-Turrax homogeniser. An aliquot of the homogenised sample was used for extraction. Residual sample material was stored in aliquots at *ca.* -20 °C.

Succulent beans and straw: At maturity (BBCH code 85-89; 29 days after last treatment), beans in their pods were cut from the plant and the succulent beans were picked from the pods. The pods were united with the rest of the plants, which were cut above the soil (straw fraction). The succulent beans were directly homogenised with liquid nitrogen using an Ultra-Turrax homogeniser. The straw fraction was processed in the same way after being cut into small pieces. A representative aliquot of the homogenised samples was used for extraction. Residual sample material was stored in aliquots at *ca.* -20 °C.

Dry beans: At maturity (BBCH code 89) beans of pods, which had already dried on the plant, were cut from the plant, and the beans were picked from the pods by hand. The pods were united with straw fraction. The beans were allowed to dry at room temperature until no loss of weight *(i.e.* water loss) was measured (11 days). A representative aliquot of the dry beans was allowed to soak overnight with water and was used for extraction. The rest of the dry beans were stored at *ca.* -20 °C.

C. Analytical Procedures

The bean RACs were extracted and the extracts were analysed by HPLC and TLC. The identification of parent compound and metabolites was based on co-chromatography experiments and HPLC-MS/MS and/or LC-NMR.

1. Extraction and fractionation:

In general, samples of the bean RACs were extracted 1–3 times with a mixture of acetonitrile/water (8:2, v/v) using an Ultra-Turrax. The extracts were separated from the solids by centrifugation. The radioactivity of the extracts was determined by volume measurement and LSC. The solids (PES) were lyophilised overnight, homogenised and weighed. Aliquots thereof were combusted and measured for radioactivity by LSC. Aliquots of the combined solvent extracts were concentrated using a Speedvac.

The ^{14}C-radioactivity of liquid samples was determined by liquid scintillation counting (LSC) using Quicksafe A containing 5% of water. The radioactivity in sold samples was measured by combustion. The released $^{14}CO_2$ was absorbed in an alkaline scintillation cocktail and radio assayed by LSC.

The total radioactive residues (TRR) in the RACs of beans were determined by summation of the radioactivity in the combined acetonitrile/water extracts and in the PES. The residue levels are expressed as parent compound equivalents per weight (mg eq/kg). The concentrated acetonitrile/water extracts were analysed by HPLC for quantification of metabolites.

2. Identification and characterisation:

First assignments to the metabolites were obtained after co-chromatography (HPLC, TLC) with authentic reference compounds, with metabolites isolated and identified in a supplemental cell culture study and/or the corresponding study in beans using the pyridyl label. Confirmation of these assignments was obtained after isolation of the major compounds out of the acetonitrile/water extract of dry beans by semipreparative HPLC. Corresponding fractions were combined, concentrated, purified in one of the secondary HPLC systems, and analysed by LC-NMR and/or HPLC-MS/MS.

3. Storage stability:

The RACs were extracted and analysed within a few days after sampling. All samples were stored at temperatures ≤ –18 °C. A few days after extraction, the earliest metabolite profiles of the combined extracts were obtained by HPLC using the profiling method for quantitation and identification of metabolites. The extracts were analysed again using HPLC approx. one month after the initial analysis. The profiles from the initial and the late analysis showed no significant differences in the compound pattern. Therefore, it was concluded that the results of this study were not negatively influenced by storage effects.

II. Results and Discussion

The metabolism of [phenyl-UL-^{14}C]fluopyram, formulated as a SC 500, was investigated in beans following two foliar applications at single rates of 268.8 g a.s./ha and 259.3 g a.s./ha and a total rate of 528 g a.s./ha.

The TRR amounted to 1.40 mg/kg in green beans, 36.66 mg/kg in the respective foliage, 0.07 mg/kg in succulent beans, 0.12 mg/kg in dry beans, and 16.55 mg/kg in bean straw (Table 6.2.1-13). The TRR of the edible RACs (green, succulent and dry beans) was significantly lower than those of the respective foliage or straw.

Table 6.2.1-13: TRR values in bean matrices after application of [phenyl-UL-^{14}C]fluopyram

Matrix	Timing and Applic. No.	PHI (days)	TRR (ppm, mg a.s. equiv./kg)
green beans	Two foliar spray applications: at growth stages BBCH 51 and BBCH 75; total 528 g a.s./ha	4	1.40
foliage	Two foliar spray applications: at growth stages BBCH 51 and BBCH 75; total 528 g a.s./ha	4	36.66
succulent beans	Two foliar spray applications: at growth stages BBCH 51 and BBCH 75; total 528 g a.s./ha	29	0.07
dry beans	Two foliar spray applications: at growth stages BBCH 51 and BBCH 75; total 528 g a.s./ha	29 + 14 days for drying	0.12
straw (including empty pods)	Two foliar spray applications: at growth stages BBCH 51 and BBCH 75; total 528 g a.s./ha	29	16.55

The bean matrices were extracted with acetonitrile/water (8:2, v/v), the extracts were analysed by HPLC and, where necessary, TLC and parent compound and metabolites were identified.

The majority of the radioactive residue in green beans, 93.9% (1.31 mg/kg) of the TRR, was extracted by acetonitrile/water (Table 6.2.1-14), whereas 6.1% (0.09 mg/kg) of the TRR remained in the solids (PES). Nearly the complete radioactive residues in bean foliage, 98.1% (35.97 mg/kg) of the TRR, was extracted by acetonitrile/water (Table 6.2.1-14), leaving only 1.9% (0.69 mg/kg) of the TRR in the solids (PES). The majority of the radioactive residue in succulent beans, 94.9% (0.067 mg/kg) of the TRR, was extracted by acetonitrile/water (Table 6.2.1-14), whereas 5.0% (< 0.01 mg/kg) of the TRR remained in the solids (PES). The majority of the radioactive residue in dry beans, 97.3% (0.117 mg/kg) of the TRR, was extracted by acetonitrile/water (Table 6.2.1-14), whereas 2.7% (< 0.01 mg/kg) of the TRR remained in the solids. The major amount of radioactivity in the bean straw, 96.7% (16.01 mg/kg) of the TRR was extracted by acetonitrile/water (Table 6.2.1-14), leaving only 3.3% (0.54 mg/kg) of the TRR with the solids (PES). Due to the low radioactive residue, the solids (PES) from all matrices were not further investigated.

Table 6.2.1-14: Distribution of radioactivity in the extracts of the bean matrices after foliar spray application of [phenyl-UL-^{14}C]AE 656948

	Green beans		Foliage		Succulent beans		Dry beans		Straw	
TRR [mg eq/kg] =	1.40		36.66		0.07		0.12		16.55	
	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Acetonitrile/water extract	93.9	1.31	98.1	35.97	94.9	0.067	97.3	0.117	96.7	16.01
Solids (PES)	6.1	0.09	1.9	0.69	5.0	0.003	2.7	0.003	3.3	0.54
Total extracted	93.9	1.31	98.1	35.97	94.9	0.067	97.3	0.117	96.7	16.01
Accountability	100.0	1.40	100.0	36.66	100.0	0.070	100.0	0.121	99.9	16.54

For the elucidation of metabolism, the solvent extracts (acetonitrile/water) were analysed by HPLC and, where required, also by TLC with radiodetection. Metabolites were either identified by co-chromatography (HPLC, TLC) with reference compounds or isolated metabolites or by LC-MS/MS and/or LC-NMR of isolated peaks.

Green beans and foliage: The parent compound (93.9%, 1.31 mg/kg of the TRR) was the only component detected in the extract of green beans (Table 6.2.1-15) and it was the predominant residue also in the extract of foliage (93.8%, 34.39 mg/kg of the TRR). The main metabolite in the concentrated extracts of foliage was the glucoside-malonic acid conjugate fluopyram-7-hydroxy-glc-MA (2.2%, 0.82 mg eq/kg of the TRR). In addition, the metabolites fluopyram-7-hydroxy (0.7%, 0.26 mg eq/kg of the TRR), fluopyram-benzamide (0.5%, 0.17 mg eq/kg of the TRR), fluopyram-7-hydroxy-glc (0.4%, 0.15 mg eq/kg of the TRR), fluopyram and fluopyram-8-hydroxy (0.3%, 0.11 mg eq/kg of the TRR) were identified (Table 6.2.1-15). Furthermore, two unknown minor compounds (each ≤0.1% of the TRR) were characterised in foliage by extraction and chromatographic behaviour.

Succulent beans: The main metabolite in the concentrated extract of succulent beans was AEC656948-benzamide (51.6%, 0.036 mg eq/kg of the TRR) whereas the parent compound was 11.4% (0.008 mg/kg) of the TRR. The metabolite identified as glucuronic acid-glycoside conjugate of the hydroxylated parent compound (fluopyram-hydroxy-glyc-gluc) accounted for 6.7% (0.005 mg eq/kg) of the TRR. Other identified minor metabolites accounted for < 0.005 mg eq/kg (Table 6.2.1-16). Furthermore, three unknown compounds (each < 10% of the TRR and ≤ 0.006 mg eq/kg) were characterized based on their extraction and chromatographic behaviour.

Dry beans: Major metabolites detected in the acetonitrile/water extract were fluopyram-benzamide (64.0%, 0.077 mg eq/kg of the TRR) and fluopyram-hydroxy-glyc-gluc (10.4%, 0.013 mg eq/kg of the TRR). Parent compound was detected as 12.6% of the TRR (0.015 mg/kg). The metabolites AE C6556948-7-hydroxy (2.5%, 0.003 mg eq/kg of the TRR) and 8-hydroxy (2.1%, 0.003 mg eq/kg of the TRR) were also detected in dry beans (Table 6.2.1-16). Additionally, two unknown components (each < 5% of the TRR and < 0.005 mg eq/kg) were characterised by extraction and chromatographic behaviour.

Straw: The parent compound fluopyram (90.2%, 14.94 mg/kg of the TRR) was the predominant residue also in the extract of straw. As observed in immature foliage, the other metabolites in the concentrated extract of straw were the fluopyram-7-hydroxy-glc-MA (4.1%, 0.68 mg/kg of the TRR) , AE C656948-7-hydroxy (0.7%, 0.12 mg/kg of the TRR), , fluopyram-8-hydroxy (0.6%, 0.09 mg/kg of the TRR) and fluopyram-7-hydroxy-glc (0.4%, 0.07 mg/kg of the TRR) and fluopyram-benzamide (0.6% TRR, 0.10 mg eq/kg) (Table 6.2.1-16).

Table 6.2.1-15: Summary of characterisation and identification of radioactive residues in green beans and bean foliage after foliar spray application of [phenyl-UL-^{14}C]fluopyram

	Green beans	Foliage
TRR [mg eq/kg] =	1.40	36.66

Compound	% of the TRR	mg eq/kg	% of the TRR	mg eq/kg
AE C656948, parent compound fluopyram	93.9	1.31	93.8	34.39
fluopyram-8-hydroxy (M18)	-	-	0.3	0.11
fluopyram-7-hydroxy (M08)	-	-	0.7	0.26
fluopyram-7-hydroxy-glc-MA (M12)	-	-	2.2	0.82
fluopyram-7-hydroxy-glc (M11)	-	-	0.4	0.15
fluopyram-hydroxy-glyc-gluc (M22)	-	-	-	-
fluopyram-benzamide (M25)	-	-	0.5	0.17
Total identified	93.9	1.31	97.9	35.90
unknown 1	-	-	0.1	0.02
unknown 2	-	-	0.1	0.04
Total characterised	-	-	0.2	0.06
Total extracted	93.9	1.31	98.1	35.97
Solids (non-extractable residue)	6.1	0.09	1.9	0.69
Accountability	100.0	1.40	100.0	36.66

Table 6.2.1-16: Summary of characterisation and identification of radioactive residues in succulent beans, dry beans and bean straw after foliar spray application of [phenyl-UL-^{14}C]fluopyram

	Succulent beans		Dry beans		Straw	
TRR [mg eq/kg] =	0.07		0.12		16.55	
Compound	% of the TRR	mg eq/kg	% of the TRR	mg eq/kg	% of the TRR	mg eq/kg
AE C656948, parent compound fluopyram	11.4	0.008	12.6	0.015	90.2	14.94
fluopyram-8-hydroxy (M18)	6.0	0.004	2.1	0.003	0.6	0.09
fluopyram-7-hydroxy (M08)	4.0	0.003	2.5	0.003	0.7	0.12
fluopyram-7-hydroxy-glc-MA (M12)	2.2	0.002	-	-	4.1	0.68
fluopyram-7-hydroxy-glc (M11)	1.7	0.001	-	-	0.4	0.07
fluopyram-hydroxy-glyc-gluc (M22)	6.7	0.005	10.4	0.013	-	-
fluopyram-benzamide (M25)	51.6	0.036	64.0	0.077	0.6	0.10
Total identified	83.6	0.059	91.6	0.111	96.7	16.01
unknown 3	1.9	0.001	3.2	0.004	-	-
unknown 4	6.1	0.001	-	-	-	-
unknown 5	8.3	0.006	2.5	0.003	-	-
Total characterised	11.3	0.008	5.7	0.007	-	-
Total extracted	94.9	0.067	97.3	0.117	96.7	16.01
Total bound residues (PES)	5.0	0.003	2.7	0.003	3.3	0.54
Accountability	100.0	0.070	100.0	0.121	99.9	16.54

III. Conclusions

After foliar spray application of [phenyl-UL^{14}C]fluopyram, most of the recovered radioactivity was detected in foliage (35.97 mg/kg) and straw (16.55 mg/kg), whereas only minor residues were detected in the respective bean samples (1.40 mg/kg for green beans, 0.07 mg/kg for succulent beans, and 0.12 mg/kg for dry beans).

Unchanged parent compound was the predominant portion of the TRR in foliage, straw and green beans, accounting for more than 90% of the TRR, and was still present (> 10% of the TRR but < 0.01 (succulent bean) and 0.02 mg/kg(dry bean)) in bean samples at maturity. In total, six metabolites were identified. The major metabolite in bean samples at maturity (succulent and dry) was fluopyram-benzamide (> 50% of the TRR). In other bean matrices, identified metabolites accounted generally for < 10% of the TRR.

The following metabolic routes were deduced:

- Hydroxylation of the ethyl linking group of the a.s. forming 7- or 8- hydroxyl metabolites
- Cleavage and oxidation leading to fluopyram-benzamide
- Conjugation of hydroxylated metabolites with hexoses and subsequent higher conjugations with malonic and glucuronic acid

On the basis of the nature and amount of metabolites found in foliage, beans and straw, the metabolic pathway of [phenyl-UL^{14}C]fluopyram in beans is proposed in Figure 6.2.1- 5.

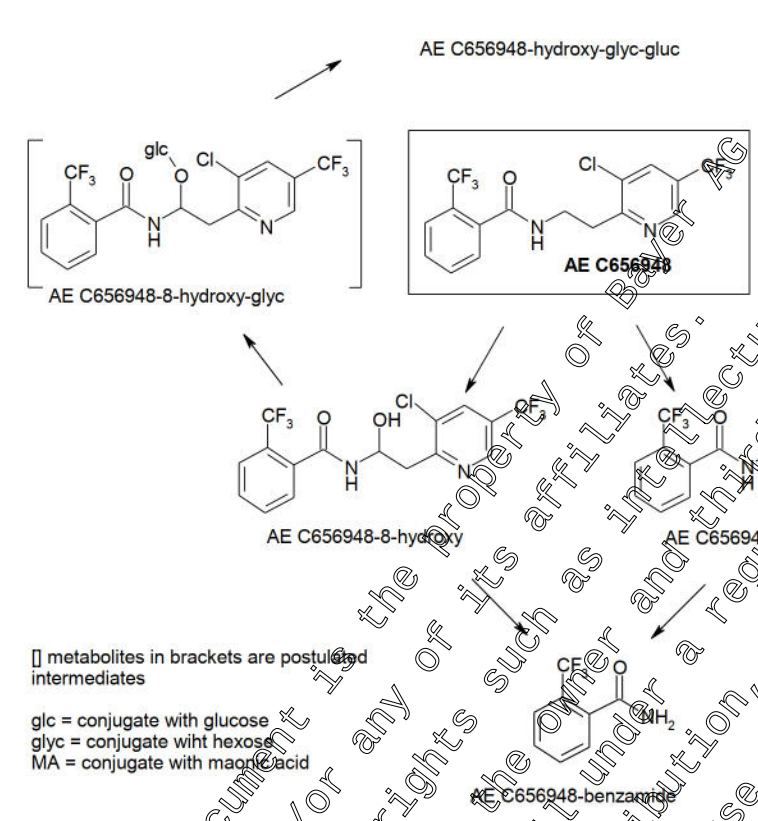


Figure 6.2.1- 5: Proposed metabolic pathway of [phenyl-UL-^{14}C]AE C656948 (fluopyram) in beans

Assessment and conclusion by applicant:

The study is valid and acceptable.

Metabolism studies in beans were conducted with [pyridyl-2,6-^{14}C]fluopyram:

Data Point:	KCA 6.2.1/06
Report Author:	[redacted]
Report Year:	2008
Report Title:	Metabolism of [pyridyl-2,6-14C]AE C656948 in beans after spray application
Report No:	MEF-06/004
Document No:	M-299067-01-1
Guideline(s) followed in study:	US EPA OPPTS 860.1300; Canadian PMRA Ref.: DACO 6.3; EU 91/414/EEC amended by 96/68/EC
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted rev. 1 to Vol.3 of DAR, B7 August 2012 (references relied on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The metabolism of [pyridyl-2,6-^{14}C]fluopyram, formulated as a SC 500 (Suspension Concentrate), was investigated in beans following two foliar spray applications. The applications were performed at growth stages BBCH 51 and BBCH 75) targeting a maximum annual application rate of 500 g a.s./ha. The single application rates were 264.6 g a.s./ha and 254.2 g a.s./ha for the first and the second application, respectively, resulting in a total actual application rate of about 519 g a.s./ha. The immature RACs investigated green beans and foliage were taken four days after the second application. The mature RACs beans ("succulent beans") and straw were harvested 29 days after the last application. A portion of the mature beans were dried for 11 days and were analyzed as edible RAC "dry beans".

The TRR of the edible RACs (green, succulent and dry beans) ranged between 0.17 mg eq/kg and 3.88 mg eq/kg and was considerably lower than those of foliage and straw (38.53 mg eq/kg and 19.02 mg eq/kg, respectively). The major amount of radioactivity (95.7–99.3% of the TRR) was effectively extracted with acetonitrile/water from all RACs leaving only minor amounts < 5% with the solids (PES). Parent compound and metabolites in the extracts were quantified by HPLC.

Regarding sampling at a PHI of 4 days, parent compound was detected at levels >90% TRR. In green beans, the parent compound was the only compound detected in the extracts, amounting to 99.3% (3.86 mg eq/kg) of the TRR. The foliage TRR comprised of 92.3% parent compound (35.53 mg eq/kg). Six other metabolites were identified in foliage, none of them exceeding 3.2% of the TRR. Regarding later sampling dates, the major part of the residues in succulent and dry beans consisted of fluopyram-pyridyl-carboxylic acid (PCA,

M43) and -acetic acid (PAA, M40), accounting each for > 20% (0.05–0.10 mg eq/kg) of the TRR. The unchanged parent compound accounted for <6% of the TRR in both RACs (≤ 0.02 mg eq/kg). Six additional metabolites were identified in succulent and dry beans, each accounting for ≤ 0.02 mg eq/kg. In the straw, the residues again comprised of approximately 87% parent compound (19.02 mg eq/kg). None of the other seven metabolites identified in straw exceeded 4.7% of the TRR/ 0.90 mg eq/kg.

A total of 99.3% of the TRR (3.86 mg eq/kg) was identified in green beans and 98.9% (38.06 mg eq/kg) in bean foliage, 79.8% (0.14 mg eq/kg) of the TRR was identified in succulent beans, 76.4% (0.24 mg eq/kg) of the TRR in dry beans, and 95.5% (18.15 mg eq/kg) of the TRR in bean straw. Only a few minor unknown components (each < 10% of the TRR and < 0.05 mg eq/kg) were characterised by their chromatographic behaviour in foliage, mature beans and straw.

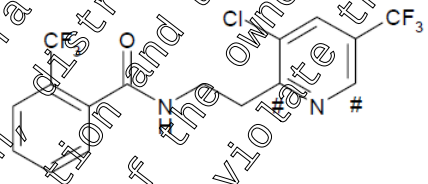
The metabolic reactions involved were:

- Hydroxylation of the ethyl linking group of the a.s. forming 7- or 8- hydroxyl metabolites
- Hydrolytic cleavage and oxidation leading to fluopyram-pyridyl-carboxylic acid and fluopyram-pyridyl acetic acid, respectively
- Conjugation of hydroxy-metabolites with hexoses and subsequent higher conjugations with malonic and glucuronic acid

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	 # positions of radiolabel
Compound	AEC656948
IUPAC name	*N*-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]ethyl}-2-(trifluoromethyl)benzamide
CAS name	Benzamide, *N*-[2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-(trifluoromethyl)-(9Cl)
CAS #	658066-35-4
Radiolabel position	Pyridyl-2,6-^{14}C
Specific radioactivity	3.85 MBq/mg (104.2 µCi/mg)
Purity Chemical Purity	> 98% (HPLC) > 98% (TLC) > 99% (HPLC)

2. Soil: "Monheim 3" (sandy loam from Germany), pH ($CaCl_2$) = 6.4, 60.7% sand, 26.3% silt and 13.0%

clay, 1.36% organic carbon, 2.34% organic material, cation exchange capacity (CEC) of 5.9 meq/100 g.

3. Plant: Bush beans, variety: "Dublette"

B. Study Design

1. Experimental conditions:

The bean plants were grown in the vegetation area (building 6682) of Bayer CropScience AG, Metabolism / Environmental Fate, Monheim, Germany, which allows plant growth under natural sunlight and temperatures. Approximately 40 bush bean plants (variety: Dublette) were cultivated in a planting container with a surface area of 1.0 m^2, filled with a sandy loam soil. The plants were sprayed with [pyridyl-2,6-^{14}C]fluopyram formulated as a SC 500 (Suspension Concentrate) and the spraying was performed using a computer controlled track sprayer.

The metabolism study simulated the envisaged use pattern and was based on the maximum proposed application rate (500 g a.s./ha). To compensate losses during applications, 10% excess of a.s. was targeted. Two applications were performed at growth stage BBCH 51 and BBCH 75 (interval 28 days), at actual application rates of 264.6 g a.s./ha and 254.2 g a.s./ha, respectively. This resulted in a total application rate of about 519 g a.s./ha.

2. Sampling

Green beans and foliage: As about 50% of the beans had a harvestable size (BBCH 75; 4 days after treatment), these green beans (whole pods) were picked by hand. The green beans were cut into small pieces and were homogenised with liquid nitrogen using an Ultra-Turrax homogeniser. The complete homogenised plant material was used for extraction. At the same growth stage, a representative sample of the leaves (foliage) was cut from the bean plants. The leaf material was cut into small pieces and homogenised with liquid nitrogen using an Ultra-Turrax homogeniser. An aliquot of the homogenised sample was used for extraction. Residual sample material was stored in aliquots at *ca.* -20 °C.

Succulent beans and straw: At maturity (BBCH code 85–89, 29 days after last treatment), beans in their pods were cut from the plant and the succulent beans were picked from the pods. The pods were united with the rest of the plants, which were cut above the soil (straw fraction). The succulent beans were directly homogenised with liquid nitrogen using an Ultra-Turrax homogeniser. The straw fraction was processed in the same way after being cut into small pieces. A representative aliquot of the homogenised samples was used for extraction. Residual sample material was stored in aliquots at *ca.* -20 °C.

Dry beans: At maturity (BBCH code 89) beans of pods, which had already dried on the plant, were cut from the plant, and the beans were picked from the pods by hand. The pods were united with straw fraction. The beans were allowed to dry at room temperature until no loss of weight *(i.e.* water loss) was measured (11 days). A representative aliquot of the dry beans was allowed to soak overnight with water and was used for extraction. The rest of the dry beans were stored at *ca.* -20 °C.

C. Analytical Procedures

The bean RACs were extracted and the extracts were analysed by HPLC and TLC. The identification of parent compound and metabolites was based on co-chromatography experiments and HPLC-MS/MS and/or LC-NMR.

1. Extraction and fractionation:

In general, samples of the bean RACs were extracted 3 times with a mixture of acetonitrile/water (8/2, v/v) using an Ultra-Turrax. The extracts were separated from the solids by centrifugation. The radioactivity of the extracts was determined by volume measurement and LSC. The solids (PES) were lyophilised overnight, homogenised and weighed. Aliquots thereof were combusted and measured for radioactivity by LSC. Aliquots of the combined solvent extracts were concentrated using a Speedvac.

The ^{14}C-radioactivity of liquid samples was determined by liquid scintillation counting (LSC) using Quicksafe A containing 5% of water. The radioactivity in solid samples was measured by combustion. The released $^{14}CO_2$ was absorbed in an alkaline scintillation cocktail and radio assayed by LSC.

The total radioactive residues (TRR) in the RACs of beans were determined by summation of the radioactivity in the combined acetonitrile/water extracts and in the PES. The residue levels are expressed as parent compound equivalents per weight. The concentrated acetonitrile/water extracts were analysed by HPLC for quantification of metabolites.

2. Identification and characterisation:

The identification of most metabolites in the bean RACs was based on identified metabolites gained from the bean foliage. These metabolites were isolated from the concentrated extracts of the foliage by semi-preparative HPLC and identified by HPLC-MS/MS and/or LC-NMR. Co-chromatography (HPLC, TLC) with authentic reference compounds, with the identified foliage metabolites, with metabolites isolated and identified in a supplemental cell culture study or a metabolism study in the rat using the phenyl label was performed to identify corresponding metabolites in the extracts of succulent and dry beans as well as in straw. Confirmation of these assignments was obtained after isolation of the major compounds out of the acetonitrile/water extract of succulent and dry beans by semipreparative HPLC. Corresponding fractions were combined, concentrated, purified in one of the secondary HPLC systems, and analysed by LC-NMR and/or HPLC-MS/MS.

3. Storage stability:

The RACs were extracted and analysed within a few days after sampling. All samples were stored at temperatures ≤ −18 °C. A few days after extraction, the earliest metabolite profiles of the combined extracts were obtained by HPLC using the profiling method for quantitation and identification of metabolites.

II. Results and Discussion

The TRR amounted to 3.88 mg eq/kg in green beans, 38.53 mg eq/kg in the corresponding foliage, 0.17 mg eq/kg in succulent beans, 0.31 mg eq/kg in dry beans, and 19.02 mg eq/kg in bean straw (Table 6.2.1-17). The TRR of the edible RACs (green, succulent and dry beans) was significantly lower than those of the corresponding foliage or straw.

Table 6.2.1-17: TRR values in bean matrices after application of [pyridyl-2,6-UL-^{14}C]fluopyram

Matrix	Applic. No. and timing, total dose	PHI (days)	TRR (ppm, mg a.s. equiv./kg)
Green beans	Two foliar spray applications: at growth stages BBCH 51 and BBCH 75; total 519 g a.s./ha	4	3.88
Foliage	Two foliar spray applications: at growth stages BBCH 51 and BBCH 75; total 519 g a.s./ha	4	38.53
Succulent beans	Two foliar spray applications: at growth stages BBCH 51 and BBCH 75; total 519 g a.s./ha	29	0.17
Dry beans	Two foliar spray applications: at growth stages BBCH 51 and BBCH 75; total 519 g a.s./ha	29 + 11 days for drying	0.31
Straw (including empty pods)	Two foliar spray applications: at growth stages BBCH 51 and BBCH 75; total 519 g a.s./ha	29	19.02

The bean matrices were extracted with acetonitrile/water (8/2; v/v), the extracts were analysed by HPLC and, where necessary, TLC and parent compound and metabolites were identified.

Nearly the complete radioactive residues in green beans, 99.3% (3.86 mg eq/kg) of the TRR, was extracted by acetonitrile/water (Table 6.2.1-18), whereas 0.7% (0.03 mg eq/kg) of the TRR remained in the solids (PES). Nearly the complete radioactive residues in bean foliage, 99.1% (38.14 mg eq/kg) of the TRR, was extracted by acetonitrile/water (Table 6.2.1-18), leaving only 1.0% (0.39 mg eq/kg) of the TRR in the solids (PES). The majority of the radioactive residue in succulent beans, 97.6% (0.170 mg eq/kg) of the TRR, was extracted by acetonitrile/water (Table 6.2.1-18), whereas 2.3% (< 0.01 mg eq/kg) of the TRR remained in the solids (PES). The majority of the radioactive residue in dry beans, 97.4% (0.300 mg eq/kg) of the TRR, was extracted by acetonitrile/water (Table 6.2.1-18), whereas 2.6% (< 0.01 mg eq/kg) of the TRR remained in the solids. The major amount of radioactivity in the bean straw, 95.7% (18.20 mg eq/kg) of the TRR was extracted by acetonitrile/water (Table 6.2.1-18), leaving only 4.3% (0.83 mg eq/kg) of the TRR with the solids (PES). Due to the low radioactive residue, the solids (PES) from all matrices were not further investigated.

Table 6.2.1-18: Distribution of radioactivity in the extracts of the bean matrices after foliar spray application of [pyridyl-2,6-UL-^{14}C]AE 656948

	Green beans		Foliage		Succulent beans		Dry beans		Straw	
TRR [mg eq/kg] =	3.88		38.53		0.17		0.31		19.02	
	% of TRR	mg eq/kg	% of TRR	mg eq/kg	% of TRR	mg eq/kg	% of TRR	mg eq/kg	% of TRR	mg eq/kg
Acetonitrile/water extract	99.3	3.86	99.1	38.14	97.6	0.170	97.4	0.300	95.7	18.20
Solids (PES)	0.7	0.03	1.0	0.39	2.3	0.003	2.6	0.008	4.3	0.83
Total extracted	99.3	3.86	99.1	38.14	97.6	0.170	97.4	0.300	95.7	18.20
Accountability	100.0	3.88	100.0	38.53	100.0	0.170	100.0	0.310	100.0	19.02

For the elucidation of metabolites, the solvent extracts (acetonitrile/water) were analysed by HPLC and, where required, also by TLC with radiodetection. Metabolites were either identified by co-chromatography (HPLC, TLC) with reference compounds or isolated metabolites or by LC-MS/MS and/or LC-NMR of isolated peaks.

Green beans and foliage: The parent compound (99.3% of the TRR, 3.86 mg eq/kg) was the only component detected in the extract of green beans (Table 6.2.1-19) and it was the predominant residue also in the extract of foliage (92.3% of the TRR, 35.53 mg eq/kg), in the concentrated extracts of foliage was also detected the glucoside-malonic acid conjugate fluopyram-7-hydroxy-glc-MA (M12), occurring at 3.2% of the TRR, 1.22 mg eq/kg. In addition, the metabolites fluopyram-7-hydroxy (M08, 1.6% of the TRR, 0.60 mg eq/kg), fluopyram-7-hydroxy-glc (M11, 0.6% of the TRR, 0.25 mg eq/kg), fluopyram-8-hydroxy (M18, 0.5% of the TRR, 0.21 mg eq/kg), fluopyram-pyridyl-carboxylic acid (0.5%, 0.19 mg eq/kg) and AE C656984-hydroxyethyl-glyc (0.2% of the TRR, 0.06 mg eq/kg) were identified (Table 6.2.1-19). Furthermore, one unknown minor compound (0.2% of the TRR, 0.08 mg eq/kg) was characterised in foliage by extraction and chromatographic behaviour.

Succulent beans: The main metabolites in the concentrated extract of succulent beans were fluopyram-pyridyl-carboxylic acid (PCA, M43, 31.0% of the TRR, 0.054 mg eq/kg) and the fluopyram-pyridyl-acetic acid (29.5% of the TRR, 0.051 mg eq/kg), whereas the parent compound was 4.8% (0.008 mg eq/kg) of the TRR. The metabolite identified as glucuronic acid-glycoside conjugate of the hydroxylated parent compound (fluopyram-hydroxy-glyc-gluc) accounted for 4.5% (0.008 mg eq/kg) of the TRR. The metabolites AE C656948-7-hydroxy (4.0% of the TRR, 0.007 mg eq/kg) and 8-hydroxy (2.7% of the TRR, 0.005 mg eq/kg) were also detected in succulent beans. The minor metabolite fluopyram-pyridyl-hydroxyethyl-glyc was also identified (1.9% of the TRR, 0.003 mg eq/kg). Conjugates of the 7-hydroxy metabolite were identified and accounted for < 0.01 mg eq/kg (Table 6.2.1-20). Furthermore, two unknown compounds (each < 5% of the TRR and ≤ 0.007 mg eq/kg) and a multiple metabolite fraction, containing higher conjugates like fluopyram-7-hydroxy-glc-MA (11.7%, 0.020 mg eq/kg), were characterized based on their extraction and chromatographic behaviour.

Dry beans: Major metabolites detected in the acetonitrile/water extract were fluopyram-pyridyl- carboxylic acid (32.5% of the TRR, 0.100 mg eq/kg) and fluopyram-pyridyl-acetic acid (22.6% of the TRR, 0.070 mg eq/kg). Parent compound was detected as minor component and accounted for 5.7% of the TRR (0.018 mg eq/kg). The metabolite fluopyram-hydroxy-glyc-gluc was also identified and accounted for 5.6% (0.017 mg eq/kg) of the TRR. The metabolites fluopyram-7-hydroxy and 8-hydroxy accounted for 4.0% (0.012 mg eq/kg) of the TRR and 1.6% (0.005 mg eq/kg) of the TRR, respectively. Also, the corresponding very minor metabolite fluopyram-7-hydroxy-glc was detected (1.3% of the TRR, 0.004 mg eq/kg). fluopyram-pyridyl-hydroxyethyl-glyc was also identified (3.1% of the TRR, 0.010 mg eq/kg).

Additionally, four unknown components (each < 5% of the TRR and < 0.05 mg eq/kg) and a multiple metabolite fraction (12.1% of the TRR, 0.037 mg eq/kg), containing higher conjugates like fluopyram-7-hydroxy-glc-MA, were characterised by extraction and chromatographic behaviour.

Straw: The parent compound fluopyram (87.1% of the TRR, 16.56 mg eq/kg) was the predominant residue also in the extract of straw. As observed in immature foliage, the main metabolite in the concentrated extract of straw was the fluopyram-7-hydroxy-glc-MA (4.7% of the TRR, 0.90 mg eq/kg). Other identified metabolites were fluopyram-7-hydroxy (1.1% of the TRR, 0.20 mg eq/kg), fluopyram-8-hydroxy (0.9% of the TRR, 0.17 mg eq/kg), fluopyram-7-hydroxy-glc (0.7% of the TRR, 0.14 mg eq/kg), fluopyram-pyridyl-carboxylic acid (0.6% of the TRR, 0.11 mg eq/kg), fluopyram-pyridyl-acetic acid (0.2% of the TRR, 0.04 mg eq/kg) and fluopyram-hydroxy-glyc-gluc (0.2% of the TRR, 0.03 mg eq/kg; Table 6.2.1-20). Additionally, one unknown compound (0.3%, 0.06 mg eq/kg) was characterized based on its extraction and chromatographic behaviour.

Table 6.2.1-19: Summary of characterisation and identification of radioactive residues in green beans and bean foliage after foliar spray application of [pyridyl-2,6-UL-^{14}C]fluopyram

	Green beans		Foliage	
TRR [mg eq/kg] =	3.88		38.53	
Compound	% of the TRR	mg eq/kg	% of the TRR	mg eq/kg
AE C656948, parent fluopyram	99.3	3.86	92.3	35.53
fluopyram-8-hydroxy (M18)	-	-	0.5	0.21
fluopyram-7-hydroxy (M08)	-	-	1.6	0.60
fluopyram-7-hydroxy-glc-MA (M12)	-	-	3.2	1.22
fluopyram-7-hydroxy-glc (M11)	-	-	0.6	0.25
fluopyram-hydroxyethyl-glyc (M35)	-	-	0.2	0.06
fluopyram-pyridyl-carboxylic acid (M43)	-	-	0.5	0.19
Total identified	99.3	3.86	98.9	38.06
unknown 1 (DO7)	-	-	0.2	0.08
Total characterised	-	-	0.2	0.08
Total extracted	99.3	3.86	99.1	38.14

Solids (non-extractable residue)	0.7	0.03	1.0	0.39
Accountability	100.0	3.88	100.0	38.53

Table 6.2.1-20: Summary of characterisation and identification of radioactive residues in succulent beans, dry beans and bean straw after foliar spray application of [pyridyl-2,6-^{14}C]-fluopyram

	Succulent beans		Dry beans		Straw	
TRR [mg eq/kg] =	0.17		0.31		19.02	
Compound	% of the TRR	mg eq/kg	% of the TRR	mg eq/kg	% of the TRR	mg eq/kg
AE C656948, parent fluopyram	4.8	0.008	5.7	0.018	87.1	16.56
fluopyram-8-hydroxy (M18)	2.7	0.005	1.6	0.005	0.9	0.17
fluopyram-7-hydroxy (M08)	4.0	0.007	4.0	0.012	1.1	0.20
fluopyram-7-hydroxy-glc-MA (M12)	-	-	-	-	4.7	0.90
fluopyram-7-hydroxy-glc (M11)	1.4	0.002	1.3	0.004	0.7	0.14
fluopyram-hydroxy-glyc-gluc (M22)	4.5	0.008	5.6	0.017	0.2	0.03
fluopyram-pyridyl-acetic acid (M40)	29.5	0.051	22.6	0.070	0.2	0.04
fluopyram-pyridyl-hydroxyethyl-glyc (M35)	1.9	0.003	3.1	0.010	-	-
fluopyram-pyridyl-carboxylic acid (M43)	31.0	0.054	32.5	0.100	0.6	0.11
Total identified	79.8	0.138	76.4	0.236	95.5	18.15
unknown 6 (ST 6)	-	-	-	-	0.3	0.06
multiple metabolite fraction[#]	11.7	0.020	12.1	0.037	-	-
unknown 5 (DB 7)	-	-	1.6	0.005	-	-
unknown 4 (DB 5)	-	-	1.3	0.004	-	-
unknown 3 (SB 4, DB 4)	2.3	0.004	2.6	0.008	-	-
unknown 2 (SB 2, DB 2)	3.9	0.007	3.4	0.011	-	-
Total characterised	17.9	0.031	21.0	0.065	0.3	0.05
Total extracted	97.6	0.170	97.4	0.300	95.7	18.20
Total bound residues (PES)	2.3	0.003	2.6	0.008	4.3	0.83
Accountability	100.0	0.170	100.0	0.310	100.0	19.02

[#] Region of approximately 10 fractions including higher conjugates like AE C656948-7-hydroxy-glc-MA

III. Conclusions

After two foliar spray applications of [pyridyl-2,6-UL^{14}C]fluopyram amounting a total of about 519 g/ha, most of the recovered radioactivity was detected in foliage (38.53 mg eq/kg) and straw (19.02 mg eq/kg), whereas significantly less were detected in the respective bean samples (3.88 mg eq/kg for green beans, 0.17 mg eq/kg for succulent beans, and 0.31 mg eq/kg for dry beans).

Unchanged parent compound was the predominant portion of the TRR in foliage, straw and green beans, accounting for more than 80% of the TRR, and was still present (< 10% of the TRR and < 0.1 mg eq/kg) in bean samples at maturity. In total, eight metabolites were identified. The major metabolite in bean samples at maturity (succulent and dry) was fluopyram-pyridyl-carboxylic acid (PCA, M43, > 30% of the TRR), followed by fluopyram-pyridyl-acetic acid (PAA, M40, > 20% of the TRR). In other bean matrices, identified metabolites accounted generally for < 10% of the TRR.

The following metabolic routes were deduced:

- Hydroxylation of the ethyl linking group of the a.s. forming 7- or 8- hydroxyl metabolites
- Hydrolytic cleavage and oxidation leading to fluopyram-pyridyl-carboxylic acid and fluopyram-pyridyl acetic acid, respectively
- Conjugation of hydroxyl-metabolites with hexoses and subsequent higher conjugations with malonic and glucuronic acid

On the basis of the nature and amount of metabolites found in foliage, beans and straw, the metabolic pathway of [pyridyl-2,6-^{14}C]fluopyram in beans is proposed in Figure 6.2.1-6.

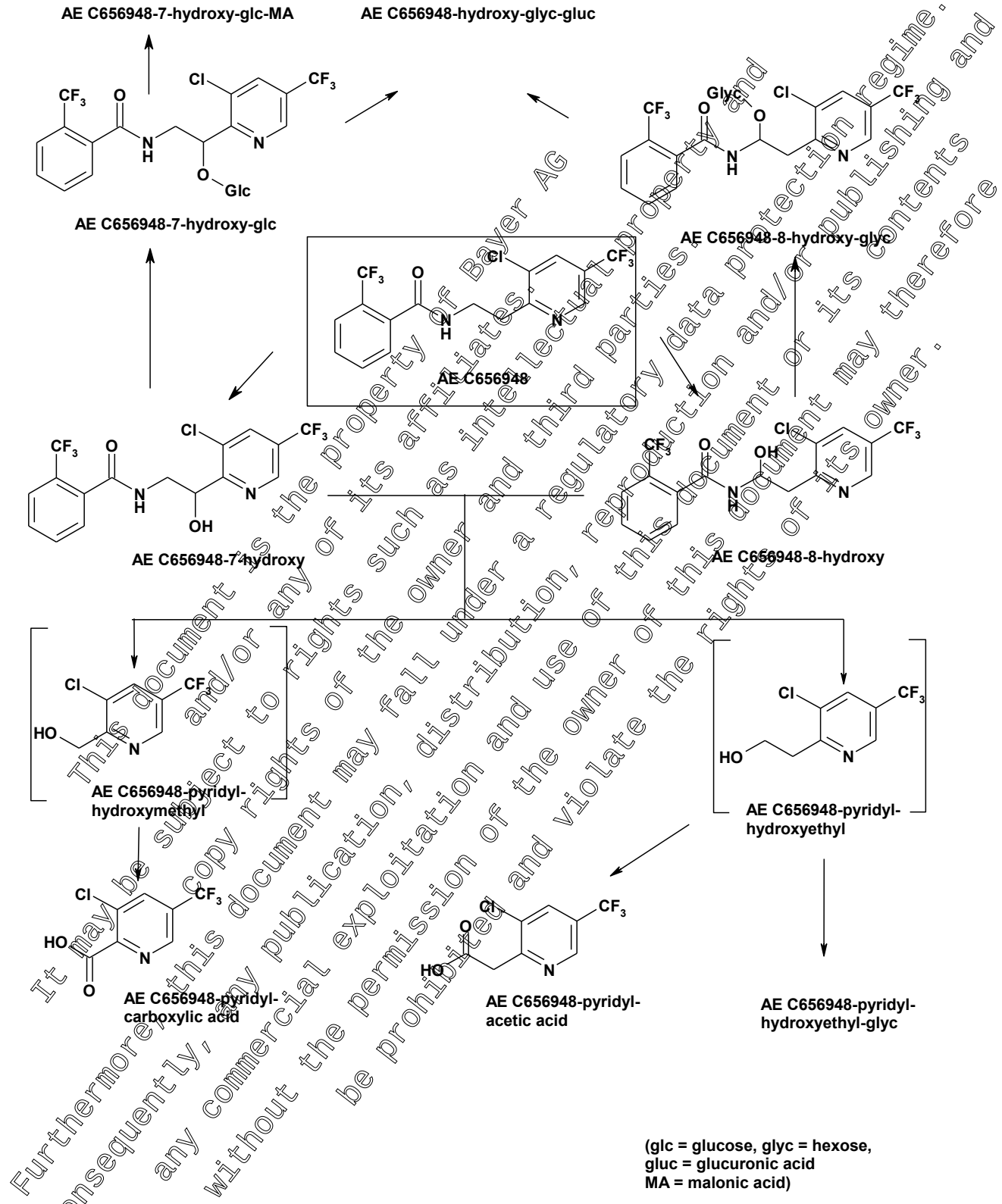


Figure 6.2.1-6: Proposed metabolic pathway of [pyridyl-2,6-^{14}C]AE C656948 (fluopyram) in beans

Assessment and conclusion by applicant:
The study is valid and acceptable.

Metabolism in red pepper (drip application)

Metabolism studies in red peppers were conducted with [phenyl-UL-^{14}C]fluopyram:

Data Point:	KCA 6.2.1/07
Report Author:	[redacted]
Report Year:	2008
Report Title:	Metabolism of [phenyl-UL-14C] AE C656948 in red pepper after drip application
Report No:	MEF-06/315
Document No:	M-298790-01-1
Guideline(s) followed in study:	US EPA OPPTS 860.1300; Canadian PMRA Rpt. DACO 6.3; Japanese MAFF, 12 Nousan 8147; EU 91/414/EEC amended by 96/68/EC
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted rev. 10 Vol 3 of DAR B7 August 2012 (references relied on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The metabolism of [phenyl-UL-^{14}C]fluopyram, formulated as a SC 500 (Suspension Concentrate), was investigated in red peppers following one drip application. The experimental design reflected the soil-less cultivation of vegetables in the greenhouse. For the envisaged use pattern and according to agricultural practice, one application was performed using 5 mg a.s./plant (1X experiment). Additionally, an exaggerated dose experiment was conducted using 20 mg a.s./plant (4X experiment). The applications were performed at growth stage BBCH 15–17.
A plant at an intermediate growth stage (BBCH 61 – first flower open) was harvested from the 4X experiment 33 days after application. Ripe fruits were picked from the 1X experiment at three time points (BBCH 89 – fully ripe). The remaining plants were sampled after the third harvest of fruits (BBCH 89).

The TRR of the edible RAC (pepper fruits) from the 1X experiment accounted for 0.038 mg eq/kg and was considerably lower than that of the rest of the plant (3.540 mg eq/kg). In the 4X experiment a TRR of 6.24 mg eq/kg was found in the intermediate plant. The major amount of radioactivity (96.2–98.5% of the

TRR) was effectively extracted with acetonitrile/water from all RACs leaving only minor amounts < 4% with the solids (PES). Parent compound and metabolites in the extracts were quantified by HPLC.

In pepper intermediate, the parent compound was most abundant compound, amounting to 86.6% (5.400 mg eq/kg) of the TRR. Additionally, four metabolites were identified, none of them exceeding 4% of the TRR. The pepper fruit TRR is comprised of 48.9% (0.019 mg eq/kg) parent compound. Other three metabolites were identified in fruits, of which the most abundant was fluopyram-benzamide (16.1%, 0.006 mg eq/kg), followed by fluopyram-7-hydroxy (9.0%, 0.003 mg eq/kg) and the corresponding conjugate fluopyram-7-hydroxy-glc (3.9%, 0.001 mg eq/kg). The major part of the residues in plants after harvest consisted of parent compound fluopyram, accounting for 64.0% (2.266 mg eq/kg) of the TRR. Five metabolites were identified in the rest of the pepper plants, of which the most abundant was fluopyram-benzamide, accounting for 10.1% (0.358 mg eq/kg) of the TRR. None of the other five metabolites identified in the rest of the plants exceeded 8.9% of the TRR.

A total of 97.4% of the TRR (6.075 mg eq/kg) was identified in pepper intermediate, 77.8% (0.030 mg eq/kg) of the TRR was identified in pepper fruits and 90.9% (3.219 mg eq/kg) of the TRR was identified in pepper plants after harvest. Only a few minor unknown components (each ≤ 18.4% of the TRR and ≤ 0.007 mg eq/kg) were characterised by their chromatographic behaviour in pepper intermediate plant, pepper fruits and plants after harvest.

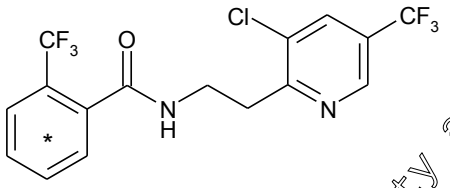
The metabolic reactions involved:

- Hydroxylation of the ethyl linking group of fluopyram forming fluopyram-7-hydoxy or fluopyram-8-hydroxy
- Hydrolytic cleavage leading to fluopyram-benzamide
- Conjugation of fluopyram-7-hydroxy with glucose and malonic acid

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	 * positions of radiolabel
Compound	AE C656948
IUPAC name	*N*-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]ethyl}-2-(trifluoromethyl)benzamide
CAS name	Benzamide, *N*-[2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-(trifluoromethyl)-(9Cl)
CAS #	658066-35-4
Radiolabel position	Phenyl-UL-^{14}C
Specific radioactivity	3.85 MBq/mg (104.2 µCi/mg)
Purity Chemical Purity	> 99% (HPLC) > 99% (TLC) > 99% (HPLC)

2. Substrate: Soil-less on Grodan® growcubes in slabs of stonewool substrate

3. Plant: Red bell pepper (*Capsicum annuum*), variety: "Feher"

B. Study Design

1. Experimental conditions:

The red pepper plants were grown in the greenhouse of Bayer CropScience AG, Metabolism / Environmental Fate, Monheim, Germany, which allows plant growth under natural sunlight and temperatures. The plants were cultivated without soil on stonewool substrate (Grodan® Growcube, Grodan BV, Roermond, The Netherlands) according to professional horticulture. Each plant was grown in one cube of Grodan® Growcube and three cubes were places together into a slab of the same material. The slab was kept in a box (container). The plants were irrigated and fertilised with a mix of a fertilizer on nitrate basis and a commercially available nutrient solution using a dripping system. A defined volume of nutrient solution was applied per plant daily. Due to the good water-retention properties of the stone wool substrate, the water content in the cubes was between 50% and 55% during the whole cultivation period. Uptake and delivery of nutrients was in a balanced state and only an excess of nutrient solution was drained into a bottle.

The radiolabelled active substance was formulated as a Suspension Concentrate (SC 500). For the 4X experiment, the radiolabelled test item was diluted with non-radiolabelled test item (ratio 1/1, w/w) prior to formulation. The application conditions of the 1X experiment simulated the anticipated use pattern for professional horticulture with 5 mg a.s./plant. The additional 4X overdose experiment was performed to support identification of metabolites. The application suspensions were prepared by diluting the formulations for the 1X experiment and the 4X experiment with water. The formulation was applied at

growth stage BBCH 15–17 (fifth to seventh leaf of the main shoot unfolded). A portion of 100 mL of application suspension were poured to each plant. One day prior to application and one day after, the dripping of the nutrient solution had been stopped in order to allow the complete uptake of the application suspension. The application solution was not drained and was quantitatively available for the plant for a long time period.

2. Sampling

Pepper intermediate (immature plant): One immature plant (intermediate plant) in the 4X experiment was harvested 33 days after application at developmental stage BBCH 61 (first flower was open). The plant was collected with its cube. The cube was removed by cutting the plant slightly above the stone wool surface. The weight of the sample was determined and the plant was cut into pieces and homogenised with liquid nitrogen using an Ultra-Turrax homogeniser. The complete homogenised plant material was used for extraction.

Pepper fruits: At maturity (BBCH 89), pepper fruits were picked from the plants at three harvest dates. The fruits of the first and second harvest were stored in a freezer. At the day of the third harvest the fruits of the three samplings were combined and homogenised with liquid nitrogen using an Ultra-Turrax homogeniser. A representative aliquot of the homogenised sample was used for extraction. Residual sample material was stored in aliquots at ≤ -18 °C.

Pepper plants after harvest: One day after the last pepper fruit has been sampled, the rest of the plants (BBCH 89) were cut above their cubes. The plants were cut into smaller pieces using scissors. The plant parts from the 4X experiment were stored in a freezer for optional analysis, whereas the plant parts of the 1X experiment were homogenised with liquid nitrogen using an Ultra-Turrax homogeniser. A representative aliquot of the homogenised sample from the 1X experiment was used for extraction. Residual homogenised plant material was stored in aliquots at ≤ -18 °C.

C. Analytical Procedures

The pepper RACs were extracted and the extracts were analysed by HPLC and TLC. The identification of parent compound and metabolites was based on co-chromatography experiments and HPLC-MS/MS and/or LC-NMR.

1. Extraction and fractionation:

In general, samples of the pepper RACs were extracted three times with a mixture of acetonitrile/water (4/1, v/v) using an Ultra-Turrax homogeniser. The extracts were separated from the solids by centrifugation. The radioactivity of the extracts was determined by volume measurement and LSC. The radioactivity in the solids (PES) was determined by combustion followed by LSC. Aliquots of the combined solvent extracts were concentrated using a rotary evaporator.

The ^{14}C-radioactivity of liquid samples was determined by liquid scintillation counting (LSC) using Quicksafe A containing 5% of water. The radioactivity in sold samples was measured by combustion. The released $^{14}CO_2$ was absorbed in an alkaline scintillation cocktail and radio assayed by LSC.

The total radioactive residues (TRR) in the RACs of peppers were determined by summation of the radioactivity in the combined acetonitrile/water extracts and in the PES. The residue levels are expressed as parent compound equivalents per weight. The concentrated acetonitrile/water extracts were analysed by HPLC for quantification of metabolites.

2. Identification and characterisation:

The identification of parent and metabolites in the pepper RACs was based on co-chromatography (HPLC, TLC) with authentic non-radiolabelled reference compounds or with metabolites isolated and identified in the plant study conducted with [pyridyl-2,6-^{14}C]fluopyram in beans (M-299067-01-1).

3. Storage stability:

Pepper intermediate (4X) and pepper plants (1X) were extracted and analysed within a day after sampling. Pepper fruits (1X) were harvested at three sampling dates. The fruits of the first two sampling dates were stored in a freezer until the last sampling date. On the day of the last sampling, the fruits were combined and extracted. The pepper fruits were stored for a maximum of 42 days prior to sample preparation. Quantitative analysis followed within a day. Thus, the metabolite profiles of all sample extracts were recorded in a minimum time frame, not exceeding 42 days after sampling.

II. Results and Discussion

The metabolism of [phenyl-UL-^{14}C]fluopyram, formulated as a SC 500 (Suspension Concentrate), was investigated in red peppers following one drip application. Two experiments were performed at single rates of 5 mg a.s./plant (1X experiment) and 20 mg a.s./plant (4X experiment).

The TRR amounted to 0.038 mg eq/kg in the pepper fruits (edible RAC) and 3.540 mg eq/kg in plants after harvest in the 1X experiment. In the 4X experiment the TRR of the intermediate plant accounted for 6.237 mg eq/kg (Table 6.2.1-21).

Table 6.2.1-21: TRR values in pepper matrices after application of [phenyl-UL-^{14}C]fluopyram

Matrix	Timing and Applic. No.	PHI (days)	TRR (ppm, mg a.s. equiv./kg)
Pepper intermediate (BBCH 61)	One application: at growth stages BBCH 15–17 1 x 20 mg a.s./plant (4X experiment)	33	6.237
Pepper fruits (BBCH 89)	One application: at growth stages BBCH 15–17 1 x 5 mg a.s./plant (1X experiment)	55-96	0.038
Pepper plants after harvest (BBCH 89)	One application: at growth stages BBCH 15–17 1 x 5 mg a.s./plant (1X experiment)	97	3.540

The pepper matrices were extracted with acetonitrile/water (4:1; v/v), the extracts were analysed by HPLC and, where necessary, TLC and parent compound and metabolites were identified.

Nearly the complete radioactive residues in pepper intermediate plant, 98.5% (6.141 mg eq/kg) of the TRR, was extracted by acetonitrile/water (Table 6.2.1-22), leaving only 1.5% (0.096 mg eq/kg) of the TRR in the solids (PES). The majority of the radioactive residues in pepper fruits, 96.2% (0.037 mg eq/kg) of the TRR, was extracted by acetonitrile/water (Table 6.2.1-22), while 3.8% (0.001 mg eq/kg) of the TRR remained in the solids (PES). The majority of the radioactive residue in the plants after harvest, 96.3% (3.410 mg eq/kg) of the TRR, was extracted by acetonitrile/water, whereas 3.7% (0.130 mg eq/kg) of the TRR remained in the solids (PES).

Table 6.2.1-22: Distribution of radioactivity in the extracts of the pepper matrices after drip application of [phenyl-UL-^{14}C]AE 656948

	Pepper intermediate (4X experiment)		Pepper fruits (1X experiment)		Pepper plants after harvest (1X experiment)	
TRR [mg eq/kg] =	6.237		0.038		3.540	
	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Acetonitrile/water extract	98.5	6.141	96.2	0.037	96.3	3.410
Solids 1 (PES)	1.5	0.096	3.8	0.001	3.7	0.130
Total extracted	98.5	6.141	96.2	0.037	96.3	3.410
Accountability	100.0	6.237	100.0	0.038	100.0	3.540

For the elucidation of metabolism, the solvent extracts (acetonitrile/water) were analysed by HPLC and, where required, also by TLC with radiodetection. Metabolites were identified by co-chromatography (HPLC, TLC) with reference compounds of isolated and identified metabolites.

Pepper intermediate: The parent compound (86.6%, 5.400 mg eq/kg of the TRR) was the predominant residue. Beside the parent compound, the following metabolites were detected in the intermediate sample in low amounts (≤ 5% of TRR): fluopyram-benzamide, fluopyram-7-hydroxy, fluopyram-8-hydroxy and the conjugate fluopyram-7-hydroxy-glc (Table 6.2.1-23). Furthermore, three unknown minor compounds

(≤ 0.7%, ≤ 0.047 mg eq/kg) were characterised in the intermediate plant by extraction and chromatographic behaviour.

Pepper fruits: The main residue in the concentrated extract of pepper fruits was the parent compound fluopyram (48.9% of the TRR, 0.019 mg eq/kg). The main metabolite detected in pepper fruits was fluopyram-benzamide (16.1% of the TRR, 0.006 mg eq/kg). Minor metabolites found in fruits were fluopyram-7-hydroxy (9.0% of the TRR, 0.003 mg eq/kg) and the conjugate fluopyram-7-hydroxy-glc (3.9% of the TRR, 0.001 mg eq/kg) (Table 6.2.1-23). Furthermore, a compound was detected eluting early from the column. The early elution time in the reversed phase system characterises the substance as very polar. The substance was not detected in the parallel study conducted with [pyridyl-2,6-^{14}C]fluopyram and was therefore considered as label-specific (18.4% of the TRR, 0.007 mg eq/kg).

Plants after harvest:

In plants after harvest again parent compound constituted the major residue found (64.0% of the TRR, 2.266 mg eq/kg), followed by fluopyram-benzamide (10.1% of the TRR, 0.358 mg eq/kg), fluopyram-7-hydroxy-glc (8.9% of the TRR, 0.314 mg eq/kg) and fluopyram-7-hydroxy (6.8% of the TRR, 0.230 mg eq/kg, Table 6.2.1-23). Metabolites found in low amounts were the conjugate fluopyram-7-hydroxy-glc-MA (0.7% of the TRR, 0.024 mg eq/kg) and fluopyram-8-hydroxy (0.5% of the TRR, 0.018 mg eq/kg). Additionally, six unknown compounds (≤ 1.1% of the TRR, ≤ 0.04 mg eq/kg) were characterized based on their extraction and chromatographic behaviour.

Table 6.2.1-23: Summary of characterisation and identification of radioactive residues in pepper matrices after drip application of [phenyl-UL-^{14}C]fluopyram

	Pepper intermediate (4X experiment)		Pepper fruits (1X experiment)		Pepper plants after harvest (1X experiment)	
TRR [mg eq/kg] =	6.237		0.038		3.540	
Compound	% of the TRR	mg eq/kg	% of the TRR	mg eq/kg	% of the TRR	mg eq/kg
AE C656948, a.s.(Fluopyram)	86.6	5.400	48.9	0.019	64.0	2.266
fluopyram-benzamide (M25)	3.8	0.235	16.1	0.006	10.1	0.358
fluopyram-7-hydroxy-glc-MA (M12)	-	-	-	-	0.7	0.024
fluopyram-7-hydroxy-glc (M11)	2.7	0.171	2.9	0.001	8.9	0.314
fluopyram-7-hydroxy (M08)	3.8	0.234	9.0	0.003	6.8	0.239
fluopyram-8-hydroxy (M18)	0.6	0.034	-	-	0.5	0.018
Total identified	97.4	6.075	77.8	0.030	90.9	3.219
unknown 1	-	-	18.4	0.007	-	-
unknown 2	-	-	-	-	0.7	0.024
unknown 3	-	-	-	-	1.1	0.038
unknown 4	-	-	-	-	0.6	0.023
unknown 5	0.1	0.007	-	-	0.7	0.026
unknown 6	-	-	-	-	1.1	0.040
unknown 7	0.7	0.047	-	-	1.1	0.040
unknown 8	0.2	0.012	-	-	-	-
Total characterised	1.1	0.066	18.4	0.007	5.4	0.191
Total extracted	98.5	6.141	96.2	0.037	96.3	3.410
Total bound residues (PES)	1.5	0.096	3.8	0.001	3.7	0.130
Accountability	100.0	6.237	100.0	0.038	100.0	3.540

III. Conclusions

After drip application of [phenyl-UL^{14}C]fluopyram, the radioactivity recovered in pepper fruits (1X experiment) was very low (0.038 mg eq/kg), whereas the recovered radioactivity recovered in plants after harvest was relatively high (3.54 mg eq/kg). The radioactivity recovered in the intermediate plant (4X overdose experiment) was in the range found for plants after harvest (6.24 mg eq/kg).

Unchanged parent compound was the predominant portion of the TRR in all investigated RACs, accounting for more than 40% of the TRR. In total, five metabolites were identified. The most abundant metabolite was fluopyram-benzamide, followed by fluopyram-7-hydroxy and fluopyram-7-hydroxy-glc. Minor metabolites (<1% of TRR) detected were fluopyram-8-hydroxy and fluopyram-7-hydroxy-glc-MA.

The following metabolic routes were deduced:

- Hydroxylation of the ethyl linking group of the parent compound leading to fluopyram-7-hydroxy or fluopyram-8-hydroxy
- Hydrolytic cleavage of the hydroxylated metabolites leading to fluopyram-benzamide
- Conjugation of hydroxyl-metabolites with glucose and malonic acid

On the basis of the nature and amount of metabolites found in an intermediate plant, pepper fruits and plants after harvest, the metabolic pathway of [phenyl-UL-^{14}C]fluopyram in red bell pepper is proposed below.

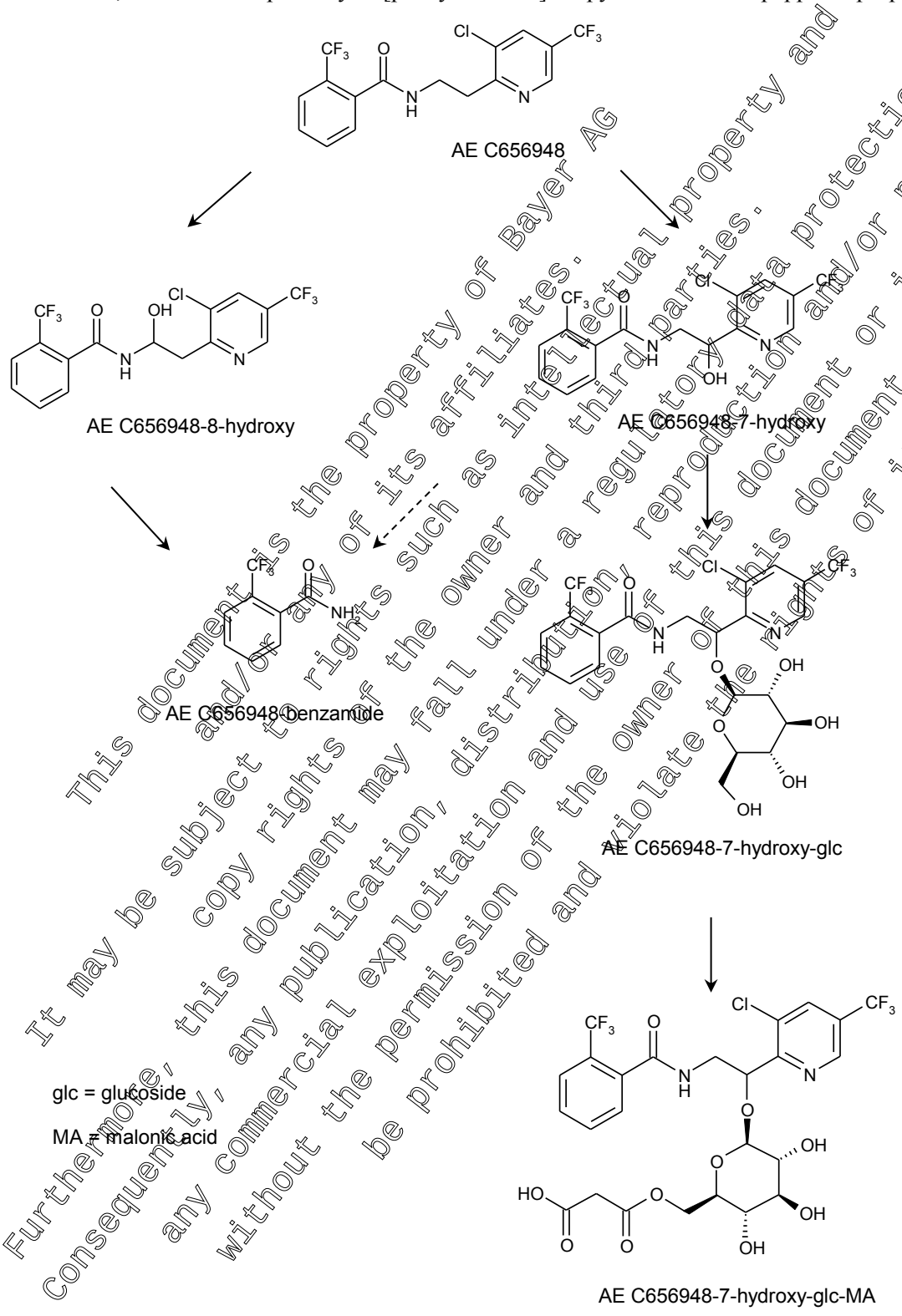


Figure 6.2.1-7: Proposed metabolic pathway of [phenyl-UL-^{14}C]AE C656948 (fluopyram) in pepper plants

Assessment and conclusion by applicant:
The study is valid and acceptable.

Metabolism in Red bell pepper (drip application)

Metabolism studies in Red pepper were conducted with [pyridyl-2,6-^{14}C]fluopyram:

Data Point:	KCA 6.2.1/08
Report Author:	[redacted]
Report Year:	2008
Report Title:	Metabolism of [pyridyl-2,6-14C]AE C656948 in red pepper after drip application
Report No:	ME-06/314
Document No:	M-298744-01-1
Guideline(s) followed in study:	US EPA OPPTS 860.1300; Canadian PMRA Re-DACO 6.3; Japanese MAFF, 12 Nousan 8147; EU 91/414/EEC amended by 94/79/EC
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted Rev. 1.0 Vol.3 of DAR B7 August 2007 (references relied on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The metabolism of [pyridyl-2,6-^{14}C]fluopyram, formulated as a SC 500 (Suspension Concentrate), was investigated in red pepper following one drip application. The experimental design reflected the soil-less cultivation of vegetables in the greenhouse. For the envisaged use pattern and according to agricultural practice, one application was performed using 5 mg a.s./plant (1X experiment). Additionally, an overdose experiment was conducted using 20 mg a.s./plant (4X experiment). The applications were performed at growth stages BBCH 15-17.
Pepper fruits were harvested at maturity (BBCH 89) for both 1X and 4X experiments. The plants were also sampled after harvest of the fruits. Moreover, an intermediate sample of the overdose experiment was additionally collected at the beginning of flowering (BBCH 61).

The TRR of the fruits at 0.060 mg eq/kg (1X dose) and 0.149 mg eq/kg (4X overdose) was considerably lower than the TRR for the 1X experiment pepper plant (2.344 mg eq/kg) and the 4X overdose experiment intermediate (18.24 mg eq/kg). The major amount of radioactivity (> 95% of the TRR) was effectively extracted with acetonitrile/water from all RACs leaving only minor amounts < 5% with the solids (PES). Parent compound and metabolites in the extracts were quantified by HPLC.

Parent compound was the predominant residue in the pepper intermediate and in the pepper plant after harvest accounting for 70% to 88% of the TRR.

In pepper fruits, fluopyram-pyridyl-carboxylic acid was the main metabolite at 43.5% (0.026 mg eq/kg) of the TRR followed by two isomeric glycosides of fluopyram-pyridyl-acetic acid (PAA) at 23.8% TRR (0.014 mg eq/kg) and 14.2% TRR (0.009 mg eq/kg), respectively. The glycosides were detected exclusively in pepper fruits, whereas traces of fluopyram-pyridyl-carboxylic acid were also detected in the intermediate sample at relatively low levels of 0.4% TRR (0.08 mg eq/kg). No further metabolites were detected in pepper fruits of the 1X experiment. Other metabolites of interest in the green plant parts were the hydroxylated metabolites fluopyram-7-hydroxy at 5.1% TRR (0.120 mg eq/kg) and its corresponding glucoside fluopyram-7-hydroxy-glc at 9.2% TRR (0.215 mg eq/kg) and the conjugate hydroxyethyl-di-glc at 7.0% TRR (0.164 mg eq/kg). Besides, small amounts of fluopyram-*N*-oxide was found in green plant parts (2.9% TRR; 0.069 mg eq/kg). The metabolite fluopyram-8-hydroxy was only found in the overdose intermediate at low levels of 0.7% TRR (0.13 mg eq/kg).

A total of 97.8% TRR (0.059 mg eq/kg) was identified in the fruits and 94.3% TRR (2.211 mg eq/kg) in the green parts of the plant of the 1X experiment, respectively. Only a few minor unknown components (each < 10% of the TRR and < 0.05 mg eq/kg) were characterised by their chromatographic behaviour in green parts of the plant of the 1X experiment.

In the overdose experiment, the amount of TRR in the intermediate was 96.7% (17.63 mg eq/kg) and 98.1% (0.146 mg eq/kg) in the fruit. 1.1% of the TRR (0.21 mg eq/kg) remained unidentified and was only characterized by their chromatographic behaviour in the intermediate of the overdose experiment.

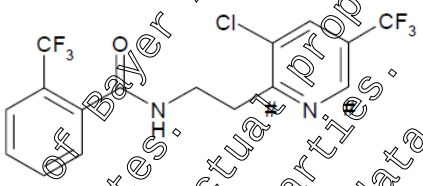
The metabolic reactions involved:

- Hydroxylation of the ethyl linking group of the a.s. forming 7- or 8-hydroxy metabolites
- Subsequent cleavage to fluopyram-pyridyl-acetic acid, pyridyl-carboxylic acid and fluopyram-pyridyl-hydroxyethyl
- Conjugation of hydroxyl-metabolites with hexoses and subsequent higher conjugations with malonic and glucuronic acid
- Oxidation of the *N*-atom of the pyridyl moiety of the parent compound resulting in fluopyram-*N*-oxide

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	
Compound	AE C656948
IUPAC name	*N*-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]ethyl}-2-(trifluoromethyl)benzamide
CAS name	Benzamide, *N*-[2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-(trifluoromethyl)-(9CI)
CAS #	658066-35-4
Radiolabel position	Pyridyl-2,6-^{14}C
Specific radioactivity	3.85 MBq/mg (104.2 µCi/mg)
Purity	99% (HPLC) > 99% (TLC) > 99% (HPLC; radiolabelled) 99.8% (HPLC; unlabelled)
Chemical Purity	

2. Soil: Soil-less on Grodan® growcubes in slabs of stonewool substrate

3. Plant: Red bell pepper (*Capsicum annuum*), variety "Feher"

B. Study Design

1. Experimental conditions:

The red pepper plants were cultivated in a greenhouse (building 6681) of Bayer CropScience AG, Metabolism / Environmental Fate, Monheim, Germany, which allows plant growth under natural sunlight and temperatures. The plants were cultivated without soil on stonewool substrate (Grodan® Growcube, Grodan BV, Roermond, The Netherlands) according to professional horticulture. Each plant was grown in one cube of Grodan® Growcube and three cubes were places together into a slab of the same material. The slab was kept in a box (container). The plants were irrigated and fertilized with a mix of a fertilizer on nitrate basis and a commercially available nutrient solution using a dripping system. A defined volume of the nutrient solution was applied daily to each plant. Due to the good water-retention properties of the stone

wool substrate, the water content in the cubes was between 50% and 55% during the whole cultivation period. Uptake and delivery of nutrients was in a balanced state and only an excess of nutrient solution was drained into a bottle.
The radiolabelled active substance was formulated as a suspension concentrate (SC 500). For the 4X experiment, the radiolabelled test item was radiodiluted with non-radiolabelled test item (ratio 1/1 w/w) prior to formulation. The application conditions of the 1X experiment simulated the anticipated use pattern for professional horticulture with 5 mg a.s./plant. The additional 4X overdose experiment was performed to support identification of metabolites. The application suspensions were prepared by diluting the formulations for the 1X experiment and the 4X experiment with water. The formulation was applied at growth stage BBCH 15–17 (fifth to seventh leaf of the main shoot unfolded). A portion of 100 mL of application suspension were poured to each plant. One day prior to application and one day after, the dripping of the nutrient solution had been stopped in order to allow the complete uptake of the application suspension. The application solution was not drained and was quantitatively available for the plant.

2. Sampling

Pepper intermediate (immature plant): One immature plant was sampled 33 days after application at BBCH 61 (first flower was open) in the 4X overdose experiment. The plant was collected with its growcube. The growcube was removed by cutting the plant slightly above the stonewool surface. The weight of the sample was determined and the plant was cut into pieces and homogenised with liquid nitrogen using an Ultra-Turrax homogeniser. The complete homogenised plant material was used for extraction.

Pepper fruits: At maturity (growth stage BBCH 89; 55–96 days after application) pepper fruits were sampled in both experiments (1X and 4X overdose) on three harvest dates. The fruits of the first and second harvest were stored in a freezer until the last harvest. On the third harvest day, the fruits of the three samplings of the 1X experiment were combined, crushed and homogenised with liquid nitrogen using an Ultra-Turrax homogeniser. A representative aliquot of the homogenised sample was used for extraction. Residual homogenised red pepper fruits were stored in aliquots at ≤ -18°C.

The fruits of the three sampling dates of the 4X experiment were also combined and stored at ≤ -18°C for optional analysis.

Pepper plants after harvest: One day after the last pepper fruit had been sampled, the rest of the plants (BBCH code 89) were cut above their cubes. The plants were cut into pieces using scissors. The plants of the 1X experiment were homogenised with liquid nitrogen using an Ultra-Turrax homogeniser. The plant parts of the 4X experiment were stored in a freezer for optional analysis, whereas a representative aliquot of the homogenised sample of the 1X experiment was used for extraction. Residual homogenised plant material was stored in aliquots at ≤ -18°C.

C. Analytical Procedures

The pepper RACs were extracted and subsequently analysed by HPLC and TLC. The identification of parent compound and metabolites was based on co-chromatography with LC-FT-MS, LC-MS/MS and LC-NMR-MS.

1. Extraction and fractionation:

The samples of the homogenised raw agricultural commodities (RACs) were extracted three times with a mixture of acetonitrile/water (4/1, v/v) using an Ultra-Turrax homogeniser. The extracts were separated from the solids by centrifugation. The radioactivity of each extract was determined by LSC measurement. The remaining solids (PES) were lyophilised, homogenised and weighed. The radioactivity in the solids was determined by combustion followed by LSC. Aliquots of the combined solvent extracts were concentrated using a rotary evaporator.

The ^{14}C-radioactivity of liquid samples was determined by liquid scintillation counting (LSC) using Quicksafe A containing 5% of water. The released $^{14}CO_2$ was trapped in an alkaline scintillation cocktail and the radioactivity was determined by LSC.

The total radioactive residues (TRR) in the RACs of red bell pepper were determined by summation of the radioactivity in the combined acetonitrile/water extracts and in the PES.

The concentrated acetonitrile/water extracts were analysed by HPLC for quantification of metabolites.

2. Identification and characterisation:

The identification of metabolites in edible RAC (pepper fruits) and green material (pepper plant) was carried out by HPLC and TLC co-chromatography or by spectroscopic investigations.

The metabolite pattern of pepper plant was used as reference sample for peak assignment. Individual compounds in pepper plant were identified by spectroscopic means and other matrices were assigned by comparison of the metabolite patterns with that of pepper plant. By HPLC co-chromatography with reference compounds or isolated (and identified) metabolites from pepper plant the assignment was confirmed in a second step.

Metabolites in pepper fruit, which were not detected in pepper plant, were isolated and purified by HPLC fractionation and were identified by LC-FT-MS, LC-MS/MS and LC-NMR-MS.

3. Storage stability:

Pepper intermediate (4X) and pepper plants (1X) were extracted and analysed within a day after sampling. Pepper fruits (1X) were harvested at three sampling dates and therefore stored in a freezer until the last sampling date. The storing period of the pepper fruits was 41 days in maximum before sample preparation. Analysis followed within a day. Thus, metabolite profiling including quantification of compounds was performed within less than two months (42 days) after sampling of the respective RAC, whereas identification of compounds required approximately 13 months.

II. Results and Discussion

The metabolism of [pyridyl-2,6-^{14}C]fluopyram, formulated as a SC 500, was investigated in red bell pepper following one application in two different experiments (1X and 4X overdose). The application rates were 5 mg a.s./plant in the 1X experiment and approximately 20 mg a.s./plant in the 4X overdose experiment.

The TRR amounted to 18.24 mg eq/kg in the 4X pepper intermediate, 0.149 mg eq/kg in the 4X pepper fruits, 0.060 mg eq/kg in the 1X pepper fruits and 2.344 mg eq/kg in the 1X pepper plants after harvest (Table 6.2.1-24). The TRR of the edible RACs (pepper fruit) was significantly lower than those of the respective plant or intermediate.

Table 6.2.1-24 TRR values in pepper matrices after application of [pyridyl-2,6-^{14}C]fluopyram

Matrix	Timing and Applic. No.	PHI (days)	TRR (ppm, mg a.s. equiv./kg)
Pepper intermediate (BBCH 61)	One drip application at growth stages BBCH 15–17; total 19.1 mg a.s./plant (4X experiment)	33	18.24
Pepper fruits (BBCH 89)	One drip application at growth stages BBCH 15–17; total 19.1 mg a.s./plant (4X experiment)	55-96	0.149
Pepper fruits (BBCH 89)	One drip application at growth stages BBCH 15–17; total 5 mg a.s./plant (1X experiment)	55-96	0.060
Pepper plants after harvest (BBCH 89)	One drip application at growth stages BBCH 15–17; total 5 mg a.s./plant (1X experiment)	97	2.344

The pepper matrices were extracted with acetonitrile/water (4/1, v/v), the extracts were analysed by HPLC and, where necessary, TLC and parent compound and metabolites were identified.

The majority of the radioactive residues in the intermediate (4X experiment), 97.8% (17.84 mg eq/kg) of the TRR, was extracted by acetonitrile/water (Table 6.2.1-25), whereas 2.2% (0.40 mg eq/kg) of the TRR remained in the solids (PES).

The majority of the radioactive residues in pepper fruits, 98.1% (0.146 mg eq/kg) of the TRR in the 4X overdose and 97.8% (0.059 mg eq/kg) of the TRR in the 1X experiment, was extracted by acetonitrile/water, leaving only 1.9% (0.003 mg eq/kg) and 2.2% (0.001 mg eq/kg) of the TRR in the solids (PES), respectively.

The majority of the radioactive residues in plants (1X experiment), 95.3 % (2.233 mg eq/kg) of the TRR, was extracted by acetonitrile/water, leaving 4.7 % (0.110 mg eq/kg) of the TRR in the solids (PES). Due to the low radioactive residue, the solids (PES) from all matrices were not further investigated.

Table 6.2.1-25 Distribution of radioactivity in the extracts of the pepper matrices after single application of [pyridyl-2,6-^{14}C]AE 656948

	Pepper intermediate (4X experiment)		Pepper fruits (4X experiment)		Pepper fruits (1X experiment)		Pepper plants after harvest (1X experiment)	
TRR [mg eq/kg] =	18.24		0.149		0.060		2.344	
	% of TRR	mg eq /kg	% of TRR	mg eq /kg	% of TRR	mg eq /kg	% of TRR	mg eq /kg
Acetonitrile/water extract	97.8	17.84	98.1	0.146	97.8	0.059	95.3	2.233
Solids (PES)	2.2	0.40	1.9	0.003	2.2	0.001	4.7	0.110
Total extracted	97.8	17.84	98.1	0.146	97.8	0.059	95.3	2.233
Accountability	100.0	18.24	100.0	0.149	100.0	0.060	100.0	2.344

For the elucidation of metabolism, the solvent extracts (acetonitrile/water) were analysed by HPLC and, where required, also by TLC with radiodetection. Metabolites were either identified by co-chromatography (HPLC, TLC) with reference compounds or isolated metabolites or by LC-FT-MS, LC-MS/MS and/or LC-NMR-MS of isolated peaks.

Pepper intermediate: Parent compound fluopyram (88.1% of the TRR; 16.07 mg eq/kg) was the most prominent compound in the sample, followed by fluopyram-7-hydroxy (3.5% of the TRR; 0.63 mg eq/kg), the corresponding conjugate fluopyram-7-hydroxy-glc (1.9% of the TRR; 0.34 mg eq/kg) and the label-specific metabolite fluopyram-hydroxyethyl-di-glc (1.5% of the TRR; 0.27 mg eq/kg). fluopyram-8-hydroxy (0.7% of the TRR; 0.13 mg eq/kg), fluopyram-*N*-oxide (0.5% of the TRR; 0.10 mg eq/kg) and fluopyram-pyridyl-carboxylic (0.4% of the TRR; 0.08 mg eq/kg) were identified as minor metabolites. Besides, three unknown compounds were characterized based on their extraction and chromatographic behaviour (total 1.1% of the TRR; 0.21 mg eq/kg).

Pepper fruits: The most prominent metabolite was identified as fluopyram-pyridyl-carboxylic acid (43.5% of the TRR; 0.026 mg eq/kg). Two isomeric hexose conjugates of fluopyram-pyridyl-acetic acid were identified as fluopyram-PAA-glycosides (23.8% of the TRR; 0.014 mg eq/kg and 14.2% of the TRR; 0.009 mg eq/kg). The parent compound fluopyram (16.2% of the TRR, 0.010 mg eq/kg) was clearly reduced in pepper fruits compared to other compartments.

In the 4X overdose experiment two further metabolites were found: fluopyram pyridyl-acetic acid (PAA; 9.8%; 0.015 mg eq/kg of the TRR) and fluopyram-7-hydroxy (3.7% of the TRR; 0.006 mg eq/kg).

Pepper plants after harvest: Parent compound fluopyram (70.1% of the TRR; 1.643 mg /kg) was the most prominent compound in the plant sample, followed by fluopyram-7-hydroxy-glc (9.2% of the TRR; 0.215 mg eq/kg), the label specific metabolite fluopyram-hydroxyethyl-di-glc (7.0% of the TRR; 0.164 mg eq/kg) and the metabolite fluopyram-7-hydroxy (5.1% of the TRR; 0.120 mg eq/kg). fluopyram-*N*-oxide (2.9% of the TRR; 0.069 mg eq/kg) was identified as a minor compound. One unknown component was characterized based on its extraction and chromatographic behaviour (1.0% of the TRR; 0.023 mg eq/kg).

Table 6.2.1-26: Summary of characterisation and identification of radioactive residues in pepper matrices after drip application of [pyridyl-2,6-^{14}C]fluopyram in the 4X experiment

	Pepper intermediate (4X experiment)		Pepper fruits (4X experiment)	
TRR [mg eq/kg] =	18.24		0.149	
Compound	% of the TRR	mg eq/kg	% of the TRR	mg eq/kg
AE C656948, a.s., fluopyram	88.1	16.07	32.8	0.049
fluopyram pyridyl-carboxylic acid (PCA) (M43)	0.4	0.08	19.5	0.029
fluopyram pyridyl-acetic acid (PAA) (M40)	-	-	9.8	0.015
fluopyram PAA-glycoside (M42) (isomer 1)	-	-	13.5	0.019
fluopyram PAA-glycoside (M42) (isomer 2)	-	-	19.7	0.029
fluopyram hydroxyethyl-di-glc (M36)	4.5	0.82	-	-
fluopyram 7-hydroxy-glc (M11)	1.9	0.34	-	-
fluopyram-*N*-oxide (M01)	0.5	0.10	-	-
fluopyram-7-hydroxy (M08)	3.5	0.63	3.7	0.006
fluopyram 8-hydroxy (M18)	0.7	0.13	-	-
Total identified	96.7	17.63	98.1	0.146
unknown 6	0.4	0.07	-	-
unknown 7	0.3	0.06	-	-
unknown 9	0.4	0.08	-	-
Total characterised	1.1	0.21	-	-
Total extracted	97.8	17.84	98.1	0.146
Solids (non-extractable residue)	2.2	0.40	1.9	0.003
Accountability	100.0	18.24	100.0	0.149

Table 6.2.1-27: Summary of characterisation and identification of radioactive residues in pepper matrices after drip application of [pyridyl-2,6-^{14}C]fluopyram in the 1X experiment

	Pepper fruits (1X experiment)		Pepper plants after harvest (1X experiment)	
TRR [mg eq/kg] =	0.060		2.344	
Compound	% of the TRR	mg eq/kg	% of the TRR	mg eq/kg
AE C656948, a.s., fluopyram	16.2	0.010	70.1	1.643
fluopyram pyridyl-carboxylic acid (PCA) (M43)	43.5	0.026	-	-
fluopyram pyridyl-acetic acid (PAA) (M40)	-	-	-	-
fluopyram PAA-glycoside (M42) (isomer 1)	23.8	0.014	-	-
fluopyram PAA-glycoside (M42) (isomer 2)	14.2	0.009	-	-
fluopyram hydroxyethyl-di-glc (M36)	-	-	7.0	0.164
fluopyram 7-hydroxy-glc (M11)	-	-	9.2	0.215
fluopyram-*N*-oxide (M01)	-	-	2.9	0.069
fluopyram-7-hydroxy (M08)	-	-	5.1	0.120
fluopyram 8-hydroxy (M18)	-	-	-	-
Total identified	97.8	0.059	94.3	2.211
unknown 6	-	-	-	-
unknown 7	-	-	1.0	0.023
unknown 9	-	-	-	-
Total characterised	-	-	1.0	0.023
Total extracted	97.8	0.059	95.3	2.233
Solids (non-extractable residue)	3.2	0.001	4.7	0.110
Accountability	100.0	0.060	100.0	2.344

III. Conclusions

After one application of [pyridyl-2,6-^{14}C]fluopyram (5 mg a.s./plant), the TRR in pepper fruits of the 1X experiment was low and amounted to 0.060 mg eq/kg. The TRR in the plants sampled after harvest of the fruits was 2.344 mg eq/kg. The TRR values in the 4X overdose experiment, performed to support identification and structure elucidation of metabolites, amounted to 18.24 mg eq/kg in the intermediate sample and to 0.149mg eq/kg in the pepper fruits, respectively.

In both experiments, the unchanged parent compound was the predominant portion of the TRR in the intermediate sample and in the plants at harvest (70% to 88% of the TRR). In pepper fruits, it was still a major compound, but represented only approximately 16–33% of the TRR.

In total, nine metabolites were identified. In the 1X experiment, the major compound in red bell pepper fruits was fluopyram-pyridyl-carboxylic acid (> 40% of the TRR), followed by two isomeric fluopyram-PAA-glycosides (24% and 14% of the TRR, respectively) and the parent fluopyram (approximately 16% of the TRR).

The following metabolic routes were deduced:

- Hydroxylation of the ethyl linking group of the a.s. forming 7- or 8-hydroxy metabolites

- Subsequent cleavage to fluopyram-pyridyl-acetic acid, pyridyl-carboxylic acid and fluopyram-pyridyl-hydroxyethyl
- Conjugation of hydroxyl-metabolites with hexoses and subsequent higher conjugations with malonic and glucuronic acid
- Oxidation of the *N*-atom of the pyridyl moiety of the parent compound resulting in fluopyram-*N*-oxide

On the basis of the nature and amount of metabolites found in the intermediate sample, the fruits and the plants at harvest, the metabolic pathway of [pyridyl-2,6-^{14}C]fluopyram in red bell pepper is proposed in Figure 6.2.1-8.

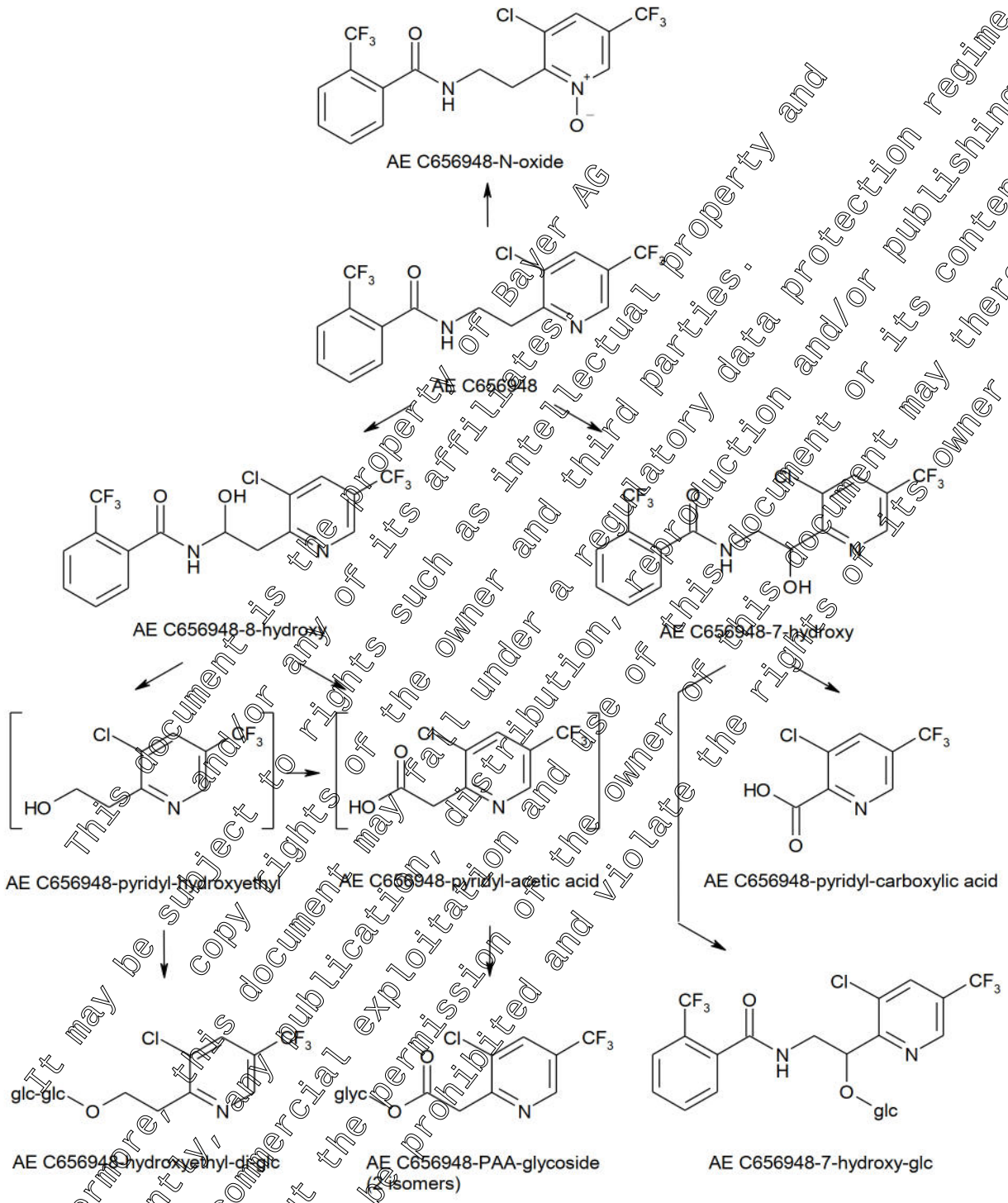


Figure 6.2.1-8 Proposed metabolic pathway of [pyridyl-2,6-^{14}C]AE C656948 (fluopyram) in red bell pepper

Assessment and conclusion by applicant:

The study is valid and acceptable.

Supplemental cell culture study

Data Point:	KCA 6.2.1/09
Report Author:	[redacted]
Report Year:	2005
Report Title:	Degradation of [phenyl-UL-14C] and [pyridyl-2,6-14C]AE C656948 by plant suspension cell cultures
Report No:	MEF-05/142
Document No:	M-259283-01-1
Guideline(s) followed in study:	Equivalent to US EPA OPPTS Guideline No. 860.1300 (Supplemental)
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted, rev. 1 to Vol.3 of DAR B7, August 2012 (reference relied on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Summary

The metabolism of the fungicide AE C656948 was investigated in heterotrophic plant cell suspension cultures originating from apple fruit following incubation with [phenyl-UL-14C] and [pyridyl-2,6-^{14}C]AE C656948, respectively. This was done to facilitate metabolite identification and to produce radiolabeled reference compounds for the identification of metabolites in plant and animal metabolism studies.

I. Materials and Methods

A. Materials

1. Test Material: [phenyl-UL-^{14}C]AE C656948
Radiochemical purity > 98% (lowest value of two lots)
Chemical purity > 99% (lowest value of two lots)
Specific Activity 3.85 MBq/mg (104.2 µCi/mg), radio-diluted with non-labeled reference compound to 0.2434 MBq/mg
[pyridyl-2,6-^{14}C]AE C656948
Radiochemical purity > 98%
Chemical purity > 99%
Specific Activity 5.48 MBq/mg (148.1 µCi/mg), radio-diluted with non-labeled reference compound to 0.2412 MBq/mg

2. Cell culture: Apple fruits (*Malus sylvestris*), cultivar Boskop

B. Study Design

Experimental conditions: The radiolabeled and non-radiolabeled a.s. were dissolved in acetonitrile. For the experiment, the radioactive compound was diluted with the non-radiolabeled substance to obtain the desired specific radioactivity (concentration: 50 µM = 794 µg / 40 mL cell suspension; radioactivity ca. 185 kBq = 5 µCi). The solution was applied directly to the plant suspension cells. The amount of the organic solvent in the cell culture did not exceed 50 µL/40 mL cell suspension (= 0.13%) avoiding phytotoxic effects of the organic solvent in cell growth. The experiments were performed with cells from the beginning of the exponential growth phase. After application of the test substance the cells were further cultivated under the normal growth conditions.

Sampling: On day seven after application, 10 flasks (of each label) were sampled. Plant cells and the nutrient medium were immediately separated by filtration. The cells were washed intensively with water to remove soluble radioactivity from the cell surfaces. This wash solution was combined with the nutrient medium. These liquid samples were concentrated at about 35°C under vacuum using a rotary evaporator and redissolved in an appropriate solvent (i.e. acetonitrile or mixtures of water with acetonitrile). The samples were stored at ca. 4°C during processing and then at ca. -20°C in a freezer.

C. Analytical Procedures

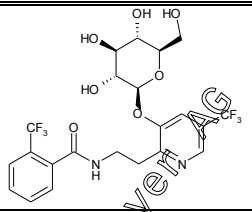
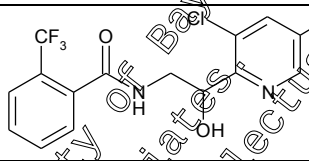
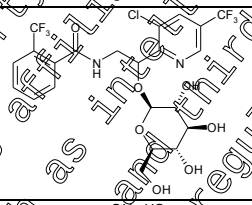
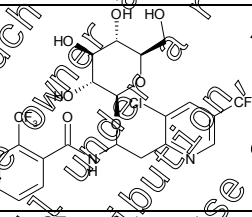
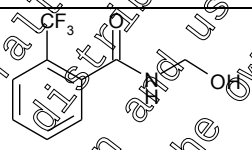
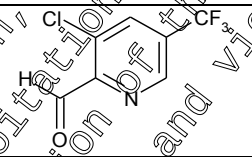
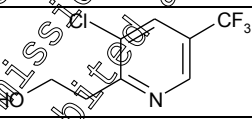
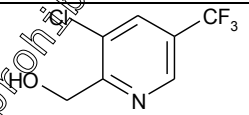
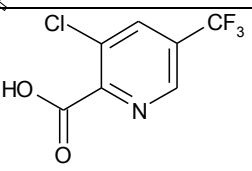
Extraction and quantitation: The cells were successively extracted with acetonitrile/water (80:20, v/v, 3x). The acetonitrile/water extracts were combined and concentrated to the aqueous remainder. The extracted fibrous solids were air dried at room temperature, weighed, and radioassayed. More drastic extraction methods for the solids were not necessary since only a low residual amount of radioactivity was found in this sample.
The nutrient medium of each label was concentrated. Cell debris and proteins were precipitated with acetonitrile and centrifuged. The clear supernatant was decanted, concentrated and again mixed with acetonitrile. After centrifugation, the supernatant was decanted and concentrated.

Identification and characterization: Metabolites were isolated and identified from the concentrated medium and from the cell extracts by HPLC fractionation and purification of the fractions. Purified metabolites were identified by LC-NMR, HPLC-MS/MS, FT-MS and different NMR techniques.

H. Results and Discussion

From the cell culture study nine metabolites were isolated and identified. Structures are given in **Table 6.2.1-28**.

Table 6.2.1-28: Identified metabolites form a cell culture study with [phenyl-UL14C] and [pyridyl-2,6-14C]fluopyram

Identified metabolite	Structure	Remark
fluopyram-deschloro-3-OH-glc		identified with the phenyl-label
fluopyram-7-hydroxy		identified with the phenyl-label
fluopyram-7-hydroxy-glc		identified with the phenyl- and the pyridyl-label
fluopyram-8-hydroxy-glc		identified with the phenyl-label
fluopyram-hydroxymethyl-benzamide		identified with the phenyl-label
fluopyram-benzamide		identified with the phenyl-label
fluopyram-pyridyl-hydroxyethyl		identified with the pyridyl-label as aglycon of a higher conjugate
fluopyram-pyridyl-hydroxymethyl		identified with the pyridyl-label as aglycon of a higher conjugate
fluopyram-pyridyl-carboxylic acid (PCA)		identified with the pyridyl-label

glc = glucoside

Assessment and conclusion by applicant:

The study is valid and acceptable.

New Data

Metabolism in wheat (seed dressing)

Metabolism studies in wheat were conducted with both [phenyl-UL-^{14}C]- and [pyridyl-2,6-^{14}C]fluopyram:

Data Point:	KCA 6.2.1/10
Report Author:	[redacted]
Report Year:	2009
Report Title:	Metabolism of AE C656948 in wheat after seed dressing
Report No:	MEF 09/124
Document No:	M-345948-01-1
Guideline(s) followed in study:	US EPA OPPTS 860.1300; Canadian PMRA Rel. DACO 6.3; OECD 501; EU 91/414/EEC amended by 96/68/EC; Japanese MAFF, 12 Nousan 8147
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The metabolism of the fungicide fluopyram was investigated in wheat after seed treatment with both [phenyl-UL-^{14}C]fluopyram and [pyridyl-2,6-^{14}C]fluopyram, formulated as a SC 500 (Suspension Concentrate).

The plants were cultivated in a vegetation area under natural temperature and light conditions. The seed treatment was performed one day before sowing the seeds (BBCH 00). Due to the low intended dressing rate of 1 g a.s./dt in agricultural practice, only an overdose experiment was performed with a dressing rate of approx. 10 g a.s./dt (10X). The actual application rates were 10.5 g a.s./dt and 10.8 g a.s./dt for the phenyl label and the pyridyl label, respectively.

Wheat forage (BBCH 30) and hay (BBCH 77–83) were collected as intermediate plant samples and wheat straw and grains (BBCH 89-92) were harvested at maturity.

The major amount of radioactivity (79.1–93.3% of the TRR) was extracted with acetonitrile/water from all RACs in both label experiments. Left amounts with the solids (PES) amounted to 6.7–12.5% of the TRR in forage, hay and straw and up to 20.4% of the TRR in grains. The TRRs in straw (phenyl label: 0.506 mg eq/kg and pyridyl label: 0.477 mg eq/kg) were higher than the TRRs in hay (phenyl label: 0.238 mg eq/kg and pyridyl label: 0.287 mg eq/kg) and forage (phenyl label: 0.132 mg eq/kg and pyridyl label: 0.142 mg eq/kg). The TRRs in grains were lower in both labels (phenyl label: 0.006 mg eq/kg and pyridyl label: 0.012 mg eq/kg).

The unchanged parent compound was the predominant residue in all plant matrices accounting for 40.6–65.3% of the TRR in both labels.

Two isomers of fluopyram-7-hydroxy-glc-MA (M12) were major metabolites in forage, hay and straw (10.4–15.2% of the TRR), followed by fluopyram-7-hydroxy (M08, 5.2–11.1% of the TRR). A major metabolite in grain of the phenyl label was fluopyram-benzamide (M25, 10.4% of the TRR). It was also found in forage, hay and straw (2.3–11.4% of the TRR). Besides, fluopyram-7-hydroxy (M08) was found in grain of the phenyl label (3.4% of the TRR, < 0.001 mg eq/kg). fluopyram-8-hydroxy-glc-MA (M19), fluopyram-7-hydroxy-glc (M11) and fluopyram-8-hydroxy (m18) and fluopyram-pyridyl-carboxylic acid (M43) were detected in minor amounts of < 5% (< 0.01 mg eq/kg) of the TRR. Fluopyram-methyl-sulfoxide (M45) was detected exclusively in wheat straw in minor amounts (2.7% of the TRR).

A total of 59.2–89.8% (0.004–0.402 mg eq/kg) of the TRR was identified in forage, hay, straw and grain. A few minor unknown components (each < 20% of the TRR, < 0.05 mg eq/kg) were characterised by their chromatographic behaviour in all RACs.

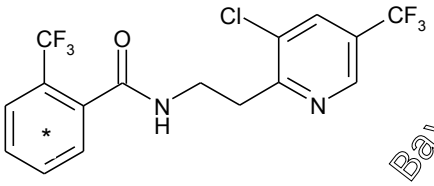
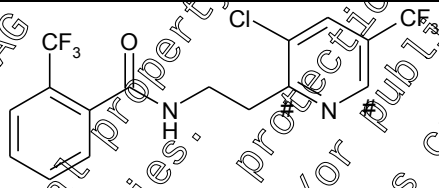
The mean metabolic reactions involved were:

- Hydroxylation of the ethyl group of the parent compound
- Conjugation of the hydroxylated metabolites with glucose and subsequently with malonic acid to two isomers
- Cleavage of the hydroxylated metabolites with and without additional oxidation

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	 *positions of radiolabel	 # positions of radiolabel
Compound	AE C656948	
IUPAC name	*N*-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]ethyl}-2-(trifluoromethyl)benzamide	
CAS name	Benzamide, *N*-[2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-(trifluoromethyl)-(9Cl)	
CAS #	658066-35-4	
Radiolabel position	Phenyl-UL-^{14}C	Pyridyl-2,6-^{14}C
Specific radioactivity	3.85 MBq/mg (104.15 µCi/mg, 231213000 dpm/mg)	
Purity Chemical Purity	> 99% (HPLC and TLC) > 98% (HPLC)	> 99% (HPLC and TLC) > 99% (HPLC)

2. Soil:

"Monheim 4" (sandy loam from Germany), pH ($CaCl_2$) = 6.9, 58.1% sand 27.9% silt and 14.0% clay, 1.23% organic carbon, 2.12% organic material, cation exchange capacity (CEC) of 8.1 meq/100 g

3. Plant:

Wheat, variety "Thasos"

B. Study Design

1. Experimental conditions:

Wheat was cultivated in the vegetation area (building 6682) of Bayer CropScience AG, Environmental Safety, Metabolism/ADME and Environmental Fate, Monheim, Germany, which allows plant growth under natural temperature and light conditions. Wheat was sown in planting containers with a surface area of approx. 1.0 m² and filled with sandy loam soil "Monheim 4". In each planting container six rows were sown with 75 wheat seeds per row.

Due to the low intended dressing rate of 1 g a.s./dt in agricultural practice, only an overdose experiment has been conducted with a dressing rate of approx. 10 g a.s./dt (10X experiment). One planting container was used for the experiment with [phenyl-UL-^{14}C]fluopyram and another for the experiment with [pyridyl-2,6-^{14}C]fluopyram. The seed treatment was performed one day before sowing the seeds (BBCH 00).

2. Sampling

Wheat forage: One row of intermediate plants was sampled at the beginning of stem elongation (BBCH 30). The plants were cut slightly above the soil surface, cut into pieces and the sample material was weighed and homogenised.

Wheat hay: Two rows were collected as intermediate plant samples (BBCH 77–83). The plants were cut slightly above the soil surface, cut into pieces and the sample material was dried at room temperature for three days before being weighed and homogenised.

Wheat straw and grains: At maturity (BBCH 89–92) the remaining plants were harvested. The plants were cut slightly above the soil surface. The ears were separated from the straw and grains were separated from the husks. The husks were combined with the straw sample and the total weight was determined.

C. Analytical Procedures

1. Extraction and fractionation:

All RAC samples were extracted either three or four times with acetonitrile/water (4/1, v/v) mixture using a Polytron homogenizer. Subsequently, extracts were purified, concentrated and analysed by HPLC analogously to the confined rotational crop (CRC) studies to facilitate comparison of corresponding metabolic profiles within the different studies. The extracts were separated from the solids by centrifugation and radioactivity was determined by volume measurement and LSC. The remaining solids were dried at room temperature, homogenised using a blender where necessary and subjected to combustion for determination of radioactivity. Aliquots of the purified solvent extracts were concentrated using a rotary evaporator prior to HPLC analyses.

The ^{14}C-radioactivity of liquid samples was determined by liquid scintillation counting (LSC) using Quicksafe A containing 5% of water. The radioactivity in solid samples was measured by combustion. The released $^{14}CO_2$ was absorbed in an alkaline scintillation cocktail and radio assayed by LSC.

The actual TRR value of each RAC was determined after extraction by summing up the radioactivity measured in the extracts and in the remaining solids. The concentrated acetonitrile/water extracts were analysed by HPLC for quantification of metabolites.

2. Identification and characterisation:

Parent compound and the metabolites detected were identified in a first step by HPLC comparison. Therefore, the metabolic profiles of each RAC were first compared with the corresponding metabolic profiles of the CRC study and assignments were confirmed by HPLC co-chromatography with corresponding extracts of the CRC study.

3. Storage stability:

The RACs were extracted and analysed within a few days (< 6 days) after sampling. All investigations concerning identification (HPLC comparison and co-chromatography) were performed within less than one month after sampling of the respective RAC. During the study, all samples and extracts were stored in a freezer or in a refrigerator. Thus, no additional storage investigations became necessary for the samples or extracts of the present study.

II. Results and Discussion

The metabolism of the fungicide fluopyram was investigated in wheat after seed treatment with [phenyl-UL-^{14}C]fluopyram (10.5 g a.s./dt) and [pyridyl-2,6-^{14}C]fluopyram (10.8 g a.s./dt) formulated as SC 500.

The final TRR values were calculated as the sum of the radioactivity determined in the extracts and the radioactivity in the remaining non-extractable solids.

The TRR was the highest in straw (phenyl label: 0.506 mg eq/kg; pyridyl label: 0.477 mg eq/kg) followed by the residues in hay (phenyl label: 0.238 mg eq/kg; pyridyl label: 0.287 mg eq/kg) and forage (phenyl label: 0.132 mg eq/kg; pyridyl label: 0.142 mg eq/kg). TRR in grain were noticeably lower (phenyl label: 0.006 mg eq/kg; pyridyl label: 0.012 mg eq/kg) (Table 6.2.1-29).

Table 6.2.1-29: TRR values in wheat matrices after application of [phenyl-UL-^{14}C]fluopyram and [pyridyl-2,6-^{14}C]fluopyram

Matrix	Timing and Applic. No.	PHI (days)	TRR (ppm, mg a.s. equiv./kg)
Wheat forage (BBCH 30)	Seed dressing (BBCH 00): Total 10.5 g a.s./dt (phenyl label)	40	0.132
	Seed dressing (BBCH 00): Total 10.8 g a.s./dt (pyridyl label)		0.142
Wheat hay (BBCH 77-83)	Seed dressing (BBCH 00): Total 10.5 g a.s./dt (phenyl label)	49	0.238
	Seed dressing (BBCH 00): Total 10.8 g a.s./dt (pyridyl label)		0.287
Wheat straw (BBCH 89-92)	Seed dressing (BBCH 00): Total 10.5 g a.s./dt (phenyl label)	112	0.506
	Seed dressing (BBCH 00): Total 10.8 g a.s./dt (pyridyl label)		0.477
Wheat grain (BBCH 89-92)	Seed dressing (BBCH 00): Total 10.5 g a.s./dt (phenyl label)	112	0.006
	Seed dressing (BBCH 00): Total 10.8 g a.s./dt (pyridyl label)		0.012

The wheat matrices were extracted with acetonitrile/water (4/1, v/v), the extracts were analysed by HPLC and parent compound and metabolites were identified.

Comparing the residues in the RACs related to the radiolabels used, only marginal differences were detected. Only for wheat grain, the residues were about doubled using [pyridyl-2,6-^{14}C]fluopyram. These results are also in very good accordance to those of the CRC studies, where the residues in grain were higher after administration of [pyridyl-2,6-^{14}C]fluopyram.

Nearly the complete radioactive residues in forage (phenyl label: 93.3% of the TRR, 0.124 mg eq/kg; pyridyl label: 92.2% of the TRR, 0.131 mg eq/kg) were extracted by acetonitrile/water, whereas post-extraction solids (PES) accounted for 6.7% (0.009 mg eq/kg) of the TRR for the phenyl label and 7.8% (0.011 mg eq/kg) of the TRR for the pyridyl label (Table 6.2.1-30).

The major amount of radioactivity in hay (phenyl label: 90.8% of the TRR, 0.216 mg eq/kg; pyridyl label: 91.8% of the TRR, 0.263 mg eq/kg) was extracted by acetonitrile/water, whereas PES accounted for 9.2% (0.022 mg eq/kg) of the TRR for the phenyl label and 8.2% (0.024 mg eq/kg) of the TRR for the pyridyl label (Table 6.2.1-30 and Table 6.2.1-31).

The majority of the TRR in straw (phenyl label: 86.8% of the TRR, 0.439 mg eq/kg; pyridyl label: 86.5% of the TRR, 0.413 mg eq/kg) was extracted by acetonitrile/water, whereas PES accounted for 12.3% (0.062 mg eq/kg) of the TRR for the phenyl label and 12.5% (0.060 mg eq/kg) of the TRR for the pyridyl label (Table 6.2.1-30 and Table 6.2.1-31).

The majority of the TRR in grain (phenyl label: 79.6% of the TRR, 0.005 mg eq/kg; pyridyl label: 79.1% of the TRR, 0.009 mg eq/kg) was extracted by acetonitrile/water, whereas PES accounted for 20.4% (0.001 mg eq/kg) of the TRR for the phenyl label and 15.6% (0.002 mg eq/kg) of the TRR for the pyridyl label (Table 6.2.1-30 and Table 6.2.1-31).

Table 6.2.1-30: Distribution of radioactivity in the extracts of the wheat matrices after seed dressing with [phenyl-UL-^{14}C]fluopyram

	Forage		Hay		Straw		Grain	
TRR [mg eq/kg] =	0.132		0.238		0.506		0.006	
	% of TRR	mg eq/kg	% of TRR	mg eq/kg	% of TRR	mg eq/kg	% of TRR	mg eq/kg
Acetonitrile/water extract	93.3	0.124	90.8	0.216	86.8	0.439	79.6	0.005
Solids (PES)	6.7	0.009	9.2	0.022	12.3	0.062	20.4	0.001
Total extracted	93.3	0.124	90.8	0.216	86.8	0.439	79.6	0.005
Accountability	100.0	0.132	100.0	0.238	100.0	0.506	100.0	0.006

Table 6.2.1-31: Distribution of radioactivity in the extracts of the wheat matrices after seed dressing with [pyridyl-2,6-^{14}C]AE 656948

	Forage		Hay		Straw		Grain	
TRR [mg eq/kg] =	0.142		0.287		0.477		0.012	
	% of TRR	mg eq/kg	% of TRR	mg eq/kg	% of TRR	mg eq/kg	% of TRR	mg eq/kg
Acetonitrile/water extract	92.2	0.131	91.8	0.263	86.5	0.413	79.1	0.009
Solids (PES)	7.8	0.011	8.2	0.024	12.5	0.060	15.6	0.002
Total extracted	92.2	0.131	91.8	0.263	86.5	0.413	79.1	0.009
Accountability	100.0	0.142	100.0	0.287	100.0	0.477	100.0	0.012

For the elucidation of metabolism, the solvent extracts (acetonitrile/water) were analysed by HPLC and the metabolic profiles were compared with those of the CRC study. Metabolite assignments were confirmed by HPLC co-chromatography with corresponding extracts of the CRC study.

Wheat forage: The parent compound fluopyram (phenyl label: 65.3% of the TRR, 0.086 mg eq/kg; pyridyl label: 62.6% of the TRR, 0.089 mg eq/kg) was the most prominent compound in the forage samples followed by fluopyram-7-hydroxy-glc-MA (phenyl label: 11.0% of the TRR, 0.015mg eq/kg; pyridyl label: 12.3% of the TRR, 0.017 mg eq/kg) and fluopyram-7-hydroxy (phenyl label: 6.2% of the TRR, 0.008mg eq/kg; pyridyl label: 5.2% of the TRR, 0.007 mg eq/kg). Other minor metabolites, namely fluopyram-pyridyl-carboxylic acid, fluopyram-8-hydroxy-glc-MA, fluopyram-7-hydroxy-glc, fluopyram-8-hydroxy and fluopyram-benzamide amounted to < 5% of the TRR (< 0.01 mg eq/kg). Moreover eight unknown components, all below 1.5% of the TRR (< 0.002 mg eq/kg), were characterized in forage (Table 6.2.1-32 and Table 6.2.1-33).

Wheat hay: The parent compound fluopyram (phenyl label: 51.4% of the TRR, 0.122 mg eq/kg; pyridyl label: 62.8% of the TRR, 0.18 mg eq/kg) was the most prominent compound in the hay samples, followed by fluopyram-7-hydroxy-glc-MA (phenyl label: 12.1% of the TRR, 0.029 mg eq/kg; pyridyl label: 11.9% of the TRR, 0.034 mg eq/kg), the phenyl label specific metabolite fluopyram-benzamide (11.4% of the TRR, 0.027 mg eq/kg) and fluopyram-7-hydroxy (phenyl label: 8.2% of the TRR, 0.020 mg eq/kg; pyridyl label: 8.3% of the TRR, 0.024 mg eq/kg). Other minor metabolites, namely fluopyram-pyridyl-carboxylic acid, fluopyram-8-hydroxy-glc-MA, fluopyram-7-hydroxy-glc, fluopyram-8-hydroxy and fluopyram-benzamide amounted each to < 5% of the TRR (< 0.01 mg eq/kg), respectively. Moreover five unknown components, each < 1.5 % of the TRR (< 0.004 mg eq/kg), were characterized in hay (Table 6.2.1-32 and Table 6.2.1-33).

Wheat straw: The parent compound fluopyram (phenyl label: 46.1% of the TRR, 0.233 mg eq/kg; pyridyl label: 40.6% of the TRR, 0.194 mg eq/kg) was the most prominent compound in the straw samples followed by fluopyram-7-hydroxy-glc-MA (phenyl label: 10.4% of the TRR, 0.053 mg eq/kg; pyridyl label: 15.2% of the TRR, 0.073 mg eq/kg), the phenyl label specific metabolite fluopyram-benzamide (8.0% of the TRR, 0.041 mg eq/kg) and fluopyram-7-hydroxy (phenyl label: 9.7% of the TRR, 0.049 mg eq/kg; pyridyl label: 11.1% of the TRR, 0.053 mg eq/kg). Other minor metabolites, namely fluopyram-8-hydroxy-glc-MA, fluopyram-7-hydroxy-glc, fluopyram-8-hydroxy and fluopyram-benzamide amounted to < 5% of the TRR (< 0.015 mg eq/kg), respectively. Fluopyram-methyl-sulfoxide was exclusively found in straw in an amount of 2.7% of the TRR (0.013 mg eq/kg). Moreover six unknown components, all below 1.5% of the TRR (< 0.004 mg eq/kg) were characterized in hay (Table 6.2.1-32 and Table 6.2.1-33).

Wheat grain: The parent compound fluopyram (phenyl label: 40.6% of the TRR, 0.003 mg eq/kg; pyridyl label: 59.2% of the TRR, 0.007 mg eq/kg) was the most prominent compound in the straw samples followed by the two phenyl label specific metabolites fluopyram-benzamide (phenyl label: 10.4% of the TRR, 0.001 mg eq/kg) and fluopyram-benzoic acid (8.0% of the TRR; < 0.001 mg eq/kg). The last was exclusively found in grains. One additional minor metabolite was fluopyram-7-hydroxy, amounting to 3.4% (< 0.001 mg eq/kg) of the TRR. Moreover, two unknown components, accounting for 17.3% (0.001 mg eq/kg) of the TRR and 19.9% (0.002 mg eq/kg) of the TRR, were characterized in grains (Table 6.2.1-32 and Table 6.2.1-33).

Table 6.2.1-32: Summary of characterisation and identification of radioactive residues in wheat after seed dressing with [phenyl-UL-^{14}C]fluopyram

	Forage		Hay		Straw		Grain	
TRR [mg eq/kg] =	0.132		0.238		0.506		0.006	
Compound	% of TRR	mg eq/kg	% of TRR	mg eq/kg	% of TRR	mg eq/kg	% of TRR	mg eq/kg
AE C656948, a.s., fluopyram	65.3	0.086	51.4	0.122	46.1	0.233	40.6	0.003
fluopyram-benzoic acid (M33)	-	-	-	-	-	-	8.0	<0.001
fluopyram-benzamide (M25)	2.3	0.003	11.4	0.027	8.0	0.041	10.4	0.001
fluopyram-7-hydroxy-glc-MA (isomer 1 & 2) (M12)	11.0	0.015	12.1	0.029	10.4	0.053	-	-
fluopyram-8-hydroxy-glc-MA (M19)	1.9	0.003	2.5	0.006	2.0	0.010	-	-
fluopyram-7-hydroxy-glc (M11)	2.3	0.003	2.1	0.005	1.7	0.009	-	-
fluopyram-7-hydroxy (M08)	6.2	0.008	8.2	0.020	9.7	0.049	3.4	<0.001
fluopyram-8-hydroxy (M18)	0.8	0.001	-	-	1.4	0.007	-	-
Total identified	89.8	0.119	87.8	0.209	79.4	0.402	62.3	0.004
unknown 1	-	-	1.5	0.003	1.2	0.006	17.3	0.001
unknown 8	0.8	0.001	-	-	-	-	-	-
unknown 11	1.0	0.001	0.7	0.002	2.0	0.010	-	-
unknown 14	1.1	0.001	0.8	0.002	4.3	0.022	-	-
unknown 17	0.6	0.001	-	-	-	-	-	-
Total characterised	3.5	0.005	3.0	0.007	7.4	0.038	17.3	0.001
Total extracted	93.3	0.124	90.8	0.216	86.8	0.439	79.6	0.005
Solids (non-extractable residue)	6.7	0.009	9.2	0.022	12.3	0.062	20.4	0.001
Fractions not analysed	-	-	-	-	0.9	0.005	-	-
Accountability	100.0	0.132	100.0	0.238	100.0	0.506	100.0	0.006

Table 6.2.1-33: Summary of characterisation and identification of radioactive residues in wheat after seed dressing with [pyridyl-2,6-^{14}C]fluopyram

	Forage		Hay		Straw		Grain	
TRR [mg eq/kg] =	0.142		0.287		0.477		0.012	
Compound	% of TRR	mg eq/kg	% of TRR	mg eq/kg	% of TRR	mg eq/kg	% of TRR	mg eq/kg
AE C656948, a.s., fluopyram	62.6	0.089	62.8	0.18	40.6	0.194	59.2	0.007
fluopyram-methyl-sulfoxide (M45)	-	-	-	-	2.7	0.013	-	-
AEC656948-pyridyl-carboxylic acid (M43)	3.6	0.005	1.7	0.005	-	-	-	-
fluopyram-7-hydroxy-glc-MA (isomer 1 & 2) (M12)	12.3	0.017	11.9	0.034	15.2	0.073	-	-
fluopyram-8-hydroxy-glc-MA (M19)	2.3	0.003	1.5	0.004	2.9	0.014	-	-
fluopyram-7-hydroxy-glc (M11)	2.4	0.003	2.2	0.006	2.2	0.010	-	-
fluopyram-7-hydroxy (M08)	5.2	0.007	8.3	0.024	11.1	0.053	-	-
fluopyram-8-hydroxy (M18)	0.6	0.001	0.7	0.002	1.8	0.008	-	-
Total identified	89.1	0.126	89.1	0.255	76.6	0.366	59.2	0.007
unknown 4	-	-	-	-	1.1	0.005	-	-
unknown 7	0.4	0.001	-	-	-	-	-	-
unknown 8	0.7	0.001	-	-	-	-	19.9	0.002
unknown 11	0.9	0.001	1.2	0.004	2.2	0.010	-	-
unknown 14	1.1	0.002	1.4	0.004	6.6	0.032	-	-
Total characterised	3.1	0.004	2.7	0.008	9.9	0.047	19.9	0.002
Total extracted	92.2	0.131	91.8	0.263	86.5	0.413	79.1	0.009
Solids (non-extractable residue)	7.8	0.011	8.2	0.024	12.5	0.060	15.6	0.002
Fractions not analysed	-	-	-	-	1.0	0.005	5.3	0.001
Accountability	100.0	0.142	100.0	0.287	100.0	0.477	100.0	0.012

III. Conclusions

The translocation and metabolism of the fungicide AE C656948 was investigated in wheat after seed treatment. Two experiments were performed in parallel, one with test item radiolabelled in the phenyl moiety and the second with test item radiolabelled in the pyridyl moiety.

In the edible plant matrix (wheat grain), the TRR values amounted to 0.006 mg eq/kg (phenyl label) and to 0.012 mg eq/kg (pyridyl label), respectively. The highest TRR values in both experiments were detected in straw at 0.506 mg eq/kg (phenyl label) and 0.477 mg eq/kg (pyridyl label), respectively.

The metabolite patterns of the RACs of the present study were comparable to those obtained in the CRC studies. Parent compound was the main constituent in all RACs, accounting for more than 40% of the TRR in the seed treatment study. In total nine metabolites were identified.

The following main metabolic routes were deduced:

- Hydroxylation of the ethyl group of the parent compound
- Conjugation of the hydroxylated metabolites with glucose and subsequently with malonic acid to two isomers
- Cleavage of the hydroxylated metabolites with and without additional oxidation

On the basis of the nature and amount of metabolites found in forage, hay, straw and grains, the metabolic pathway of fluopyram in wheat after seed treatment is proposed Figure 6.2.1-9.

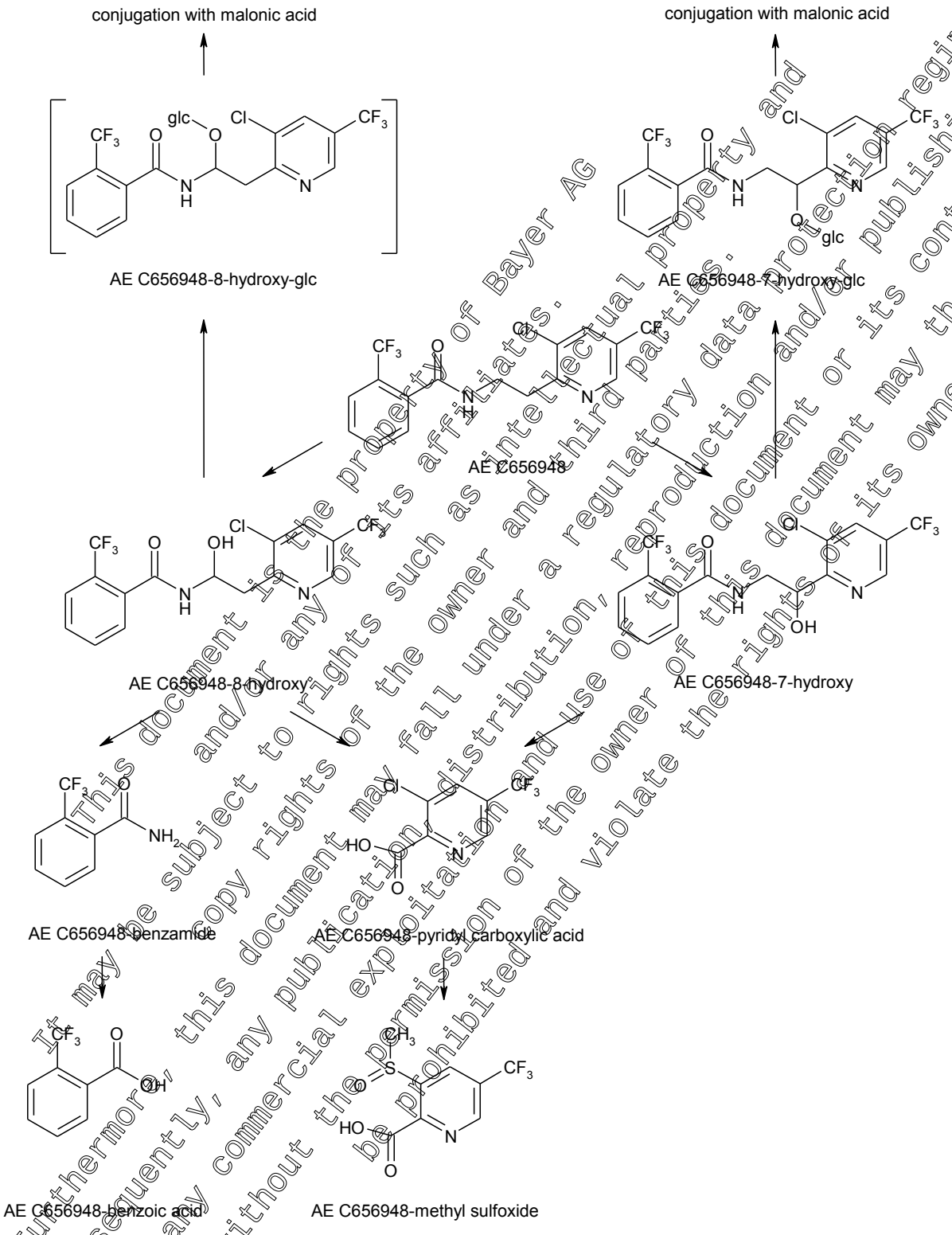


Figure 6.2.1-9 Proposed metabolic pathway of AE C656948 (fluopyram) in wheat after seed dressing

Assessment and conclusion by applicant:
The study is valid and acceptable.

Metabolism in paddy rice (foliar spray application)

Data Point:	KCA 6.2.1/11
Report Author:	[redacted]
Report Year:	2018
Report Title:	Metabolism of [phenyl-UL-14C]fluopyram (AE C650248) in paddy rice after foliar treatment
Report No:	M17304953-2
Document No:	M-615284-01-1
Guideline(s) followed in study:	OECD Test Guideline No. 501; Commission Regulation (EU) No. 283/2013 in accordance with Regulation (EC) No. 1107/2009; US EPA OCSPP Test Guideline No. 860.1300; JMP FAMIC-ACIS Notification 13 Nousan 8147
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The metabolism of [phenyl-UL-^{14}C]fluopyram formulated as an SC 400 was investigated in paddy rice following two foliar spray applications. The applications were performed at BBCH 49 (flag leaf sheath open) and BBCH 65-73 (between full flowering period and development of fruit - early milk). The single application rates were 118.9 and 120.7 g a.s./ha, the total application rate was 239 g a.s./ha.

The rice straw with panicles were harvested at maturity (BBCH 89-92), 30 days after the last treatment and dried. After drying, the rice panicles were separated into hulls and kernels. The straw, hulls and kernels were extracted at least three times with mixtures of acetonitrile/water, the concentrated extracts were analysed by HPLC and TLC and parent compound and metabolites identified. In total 96.9% (5.423 mg eq/kg), 91.1% (4.888 mg eq/kg) and 93.3% (0.189 mg/kg) of the TRR was extracted from the rice straw, hulls and kernels respectively.

The TRRs in paddy rice straw and hulls were highest at 5.599 mg eq/kg and 5.362 mg eq/kg respectively due to the foliar application. Significantly lower residues were observed in the rice kernels with a concentration at 0.203 mg eq/kg.

Parent compound was the main component detected in all the matrices amounting to 76.3% (4.273 mg eq/kg) of the TRR in the rice straw, 86.5% of the TRR (4.636 mg eq/kg) in the hulls and 85.0% (0.172 mg eq/kg) of the TRR in the kernels. Besides parent, three further metabolites were identified fluopyram-7 hydroxy, fluopyram-8 hydroxy and fluopyram-benzamide. All three were minor accounting for a maximum of 8.9% of the TRR or 0.496 mg eq/kg. The largest at 8.9% of the TRR was fluopyram-8 hydroxy and was seen in the rice straw. Up to eight additional minor metabolites (each ≤ 1% of the TRR) were characterised based on the extraction procedure, partitioning behaviour and retention time. In total 93.8% of the TRR in the straw, 90.3% of the TRR in the hulls and 91.6% of the TRR in the kernels were identified.

The following two metabolic reactions occurred in the plants :

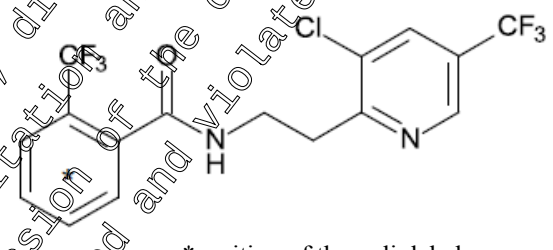
- hydroxylation of the ethyl linking group of the parent compound forming 7- and 8- hydroxy metabolites.
- hydrolytic cleavage of fluopyram-8-hydroxy leading to formation of fluopyram-benzamide.

Based on these results, the metabolism of [phenyl-UL-^{14}C]fluopyram in paddy rice after foliar treatment can be considered as well understood and a metabolic pathway proposed. This metabolism study successfully fulfils all of the requirements of OECD test guideline 501 (2007).

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	 *position of the radiolabel
Radiolabelled test material	[phenyl-UL-^{14}C]fluopyram
Specific radioactivity	5.15 MBq/mg
Radiochemical purity	> 98%
Batch	KML 10175

2. Soil: "Monheim 4" (sandy loam from Germany), pH ($CaCl_2$) = 6.7, 72% sand, 18% silt and 10% clay, 1.1% organic carbon, cation exchange capacity (CEC) of 8.5 meq/100 g

3. Plant: Paddy rice, variety: "*Balilla*"

B. Study Design

1. Experimental conditions:

The paddy rice was cultivated under artificial temperature and light conditions in a greenhouse at the test facility. Plants were pre-grown in a Japanese nursey box. The pre-grown seedlings were transplanted into a plant container (surface of 0.5 m^2) filled with a sandy loam soil in eleven planting holes. Each planting hole contained four seedlings. Water was then added to the plant container to a level of *ca.* 2 cm above the soil. Evaporated water was regularly renewed during cultivation until 21 days before harvest (the last irrigation was performed on the 20th March 2017). Hence, 88 plants were sprayed with [phenyl-UL-^{14}C]fluopyram formulated as an SC 400 with a hand pump sprayer. The envisaged use pattern at initiation of the study included two spray applications each 140 g a.s./ha, resulting in a maximum annual field rate of 220 g a.s./ha. The first field application was proposed at flag leaf sheath open (growth stage BBCH 49; 10th February 2017) and the second application at between full flowering and fruit development - early milk (growth stage BBCH 65-73; 1st March 2017). The metabolism study simulated the envisaged use pattern according to agricultural practice and were based on the maximum proposed application rate. The time interval between the first and the second application was 19 days.

All experiments were performed in the greenhouse with a day/night rhythm of 14/10 hours and an average temperature of 25 °C (day) and 20 °C (night) and a relative humidity of 80%.

2. Sampling

The rice plants were cut off just above the soil surface at BBCH growth stage 89-92 (31st March 2017), 30 days after the last treatment and dried for 4 days. After four days of drying in the greenhouse the grains were separated from the panicles, and the empty panicles added to the straw sample. The straw was cut into 1-2 cm lengths and an aliquot homogenised with liquid nitrogen in a highspeed blender. The rice grain was passed through a rice husker to remove the hulls and an aliquot of the hulls homogenised with liquid nitrogen using a polytron homogeniser. The husked grains (kernels) were again homogenised with liquid nitrogen using a polytron homogeniser. All samples stored at ≤ -18 °C until analysed.

C. Analytical Procedures

Straw, rice hulls and kernels were extracted and the extracts were analysed by HPLC and TLC.

1. Extraction and fractionation:

An aliquot of the homogenised rice straw was soaked in acetonitrile/water (8:2, v/v) before being extracted using a high-speed blender. The extraction was repeated using acetonitrile/water (8:2, v/v) twice more and then finally with acetonitrile/water (1:1, v/v). The suspensions were vacuum filtered and the three acetonitrile/water (8:2, v/v) extracts combined and purified by SPE. The acetonitrile/water extract was passed through a pre-conditioned C18 solid phase extraction cartridge. The cartridge was then rinsed with an aliquot of acetonitrile and eluted with a mixture of methanol and THF. The resultant percolate and rinse

fractions were then mixed with the fourth extract (acetonitrile/water (1:1, v/v) subsequently concentrated by rotary evaporation and analysed by HPLC.

An aliquot of the homogenised rice hulls was treated in the same manner as the straw. The hulls were soaked in acetonitrile/water (8:2, v/v) before being extracted using a high-speed blender. The extraction was repeated using acetonitrile/water (8:2, v/v) twice more and then finally with acetonitrile/water (1:1, v/v). The suspensions were vacuum filtered and the three acetonitrile/water (8:2, v/v) extracts combined and purified by SPE. The acetonitrile/water extract was passed through a pre-conditioned C18 solid phase extraction cartridge. The cartridge was then rinsed with an aliquot of acetonitrile and eluted with a mixture of methanol and THF. The resultant percolate and rinse fractions were then mixed with the fourth extract (acetonitrile/water (1:1, v/v) subsequently concentrated by rotary evaporation and analysed by HPLC.

An aliquot of the homogenised rice kernels was soaked in acetonitrile/water (8:2, v/v) before being extracted using a high speed-blender. The extraction was repeated using acetonitrile/water (8:2, v/v) three further times. The suspensions were vacuum filtered and the four acetonitrile/water (8:2, v/v) extracts combined and purified by SPE. The acetonitrile/water extract was passed through a pre-conditioned C18 solid phase extraction cartridge. The cartridge was then rinsed with an aliquot of acetonitrile and eluted with a mixture of methanol and THF. The resultant percolate and rinse fractions were concentrated by rotary evaporation and analysed by HPLC.

The radioactivity in liquid samples was determined by liquid scintillation counting (LSC). Solid samples were combusted. The $^{14}CO_2$ produced by combustion was absorbed in a CO_2 absorbent/ scintillation cocktail mixture and the radioactivity was measured by LSC.

The total radioactive residue (TRR) was determined by summation of the radioactivity of the combined extract(s) and of the remaining solids. The TRR was expressed in mg a.s. equivalents per kg sample weight. Amounts of radioactive residues in the extracts were expressed as percentage of the TRR and also as mg a.s. equivalents per kg sample weight.

2. Identification and characterisation:

For elucidation of metabolism, extracts and phases were analysed by HPLC and/or TLC with radio detection. Metabolites were either identified by LC/MS/MS of isolated peaks (in some cases supported by NMR) or by co-chromatography with authentic reference compounds using two independent chromatographic methods with different selectivity (e.g. HPLC and TLC).

3. Storage stability:

All extraction experiments for metabolism investigations and the first HPLC analyses were performed within two months after sampling of the individual rice samples. The conventional extracts were analysed by HPLC on the day of the extraction. It is 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 at harvest. All samples were stored at ≤-18 °C.

Determination of the extraction efficiency of the residue method

Validation of the residue analytical method 00984/M003 was performed in this study. Aliquots of rice straw and kernels were extracted by shaking with a mixture acetonitrile/water (8/2, v/v). The suspensions were vacuum filtered and the extracts purified by SPE, concentrated by rotary evaporation and analysed by HPLC. The resulting chromatograms were compared to the HPLC chromatograms from the corresponding sample extracts performed in this study and the extraction efficiency of the residue analytical method 00984/M003 calculated.

II. Results and Discussion

The metabolism of [phenyl-UL-^{14}C]fluopyram formulated as an SC 400 was investigated in paddy rice following two foliar spray applications, at single rates of 118.3 and 120.7 g a.s./ha and a total rate of 239.0 g a.s./ha.

The TRRs in straw and hulls were relatively high amounting to 5.599 mg eq/kg and 5.362 mg eq/kg (Table 6.2.1- 34). Significantly lower residues were observed in the edible RAC (raw agricultural commodity), rice kernels (0.203 mg eq/kg) due to protection by the hulls.

Table 6.2.1- 34: TRR values in paddy rice matrices after application of [phenyl-UL-^{14}C]fluopyram

Matrix	Timing and Application	Growth stage at harvest	PHI (days)	TRR (mg a.s. equiv./kg)
Straw	1st foliar treatment at growth stage BBCH 49, 118.3 g a.s./ha 2nd foliar treatment at growth stage BBCH 65-73, with 118.3 g a.s./ha	BBCH 88-92	30	5.599
Hulls		BBCH 88-92	30	5.362
Kernels		BBCH 88-92	30	0.203

PHI = pre-harvest interval, The TRR values were determined by summing up the radioactivity measured in the extract and in the remaining solids

The straw, hulls and kernels were extracted at least three times with mixtures of acetonitrile/water (8/2;v/v), the concentrated extracts were analysed by HPLC and TLC and parent compound and metabolites were identified.

From rice straw, a portion of 96.9% of the TRR (5.423 mg eq/kg) was extractable with acetonitrile/water (Table 6.2.1-35) and 3.1% of the TRR (0.176 mg eq/kg) remained in the post extraction solids. From rice hulls, 91.1% of the TRR (4.888 mg eq/kg) was extractable with acetonitrile/water, 8.9% of the TRR (0.475 mg eq/kg) remained in the post extraction solids. Acetonitrile/water extracted 93.3% of the TRR (0.189 mg/kg) from the rice kernels leaving 6.7% of the TRR (0.014 mg eq/kg) in the post extraction solids (PES).

Virtually no radioactivity was lost during concentration of the extracts <0.1% (0.002 mg eq/kg). Only a small amount of radioactivity <0.2% (0.012 mg eq/kg) of the TRR was detected in the methanol/THF eluates of the SPE purification procedures from the rice straw and hulls.

Table 6.2.1-35: Distribution of radioactivity in the extracts of the paddy rice matrices after foliar spray application of [phenyl-UL-^{14}C]fluopyram

	straw		hulls		kernels	
TRR [mg/kg] =	5.599		5.362		0.203	
	% of TRR	mg a.s. equiv./kg	% of TRR	mg a.s. equiv./kg	% of TRR	mg a.s. equiv./kg
Acetonitrile/water extract	96.9	5.423	91.1	4.88	93.3	0.189
Concentrate used for quantitation of metabolites	96.9	5.423	91.1	4.88	93.3	0.189
Not analysed fraction (methanol / THF)	0.2	0.012	0.1	0.006	-	-
Not analysed fraction (condensate)	-	-	-	-	-	-
Not analysed fraction (distillate)	<0.1	0.002	<0.1	0.002	-	-
Total extracted	96.9	5.423	91.1	4.88	93.3	0.189
Post extraction solids (PES)	3.1	0.176	8.9	0.475	6.7	0.014
Accountability	100.0	5.599	100.0	5.362	100.0	0.203

The radioactive residues in the acetonitrile/water extract from the paddy rice straw (Table 6.2.1-36), mainly consisted of parent compound amounting to 76.3% (4.273 mg eq/kg) of the TRR. Besides parent a further three metabolites were identified as fluopyram-7-hydroxy, fluopyram-8-hydroxy and fluopyram-benzamide. All were minor accounting for a maximum of 8.9% of the TRR. Seven additional minor metabolites (each <1% of the TRR) were characterised based on the extraction procedure, partitioning behaviour and retention time. In total 93.8% (5.250 mg eq/kg) of the TRR in the rice straw was identified and 3.1% (0.173 mg eq/kg) of the TRR characterised based on the extraction and chromatographic behaviour.

In the rice hulls, 86.5% of the TRR (4.636 mg eq/kg) was parent compound. As with the rice straw the three minor metabolites fluopyram-7-hydroxy, fluopyram-8-hydroxy and fluopyram-benzamide were all identified with each accounting for ≤2.2% (≤0.120 mg eq/kg) of the TRR. Five additional very minor metabolites each at ≤0.2% (≤0.010 mg eq/kg) of the TRR were characterised based on the extraction procedure, partitioning behaviour and retention time. In total 90.3% (4.845 mg eq/kg) of the TRR in the rice straw was identified and 0.8% (0.043 mg eq/kg) of the TRR characterised based on the extraction and chromatographic behaviour.

In the edible RAC rice kernels, parent compound was the main component amounting to 85.0% (0.172 mg eq/kg) of the TRR. Besides parent the three metabolites fluopyram-7 hydroxy, fluopyram-8 hydroxy and fluopyram-benzamide were all identified each accounting for ≤4.8% (≤0.010 mg eq/kg) of the TRR. In total 91.0% (0.186 mg eq/kg) of the TRR in the kernels was identified. Only two minor peaks accounting for ≤1.0% (0.002 mg eq/kg) of the TRR were characterised based on the extraction and chromatographic behaviour.

Table 6.2.1-36: Distribution of parent compound and metabolites in the extracts of paddy rice matrices after foliar spray application of [phenyl-UL-^{14}C]fluopyram

	straw		hulls		kernels	
TRR [mg/kg] =	5.599		5.362		0.203	
Compound	% of TRR	mg a.s. equiv./kg	% of TRR	mg a.s. equiv./kg	% of TRR	mg a.s. equiv./kg
Acetonitrile/water extract						
AE C656948 (fluopyram)	76.3	4.273	86.5	4.636	85.0	0.172
fluopyram-benzamide (M25)	6.6	0.368	2.2	0.120	4.8	0.010
fluopyram-7-hydroxy (M08)	2.0	0.111	0.9	0.047	1.1	0.002
fluopyram-8-hydroxy (M18)	8.9	0.496	0.8	0.042	0.7	0.001
Total identified	**93.8**	**5.250**	**90.3**	**4.845**	**91.6**	**0.186**
Unknown 1	0.1	0.007	-	-	1.0	0.002
Unknown 2	0.3	0.015	0.1	0.008	0.6	0.001
Unknown 5	0.5	0.029	0.1	0.004	-	-
Unknown 6	0.3	0.016	-	-	-	-
Unknown 5	0.9	0.051	0.2	0.010	-	-
Unknown 6	0.3	0.014	-	-	-	-
Unknown 7	-	-	0.1	0.003	-	-
Unknown 8	0.5	0.027	0.2	0.010	-	-
Sum of unknowns (characterised by HPLC)	**2.8**	**0.159**	**0.7**	**0.036**	**1.7**	**0.003**
Not analysed fractions (total)	0.3	0.015	0.1	0.007	-	-
- not analysed fraction (methanol, THF)	0.2	0.012	0.1	0.006	-	-
- not analysed fraction (condensate)	-	-	-	-	-	-
- not analysed fraction (distillate)	0.1	0.002	<0.1	0.002	-	-
Total characterised (HPLC and not analysed)	**3.1**	**0.173**	**0.8**	**0.043**	**1.7**	**0.003**
Total extracted	**96.9**	**5.423**	**91.1**	**4.888**	**93.3**	**0.189**
Unextractable (PES)	3.1	0.176	8.9	0.475	6.7	0.014
Accountability	100.0	5.599	100.0	5.362	100.0	0.203

Peak assignments (M3, 5, 6, 7, 8, 9, 10, 11 and 14) were not assigned in this study

The extraction efficiency of the residue method 00984/M003 was checked with aliquots of rice straw and rice kernels. The extraction efficiency of rice straw for the parent compound and fluopyram-benzamide amounted to 98.6% and 100.0% respectively and for rice kernels 96.5% for parent and 77.1% for fluopyram-benzamide (Table 6.2.1-37).

Table 6.2.1-37: Extraction efficiencies for the relevant residues in rice straw after extraction according to the residue method

	Rice straw			Rice kernels		
	Metabolism method	Residue method	Extraction efficiency	Metabolism method	Residue method	Extraction efficiency
	%TRR	%TRR	[%]	%TRR	%TRR	[%]
Total extracted	96.9	94.1		93.3	88.0	
Unextracted (PES)	3.1	5.9		6.7	12.0	
TRR	**100.0**	**100.0**		**100.0**	**100.0**	
AE C656948 (fluopyram)	76.3	75.2	98.6	85.0	82.0	96.5
fluopyram-benzamide	6.6	6.6	100.0	4.8	3.7	77.1
TRR of relevant residues	**82.9**	**81.8**	**98.7**	**89.8**	**85.7**	**95.4**

III. Conclusions

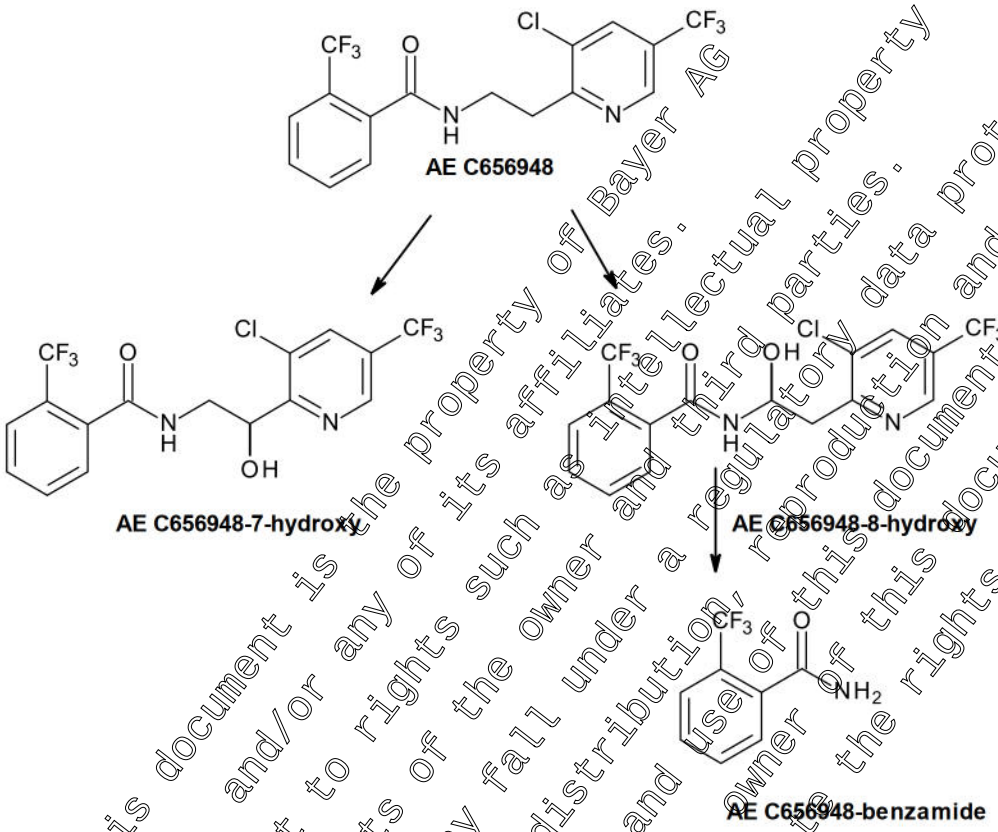
After foliar spray application of [phenyl-ring-UL-^{14}C]fluopyram, the highest residues were in paddy rice straw (5.599 mg eq/kg) and hulls (5.362 mg eq/kg) and mainly consisted of parent compound, amounting to 76.3% and 86.5% of the TRR respectively due to the direct exposure from foliar treatment. The TRR found in the edible RAC kernels amounted to 0.203 mg eq/kg and again consisted of mainly parent compound at 85.0% of the TRR. Three further identified metabolites fluopyram-7-hydroxy, fluopyram-8 hydroxy and fluopyram-benzamide were all minor accounting for less than 8.9% of the TRR. Up to eight additional unknown metabolites were characterised by HPLC but represented <1% each of the TRR in all plant parts.

Two metabolic reactions occurred in the plants:

- hydroxylation of the ethyl linking group of the parent compound forming 7- and 8- hydroxy metabolites.
- hydrolytic cleavage of fluopyram-8-hydroxy leading to formation of fluopyram-benzamide.

Based on these results, the metabolism of [phenyl-UL-^{14}C]fluopyram in paddy rice after foliar treatment can be considered as well understood and a metabolic pathway proposed is proposed in Figure 6.2.1-10.

Figure 6.2.1-10: Proposed metabolic pathway of [phenyl-UL-^{14}C]fluopyram in paddy rice



Assessment and conclusion by applicant:

The study is valid and acceptable.

Data Point:	KCA 6.2.1/12
Report Author:	[redacted]
Report Year:	2018
Report Title:	Metabolism of [pyridyl-2,6-14C]fluopyram (AE C656948) in paddy rice after foliar treatment
Report No:	M17304952-1
Document No:	M-615282-01-1
Guideline(s) followed in study:	OECD Test Guideline No. 501; Commission Regulation (EU) No. 283/2013 in accordance with Regulation (EC) No. 1107/2009; US EPA OCSPP Test Guideline No. 860.1300; JAP FAMIC-ACIS Notification 12 Nousan 8147
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The metabolism of [pyridyl-2,6-^{14}C]fluopyram formulated as an SC 400 was investigated in paddy rice following two foliar spray applications. The applications were performed at BBCH 49 (flag leaf sheath open) and BBCH 65-73 (between full flowering period and development of fruit - early milk). The single application rates were 123.3 and 126.3 g a.s./ha; the total application rate was 249.6 g a.s./ha.

The rice straw with panicles were harvested at maturity (BBCH 89-92), 30 days after the last treatment and dried. After drying the rice panicles were separated into hulls and kernels. The straw, hulls and kernels were extracted at least three times with mixtures of acetonitrile/water, the concentrated extracts were analysed by HPLC and TLC and parent compound and metabolites identified. In total 93.9% (4.344 mg eq/kg), 88.5% (3.819 mg eq/kg) and 92.7% (0.242 mg/kg) of the TRR was extracted from the rice straw, hulls and kernels respectively.

The TRRs in paddy rice straw and hulls were highest at 4.626 mg eq/kg and 4.316 mg eq/kg respectively due to the foliar application. Significantly lower residues were observed in the rice kernels at a concentration of 0.261 mg eq/kg.

Parent compound was the main component detected in all the matrices amounting to 76.2% (3.525 mg eq/kg) of the TRR in the rice straw, 86.3% of the TRR (3.724 mg eq/kg) in the hulls and 88.3% (0.230 mg eq/kg) of the TRR in the kernels. Besides parent three further metabolites were identified :fluopyram-7 hydroxy, fluopyram-8 hydroxy and fluopyram-pyridyl-carboxylic acid. All three accounted for ≤9.8% of

the TRR or ≤0.455 mg eq/kg in rice straw, <1% TRR and <0.05 mg eq/kg in hull and <5% TRR (<0.01 mg eq/kg) in kernel. The largest at 9.8% of the TRR was fluopyram-8 hydroxy and was seen in the rice straw. Up to eleven additional minor metabolites (each ≤ 1% of the TRR) were characterised based on the extraction procedure, partitioning behaviour and retention time. In total 89.6% of the TRR in the straw, 88.2% of the TRR in the hulls and 92.7% of the TRR in the kernels was identified.

The following two metabolic reactions occurred in the plants:

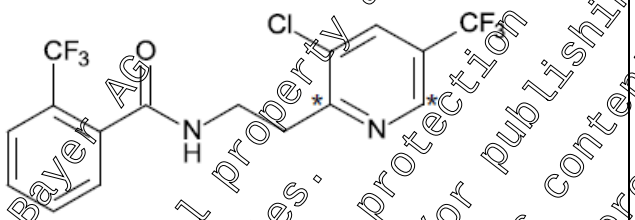
- hydroxylation of the ethyl linking group of the parent compound forming 7- and 8- hydroxy metabolites.
- cleavage of fluopyram-7-hydroxy followed by oxidation leading to formation of formation of fluopyram-pyridyl-carboxylic acid.

Based on these results, the metabolism of [pyridyl-2,6-^{14}C]fluopyram in paddy rice after foliar treatment can be considered as well understood and a metabolic pathway proposed. This metabolism study successfully fulfils all the requirements of OECD test guideline 501 (2007).

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	 *position of the radiolabel
Radiolabelled test material	[pyridyl-2,6-^{14}C]fluopyram
Specific radioactivity	3.64 MBq/mg
Radiochemical purity	> 99%
Batch	KML 10174

2. Soil: "Monheim 4" (sandy loam from Germany), pH ($CaCl_2$) = 6.7, 72% sand, 18% silt and 10% clay, 1.1% organic carbon, cation exchange capacity (CEC) of 8.5 meq/100 g.

3. Plant: Paddy rice, variety: "*Balilla*"

B. Study Design

1. Experimental conditions:

The paddy rice was cultivated under artificial temperature and light conditions in a greenhouse at the test facility. Plants were pre-grown in a Japanese nursey box. The pre-grown seedlings were transplanted into a plant container (surface of 0.5 m^2) filled with a sandy loam soil in eleven planting holes. Each planting hole contained four seedlings. Water was then added to the plant container to a level of *ca.* 2 cm above the soil. Evaporated water was regularly renewed during cultivation until 11 days before harvest (the last irrigation was performed on the 20th March 2017). Hence, 88 plants were sprayed with [pyridyl-2,6-^{14}C]fluopyram formulated as an SC 400 with a hand pump sprayer. The envisaged use pattern at initiation of the study included two spray applications, each 110 g a.s./ha, resulting in a maximum annual field rate of 220 g a.s./ha. The first field application was proposed at flag leaf sheath open (growth stage BBCH 49; 9th February 2017) and the second application at between full flowering and fruit development - early milk (growth stage BBCH 65-73; 28th February 2017). The metabolism study simulated the envisaged use pattern according to agricultural practice and were based on the maximum proposed application rate. The time interval between the first and the second application was 19 days.

All experiments were performed in the greenhouse with a day/night rhythm of 14/10 hours and an average temperature of 25°C (day) and 20°C (night) and a relative humidity of 80%.

2. Sampling

The rice plants were cut off just above the soil surface at BBCH growth stage 89-92 (30th March 2017), 30 days after the last treatment and dried for 4 days. After four days of drying in the greenhouse the grains were separated from the panicles, and the empty panicles added to the straw sample. The straw was cut into 1-2 cm lengths and an aliquot homogenised with liquid nitrogen in a highspeed blender. The rice grain was passed through a rice husker to remove the hulls and an aliquot of the hulls homogenised with liquid nitrogen using a polytron homogeniser. The husked grains (kernels) were again homogenised with liquid nitrogen using a polytron homogeniser. All samples stored at ≤ -18 °C until analysed.

C. Analytical Procedures

Straw, rice hulls and kernels were extracted and the extracts were analysed by HPLC and TLC.

1. Extraction and fractionation:

An aliquot of the homogenised rice straw was soaked in acetonitrile/water (8:2, v/v) before being extracted using a high-speed blender. The extraction was repeated using acetonitrile/water (8:2, v/v) twice more and then finally with acetonitrile/water (1:1, v/v). The suspensions were vacuum filtered and the four acetonitrile/water (8:2, v/v) extracts combined and purified by SPE. The combined acetonitrile/water extract was passed through a pre-conditioned C18 solid phase extraction cartridge. The cartridge was then rinsed with an aliquot of acetonitrile and eluted with a mixture of methanol and THF. The resultant percolate and rinse fractions were then combined subsequently concentrated by rotary evaporation and analysed by HPLC.

An aliquot of the homogenised rice hulls was treated in a similar manner to the straw. The hulls were soaked in acetonitrile/water (8:2, v/v) before being extracted using a high-speed blender. The extraction was repeated using acetonitrile/water (8:2, v/v) twice more and then finally with acetonitrile/water (1:1, v/v). The suspensions were vacuum filtered and the three acetonitrile/water (8:2, v/v) extracts combined and purified by SPE. The acetonitrile/water extract was passed through a pre-conditioned C18 solid phase extraction cartridge. The cartridge was then rinsed with an aliquot of acetonitrile and eluted with a mixture of methanol and THF. The resultant percolate and rinse fractions were then mixed with the fourth extract (acetonitrile/water (1:1, v/v) subsequently concentrated by rotary evaporation and analysed by HPLC.

An aliquot of the homogenised rice kernels was soaked in acetonitrile/water (8:2, v/v) before being extracted using a high-speed blender. The extraction was repeated using acetonitrile/water (8:2, v/v) three further times. The suspensions were vacuum filtered and the four acetonitrile/water (8:2, v/v) extracts combined and purified by SPE. The acetonitrile/water extract was passed through a pre-conditioned C18 solid phase extraction cartridge. The cartridge was then rinsed with an aliquot of acetonitrile and eluted with a mixture of methanol and THF. The resultant percolate and rinse fractions were concentrated by rotary evaporation and analysed by HPLC.

The radioactivity in liquid samples was determined by liquid scintillation counting (LSC). Solid samples were combusted. The CO_2 produced by combustion was absorbed in a CO_2 absorbent/ scintillation cocktail mixture and the radioactivity was measured by LSC.

The total radioactive residue (TRR) was determined by summation of the radioactivity of the combined extract(s) and of the remaining solids. The TRR was expressed in mg a.s. equivalents per kg sample weight. Amounts of radioactive residues in the extracts were expressed as percentage of the TRR and also as mg a.s. equivalents per kg sample weight.

2. Identification and characterisation:

For elucidation of metabolism, extracts and phases were analysed by HPLC and/or TLC with radio detection. Metabolites were identified by isolation of peaks followed by co-chromatography with authentic reference compounds using two independent chromatographic methods with different selectivity (e.g. HPLC and TLC).

3. Storage stability:

All extraction experiments for metabolism investigations and the first HPLC analyses were performed within two months after sampling of the individual rice samples. The conventional extracts were analysed by HPLC on the day of the extraction or the day after. It is 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 at harvest. All samples were at ≤ -18 °C.

II. Results and Discussion

The metabolism of [pyridyl-2,6-^{14}C]fluopyram formulated as an SC 400 was investigated in paddy rice following two foliar spray applications, at single rates of 123.3 and 126.3g a.s./ha and a total rate of 249.6 g a.s./ha.

The TRR values in straw and hulls were high amounting to 4.626 mg eq/kg and 4.316 mg eq/kg (Table 6.2.1-38). Significantly lower residues were observed in the edible RAC (raw agricultural commodity), rice kernels (0.261 mg eq/kg), due to protection from the hulls.

Table 6.2.1-38: TRR values in paddy rice matrices after application of [pyridyl-2,6-^{14}C]fluopyram

Matrix	Timing and Application	Growth stage at harvest	PHI (days)	TRR (mg a.s. equiv./kg)*
Straw	1st foliar treatment at growth stage BBCH 49, 123.3 g a.s./ha 2nd foliar treatment at growth stage BBCH 65-73 with 126.3 g a.s./ha	BBCH 88-92	30	4.626
Hulls		BBCH 88-92	30	4.316
Kernels		BBCH 88-92	30	0.261

PHI = pre-harvest interval, * the TRR values were determined by summing up the radioactivity measured in the extract and in the remaining solids

The straw, hulls and kernels were extracted at least three times with mixtures of acetonitrile/water (8/2, v/v), the concentrated extracts were analysed by HPLC and TLC and parent compound and metabolites were identified.

From rice straw, a portion of 93.9% of the TRR (4.344 mg eq/kg) was extractable with acetonitrile/water (Table 6.2.1-39) and 6.1% of the TRR (0.282 mg eq/kg) remained in the post extraction solids. From rice hulls, 88.5% of the TRR (3.819 mg eq/kg) was extractable with acetonitrile/water, 11.5% of the TRR (0.497 mg eq/kg) remained in the post extraction solids. Acetonitrile/water extracted 92.7% of the TRR (0.242 mg/kg) from the rice kernels leaving 7.3% of the TRR (0.019 mg eq/kg) in the post extraction solids (PES).

Virtually no radioactivity was lost during concentration of the extracts <0.3% (0.013 mg eq/kg). Only a small amount of radioactivity <0.2% (0.009 mg eq/kg) of the TRR was detected in the methanol/THF eluates of the SPE purification procedures from the rice straw and hulls.

Table 6.2.1-39: Distribution of radioactivity in the extracts of the paddy rice matrices after foliar spray application of [pyridyl-2,6-^{14}C]fluopyram

	straw		hulls		kernels	
TRR [mg/kg] =	4.626		4.316		0.261	
	% of TRR	mg a.s. equiv./kg	% of TRR	mg a.s. equiv./kg	% of TRR	mg a.s. equiv./kg
Acetonitrile/water extract	93.9	4.344	88.5	3.819	92.7	0.242
Concentrate used for quantitation of metabolites	93.4	4.321	88.3	3.813	92.7	0.242
Not analysed fraction (methanol / THF)	0.2	0.009	0.1	0.0064	-	-
Not analysed fraction (condensate)	<0.1	0.001	-	-	-	-
Not analysed fraction (distillate)	0.3	0.013	0.1	0.003	-	-
Total extracted	93.9	4.344	88.5	3.819	92.7	0.242
Post extraction solids (PES)	6.1	0.282	11.5	0.497	7.3	0.019
Accountability	100.0	4.626	100.0	4.316	100.0	0.261

The radioactive residues in the acetonitrile/water extract from the paddy rice straw (Table 6.2.1-40), mainly consisted of parent compound amounting to 76.2% (3.525 mg eq/kg) of the TRR. Besides parent a further three metabolites were identified as fluopyram-7 hydroxy, fluopyram-8 hydroxy and fluopyram-pyridyl-carboxylic acid. All were minor accounting for less than ≤9.8% of the TRR. Ten additional minor metabolites (each < 1% of the TRR) were characterised based on the extraction procedure, partitioning behaviour and retention time. In total 89.6% (4.142 mg eq/kg) of the TRR in the rice straw was identified and 4.3% (0.1201 mg eq/kg) of the TRR characterised based on the extraction and chromatographic behaviour.

In the rice hulls, 86.3% of the TRR (3.724 mg eq/kg) was parent compound. As with the rice straw the three minor metabolites fluopyram-7 hydroxy, fluopyram-8 hydroxy and fluopyram-pyridyl-carboxylic acid were all identified with each accounting for ≤0.8% (≤0.033 mg eq/kg) of the TRR. One additional very minor metabolite at 0.1% (0.005 mg eq/kg) of the TRR were characterised based on the extraction procedure, partitioning behaviour and retention time. In total 88.2% (3.808 mg eq/kg) of the TRR in the rice straw was identified and 0.3% (0.012 mg eq/kg) of the TRR characterised based on the extraction and chromatographic behaviour.

In the edible RAC rice kernels parent compound was the main component amounting to 88.3% (0.230 mg eq/kg) of the TRR. Besides parent the three metabolites fluopyram-7 hydroxy, fluopyram-8 hydroxy and fluopyram-pyridyl-carboxylic acid were all identified each accounting for ≤2.8% (≤0.007 mg eq/kg) of the TRR. In total 92.7% (0.242 mg eq/kg) of the TRR in the kernels was identified and unextractable residues constituted 7.3% (0.019 mg eq/kg) of the TRR.

Table 6.2.1-40: Distribution of parent compound and metabolites in the extracts of paddy rice matrices after foliar spray application of [pyridyl-2,6-^{14}C]fluopyram

	straw		hulls		kernels	
TRR [mg/kg] =	4.626		4.316		0.216	
Compound	% of TRR	mg a.s. equiv./kg	% of TRR	mg a.s. equiv./kg	% of TRR	mg a.s. equiv./kg
Acetonitrile/water extract						
AE C656948 (fluopyram)	76.2	3.525	86.3	3.724	88.3	0.230
fluopyram-pyridyl-carboxylic acid (M43)	1.3	0.058	0.4	0.019	2.8	0.007
fluopyram-7-hydroxy (M08)	2.3	0.105	0.8	0.033	0.9	0.002
fluopyram-8-hydroxy (M18)	9.8	0.455	0.7	0.031	0.7	0.002
Total identified	**89.6**	**4.142**	**88.2**	**3.808**	**92.7**	**0.242**
Unknown 1	0.3	0.013	-	-	-	-
Unknown 2	0.3	0.016	-	-	-	-
Unknown 3	0.2	-	0.2	0.005	-	-
Unknown 4	0.2	0.012	-	-	-	-
Unknown 5	0.9	0.040	-	-	-	-
Unknown 6	0.1	0.005	-	-	-	-
Unknown 7	0.3	0.012	-	-	-	-
Unknown 8	0.3	0.012	-	-	-	-
Unknown 9	0.4	0.020	-	-	-	-
Unknown 10	0.6	0.026	-	-	-	-
Unknown 11	0.5	0.022	-	-	-	-
Sum of unknowns (characterised by HPLC)	**3.9**	**0.178**	**0.1**	**0.005**	**-**	**-**
Not analysed fractions (total)	0.5	0.023	0.1	0.006	-	-
- not analysed fraction (methanol / THF)	0.2	0.009	0.1	0.004	-	-
- not analysed fraction (condensate)	<0.1	0.001	-	-	-	-
- not analysed fraction (distillate)	0.3	0.013	0.1	0.003	-	-
Total characterised (HPLC and not analysed)	**4.3**	**0.201**	**0.3**	**0.012**	**-**	**-**
Total extracted	**93.9**	**4.344**	**88.5**	**3.819**	**92.7**	**0.242**
Unextractable (PES)	6.1	0.282	11.5	0.497	7.3	0.019
Accountability	100.0	4.626	100.0	4.316	100.0	0.261

Peak assignments (M1, 2, 4, 11, 16, 20 and 21) were not assigned in this study

III. Conclusions

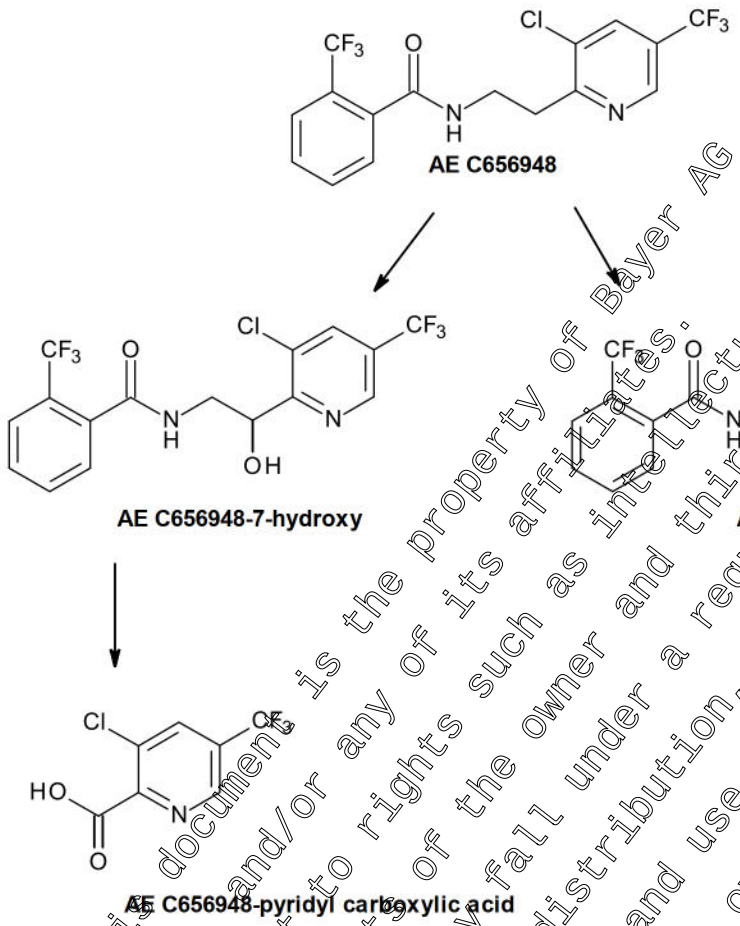
After foliar spray application of [pyridyl-2,6-^{14}C]fluopyram, the highest residues were in paddy rice straw (4.626 mg eq/kg) and hulls (4.316 mg eq/kg) and mainly consisted of parent compound, amounting to 76.2% and 86.3% of the TRR respectively due to the direct exposure from foliar treatment. The TRR found in the edible RAC kernels was relatively low and amounted to 0.261 mg eq/kg and again consisted of mainly parent compound at 88.3% of the TRR. Three further identified metabolites fluopyram-pyridyl-carboxylic, fluopyram-7 hydroxy and fluopyram-8 hydroxy accounting for less than ≤9.8% of the TRR. Up to eleven additional unknown metabolites were characterised by HPLC but each represented <1% of the TRR in all plant parts.

Two metabolic reactions occurred in the plants :

- hydroxylation of the ethyl linking group of the parent compound forming 7- and 8- hydroxy metabolites.
- cleavage of fluopyram-7-hydroxy followed by oxidation leading to formation of formation of fluopyram-pyridyl-carboxylic acid.

Based on these results, the metabolism of [pyridyl-2,6-^{14}C]fluopyram in paddy rice after foliar treatment can be considered as well understood and a metabolic pathway is proposed in Figure 6.2.1-11.

Figure 6.2.1-11: Proposed metabolic pathway of [pyridyl-2,6-^{14}C]fluopyram in paddy rice



Assessment and conclusion by applicant:

The study is valid and acceptable.

Data Point:	KCA 6.2.1/13
Report Author:	[redacted]
Report Year:	2016
Report Title:	Metabolic and dynamic profiling for risk assessment of fluopyram, a typical phenylamide fungicide widely applied in vegetable ecosystem
Report No:	M-763231-01-1
Document No:	M-763231-01-1
Guideline(s) followed in study:	not applicable
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

This is an article from the literature. Although it is not relevant for risk assessment, it is summarised here as supportive information.

Executive Summary

Fluopyram, a typical phenylamide fungicide, was widely applied to protect fruit vegetables from fungal pathogens-responsible yield loss. A modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) extraction combined with GC-MS/MS analysis was developed to investigate fluopyram fate in the typical fruit vegetables including tomato, cucumber, pepper under the greenhouse environment. Fluopyram dissipated in accordance with the first-order rate dynamics equation with the maximum half-life of 5.7 d. Cleavage of fluopyram into fluopyram-benzamide (2-trifluoromethyl benzamide in article) and subsequent formation of fluopyram-pyridyl-acetic acid (3-chloro-5-(trifluoromethyl) pyridine-2-acetic acid in article) and fluopyram-pyridyl-carboxylic acid (3-chloro-5-(trifluoromethyl) picolinic acid in article) was elucidated to be its ubiquitous metabolic pathway. Moreover, the occurrence of fluopyram at the pre-harvest interval (PHI) of 7–20 d was between 0.0108 and 0.1603 mg/kg, and the Hazard Quotients (HQs) were calculated to be less than 1, indicating temporary safety on consumption of the fruit vegetables incurred with fluopyram, irrespective of the uncertain toxicity of the metabolites.

I. Materials and Methods

Chemicals and reagents

Fluopyram (purity 99.4%) was purchased from Dr. Ehrenstorfer (Augsburg, Germany). Fluopyram&Trifloxystrobin SC (FLU+TFS, 500 g/L) used for field trial as well as acetonitrile (ACN, HPLC grade), ethyl acetate (EtOAc, GC grade) and other organic solvent, magnesium sulfate, sodium acetate and PSA (primary secondary amine) were purchased from commercial suppliers.

Soil characteristics: 50% sand, 17% clay, 31% silt, 2% organic matter, pH 6.1, classified as sandy loam.

Plants: tomato (*Lycopersicon esculentum* cv. Dahongyihao), cucumber (*Cucumis sativus L.* cv.

Shandongmici) and pepper (*Capsicum annuum L.* cv. Qingjiaowang).

Study design and sample collection

In 2015, a field trial was conducted in fruit vegetables (tomato, cucumber and pepper) in greenhouses in China (Institute of Pesticide and Environmental Toxicology, Zhejiang University, Jiangsu Province, China). For initial deposition, dynamics and metabolism, FLU+TFS SC was sprayed at rate of 62.4 g a.s./ha (2xGAP obtained from Institute for the Control of Agrochemicals, Ministry of Agriculture, China) when the first fruit on the main stem reached the typical size and form. Edible parts and soils samples were taken at 2 h, 1, 3, 7, 14, 21 and 28 days after last application. For monitoring of the occurrence, FLU+TFS SC was sprayed three times at a rate of 31.2 g a.s./ha (1xGAP) with an interval of 7 days. Mature edible part samples were collected at pre-harvest interval of 7, 14 and 21 days after the last application.
Plant samples were stored in a freezer after quartering and homogenized into the sample vial. Soil samples were cleaned and screened. All field samples were stored in a freezer at − 20 °C until analysis.
All processes and operations in the supervised trials were carried out per Good Agricultural Practices (GAPs) issued by the Institute for the Control of Agrochemicals, Ministry of Agriculture, China.

Sample extraction

The samples were analysed for fluopyram using a modified QuEChERS method. A homogenized vegetable sample (20 g or 10 g of soil sample) was mixed in a 250 mL-centrifuge bottle with 50 mL ACN and 10 mL deionized water. The mixture was shaken for 30 min and then centrifugated. Afterwards, an aliquot of the extract (25 mL) was transferred into a 50-mL centrifuge tube. After the addition of 6 g magnesium sulfate and 1.5 g sodium acetate, each mixture was shaken vigorously for 1 min and then centrifuged. The supernatant (25 mL) was concentrated. The resulting concentrates were re-dissolved with ethyl acetate (2 mL) and pipetted into a 2 mL-clean up tube filled with 50 mg PSA and 150 mg magnesium sulfate. After shaking vigorously for 30 s, the tube was centrifuged. After filtration through a 0.22 µ m filter, the resulting filtrates were subjected to quantitative analysis of fluopyram.

Quantification of fluopyram

For quantification of fluopyram, an Agilent HP capillary column (Agilent HP-5 MS) was installed in a GC-MS/MS (Agilent 7000 C). MS/MS was operated in electron ionization mode with a mass range of *m/z* 50~500. Multiple reaction monitoring (MRM) transitions were 173.0 > 145.4 and 173.0 > 95.2. Series of dilutions of fluopyram in EtOAc spiked at the concentrations 0.001, 0.01, 0.1, 1 and 5 mg/kg in the matrices were used as the working solutions for quantification of the fluopyram using external standard method.

Validation was carried out using control matrix tomato, cucumber and pepper fruits as well as control soil samples fortified with fluopyram at 0.001, 0.05 and 0.5 mg/kg with 5 replicates. Recovery was calculated for evaluation of the method performance. Spiked samples were left to stand for 60 min to allow pesticide absorption onto the sample adequately and then subjected to treatment and analysis under the same conditions as described above. For reliability, RSDR (intra-day precision) was measured by comparing standard deviation of the recoveries of five replicates in the same day, and RSDR (inter-day precision) was determined by analyzing spiked samples in three alternate days for reproducibility. Intra- and inter-day precisions tests were also done at 0.001, 0.05 and 0.5 mg/kg with nine replicates on 3 different days.

Dynamics fitting.

The dynamics of fluopyram in various samples was analysed by plotting the residue concentration against time via the first-order rate equation: $C_t = C_0 x e^{-kt}$. C_t and C_0 represent the concentrations of the residual fluopyram at the day t and day 0 (2h), respectively, and k is the dissipation rate constant. The half-life ($t_{1/2}$)

is defined as the time required for the pesticide residue level to fall to the half of the initial residual level of day 0 (i.e., C_0) and calculation was done using the following equation: $t_{1/2} = (\ln 2)/k$.

Track of fluopyram metabolism

Samples collected from multiple application trial were subjected to GC-MS/MS using the full scan mode. In the total ion chromatogram (TIC) peaks of interest were further isolated for characterization of the molecular structure. The parent ions of the tentative metabolites were selected and further subjected to MS/MS using daughter scan mode for characteristic daughter ion fragmentation. Finally, identification of the metabolites' structure was done by parent ion deconvolution and the daughter ion fragmentation assignment through the Agilent Mass-hunter Library NIST11.L database. The same analytical method as for fluopyram was used for extraction and purification of the metabolites. For each metabolite, a pair of quantitative ions were selected for quantification with the use of p-tert-butylphenol as an internal standard.

Dietary risk assessment.

For assessment of the dietary risk of fluopyram, the estimated daily intake (EDI) as a percentage of acceptable daily intake (ADI) for a 60-kg adult person is 0.01 mg/kg body weight (bw)/day for fluopyram. EDI was calculated by multiplying the highest residue (HR) in each sample (mg/kg) with the average daily per capita consumption estimated for fruit vegetables (58.291 g/day in China). The risk assessment of intakes compared to pesticide toxicological data was conducted via calculation of the hazard quotient (HQ), where EDI was divided by the relevant ADI (HQ = EDI/ADI × 100%).

II. Results and Discussion

Analytical approach performance

The matrix-matched calibration was done by the standard addition method in matrix extracts and the calibration curve of fluopyram was constructed by plotting analyte concentration against peak area. In the range of concentrations (from 1 to 5000 µg/kg), good linearity was achieved for the target compound with correlation coefficients higher than 0.99. The LOQ was 0.1 µg/kg in all matrixes. Using the modified QuEChERS, spiked recoveries for tomato, cucumber, pepper and soil sample matrices at all spiked levels were obtained as 90.5–95.6%, 92.0–93.2%, 89.0–95.4% and 88.5–95.1%, respectively with all RSD values < 20% (Table 6.2.1-41 A). Besides, the intra-day and inter-day variability were lower than 10.7 and 13.5%, respectively, at all spiked level (Table 6.2.1-41 B). These data suggested the method established was of favorable performance and reliability.

Table 6.2.1-41: Validation parameters of fluopyram (A). with various matrices and levels and (B). with intra-day and inter-day variability

A	Tomato		Cucumber		Pepper		Soil	
Spiking level (mg/kg)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
0.001/0.005*	90.5 ± 7.8	8.6	92.0 ± 2.7	3.0	89.0 ± 2.3	2.5	90.7 ± 8.6	9.5
0.01/0.05	95.6 ± 3.5	3.7	92.2 ± 2.3	2.5	89.5 ± 2.4	2.6	88.5 ± 4.0	4.6
0.5/1	91.9 ± 4.3	4.7	93.2 ± 3.6	3.9	95.4 ± 4.3	4.5	95.1 ± 2.2	2.3

*0.001/0.005 expresses the fortified level of three vegetables/the fortified level of soil (n = 6).

D	Spiking 0.001/0.005* mg/kg		Spiking 0.01/0.05 mg/kg		Spiking 0.5 /1 mg/kg	
RSD_R (%)	Intra-day[a]	Inter-day[b]	Intra-day[a]	Inter-day[b]	Intra-day[a]	Inter-day[b]
Tomato	5.9	9.7	6.1	10.2	4.1	8.5
Cucumber	4.6	8.5	6.7	13.3	7.0	7.9
Pepper	6.2	11.9	4.8	9.1	7.2	6.3
Soil	5.3	10.6	3.6	8.8	10.7	5.1

*0.001/0.005 expresses the fortified level of three vegetables/the fortified level of soil (a n = 3, b n = 9).

Initial deposition and dynamics of fluopyram

In edible parts samples collected from greenhouse plants after the foliar application, initial deposits of fluopyram followed the decreasing order of pepper > tomato > cucumber. The initial deposit of fluopyram was 1.913 mg/kg in pepper, while the initial deposits in tomato and cucumber were 1.391 mg/kg and 1.172 mg/kg, respectively. Compared with the edible part of vegetables, almost 2-fold lower initial deposits of fluopyram were observed in the related greenhouse soil, which were 0.637, 0.738 and 0.872 mg/kg in tomato soil, cucumber soil and pepper soil. The dynamics curves demonstrated that the residual fluopyram dissipated rapidly within 7 days (Figure 6.2.1-12). Half-lives of fluopyram were similar in the three vegetables (3.8 d in pepper, 3.9 d in cucumber and 4.4 d in tomato, Table 6.2.1-42). In soils, fluopyram declined constantly with half-lives of 4.2, 5.7 and 4.3 d for tomato, cucumber and pepper, respectively.

Table 6.2.1-42: Dynamic equations, correlation coefficients and half-lives of fluopyram in vegetables and soils.

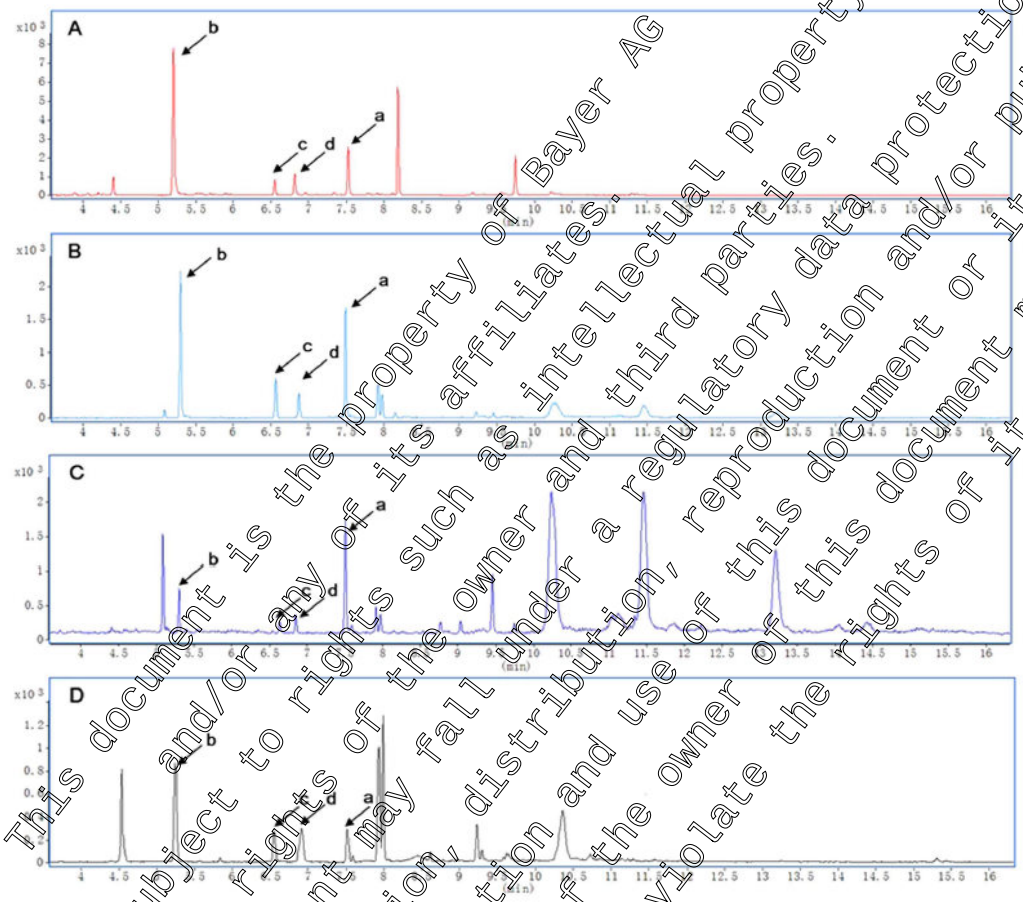
Sample	Dynamic equation	Correlation coefficient (R^2)	Half-live(d)
Tomato	$C_t = 1.532e^{-0.158t}$	0.8799	4.4
Cucumber	$C_t = 1.165e^{-0.177t}$	0.8820	3.9
Pepper	$C_t = 2.368e^{-0.181t}$	0.9056	3.8
Tomato soil	$C_t = 3.696e^{-0.16t}$	0.9619	4.2
Cucumber soil	$C_t = 0.814e^{-0.121t}$	0.8789	5.7
Pepper soil	$C_t = 1.102e^{-0.163t}$	0.8964	4.3

Metabolic pathway of fluopyram

Using full scan mode of GC-MS/MS, a wide array of peaks was observed in the different samples (see figure below). Several peaks not observed in the control group, increased regularly along with the decline

of fluopyram. These relevant peaks were further selected and subjected to GC-MS/MS analysis per characteristic fragmentation and assigned by Agilent Mass-hunter Library NIST11.L database.

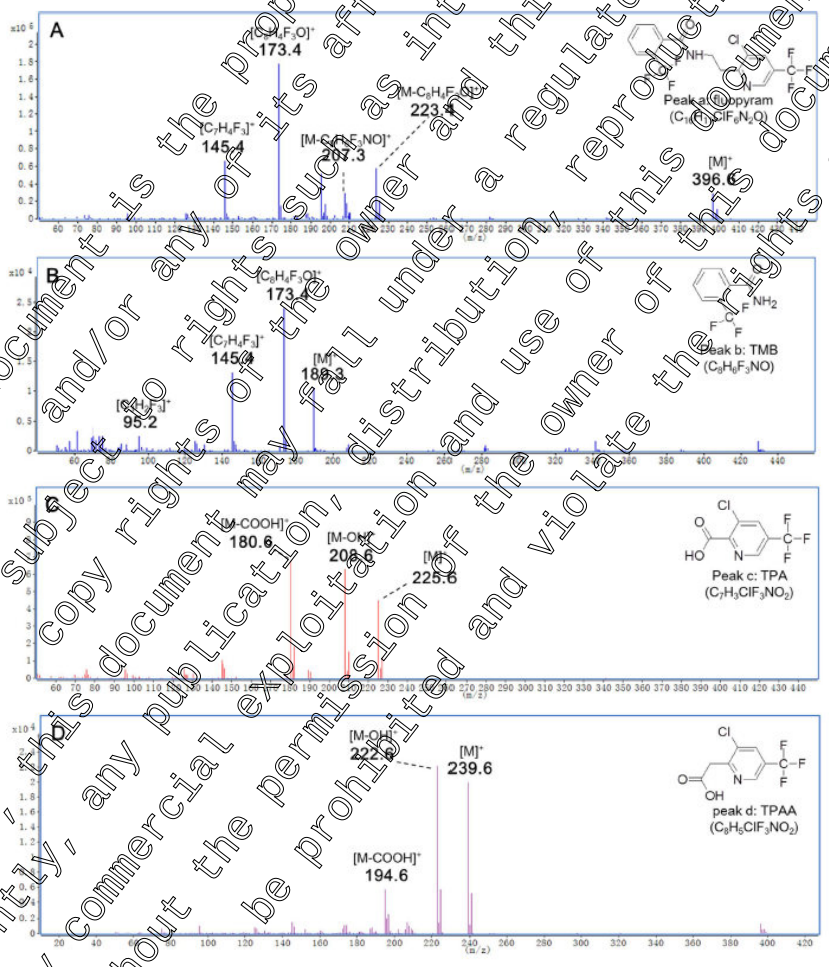
Figure 6.2.1-12: TIC of different samples obtained from GC-MS/MS using full scan mode in tomato (A), cucumber (B), pepper (C) and greenhouse soil (D).



Three peaks were identified as fluopyram metabolites (Figure 6.2.1-13). The mass fragments of *m/z* 173.4 and 145.4 existed in both of peak a and b, indicating that they had identical functional groups (Figure 6.2.1-13, A, B), suggesting peak b originated from peak a. Parallel to the absence of *m/z* 396.6 and *m/z* 207.3 peaks a peak at *m/z* 189.3 emerged (Figure 6.2.1-13B), which showed the loss of a moiety of *m/z* 207.3 from *m/z* 396.6. A cleavage of fluopyram into a trifluoromethyl benzamide molecule (peak b) and a trifluoromethyl pyridine-containing molecule occurred. Peak b was further identified as fluopyram-benzamide via the characteristic fragmentation mentioned above and database deconvolution (Figure 6.2.1-13B). Interestingly, the trifluoromethyl pyridine-containing molecule speculated as 2-(3-chloro-5-(trifluoromethyl)pyridin-2-yl)ethanol (TPE) was not observed in all samples collected, while the peak c and d identified as fluopyram-pyridine-carboxylic acid (Figure 6.2.1-13C) and fluopyram-pyridine-acetic acid Figure 6.2.1-13D) seemed to be degradation products of TPE.

Figure 6.2.1-13: Fragmentations of fluopyram (A) and three metabolites fluopyram-benzamide (B), fluopyram-pyridine-carboxylic acid (C) and fluopyram-pyridine-acetic acid (D)

Based on the qualitative and quantitative analyses mentioned above, it was deduced that the parent molecule was split into TMB and potentially into TPE in the initial step of fluopyram metabolic pathway. Interestingly, we found that fluopyram was degraded in the sterilized soil with a 40-fold higher half-life than the field trial, and neither fluopyram-benzamide nor TPE was detected (data not shown). It was considered likely that microbial enzymatic catalysis in the vegetable ecosystem was responsible for this cleavage reaction at the single carbon-nitrogen bond of fluopyram. The missing link converting fluopyram to fluopyram-pyridine-acetic acid mediated by TPE could be explained by the rapid oxidative transformation of TPE to fluopyram-pyridine-acetic acid, which was further decarboxylated and oxidized to generate fluopyram-pyridine-carboxylic acid. Cleavage of the molecule fluopyram to produce fluopyram-benzamide, fluopyram-pyridine-carboxylic acid and fluopyram-pyridine-acetic acid was the ubiquitous metabolic pathway of fluopyram in the three vegetables, among which fluopyram-benzamide was the primary metabolite whereas fluopyram-pyridine-carboxylic acid and fluopyram-pyridine-acetic

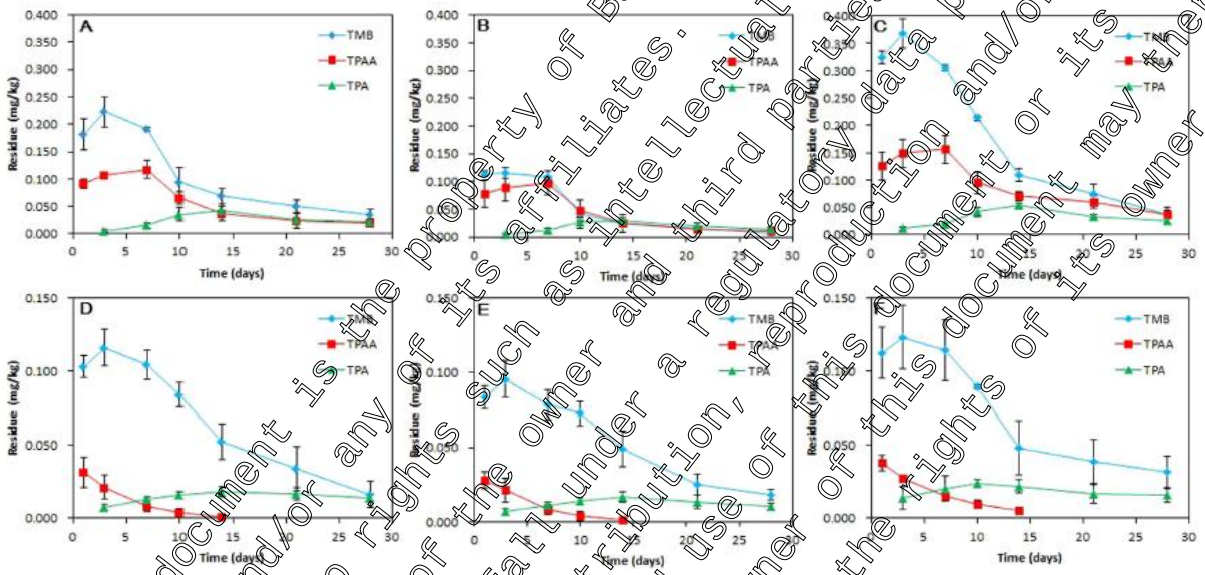


acid were secondary metabolites.

For the further elucidation of the metabolic pathway of fluopyram, the distribution and formation of these metabolites was also quantitatively analysed in plant and soil samples. fluopyram-benzamide began to occur at 1 d and then gradually dissipated from 3 d to 28 d in both plant and soil samples, and finally stayed

at level of 0.008 - 0.036 mg/kg (Figure 6.2.1-14). Similar to fluopyram-benzamide, the dynamics of fluopyram-pyridine-acetic acid showed an increasing tendency from 1d to 7 d in all vegetables followed by a constant decline until 28 d. In the soils, fluopyram-pyridine-acetic acid exhibited a constant decline curve and became undetectable (< 0.001 mg/kg) at 14 d. Highly associated with fluopyram-pyridine-acetic acid, fluopyram-pyridine-carboxylic acid occurred when fluopyram-pyridine-acetic acid significantly declined within 14 d, followed by a slight decline stage and finally stayed at a relatively stable level until 28 d.

Figure 6.2.1-14: Distribution and formation dynamics of fluopyram metabolites in the fruit vegetable ecosystem in tomato (A), cucumber (B), pepper (C), tomato soil (D), cucumber soil (E) and pepper soil (F). Values are means ± standard deviations (shown by error bars) (n = 3).



Dietary risk of fluopyram

According to the calculation of hazard quotient (HQ) based on HR value (Table 6.2.1-43) and GEMS/Food consumption database (fruit vegetable consumption in China), HQs in three fruit vegetables were all lower than 1, indicating safety of the fruit vegetable incurred with residues of this fungicide to the consumers by daily consumption. However, it was found that the edible parts of three vegetables were incurred with fluopyram-benzamide, fluopyram-pyridine-carboxylic acid and fluopyram-pyridine-acetic acid at trace level. It still remained unclear whether the three metabolites of fluopyram should be considered in the dietary risk assessment due to the unknown toxicological effect of these metabolites.

Table 6.2.1-43: Occurrence, HR and HQ of fluopyram in edible parts of the three fruit vegetables.

Sample	Crop group*	Incurrence (mg/kg)			HR (mg/kg)	HQ
		PHI 7 d	PHI 14 d	PHI 21 d		
Tomato	8–10B	0.0769	0.0331	0.0058	0.0769	0.4483
Cucumber	8–10B	0.0571	0.0303	0.0043	0.0571	0.3328
Pepper	8–10B	0.1603	0.0861	0.0108	0.1603	0.9344

* Code of Federal Regulations Group 8–10B and 8–10C indicate the fruit vegetable group.

III. Conclusions

In this study, an effective and sensitive analytical method using a modified QuEChERS extraction with GC-MS/MS for the detection of fluopyram in three fruit vegetables (tomato, cucumber, pepper) and relevant soils was developed and validated. After a single application at 62.4 g a.s./ha, fluopyram dissipated rapidly in the vegetable greenhouse ecosystem in accordance with the first-order rate dynamics with the maximum half-life as 5.7 d. Fluopyram was split into a primary metabolite fluopyram-benzamide and followed by formation of two secondary metabolites fluopyram-pyridine-acetic acid and fluopyram-pyridine-carboxylic acid along with the decline process. After the multi-application, the occurrence of fluopyram in tomato, cucumber and pepper at PHI 7–21 d ranged from 0.0108 to 0.1603 mg/kg, and all the related HQs were below 1. Taken together, a highly compatible tool to monitor fluopyram in plant- and environmental origin is provided.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The first purpose of the publication was to describe and discuss the performance of a modified QuEChERs analytical method for fluopyram in food of plant origin and soil. Based on the provided validation results, the method is considered reliable, however, a full data package on valid methods for data generation and monitoring is already available and provided with the dossier.

The second purpose of the publication was the identification of possible metabolites of fluopyram in the matrices of tomato, cucumber and pepper fruits as well as in soil. Metabolites such as fluopyram-benzamide, fluopyram-pyridine-acetic acid and fluopyram-pyridine-carboxylic acid have been identified. However, these metabolites have been already described in studies submitted in this AIR dossier and no new information is gained. Therefore, as such, the publication is relevant but do not affect risk assessment.

CA 6.2.2 Poultry

Data to address this point were presented in the dossier submitted for first inclusion in Annex and were deemed acceptable following evaluation and peer review at EU level (2013).

For details of data submitted previously please refer also to the Baseline dossier CA 6.2. For completeness, a summary of these previously submitted studies are included below.

Data already evaluated during the first EU review process for inclusion on Annex I.(no new studies)

Data Point:	KCA 6.2.2/01
Report Author:	[redacted]
Report Year:	2008
Report Title:	Metabolism of [phenyl-UL-14C]AE C656948 in the laying hen
Report No:	MEF-06/329
Document No:	M-297093-01-1
Guideline(s) followed in study:	US EPA OPPTS 860.1300; Health Canada PMRA Ref.: DACO 6.2; EU 91/414/EEC, amended by 96/68/EC, Appendix F
Deviations from current test guideline:	Deviation to OECD 503: 6 birds instead of 10. No impact, because sufficient amount of sample material was available for characterization and identification of metabolites.
Previous evaluation:	yes, evaluated and accepted rev. 1 to Vol.3 of DAR 07 August 2012 (references relied on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The metabolism of [phenyl-UL-^{14}C]fluopyram was investigated in six laying hens, which where orally dosed for 14 consecutive days at a rate of 2.03 mg a.s./kg of body weight per day (equivalent to 26.42 mg a.s./kg feed/day). Sacrifice was made 24 h after the 14th administration. Radioactivity was measured in the excreta and eggs collected daily, as well as in the kidney, liver, eggs from the ovary and oviduct, skin without subcutaneous fat, muscle and fat at sacrifice. The collected eggs and the edible tissues and organs were analysed for parent compound and metabolites by extraction, chromatographic separation techniques and spectroscopic methods.

Until sacrifice, the excretion amounted to 82.67% of the totally administered radioactivity. An amount of 4.34% of the total dose was found in eggs. At sacrifice, the calculated or estimated total residue in the tissues and organs dissected from the body was approx. 7.83% of the total dose. More than half of this amount was detected in the muscle (4.94%).

The mean active substance equivalent concentrations in eggs increased from 0.462 mg/kg (day 1) to 3.901 mg/kg (day 14). A further but slower increase was measured up to the test end, indication that a residue plateau-level was nearly reached.

At sacrifice, highest mean equivalent concentrations occurred in liver with 9.536 mg/kg and in kidney (5.759 mg/kg). The residue-level of eggs collected from the ovary and oviduct (5.771 mg/kg) was by a factor 1.5 higher compared to the laid eggs at the test end (3.901 mg/kg), indicating that the egg yolk was assumedly the preferential site for the excretion. These values were followed in decreasing order by the mean concentrations determined in the muscle (3.290 mg/kg), the skin (2.533 mg/kg) and the subcutaneous fat (1.696 mg/kg).

The radioactive residues were extracted with high efficiencies (92-99%) from eggs and edible organs or tissues with acetonitrile/water mixtures and identification was achieved by co-chromatography with reference compounds.

In all edible matrices fluopyram-benzamide (M25) was the major metabolite representing 68.6% to 98.6% of the TRR (1.126 mg eq/kg to 8.737 mg eq/kg). Other metabolites identified were fluopyram-*Z*-olefin (M03), fluopyram-*E*-olefin (M02) and fluopyram-benzoic acid (M33). fluopyram-*Z*-olefin was identified in all edible matrices and ranged from 0.2% to 25.9% of the TRR (0.010 mg eq/kg to 0.425 mg eq/kg). fluopyram-*E*-olefin was detected only in fat (2.3% TRR or 0.037 mg eq/kg) and liver (0.3% TRR or 0.028 mg eq/kg). fluopyram-benzoic acid was detected only in liver (0.3% TRR or 0.024 mg eq/kg).

The parent compound fluopyram was detected as minor component only in eggs and fat (0.7% to 2.5% of the TRR or 0.024 mg/kg to 0.042 mg/kg).

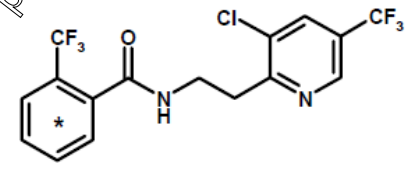
Based on the identified metabolites, the following metabolic routes were deduced:

- cleavage of the aliphatic chain as major biochemical reaction
- hydroxylation of the aliphatic chain followed by elimination
- hydrolysis of the benzamide to the corresponding benzoic acid

I. Materials and Methods

A. Materials

1. Test Material:

IUPAC Name	N-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]ethyl}-2-(trifluoromethyl)benzamide
CAS Name	Benzamide, N-[2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-(trifluoromethyl)- (9CI)
Code name	AE C656948
Common name	Fluopyram
Empirical formula	$C_{16}H_{11}ClF_6N_2O$
CAS Number	658066-35-4
Molar mass	396.72 g/mol
Chemical structure	 * position of the ^{14}C radiolabel
Radiolabelled test material	[phenyl-UL-^{14}C]AE C656948

Batch number	BECH 1920
Original specific radioactivity	1.93 MBq/mg = 1.16 x 10^8 dpm/mg = 52.1 µCi/mg = 20.67 Ci/mol
Radiochemical purity	> 99% by radio-TLC > 99% by radio-HPLC The radiochemical purity check was performed before dilution with the authentic non-radiolabelled test compound.
Chemical purity	99% (HPLC)
Specific radioactivity after radiodilution used for the study	32.7 MBq/mL (19.6x10^8 dpm/mL)
Stability of test compound	The administration suspensions were freshly prepared on the day before their use and each suspension was applied for three to four dosages. The radiolabelled parent compound was proved to be stable in the 0.5% aqueous tragacanth suspension for at least four days at +4 °C as shown by radio-HPLC analysis. The evaluation of the chromatogram revealed a radiochemical purity of > 99% in tragacanth suspension.

2. Test Animals

Species	Hen (*Gallus gallus domesticus*)
Breed	White Leghorn
Breeding facility	[redacted]
Sex, number	Six female laying hens
Mean body weight	1.53 kg at test start (1.43 – 1.64 kg) 1.64 kg at test end (1.52 – 1.75 kg)
Age	24 week old at experiment start
Acclimatization	18 days before administration, the egg production was recorded
Identification	Individual cages, wing tags
Housing	Each 1 bird per stainless steel metabolism cage, approx. 20–25 °C, 40–58% rel. humidity, 16/8 hours light/dark cycle
Feed and water	Commercial pulverized hen feed, "Union Legemehl" (LS 211) at 200 g per day per animal, supplemented with eggshells and crushed marine shells Tap water *ad libitum*
Health status	Acceptable

B. Study Design

Preparation of the dosing mixtures and administration:

The stock solution was prepared by dissolving the solid radiolabelled test compound (certified specific radioactivity: 1.93 MBq/mg) in 25 mL acetonitrile and radioassayed. The radioactivity concentration was determined to be 32.7 MBq/mL, corresponding to 16.9 mg compound/mL based on the specific radioactivity.

Four administration suspensions were prepared on the day before their use and each suspension was applied for three to four dosages. Definite volumes of [phenyl-UL-^{14}C]fluopyram stock solution in acetonitrile were taken to prepare the administration suspensions (suspensions 1–2: 5.106 mL, corresponding to 86.4 mg fluopyram; suspensions 3–4: 4.149 mL, corresponding to 70.2 mg fluopyram). The solvent was removed by a nitrogen stream. Definite volumes of 0.5% aqueous Tragacanth (43.2 mL for suspensions 1–2 and 35.1 mL for suspensions 3–4) were added and the samples were stirred constantly until administration. The radioactivity of the suspensions was calibrated by liquid scintillation counting and the animals were dosed related to their individual body weights at test start. The administration volume was 1 mL/kg bw, corresponding to a treatment rate of 2.03 mg/kg bw per day. In relation to a mean body weight of 1.53 kg, the laying hens received a total mean dose of 43.47 mg per animal.

The oral administration procedure was carried out with a knob cannula attached to a glass syringe. Directly after dosage, the act of swallowing was sustained by a gentle massage of the throat in direction of the crop.

The radiolabelled parent compound was proved to be stable in the 0.5% aqueous tragacanth suspension for at least four days at +4 °C as shown by radio-HPLC analysis, and thus for the time from preparation of the administration suspension until dosing. The evaluation of the chromatogram revealed a radiochemical purity of > 99% in tragacanth suspension. The identity of the test compound was confirmed by LC-MS/MS (ionisation by electron-spray ionization).

Sampling:

Collection and processing of eggs and excreta

The eggs were collected each day (day 1–14), numbers and weight of eggs were recorded for all hens. For sampling, the egg-shells were discarded, and the white and yolk were thoroughly mixed. An aliquot sample of each egg mix was taken for the determination of total radioactivity by LSC. Two pools of eggs (day 1 to 6 and day 7 to 14) were prepared and stored at about -18 °C until extraction for metabolite analysis. One sample of the combined egg-mix was taken for radioactivity measurement.

The faecal-urine excreta were collected individually as quantitatively as possible at room temperature in intervals of 24 hours until sacrifice. All fractions were homogenised after adding water, before the total

weights were recorded. The total radioactivity was determined by combustion. The $^{14}CO_2$ absorbed was measured by liquid scintillation counting.

Sacrifice and collection of organs and tissues

The treated laying hens were weighed at the end of the test. They were sacrificed ca. 24 hours after the last dose. The animals were anaesthetised using carbon dioxide gas, sacrificed by decapitation and exsanguinated. Immediately after sacrifice, the following organs and tissues were dissected: liver without bile bladder, kidneys, leg muscle, breast muscle, skin without subcutaneous fat, subcutaneous fat, eggs from the ovary and oviduct, and the bile bladder.

All tissues were individually weighed, and liver, kidneys, muscle samples as well as the eggs dissected from the ovary and oviduct were thoroughly homogenized in half-frozen state. One aliquot of each resulting homogenized tissue was weighed, combusted and radioassayed by liquid scintillation counting (LSC). Aliquots of the skin without fat were weighted, solubilised with tissue solubiliser and taken for radioactivity measurement by LSC. The two types of muscle (leg and breast) of all laying hens were combined, as well as their livers and fat samples and thoroughly homogenized.

Samples from the organs and other liquid samples were kept frozen at about -18 °C at all times until extraction. During the analytical work, the samples were stored either at approx. +4 °C in a refrigerator or at approx. -18 °C in a freezer.

C. Analytical Procedures

Extraction and fractionation:

Composite samples were conventionally extracted four times with acetonitrile/water (4/1; v/v) using a Polytron homogeniser.

The combined acetonitrile/water extracts were purified by SPE using RP18 cartridge. Flow-through ("sample passage") and rinse solutions of the extracts with acetonitrile/water (4:1, v/v) were combined and, as they contained the main portion of radioactivity, were concentrated and used for HPLC analysis. The methanol/dichloromethane (1:1; v/v) eluate and the dried RP18 material were assayed for radioactivity by LSC. Aliquots of the solid phase were submitted to combustion for determination of radioactivity.

Analytical methods:

The radioactivity of all liquid samples was measured using a liquid scintillation spectrometer after mixing a known amount of each sample with scintillation fluid. Solid samples were combusted using an oxidizer. The resulting $^{14}CO_2$ was trapped in Carbosorb E or Oxysolve C400. The TRR in the samples was calculated by summing up the radioactivity measured in different extracts and remaining solids after solvent extraction. The TRR was expressed in mg a.s. equivalents per kg sample weight. Amounts of radioactive

residues in the extracts were expressed as percentage of the TRR and also as mg a.s. equivalents per kg sample weight.

Aliquots of all acetonitrile/water extracts were analysed by HPLC. Four metabolites as well as parent compound were identified by HPLC and TLC co-chromatography with reference compounds.

The HPLC was conducted using a reversed-phase column (RP18-endcapped, 250 x 4.6 mm) that was operated with a gradient mixture of water + 25% (v/v) aqueous ammonia + formic acid (1000/1.54/0.8; v/v/v) and acetonitrile + methanol (1/1; v/v). The HPLC system was equipped with a radiodetector and a UV detector with variable wavelengths. The LOQ was derived from the average background level and the specific radioactivity of the radiolabelled test compound.

TLC was performed on HPTLC silica 60 F254 plates, 20x20 cm, using two different mobile solvent systems (dichloromethane/ethyl acetate (3:1; v/v) or dichloromethane). Non-radioactive standards were visualized using UV light. Radioactive zones were detected by radioluminography.

Storage stability

Samples from the organs and other liquid samples were kept frozen at about -18 °C during the entire study. During the analytical work, extracts of tissues and eggs were stored either at approx. +4 °C in a refrigerator or at approx. -18 °C in a freezer. The samples of eggs, muscle, liver and fat were analysed 27–33 days, 84 days, 124 days and 130 days after sample collection, respectively, *i.e.* within a maximum of *ca.* 4 months after sample collection.

A second HPLC analysis was conducted at later intervals (eggs: 4–4.5 months, liver: 1 month, muscle: 2.5 months, fat: 10 months). Comparing the metabolite patterns, no significant change could be observed regarding the qualitative distribution. Overall, the radioactive residues in all extracts were found to be stable under storage conditions.

H. Results and Discussion

The metabolism in laying hens of [phenyl-UL-^{14}C]fluopyram administrated at a daily dose of 26.42 mg/kg in the feed corresponding to a daily intake of 2.03 mg/kg body weight for 14 consecutive days was investigated.

Until sacrifice 24 hours after the last dose, the mean excretion amounted on average to 82.67% of the radioactivity totally administered (Table 6.2.2- 1). The time course of the excretion was characterised by a relatively constant rate starting at day 2 until test end (Table 6.2.2- 2). Only 4.34% of the dose totally administered was measured in the eggs produced within the whole test period. At sacrifice, 24 h after the

last administration, the total residue in the tissues and organs dissected from the body was calculated and estimated to about 7.83% of the total dose. Based on these values, the recovery amounted to 94.83% (Table 6.2.2- 1).

The total radioactive residues were determined in eggs and excreta produced from the first dose until sacrifice and in edible tissues and organs at sacrifice. The highest mean active substance equivalent concentrations were measured in the liver (9.536 mg/kg, corresponding to 0.86% of the total dose) and the eggs dissected from the ovary and oviduct (5.771 mg/kg, corresponding to 0.73% of the total dose). These values were followed in decreasing order by the mean concentrations determined in the kidney (5.759 mg/kg), the muscle (3.290 mg/kg), the skin (2.533 mg/kg) and the fat (1.696 mg/kg). For details see Table 6.2.2- 4. The mean radioactive residue in the total body skin amounted to about 0.38%, in the total body fat to about 0.76%, and in the body muscle to about 4.94% of the dose totally administered. The calculation of these values was based on the experimentally confirmed assumption that total body skin (without subcutaneous fat), fat, and muscle account for 4%, 12%, and 40% of the body weight, respectively (Table 6.2.2- 1).

Table 6.2.2- 1: Recovery of radioactivity following oral administration of [phenyl-UL-^{14}C]fluopyram at a daily dose rate of 2.03 mg/kg body weight for 14 consecutive days

Matrix	Recovery of radioactivity (% of the totally administrated radioactivity)
Excreta[1)]	82.67
Eggs[2)]	4.34
Totally excreted	87.01
Tissues[3)]	7.83
Recovery	94.83

1) Recovery in excreta obtained by adding recovery values of samples collected within the observation period of 14 days
2) Recovery in eggs calculated by adding recovery values of samples produced within the observation period of 14 days
3) Recovery in tissues estimated from the body weight, assuming 40, 12 or 4% of the body weight for total body muscle, dissectible body fat or body skin (without subcutaneous fat), and from the sum of percentage values of the other organs prepared.

Table 6.2.2- 2: Time course of total radioactivity in excreta following oral administration of [phenyl-UL-^{14}C]fluopyram at a daily dose rate of 2.03 mg/kg body weight for 14 consecutive days

Matrix	Time after the first dosage (day)	Administration number	TRR (% of totally administrated radioactivity)
Excreta	0	1	-
	1	2	4.25
	2	3	8.93
	3	4	14.53
	4	5	20.13
	5	6	25.80
	6	7	31.95
	7	8	37.85
	8	9	43.93
	9	10	50.08
	10	11	56.61
	11	12	62.88
	12	13	69.34
	13	14	76.20
	14		82.67

The equivalent concentration of radioactivity in the eggs showed an increase from 0.462 mg/kg obtained on day 2 (24 hours after the first dosage) to 3.901 mg/kg at sacrifice (14 days after the first dosage). For details see Table 6.2.2- 3. A further but slower increase was measured up to the test end, indication that a residue plateau-level was nearly reached.

Table 6.2.2- 3: Time course of total radioactivity in eggs following oral administration of [phenyl-UL-^{14}C]fluopyram at a daily dose rate of 2.03 mg/kg body weight for 14 consecutive days

Matrix	Time after the first dosage (days)	Administration number	TRR (mg a.s. equiv./kg)
Eggs	0	1	-
	1	2	0.462
	2	3	1.017
	3	4	1.652
	5	5	2.094
	6	6	2.445
	7	7	2.872
	8	8	3.243
	9	9	3.223
	10	10	3.383
	11	11	3.565
	12	12	3.719
	13	13	3.802
	14	14	3.827
			3.901

mg a.s. equiv./kg: mg parent equivalents per kg matrix

Table 6.2.2- 4: Distribution of residues in eggs, tissues and organs of laying hens following oral administration of [phenyl-UL-^{14}C]fluopyram at a daily dose rate of 2.03 mg/kg body weight for 14 consecutive days

Matrix	Interval (day)	TRR (mg a.s. equiv./kg)
Liver (composite)	14	9.536
Kidney	14	5.759
Eggs (composite)	14	2.870
Eggs from ovary/oviduct	14	5.771
Muscle, leg	14	3.390
Muscle, breast	14	3.281
Composite muscle (leg and breast)	14	3.290
Skin without subcutaneous fat	14	2.533
Subcutaneous fat (composite)	14	1.696

mg a.s. equiv./kg: mg parent equivalents per kg matrix

In composite egg pool (day 1–6), 98.8% of the radioactivity (1.791 mg/kg) was extracted with solvent extraction (acetonitrile/water). Following solid phase extraction purification, the main radioactivity was contained in the combined sample passage and acetonitrile/water rinse solution ("purified acetonitrile/water extract", 98.8% of the TRR, 1.790 mg/kg) and, after the concentration step, the concentrated acetonitrile/water extract contained 97.7% of the TRR (1.770 mg/kg). In total, 98.9% of the TRR (1.791 mg/kg) was extracted, whereas 1.1% of the TRR (0.020 mg/kg) remained non-extractable in solids (Table 6.2.2-5).

In composite egg pool (day 7–14), 98.6% of the radioactivity (3.530 mg/kg) was extracted with solvent extraction (acetonitrile/water). Following solid phase extraction purification, the main radioactivity was contained in the purified acetonitrile/water extract (98.5% of the TRR, 3.527 mg/kg) and, after the concentration step, the concentrated acetonitrile/water extract contained 98.2% of the TRR (3.515 mg/kg). In total, 98.6% of the TRR (3.530 mg/kg) was extracted, whereas 1.4% of the TRR (0.051 mg/kg) remained non-extractable in solids (Table 6.2.2-6)

In muscle, the major part of the radioactive residues (99.6% of the TRR, 3.265 mg/kg) was extracted with acetonitrile/water. After solid phase extraction purification, the main radioactivity was contained in the purified acetonitrile/water extract (99.5% of the TRR, 3.265 mg/kg). After the concentration step, the radioactivity in the concentrated extract amounted to 99.1% of the TRR (3.249 mg/kg). In total, 99.6% of the TRR (3.265 mg/kg) was extracted, whereas only 0.4% of the TRR (0.015 mg/kg) remained non-extractable in solids (Table 6.2.2-7)

In fat, the radioactive residues extractable with acetonitrile/water amounted to 99.4% of the TRR (1.631 mg/kg) and, after clean-up procedure by solid phase extraction, to 99.3% of the TRR (purified

acetonitrile/water extract, 1.630 mg/kg). After the concentration step the radioactivity in the concentrated extract amounted to 99.1% of the TRR (3.249 mg/kg). In total, 99.4% of TRR (1.631 mg/kg) was extracted and only 0.6% of the TRR (0.010 mg/kg) remained non-extractable in solids (Table 6.2.2- 8).

In liver, the radioactive residues extractable with acetonitrile/water amounted to 93.6% of the TRR (8.861 mg/kg) and, after clean-up procedure by solid phase extraction, to 93.5% of the TRR (purified acetonitrile/water extract, 8.850 mg/kg). After the concentration step the radioactivity in the concentrated extract amounted to 93.0% of the TRR (8.805 mg/kg). In total, 93.6% of the TRR (8.861 mg/kg) was extracted and 6.4% of the TRR (0.605 mg/kg) remained non extractable in solids (Table 6.2.2-9).

Table 6.2.2- 5: Extraction of radioactive residues from the hen's egg pool (day 1-6) after dosing of [phenyl-UL-^{14}C]fluopyram at a daily dose rate of 2.03 mg/kg body weight for 14 consecutive days

	Egg pool (day 1–6)	
TRR, mg eq/kg	1.815	
Fraction	% TRR	mg a.s. equiv./kg
Solvent extract[1)]	98.9	1.791
Purification via RP18		
Purified acetonitrile/water extract	98.8	1.790
Concentration		
Concentrated acetonitrile/water extract	**97.7**	**1.770**
Distillate	1.1	0.020
Methanol/dichloromethane eluate	0.1	0.001
Solids	1.1	0.020
PES[2)]	1.1	0.020
Total extracted	98.9	1.791
Accountability / Total[3)]	100.0	1.811

1) Solvent extraction was done with 4x with acetonitrile/water (4:1, v/v).
2) PES: post extraction solids = non-extractable radioactivity.
3) Sum of extracts and PES.
Fractions given in **bold** font were analysed by radio-HPLC.

Table 6.2.2- 6: Extraction of radioactive residues from the hen's egg pool (7–14 days) after dosing of [phenyl-UL-^{14}C]fluopyram at a daily dose rate of 2.03 mg/kg body weight for 14 consecutive days

	Egg pool (day 7–14)	
TRR, mg eq/kg	3.581	
Fraction	% TRR	mg a.s. equiv./kg
Solvent extract[1)]	98.6	3.530
Purification via RP18		
Purified acetonitrile/water extract	98.5	3.527
Concentration		
Concentrated acetonitrile/water extract	**98.2**	**3.515**
Distillate	0.3	0.012
Methanol/dichloromethane eluate	0.1	0.003
Solids	1.1	0.020
PES[2)]	1.4	0.051
Total extracted	98.6	3.530
Accountability / Total[3)]	100.0	3.581

1) Solvent extraction was done with 4x with acetonitrile/water (4:1, v/v).
2) PES: post extraction solids = non-extractable radioactivity.
3) Sum of extracts and PES.
Fractions given in **bold** font were analysed by radio-HPLC.

Table 6.2.2- 7: Extraction of radioactive residues from the hen's muscle after dosing of [phenyl-UL-^{14}C]fluopyram at a daily dose rate of 2.03 mg/kg body weight for 14 consecutive days

	Muscle (composite)	
TRR, mg eq/kg	3.280	
Fraction	% TRR	mg a.s. equiv./kg
Solvent extract[1)]	99.6	3.265
Purification via RP18		
Purified acetonitrile/water extract	99.5	3.265
Concentration		
Concentrated acetonitrile/water extract	**99.1**	**3.249**
Distillate	0.5	0.016
Methanol/dichloromethane eluate	n.q.	n.q.
Solids	1.1	0.020
PES[2)]	0.4	0.015
Total extracted	99.6	3.265
Accountability / Total[3)]	100.0	3.280

1) Solvent extraction was done with 4x with acetonitrile/water (4:1, v/v).
2) PES: post extraction solids = non-extractable radioactivity.
3) Sum of extracts and PES.
Fractions given in **bold** font were analysed by radio-HPLC. n.q.: not quantified

Table 6.2.2- 8: Extraction of radioactive residues from the hen's fat after dosing of [phenyl-UL-^{14}C]fluopyram at a daily dose rate of 2.03 mg/kg body weight for 14 consecutive days

	Fat (composite)	
TRR, mg eq/kg	1.641	
Fraction	% TRR	mg a.s. equiv./kg
Solvent extract[1)]	99.4	1.631
Purification via RP18		
Purified acetonitrile/water extract	99.3	1.630
Concentration		
Concentrated acetonitrile/water extract	**99.3**	**1.630**
Distillate	n.q.	n.q.
Methanol/dichloromethane eluate	n.q.	n.q.
Solids	0.6	0.010
PES[2)]	0.6	0.010
Total extracted	99.4	1.631
Accountability / Total[3)]	100.0	1.641

1) Conventional extraction was done with 4x with acetonitrile/water (4:1, v/v).
2) PES: post extraction solids = non-extractable radioactivity.
3) Sum of extracts and PES.
Fractions given in **bold** font were analysed by radio-HPLC. n.q.: not quantified

Table 6.2.2- 9: Extraction of radioactive residues from the hen's liver after dosing of [phenyl-UL-^{14}C]fluopyram at a daily dose rate of 2.03 mg/kg body weight for 14 consecutive days

	Liver (composite)	
TRR, mg eq/kg	9.466	
Fraction	% TRR	mg a.s. equiv./kg
Solvent extract[1)]	93.6	8.861
Purification via RP18		
Purified acetonitrile/water extract	93.5	8.855
Concentration		
Concentrated acetonitrile/water extract	**93.0**	**8.805**
Distillate	0.5	0.050
Methanol/dichloromethane eluate	n.q.	n.q.
Solids	6.4	0.605
PES[2)]	6.4	0.605
Total extracted	93.6	8.861
Accountability / Total[3)]	100.0	9.466

1) Conventional extraction was done with 4x with acetonitrile/water (4:1, v/v).
2) PES: post extraction solids = non-extractable radioactivity.
3) Sum of extracts and PES.
Fractions given in **bold** font were analysed by radio-HPLC. n.q.: not quantified

For elucidation of metabolism, all acetonitrile/water extracts were analysed by HPLC with radiodetection. Metabolites were identified by co-chromatography with authentic reference compounds using HPLC and TLC co-chromatographic methods.

The parent compound fluopyram was detected as minor component only in eggs and fat (0.7% to 2.5% of the TRR or 0.024 mg/kg to 0.042 mg/kg). A total of four metabolites was identified. The identification rate was high (93.0–99.3% of the TRR).

In egg, the major metabolite was fluopyram-benzamide (M25), representing 95.8% and 96.3% of the TRR (1.735 and 3.447 mg eq/kg) for the pool day 1–6 and the pool day 7–14, respectively. fluopyram-*Z*-olefin (M03) was detected at a lower level (0.5% or 0.010 mg eq/kg and 1.2% of the TRR or 0.044 mg eq/kg for pool day 1–6 and pool day 7–14, respectively). In total 97.7% and 98.2% of the TRR was identified for egg pool day 1–6 (Table 6.2.2- 10) and egg pool day 7–14 (Table 6.2.2- 11), respectively.

In muscle, fluopyram-benzamide was the only major component (98.6% of the TRR or 3.233 mg eq/kg). fluopyram-*Z*-olefin (M03) was the sole other metabolite detected (0.5% TRR or 0.015 mg eq/kg). In total 99.1% of the TRR was identified (Table 6.2.2- 12).

In fat, fluopyram-benzamide (M25) was the major component with 68.6% of the TRR (1.126 mg eq/kg), followed by both fluopyram-*Z*-olefin (M03) (25.9% of the TRR or 0.425 mg eq/kg) and fluopyram-*E*-olefin (M02) (2.3% of the TRR or 0.037 mg/kg). In total 99.3% of the TRR was identified (Table 6.2.2- 13).

In liver, fluopyram-benzamide (M25) was the major component with 92.3% of the TRR (8.737 mg eq/kg), followed by fluopyram-*E*-olefin (M02) (0.3% TRR or 0.028 mg eq/kg), fluopyram-benzoic acid (M33) (0.3% TRR or 0.024 mg eq/kg) and fluopyram-*Z*-olefin (M03) (0.2% TRR or 0.016 mg eq/kg). In total 93.0% of the TRR was identified (Table 6.2.2- 14).

Table 6.2.2- 10: Summary of identification and characterization of radioactive residues in the hen's egg pool (day 1–6) after dosing of [phenyl-UL-^{14}C] fluopyram at a daily dose rate of 2.03 mg/kg body weight for 14 consecutive days

	Egg pool (day 1–6)	
TRR [mg eq/kg] =	1.811	
Compound	% of TRR	mg a.s. equiv./kg
Acetonitrile/water extract		
AE C656948, fluopyram	1.4	0.026
fluopyram-benzoic acid (M33)	-	-
fluopyram-benzamide (M25)	95.8	1.735
fluopyram-*E*-olefin (M02)	-	-
fluopyram-*Z*-olefin (M03)	0.5	0.010
Total identified in conventional extract	**97.7**	**1.770**
Analysed extract(s)	97.7	1.770
Volatiles in distillates	1.1	0.020
Extracts not analysed	0.1	0.001
Total extractable	98.9	1.791
Unextractable residues (solids)	1.1	0.020
Accountability / Total	100.0	1.811

Table 6.2.2- 11: Summary of identification and characterization of radioactive residues in the hen's egg pool (day 7–14) after dosing of [phenyl-UL-^{14}C]fluopyram at a daily dose rate of 2.03 mg/kg body weight for 14 consecutive days

	Egg pool (day 7–14)	
TRR [mg/kg] =	3.581	
Compound	% of TRR	mg a.s. equiv./kg
Acetonitrile/water extract		
AE C656948, fluopyram	0.7	0.024
fluopyram-benzoic acid (M33)	-	-
fluopyram-benzamide (M25)	96.3	3.447
fluopyram-*E*-olefin (M02)	-	-
fluopyram-*Z*-olefin (M03)	1.2	0.044
Total identified in conventional extract	**98.2**	**3.515**
Analysed extract(s)	98.2	3.515
Volatiles in distillates	0.3	0.012
Extracts not analysed	0.1	0.003
Total extractable	98.6	3.530
Unextractable residues (solids)	1.4	0.051
Accountability / Total	100.0	3.581

Table 6.2.2- 12: Summary of identification and characterization of radioactive residues in hen's muscle after dosing of [phenyl-UL-^{14}C] fluopyram at a daily dose rate of 2.03 mg/kg body weight for 14 consecutive days

	Muscle	
TRR [mg/kg] =	3.280	
Compound	% of TRR	mg a.s. equiv./kg
Acetonitrile/water extract		
AE C656948, fluopyram	-	-
fluopyram-benzoic acid (M33)	-	-
fluopyram-benzamide (M25)	98.6	3.233
fluopyram-*E*-olefin (M02)	-	-
fluopyram-*Z*-olefin (M03)	0.5	0.015
Total identified in conventional extract	**99.1**	**3.249**
Analysed extract(s)	99.1	3.249
Volatiles in distillates	0.5	0.016
Extracts not analysed	-	-
Total extractable	99.6	3.265
Unextractable residues (solids)	0.4	0.015
Accountability / Total	100.0	3.280

Table 6.2.2- 13: Summary of identification and characterization of radioactive residues in hen's fat after dosing of [phenyl-UL-^{14}C] fluopyram at a daily dose rate of 2.03 mg/kg body weight for three consecutive days

	Fat	
TRR [mg/kg] =	1.641	
Compound	% of TRR	mg a.s. equiv./kg
Acetonitrile/water extract		
AE C656948, fluopyram	2.5	0.042
fluopyram-benzoic acid (M33)	-	-
fluopyram-benzamide (M25)	68.6	1.126
fluopyram-*E*-olefin (M02)	2.3	0.037
fluopyram-*Z*-olefin (M03)	25.9	0.425
Total identified in conventional extract	**99.3**	**1.630**
Analysed extract(s)	99.3	1.630
Volatiles in distillates	-	-
Extracts not analysed	0.1	0.001
Total extractable	99.4	1.631
Unextractable residues (solids)	0.6	0.010
Accountability / Total	100.0	1.641

Table 6.2.2- 14: Summary of identification and characterization of radioactive residues in hen's liver after dosing of [phenyl-UL-^{14}C] fluopyram at a daily dose rate of 2.03 mg/kg body weight for three consecutive days

	Liver	
TRR [mg/kg] =	9.466	
Compound	% of TRR	mg a.s. equiv./kg
Acetonitrile/water extract		
AE C656948, fluopyram	-	-
fluopyram-benzoic acid (M33)	0.3	0.024
fluopyram-benzamide (M25)	92.3	8.737
fluopyram-*E*-olefin (M02)	0.3	0.028
fluopyram-*Z* -olefin (M03)	0.2	0.016
Total identified in conventional extract	**93.0**	**8.805**
Analysed extract(s)	93.0	8.805
Volatiles in distillates	0.5	0.050
Extracts not analysed	0.1	0.006
Total extractable	93.6	8.861
Unextractable residues (solids)	6.4	0.605
Accountability / Total	100.0	9.466

III. Conclusion

Six laying hens were dosed orally for 14 consecutive days with [phenyl-UL-^{14}C]fluopyram at a daily dose of 2.03 mg/kg of body weight and sacrificed 24 hours after the last dose.

Laying hens extensively metabolised AE C565948 and the major compound in all edible matrices was metabolite AE C565948-benzamide (M25), representing 68.6% to 98.6% of the TRR. Other metabolites identified were fluopyram-*Z*-olefin(M03), fluopyram-*E*-olefin (M02) and fluopyram-benzoic acid (M33). fluopyram-Z-olefin was identified in all edible matrices and ranged from 0.2% to 25.9% of the TRR. fluopyram-E-olefin was detected only in fat (2.3% TRR) and liver (0.3% TRR). fluopyram-benzoic acid was detected only in liver (0.3% TRR). The parent compound fluopyram was detected as minor component only in egg pools and fat (0.7% to 2.5% of the TRR).

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

- cleavage of the aliphatic chain as major biochemical reaction
- hydroxylation of the aliphatic chain followed by elimination

- hydrolysis of the benzamide to the corresponding benzoic acid

The metabolic pathway is proposed in Figure 6.2.2- 1.

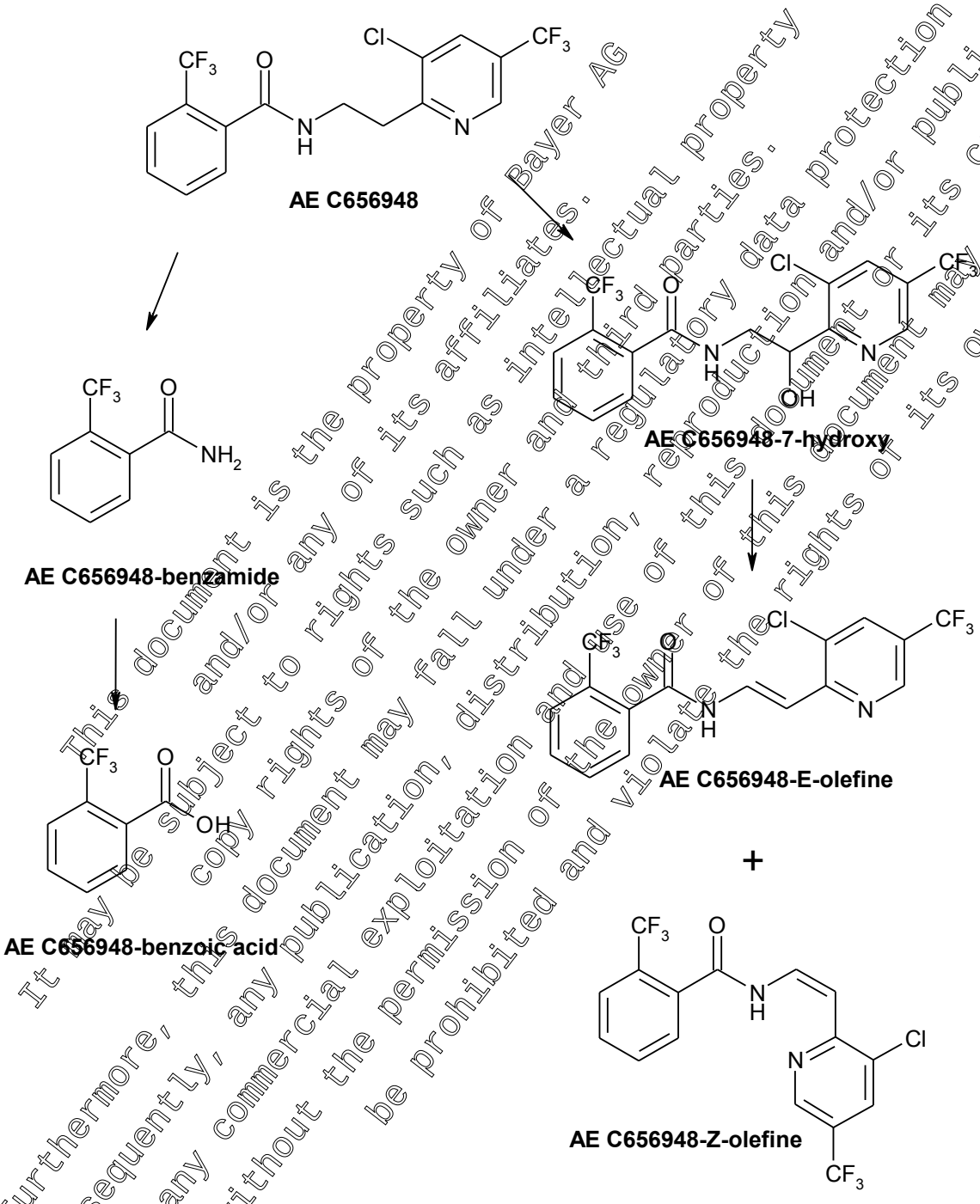


Figure 6.2.2- 1: Proposed metabolic pathway of AE C656948 (fluopyram) in hen after treatment with [phenyl-UL-^{14}C] fluopyram

Assessment and conclusion by applicant:

The study is valid and acceptable.

Data Point:	KCA 6.2.2/02
Report Author:	[redacted]
Report Year:	2008
Report Title:	Metabolism of [pyridyl-2,6-^{14}C]AE C656948 in the laying hen
Report No:	MEF-06/405
Document No:	M-298190-01-1
Guideline(s) followed in study:	US EPA OPPTS 860.1300, Health Canada PMRA ref.: DACO 6.2, EU 91/414/EEC amended by 96/68/EC, appendix F
Deviations from current test guideline:	Deviation to OECD 502, 6 birds instead of 10. No impact, because sufficient amount of sample material was available for characterization and identification of metabolites.
Previous evaluation:	Yes, evaluated and accepted rev. 4.0 Vol 3 of DAR B7 August 2012 (references cited on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP, officially recognised testing facilities
Acceptability/Reliability:	Yes

Summary

The metabolism of [pyridyl-2,6-^{14}C]fluopyram was investigated in six laying hens, which where orally dosed for 14 consecutive days at a rate of 2.02 mg a.s./kg of body weight per day (corresponding to 25.96 mg a.s./kg feed/day). Sacrifice was made 24 h after the 14th administration. Radioactivity was measured in the excreta and eggs collected daily as well as in the kidney, liver, eggs from the ovary and oviduct, skin without subcutaneous fat, muscle and fat at sacrifice. The collected eggs and the edible tissues and organs were analysed for parent compound and metabolites by extraction, chromatographic separation techniques and spectroscopic methods.

The overall recovery (sum of radioactivity in the excreta, eggs as well as organs and tissues) was 95.6% of the total administered dose. The residual balance of only 4.4% at sacrifice may be still present in the gastro-intestinal tract. Until sacrifice, the excretion amounted to 94.71% of the totally administered radioactivity. An amount of 0.36% of the total dose was found in eggs. At sacrifice, the calculated or estimated total

residue in the tissues and organs dissected from the body was approx. 0.48% of the total dose. About half of this amount was detected in the fat (0.22%).

The mean TRRs in eggs increased from 0.047 mg eq/kg (day 1) to 0.321 mg eq/kg (day 8) and slowly decreased to 0.262 mg eq/kg (day 14). This showed that a residue plateau-level was clearly reached.

At sacrifice, highest mean equivalent concentrations occurred eggs dissected from the ovary and oviduct (0.831 mg eq/kg) and was by a factor 3 higher compared to the laid eggs at the test end (0.262 mg eq/kg), indicating that the egg yolk was assumedly the preferential site for the secretion. These values were followed in decreasing order by the mean concentrations determined in the liver (0.538 mg eq/kg), subcutaneous fat (0.498 mg eq/kg), the kidney (0.242 mg eq/kg), skin without subcutaneous fat (0.152 mg eq/kg) and total body muscle (0.048 mg eq/kg). The radioactive residues were extracted with efficiencies of 32.3–99.2% from eggs and edible organs or tissues with acetonitrile/water mixtures and *n*-heptane, and identification was achieved by co-chromatography with reference compounds.

For elucidation of parent compound and metabolites, eggs, organs and tissues as well as excreta were extracted with acetonitrile/water mixtures. Fat was extracted with n-heptane. After purification and concentration steps, the resulting extracts of eggs, muscle, fat and excreta represented between ca. 28% and 95% of the total radioactive residue (TRR). To increase the extraction efficiency an alternative approach was applied to the two egg pools and the liver pool. Aliquots of these were extracted by enzymatic digestion using proteolytic enzymes. The resulting efficiency was slightly higher to that of the conventional extraction. To solubilize even more of the radioactive residues the extracted solids of the eggs (from day 7 to 14) after conventional extraction and the solids of the liver after enzymatic digestion were additionally extracted with microwave at increased temperature. With this procedure, the radioactivity remaining in the final solids was below 0.05 mg/kg. Thus a total extraction efficiency of approx. 99% and 90% of the TRR was achieved for the eggs (7-14 days) and the liver, respectively.

AE C565948 was extensively metabolised, but was still the major compound in egg pool 1–6 days (14.7–17.9% of the TRR) and represented 1%–12% in the other matrices excluding liver. fluopyram-*Z*-olefin was the main metabolite in egg pool 7–14 days, muscle and fat and accounted for 15.4% to 70.5% of the TRR, and was also found at 4.1% in egg pool 1–6 days and at 1.9%–3.1% of the TRR in liver. fluopyram-*E*-olefin was the major metabolite in liver (11.8% -13.9% of the TRR) and represented 1.0% to 12.4% of the TRR in the other matrices. Further detected metabolites were fluopyram-7-hydroxy (M08) found in eggs, liver and excreta (0.8–5.8% of the dose) and fluopyram-pyridyl-acetic acid found in eggs and liver (1.3–6.4% of the TRR).

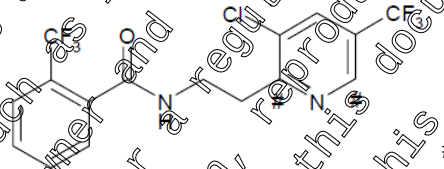
Based on the identified metabolites, the following metabolic routes were deduced:

- hydroxylation of the aliphatic chain followed by elimination
- oxidative cleavage of the aliphatic chain

I. Materials and Methods

A. Materials

1. Test Material:

IUPAC Name	*N*-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]ethyl}-2-(trifluoromethyl)benzamide
CAS Name	Benzamide, *N*-[2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-(trifluoromethyl)- (9Cl)
Code name	AE C656948
Common name	Fluopyram
Empirical formula	$C_{16}H_{11}ClF_6N_2O$
CAS Number	658066-35-4
Molar mass	396.72 g/mol
Chemical structure	 # positions of radiolabel
Radiolabelled test material	[pyridyl-2,6-^{14}C]AE C656948
Batch number	BECH 1976 + BECH 1939
Original specific radioactivity	1.93 MBq/mg = 1.16 x 10^8 dpm/mg = 52.1 µCi/mg = 20.67 Ci/mol
Radiochemical purity	> 98% (HPLC); > 98% (TLC) for BECH 1976 > 98% (HPLC); > 99% (TLC) for BECH 1939
Chemical purity	> 99% (HPLC) for BECH 1976 and BECH 1939
Stability of test compound	The administration suspensions were freshly prepared on the day before their use and each suspension was applied for three to four dosages. The radiolabelled parent compound was proved to be stable in the 0.5% aqueous tragacanth suspension for at least four days at +4 °C as shown by radio-HPLC analysis. The evaluation of the chromatogram revealed a radiochemical purity of > 98% in tragacanth suspension.

2. Test Animals

Species	Hen (*Gallus gallus domesticus*)
Breed	White Leghorn
Breeding facility	[redacted]
Sex, number	Six female laying hens
Mean body weight	1.53 kg at test start (1.46–1.61 kg) 1.62 kg at test end (1.57–1.67 kg)
Age	*ca.* 20 week old at experiment start
Acclimatization	8 days
Identification	Individual cage cards and wing tags
Housing	Each 1 bird per stainless steel metabolism cage, approx. 18–30 °C, 53–80% rel. humidity, 16/8 hours light/dark cycle
Feed and water	Commercial pulverized hen feed, "Legemehl" (REG-Lm) at 200 g per day per animal, supplemented with eggshells and crushed marine shells Tap water *ad libitum*
Health status	Acceptable

B. Study Design

Preparation of the dosing mixtures and administration:

The stock solution was prepared by dissolving both batches of the solid radiolabelled test compound (certified specific radioactivity: 1.93 MBq/mg) in 50 mL acetonitrile and radioassayed. The radioactivity concentration was determined to be 14.6 MBq/mL, corresponding to 7.56 mg compound/mL based on the specific radioactivity.

Four administration suspensions were prepared on the day before their use and each suspension was applied for three to four dosages. Definite volumes of [pyridyl-2,6-^{14}C]fluopyram stock solution in acetonitrile were taken to prepare the administration suspensions (suspensions 1–2: 12.381 mL, corresponding to 93.6 mg fluopyram; suspensions 3–4: 9.286 mL, corresponding to 70.2 mg fluopyram). The solvent was removed by a nitrogen stream. Definite volumes of 0.5% aqueous Tragacanth (46.8 mL for suspensions 1–2 and 35.1 mL for suspensions 3–4) were added and the samples were stirred constantly until administration. The radioactivity of the suspensions was calibrated by liquid scintillation counting. The administration volume was 1 mL/kg bw, corresponding to a treatment rate of 2.02 mg a.s./kg bw per day. In relation to a mean body weight of 1.53 kg, the laying hens received a total mean dose of 43.24 mg per animal.

The oral administration procedure was carried out with a knob cannula attached to a glass syringe. Directly after dosage, the act of swallowing was sustained by a gentle massage of the throat in direction of the crop.

The radiolabelled parent compound was proved to be stable in the 0.5% aqueous tragacanth suspension for at least four days at +4 °C as shown by radio-HPLC analysis, and thus for the time from preparation of the administration suspension until dosing. The evaluation of the chromatogram revealed a radiochemical purity of > 98% in tragacanth suspension. The identity of the test compound was confirmed by LC/MS and LC-MS/MS.

Sampling:

Collection and processing of eggs and excreta

The eggs were collected each day (day 1–14), number and weight of eggs were recorded for all hens. For sampling, the egg-shells were discarded, and the white and yolk were thoroughly mixed. An aliquot sample of each egg mix was taken for the determination of total radioactivity by LSC. Two pools of eggs (day 1 to 6 and day 7 to 14) were prepared, thoroughly homogenized and stored at about -18 °C until extraction for metabolite analysis. One sample of the combined egg-mix was taken for radioactivity measurement.

The faecal-urine excreta were collected individually as quantitatively as possible at room temperature in intervals of 24 hours until sacrifice. All fractions were homogenised after adding water, before the total weights were recorded. The total radioactivity was determined by combustion.

Sacrifice and collection of organs and tissues

The treated laying hens were weighed and sacrificed *ca.* 24 hours after the last dose. The animals were anaesthetised using carbon dioxide gas, sacrificed by decapitation and exsanguinated. The following organs and tissues were dissected: liver without gall bladder, kidneys, leg and breast muscle, skin without subcutaneous fat, subcutaneous fat, eggs from the ovary and oviduct, and the gall bladder.

All tissues were individually weighed, and liver, kidneys, muscle samples as well as the eggs dissected from the ovary and oviduct were thoroughly homogenized in half-frozen state. One aliquot of each resulting homogenized tissue was weighed, combusted and radioassayed by liquid scintillation counting (LSC). Aliquots of the skin without fat were weighted, solubilised with tissue solubiliser and taken for radioactivity measurement by LSC. The gall bladders were punctured for the collection of the bile fluid that was stored at ≤ -18 °C for the (optional) metabolite analysis. The two types of muscle (leg and breast) of all laying hens were combined, as well as their livers and fat samples and thoroughly homogenized.

Samples from the organs and other liquid samples were divided into equal portions and kept frozen at about -18 °C at all times until extraction. During the analytical work, the samples were stored either at approx. +4 °C in a refrigerator or at approx. -18 °C in a freezer.

C. Analytical Procedures

Extraction and fractionation:

Aliquot samples from egg, combined muscle, liver and excreta pools were conventionally extracted four times with acetonitrile/water (4/1; v/v) using a Polytron homogeniser. The resulting extracts and solids were separated by filtration or centrifugation.

The combined acetonitrile/water extracts were purified by SPE using RP18 cartridge. The effluents which contained the major part of the applied radioactivity were concentrated using a rotary evaporator. The solids after extraction of the egg pool day 7–14 were further extracted twice with acetonitrile/water (1/1; v/v) and with acetonitrile/1*N* hydrochloric acid (1/1; v/v) with microwave assistance at increased temperature (approx. +120 °C) for solubilisation of remaining radioactivity.

The distillate from the concentration of the excreta extract was applied on a reversed phase SPE-cartridge. The effluent was then collected and the cartridge was eluted 3 times with a small volume of methanol. The second and third methanol eluate were combined and concentrated prior to HPLC chromatography.

An aliquot of the fat pool was extracted twice with a mixture of acetonitrile/water (4/1; v/v) and *n*-heptane. After each extraction the acetonitrile/water and the *n*-heptane phases were separated. The acetonitrile/water extracts were combined and subjected to a clean-up step using a reversed phase SPE-cartridge. The effluent which contained the major part of the applied radioactivity was concentrated using a rotary evaporator, yielding the fat acetonitrile extract. The *n*-heptane extracts were combined and partitioned against acetonitrile. The acetonitrile phase was concentrated, yielding the fat heptane extract.

Aliquots of the two egg pools and the liver pool were enzymatically digested by the proteolytic enzyme subtilisin Carlsberg (type VIII) and subsequently with tartaric acid. The extracts were separated from the solids by centrifugation and the remaining solids were extracted successively three times with acetonitrile/water (4/1; v/v) using a Polytron homogeniser. The resulting extracts and solids were separated by centrifugation. All individual extracts were combined and subjected to a clean-up step using a reversed phase SPE-cartridge. The effluents which contained the major part of the applied radioactivity were concentrated using a rotary evaporator. The solids after extraction of the liver pool were further extracted twice with acetonitrile/water (1/1; v/v) with microwave assistance at increased temperature (approx. +120 °C) for solubilisation of remaining radioactivity.

Analytical methods:

The radioactivity of all liquid samples was measured using a liquid scintillation counter after mixing a known amount of each sample with scintillation fluid. Solid samples were combusted using an oxidizer. The resulting $^{14}CO_2$ was trapped in an alkaline scintillation cocktail. The TRR in the samples was calculated by summing up the radioactivity measured in different extracts and remaining solids after solvent extraction. The TRR was expressed in mg a.s. equivalents per kg sample weight (mg eq/kg). Amounts of radioactive residues in the extracts were expressed as percentage of the TRR and also as mg a.s. equivalents per kg sample weight (mg eq/kg).

Aliquots of all acetonitrile/water and n-heptane extracts were analysed by HPLC. Four metabolites as well as parent compound were identified by HPLC co-chromatography with reference compounds.

The HPLC was conducted using a reversed-phase column using a reversed phase column and a buffered acetonitrile/water gradient. The HPLC system was equipped with a radiodetector and a UV detector with variable wavelengths. The LOQ was derived from the average background level and the specific radioactivity of the radiolabelled test compound.

Storage stability:

Samples from the organs and other liquid samples were kept frozen at about -18 °C during the entire study. During the analytical work, extracts of tissues and eggs were stored either at approx. +4 °C in a refrigerator or at approx. -18 °C in a freezer. All solvent extraction experiments and first HPLC analyses of the extracts from eggs, muscle, fat, liver and excreta were performed within *ca.* 6 months after sacrifice of the hens.

The conventional extract of egg pool day 1–6 was analysed again 1 year later, the muscle extract *ca.* 10 months later, the fat extract *ca.* 5 months later. No significant change was observed in the metabolic profiles. Overall, the radioactive residues in all extracts were found to be stable under storage conditions.

II. Results and Discussion

The metabolism in laying hens of [pyridyl-2,6-^{14}C]fluopyram administrated at a daily dose of 25.96 mg eq/kg in the feed corresponding to a daily intake of 2.02 mg eq/kg body weight for 14 consecutive days was investigated.

Until sacrifice 24 hours after the last dose, the mean excretion amounted on average to 94.71% of the radioactivity totally administered (Table 6.2.2- 15). The time course of the excretion was characterised by a relatively constant rate starting at day 1 until test end (Table 6.2.2- 16). Only 0.36% of the dose totally administered was measured in the eggs produced within the whole test period. At sacrifice, 24 h after the last administration, the total residue in the tissues and organs dissected from the body was calculated and estimated to about 0.48% of the total dose. Based on these values, the recovery amounted to 95.55% (Table 6.2.2- 15).

The total radioactive residues were determined in eggs and excreta produced from the first dose until sacrifice and in edible tissues and organs at sacrifice. The highest mean active substance equivalent concentrations were measured in the eggs dissected from the ovary and oviduct (0.831 mg eq/kg) and liver (0.532 mg eq/kg). These values were followed in decreasing order by the mean concentrations determined in the subcutaneous fat (0.498 mg eq/kg), the kidney (0.242 mg eq/kg), skin without subcutaneous fat (0.152 mg eq/kg) and total body muscle (0.048 mg eq/kg). For details see Table 6.2.2- 18).

Table 6.2.2- 15: Recovery of radioactivity following oral administration of [pyridyl-2,6-^{14}C]fluopyram at a daily dose rate of 2.02 mg eq/kg body weight for 14 consecutive days

Matrix	Recovery of radioactivity (% of the totally administrated radioactivity)
Excreta[1)]	94.71
Eggs[2)]	0.36
Totally excreted	*95.01*
Tissues[3)]	0.48
Recovery	95.55

1) Recovery in excreta obtained by adding recovery values of samples collected within the observation period of 14 days
2) Recovery in eggs calculated by adding recovery values of samples produced within the observation period of 14 days
3) Recovery in tissues estimated from the body weight, assuming 40, 12 or 4% of the body weight for total body muscle, dissectible body fat or body skin (without subcutaneous fat), and from the sum of percentage values of the other organs prepared.

Values in *italics* were calculated from the values in the report

Table 6.2.2- 16: Time course of total radioactivity in excreta following oral administration of [pyridyl-2,6-^{14}C]fluopyram at a daily dose rate of 2.02 mg eq/kg body weight for 14 consecutive days

Matrix	Time after the first dosage (day)	Administration number	TRR (mg a.s. equiv./kg)
Excreta	0	1	-
	1	2	11.810
	2	3	15.694
	3	4	15.212
	4	5	13.591
	5	6	13.177
	6	7	13.650
	7	8	12.189
	8	9	11.672
	9	10	11.693
	10	11	12.411
	11	12	12.294
	12	13	12.277
	13	14	12.506
	14	-	11.889

The equivalent concentration of radioactivity in the eggs showed an increase from 0.047 mg eq/kg obtained on day 1 (24 hours after the first dosage) to 0.321 mg eq/kg at day 8. For details see Table 6.2.2- 17. Radioactivity concentrations decreased slowly after day 8 to 0.262 mg eq/kg at day 14. This showed that a residue plateau-level was clearly reached.

Table 6.2.2- 17: Time course of total radioactivity in eggs following oral administration of [pyridyl-2,6-^{14}C]fluopyram at a daily dose rate of 2.02 mg eq/kg body weight for 14 consecutive days

Matrix	Time after the first dosage (days)	Administration number	TRR (mg a.s. equiv./kg)
Eggs	0	1	-
	1	2	0.047
	2	3	0.093
	3	4	0.154
	4	5	0.177
	5	6	0.223
	6	7	0.272
	7	8	0.284
	8	9	0.323
	9	10	0.304
	10	11	0.293
	11	12	0.289
	12	13	0.282
	13	14	0.262
	14	-	0.262

Table 6.2.2- 18: Distribution of residues in eggs, tissues and organs of laying hens following oral administration of [pyridyl-2,6-^{14}C]fluopyram at a daily dose rate of 2.02 mg eq/kg body weight for 14 consecutive days

Matrix	Interval (day)	TRR (mg a.s. equiv./kg)
Liver (composite)	14	0.538
Kidney	14	0.242
Eggs from ovary/oviduct	14	0.831
Muscle, leg *	14	0.061
Muscle, breast *	14	0.035
Total body muscle	14	0.048
Skin without subcutaneous fat	14	0.152
Subcutaneous fat (composite)	14	0.498
Total body fat	14	0.498

mg a.s. equiv./kg: mg parent equivalents per kg matrix
* sample

In the composite egg pool (day 1–6) with conventional extraction, 53.6% of the radioactivity (0.084 mg eq/kg) was extracted with solvent extraction (acetonitrile/water). Following solid phase extraction purification, most of the radioactivity was contained in the combined sample passage and acetonitrile/water rinse solution ("purified acetonitrile/water extract": 49.9% or 0.078 mg eq/kg) and, after the concentration step, the concentrated acetonitrile/water extract contained 47.8% of the TRR (0.075 mg eq/kg). In total, 53.6% of the TRR (0.084 mg eq/kg) was extracted, whereas 46.4% of the TRR (0.072 mg eq/kg) remained non-extractable in solids (Table 6.2.2- 19).

In composite egg pool (day 1–6) with enzymatic treatment, 69.7% of the radioactivity (0.109 mg eq/kg) was extracted with solvent extraction (acetonitrile/water). Following solid phase extraction purification, most of the radioactivity was contained in the combined sample and acetonitrile/water rinse solution ("purified acetonitrile/water extract": 65.9% or 0.103 mg eq/kg) and, after the concentration step, the concentrated acetonitrile/water extract contained 57.7% of the TRR (0.090 mg eq/kg). In total, 69.7% of the TRR (0.109 mg eq/kg) was extracted, whereas 30.3% of the TRR (0.047 mg eq/kg) remained non-extractable in solids (Table 6.2.2- 19). With this procedure, the radioactivity remaining in the final solids was therefore below 0.05 mg/kg.

In the composite egg pool (day 7–14) with conventional extraction, 47.8% of the radioactivity (0.137 mg eq/kg) was extracted with solvent extraction (acetonitrile/water). Following solid phase extraction purification, the main radioactivity was contained in the purified acetonitrile/water extract (39.9% of the TRR, 0.114 mg eq/kg) and, after the concentration step, the concentrated acetonitrile/water extract contained 38.0% of the TRR (0.109 mg eq/kg).

After further extraction of the solids with acetonitrile/water (1/1, v/v) using a microwave and temperature gradient, the microwave extracts contained further 51.2% of the radioactivity (0.146 mg eq/kg) and only 1.0% (0.003 mg eq/kg) remained in the solids.

In total, 99.0% of the TRR (0.283 mg eq/kg) was extracted, whereas 1.0% of the TRR (0.003 mg eq/kg) remained non-extractable in solids (Table 6.2.2-20)

In the composite egg pool (day 7–14) with enzymatic treatment, 64.1% of the radioactivity (0.183 mg eq/kg) was extracted with solvent extraction (acetonitrile/water). Following solid phase extraction purification, the main radioactivity was contained in the purified acetonitrile/water extract (63.7% of the TRR, 0.182 mg eq/kg) and, after the concentration step, the concentrated acetonitrile/water extract contained 55.5% of the TRR (0.159 mg eq/kg). In total, 64.1% of the TRR (0.183 mg eq/kg) was extracted, whereas 35.9% of the TRR (0.103 mg eq/kg) remained non-extractable in solids (Table 6.2.2-20).

In muscle, the major part of the radioactive residues (59.3% of the TRR, 0.029 mg eq/kg) was extracted with acetonitrile/water. After solid phase extraction purification, the main radioactivity was contained in the purified acetonitrile/water extract (58.5% of the TRR, 0.029 mg eq/kg). After the first concentration step, the radioactivity in the concentrated extract amounted to 55.7% of the TRR (0.027 mg eq/kg) and to 54.6% of the TRR (0.027 mg eq/kg) in the second concentration step. In total, 59.3% of the TRR (0.029 mg eq/kg) was extracted, whereas 40.7% of the TRR (0.020 mg eq/kg) remained non-extractable in solids (Table 6.2.2-21).

In fat, the radioactive residues extractable with acetonitrile/water amounted to 71.1% of the TRR (0.353 mg eq/kg) and, after clean-up procedure by solid phase extraction, to 70.8% of the TRR (purified acetonitrile/water extract, 0.351 mg eq/kg). After the concentration step the radioactivity in the concentrated extract amounted to 69.6% of the TRR (0.345 mg eq/kg).

The radioactive residues extractable with *n*-heptane amounted to 28,1% of the TRR (0.139 mg eq/kg) and, after partition against acetonitrile, to 25.5% of the TRR (0.127 mg eq/kg) in the acetonitrile phase. After the concentration step, the radioactivity in the concentrated extract amounted to 25.5% of the TRR (0.127 mg eq/kg).

In total, 99.2% of TRR (0.492 mg eq/kg) was extracted and only 0.8% of the TRR (0.004 mg eq/kg) remained non-extractable in solids (Table 6.2.2- 22).

In liver with conventional extraction, 32.3% of the radioactivity (0.172 mg eq/kg) was extracted with solvent extraction (acetonitrile/water). Following solid phase extraction purification, the main radioactivity was contained in the purified acetonitrile/water extract (28.9% of the TRR, 0.154 mg eq/kg) and, after the concentration step, the concentrated acetonitrile/water extract contained 28.2% of the TRR (0.150 mg eq/kg).

In total, 32.3% of the TRR (0.172 mg eq/kg) was extracted, whereas 67.7% of the TRR (0.360 mg eq/kg) remained non-extractable in solids (Table 6.2.2- 23).

In liver with enzymatic treatment, 82.9% of the radioactivity (0.441 mg eq/kg) was extracted with solvent extraction (acetonitrile/water). Following solid phase extraction purification, the main radioactivity was contained in the purified acetonitrile/water extract (75.9% of the TRR, 0.404 mg eq/kg) and, after the concentration step, the concentrated acetonitrile/water extract contained 67.3% of the TRR (0.358 mg eq/kg).

After further extraction of the solids with acetonitrile/water (1/1, v/v) using a microwave and temperature gradient, the microwave extract contained further 7.4% of the radioactivity (0.039 mg eq/kg) and 9.7% (0.052 mg eq/kg) remained in the solids.

In total, 90.3% of the TRR (0.480 mg eq/kg) was extracted, whereas 9.7% of the TRR (0.052 mg eq/kg) remained non-extractable in solids (Table 6.2.2-20).

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Table 6.2.2- 19: Extraction of radioactive residues from the hen's egg pool (day 1–6) after dosing of [pyridyl-2,6-^{14}C]fluopyram at a daily dose rate of 2.02 mg eq/kg body weight for 14 consecutive days

	Egg pool (day 1–6)			
	Conventional extraction		Enzymatic treatment	
TRR, mg eq/kg	0.156			
Fraction	% TRR	mg a.s. equiv./kg	% TRR	mg a.s. equiv./kg
Solvent extract[1)]	53.6	0.084	69.7	0.109
Purification via RP18				
Purified acetonitrile/water extract	49.8	0.078	65.9	0.103
Concentration				
Concentrated acetonitrile/water extract	**47.8**	**0.075**	**57.7**	**0.090**
Distillate	2.1	0.003	8.2	0.013
Methanol/dichloromethane eluate	3.7	0.006	-	-
Solid phase	-	-	3.8	0.006
Solids	46.4	0.072	30.3	0.047
PES[2)]	46.4	0.072	30.3	0.047
Total extracted	53.6	0.084	69.7	0.109
Accountability / Total[3)]	100.0	0.156	100.0	0.156

1) Solvent extraction was done with 4x with acetonitrile/water (4/1, v/v).
2) PES: post extraction solids = non-extractable radioactivity.
3) Sum of extracts and PES.
Fractions given in **bold** font were analysed by radio-HPLC.

Table 6.2.2- 20: Extraction of radioactive residues from the hen's egg pool (day 7–14) after dosing of [pyridyl-2,6-^{14}C]fluopyram at a daily dose rate of 2.02 mg eq/kg body weight for 14 consecutive days

	Egg pool (day 7–14)			
	Conventional extraction		Enzymatic treatment	
TRR, mg eq/kg	0.286			
Fraction	% TRR	mg a.s. equiv./kg	% TRR	mg a.s. equiv./kg
Solvent extract[1)]	47.8	0.137	64.1	0.183
Purification via RP18				
Purified acetonitrile/water extract	39.9	0.114	*63.7*	*0.182*
Concentration				
Concentrated acetonitrile/water extract	**38.0**	**0.109**	**55.5**	**0.159**
Distillate	1.9	0.006	6.2	0.018
Concentrated Methanol/dichloromethane eluate	2.8	0.008	2.0	0.006
Solid phase	-	-	0.4	0.001
Solids	52.2	0.149	35.9	0.103
Microwave solvent extraction [2)]				
Microwave extract	51.2	0.146	-	-
Solids	1.0	0.003	-	-
PES[3)]	1.0	0.003	35.9	0.103
Total extracted	99.0	0.283	64.1	0.183
Accountability / Total[4)]	100.0	0.286	100.0	0.286

1) Solvent extraction was done with 4x with acetonitrile/water (4/1, v/v).
2) Microwave extraction was performed with acetonitrile/water (1/1, v/v) using a temperature gradient
3) PES: post extraction solids = non-extractable radioactivity 4) Sum of extracts and PES.
Fractions given in **bold** font were analysed by radio-HPLC; Values given in *italics* were calculated from the values in the report.

Table 6.2.2- 21: Extraction of radioactive residues from the hen's muscle after dosing of [pyridyl-2,6-^{14}C]fluopyram at a daily dose rate of 2.02 mg eq/kg body weight for 14 consecutive days

	Muscle (composite)	
TRR, mg eq/kg	0.049	
Fraction	% TRR	mg a.s. equiv./kg
Solvent extract[1)]	59.3	0.029
Purification via RP18		
Purified acetonitrile/water extract	58.3	0.029
Concentration and repeated purification via RP18		
Concentrated acetonitrile/water extract 1	55.7	0.027
Concentration		
Concentrated acetonitrile/water extract 2	**54.6**	**0.027**
Distillate	1.1	0.001
Concentrated Methanol/dichloromethane eluate	0.8	0.001
Distillate	1.8	0.001
Solid phase	-	-
Solid phase	-	-
Methanol/dichloromethane eluate	1.0	<0.001
Solids	40.7	0.020
PES[2)]	40.7	0.020
Total extracted	59.3	0.029
Accountability / Total[3)]	100.0	0.049

1) Solvent extraction was done with 1x acetonitrile and 2x with acetonitrile/water (4/1, v/v).
2) PES: post extraction solids = non-extractable radioactivity.
3) Sum of extracts and PES.
Fractions given in **bold** font were analysed by radio-HPLC.

Table 6.2.2- 22: Extraction of radioactive residues from the hen's fat after dosing of [pyridyl-2,6-^{14}C]fluopyram at a daily dose rate of 2.02 mg eq/kg body weight for 14 consecutive days

	Fat (composite)	
TRR, mg eq/kg	0.496	
Fraction	% TRR	mg a.s. equiv./kg
Acetonitrile/water extract[1)]	71.1	0.352
Purification via RP18		
Purified acetonitrile/water extract	70.8	0.351
Concentration		
Concentrated acetonitrile/water extract	**69.6**	**0.345**
Distillate	1.1	0.006
Solid phase	0.4	0.002
Methanol/dichloromethane eluate	-	
n-Heptane extract [1)]	28.1	0.140
Partition against acetonitrile		
Acetonitrile phase	25.5	0.127
Concentration		
Concentrated acetonitrile phase of the n-heptane extract	**25.5**	**0.127**
Distillate	-	-
n-Heptane phase	2.6	0.013
Solids	0.8	0.004
PES[2)]	0.8	0.004
Total extracted	99.2	0.492
Accountability / Total[3)]	100	0.496

1) Solvent extraction was done 2x with n-heptane and acetonitrile/water (4/1, v/v) the n-heptane and the acetonitrile/water phases were each separated.
2) PES: post extraction solids = non-extractable radioactivity.
3) Sum of extracts and PES.

Fractions given in **bold** font were analysed by radio-HPLC.

Table 6.2.2- 23: Extraction of radioactive residues from the hen's liver after dosing of [pyridyl-2,6-^{14}C]fluopyram at a daily dose rate of 2.02 mg eq/kg body weight for 14 consecutive days

	Liver (composite)			
	Conventional extraction		Enzymatic treatment	
TRR, mg eq/kg	0.532			
Fraction	% TRR	mg a.s. equiv./kg	% TRR	mg a.s. equiv./kg
Solvent extract[1)]	32.3	0.172	82.9	0.441
Purification via RP18				
Purified acetonitrile/water extract	28.9	0.154	75.9	0.404
Concentration				
Concentrated acetonitrile/water extract	**28.2**	**0.150**	**67.3**	**0.358**
Distillate	0.7	0.004	8.6	0.046
Solid phase	0.7	0.004	1.4	0.007
Concentrated Methanol/dichloromethane eluate	2.7	0.014	5.7	0.030
Solids	67.7	0.360	17.1	0.091
Microwave solvent extraction [2)]				
Microwave extract	-	-	7.4	0.039
Solids	-	-	9.7	0.052
PES [3)]	67.7	0.360	9.7	0.052
Total extracted	32.3	0.172	90.3	0.480
Accountability / Total [4)]	100.0	0.532	100.0	0.532

1) Solvent extraction was done with 4x with acetonitrile/water (4/1, v/v).
2) Microwave extraction was performed 2x with acetonitrile/water (1/1, v/v) using a temperature gradient
3) PES: post extraction solids = non-extractable radioactivity.
4) Sum of extracts and PES.
Fractions given in **bold** font were analysed by radio-HPLC

For structure elucidation, all acetonitrile/water extracts and *n*-heptane extracts were analysed by HPLC with radiodetection. Metabolites were identified by co-chromatography with authentic reference compounds.

In egg pool 1–6 days, the main compound was the parent amounting for 14.7% - 17.9% of the TRR (0.023 mg eq/kg - 0.028 mg eq/kg with conventional extraction and enzymatic treatment, respectively). The main metabolite in the conventionally extracted eggs was fluopyram-pyridyl-acetic acid (PAA, M40), representing 6.4% of the TRR (0.010 mg eq/kg). The main metabolite with enzymatic treatment was fluopyram-7-hydroxy (M08) (5.8% of the TRR or 0.009 mg eq/kg), which also amounted to 3.9% of the TRR (0.006 mg eq/kg) in the conventionally extracted eggs. fluopyram-*Z*-olefin was found in equal amounts in eggs extracted conventionally and after enzymatic treatment (4.1% of the TRR or 0.006 mg eq/kg), while fluopyram-*E*-olefin was only found in low amounts in conventionally extracted eggs (1.0% of the TRR or 0.001 mg eq/kg). In total 29.8% (enzymatic) - 30.2% (conventional) of the TRR was identified for egg pool day 1–6 (Table 6.2.2- 24).

In egg pool 7–14 days, the parent compound amounted to 6.0% - 9.5% of the TRR (0.017 mg eq/kg to 0.027 mg eq/kg with conventional extraction and enzymatic treatment, respectively). The main compound was fluopyram-*Z*-olefin and amounted to 15.4% (0.004 mg eq/kg) 19.3% (0.055 mg eq/kg) of the TRR with conventional extraction and enzymatic treatment, respectively. fluopyram-pyridyl-acetic acid (PAA, M40) was found only in conventionally extracted eggs at 3.7% of the TRR (0.011 mg eq/kg). fluopyram-7-hydroxy (M08) and fluopyram-*E*-olefin were found in low amounts in egg pool 7–14 days at less than 2% of the TRR (≤ 0.005 mg eq/kg). In total, 26.9% (enzymatic) 32.2% (conventional) of the TRR was identified egg pool day 7–14 (Table 6.2.2- 25).

Due to a high amount of matrix components and the low concentration of radioactivity, no HPLC profile of the microwave extracts could be recorded. However a total extraction efficiency of approx. 99% and 90% of the TRR was achieved for the eggs (7-14 days). Though representing a higher amount of the TRR, the profile of the extract after enzymatic digestion showed neither significant amounts of further metabolites nor different amounts of metabolites compared to the profile of the conventional extract. This indicates, that the radioactivity released by microwave extraction from the solids did not consist of any further unknown metabolites.

In muscle, fluopyram-*Z*-olefin was the major component (33.0% of the TRR or 0.016 mg eq/kg). The only other components detected were fluopyram-*E*-olefin at 3.9% (0.002 mg eq/kg) and the parent compound accounting for only 1.0% (0.001 mg eq/kg). In total 37.9% of the TRR was identified (Table 6.2.2- 26).

In fat fluopyram-*Z*-olefin was also the major component with 70.5% of the TRR (0.350 mg eq/kg), followed by both fluopyram-*E*-olefin (12.4% of the TRR or 0.062 mg eq/kg) and the parent compound (12.2% of the TRR or 0.061 mg eq/kg). In total 95.2% of the TRR was identified (Table 6.2.2- 27).

In liver, fluopyram-*E*-olefin was the major component accounting for 11.8%–13.9% of the TRR (0.063 mg eq/kg–0.074 mg eq/kg, with conventional extraction and enzymatic treatment, respectively). In liver pool with enzymatic treatment, fluopyram-*Z*-olefin accounted for 3.1% of the TRR (0.017 mg eq/kg) and fluopyram-7-hydroxy (M08) accounted for 2.9% of the TRR (0.016 mg eq/kg). In liver pool with conventional extraction fluopyram-pyridyl-acetic acid (PAA, M40), fluopyram-7-hydroxy and fluopyram-*Z*-olefin accounted each for < 2% of the TRR (≤ 0.01 mg eq/kg).

In total 15.7% -20.0% of the TRR was identified with conventional extraction and enzymatic treatment, respectively (Table 6.2.2-28).

Due to a high amount of matrix components and the low concentration of radioactivity, no HPLC profile of the microwave extracts could be recorded. However, a total extraction efficiency of approx. 90% of the TRR was achieved for liver.

Table 6.2.2- 24: Summary of identification and characterization of radioactive residues in the hen's egg pool (day 1–6) after dosing of [pyridyl-2,6-^{14}C] fluopyram at a daily dose rate of 2.02 mg eq/kg body weight for 14 consecutive days

	Egg pool (day 1–6)			
TRR [mg eq/kg] =	0.156			
	Conventional extraction		Enzymatic treatment	
Compound	% of TRR	mg a.s. equiv./kg	% of TRR	mg a.s. equiv./kg
AE C656948, fluopyram	14.7	0.023	17.9	0.028
fluopyram-pyridyl-acetic acid (M40)	6.4	0.010	-	-
fluopyram-7-hydroxy (M08)	3.9	0.006	5.8	0.009
fluopyram-*E*-olefin (M02)	1.0	0.001	-	-
fluopyram-*Z*-olefin (M03)	4.1	0.006	4.1	0.006
Total identified	**30.2**	**0.047**	**27.8**	**0.043**
Sum of unknowns	17.6	0.028	29.9	0.047
Analysed extract(s)	47.8	0.075	57.7	0.090
Volatiles in distillates	2.1	0.003	8.2	0.013
Microwave extracts	-	-	-	-
Extracts not analysed	3.7	0.006	3.8	0.006
Total extractable	53.6	0.084	69.7	0.109
Unextractable residues (solids)	46.4	0.072	30.3	0.047
Accountability	100.0	0.156	100.0	0.156

Table 6.2.2- 25: Summary of identification and characterization of radioactive residues in the hen's egg pool (day 7–14) after dosing of [pyridyl-2,6-^{14}C] fluopyram at a daily dose rate of 2.02 mg eq/kg body weight for 14 consecutive days

	Egg pool (day 7–14)			
TRR [mg eq/kg] =	0.286			
	Conventional extraction		Enzymatic treatment	
Compound	% of TRR	mg a.s. equiv./kg	% of TRR	mg a.s. equiv./kg
AE C656948, fluopyram	6.0	0.017	9.5	0.027
fluopyram-pyridyl-acetic acid (M40)	3.7	0.011	-	-
fluopyram-7-hydroxy (M08)	0.9	0.002	1.6	0.005
fluopyram-*E*-olefin (M02)	1.0	0.003	1.8	0.005
fluopyram-*Z*-olefin (M03)	15.4	0.044	19.3	0.055
Total identified	**26.9**	**0.077**	**32.2**	**0.092**
Sum of unknowns	11.1	0.032	23.5	0.067
Analysed extract(s)	38.0	0.109	55.5	0.159
Volatiles in distillates	1.9	0.006	6.2	0.018
Microwave extracts	51.2	0.146	-	-
Extracts not analysed	7.9	0.023	2.4	0.007
Total extractable	99.0	0.283	64.1	0.183
Unextractable residues (solids)	1.0	0.003	35.9	0.103
Accountability	100.0	0.286	100.0	0.286

Table 6.2.2- 26: Summary of identification and characterization of radioactive residues in hen's muscle after dosing of [pyridyl-2,6-^{14}C] fluopyram at a daily dose rate of 2.02 mg eq/kg body weight for 14 consecutive days

	Muscle pool	
TRR [mg/kg] =	0.049	
Compound	% of TRR	mg a.s. equiv./kg
AE C656948, fluopyram	1.0	0.001
fluopyram-pyridyl-acetic acid (M40)	-	-
fluopyram-7-hydroxy (M08)	-	-
fluopyram-*E*-olefin (M02)	3.9	0.002
fluopyram-*Z*-olefin (M03)	33.0	0.016
Total identified	**37.9**	**0.019**
Sum of unknowns	16.7	0.008
Analysed extract(s)	54.6	0.027
Volatiles in distillates	2.9	0.001
Microwave extracts	-	-
Extracts not analysed	1.8	0.001
Total extractable	59.3	0.029
Unextractable residues (solids)	40.7	0.020
Accountability	100.0	0.049

Table 6.2.2- 27: Summary of identification and characterization of radioactive residues in hen's fat after dosing of [pyridyl-2,6-^{14}C] fluopyram at a daily dose rate of 2.02 mg eq/kg body weight for 14 consecutive days

	Fat pool	
TRR [mg/kg] =	0.496	
Compound	% of TRR	mg a.s. equiv./kg
AE C656948, fluopyram	12.2	0.061
fluopyram-pyridyl-acetic acid (M40)	-	-
fluopyram-7-hydroxy (M08)	-	-
fluopyram-*E*-olefin (M02)	12.4	0.062
fluopyram-*Z*-olefin (M03)	70.5	0.350
Total identified	**95.2**	**0.472**
Sum of unknowns	0.0	0.000
Analysed extract(s)	95.2	0.472
Volatiles in distillates	1.4	0.006
Microwave extracts	-	-
Extracts not analysed	2.9	0.014
Total extractable	99.2	0.492
Unextractable residues (solids)	0.8	0.004
Accountability	100.0	0.496

Table 6.2.2-28: Summary of identification and characterization of radioactive residues in hen's liver after dosing of [pyridyl-2,6-^{14}C] fluopyram at a daily dose rate of 2.02 mg eq/kg body weight for 14 consecutive days

	Liver			
TRR [mg/kg] =	0.532			
	Conventional extraction		Enzymatic treatment	
Compound	% of TRR	mg a.s. equiv./kg	% of TRR	mg a.s. equiv./kg
AE C656948, fluopyram	-	-	-	-
fluopyram-pyridyl-acetic acid (M40)	1.3	0.007	-	-
fluopyram-7-hydroxy (M08)	0.8	0.004	2.9	0.016
fluopyram-*E*-olefin (M02)	11.8	0.063	13.9	0.074
fluopyram-*Z*-olefin (M03)	1.9	0.010	3.1	0.017
Total identified	**15.7**	**0.084**	**20.0**	**0.106**
Sum of unknowns	12.5	0.066	47.3	0.252
Analysed extract(s)	28.2	0.150	67.3	0.358
Volatiles in distillates	0.7	0.004	8.6	0.046
Microwave extracts	-	-	7.4	0.039
Extracts not analysed	3.4	0.018	7.0	0.037
Total extractable	32.3	0.172	90.3	0.480
Unextractable residues (solids)	67.7	0.360	9.7	0.052
Accountability	100.0	0.532	100.0	0.532

III. Conclusion

Six laying hens were dosed orally for 14 consecutive days with [pyridyl-2,6-^{14}C]fluopyram at a daily dose of 2.02 mg eq/kg of body weight and sacrificed 24 hours after the last dose.

The major component and metabolite in eggs, muscle and fat was fluopyram-*Z*-olefin (up to 70.5% of the TRR). The other isomer fluopyram-*E*-olefin was the major metabolite in liver (up to 13.9% of the TRR) and represented 1.0% to 12.4% of the TRR in the other matrices. Parent compound fluopyram was detected at up to 17.9% of the TRR. Further detected minor metabolites were fluopyram-7-hydroxy and fluopyram-pyridyl-acetic acid found in eggs and liver at up to 6.4% of the TRR.

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

- hydroxylation of the aliphatic chain followed by elimination
- oxidative cleavage of the aliphatic chain

The metabolic pathway is proposed in Figure 6.2.2-2.

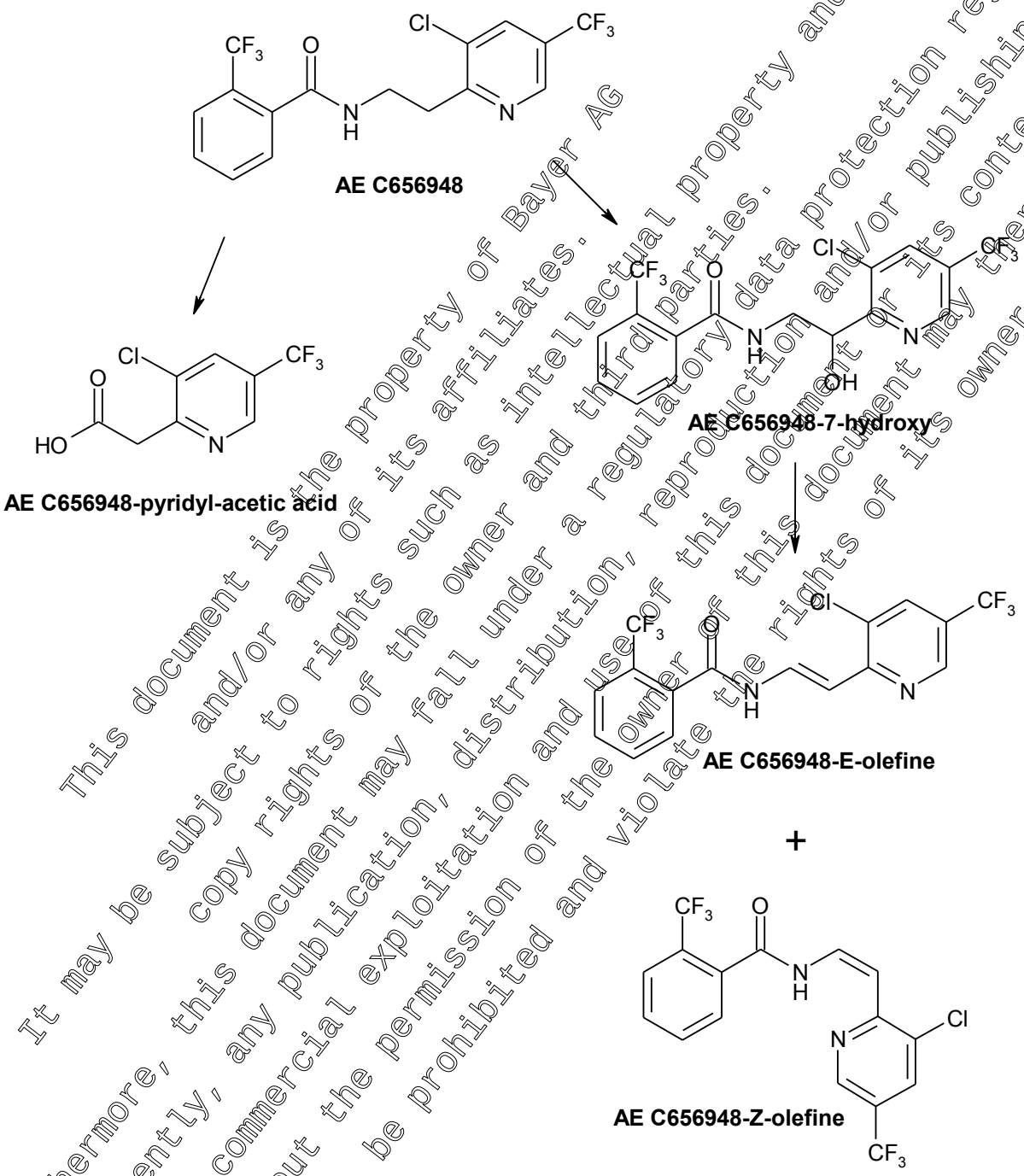


Figure 6.2.2-2: Proposed metabolic pathway of AE C656948 (fluopyram) in hen after treatment with [pyridyl-2,6-^{14}C] fluopyram

Assessment and conclusion by applicant:

The study is valid and acceptable.

CA 6.2.3 Lactating ruminants

Data to address this point were presented in the dossier submitted for first inclusion in Annex and were deemed acceptable following evaluation and peer review at EU level (2013).

For details of data submitted previously please refer also to the Baseline dossier CA 6.2. For completeness, a summary of these previously submitted studies are included below.

Data already evaluated during the first EU renew process for inclusion on Annex I (no new studies)

Data Point:	IIA 6.2.3/01
Report Author:	[redacted]
Report Year:	2008
Report Title:	Metabolism of [phenyl-UL-14C]-C65048 in the lactating goat
Report No:	MEF-08/019
Document No:	M-299111-01-1
Guideline(s) followed in study:	US EPA Residue Chemistry Test Guidelines OPPTS 860.1300 Nature of the Residue – Plants, Livestock PMRA Ref.: DACO 6.2 Metabolism in Livestock EU Council Directive 91/414/EEC as amended by the Commission Directive 96/68/EC
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted refer to Vol 3 of DAR BG August 2012 (references relied on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The metabolism of [phenyl-UL-^{14}C]fluopyram was investigated in one lactating goat, which was orally dosed for 5 consecutive days at a rate of 1.91 mg a.s./kg of body weight per day corresponding to 46.26 mg a.s./kg feed/day. The goat was sacrificed 24 h after the fifth administration. Radioactivity was measured in the excreta, milk and plasma collected at timed sampling intervals, as well as in the liver, kidney, muscle and fat at sacrifice. The milk, edible organs and tissues and excreta (urine and faeces) were

analysed for parent compound and metabolites by extraction, chromatographic separation techniques and spectroscopic methods.

Until sacrifice, the excretion amounted to 88.31% of the totally administered radioactivity. An amount of 0.56% of the total dose was found in milk. At sacrifice, the calculated total residue in the tissues and organs dissected from the body was approximately 4.58% of the total dose. About half of this amount was detected in the muscle (2.31%).

The mean active substance equivalent concentrations in plasma increased from 0.189 mg eq/kg at 24 h to 0.720 mg eq/kg at 120 h. Also mean active substance equivalent concentrations in milk increased from 24 h to 120 h. This showed that a residue plateau-level was not yet reached.

At sacrifice, highest mean equivalent concentrations occurred in liver (8.379 mg eq/kg). These values were followed in decreasing order by the mean concentrations determined in the kidney (2.295 mg eq/kg), total body muscle (0.737 mg eq/kg) and total body fat (0.399 mg eq/kg). The radioactive residues were extracted with efficiencies of 91.8–99.5% from milk, edible organs or tissues and excreta with acetonitrile/water mixtures and n-heptane, and identification was achieved by LC-MS/MS, co-chromatography or comparison of profiles with those from the lactating goat study with [pyridyl-2,6-14C]fluopyram.

Fluopyram was extensively metabolised, but was still the major compound in faeces (13.18% of the dose) and represented 0.4%–18% of the TRR in the other matrices except muscle. The label-specific metabolite fluopyram-benzamide (M25) was the main compound in milk and edible organs and tissues, accounting for 49.1–97.6% of the TRR, but accounted for <5% of the dose in excreta. Fluopyram-Z-olefin (M03) was found in significant amounts in goat fat (13.1% of the TRR) and fluopyram-*E*-olefin (M02) was found at 8.6% of the TRR in fat. Other minor metabolites accounted for < 5% of the TRR in milk and edible organs and tissues.

Further relevant metabolites were fluopyram-7-OH-GA (isomer 1) and fluopyram-8-OH-GA (isomer 2) found in urine at amounts of 17.18% and 15.75% of the dose, respectively. Furthermore, in faeces fluopyram-7-hydroxy accounted for 10.32% of the dose. Other minor metabolites accounted for < 5% of the dose in excreta.

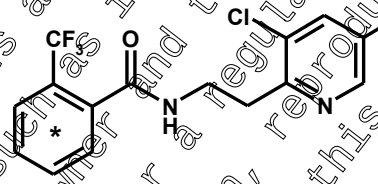
Based on the identified metabolites, the following metabolic routes were deduced:

- hydroxylation of the ethylene bridge of the molecule resulting in fluopyram-7-hydroxy, fluopyram-8-hydroxy, and a dihydroxylated compound
- hydroxylation of the phenyl ring leading to fluopyram-phenol
- conjugation of the hydroxylated metabolites with glucuronic acid
- elimination of water from compounds hydroxylated in the ethylene bridge leading to fluopyram-*Z*-olefin and *E*-olefin
- molecular cleavage to fluopyram-benzamide
- hydroxylation of fluopyram-benzamide followed by conjugation with sulphate

I. Materials and Methods

A. Materials

1. Test Material:

IUPAC Name	N-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]ethyl}-2-(trifluoromethyl)benzamide
CAS Name	Benzamide, N-[2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-(trifluoromethyl)- (9Cl)
Code name	AE C656948
Common name	Fluopyram
Empirical formula	$C_{16}H_{11}ClF_6N_2O$
CAS Number	658066-35-4
Molar mass	396.72 g/mol
Chemical structure	 * position of the ^{14}C radiolabel
Radiolabelled test material	[phenyl-UL-^{14}C]AE C656948
Batch number	BECH 2013 (radiolabelled) RFC633-04 (non-radiolabelled)
Original specific radioactivity	5.15 MBq/mg = 139.19 µCi/mg
Radiochemical purity	> 99.8% (HPLC)
Chemical purity (non-radiolabelled)	99.8% (potentiometric titration)
Specific radioactivity after radiodilution used for the study	1.98 MBq/mg = 1.188 x 108 dpm/mg = 53.5 µCi/mg = 21.22 Ci/mol
Stability of test compound	11 days at -18 °C

2. Test Animals

Species	Goat (*Capra hircus*)
Breed	"Bunte deutsche Edelziege"
Breeding facility	[redacted]
Sex, number	One female lactating goat
Body weight	36 kg at test start
Age	*ca.* 32 months old at experiment start
Acclimatization	8 days
Housing	Stainless steel metabolism cage, approx. 18–23 °C, 45–5% rel. humidity, 12/12 hours light/dark cycle, air changes: 10–15 changes/h
Identification	Skin marking
Feed and water	Ruminant feed, hay, apples (ad libitum) Tap water *ad libitum*
Health status	Acceptable

B. Study Design

Preparation of the dosing mixtures and administration:

The radiolabelled test compound (certified specific radioactivity: 5.15 MBq/mg) was radiodiluted with the non-radiolabelled test item to a specific radioactivity of 1.98 MBq/mg. In total, five gelatine capsules containing 66 mg each of the radiodiluted solid test item were prepared immediately before the first administration and stored at -18 °C. In relation to a body weight of 36 kg, this amount of radioactivity corresponded to an actual dose of 1.91 mg a.s./kg body weight (bw). Based on the experimentally determined food consumption this corresponds to 46.26 mg a.s./kg feed/day.

The goat received daily one gelatine capsule administered orally using a capsule applicator. The administration was performed on five consecutive days in the morning after milking.

The radiolabelled parent compound was proved to be stable for at least eleven days at -18 °C as shown by radio-HPLC analysis, and thus for the time from preparation of the gelatine capsules until dosing. The evaluation of the chromatogram revealed a radiochemical purity of > 99%. The identity of the test compound was confirmed by LC-MS/MS.

Sampling:

Collection and processing of blood

Micro-samples of blood were taken from the ear veins at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 24, 32, 48, 56, 72, 80, 96, 104 and 120 hours after starting the experiment. The blood was collected in heparinized capillaries which were centrifuged afterwards using a haematocrit centrifuge for separation of blood cells and plasma. The plasma samples were weighed and prepared for radioactivity measurement by LSC.

Collection and processing of milk

The goat was milked in the morning immediately prior to each administration, about 8 hours later in the afternoon, and directly before sacrifice (time schedule: 8, 24, 32, 48, 56, 72, 80, 96, 104, and 120 hours after the first administration). After weighing, one aliquot was taken from each sample for radioactivity measurement by LSC.

Collection and processing of urine

Urine samples were collected in plastic vessels as quantitatively as possible under dry ice cooling in intervals of 24 hours after each administration. The vessels were changed immediately before the next administration. The collection funnel was rinsed with deionised water into the same urine vessel of the respective collection period. After recording the total volumes, one aliquot was taken from each sample for radioactivity measurement by LSC.

Collection and processing of faeces

Faeces samples were collected as quantitatively as possible at room temperature in intervals of 24 hours after each administration, i.e. immediately before the next administration. The collecting grid was cleaned prior to each administration. No samples of the rinsing water were taken for radioactivity measurement. Each faeces fraction was homogenized after addition of water to get a wet paste before the total weight was recorded. One aliquot of each wet sample was weighed and prepared for radioactivity measurement by combustion/LSC.

Sacrifice and collection of organs and tissues

For the collection of organs and tissues, the animal was sacrificed *ca.* 24 hours after the fifth administration, a time distance that is consistent with normal slaughtering practices. The animal was anaesthetised by an intravenous dose of about 40 mg eq/kg bw Pentobarbital-Na (Narcoren®), exsanguinated by cannulating the jugular veins and sacrificed by intracardiac injection with ca. 10 mL of the sacrificing agent "T 61®". The following organs and tissues were dissected: muscle (round and loin), fat (omental and perirenal), liver without gall bladder, kidneys, and the gall bladder.
The organs or tissue samples were then transferred into tarred plastic vessels and after determination and recording of the individual weights, liver, kidneys, fat and muscle samples were passed several times through a mincing machine in half-frozen state. One aliquot of each resulting tissue pulp was weighed and prepared for radioactivity measurement by combustion/LSC.

The gall bladder was punctured for the collection of the bile fluid that was stored in a freezer for the (optional) metabolite analysis.

C. Analytical Procedures

Extraction and fractionation:

Samples of milk and edible tissues were extracted three times with acetonitrile/water mixtures (*ca.* 8/2, v/v). The combined extracts were applied to C18 SPE cartridges to remove the lipid fraction of the matrix. The SPE percolate and the column wash with acetonitrile/water were combined and concentrated to the aqueous remainder for HPLC analysis. After the wash step, the SPE cartridge was eluted with dichloromethane/methanol to recover very lipophilic radioactivity, if present. All eluates were discarded since no significant amount of radioactivity was detected.

For muscle, liver, and kidney an additional solvent step was performed with acetonitrile/water (1/1, v/v) under microwave assistance at 120 °C. The acetonitrile/water extracts from microwave assisted extraction were concentrated to the aqueous remainder and then analysed by HPLC.

An alternative extraction of muscle, liver, and kidney was conducted under the conditions of the residue analytical method developed for analysis of sample materials from a dairy cow feeding study (denoted as "residue method"). The samples were homogenised with acetonitrile/water (8/2; v/v) using a Polytron Typ PT 6000 homogenizer and then extracted twice in a microwave oven for 30 min at 120 °C. Extracts and solids were separated each time by centrifugation.

The combined extracts were purified by SPE on C18 cartridges and then concentrated for HPLC profiling.

Analytical methods:

The radioactivity of all liquid samples was measured using a liquid scintillation counter after mixing a known amount of each sample with scintillation fluid. Solid samples were combusted using an oxidizer. The resulting $^{14}CO_2$ was trapped in an alkaline scintillation cocktail. The TRR in the samples was calculated by summing up the radioactivity measured in different extracts and remaining solids after solvent extraction. The TRR was expressed in mg a.s. equivalents per kg sample weight. Amounts of radioactive residues in the extracts were expressed as percentage of the TRR and also as mg a.s. equivalents per kg sample weight.

Aliquots of all extracts were analysed by HPLC. Nine metabolites as well as parent compound were identified by LC-MS/MS, HPLC co-chromatography and comparison of profiles with those from the lactating goat study with [pyridyl-2,6-14C]fluopyram.

The HPLC was conducted using a reversed-phase column and a buffered acetonitrile/water gradient. The HPLC system was equipped with a radiodetector and a UV detector with variable wavelengths. The LOQ was derived from the average background level and the specific radioactivity of the radiolabelled test compound.

Storage stability

Extraction and first HPLC-analyses for quantification of extracts from morning milk, evening milk, muscle, fat, liver and kidney were performed within 6 weeks after sacrifice of the goat.

A second extraction and analysis of muscle, liver, and kidney with the procedure of the residual analytical method was conducted between ca. 3–3.5 months after sacrifice. These second extractions showed very similar extraction rates as the extractions procedures for the first quantification. The metabolic profiles were also very similar to profiles of the first analyses.

All experimental work in the study for extraction, quantification, and identification of residues was completed within less than 4 months.

An additional experiment for solvent extraction of muscle under modified conditions was conducted *ca.* 8 months after sacrifice. In this experiment only the balance for radioactivity was determined.

Extracts were measured by HPLC for quantification of residues within two to six days after start of the extraction, i.e. normally on the day when the sample preparation was completed.

Investigations on storage stability of radioactive residues in edible samples were therefore not required.

II. Results and Discussion

The metabolism in lactating goat of [phenyl-UL-^{14}C]fluopyram administrated at a daily dose of 46.26 mg eq/kg in the feed corresponding to a daily intake of 1.91 mg eq/kg bw for 5 consecutive days was investigated.

Until sacrifice 24 hours after the last dose, the mean excretion amounted on average to 88.31% of the radioactivity totally administered (Table 6.2.3-1). The time course of the excretion was characterised by a relatively constant rate starting at day 4 until test end (Table 6.2.3-2). Only 0.56% of the dose totally administered was measured in the milk secreted within the whole test period. At sacrifice, 24 h after the last administration, the total residue in the tissues and organs dissected from the body was calculated and estimated to about 4.58% of the total dose. Based on these values, the recovery amounted to 93.46% (Table 6.2.3-1).

The TRR-values in plasma, determined over the whole testing period in timed sampling intervals increased continuously until the test end (Table 6.2.3-3). During the 8-hour period after each dosing, a significant increase was observed. In the following 16-hour time range until delivery of the next dose the TRR-values increased or decreased only slightly. This indicated an ongoing absorption of the test item, a rapid distribution of the systemically bioavailable compound-related radioactivity within the body and a delayed excretion. A plateau level of plasma concentration was not reached during the observation period.

The total radioactive residues were determined in milk, faeces and urine produced from the first dose until sacrifice and in edible tissues and organs at sacrifice. The highest mean active substance equivalent concentrations were measured in liver (8.379mg eq/kg) and kidney (2.295 mg eq/kg). These values were

followed in decreasing order by total body muscle (0.737 mg eq/kg) and total body fat (0.399 mg eq/kg). For details see Table 6.2.3-5.

Table 6.2.3-1: Recovery of radioactivity following oral administration of [phenyl-UL-^{14}C]fluopyram at a daily dose rate of 1.91 mg eq/kg body weight for 5 consecutive days

Matrix	Recovery of radioactivity (% of the totally administrated radioactivity)
Excreta[1)]	88.31
Milk[2)]	0.56
Totally excreted	*88.87*
Tissues	4.58
Recovery	93.46

1) Recovery in excreta obtained by adding recovery values of samples collected within the observation period of 5 days
2) Recovery in milk calculated by adding recovery values of samples produced within the observation period of 5 days

Values in *italics* were calculated from the values in the report.

Table 6.2.3-2: Time course of total radioactivity in excreta following oral administration of [phenyl-UL-^{14}C]fluopyram at a daily dose rate of 1.91 mg eq/kg body weight for 5 consecutive days

Matrix	Time after the first dosage (hours)	Administration number	Excretion per day (% of totally administrated radioactivity)
Urine	24	2	5.52
	48	3	11.73
	72	4	9.13
	96	5	13.51
	120	Sacrifice	12.69
Faeces	24	2	2.36
	48	3	10.99
	72	4	7.71
	96	5	6.07
	120	Sacrifice	8.55

The equivalent concentration of radioactivity in the milk showed an increase from 0.009 mg eq/kg (morning milk) or 0.029 mg eq/kg (evening milk) obtained on day 1 (24 hours after the first dosage) to 0.057 mg eq/kg (morning milk) or 0.105 mg eq/kg (evening milk) at day 5. For details see Table 6.2.3-4.

Table 6.2.3-3: **Time course of total radioactivity in plasma following oral administration of [phenyl-UL-^{14}C]fluopyram at a daily dose rate of 1.91 mg eq/kg body weight for 5 consecutive days**

Matrix	Time after the first dosage (hours)	Administration number	[mg eq/kg plasma]
Plasma	0.25	1	0.012
	0.5		0.016
	1		0.054
	2		0.098
	3		0.112
	4		0.107
	6		0.113
	8		0.117
	24	2	0.189
	32		0.348
	48	3	0.364
	56		0.473
	72	4	0.459
	80		0.577
	96	5	0.629
	104		0.716
	120		0.720

Table 6.2.3-4: Time course of total radioactivity in milk following oral administration of [phenyl-UL-^{14}C]fluopyram at a daily dose rate of 1.91 mg eq/kg body weight for 5 consecutive days

Matrix	Time after the first dosage (hours)	Administration number	Secretion per period (% of totally administrated radioactivity)
Milk	8	1	0.009
	24		0.020
	32	2	0.026
	48		0.060
	56	3	0.039
	72		0.088
	80	4	0.049
	96		0.103
	104	5	0.055
	120		0.105

Table 6.2.3-5: Distribution of residues in tissues and organs of lactating goats following oral administration of [phenyl-UL-^{14}C]fluopyram at a daily dose rate of 1.91 mg eq/kg body weight for 5 consecutive days

Matrix	Interval (day)	TRR (mg a.s. equiv./kg)
Liver	5	8.379
Kidney	5	2.295
Total body muscle	5	0.737
Total body fat	5	0.399

mg a.s. equiv./kg: mg parent equivalents per kg matrix

In milk pool, 99.4–99.5% of the TRR (0.227–0.275 mg eq/kg) was extracted with solvent extraction (acetonitrile/water). Following degreasing by SPE, most of the radioactivity was contained in the percolate and wash (98.4–99.0% of the TRR or 0.224–0.273 mg eq/kg) and, after the concentration step, the aqueous remainder contained 96.8–97.9% of the TRR (0.223–0.273 mg eq/kg). In total, 99.4–99.5% of the TRR (0.227–0.275 mg eq/kg) was extracted, whereas 0.5–0.6% of the TRR (0.001 mg eq/kg) remained non-extractable in solids (Table 6.2.3-6).

In goat muscle with conventional extraction, 67.7% of the TRR (0.499 mg eq/kg) was extracted with solvent extraction (acetonitrile/water). Following degreasing by SPE, most of the radioactivity was

contained in the percolate and wash (67.5% of the TRR or 0.497 mg eq/kg) and, after the concentration step, the aqueous remainder contained all of the radioactivity.

After further extraction of the solids with acetonitrile/water (1/1; v/v) using a microwave at 120 °C, the microwave extract contained further 31.7% of the radioactivity (0.234 mg eq/kg). After the concentration step, the aqueous remainder contained 31.4% of the radioactivity (0.231 mg eq/kg).

In total, 99.4% of the TRR (0.733 mg eq/kg) was extracted, whereas 0.6% of the TRR (0.004 mg eq/kg) remained non-extractable in solids (Table 6.2.3-7).

In goat muscle extracted by residue method, 96.6% of the TRR (0.712 mg eq/kg) was extracted with solvent extraction (acetonitrile/water). Following degreasing by SPE, most of the radioactivity was contained in the percolate and wash (96.3% of the TRR or 0.710 mg eq/kg) and, after the concentration step, the aqueous remainder contained all of the radioactivity.

In total, 96.6% of the TRR (0.712 mg eq/kg) was extracted, whereas 3.4% of the TRR (0.025 mg eq/kg) remained non-extractable in solids (Table 6.2.3-7).

In goat fat, 96.9% of the radioactivity (0.387 mg eq/kg) was extracted with acetonitrile/water, while the n-heptane phase contained only 2.3% of the TRR (0.009 mg eq/kg). Following degreasing of the acetonitrile/water phase by SPE, most of the radioactivity was contained in the percolate and wash (96.7% of the TRR or 0.386 mg eq/kg) and, after the concentration step, the aqueous remainder contained all of the radioactivity.

In total, 99.2% of the TRR (0.396 mg eq/kg) was extracted, whereas 0.8% of the TRR (0.003 mg eq/kg) remained non-extractable in solids (Table 6.2.3-8).

In goat liver with conventional extraction, 76.6% of the TRR (6.416 mg eq/kg) was extracted with solvent extraction (acetonitrile/water). Following degreasing by SPE, most of the radioactivity was contained in the percolate and wash (76.5% of the TRR or 6.409 mg eq/kg) and, after the concentration step, the aqueous remainder contained 76.2% of the TRR (6.384 mg eq/kg).

After further extraction of the solids with acetonitrile/water (1/1; v/v) using a microwave at 120 °C, the microwave extract contained further 17.4% of the TRR (1.456 mg eq/kg). After the concentration step, the aqueous remainder contained 17.3% of the TRR (1.449 mg eq/kg).

In total, 94.0% of the TRR (7.872 mg eq/kg) was extracted, whereas 6.0% of the TRR (0.507 mg eq/kg) remained non-extractable in solids (Table 6.2.3-9).

In goat liver extracted by residue method, 93.6% of the TRR (7.846 mg eq/kg) was extracted with solvent extraction (acetonitrile/water). Following degreasing by SPE, most of the radioactivity was contained in the percolate and wash (93.3% of the TRR or 7.814 mg eq/kg) and, after the concentration step, the aqueous remainder contained all of the radioactivity.

In total, 93.6% of the TRR (7.846 mg eq/kg) was extracted, whereas 6.4% of the TRR (0.533 mg eq/kg) remained non-extractable in solids (Table 6.2.3-9).

In goat kidney with conventional extraction, 68.6% of the TRR (1.573 mg eq/kg) was extracted with solvent extraction (acetonitrile/water). Following degreasing by SPE, all of the radioactivity was contained in the percolate and wash and, after the concentration step, the aqueous remainder contained 68.1% of the TRR (1.564 mg eq/kg).

After further extraction of the solids with acetonitrile/water (1/1; v/v) using a microwave at 120 °C, the microwave extract contained further 29.8% of the TRR (0.684 mg eq/kg). After the concentration step, the aqueous remainder contained 29.5% of the TRR (0.677 mg eq/kg).

In total, 98.4% of the TRR (2.258 mg eq/kg) was extracted, whereas 1.6% of the TRR (0.037 mg eq/kg) remained non-extractable in solids (Table 6.2.3-10).

In goat kidney extracted by residue method, 97.4% of the TRR (2.236 mg eq/kg) was extracted with solvent extraction (acetonitrile/water). Following degreasing by SPE, most of the radioactivity was contained in the percolate and wash (97.1% of the TRR or 2.228 mg eq/kg) and, after the concentration step, the aqueous remainder contained all of the radioactivity.

In total, 97.4% of the TRR (2.236 mg eq/kg) was extracted, whereas 6.4% of the TRR (0.533 mg eq/kg) remained non-extractable in solids (Table 6.2.3-10).

Table 6.2.3-6: Extraction of radioactive residues from the goat milk pool after dosing of [phenyl-UL-^{14}C]fluopyram at a daily dose rate of 1.91 mg eq/kg body weight for 5 consecutive days

	Milk pool			
	Morning milk		Evening milk	
TRR, mg eq/kg	0.276		0.228	
Fraction	% TRR	mg a.s. equiv./kg	% TRR	mg a.s. equiv./kg
Solvent extract[1)]	99.5	0.275	99.4	0.227
Degreasing via SPE				
Percolate and wash	99.0	0.273	98.4	0.224
Concentration				
Aqueous remainder	**96.8**	**0.267**	**97.9**	**0.223**
Distillate	2.2	0.006	0.5	0.001
Eluate	0.6	0.002	1.0	0.002
Solids	0.5	0.001	0.6	0.001
PES[2)]	0.5	0.001	0.6	0.001
Total extracted	99.5	0.275	99.4	0.227
Accountability / Total[3)]	100.0	0.276	100.0	0.228

1) Solvent extraction was done with 3x with acetonitrile/water (4/1, v/v).
2) PES: post extraction solids = non-extractable radioactivity.
3) Sum of extracts and PES.
Fractions given in **bold** font were analysed by radio-HPLC.

Table 6.2.3-7: Extraction of radioactive residues from the goat muscle after dosing of [phenyl-UL-^{14}C]fluopyram at a daily dose rate of 1.91 mg eq/kg body weight for 5 consecutive days

	Goat muscle			
	Conventional extraction		Residue method	
TRR, mg eq/kg	0.737			
Fraction	% TRR	mg a.s. equiv./kg	% TRR	mg a.s. equiv./kg
Solvent extract[1)]	67.7	0.499	96.6	0.712
Degreasing via SPE				
Percolate and wash	67.5	0.497	96.3	0.710
Concentration				
Aqueous remainder	**67.5**	**0.497**	**96.3**	**0.710**
Eluate	0.2	0.002	0.3	0.002
Solids 1	32.3	0.238	3.4	0.025
Microwave solvent extraction [2)]				
Microwave extract	31.7	0.234	-	-
Concentration				
Aqueous remainder	**31.4**	**0.231**	-	-
Distillate	0.3	0.002	-	-
Solids 2	0.6	0.004	-	-
PES[2)]	0.6	0.004	3.4	0.025
Total extracted	99.4	0.733	96.6	0.712
Accountability / Total[4)]	100.0	0.737	100.0	0.737

1) Solvent extraction was done with 3x with acetonitrile/water (4/1, v/v)
2) Microwave extraction was performed with acetonitrile/water (1/1, v/v) at 120 °C
3) PES: post extraction solids = non-extractable radioactivity
4) Sum of extracts and PES.

Fractions given in **bold** font were analysed by radio-HPLC.

Table 6.2.3-8: Extraction of radioactive residues from the goat fat after dosing of [phenyl-UL-^{14}C]fluopyram at a daily dose rate of 1.91 mg eq/kg body weight for 5 consecutive days

	Goat fat	
TRR, mg eq/kg	0.399	
Fraction	% TRR	mg a.s. equiv./kg
Acetonitrile/water extract[1)]	96.9	0.387
Degreasing via SPE		
Percolate and wash	96.7	0.386
Concentration		
Aqueous remainder	**96.7**	**0.386**
Eluate	0.2	0.001
Heptane phases	2.3	0.009
Solids	0.8	0.003
PES[2)]	0.8	0.003
Total extracted	99.2	0.396
Accountability / Total[3)]	100.0	0.399

1) Solvent extraction was performed by twice 200 mL n-heptane and acetonitrile/water (4/1, v+v) and the phases were separated.
2) PES: post extraction solids = non-extractable radioactivity.
3) Sum of extracts and PES.
Fractions given in **bold** font were analysed by radio-HPLC.

Table 6.2.3-9: Extraction of radioactive residues from the goat liver after dosing of [phenyl-UL-^{14}C]fluopyram at a daily dose rate of 1.91 mg eq/kg body weight for 5 consecutive days

	Goat liver			
	Conventional extraction		Residue method	
TRR, mg eq/kg	8.379			
Fraction	% TRR	mg a.s. equiv./kg	% TRR	mg a.s. equiv./kg
Solvent extract[1)]	76.6	6.416	93.6	7.846
Degreasing via SPE				
Percolate and wash	76.5	6.409	93.3	7.814
Concentration				
Aqueous remainder	**76.2**	**6.384**	**93.3**	**7.814**
Distillate	0.3	0.024	-	-
Eluate	0.1	0.008	0.4	0.031
Solids 1	23.4	1.963	6.4	0.533
Microwave solvent extraction [2)]				
Microwave extract	17.4	1.456	-	-
Concentration				
Aqueous remainder	**17.3**	**1.449**	-	-
Distillate	0.1	0.007	-	-
Solids 2	6.0	0.507	-	-
PES[2)]	6.0	0.507	6.4	0.533
Total extracted	94.0	7.872	93.6	7.846
Accountability / Total[4)]	100.0	8.379	100.0	8.379

1) Solvent extraction was done with 3x with acetonitrile/water (4/1, v/v).
2) Microwave extraction was performed with acetonitrile/water (1/1, v/v) at 150 °C
3) PES: post extraction solids = non-extractable radioactivity.
4) Sum of extracts and PES
Fractions given in **bold** font were analysed by radio-HPLC.

Table 6.2.3-10: Extraction of radioactive residues from the goat kidney after dosing of [phenyl-UL-^{14}C]fluopyram at a daily dose rate of 1.91 mg eq/kg body weight for 5 consecutive days

	Goat kidney			
	Conventional extraction		Residue method	
TRR, mg eq/kg	2.295			
Fraction	% TRR	mg a.s. equiv./kg	% TRR	mg a.s. equiv./kg
Solvent extract[1)]	68.6	1.573	97.4	2.236
Degreasing via SPE				
Percolate and wash	68.6	1.573	97.1	2.228
Concentration				
Aqueous remainder	**68.1**	**1.564**	**97.1**	**2.228**
Distillate	0.4	0.009	-	-
Eluate	-	-	0.4	0.009
Solids 1	31.4	0.722	2.6	0.059
Microwave solvent extraction [2)]				
Microwave extract	29.8	0.684	-	-
Concentration				
Aqueous remainder	**29.5**	**0.677**	-	-
Distillate	0.3	0.007	-	-
Solids 2	1.6	0.037	-	-
PES[2)]	1.6	0.037	2.6	0.059
Total extracted	98.4	2.258	97.4	2.236
Accountability / Total[4)]	100.0	2.295	100.0	2.295

1) Solvent extraction was done with 3x with acetonitrile/water (4/1, v/v).
2) Microwave extraction was performed with acetonitrile/water (1/1, v/v) at 120 °C
3) PES: post extraction solids = non-extractable radioactivity.
4) Sum of extracts and PES
Fractions given in **bold** font were analysed by radio-HPLC.

For elucidation of metabolism, all acetonitrile/water extracts were analysed by HPLC with radiodetection. Metabolites were identified by LC-MS/MS co-chromatography or comparison of profiles with those from the lactating goat study with [pyridyl-2,6-^{14}C]fluopyram.

In morning milk, the parent compound accounted for only 0.7% of the TRR (0.002 mg eq/kg). The main component was the label specific metabolite fluopyram-benzamide, amounting to 89.2% of the TRR (0.246 mg eq/kg). The second most abundant compound was fluopyram-benzamide-SA and accounted for 4.3% of the TRR (0.012 mg eq/kg). The metabolites fluopyram-7-OH-GA (isomer 1 and 2), fluopyram-7-hydroxy and fluopyram-Z-olefin were minor compounds and accounted each for < 2% of the TRR (< 0.005 mg eq/kg). In total, 96.3% of the TRR was identified in morning milk (Table 6.2.3-11).

In evening milk, the parent compound accounted for only 1.7% of the TRR (0.004 mg eq/kg). The main component was the label specific metabolite fluopyram-benzamide, amounting to 88.4% of the TRR

(0.202 mg eq/kg). The second most abundant compound was fluopyram-benzamide-SA and accounted for 4.3% of the TRR (0.010 mg eq/kg). The metabolites fluopyram-7-OH-GA (isomer 1 and 2), fluopyram-7-hydroxy and fluopyram-*Z*-olefin were minor compounds and accounted each for < 2% of the TRR (< 0.005 mg eq/kg;). In total, 96.8% of the TRR was identified in milk pool (Table 6.2.3-12).

In goat muscle, no parent compound was found. The label-specific metabolite fluopyram-benzamide accounted for the majority of the TRR and accounted for 97.6% of the TRR (0.719 mg eq/kg). Other metabolites found were fluopyram-benzamide-SA, fluopyram-7-OH-GA (isomer 1) and fluopyram-7-hydroxy, which accounted each for < 1% of the TRR (≤ 0.005 mg eq/kg). In total, 98.9% of the TRR was identified in goat muscle (Table 6.2.3-13).

In goat fat, the parent compound accounted for 13.1% of the TRR (0.052 mg eq/kg). Again the major component was fluopyram-benzamide and accounted for 49.1% of the TRR (0.196 mg eq/kg). The second most abundant metabolite was fluopyram-*Z*-olefin (13.1% of the TRR or 0.052 mg eq/kg), followed by fluopyram-*E*-olefin (8.6% of the TRR or 0.034 mg eq/kg) and fluopyram-7-hydroxy (7.7% of the TRR or 0.031 mg eq/kg). In total, 96.7% of the TRR was identified in goat fat (Table 6.2.3-14).

In goat liver, the parent compound was identified in traces of < 1% of the TRR (<0.05 mg eq/kg). fluopyram-benzamide accounted for the majority of the TRR and accounted for 82.8% of the TRR (6.941 mg eq/kg). The second most abundant metabolite was fluopyram-7-OH-GA (isomer 1) and accounted for 4.3% of the TRR (0.363 mg eq/kg). Further components found were fluopyram-8-OH-GA (isomer 2), fluopyram-benzamide-SA, fluopyram-phenol-GA, fluopyram-7-hydroxy, fluopyram-*E*-olefin and fluopyram-*Z*-olefin, each amounting to ≤2.1% of the TRR (≤0.174mg eq/kg). In total, 93.5% of the TRR was identified in goat liver (Table 6.2.3-15).

In goat kidney, the parent compound was identified in traces of < 1% of the TRR (<0.01 mg eq/kg). The main component was again fluopyram-benzamide and amounted to 77.1% of the TRR (1.769 mg eq/kg). The second most abundant metabolite was fluopyram-7-OH-GA (isomer 1), accounting for 7.3% of the TRR (0.168 mg eq/kg), followed by fluopyram-8-OH-GA (isomer 2; 7.3% of the TRR or 0.168 mg eq/kg). Further components identified were fluopyram-benzamide-SA, fluopyram-7-OH-GA (isomer 2), fluopyram-di-OH-GA, fluopyram-phenol-GA, fluopyram-7-hydroxy and the parent compound, each amounting to ≤ 2.5% of the TRR (≤ 0.058 mg eq/kg). In total, 95.4% of the TRR were identified in goat kidney (Table 6.2.3-16).

Table 6.2.3-11: Summary of identification and characterization of radioactive residues in the morning milk of lactating goat after dosing of [phenyl-UL-^{14}C] fluopyram at a daily dose rate of 1.91 mg eq/kg body weight for 5 consecutive days

	Morning milk	
TRR [mg eq/kg] =	**0.276**	
Compound	**% of the TRR**	**mg a.s. equiv./kg**
AE C656948, a.s., fluopyram	0.7	0.002
fluopyram-benzamide-SA (M31)	4.3	0.012
fluopyram-benzamide (M25)	89.2	0.246
fluopyram-7-OH-GA (isomer 1) (M09)	0.4	0.001
fluopyram-7-OH-GA (isomer 2) (M09)	0.2	0.001
fluopyram-di-OH-GA (M21)	-	-
fluopyram-phenol-GA (M07)	-	-
fluopyram-8-OH-GA (M20), isomer 2	-	-
fluopyram-7-hydroxy (M08)	0.8	0.002
fluopyram-*E*-olefin (M02)	-	-
fluopyram-*Z*-olefin (M03)	0.7	0.002
Total identified	96.3	0.266
Sum of unknowns	0.5	0.001
Analysed extracts	96.8	0.267
Losses *	0.6	0.002
Volatiles **	2.2	0.006
Total extractable	99.5	0.275
Unextractable residues (solids)	0.5	0.001
Accountability	100.0	0.276

* dichloromethane/methanol eluates of SPE column during clean up SPE eluate
** distillates from concentration steps

Table 6.2.3-12: Summary of identification and characterization of radioactive residues in the evening milk of lactating goat after dosing of [phenyl-UL-^{14}C] fluopyram at a daily dose rate of 1.91 mg eq/kg body weight for 5 consecutive days

	Evening milk	
TRR [mg eq/kg] =	**0.276**	
Compound	% of the TRR	mg a.s. equiv./kg
AE C656948, a.s., fluopyram	1.7	0.004
fluopyram-benzamide-SA (M31)	4.3	0.010
fluopyram-benzamide (M25)	88.4	0.202
fluopyram-7-OH-GA (M09), isomer 1	0.3	0.001
fluopyram-7-OH-GA (M09), isomer 2	0.3	0.001
fluopyram-di-OH-GA (M21)	-	-
fluopyram-phenol-GA (M07)	-	-
fluopyram-8-OH-GA (M20), isomer 2	-	-
fluopyram-7-hydroxy (M08)	1.8	0.004
fluopyram-*E*-olefin (M02)	-	-
fluopyram-*Z*-olefin (M03)	-	-
Total identified	96.8	0.221
Sum of unknowns	1.1	0.003
Analysed extracts	97.9	0.223
Losses *	1.0	0.002
Volatiles **	0.5	0.001
Total extractable	99.4	0.001
Unextractable residues (solids)	0.6	0.001
Accountability	100.0	0.228

* dichloromethane/methanol eluates of SPE column during clean up SPE eluate
** distillates from concentration steps

Table 6.2.3-13: Summary of identification and characterization of radioactive residues in muscle of lactating goat after dosing of [phenyl-UL-^{14}C] fluopyram at a daily dose rate of 1.91 mg eq/kg body weight for 5 consecutive days

	Goat muscle	
TRR [mg/kg] =	**0.737**	
Compound	% of TRR	mg a.s. equiv./kg
AE C656948, a.s., fluopyram	-	-
fluopyram-benzamide-SA (M31)	0.7	0.005
fluopyram-benzamide (M25)	97.6	0.719
fluopyram-7-OH-GA (M09), isomer 1	0.3	0.002
fluopyram-7-OH-GA (M09), isomer 2	-	-
fluopyram-di-OH-GA (M21)	-	-
fluopyram-phenol-GA (M07)	-	-
fluopyram-8-OH-GA (M20), isomer 2	-	-
fluopyram-7-hydroxy (M08)	0.3	0.002
fluopyram-*E*-olefin (M02)	-	-
fluopyram-*Z*-olefin (M03)	-	-
Total identified	98.9	0.729
Sum of unknowns	-	-
Analysed extracts	98.9	0.729
Losses *	0.2	0.002
Volatiles **	0.3	0.002
Total extractable***	99.4	0.733
Unextractable residues (solids)	0.6	0.004
Accountability	100.0	0.737

* dichloromethane/methanol eluates of SPE column during clean up SPE eluate and heptane phase for fat
** distillates from concentration steps
*** Total extracted for fat = sum of solvent extract (96.9% of TRR) and heptane phase (2.3% of TRR)

Table 6.2.3-14: Summary of identification and characterization of radioactive residues in fat of lactating goat after dosing of [phenyl-UL-^{14}C] fluopyram at a daily dose rate of 1.91 mg eq/kg body weight for 5 consecutive days

	Goat fat	
TRR [mg/kg] =	**0.399**	
Compound	% of TRR	mg a.s. equiv./kg
AE C656948, a.s., fluopyram	18.2	0.073
fluopyram-benzamide-SA (M31)	-	-
fluopyram-benzamide (M25)	49.1	0.196
fluopyram-7-OH-GA (M09), isomer 1	-	-
fluopyram-7-OH-GA (M09), isomer 2	-	-
fluopyram-di-OH-GA (M21)	-	-
fluopyram-phenol-GA (M07)	-	-
fluopyram-8-OH-GA (M20), isomer 2	-	-
fluopyram-7-hydroxy (M08)	7.7	0.031
fluopyram-*E*-olefin (M02)	8.6	0.034
fluopyram-*Z*-olefin (M03)	13.1	0.052
Total identified	96.7	0.386
Sum of unknowns	-	-
Analysed extracts	96.7	0.386
Losses *	2.5	0.010
Volatiles **	-	-
Total extractable***	99.2	0.396
Unextractable residues (solids)	0.8	0.003
Accountability	100.0	0.399

* dichloromethane/methanol eluates of SPE column during clean up SPE eluate and heptane phase for fat
** distillates from concentration steps
*** Total extracted for fat = sum of solvent extract (96.9% of TRR) and heptane phase (2.3% of TRR)

Table 6.2.3-15: Summary of identification and characterization of radioactive residues in liver of lactating goat after dosing of [phenyl-UL-^{14}C] fluopyram at a daily dose rate of 1.91 mg eq/kg body weight for 5 consecutive days

	Goat liver	
TRR [mg/kg] =	**8.379**	
Compound	% of TRR	mg a.s. equiv./kg
AE C656948, a.s., fluopyram	0.6	0.047
fluopyram-benzamide-SA (M31)	1.6	0.133
fluopyram-benzamide (M25)	82.8	6.941
fluopyram-7-OH-GA (M09), isomer 1	4.3	0.363
fluopyram-7-OH-GA (M09), isomer 2	-	-
fluopyram-di-OH-GA (M21)	-	-
fluopyram-phenol-GA (M07)	0.8	0.068
fluopyram-8-OH-GA (M20), isomer 2	2.1	0.174
fluopyram-7-hydroxy (M08)	0.9	0.073
fluopyram-*E*-olefin (M02)	0.3	0.021
fluopyram-*Z*-olefin (M03)	0.2	0.013
Total identified	93.5	7.833
Sum of unknowns	-	-
Analysed extracts	93.5	7.833
Losses *	0.1	0.008
Volatiles **	0.4	0.031
Total extractable***	94.0	7.872
Unextractable residues (solids)	6.0	0.507
Accountability	100.0	8.379

* dichloromethane/methanol eluates of SPE column during clean up SPE eluate and heptane phase for fat
** distillates from concentration steps
*** Total extracted for fat = sum of solvent extract (96.9% of TRR) and heptane phase (2.3% of TRR)

Table 6.2.3-16: Summary of identification and characterization of radioactive residues in kidney of lactating goat after dosing of [phenyl-UL-^{14}C] fluopyram at a daily dose rate of 1.91 mg eq/kg body weight for 5 consecutive days

	Goat kidney	
TRR [mg/kg] =	**2.295**	
	% of TRR	mg a.s. equiv./kg
AE C656948, a.s., fluopyram	0.4	0.009
fluopyram-benzamide-SA (M31)	2.5	0.058
fluopyram-benzamide (M25)	77.1	1.769
fluopyram-7-OH-GA (M09), isomer 1	7.3	0.168
fluopyram-7-OH-GA (M09), isomer 2	2.1	0.048
fluopyram-di-OH-GA (M21)	0.7	0.015
fluopyram-phenol-GA (M07)	1.2	0.027
fluopyram-8-OH-GA (M20), isomer 2	3.6	0.082
fluopyram-7-hydroxy (M08)	0.6	0.014
fluopyram-*E*-olefin (M02)	-	-
fluopyram-*Z*-olefin (M03)	-	-
Total identified	95.4	2.190
Sum of unknowns	2.2	0.051
Analysed extracts	97.7	2.241
Losses *	-	-
Volatiles **	0.7	0.016
Total extractable***	98.4	2.258
Unextractable residues (solids)	1.6	0.037
Accountability	100.0	2.295

* dichloromethane/methanol eluates of SPE column during clean up SPE eluate and heptane phase for fat
** distillates from concentration steps
*** Total extracted for fat = sum of solvent extract (96.9% of TRR) and heptane phase (2.3% of TRR)

III. Conclusion

One lactating goat was dosed orally for 5 consecutive days with [phenyl-UL-^{14}C]fluopyram at a daily dose of 1.91 mg eq/kg of body weight and sacrificed 24 hours after the last dose.

The lactating goat extensively metabolised AE C656948, which represented 0.4%–18% of the TRR in all matrices except muscle. The label-specific metabolite fluopyram-benzamide was the main compound in milk and edible organs and tissues, accounting for 49.1–97.6% of the TRR. fluopyram-*Z*-olefin was found in significant amounts only in goat fat (13.1% of the TRR). Other minor metabolites accounted for < 5% of the TRR in milk and edible organs and tissues.

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

- hydroxylation of the ethylene bridge of the molecule resulting in fluopyram-7-hydroxy, fluopyram-8-hydroxy and a dihydroxylated compound
- hydroxylation of the phenyl ring leading to fluopyram-phenol
- conjugation of the hydroxylated metabolites with glucuronic acid

- elimination of water from compounds hydroxylated in the ethylene bridge leading to fluopyram-*Z*-olefine and *E*-olefin
- molecular cleavage to fluopyram-benzamide
- hydroxylation of fluopyram-benzamide followed by conjugation with sulphate

The metabolic pathway is proposed in Figure 6.2.3-1.

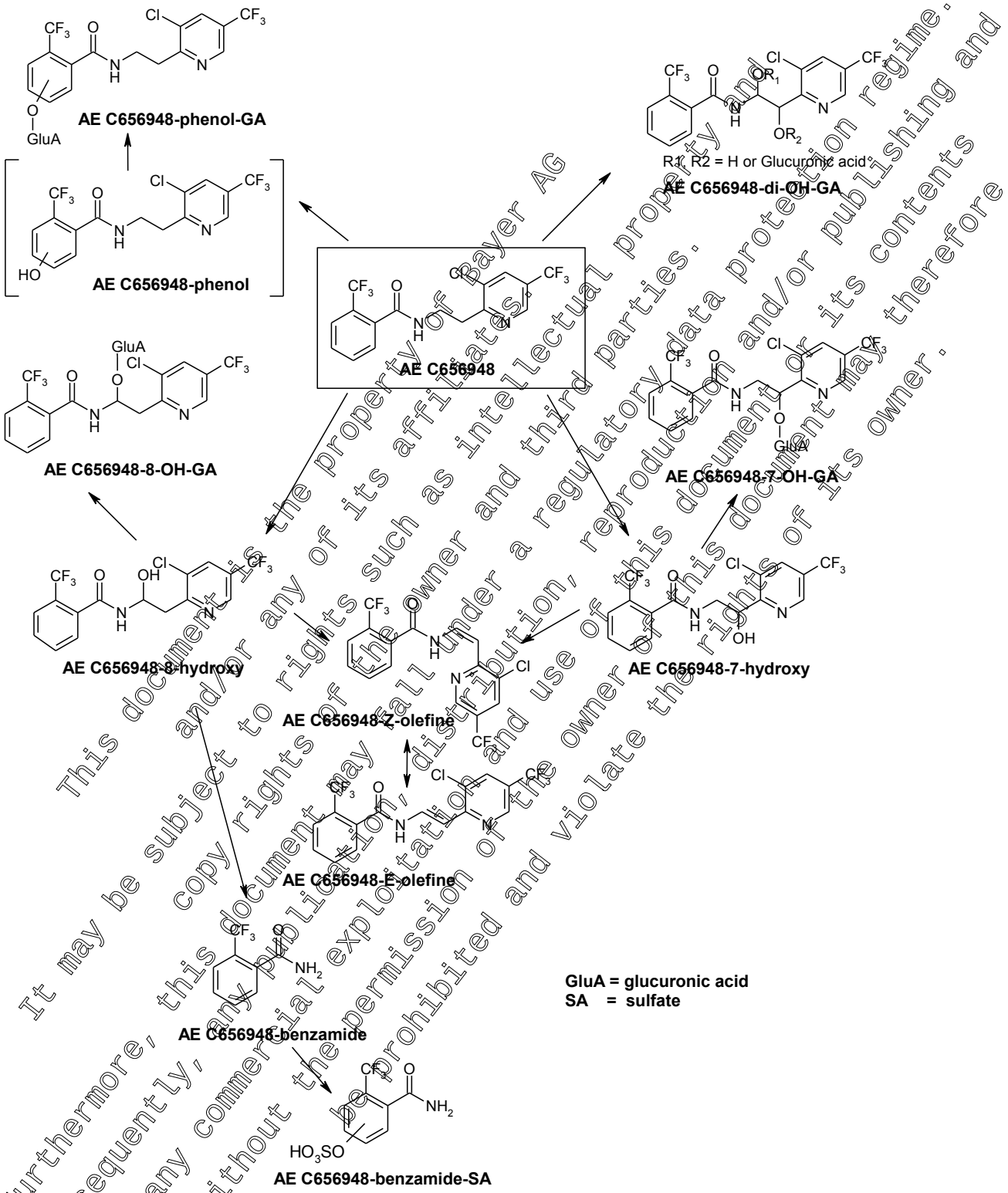


Figure 6.2.3-1: Proposed metabolic pathway of fluopyram in goat after treatment with [phenyl-UL-^{14}C] fluopyram

Assessment and conclusion by applicant:

The study is valid and acceptable.

Data Point:	KCA 6.2.3/02
Report Author:	[redacted]
Report Year:	2008
Report Title:	Metabolism of [pyridyl-2,6-14C]AE C656748 in the lactating goat
Report No:	MEF-06/327
Document No:	M-297849-01-1
Guideline(s) followed in study:	US EPA OPPTS 860.1300, Health Canada PMRA Ref.: DACO 6.2, EU 96/54/EC amended by 96/46/EC, Appendix F
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted rev. 1 to Vol.3 of DAR of 27 August 2010 (references relied on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The metabolism of [pyridyl-2,6-^{14}C]fluopyram was investigated in one lactating goat, which was orally dosed for 5 consecutive days at a rate of 2.0 mg a.s./kg of body weight per day (representing 44.62 mg a.s./kg feed/day). The goat was sacrificed 24 h after the 5th administration. Radioactivity was measured in the excreta, milk and plasma collected at timed sampling intervals, as well as in the liver, kidney, muscle and fat at sacrifice. The milk, edible organs and tissues and excreta (urine and faeces) were analysed for parent compound and metabolites by extraction, chromatographic separation techniques and spectroscopic methods.

Until sacrifice, the excretion amounted to 80.95% of the totally administered radioactivity. An amount of 0.08% of the total dose was found in milk. At sacrifice, the calculated total residue in the tissues and organs dissected from the body was approximately 0.85% of the total dose.

The TRR-values in plasma followed a diurnal pattern. During the 8-hour period after each dosing, a significant increase was obtained followed by a decrease measured prior to the delivery of the next dose. It was shown that the radioactive residues reached a peak of about 0.19 mg/kg, corresponding to 9.5% of the equidistribution concentration of the dose at 4 hours after dosing. This indicated on the one hand a fast

absorption of the test item, a fast distribution of the systemically bioavailable compound-related radioactivity within and a quick elimination from the animal's body. The radioactive residues in plasma reached a plateau-level at about 50–60 hours after the first dosing. The equivalent concentration of radioactivity in the milk showed a constant low rate of 0.006–0.010 mg eq/kg from day 1 to the day of sacrifice. This showed that a plateau level was clearly reached. At sacrifice, highest mean equivalent concentrations occurred in liver (1.427 mg eq/kg). These values were followed in decreasing order by the mean concentrations determined in the kidney (0.403 mg eq/kg), total body fat (0.372 mg eq/kg) and total body muscle (0.042 mg eq/kg). The radioactive residues were extracted with efficiencies of 75.8–97.1% from milk, edible organs or tissues and excreta with acetonitrile/water mixtures and acetonitrile/water/aqueous ammonia mixtures, and identification was achieved by LC-MS/MS, LC-^{1}H-NMR, HPLC co-chromatography or comparison of HPLC profiles.

The lactating goat extensively metabolised fluopyram but parent compound was still the major compound in milk, muscle and fat (27.3%–46.4% of the TRR) and represented *ca.* 7.7% of the TRR in liver. The main metabolites in milk, muscle and fat were fluopyram-7-hydroxy (M08) and fluopyram-*Z*-olefin (M03), accounting each for more than 10% of the TRR, and represented up to 6.1% and 5.7% of the TRR in the other matrices, respectively. The main compound in liver and kidney was fluopyram-7-OH-GA (M09, isomer 1), accounting for 24.2–35.1% of the TRR, but only up to 6.6% of the TRR in the other matrices. Further important metabolites in liver in kidney were fluopyram-8-OH-GA (M20, isomer 2; up to 17.7% of the TRR and up to 2.5% in other matrices) and fluopyram-7-OH-GA (M09, isomer 2; up to 16.3% of the TRR and up to 5.5% in other matrices). The label-specific metabolites fluopyram-pyridyl-acetic acid (M40) and fluopyram-hydroxyethyl-GA (M37) were only found in kidney at 8.6% and 4.3% of the TRR, respectively.

Further detected minor metabolites were fluopyram-*E*-olefin (M02), fluopyram-di-OH-GA (M21) and fluopyram-phenol-GA (M07) at <5% of the TRR.

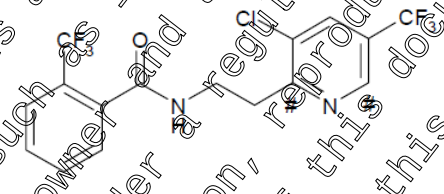
Based on the identified metabolites, the following metabolic routes were deduced:

- hydroxylation of the ethylene bridge of the molecule resulting in fluopyram-7-hydroxy, fluopyram-8-hydroxy and a dihydroxylated compound
- hydroxylation of the phenyl ring leading to fluopyram-phenol
- conjugation of the hydroxylated metabolites with glucuronic acid
- elimination of water from compounds hydroxylated in the ethylene bridge leading to fluopyram-*Z*-olefin and *E*-olefin; *E*- and *Z*-olefin can isomerise into each other
- molecular cleavage to fluopyram-pyridyl-hydroxyethyl followed by conjugation with glucuronic acid
- oxidation of fluopyram-pyridyl-hydroxyethyl to fluopyram-pyridyl-acetic acid.

I. Materials and Methods

A. Materials

1. Test Material:

IUPAC Name	*N*-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]ethyl}-2-(trifluoromethyl)benzamide
CAS Name	Benzamide, N-[2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-(trifluoromethyl)- (9Cl)
Code name	AE C656948
Common name	Fluopyram
Empirical formula	$C_{16}H_{11}ClF_6N_2O$
CAS Number	658066-35-4
Molar mass	396.72 g/mol
Chemical structure	 # positions of radiolabel
Radiolabelled test material	[pyridyl-2,6-^{14}C]AE C656948
Batch number	BECH 1939 (radiolabelled)
Original specific radioactivity	1.93 MBq/mg = 52.1 µCi/mg
Radiochemical purity	> 98% (HPLC); > 99% (TLC)
Chemical purity	> 99% (HPLC)
Stability of test compound	4 months at -18 °C

2. Test Animals

Species	Goat (*Capra hircus*)
Breed	"Bunte deutsche Edelziege"
Breeding facility	[redacted]
Sex, number	One female lactating goat
Body weight	40 kg at test start
Age	*ca.* 27 months old at experiment start
Acclimatization	9 days
Housing	Stainless steel metabolism cage, approx. 20–25 °C, 45–62% rel. humidity, 12/12 hours light/dark cycle, air changes: 10–15 changes/h
Identification	Skin marking
Feed and water	Ruminant feed, hay, apples (*ad libitum*) Tap water *ad libitum*
Health status	Acceptable

B. Study Design

Preparation of the dosing mixtures and administration:

In total, five gelatine capsules containing 84 mg each of the solid powder were prepared one day before the first administration. In relation to a body weight of 40 kg, this amount of radioactivity corresponded to an actual dose of 2.0 mg a.s./kg bw. Based on the experimentally determined food consumption this corresponds to 4462 mg a.s./kg feed/day.

The goat received daily one gelatine capsule administered orally using a capsule applicator. The administration was performed on five consecutive days in the morning after milking.

The identity of the test compound was confirmed by LC-MS/MS. The major remaining amount of the test item was stored in solid form in a freezer at ≤ -18 °C and used in a respective laying metabolism study which was performed about 4 months later. At that time the radiochemical purity was checked by HPLC analysis in order to demonstrate the stability of the test item during storage.

Sampling:

Collection and processing of blood

Micro-samples of blood were taken from the ear veins at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 24, 32, 48, 56, 72, 80, 96, and 104 hours after starting the experiment. The blood was collected in heparinized capillaries which were centrifuged afterwards using a haematocrit centrifuge for separation of blood cells and plasma. The plasma samples were weighed and prepared for radioactivity measurement by LSC.

Collection and processing of milk

The goat was milked in the morning immediately prior to each administration, about 8 hours later in the afternoon, and directly before sacrifice (time schedule: 8, 24, 32, 48, 56, 72, 80, 96, 104, and 120 hours after the first administration). After weighing, one aliquot was taken from each sample for radioactivity measurement by LSC.

Collection and processing of urine

Urine samples were collected in plastic vessels as quantitatively as possible under dry ice cooling in intervals of 24 hours after each administration. The vessels were changed immediately before the next administration. The collection funnel was rinsed with deionised water into the same urine vessel of the respective collection period. After recording the total volumes, one aliquot was taken from each sample for radioactivity measurement by LSC.

Collection and processing of faeces

Faeces samples were collected as quantitatively as possible at room temperature in intervals of 24 hours after each administration, i.e. immediately before the next administration. The collecting grid was cleaned prior to each administration. No samples of the rinsing water were taken for radioactivity measurement. Each faeces fraction was homogenized after addition of water to get a wet paste before the total weight was recorded. One aliquot of each wet sample was weighed and prepared for radioactivity measurement by combustion/LSC.

Sacrifice and collection of organs and tissues

For the collection of organs and tissues, the animal was sacrificed *ca.* 24 hours after the fifth administration, a time distance that is consistent with normal slaughtering practices. The animal was anaesthetised by an intravenous dose of about 40 mg eq/kg bw Pentobarbital-Na (Narcoren®), exsanguinated by cannulating the jugular veins and sacrificed by intracardiac injection with *ca.* 10 mL of the sacrificing agent "T 61®". The following organs and tissues were dissected: muscle (round and loin), fat (omental and perirenal), liver without gall bladder, kidneys, and the gall bladder.

The organs or tissue samples were then transferred into tarred plastic vessels and recording of the individual weights, liver, kidneys, fat and muscle samples were passed several times through a mincing machine in half-frozen state. One aliquot of each resulting tissue pulp was weighed and prepared for radioactivity measurement by combustion/LSC.

The gall bladder was punctured for the collection of the bile fluid that was stored in a freezer for the (optional) metabolite analysis.

C. Analytical Procedures

Extraction and fractionation:

Samples of milk and edible tissues were extracted two or three times with acetonitrile/water mixtures (*ca.* 8/2; v/v).

The combined extracts were applied to C18 SPE cartridges to remove the lipid fraction of the matrix. The SPE percolate and the column wash with acetonitrile/water were combined and concentrated to the aqueous remainder for HPLC analysis.

For liver two additional solvent extractions were performed with acetonitrile/water (1/1; v/v) and with acetonitrile/water/aqueous ammonia (25%) (2/1/1; v/v/v). These extracts were discarded due to low content of radioactivity.

For muscle and liver two further extraction steps were conducted with microwave assistance at elevated temperature. Solids from solvent extraction were extracted first with acetonitrile/water (1/1; v/v) and then with acetonitrile/water/aqueous ammonia (25%) (2/1/1; v/v/v). Both microwave extracts from muscle were discarded due to very low residues. The acetonitrile/water extract from microwave assisted extraction of liver was purified by SPE for chromatographic analysis. For the acetonitrile/water/aqueous ammonia extract from liver several partition and SPE procedures were conducted but a chromatographic analysis was not possible due to low residues in the fractions obtained or interference of matrix.

Analytical methods:

The radioactivity of all liquid samples was measured using a liquid scintillation counter. Solid samples were combusted using an oxidizer. The resulting $^{14}CO_2$ was trapped in an alkaline scintillation cocktail. The TRR in the samples was calculated by summing up the radioactivity measured in different extracts and remaining solids after solvent extraction. The TRR was expressed in mg a.s. equivalents per kg sample weight. Amounts of radioactive residues in the extracts were expressed as percentage of the TRR and also as mg a.s. equivalents per kg sample weight.

Aliquots of all acetonitrile/water extracts were analysed by HPLC. Ten metabolites as well as parent compound were identified by LC-MS/MS, LC-^{1}H-NMR, HPLC co-chromatography or comparison of HPLC profiles.

The HPLC was conducted using a reversed-phase column and a gradient elution using a neutral water phase and an acetonitrile/methanol organic phase. The HPLC system was equipped with a radiodetector and a UV detector with variable wavelengths. The LOQ was derived from the average background level and the specific radioactivity of the radiolabelled test compound.

Storage stability

Solvent extractions and first HPLC-analyses of the extracts from milk, muscle, fat, liver and kidney were performed within 1- 6 weeks after sacrifice of the lactating goat. Investigations on storage stability of radioactive residues in edible samples were therefore not required.

A second extraction of liver with a different methodology was performed after ca. 6 months of storage. These extracts showed mainly the same metabolites but differences in the proportions of compounds compared to the first extraction. The differences can be explained by partial degradation of metabolites caused by the procedures of the second extraction and are probably not related to storage of samples.

Storage stability of extracts was demonstrated for the extracts of milk, fat, the solvent extract of liver and the extract of kidney for a storage time of ca. 3 to 5 months.

II. Results and Discussion

The metabolism in lactating goat of [pyridyl-2,6-^{14}C]fluopyram administrated at a daily dose of 44.62 mg eq/kg in the feed corresponding to a daily intake of 2.0 mg eq/kg body weight for 5 consecutive days was investigated.

Until sacrifice 24 hours after administration of the last dose, the mean excretion amounted on average to 80.95% of the radioactivity totally administered (Table 6.2.3-17Table 6.2.2- 1). The time course of the excretion was characterised by a relatively constant rate starting at day 3 until test end (Table 6.2.3-18). Only 0.08% of the dose totally administered was measured in the milk secreted within the whole test period. At sacrifice, 24 h after the last administration, the total residue in the tissues and organs dissected from the body was calculated to about 0.85% of the total dose. Based on these values, the recovery amounted to 91.89% (Table 6.2.3-17Table 6.2.2- 1).

The TRR-values in plasma, determined over the whole testing period in timed sampling intervals followed a diurnal pattern (Table 6.2.3-19). During the 8-hour period after each dosing, a significant increase was obtained followed by a decrease measured prior to the delivery of the next dose. For the 8-hour period after the first dosing, blood samples were collected at shorter intervals in order to determine the exact course of the TRR-values in plasma. It was shown that the radioactive residues reached a peak of about 0.19 mg/kg, corresponding to 9.5% of the equidistribution concentration of the dose at 4 hours after dosing. This indicated an ongoing absorption of the test item, a rapid distribution of the systemically bioavailable compound-related radioactivity within the body and a quick excretion. A plateau-level was reached at about 50–60 hours after the first dosing.

The total radioactive residues were determined in milk, faeces and urine produced from the first dose until sacrifice and in edible tissues and organs at sacrifice. The highest mean active substance equivalent concentrations were measured in liver (1.427 mg eq/kg) and kidney (0.403 mg eq/kg). These values were followed in decreasing order by total body fat (0.372 mg eq/kg) and total body muscle (0.042 mg eq/kg). For details see Table 6.2.3-20.

Table 6.2.3-17: Recovery of radioactivity following oral administration of [pyridyl-2,6-^{14}C]fluopyram at a daily dose rate of 2.0 mg eq/kg body weight for 5 consecutive days

Matrix	Recovery of radioactivity (% of the totally administrated radioactivity)
Excreta[1)]	80.95
Milk[2)]	0.08
Totally excreted	*81.03*
Tissues	0.85
Recovery	91.89

1) Recovery in excreta obtained by adding recovery values of samples collected within the observation period of 5 days
2) Recovery in milk calculated by adding recovery values of samples produced within the observation period of 5 days
Values in *italics* were calculated from the values in the report.

Table 6.2.3-18: Time course of total radioactivity in excreta following oral administration of [pyridyl-2,6-^{14}C]fluopyram at a daily dose rate of 2.0 mg eq/kg body weight for 5 consecutive days

Matrix	Time after the first dosage (hours)	Administration number	Excretion per day (% of totally administrated radioactivity)
Urine	24	2	9.08
	48	3	11.14
	72	4	10.17
	96	5	9.42
	120	Sacrifice	12.53
Faeces	24	2	3.37
	48	3	5.67
	72	4	7.21
	96	5	6.06
	120	Sacrifice	6.31

Table 6.2.3-19: Time course of total radioactivity in plasma following oral administration of [pyridyl-2,6-^{14}C]fluopyram at a daily dose rate of 2.0 mg eq/kg body weight for 5 consecutive days

Matrix	Time after the first dosage (hours)	Administration number	[mg eq/kg plasma]
Plasma	0.25	1	0.007
	0.5		0.044
	1		0.113
	2		0.164
	3		0.182
	4		0.191
	6		0.182
	8		0.155
	24	2	0.078
	32		0.127
	48	3	0.095
	56		0.200
	72	4	0.108
	80		0.199
	96	5	0.133
	104		0.218
	120		- **

** no sample collected

The equivalent concentration of radioactivity in the milk showed a constant low rate of 0.006–0.010 mg eq/kg from day 1 to the day of sacrifice. This showed that a plateau level was clearly reached. For details see Table 6.2.3-20.

Table 6.2.3-20: Time course of total radioactivity in milk following oral administration of [pyridyl-2,6-^{14}C]fluopyram at a daily dose rate of 2.0 mg eq/kg body weight for 5 consecutive days

Matrix	Time after the first dosage (hours)	Administration number	Secretion per period (% of totally administrated radioactivity)
Milk	8	1	0.009
	24		0.006
	32	2	0.010
	48		0.007
	56	3	0.010
	72		0.007
	80	4	0.009
	96		0.008
	104	5	0.010
	120		0.006

Table 6.2.3-21: Distribution of residues in tissues and organs of lactating goats following oral administration of [pyridyl-2,6-^{14}C]fluopyram at a daily dose rate of 2.0 mg eq/kg body weight for 5 consecutive days

Matrix	Interval (day)	TRR (mg a.s. equiv./kg)
Liver	5	1.427
Kidney	5	0.403
Total body muscle	5	0.042
Total body fat	5	0.372

mg a.s. equiv./kg: mg parent equivalents per kg matrix

In evening milk pool, 93.4% of the TRR (0.050 mg eq/kg) was extracted with solvent extraction (acetonitrile/water). Following degreasing by SPE, most of the radioactivity was contained in the percolate and wash (91.1% of the TRR or 0.048 mg eq/kg) and, after the concentration step, the aqueous remainder contained all of the radioactivity. In total, 93.4% of the TRR (0.050 mg eq/kg) was extracted, whereas 6.6% of the TRR (0.003 mg eq/kg) remained non-extractable in solids (Table 6.2.3-22).

In goat muscle, 86.7% of the TRR (0.036 mg eq/kg) was extracted with solvent extraction (acetonitrile/water). Following degreasing by SPE, all of the radioactivity was contained in the percolate and wash and, after the concentration step, the aqueous remainder again contained all of the radioactivity.

After further extraction of the solids with acetonitrile/water (1/1; v/v) and acetonitrile/water/aqueous ammonia (25%) (2/1/1; v/v/v) using a microwave at 120 °C, the alkaline microwave extract contained further 5.8% of the TRR (0.002 mg eq/kg) and the neutral microwave extract contained 1.7% of the TRR (0.001 mg eq/kg).

In total, 89.2.0% of the TRR (0.037 mg eq/kg) was extracted (Table 6.2.3-23).

In goat fat, 97.1% of the TRR (0.361 mg eq/kg) was extracted with acetonitrile/water. Following degreasing of the acetonitrile/water phase by SPE, most of the radioactivity was contained in the percolate and wash (96.1% or 0.357 mg eq/kg) and, after the concentration step, the aqueous remainder contained 95.1% of the TRR (0.354 mg eq/kg). In total, 97.1% of the TRR (0.361 mg eq/kg) was extracted, whereas 2.9% of the TRR (0.011 mg eq/kg) remained non-extractable in solids (Table 6.2.3-24).

In goat liver with conventional extraction, 54.7% of the TRR (0.780 mg eq/kg) was extracted with solvent extraction (acetonitrile/water). Following degreasing by SPE, most of the radioactivity was contained in the percolate and wash (53.7% or 0.766 mg eq/kg) and, after the concentration step, the aqueous remainder contained 50.6% of the TRR (0.723 mg eq/kg).

After further solvent extraction of the solids with acetonitrile/water (1/1, v/v) with acetonitrile/water and acetonitrile/water/aqueous ammonia (25%) 2:1:1 (v/v/v), the majority of the TRR remained in the solids (39.0% of the TRR or 0.556 mg eq/kg).

After further microwave extraction with acetonitrile/water (1/1; v/v) at 120 °C, the neutral microwave extract contained further 14.7% of the TRR (0.210 mg eq/kg). After the concentration step, the aqueous remainder contained 14.2% of the TRR (0.202 mg eq/kg). The majority of the TRR after microwave extraction was however still contained in solids (24.2% of the TRR or 0.346 mg eq/kg). After a second microwave extraction with acetonitrile/water/aqueous ammonia (25%) (2/1/1; v/v/v) at 120 °C all of the TRR was contained in the alkaline microwave extract.In total, 75.8% of the TRR (1.082 mg eq/kg) was extracted (Table 6.3-25).

In goat kidney, 91.4% of the TRR (0.368 mg eq/kg) was extracted with solvent extraction (acetonitrile/water). Following degreasing by SPE, most of the radioactivity was contained in the percolate and wash (90.9% of the TRR or 0.366 mg eq/kg) and, after the concentration step, the aqueous remainder contained 88.9% of the TRR (0.358 mg eq/kg). In total, 91.4% of the TRR (0.368 mg eq/kg) was extracted, whereas 8.6% of the TRR (0.035 mg eq/kg) remained non-extractable in solids (Table 6.2.3-26).

Table 6.2.3-22: Extraction of radioactive residues from the evening milk pool after dosing of [pyridyl-2,6-^{14}C]fluopyram at a daily dose rate of 2.0 mg eq/kg body weight for 5 consecutive days

	Evening milk pool	
TRR, mg eq/kg	0.053	
Fraction	% TRR	mg a.s. equiv./kg
Solvent extract[1)]	93.4	0.050
Degreasing via SPE		
Percolate and wash	91.1	0.048
Concentration		
Aqueous remainder	**91.1**	**0.048**
Eluate	2.4	0.001
Solids	6.6	0.003
PES[2)]	6.6	0.003
Total extracted	93.4	0.050
Accountability / Total[3)]	100.0	0.053

1) Solvent extraction was done with 3x with acetonitrile/water (4/1, v/v).
2) PES: post extraction solids = non-extractable radioactivity.
3) Sum of extracts and PES.
Fractions given in **bold** font were analysed by radio-HPLC.

Table 6.2.3-23: Extraction of radioactive residues from the goat muscle after dosing of [pyridyl-2,6-^{14}C]fluopyram at a daily dose rate of 2.0 mg eq/kg body weight for 5 consecutive days

	Goat muscle	
TRR, mg eq/kg	0.042	
Fraction	% TRR	mg a.s. equiv./kg
Solvent extract 1-2 [1)]	86.7	0.036
Degreasing via SPE		
Percolate and wash	86.7	0.036
Concentration		
Aqueous remainder	**86.7**	**0.036**
Solvent extract 3 [1)]	2.5	0.001
Solids 1	10.8	0.005
Microwave solvent extraction [2)]		
Microwave extract (alkaline)	5.8	0.002
Microwave extract (neutral)	1.7	0.001
Loss	3.3	0.001
PES[3)]		-
Total extracted	100.0	0.042
Accountability / Total[4)]	100.0	0.042

1) Solvent extraction was done with 3x with acetonitrile/water (4/1, v/v).
2) Microwave extraction was performed with acetonitrile/water (1/1, v/v) and acetonitrile/water/aqueous ammonia (25%) 2:1:1 (v/v/v) at 120 °C
3) PES: post extraction solids = non-extractable radioactivity.
4) Sum of extracts and PES.
Fractions given in **bold** font were analysed by radio-HPLC.

Table 6.2.3-24: Extraction of radioactive residues from the goat fat after dosing of [pyridyl-2,6-^{14}C]fluopyram at a daily dose rate of 2.0 mg eq/kg body weight for 5 consecutive days

	Goat fat	
TRR, mg eq/kg	0.372	
Fraction	% TRR	mg a.s. equiv./kg
Solvent extracts [1)]	97.1	0.361
Degreasing via SPE		
Percolate and wash	96.1	0.357
Concentration		
Aqueous remainder	**95.1**	**0.354**
Distillate	1.0	0.004
Eluate	1.1	0.004
Solids	2.9	0.011
PES[2)]	2.9	0.011
Total extracted	97.1	0.361
Accountability / Total[3)]	100.0	0.372

1) Solvent extraction was done with 2x with acetonitrile/water (4/1 v/v).
2) PES: post extraction solids = non-extractable radioactivity.
3) Sum of extracts and PES.
Fractions given in **bold** font were analysed by radio-HPLC.

Table 6.2.3-25: Extraction of radioactive residues from the goat liver after dosing of [pyridyl-2,6-^{14}C]fluopyram at a daily dose rate of 2.0 mg eq/kg body weight for 5 consecutive days

	Goat liver	
TRR, mg eq/kg	1.427	
Fraction	% TRR	mg a.s. equiv./kg
Solvent extract[1)]	54.7	0.780
Degreasing via SPE		
Percolate and wash	53.7	0.766
Concentration		
Aqueous remainder	**50.6**	**0.723**
Distillate	3.1	0.044
Eluate	1.0	0.014
Solids 1	45.3	0.647
Further solvent extraction [2)]		
Acetonitrile/water extract	2.9	0.041
Acetonitrile/water/ammonia extract	3.5	0.050
Solids 2	39.0	0.556
Microwave solvent extraction [3)]		
Microwave extract (neutral)	14.7	0.210
Concentration		
Aqueous remainder	**14.2**	**0.202**
Distillate	0.6	0.008
Solids 3	24.2	0.346
Microwave solvent extraction [4)]		
Microwave extract (alkaline)	24.2	0.346
PES [5)]	-	-
Total extracted	100.0	1.427
Accountability / Total [6)]	100.0	1.427

1) Solvent extraction was done with 3x with acetonitrile/water (4/1, v/v).
2) Further solvent extraction was performed with acetonitrile/water 1:1 (v/v) followed by acetonitrile/water/aqueous ammonia (25%) 2:1:1 (v/v/v).
3) Microwave extraction was performed with acetonitrile/water (1/1, v/v) at 120 °C
4) Microwave extraction was performed with acetonitrile/water/aqueous ammonia (25%) 2:1:1 (v/v/v) at 120 °C
5) PES: post extraction solids = non-extractable radioactivity.
6) Sum of extracts and PES.

Fractions given in **bold** font were analysed by radio-HPLC.

Table 6.2.3-26: Extraction of radioactive residues from the goat kidney after dosing of [pyridyl-2,6-^{14}C]fluopyram at a daily dose rate of 2.0 mg eq/kg body weight for 5 consecutive days

	Goat kidney	
TRR, mg eq/kg	0.403	
Fraction	% TRR	mg a.s. equiv./kg
Solvent extract[1)]	91.4	0.368
Degreasing via SPE		
Percolate and wash	90.9	0.366
Concentration		
Aqueous remainder	**88.9**	**0.358**
Distillate	2.0	0.008
Eluate	0.5	0.002
Solids	8.6	0.035
PES[2)]	8.6	0.035
Total extracted	91.4	0.368
Accountability / Total[3)]	100.0	0.403

1) Solvent extraction was done with 2x with acetonitrile/water (4/1, v/v).
2) PES: post extraction solids = non-extractable radioactivity.
3) Sum of extracts and PES.
Fractions given in **bold** font were analysed by radio-HPLC.

For elucidation of metabolism, all acetonitrile/water extracts were analysed by HPLC. Ten metabolites as well as parent compound were identified by LC-MS/MS, LC-^{1}H-NMR, HPLC co-chromatography or comparison of HPLC profiles.

In evening milk pool, the parent compound was the main compound and accounted for 42.5% of the TRR (0.023 mg eq/kg). The main metabolite was fluopyram-7-hydroxy, amounting to 16.2% of the TRR (0.009 mg eq/kg), followed by fluopyram-*Z*-olefin, accounting for 12.9% of the TRR (0.007 mg eq/kg). The metabolites fluopyram-7-OH-GA (isomer 1 and 2), fluopyram-8-OH-GA (isomer 2) and fluopyram-*E*-olefin minor compounds and accounted each for < 5% of the TRR (< 0.005 mg eq/kg). In total, 79.4% of the TRR was identified in evening milk pool (Table 6.2.3-27).

In goat muscle, the parent compound was the main component and accounted for 27.3% of the TRR (0.011 mg eq/kg). The main metabolites were fluopyram-7-hydroxy and fluopyram-*Z*-olefin (each 21.6% of the TRR or 0.009 mg eq/kg). Fluopyram-7-OH-GA accounted for 6.6% (0.003 mg eq/kg) and 5.1% (0.002 mg eq/kg) of the TRR for isomer 1 and 2, respectively. Other metabolites found were fluopyram-8-OH-GA (isomer 2), and fluopyram-*E*-olefin, which accounted each for < 5% of the TRR (0.001 mg eq/kg). In total, 86.7% of the TRR was identified in goat muscle (Table 6.2.3-28).

In goat fat, the parent compound was the main component and accounted for 46.4% of the TRR (0.173 mg eq/kg). The main metabolite was fluopyram-*Z*-olefin and accounted for 33.7% of the TRR (0.125 mg eq/kg). The second most abundant metabolite was fluopyram-7-hydroxy (12.8% of the TRR or

0.048 mg eq/kg). fluopyram-*E*-olefin was a minor metabolite (2.2% of the TRR, 0.008 mg eq/kg). In total, 95.1% of the TRR was identified in goat fat (Table 6.2.3-29).

In goat liver, the parent compound accounted for 7.7% of the TRR (0.110 mg eq/kg). The main component was fluopyram-7-OH-GA (isomer 1), accounting for 24.2% of the TRR (0.345 mg eq/kg). The second most abundant metabolite was fluopyram-8-OH-GA (isomer 2) and accounted for 9.5% of the TRR (0.136 mg eq/kg), followed by fluopyram-7-hydroxy (6.1% of the TRR or 0.086 mg eq/kg) and fluopyram-*Z*-olefin (5.7% of the TRR and 0.081 mg eq/kg). Further components found were fluopyram-7-OH-GA (isomer 2), fluopyram-di-OH-GA, fluopyram-phenol-GA and fluopyram-*E*-olefin, each amounting to <5% of the TRR (≤0.058mg eq/kg). In total, 62.4% of the TRR was identified in goat liver (Table 6.2.3-30).

In goat kidney, the parent compound was not found. The main component was fluopyram-7-OH-GA (isomer 1) and amounted to 35.1% of the TRR (0.142 mg eq/kg). The second most abundant metabolite was fluopyram-8-OH-GA (isomer 2), accounting for 17.7% of the TRR (0.071 mg eq/kg), followed by fluopyram-7-OH-GA (isomer 2; 16.3% of the TRR or 0.066 mg eq/kg). The label-specific compound fluopyram-pyridyl-acetic acid accounted for 8.6% of the TRR (0.035 mg eq/kg). Further components identified were fluopyram-hydroxyethyl-GA, fluopyram-di-OH-GA, fluopyram-phenol-GA and fluopyram-7-hydroxy, each amounting to < 5% of the TRR (≤ 0.017 mg eq/kg). In total, 87.9% of the TRR were identified in goat kidney (Table 6.2.3-31).

Table 6.2.3-27: Summary of identification and characterization of radioactive residues in the milk of lactating goat after dosing of [pyridyl-2,6-^{14}C] fluopyram at a daily dose rate of 2.0 mg eq/kg body weight for 5 consecutive days

	Evening milk pool	
TRR [mg/kg] =	**0.053**	
Compound	% of TRR	mg a.s. equiv./kg
AE C656948, a.s., fluopyram	42.5	0.023
fluopyram- pyridyl-acetic acid (M40)	-	-
fluopyram-hydroxyethyl-GA (M37)	-	-
fluopyram-7-OH-GA (M09), isomer 1	1.7	0.001
fluopyram-7-OH-GA (M09), isomer 2	3.1	0.002
fluopyram-di-OH-GA (M21)	-	-
fluopyram-phenol-GA (M07)	-	-
fluopyram-8-OH-GA (M20), isomer 2	1.1	0.001
fluopyram-7-hydroxy (M08)	16.2	0.009
fluopyram-*E*-olefin (M02)	2.0	0.001
fluopyram-*Z*-olefin (M03)	12.9	0.007
Total identified	79.4	0.042
Sum of unknowns	11.6	0.006
Analysed extract	91.1	0.048
Losses *	2.4	0.001
Volatiles **	-	-
Not analysed	-	-
Total extractable	93.4	0.050
Unextractable residues (solids)	6.6	0.003
Accountability	100.0	0.053

* dichloromethane/methanol eluates of SPE column during clean up
** distillates from concentration steps

Table 6.2.3-28: Summary of identification and characterization of radioactive residues in muscle of lactating goat after dosing of [pyridyl-2,6-^{14}C] fluopyram at a daily dose rate of 2.0 mg eq/kg body weight for 5 consecutive days

	Goat muscle	
TRR [mg/kg] =	**0.042**	
Compound	**% of TRR**	**mg a.s. equiv./kg**
AE C656948, a.s., fluopyram	27.3	0.011
fluopyram- pyridyl-acetic acid (M40)	-	-
fluopyram-hydroxyethyl-GA (M37)	-	-
fluopyram-7-OH-GA (M09), isomer 1	6.6	0.003
fluopyram-7-OH-GA (M09), isomer 2	5.1	0.002
fluopyram-di-OH-GA (M21)	-	-
fluopyram-phenol-GA (M07)	-	-
fluopyram-8-OH-GA (M20), isomer 2	2.5	0.001
fluopyram-7-hydroxy (M08)	21.6	0.009
fluopyram-*E*-olefin (M02)	1.9	0.001
fluopyram-*Z*-olefin (M03)	21.6	0.009
Total identified	86.7	0.036
Sum of unknowns	-	-
Analysed extract	86.7	0.036
Losses *	-	-
Volatiles **	-	-
Not analysed ***	2.5	0.001
Total extractable ****	89.2	0.037
Unextractable residues (solids) *****	10.8	0.005
Accountability	100.0	0.042

* dichloromethane:methanol eluates of SPE column during clean up
** distillates from concentration steps
*** Muscle: solvent extract 3
**** Muscle: by solvent extraction
***** Muscle: PES after solvent extraction (solids I)

Table 6.2.3-29: Summary of identification and characterization of radioactive residues in fat of lactating goat after dosing of [pyridyl-2,6-^{14}C] fluopyram at a daily dose rate of 2.0 mg eq/kg body weight for 5 consecutive days

	Goat fat	
TRR [mg/kg] =	**0.372**	
Compound	**% of TRR**	**mg a.s. equiv./kg**
AE C656948, a.s., fluopyram	46.4	0.173
fluopyram- pyridyl-acetic acid (M40)	-	-
fluopyram-hydroxyethyl-GA (M37)	-	-
fluopyram-7-OH-GA (M09), isomer 1	-	-
fluopyram-7-OH-GA (M09), isomer 2	-	-
fluopyram-di-OH-GA (M21)	-	-
fluopyram-phenol-GA (M07)	-	-
fluopyram-8-OH-GA (M20), isomer 2	-	-
fluopyram-7-hydroxy (M08)	12.8	0.048
fluopyram-*E*-olefin (M02)	2.2	0.008
fluopyram-*Z*-olefin (M03)	33.7	0.125
Total identified	95.1	0.354
Sum of unknowns	-	-
Analysed extract	95.1	0.354
Losses *	1.1	0.004
Volatiles **	1.0	0.004
Not analysed	-	-
Total extractable	97.1	0.361
Unextractable residues (solids)	2.9	0.011
Accountability	100.0	0.372

* dichloromethane:methanol eluates of SPE column during clean up

** distillates from concentration steps

Table 6.2.3-30: Summary of identification and characterization of radioactive residues in liver of lactating goat after dosing of [pyridyl-2,6-^{14}C] fluopyram at a daily dose rate of 2.0 mg eq/kg body weight for 5 consecutive days

	Goat liver	
TRR [mg/kg] =	**1.427**	
Compound	% of TRR	mg a.s. equiv./kg
AE C656948, a.s., fluopyram	7.7	0.110
fluopyram-pyridyl-acetic acid (M40)		-
fluopyram-hydroxyethyl-GA (M37)		-
fluopyram-7-OH-GA (M09), isomer 1	24.2	0.345
fluopyram-7-OH-GA (M09), isomer 2	2.1	0.030
fluopyram-di-OH-GA (M21)	1.0	0.014
fluopyram-phenol-GA (M07)	2.0	0.029
fluopyram-8-OH-GA (M20), isomer 2	9.5	0.136
fluopyram-7-hydroxy (M08)	6.1	0.086
fluopyram-*E*-olefin (M02)	4.1	0.058
fluopyram-*Z*-olefin (M03)	5.7	0.081
Total identified	62.4	0.890
Sum of unknowns	2.4	0.035
Analysed extract	64.8	0.925
Losses *	1.0	0.014
Volatiles **	3.7	0.052
Not analysed ***	6.4	0.091
Total extractable ****	75.8	1.082
Unextractable residues (solids)*****	24.2	0.346
Accountability	**100.0**	**1.427**

* dichloromethane/methanol eluates of SPE column during clean up
** distillates from concentration steps
*** Liver: solvent extracts 4 and 5
**** Liver: by solvent extraction and first microwave extraction
***** Liver: PES after first microwave extraction step (solids 3, calculated value)

Table 6.2.3-31: Summary of identification and characterization of radioactive residues in kidney of lactating goat after dosing of [pyridyl-2,6-^{14}C] fluopyram at a daily dose rate of 2.0 mg eq/kg body weight for 5 consecutive days

	Goat kidney	
TRR [mg/kg] =	**0.403**	
Compound	% of TRR	mg a.s. equiv./kg
AE C656948, a.s.,	-	-
fluopyram-pyridyl-acetic acid (M40)	8.6	0.035
fluopyram-hydroxyethyl-GA (M37)	4.3	0.017
fluopyram-7-OH-GA (M09), isomer 1	35.1	0.142
fluopyram-7-OH-GA (M09), isomer 2	16.3	0.066
fluopyram-di-OH-GA (M21)	1.7	0.007
fluopyram-phenol-GA (M07)	3.2	0.013
fluopyram-8-OH-GA (M20), isomer 2	17.7	0.071
fluopyram-7-hydroxy (M08)	1.0	0.004
fluopyram-*E*-olefin (M02)	-	-
fluopyram-*Z*-olefin (M03)	-	-
Total identified	87.9	0.354
Sum of unknowns	1.0	0.004
Analysed extract	88.9	0.358
Losses *	0.5	0.002
Volatiles **	2.0	0.008
Not analysed	-	-
Total extractable	91.4	0.368
Unextractable residues (solids)	8.6	0.035
Accountability	100.0	0.403

* dichloromethane:methanol eluates of SPE column during clean up
** distillates from concentration steps

III. Conclusion

One lactating goat was dosed orally for 5 consecutive days with [pyridyl-2,6-^{14}C]fluopyram at a daily dose of 2.0 mg eq/kg of body weight and sacrificed 24 hours after the last dose.

The lactating goat extensively metabolised AE C656948, but it was still the major compound in milk, muscle and fat (27.3–46.4% of the TRR) and represented ca. 7.7% of the TRR in liver. The main metabolite in milk, muscle and fat were fluopyram-7-hydroxy and fluopyram-*Z*-olefin, accounting each for up to 21.6 of the TRR, respectively, and represented up to 6.1% and 5.7% of the TRR in the other matrices, respectively. The main compound in liver and kidney was fluopyram-7-OH-GA (isomer 1), accounting for 24.2–35.1% of the TRR, but only up to 6.6% of the TRR in the other matrices. Further important metabolites in liver in kidney were fluopyram-8-OH-GA (isomer 2; up to 17.7% of the TRR and up to 2.5% in other matrices) and fluopyram-7-OH-GA (isomer 2; up to 16.3% of the TRR and up to 5.5% in other matrices). The label-specific metabolites fluopyram-pyridyl-acetic acid and fluopyram-hydroxyethyl-GA were only found in kidney at 8.6% and 4.3% of the TRR, respectively.

Further detected minor metabolites were fluopyram-*E*-olefin, fluopyram-di-OH-GA and fluopyram-phenol-GA at <5% of the TRR.

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

- hydroxylation of the ethylene bridge of the molecule resulting in fluopyram-7-hydroxy, fluopyram-8-hydroxy, and a dihydroxylated compound
- hydroxylation of the phenyl ring leading to fluopyram-phenol
- conjugation of the hydroxylated metabolites with glucuronic acid
- elimination of water from compounds hydroxylated in the ethylene bridge leading to fluopyram-*Z*-olefin and *E*-olefin; *E*- and *Z*-olefin can isomerise into each other
- molecular cleavage to fluopyram-pyridyl-hydroxyethyl followed by conjugation with glucuronic acid
- oxidation of fluopyram-pyridyl-hydroxyethyl to fluopyram-pyridyl-acetic acid

The metabolic pathway is proposed in Figure 6.2.3-2.

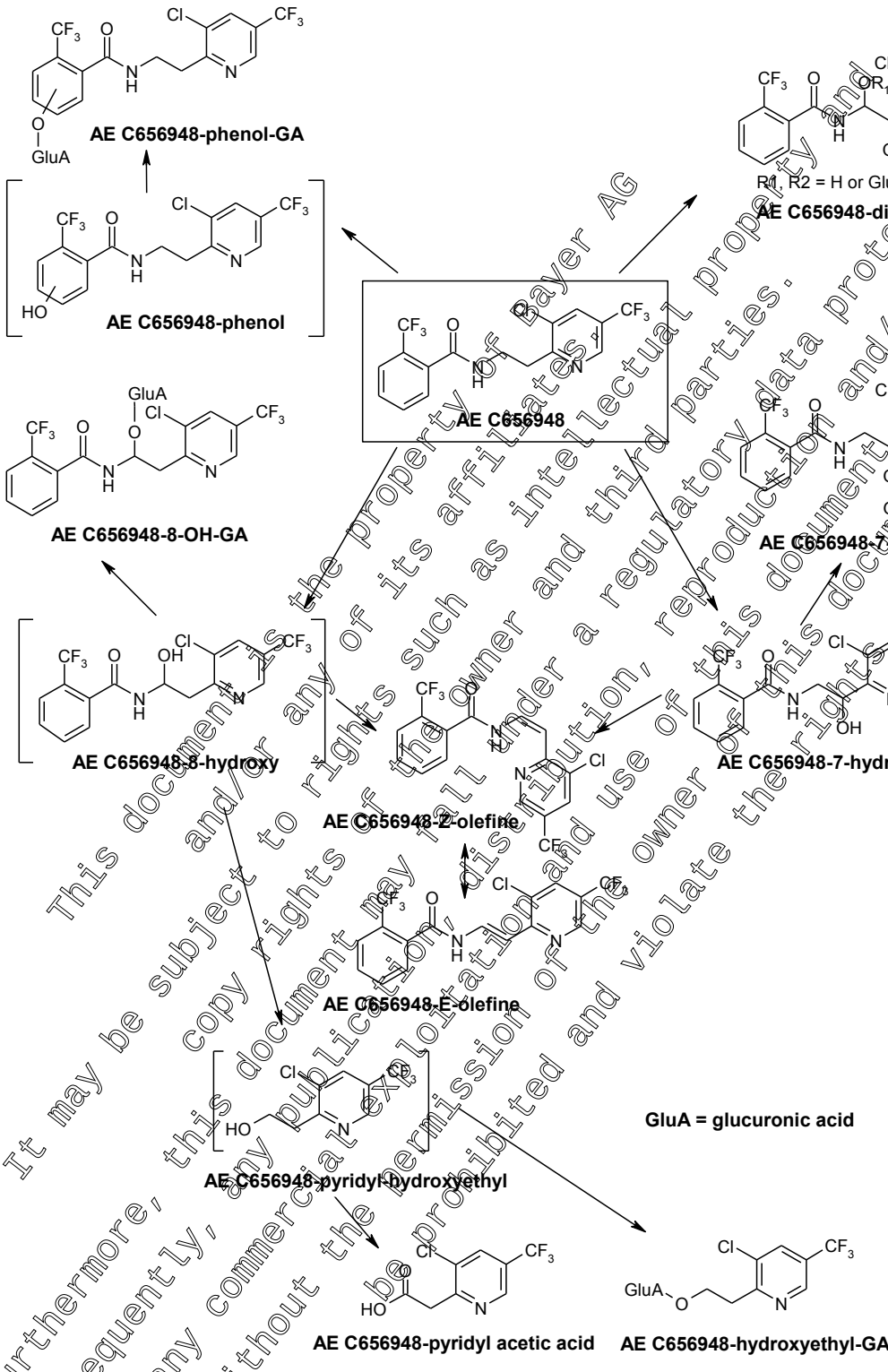


Figure 6.2.3-2: Proposed metabolic pathway of AE C656948 (fluopyram) in goat after treatment with [pyridyl-2,6-^{14}C] AE C656948

Assessment and conclusion by applicant:

The study is valid and acceptable.

CA 6.2.4 Pigs

Not triggered.

CA 6.2.5 Fish

Not triggered.

Data Point:	KCA 6.2.5/01
Report Author:	[redacted]
Report Year:	2008
Report Title:	[pyridyl-2,6-^{14}C]-fluopyram bioconcentration and biotransformation in fish *Lepomis macrochirus*
Report No:	EBC/MP116
Document No:	M-298506-01-1
Guideline(s) followed in study:	OECD 305 (1996), EPA-FIFRA 72-6 (1982), EPA-FIFRA § 165-4 (1982), OPPTS 850.1730 (1996)
Deviations from current test guideline:	Current Guideline: 305 (2012) Deviations: The particulate matter before test start was not reported. The length measurement was only reported for day 0 and not at sampling time as recommended in OECD 305. The missing information was not expected to have impacted the study results. All validity criteria were met.
Previous evaluations:	Yes, evaluated and accepted in Addendum 3 to the DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The bioconcentration in fish (BCF) study is presented in section MCA 8. Please refer to CA 8.2.2.3/01.

CA 6.3 Magnitude of residue trials in plants

CA 6.3.1 Grape

Information on the intended use pattern (GAP) is summarised in Table 6.3.1- 1.

Table 6.3.1- 1: Use patterns (critical GAP) for the spray application of Fluopyram in/on grape in European fields (northern and southern residue regions)

Formulation	F/ GH	No. of appl.	Growth stage at application (BBCH Code)	Application rate per treatment (g a.s./ha)	Water volume (L/ha)	Interval (days)	PHI (days)
FLU+TFS SC500	F*	2	53-73	50	500-750	14	

F field

Baseline dossier

Residue trials submitted during the first inclusion of fluopyram are part of the baseline dossier but do not fulfil the intended GAP anymore.

Data Point:	KCA 6.3.1/08
Report Author:	[redacted]
Report Year:	2008
Report Title:	Determination of the residues of AE C656948 in/on grape after spraying of AE C656948 (500 SC) in the field in Northern France and Germany
Report No:	RA-2611/07
Document No:	M-296857-01-1
Guideline(s) followed in study:	EU-Ref. Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC Guidance Working Document 7029/VI/95 rev. 5 (19 July 07-22); US-EPA OPPTS Guideline No. 860.1500 Supplement A
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted review to Vol 3 of DAR B7 August 2012 (references relied on)
GLP/Officially recognized testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

This EUN study is not summarized here as it does not support the intended GAP anymore (dose too high, application too late). It is overruled by study KCA 6.3.1-08 (M-614532-02-1)

Data Point:	KCA 6.3.1/02
Report Author:	[redacted]; [redacted]
Report Year:	2008
Report Title:	Determination of the residues of AE C656948 in/on grape after low-volume spraying of AE C656948 (500 SC) in the field in Southern France
Report No:	RA-2647/06
Document No:	M-296564-01-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC Guidance Working Document 7029/VI/95 rev. 5 (1997-07-22); Equivalent to US EPA OPPTS Guideline No. 860.1500 (SUPP)
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted rev. 1 to Vol.3 of DAR B7 August 2012 (references relied on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

This EUS study is not summarized here as it does not support the intended GAP anymore (dose too high, application too late). It is overruled by studies KCA 6.3.1-10 (M-623354-02-1) and KCA 6.3.1-11 (M-643609-02-1).

Data Point:	KCA 6.3.1/03
Report Author:	[redacted]; [redacted]
Report Year:	2008
Report Title:	Determination of the residues of AE C656948 in/on table grape after spraying of AE C656948 (500 SC) in the field in Spain, Portugal, Italy and Greece
Report No:	RA-2615/06
Document No:	M-296514-01-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC Guidance Working Document 7029/VI/95 rev. 5 (1997-07-22); Equivalent to US EPA OPPTS Guideline No. 860.1500 (SUPP)
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted rev. 1 to Vol.3 of DAR B7 August 2012 (references relied on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

This EUS study is not summarized here as it does not support the intended GAP anymore (dose too high, application too late). It is overruled by studies KCA 6.3.1-10 (M-653354-02-1) and KCA 6.3.1-11 (M-643609-02-1).

Data Point:	KCA 6.3.1/04
Report Author:	[redacted].
Report Year:	2008
Report Title:	Determination of the residues of AE C656948 in/on grape after low-volume spraying and spraying of AE C656948 (500 SC) in the field in Northern France and Germany
Report No:	RA-2500/07
Document No:	M-298193-01-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8; Residues in/or on Treated Products, Food and Feed; Equivalent to US EPA OPPTS Guideline No. 860.1500 (SUPP)
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted rev. 1 to Vol.3 of DAR B7 August 2012 (references relied on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

This EUN study is not summarized here as it does not support the intended GAP anymore (dose too high, application too late). It is overruled by study KCA 6.3.1-08 (M-614532-02-1)

Data Point:	KCA 6.3.1/06
Report Author:	[redacted]
Report Year:	2008
Report Title:	Determination of the residues of AE C656948 in/on grape after low-volume spraying of AE C656948 (500 SC) in the field in Southern France
Report No:	RA-2501/07
Document No:	M-298199-01-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8; Residues in or on Treated Products, Food and Feed; Equivalent to US EPA OPPTS Guideline No. 860.1500 (SUPP)
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted rev. 1 to Vol.3 of DAR B7 August 2012 (references relied on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

This EUS study is not summarized here as it does not support the intended GAP anymore (dose too high, application too late). It is overruled by studies KCA 6.3.1-10 (M-653354-02-1) and KCA 6.3.1-11 (M-643609-02-1).

Data Point:	KCA 6.3.1/06
Report Author:	[redacted]
Report Year:	2008
Report Title:	Determination of the residues of AE C656948 on/on table grapes after spraying of AE C656948 (500 SC) in the field in Spain, Portugal and Italy
Report No:	RA-2502/07
Document No:	M-298633-01-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of June 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8; Residues in or on Treated Products, Food and Feed; Equivalent to US EPA OPPTS Guideline No. 860.1500 (SUPP)
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted rev. 1 to Vol.3 of DAR B7 August 2012 (references relied on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

This EUS study is not summarized here as it does not support the intended GAP anymore (dose too high, application too late). It is overruled by studies KCA 6.3.1-10 (M-653354-02-1) and KCA 6.3.1-11 (M-643609-02-1).

Data Point:	KCA 6.3.1/07
Report Author:	[redacted]
Report Year:	2008
Report Title:	AE C656948 500 SC - Magnitude of the residue on small fruit vine climbing subgroup 13F, except fuzzy kiwifruit
Report No:	RAGDP043-1
Document No:	M-299437-01-1
Guideline(s) followed in study:	EPA Reference: OPPTS 860.1500, Crop Field Trials PMRA Reference: DACO 7.4.1, Supervised Residue Trial Study PMRA Reference: DACO 7.4.2, Residue Decline Study
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted rev. 1 to Vol.3 of DAR B7 August 2012 (references relied on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

This US study is not summarized here as EU data are required for this renewal dossier. It is overruled by studies KCA 6.3.1-08 (M-614532-02-1), KCA 6.3.1-10 (M-653354-02-1) and KCA 6.3.1-11 (M-643609-02-1).

Supplementary dossier

Residue trials were conducted in Europe (north and south) at the intended or at a similar GAP.

In the Northern zone, eight residue trials were conducted in the 2016 growing season with a higher dose rate compared to the supported GAP. As all other GAP parameter respect the GAP, an downscaling factor is applied to the residue results. According to EFSA Technical Report (EFSA supporting publication 2018-EN-1503), *the proportionality concept can be applied to residue data from field trials conducted within a dose rate range of between 0.3x and 4x the GAP dose rate*.

As the northern zone trials were applied at a BBCH growth stage of 69 (instead of 73 in the representative GAP), it is demonstrated below that the residues obtained from trials treated at BBCH 69 are comparable with residues from trials treated at BBCH 73. The comparability is shown below with the help of SEU data.

In the Southern zone, eight residue trials were conducted in the 2017 growing season

- Study 17-2112 : 2x37.5g ai/ha, last application at BBCH 73, interval 7-12 days
- Study 17-2128 : 2x50g ai/ha, last application at BBCH 73, interval 11-12 days

Table 6.3.1- 2: Overview of European residue trials conducted in grape per geographical "residue region" and vegetation period

Crop	Region	No. of independent trials			Report No. (Formulation)	Document number	Reference
		Vegetation period		Σ			
		2016	2017				
Wine grape	NEU	8		8	16-2157 (FLU SC500)	M-614532-02-1	KCA 6.3.1/08
	SEU	-	8	8	17-2112, 17-2128 (FLU+TFS SC500)	M-653354-02-1 M-643609-02-1	KCA 6.3.1/10 KCA 6.3.1/11

NEU = northern EU field SEU = southern EU field

Northern zone

For the comparison of data sets obtained after applications of fluopyram on vines at BBCH 73 Vs BBCH 69, two sets are taken from Southern European trials.

- Study 16-2197 (M-616678-01-1, KCA 6.3.1/09): 2x100 g ai/ha, last application at BBCH 69, interval 12 days
- Study 17-2112 (M-653354-02-1, KCA 6.3.1/10) : 2x37.5 g ai/ha, last application at BBCH 73, interval 7-12 days
- Study 17-2128 (M-643609-02-1, KCA 6.3.1/11): 2x50 g ai/ha, last application at BBCH 73, interval 11-12 days

As the dose rate is the only GAP parameter which differentiate the studies, the study results from 17-2112 and 17-2128 are upscaled to make the residue data comparable with study 16-2197 which is applied at twice 100 g ai/ha.

Table 6.3.1- 3: Upscaled residues of SEU trials conducted at BBCH 73 to simulate 2 application at 100 g ai/ha

Study number	Trial number	Dose rate (g ai/ha)	Interval (days)	BBCH at application	Residues at harvest (mg/kg)		Upscaled residues x 2.6
					reported	unrounded	
17-2112	17-2112-01	2 x 37.5	7	73	<0.01	0.006	0.016
	17-2112-02	2 x 37.5	12	73	0.030	0.030	0.078
	17-2112-03	2 x 37.5	12	73	0.014	0.014	0.036
	17-2112-04	2 x 37.5	9	73	<0.01	0.0009	0.002
Study number	Trial number	Dose rate (g ai/ha)	Interval (days)	BBCH at application	Residues at harvest (mg/kg)		Upscaled residues x2
					reported	unrounded	
17-2128	17-2128-01	2 x 50	12	73	0.01	0.01	0.02
	17-2128-02	2 x 50	12	73	0.01	0.01	0.02
	17-2128-03	2 x 50	12	73	0.038	0.038	0.076
	17-2128-04	2 x 50	11	73	0.033	0.033	0.066

Table 6.3.1- 4: Residues of SEU trials conducted at BBCH 69 with 2 appl. at 100 g ai/ha

Study number	Trial number	Dose rate (g ai/ha)	Interval (days)	BBCH at application	Residues at harvest (mg/kg)
16-2197	16-2197-01	2 x 100	12	69	0.030
	16-2197-02	2 x 100	12	69	0.030
	16-2197-03	2 x 100	12	69	0.015
	16-2197-04	2 x 100	12	69	<0.01
	16-2197-05	2 x 100	12	69	0.035
	16-2197-06	2 x 100	12	69	0.015
	16-2197-07	2 x 100	12	69	<0.01
	16-2197-08	2 x 100	12	69	<0.01

The two data sets are submitted to statistical tests to show if it can be considered that they belong to the same population. The comparability test Whitney Mann Willcoxon show that the two data sets are comparable. The Kruskal Wallis test (appropriate test according to FAO report 2009) concludes that the data samples are likely to come from the same population.

As a conclusion, residues in grapes at harvest are similar after two applications of fluopyram when the last application is done either at BBCH 69 or BBCH 73 in southern Europe.

By extrapolation, we can consider that residue data at harvest are similar also in Northern Europe after two applications of fluopyram when the last application is done either at BBCH 69 or BBCH 73. Thus, the eight residue trials from the study 16-2157 (M-614532-02-1, KCA 6.3.1/08) and conducted with the last application at BBCH 69 are considered acceptable.

Nevertheless, the northern study 16-2157 was conducted at a higher application rate of 2x100 g/ha compared to the representative GAP. As all other GAP parameter respect the GAP, a downscaling factor is applied to the residue results. According to EFSA Technical Report (EFSA supporting publication 2018-EN-1503), *the proportionality concept can be applied to residue data from field trials conducted within a dose rate range of between 0.3x and 4x the GAP dose rate*.

Data Point:	KCA 6.3.1/08
Report Author:	[redacted]
Report Year:	2019
Report Title:	Amendment no. 1 to final report - Determination of the residues of fluopyram in/on grape after spray application of fluopyram SC 500 in Germany, the United Kingdom, Hungary and Northern France
Report No:	16-2157
Document No:	M-614532-02-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted Study was not found in DAR/RAR and the Addenda
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and Methods

Eight residue trials on grapes were conducted in northern Europe (in Germany, the United Kingdom, Hungary, and northern France) during the 2016 season. Two spray applications (each) for treated plot 1 (T1) and treated plot 2 (T2) were carried out with Fluopyram SC 500 (SC 500). These applications were conducted with the interval between applications of 12 days and at the application rates of 0.2 L/ha corresponding to the application rate of 0.1 kg a.s./ha and water rates between 500 – 1000 L/ha.

As the dose rate is higher than the intended GAP (2x100g as/ha Vs 2x50g as/ha), the residues are downscaled by applying the scaling factor of 0.5.

Each trial contained two plots representing applications in different BBCH stages: Plot 1 (T1) includes last (2nd) application at BBCH 69 and plot 2 (T2) includes last (2nd) application at BBCH 79. The aim was to verify the impact of the BBCH stage of the latest application in the final residues at harvest.

For both plots, samples were collected at BBCH 79, BBCH 85, and BBCH 89.

All field samples were shipped deep-frozen under monitored conditions during shipment. The field samples were stored in a freezer at ≤ -18 °C until preparation of the examination samples.

For the preparation of examination samples, the deep-frozen field samples were shredded and homogenised with dry ice in a cutter and transferred into polystyrene boxes and stored at ≤ -18 °C until analysis.

Samples were analysed according to the analytical method 00984/M003 ([redacted], 2015, M-467323-03-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

However, slight adaptions were made to the extraction procedure described within the analytical method modification 00984/M003 which are as follows: residues were extracted from 5 g of sample material by extraction using a shaker (15 min) with a mixture of acetonitrile/water (4+1, v+v). The filtering procedure under low vacuum was replaced by a centrifugation step. Then, 0.5 mL of internal standard solution (1 mg/L) were added to the extract followed by 5 min centrifugation at 4750 rpm at 10 °C and further proceeded to the HPLC-MS/MS analysis

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

All final extracts were analysed within two days after the preparation. Fluopyram and fluopyram-benzamide were found to be stable in extracts of matrices of plant origin for at least four weeks at 4 ± 3 °C which was tested within the validation of method 00984/M003.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.01 mg/kg.

Findings

In order to check the performance of the method, concurrent recovery determinations were included in each set of analyses (at least one recovery for ten study samples). Control samples from the study were fortified to be used as the recovery samples. The apparent residues in the control samples used for the performance of recoveries were below 30 % of the LOQ. The recoveries were not corrected for apparent residues in the control samples.

The mean recoveries per fortification level were within the range of 70 % – 110 % except for the LOQ level for fluopyram (116 %) and for the LOQ level for fluopyram-benzamide (111 %). However, these recoveries were within the criteria of the OECD guidance document ENV/JM/MONO(2007)17. Thus, these results were considered acceptable. The RSD values were below 20 %. The overall mean concurrent recoveries were in the acceptable range of 70 % and 110 %, with the RSD < 20 %.

The storage period of deep-frozen samples intended for the analysis of fluopyram and its metabolite fluopyram-benzamide ranged between 82 and 172 days. The detailed results obtained for bunch of grapes samples in northern Europe are summarized in the Table 6.3.1- 6, where the scaling factor and the total residue downscaled are also presented.

Conclusions

Eight supervised residue trials on grapes were conducted in northern Europe with the application of Fluopyram SC 500 (SC 500) at a higher rate than required in the supported GAP (2x100g as/ha Vs 2x50g as/ha), and according to GLP. Samples of bunch of grapes were analyzed for the residues of fluopyram and its metabolite fluopyram-benzamide. The results of the trials presented above show residues at harvest for the plots with last application at BBCH 69 :

- Fluopyram residues range between <0.01 and 0.04 mg/kg.

- Fluopyram-benzamide residues were <0.01 mg/kg when the last application was done at BBCH 69.
- The total residue of fluopyram (sum of FLU + FLU-benzamide, expressed as fluopyram) after downscaling to the right dose (2x50g Vs 2x100g ai/ha)), range between <0.01 and 0.049 mg/kg.

Assessment and conclusion by applicant:

The study is acceptable.
The residue level in these GLP trials was corrected with a proportionality factor according to EFSA Technical Report (EFSA supporting publication 2018-EN-1503).

Table 6.3.1- 5: Concurrent recovery data for Fluopyram (study 16-2157)

Portion analysed	Fortification level (mg/kg)	Recovery (%)				
		Individual recoveries	Min	Max	Mean	RSD
Fluopyram (AE C656948)						
grape / bunch of grapes	0.01	112; 115; 115; 116; 117; 119	112	119	116	2.0
	0.10	109; 109; 110; 111	109	111	110	0.9
	2.0	99; 101; 102	99	102	101	1.5
		Overall recovery (n=13)			**110**	**5.8**
Fluopyram-benzamide						
grape / bunch of grapes	0.01	109; 110; 111; 111; 112; 112; 113; 113	109	113	111	1.3
	0.10	104; 105; 106; 107	104	107	106	1.2
	2.0	98; 99; 100	98	100	99	1.0
		Overall recovery (n= 15)			**107**	**4.8**

RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with fluopyram, determined as fluopyram and calculated as fluopyram
Fortified with fluopyram-benzamide, determined as fluopyram-benzamide and calculated as fluopyram
These recoveries were performed during the conduct of the study 16-2157

Table 6.3.1- 6: Summary of the 16-2157 (M-614532-02-1) study on grapes

Total residue calculated : sum of fluopyram+FLU-benzamide

Trial No. / Location / EU zone / Year	Commodity / Variety (a)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting (b)	Application rate per treatment: g a.s./ha	Water (L/ha)	g a.s./hL	Dates of treatment / Application interval (c)	Growth stage at last treatment (d)	Portion analyzed	Growth stage at sampling (d)	Residues (mg/kg): AE C656948	AE C656948-benzamide	Total residue calc.	Scaling factor	Scaled residue	PHI (days) (e)
16-2157-01 16-2157-01-T1 Germany 77704 Oberkirch Europe, North F 2016	Grape Muscat bleu; table grapes	1) 2003 2) 12.06.2016 - 22.06.2016 3) 25.08.2016 - 02.09.2016	100 100	800 800	12.5 12.5	10.06.2016/0 22.06.2016/12	69	bunch of grapes	79 85 89	0.039 0.024 0.014	<0.01 <0.01 <0.01	0.049 0.034 0.024	0.5	0.025 0.017 0.012	0 51 65
16-2157-01 16-2157-01-T2 Germany 77704 Oberkirch Europe, North F 2016	Grape Muscat bleu; table grapes	1) 2003 2) 12.06.2016 - 22.06.2016 3) 25.08.2016 - 02.09.2016	100 100	800 800	12.5 12.5	09.07.2016/0 21.07.2016/12	79	bunch of grapes	79 79 85 89	0.12 0.30 0.13 0.085	<0.01 <0.01 <0.01 <0.01	0.13 0.31 0.14 0.095	0.5	0.065 0.16 0.070 0.048	0* 0 22 36
16-2157-02 16-2157-02-T1 Germany 79356 Eichstetten Europe, North F 2016	Grape Acolon	1) 2009 2) 13.06.2016 - 23.06.2016 3) 05.09.2016 - 15.09.2016	100 100	800 800	12.5 12.5	10.06.2016/0 22.06.2016/12	69	bunch of grapes	79 85 89	0.028 0.021 <0.01	<0.01 <0.01 <0.01	0.038 0.031 <0.02	0.5	0.019 0.016 <0.01	0 51 78

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)				PHI (days)
			g a.s./ha	Water (L/ha)	g a.s./hL					AE C656948	AE C656948-benzamide	Total residue calc.	Scaling factor	Scaled residue
	(a)	(b)				(c)	(d)		(d)					(e)
16-2157-02 16-2157-02-T2 Germany 79356 Eichstetten Europe, North F 2016	Grape Acolon	1) 2009 2) 13.06.2016 - 23.06.2016 3) 05.09.2016 - 15.09.2016	100 100	800 800	12.5 12.5	15.07.2016/0 27.07.2016/12	79	bunch of grapes	79 79 85 89	0.33 0.63 0.28 0.12	0.042 0.044 0.046 0.026	0.37 0.67 0.33 0.15	0.5	0.19 0.34 0.17 0.075	0* 0 16 43
16-2157-03 16-2157-03-T1 United Kingdom GL181LS Newent Europe, North F 2016	Grape Sauvignon Blanc; White grape	1) 1997 2) 11.07.2016 - 25.07.2016 3) 12.10.2016 - 17.10.2016	100 100	560.54422 600	17.839 16.666	19.07.2016/0 31.07.2016/12	69	bunch of grapes	79 85 89	0.15 0.052 0.087	<0.01 <0.01 <0.01	0.16 0.062 0.097	0.5	0.080 0.031 0.049	18 44 74
16-2157-03 16-2157-03-T2 United Kingdom GL181LS Newent Europe, North F 2016	Grape Sauvignon Blanc; White grape	1) 1997 2) 11.07.2016 - 25.07.2016 3) 12.10.2016 - 17.10.2016	100 90.751	600 933.83459	16.666 16.999	06.08.2016/0 18.08.2016/12	79	bunch of grapes	79 79 85 89	0.16 0.38 0.070 0.062	<0.01 <0.01 <0.01 <0.01	0.17 0.39 0.080 0.072	0.5	0.086 0.20 0.04 0.036	0* 0 26 56

Trial No. / Location / EU zone / Year	Commodity / Variety (a)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting (b)	Application rate per treatment			Dates of treatment / Application interval (c)	Growth stage at last treatment (d)	Portion analyzed	Growth stage at sampling (d)	Residues (mg/kg)				PHI (days) (e)
			g a.s./ha	Water (L/ha)	g a.s./hL					AE C656948	AE C656948-benzamide	Total residue calc.	Scaling factor	Scaled residue
16-2157-04 16-2157-04-T1 Hungary H-4461 Nyirtelek - Ferenctanya Europe, North F 2016	Grape Cserszegi fuszeres; White grape	1) 2001 2) 16.05.2016 - 30.05.2016 3) 10.09.2016 - 25.09.2016	100 100	700 700	14.285 14.285	02.06.2016/0 - 14.06.2016/12	69	bunch of grapes	79 85 89	0.22 0.070 0.052	<0.01 <0.01 <0.01	0.23 0.080 0.062	0.5	0.12 0.04 0.031	0 85 94
16-2157-04 16-2157-04-T2 Hungary H-4461 Nyirtelek - Ferenctanya Europe, North F 2016	Grape Cserszegi fuszeres; White grape	1) 2001 2) 16.05.2016 - 30.05.2016 3) 10.09.2016 - 25.09.2016	100 100	700 700	14.285 14.285	04.07.2016/0 - 16.07.2016/12	79	bunch of grapes	79 85 89	0.20 0.46 0.13 0.11	<0.01 <0.01 <0.01 <0.01	0.21 0.47 0.14 0.12	0.5	0.11 0.24 0.07 0.06	0* 0 53 62
16-2157-05 16-2157-05-T1 Hungary H-3905 Monok Europe, North F 2016	Grape Furmint; White grape	1) 1986 2) 12.05.2016 - 25.05.2016 3) 01.09.2016 - 15.09.2016	100 100	700 700	14.285 14.285	03.06.2016/0 - 15.06.2016/12	69	bunch of grapes	79 85 89	0.10 0.054 0.041	<0.01 <0.01 <0.01	0.11 0.064 0.051	0.5	0.055 0.032 0.026	0 84 93

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)				PHI (days)
			g a.s./ha	Water (L/ha)	g a.s./hL					AE C656948	AE C656948-benzamide	Total residue calc.	Scaling factor	Scaled residue
	(a)	(b)				(c)	(d)		(d)					(e)
16-2157-05 16-2157-05-T2 Hungary H-3905 Monok Europe, North F 2016	Grape Furmint; White grape	1) 1986 2) 12.05.2016 - 25.05.2016 3) 01.09.2016 - 15.09.2016	100 100	700 700	14.285 14.285	04.07.2016/0 16.07.2016/12	79	bunch of grapes	79 79 85 89	0.16 0.36 0.14 0.14	<0.01 <0.01 <0.01 <0.01	0.17 0.37 0.15 0.15	0.5	0.085 0.19 0.075 0.075	0* 0 53 62
16-2157-06 16-2157-06-T1 France, north 51220 BRIMONT Europe, North F 2016	Grape pinot noir	1) 1999 2) 21.06.2016 - 03.07.2016 3) 20.09.2016 - 20.09.2016	100 100	720 720	13.888 13.888	21.06.2016/0 03.07.2016/12	69	bunch of grapes	79 85 89	0.073 0.035 0.049	<0.01 <0.01 <0.01	0.083 0.045 0.059	0.5	0.042 0.023 0.030	0 57 79
16-2157-06 16-2157-06-T2 France, north 51220 BRIMONT Europe, North F 2016	Grape pinot noir	1) 1999 2) 21.06.2016 - 03.07.2016 3) 20.09.2016 - 20.09.2016	100 100	720 720	13.888 13.888	27.07.2016/0 08.08.2016/12	79	bunch of grapes	79 79 85 89	0.15 0.30 0.18 0.12	<0.01 <0.01 <0.01 <0.01	0.16 0.31 0.19 0.13	0.5	0.08 0.16 0.095 0.065	0* 0 21 43

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)				PHI (days)
			g a.s./ha	Water (L/ha)	g a.s./hL					AE C656948	AE C656948-benzamide	Total residue calc.	Scaling factor	Scaled residue
	(a)	(b)				(c)	(d)		(d)					(e)
16-2157-07 16-2157-07-T1 France, north 41150 Mesland Europe, North F 2016	Grape Chardonnay; white vine	1) 1956 2) 22.06.2016 - 06.07.2016 3) 05.10.2016 - 07.10.2016	100 100	500 500	20 20	24.06.2016/0 06.07.2016/12	69	bunch of grapes	79 85 89	0.11 0.085 0.056	<0.01 <0.01 <0.01	0.12 0.095 0.060	0.5	0.06 0.048 0.033	0 68 82
16-2157-07 16-2157-07-T2 France, north 41150 Mesland Europe, North F 2016	Grape chardonnay; white vine	1) 1956 2) 22.06.2016 - 06.07.2016 3) 05.10.2016 - 07.10.2016	100 108.89	500 542.22222	20 20.083	22.07.2016/0 04.08.2016/13	79	bunch of grapes	79 85 89	0.080 0.21 0.13 0.098	<0.01 <0.01 0.011 0.011	0.099 0.22 0.14 0.11	0.5	0.050 0.11 0.07 0.055	0* 0 39 53
16-2157-08 16-2157-08-T1 France, north 72340 Beaumont Sur Dême Europe, North F 2016	Grape Chenin; white vine	1) 2001 2) 27.06.2016 - 10.07.2016 3) 29.09.2016 - 07.10.2016 4) 21.01.2001	100 100	500 500	20 20	28.06.2016/0 10.07.2016/12	69	bunch of grapes	79 85 89	0.11 0.065 0.030	<0.01 <0.01 <0.01	0.12 0.075 0.040	0.5	0.06 0.038 0.02	0 61 88

Trial No. / Location / EU zone / Year	Commodity / Variety (a)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting (b)	Application rate per treatment			Dates of treatment / Application interval (c)	Growth stage at last treatment (d)	Portion analyzed	Growth stage at sampling (d)	Residues (mg/kg)				PHI (days) (e)
			g a.s./ha	Water (L/ha)	g a.s./hL					AE C656948	AE C656948-benzamide	Total residue calc.	Scaling factor	Scaled residue	
16-2157-08 16-2157-08-T2 France, north 72340 Beaumont Sur Dême Europe, North F 2016	Grape Chenin; white vine	1) 2001 2) 27.06.2016 - 10.07.2016 3) 29.09.2016 - 07.10.2016 4) 21.01.2001	100 100	500 500	20 20	28.07.2016/0 09.08.2016/12	79	bunch of grapes	79 79 85 89	0.091 0.12 0.11 0.096	<0.01 <0.01 <0.01 <0.01	0.10 0.13 0.12 0.101	0.5	0.05 0.065 0.06 0.055	0* 0 31 58

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

- G greenhouse F field
-
-

- (g) Study reference
- (h) Formulation type
- (i) Application method
- (j) Method information
- (k) LOQ
- (l) Method validation
- (m) Storage (max) ! based on date of analysis, P based on production date
- * prior to last treatment
- ** residue or control
- # no data available

Southern zone

Data Point:	KCA 6.3.1/10
Report Author:	[redacted]
Report Year:	2020
Report Title:	Amendment no. 01: Determination of the residues of trifloxystrobin and AE C656948 in/on grape after spray application (high-volume and low-volume) of AE C656948 & CGA279202 SC 500 in Italy, southern France and Spain
Report No:	17-2112
Document No:	M-653354-02-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509, published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted Study was not found in DAR/RAR and the Addenda
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and Methods

Four residue trials on grapes were conducted in southern Europe (in Italy, in southern France, in Spain) during the 2017 season. Two spray applications were carried out with PLU+TFS SC 500, a SC (suspension concentrate) formulation containing 250 g/L of fluopyram and 250 g/L of trifloxystrobin.

For the purpose of this renewal, only results for fluopyram and its metabolite fluopyram-benzamide will be presented discussed.

The applications were conducted with the interval between applications of 12 days (except for the trial 17-2112-01 and 17-2112-04 for which the application interval was 7 and 9 days, respectively) and at the application rates of 0.15 L/ha corresponding to the application rate of 0.038 kg a.s./ha and water rates between 200 – 1000 L/ha.

The last application was performed at BBCH 73 (2nd application).

Samples of grapes were collected at the BBCH stage of 73, 79, 85, and 89.

All field samples were shipped deep-frozen under monitored conditions during shipment. The field samples were stored in a freezer at ≤ -18 °C until preparation of the examination samples.

For the preparation of examination samples, the deep-frozen field samples were shredded and homogenized with dry ice in a cutter and transferred into polystyrene boxes and stored at ≤ -18 °C until analysis.

Samples were analysed according to the analytical method 00984/M003 ([redacted], 2015, M-467323-03-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented

within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues were extracted from 5 g of sample material by extraction with a mixture of acetonitrile-water (4:1, v:v). The final determination was performed with HPLC-MS/MS.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.01 mg/kg.

The time between the beginning of the specimen preparation (extraction) and the specimen analysis did not exceed 16 days while stored at 1 – 9 °C in the dark. Fluopyram and fluopyram-benzamide were found to be stable in extracts of matrices of plant origin for at least four weeks at 4 ± 3 °C which was tested within the validation of method 00984/M003.

Findings

The apparent residues in the control sample used for the determination of recoveries were below 30 % of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these determinations.

In order to check the performance of the method, recovery determinations were included in each set of analyses (at least one recovery for ten study samples). Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study.

The mean recoveries per fortification level were within the range of 70 % – 110 % and the RSD values were below 20 %. The overall mean concurrent recoveries were also in the acceptable range of 70 % and 110 %, with the RSD < 20 %.

The storage period of deep-frozen samples intended for the analysis of fluopyram and fluopyram-benzamide ranged between 263 and 382 days. The detailed results obtained for bunch of grapes samples in southern Europe are summarized in the Table 6.3.1- 8.

Conclusions

Four supervised residue trials on grapes were conducted in southern Europe with the application of FLU+TFS SC 500 at the required rates (within ± 25% of the supported GAP) and according to GLP. After a last application at BBCH 73, the samples of bunch of grapes were analyzed for the residues of fluopyram and its metabolite fluopyram-benzamide. The results of the trials presented above show residues at harvest.

- Fluopyram residues range between <0.01 and 0.03 mg/kg.
- Fluopyram-benzamide residues were <0.01 mg/kg.
- The total residue of fluopyram (sum of FLU + FLU-benzamide, expressed as fluopyram) range between 0.02 and 0.04 mg/kg.

Assessment and conclusion by applicant:

The study is acceptable.

Table 6.3.1- 7: Concurrent recovery data for Fluopyram (study 17-2112)

Portion analysed	Fortification level (mg/kg)	Recovery (%)				
		Individual recoveries	Min	Max	Mean	RSD
Fluopyram (AE C656948)						
grape / bunch of grapes	0.01	79; 80; 89; 90; 90; 109	79	109	90	12.0
	0.10	82; 83; 87; 87; 88; 89	82	89	86	3.3
	0.30	85	-	-	85	
		Overall recovery (n=13)			**88**	**8.5**
Fluopyram-benzamide						
grape / bunch of grapes	0.01	80; 84; 90; 90; 104	80	104	90	10.2
	0.10	77; 82; 82; 86; 111	77	111	88	15.4
		Overall recovery (n =10)			**89**	**12.3**

RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with fluopyram, determined as fluopyram and calculated as fluopyram
Fortified with fluopyram-benzamide, determined as fluopyram-benzamide and calculated as fluopyram
These recoveries were performed during the conduct of the study 17-2112.

Table 6.3.1- 8: Summary of the 17-2112 (M-653354-02-1) study on grape

Total residue calculated : sum of fluopyram+FLU-benzamide

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)			PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					fluopyram as AE c.656948	FLU-benzamide as AE c.656948	Total residue calc.		
	(a)	(b)				(c)	(d)		(d)				(e)	(f)
17-2112-01 Italy 95045 C. da Incarrozza; Misterbianco (CT) Europe, South F 2017	Grape Alicante; Red berries	1) 2007 2) 10.05.2017 - 25.05.2017 3) 01.09.2017 - 30.09.2017	37.5 37.5	600 600	6.25 6.25	19.05.2017/0 26.05.2017/7	73	bunch of grapes	73 73 79 85 89	0.076 0.22 0.028 <0.01 <0.01	<0.01 0.015 <0.01 <0.01 <0.01	0.088 0.24 0.038 <0.02 <0.02	0* 0 13 80 104	(g) 17-2112 (h) SC (fluopyram 250 g/L ,trifloxystrobin 250 g/L) (i) Spraying (j) Analytical method: 00984/M003 (k) LOQ: 0.01 mg/kg (l) Method Validation Data : 00984/M003, 17-2112 (m) Storage: bunch of grapes: 382 days
17-2112-02 Italy 76123 Andria Europe, South F 2017	Grape Sangiovese; Red variety	1) 15.02.1994 2) 20.05.2017 - 06.06.2017 3) 01.09.2017 - 15.09.2017	37.5 37.5	800 800	4.69 4.69	16.06.2017/0 23.06.2017/7	73	bunch of grapes	73 73 79 85 89	0.075 0.19 0.11 0.043 0.030	<0.01 <0.01 <0.01 <0.01 <0.01	0.085 0.20 0.12 0.153 0.040	0* 0 15 44 72	(g) 17-2112 (h) SC (fluopyram 250 g/L ,trifloxystrobin 250 g/L) (i) Spraying (j) Analytical method: 00984/M003 (k) LOQ: 0.01 mg/kg (l) Method Validation Data : 00984/M003, 17-2112 (m) Storage: bunch of grapes: 349 days

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)			PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					fluopyram as AE C656948	fluopyram-benzamide as AE C656948	Total residue calc.		
	(a)	(b)				(c)	(d)		(d)				(e)	(f)
17-2112-03 France, south 30290 Saint Victor la Coste Europe, South F 2017	Grape Viognier; Grape White	1) 01.04.1990 2) 20.05.2017 - 31.05.2017 3) 10.09.2017 - 20.09.2017	37.5 37.5	200 200	18.8 18.8	31.05.2017/0 12.06.2017/12	73	bunch of grapes	73 73 79 85 89	0.052 0.11 0.024 0.016 0.014	<0.01 <0.01 <0.01 <0.01 <0.01	0.062 0.12 0.034 0.026 0.024	0* 0 45 67 93	(g) 17-2112 (h) SC (fluopyram 250 g/L ,trifloxystrobin 250 g/L) (i) Spraying (j) Analytical method: 00984/M003 (k) LOQ: 0.01 mg/kg (l) Method Validation Data : 00984/M003, 17-2112 (m) Storage: bunch of grapes: 357 days
17-2112-04 Spain 46842 Rugat Europe, South F 2017	Grape Macabeo; white variety	1) 01.01.2007 2) 21.05.2017 - 29.05.2017 3) 20.08.2017 - 10.09.2017	37.5 37.5	1000 1000	3.75 3.75	31.05.2017/0 09.06.2017/9	73	bunch of grapes	73 73 79 85 89	0.020 0.078 0.018 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01	0.030 0.088 0.028 <0.02 <0.02	0* 0 12 62 76	(g) 17-2112 (h) SC (fluopyram 250 g/L ,trifloxystrobin 250 g/L) (i) Spraying (j) Analytical method: 00984/M003 (k) LOQ: 0.01 mg/kg (l) Method Validation Data : 00984/M003, 17-2112 (m) Storage: bunch of grapes: 360 days

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH code
G greenhouse F field
(e) Days after last application (*label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) Study reference
* prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Data Point:	KCA 6.3.1/11
Report Author:	[redacted]
Report Year:	2019
Report Title:	Amendment no. 1 to final report - Determination of the residues of trifloxystrobin and AE C656948 in/on grape after spray application of AE C656948 & CGA279202 SC 500 in southern France, Italy, Spain and Bulgaria
Report No:	17-2128
Document No:	M-643609-02-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP Guideline No. 860.1500 on Crop Field Trial
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted Study was not found in DAR/RAR and the Addenda
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and methods

Four residue trials on grapes were conducted in southern Europe (in Italy, southern France, Spain, and Bulgaria) during the 2017 season. Two spray applications on plot T1 (2nd application at BBCH 73, interval 21 days) and two spray applications on plot T2 (2nd application at BBCH 73, interval 12 days) with FLU+TFS, SC 500, a suspension concentrate formulation containing 250 g/L fluopyram and 250 g/L trifloxystrobin.

For the purpose of this renewal, only results for fluopyram and its metabolite fluopyram-benzamide will be presented discussed.

The applications were conducted at the application rates of 0.2 L/ha corresponding to the application rate of 0.05 kg a.s./ha and water rates between 500 – 1000 L/ha.

The last application was performed at BBCH 73 (2nd application). Samples of grapes were collected at the BBCH stage of 73, 79, 85, and 89.

All field samples were shipped deep-frozen under monitored conditions during shipment and arrived at PVTL in good condition. For the trials 17-2128-01 and 17-2128-03 the 24 h mean temperature was above -18 °C (T=-17°C, no impact on the residues). The field samples were stored in a freezer at ≤-18 °C until preparation of the examination samples.

For the preparation of examination samples, the deep-frozen field samples were shredded and homogenized with dry ice in a cutter and transferred into polystyrene boxes and stored at ≤ -18 °C until analysis.

Samples were analysed according to the analytical method 00984/M003 ([redacted], 2015, M-467323-03-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues were extracted from 5 g of sample material by extraction using a shaker (15 min) with a mixture of acetonitrile/water (4:1, v:v). After filtration the extracts of the solutions were diluted by adding the internal standard and made up to volume. An aliquot of the extracts was injected into a HPLC-(MS)MS.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.01 mg/kg.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels.

The time between the beginning of the specimen preparation (extraction) and the specimen analysis did not exceed 7 days while stored at 1 – 9 °C in the dark. Fluopyram and fluopyram-benzamide were found to be stable in extracts of matrices of plant origin for at least four weeks at 4 ± 3 °C which was tested within the validation of method 00984/M003.

Findings

The apparent residues in the control sample used for the determination of recoveries were below 30 % of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these determinations.

In order to check the performance of the method, recovery determinations were included in each set of analyses (at least one recovery for ten study samples). Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study.

The mean recoveries per fortification level were within the range of 70 % – 110 % and the RSD values (when applicable, n ≥3) were below 20 %. The overall mean concurrent recoveries were also in the acceptable range of 70 % and 110 %, with the RSD < 20 %.

The storage period of deep-frozen samples intended for the analysis of fluopyram and fluopyram-benzamide ranged between 258 and 356 days. The detailed results obtained for bunch of grapes samples in southern Europe are summarized in the Table 6.3.1-10

Conclusions

Four supervised residue trials on grapes were conducted in southern Europe with the application of AE FLU+TFS SC 500 at the required rates and according to GLP. After a last application at BBCH 73, the samples of bunch of grapes were analyzed for the residues of fluopyram and its metabolite fluopyram-benzamide. The results of the trials presented above show residues at harvest.

- Fluopyram residues range between <0.01 and 0.04 mg/kg.
- Fluopyram-benzamide residues were <0.01 mg/kg.
- The total residue of fluopyram (sum of FLU + FLU-benzamide, expressed as fluopyram) range between 0.02 and 0.048 mg/kg.

Assessment and conclusion by applicant:

The study is acceptable.

Table 6.3.1- 9: Concurrent recovery data for Fluopyram (study 17-2128)

Portion analysed	Fortification level (mg/kg)	Recovery (%)				
		Individual recoveries	Min	Max	Mean	RSD
Fluopyram (AE C656948)						
grape / bunch of grapes	0.01	93; 100; 100; 100	93	100	98	3.6
	0.10	87; 90; 91; 94	87	94	91	32
	0.70	90	-	-	90	-
		Overall recovery (n=9)			**94**	**5.3**
Fluopyram-benzamide						
grape / bunch of grapes	0.01	86; 90; 100	86	100	92	7.8
	0.10	97; 102; 108	97	108	102	5.4
		Overall recovery (n =6)	-	-	**97**	**8.3**

RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with fluopyram, determined as fluopyram and calculated as fluopyram
Fortified with fluopyram-benzamide, determined as fluopyram-benzamide and calculated as fluopyram
These recoveries were performed during the conduct of the study 17-2128

Table 6.3.1- 10: Summary of the 17-2128 (M-480441-06-1) study on grape
Total residue calculated : sum of fluopyram+FLU-benzamide

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)			PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					fluopyram as AE C656948	FLU-benzamide as AE C656948	Total residue calc.		
	(a)	(b)				(c)	(d)		(d)				(e)	(f)
17-2128-01 17-2128-01-T1 France, south 66200 Elne Europe, South F, 2017	Grape Cabernet Sauvignon	1) 2000 2) 21.05.2017 - 30.05.2017 3) 03.09.2017 - 09.09.2017	50 50	500 500	10 10	07.06.2017/0 28.06.2017/21	73	bunch of grapes	73 79 85 89	0.068 0.035 0.017 0.021	<0.01 <0.01 <0.01 <0.01	0.078 0.045 0.027 0.031	0 26 57 68	(g) 17-2128 (h) SC (fluopyram 250 g/L ,trifloxystrobin 250 g/L) (i) Spraying (j) Analytical method: 00984/M003, 17-2118 (k) LOQ: 0.01 mg/kg (l) Method Validation Data : 00984/M003, 17-2118 (m) Storage: bunch of grapes: 356 days
17-2128-01 17-2128-01-T2 France, south 66200 Elne Europe, South F, 2017	Grape Cabernet Sauvignon	1) 2000 2) 21.05.2017 - 30.05.2017 3) 03.09.2017 - 09.09.2017	50 50	500 500	10 10	16.06.2017/0 28.06.2017/12	73	bunch of grapes	73 79 85 89	0.094 0.023 <0.01 0.010	<0.01 <0.01 <0.01 <0.01	0.10 0.033 <0.02 0.020	0 26 57 68	
17-2128-02 17-2128-02-T1 Italy 40053 Bazzano Europe, South F, 2017	Grape Pignoletto	1) 2016 2) 30.05.2017 - 15.06.2017 3) 06.09.2017 - 06.09.2017	50 50	600 600	8.3 8.3	20.06.2017/0 11.07.2017/21	73	bunch of grapes	73 79 85 89	0.031 0.018 0.011 <0.01	<0.01 <0.01 <0.01 <0.01	0.041 0.028 0.021 <0.02	0 21 43 57	(g) 17-2128 (h) SC (fluopyram 250 g/L ,trifloxystrobin 250 g/L) (i) Spraying (j) Analytical method: 00984/M003, 17-2118 (k) LOQ: 0.01 mg/kg

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)			PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					fluopyram as AE C656948	FLU-benzamide as AE C656948	Total residue, calc.		
	(a)	(b)				(c)	(d)		(d)			(e)		(f)
17-2128-02 17-2128-02-T2 Italy 40053 Bazzano Europe, South F, 2017	Grape Pignoletto	1) 2011 2) 30.05.2017 - 15.06.2017 3) 06.09.2017 - 06.09.2017	50 50	600 600	8.3 8.3	29.06.2017/0 11.07.2017/**12**	73	bunch of grapes	73 79 85 89	0.036 0.016 0.011 0.010	<0.01 <0.01 <0.01 <0.01	0.046 0.026 0.021 0.020	0 21 43 71	(l) Method Validation Data : 00984/M003, 17-2128 (m) Storage: bunch of grapes: 343 days
17-2128-03 17-2128-03-T1 Spain 50549 Malejan Europe, South F, 2017	Grape Garnacha	1) 1997 2) 15.05.2017 - 10.06.2017 3) 25.09.2017 - 17.10.2017	50 50	500 500	10 10	06.06.2017/0 28.06.2017/**22**	73	bunch of grapes	73 79 85 89	0.110.010 0.063 0.038 0.028	<0.01 <0.01 <0.01 <0.01	0.12 0.073 0.048 0.038	0 14 56 98	(g) 17-2128 (h) SC (fluopyram 250 g/L ,trifloxystrobin 250 g/L) (i) Spraying (j) Analytical method: 00984/M003, 17-2118 (k) LOQ: 0.01 mg/kg (l) Method Validation Data : 00984/M003, 17-2128 (m) Storage: bunch of grapes: 356 days
17-2128-03 17-2128-03-T2 Spain 50549 Malejan Europe, South F 2017	Grape Garnacha	1) 1997 2) 15.05.2017 - 10.06.2017 3) 25.09.2017 - 17.10.2017	50 50	500 500	10 10	14.06.2017/0 28.06.2017/**12**	73	bunch of grapes	73 79 85 89	0.21/0.010 0.11 0.074 0.038	<0.01 <0.01 <0.01 <0.01	0.22 0.12 0.084 0.048	0 14 56 98	

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)			PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					fluopyram as AE C656948	FLU-benzamide as AE C656948	Total residue, calc.		
	(a)	(b)				(c)	(d)		(d)				(e)	(f)
17-2128-04 17-2128-04-T1 Bulgaria 4400 Pazardjik Europe, South F 2017	Grape Merlo	1) 2005 2) 09.06.2017 - 20.06.2017 3) 15.09.2017 - 30.09.2017	50 50	1000 1000	5.0 5.0	09.06.2017/0 30.06.2017/21	73	bunch of grapes	73 79 85 89	0.02 0.057 0.024 0.027	<0.01 <0.01 <0.01 <0.01	0.33 0.067 0.34 0.037	0 32 53 80	(g) 17-2128 (h) SC (fluopyram 250 g/L ,trifloxystrobin 250 g/L) (i) Spraying (j) Analytical method: 00984/M003, 17-2118 (k) LOQ: 0.01 mg/kg (l) Method Validation Data : 00984/M003, 17-2128 (m) Storage: bunch of grapes: 354 days
17-2128-04 17-2128-04-T2 Bulgaria 4400 Pazardjik Europe, South F 2017	Grape Merlo	1) 2005 2) 09.06.2017 - 20.06.2017 3) 15.09.2017 - 30.09.2017	50 50	1000 1000	5.0 5.0	19.06.2017/0 30.06.2017/11	73	bunch of grapes	73 79 85 89	0.67 0.074 0.030 0.033	<0.01 <0.01 <0.01 <0.01	0.68 0.084 0.040 0.043	0 32 53 80	

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks (may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) Study reference
* prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Based on the residue definition for risk assessment, the sum of fluopyram and fluopyram -benzamide expressed as fluopyram, the total residues for grapes are summarized in the Table 6.3.1- 11.

Table 6.3.1- 11: Summary of fluopyram total residue data for grape trials (after results scaling for NEU)

Crop	Region/Indoor (a)	Trial results relevant to the critical GAP (mg/kg)	STMR (b)	HR (c)
Grape	NEU	<0.01 ; 0.012 ; 0.020 ; 0.026 ; 0.030 ; 0.031 ; 0.033 ; 0.049	0.028	0.049
	SEU	2x <0.02 ; 2x 0.020 ; 0.024 ; 0.040 ; 0.043 ; 0.048	0.022	0.048
	NEU/SEU	<0.01 ; 2x<0.02 ; 0.012 ; 3x 0.020 ; 0.024 ; 0.026 ; 0.030 ; 0.031 ; 0.033 ; 0.040 ; 0.043 ; 0.048 ; 0.049	0.025	0.049

(a) **NEU** or **SEU** for northern or southern outdoor trials in EU member states,
(b) **STMR**: Supervised Trials Median Residue
(c) **HR**: Highest residue

CA 6.3.2 Strawberry

Data on residues in strawberry were submitted for the first inclusion of fluopyram under Regulation (EC) N°. 1107/2009.

For strawberry, a total of 26 European supervised residue trials (9 from Northern Europe, 9 from Southern Europe and 8 in greenhouse) and 10 North American trials performed from 2006 to 2007 were available to support these representative uses.

As strawberry is not representative uses for the current active substance renewal dossier, these below listed trials will not be summarized for a sake of clarity.

Table 6.3.2- 1: First inclusion fluopyram residue trials conducted on strawberry

Residue trials				
Zone	Year		References Report No. Authors, year	Total number of trials
	2006	2007		
NEU	5	-	M-295412-01-1 [redacted] 2007	9
	-	4	M-298093-01-1 [redacted] 2008	
SEU	-	-	M-295419-01-1 [redacted] 2007	9
	-	4	M-298098-01-1 [redacted] 2008	

Residue trials				
Zone	Year		References Report No. Authors, year	Total number of trials
	2006	2007		
GH	8	-	M-295155-01-1 [redacted] 2007	8
US	-	10	M-300045-01-1 [redacted] 2008	10

CA 6.3.3 Tomato

Data on residues in tomato were submitted for the first inclusion of fluopyram under Regulation (EC) N°. 1107/2009.

For tomato, a total of 22 European supervised residue trials (10 field trials from Southern Europe and 12 in greenhouse) performed from 2006 to 2007 were available to support these representative uses.

As tomato is not representative uses for the current active substance renewal dossier, these below listed trials will not be summarized for a sake of clarity.

Table 6.3.3- 1: First inclusion fluopyram residue trials conducted on tomato

Residue trials				
Zone	Year		References Report No. Authors, year	Total number of trials
	2006	2007		
SEU	5	-	M-295816-01-1 [redacted] 2007	10
		5	M-298074-01-1 [redacted] 2008	
MGH	8		M-290788-01-1 [redacted] 2007	12
GH	-	4	M-297499-01-1 [redacted] 2008	
US	-	11	M-299989-01-1 [redacted] 2008	11

CA 6.3.5 Apple

Information on the intended use pattern (GAP) is summarised in Table 6.3.4- 1.

Table 6.3.4- 1: Use patterns (critical GAP) for the spray application of Fluopyram in/on apple in European fields (northern and southern residue regions)

GAP number	Formulation	F/ GH	No. of appl.	Growth stage at application (BBCH Code)	Application rate per treatment (g a.s./ha)	Water volume (L/ha)	Interval (days)	PHI (days)
1	FLU SC500	F*	1	71-89	75	500-1000		14

F field

Residue trials supporting GAP 1 were conducted in Europe (north and south) on apple and pear at a similar GAP during the 2019 growing season.

Table 6.3.4- 2: Overview of European residue trials conducted in apple & pear per geographical "residue region" and vegetation period to support GAP 1

Crop	Region	No. of independent trials Harvest 2019 apple	pear	Σ	Report No. (Formulation)	Document number	Reference
Pome fruits	NEU	4	4	8	E19RP062 E19RP083 (FLU SC250)	M-757113-01-1 M-755638-01-1	KCA 6.3.4/01 KCA 6.3.4/02
	SEU	4	4	8	E19RP105 E19RP106 (FLU SC250)	M-757080-01-1 M-755520-01-1	KCA 6.3.4/03 KCA 6.3.4/04

NEU = northern EU field SEU = southern EU field

Northern zone

Data Point:	KCA 6.3.4/01
Report Author:	[redacted]
Report Year:	2020
Report Title:	Determination of the residues of AE C656948 in/on apple after a spray application of fluopyram SC 250 in Germany, United Kingdom, The Netherlands and Belgium
Report No:	E19RP062
Document No:	M-757113-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and methods

Four residue trials on apples were conducted in northern Europe (in Germany, United Kingdom, the Netherlands and Belgium) during the 2019 season.

One spray application was conducted with Fluopyram SC 250, a SC formulation containing 250 g/L of fluopyram.

The target application rate was 0.03 kg as/ha x m height with a pre harvest interval of 14 days.

As the canopies height ranges between 2.5 and 3.5 m, these applications rates corresponds to a range of 0.070 – 0.092 kg as/ha.

At harvest, each field sample was placed in doubled labelled bags and stored deep-frozen within 24 hours after sampling and until dispatch. All field samples were shipped deep-frozen under monitored conditions during shipment and arrived in good condition. The field samples were stored in a freezer at ≤- 18 °C until preparation of the examination samples.

For the preparation of examination samples, the deep-frozen field samples were shredded and homogenized with dry ice in a cutter. Representative parts of the shredded samples were transferred into polystyrene boxes and stored at ≤ -18 °C.

Samples were analysed according to the analytical method 00984 ([redacted]., 05/02/2007, M-283301-01-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev.4.

Briefly, residues were extracted from 5 g of sample material by two successive extractions using a high speed blender with a mixture of acetonitrile:water (80:20; *v:v*). After centrifugation the extract volume was adjusted. Then, the extracts were diluted ten times by adding the internal standards:

- dilution performed under acidic conditions and measured in negative electrospray ionization for the determination of FLU-PCA and positive ion mode for FLU-methyl-sulfoxide.

- dilution performed under basic conditions and measured in positive electrospray ionization for the determination of fluopyram, FLU-benzamide, FLU-PAA and FLU-7-hydroxy.

*Due to its instability, the analytical standard of fluopyram-pyridyl-acetic acid (BCS-AA10139) was made available under its sodium salt form (BCS-AA10189) which was used as reference item.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels. For each calibration curve, the correlation coefficient R was above 0.99.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.01 mg/kg.

The examination samples were kept deep-frozen until their analysis. The quantity necessary for analysis was weighed while the sample was still deep-frozen, and the remaining sample was immediately returned to the freezer. All final extracts were analysed within 24 hours.

Findings

In order to check the performance of the method, recovery determinations were included in each set of analyses. Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study.

The apparent residues in the control sample used for the determination of recoveries were below 30 % of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these determinations.

The mean recoveries per fortification level were within the range of 70 % - 110 % and the RSD values were below 20 %. The overall mean concurrent recoveries were also in the acceptable range of 70 % and 110 %, with the RSD < 20 %.

The storage period of deep-frozen samples intended for the analysis of fluopyram and its metabolites ranged between 199 and 257 days.

The detailed results obtained for apple samples in northern Europe are summarized in the Table 6.3.4- 4.

Conclusion

Four supervised residue trials on apples were conducted in northern Europe with the application of Fluopyram SC 250 at the required rate corresponding to the supported GAP (1x75 g as/ha, PHI=14 days), and according to GLP. Samples of apples were analysed for the residues of fluopyram and its metabolite fluopyram-benzamide (AEF148815, alias FLU-benzamide), fluopyram-pyridyl-acetic-acid (BCS-AA10139, alias FLU-PAA), fluopyram-pyridyl-carboxylic acid (AE C657188, alias FLU-PCA), fluopyram-7-hydroxy (BCS-AA10065, alias FLU-7-OH) and fluopyram-methyl-sulfoxide (AE1344122, alias FLU-methyl-sulfoxide). The results of the trials presented above show the following residues 14 days after last application.

- Fluopyram residues range between 0.02 and 0.04 mg/kg.
- Fluopyram-benzamide residues were <0.01 mg/kg
- FLU-7-OH residues were <0.01 mg/kg
- FLU-PAA residues were <0.01 mg/kg
- FLU-PCA residues were <0.01 mg/kg
- FLU-methyl-sulfoxide residues were <0.01 mg/kg
- The total residue of fluopyram (sum of FLU + FLU-benzamide, expressed as fluopyram) range between 0.03 and 0.05 mg/kg.

Assessment and conclusion by applicant:

The study is acceptable.

Table 6.3.4- 3: Concurrent recovery data for Fluopyram (study E19RP062)

Portion analysed	Fortification level (mg/kg)	Recovery (%)				
		Individual recoveries	Min	Max	Mean	RSD
Fluopyram (AE C656948)						
apple / fruit	0.01	95, 102, 108	95	103	100	4.4
	0.10	94, 95, 96	94	96	95	1.0
		Overall recovery (n=6)			**98**	**4.0**
Fluopyram-benzamide						
apple / fruit	0.01	96, 100, 102	96	102	99	3.1
	0.10	93, 94, 98	93	98	95	3.0
		Overall recovery (n=6)			**97**	**3.6**
Fluopyram-7-OH (BCS-AA10065)						
apple / fruit	0.01	96, 100, 108	96	108	101	6.0
	0.10	95, 95, 96	95	96	95	0.6
		Overall recovery (n=6)			**98**	**5.2**
Fluopyram-PAA (BCS-AA10139)						
apple / fruit	0.01	95, 100, 103	95	103	99	4.1
	0.10	93, 96, 106	92	106	98	7.4
		Overall recovery (n=6)			**99**	**5.4**
Fluopyram-PCA (AE C657188)						
apple / fruit	0.01	80, 82, 95	80	95	86	9.5
	0.10	82, 97, 100	82	100	93	10.4
		Overall recovery (n=6)			**89**	**10.0**
Fluopyram-methylsulfoxide (AE1344122)						
apple / fruit	0.01	96, 97, 106	96	106	100	5.5
	0.10	87, 94, 94	87	94	92	4.4
		Overall recovery (n=6)			**69**	**6.4**

RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with fluopyram, determined as fluopyram and calculated as fluopyram
Fortified with fluopyram-benzamide, determined as fluopyram-benzamide and calculated as fluopyram
Fortified with fluopyram-7-OH, determined as fluopyram-7-OH and calculated as fluopyram
Fortified with fluopyram-PAA, determined as fluopyram-PAA and calculated as fluopyram
Fortified with fluopyram-PCA, determined as fluopyram-PCA and calculated as fluopyram
Fortified with fluopyram-methulsulfoxide, determined as fluopyram-methulsulfoxide and calculated as fluopyram
These recoveries were performed during the conduct of the study E19RP062

Table 6.3.4- 4: Summary of the E19RP062 (M-757113-01-1) study on apple

Total residue calculated : sum of fluopyram+FLU-benzamide

Trial No. / Location / EU zone / Year	Commodity / Variety (a)	Date of 1. planting 2. Flowering 3. Harvest 4. Transplanting (b)	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment (d)	Portion analyzed	Growth stage at sampling (d)	Residues (mg/kg)							PHI (days) €
			g a.s./ha	Water (L/ha)	g a.s./hL					fluopyram as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-PAA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C65694	Total residues calc	
E19RP062-01 Germany 51399 Burscheid Europe, North F, 2019	Apple Jonagold	1) 2012 2) 19.04.2019 - 29.04.2019 3) 25.09.2019 - 18.10.2019	72.8 *29.1 g/(ha*m)*	849 *340 L/(ha*m)*	8.57	30.08.2019/ 0	79	fruit	79 81 85 87 89	0.0578 0.0427 0.0225 0.0284 0.0223	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 0.0384 < 0.01	0.0678 0.0527 0.0325 0.0384 0.0323	0 7 14 21 28
E19RP062-02 United kingdom SG8 8SS Great Chishill, near Royston Europe, North F, 2019	Apple Jonothan	1) 2008 2) 18.04.2019 - 10.05.2019 3) 23.09.2019 - 07.10.2019	69.6 *27.8 g/(ha*m)*	580 *232 L/(ha*m)*	12	10.09.2019/ 0	81	fruit	81 85 89 89 89	0.0455 0.0341 0.0279 0.0215 0.0141	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 0.0379 < 0.01 < 0.01	0.0535 0.0441 0.0379 0.0315 0.0241	0 7 13 20 27
E19RP062-03 Netherlands 1608 HG Wijdenes Europe, North	Apple Elstar	1) 2008 2) 15.04.2019 - 01.05.2019 3) 26.08.2019 - 15.09.2019	91.9 *30.6 g/(ha*m)*	917 *306 L/(ha*m)*	10	14.08.2019/ 0	81	fruit	81 85 85 87 87	0.0681 0.0347 0.0258 0.0232 0.0252	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 0.0358 < 0.01 < 0.01	0.0781 0.0447 0.0358 0.0332 0.0352	0 7 14 21 28

Trial No. / Location / EU zone / Year	Commodity / Variety (a)	Date of 1. planting 2. Flowering 3. Harvest 4. Transplanting (b)	Application rate per treatment			Dates of treatment / Application interval (d)	Growth stage at last treatment (d)	Portion analyzed	Growth stage at sampling (d)	Residues (mg/kg)							PHI (days) €
			g a.s./ha	Water (L/ha)	g a.s./hL					fluopyram as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-PAA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C65694	Total residues calc	
F, 2019																	
E19RP062-04 Belgium 6220 Fleurus Europe, North F, 2019	Apple Karmijn	2) 17.04.2019 - 30.04.2019 3) 23.09.2019 - 10.10.2019	74 *29.6 g/(ha*m)*	494 *197 L/(ha*m)*	15	09.09.2019/ 0	85	fruit	85	0.0647	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.0747	0
									85	0.0577	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.0677	7
									89	0.0442	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.0542	14
									89	0.0337	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.0437	21
									89	0.0325	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.0425	28

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions, Reference to analytical method information which metabolites are included
(g) Study reference
* prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Data Point:	KCA 6.3.4/02
Report Author:	[redacted]
Report Year:	2020
Report Title:	Determination of the residues of AE C656948 in/on pear after spray application of fluopyram SC 250 in Germany and Hungary
Report No:	E19RP083
Document No:	M-755638-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market US EPA OCSPP 860.1500, Crop Field Trial OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009)
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and methods

Four residue trials on apples were conducted in northern Europe (in Germany and Hungary) during the 2019 season.

One spray application was conducted with fluopyram SC 250, a SC formulation containing 250 g/L of fluopyram.

The target application rate was 0.03 kg as/ha x m height with a pre harvest interval of 14 days.

As the canopies height ranges between 2.5 and 3.2 m, these applications rates corresponds to a range of 0.075 – 0.096 kg as/ha.

At harvest, each field sample was placed in doubled labelled bags and stored deep-frozen within 24 hours after sampling and until dispatch. All field samples were shipped deep-frozen under monitored conditions during shipment and arrived in good condition. The field samples were stored in a freezer at ≤- 18 °C until preparation of the examination samples.

For the preparation of examination samples, the deep-frozen field samples were shredded and homogenized with dry ice in a cutter. Representative parts of the shredded samples were transferred into polystyrene boxes and stored at ≤-18 °C.

Samples were analysed according to the analytical method 00984 ([redacted], 05/02/2007, M-283301-01-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Briefly, residues were extracted from 5 g of sample material by two successive extractions using a high speed blender with a mixture of acetonitrile:water (80:20; *v:v*). After centrifugation the extract volume was adjusted. Then, the extracts were diluted ten times by adding the internal standards:

- dilution performed under acidic conditions and measured in positive electrospray ionization for the determination of FLU-PCA.
- dilution performed under basic conditions and measured in positive electrospray ionization for the determination of fluopyram, FLU-benzamide and FLU-PAA.

*Due to its instability, the analytical standard of fluopyram-pyridyl-acetic acid (BCS-AA10139) was made available under its sodium salt form (BCS-AA10189) which was used as reference item.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels. For each calibration curve, the correlation coefficient R was above 0.99.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.01 mg/kg.

The examination samples were kept deep-frozen until their analysis. The quantity necessary for analysis was weighed while the sample was still deep-frozen, and the remaining sample was immediately returned to the freezer. All final extracts were analysed within 24 hours.

Findings

In order to check the performance of the method, recovery determinations were included in each set of analyses. Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study.

The apparent residues in the control sample used for the determination of recoveries were below 30 % of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these determinations.

The mean recoveries per fortification level were within the range of 70 % – 110 % and the RSD values were below 20 %. The overall mean concurrent recoveries were also in the acceptable range of 70 % and 110 %, with the RSD < 20 %.

The storage period of deep-frozen samples intended for the analysis of fluopyram and its metabolites ranged between 315 and 353 days.

The detailed results obtained for apple samples in northern Europe are summarized in the Table 6.3.4- 6.

Conclusion

Four supervised residue trials on apples were conducted in northern Europe with the application of Fluopyram SC 250 at the required rate corresponding to the supported GAP (1x75 g as/ha, PHI=14 days), and according to GLP. Samples of apples were analysed for the residues of fluopyram and its metabolite fluopyram-benzamide (AEF148815, alias FLU-benzamide), fluopyram-pyridyl-acetic-acid (BCS-AA10139, alias FLU-PAA) and fluopyram-pyridyl-carboxylic acid (AE C657188, alias FLU-PCA). The results of the trials presented above show the following residues 14 days after last application.

- Fluopyram residues range between 0.05 and 0.09 mg/kg.
- Fluopyram-benzamide residues were <0.01 mg/kg
- FLU-PAA residues were <0.01 mg/kg
- FLU-PCA residues were <0.01 mg/kg
- The total residue of fluopyram (sum of FLU + FLU-benzamide, expressed as fluopyram) range between 0.06 and 0.10 mg/kg.

Assessment and conclusion by applicant:

The study is acceptable.

Table 6.3.4- 5: Concurrent recovery data for Fluopyram (study E19RP083)

Portion analysed	Fortification level (mg/kg)	Recovery (%)				
		Individual recoveries	Min	Max	Mean	RSD
Fluopyram (AE C656948)						
pear / fruit	0.01	96, 100, 101	96	101	99	2.6
	0.10	93, 97, 98	93	98	96	2.8
		Overall recovery (n=6)			**98**	**3.0**
Fluopyram-benzamide						
pear / fruit	0.01	106, 108, 113	106	113	109	3.2
	0.10	94, 94, 95	94	95	94	0.6
		Overall recovery (n=6)			**102**	**8.2**
Fluopyram-PAA (BCS-AA10139)						
pear / fruit	0.01	99, 100, 111	99	111	103	6.4
	0.10	94, 97, 98	94	98	96	2.2
		Overall recovery (n=6)			**100**	**5.9**
Fluopyram-PCA (AE C657188)						
apple / fruit	0.01	94, 96, 99	94	99	96	2.6
	0.10	96, 101, 101	96	101	99	2.9
		Overall recovery (n=6)			**98**	**3.0**

RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with fluopyram, determined as fluopyram and calculated as fluopyram
Fortified with fluopyram-benzamide, determined as fluopyram-benzamide and calculated as fluopyram
Fortified with fluopyram-PAA, determined as fluopyram-PAA and calculated as fluopyram
Fortified with fluopyram-PCA, determined as fluopyram-PCA and calculated as fluopyram
These recoveries were performed during the conduct of the study E19RP083

Table 6.3.4- 6: Summary of the E19RP083 (M-755638-01-1) study on pear

Total residue calculated : sum of fluopyram+FLU-benzamide

Trial No. / Location / EU zone / Year	Commodity / Variety (a)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting (b)	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment (d)	Portion analyzed	Growth stage at sampling (d)	Residues (mg/kg)					PHI (days) (e)
			g a.s./ha	Water (L/ha)	g a.s./hL					fluopyram as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-PAA as AE C656948	Total residues calc	
E19RP083-01 Germany 79346 Endingen-Königschaffhausen Europe, North F, 2019	Pear Williams christ; table pears	1) 14.12.2013 2) 01.04.2019 - 15.04.2019 3) 13.08.2019 - 27.08.2019	92.1 *30.7 g/(ha*m)*	767 *256 L/(ha*m)*	12.0	31.07.2019/0	81	fruit	81 85 87 87 89	0.0913 0.0937 0.0462 0.0402 0.0298	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01	0.1013 0.1037 0.0562 0.0502 0.0398	0 6 14 22 28
E19RP083-02 Germany77704 Oberkirchnussbach Europe, North F, 2019	Pear Williams Christ	1) 1995 2) 01.04.2019 - 15.04.2019 3) 14.08.2019 - 30.08.2019	96.1 *30.0 g/(ha*m)*	641 *200 L/(ha*m)*	15.0	31.07.2019/0	81	fruit	81 85 87 87 89	0.104 0.100 0.0738 0.0619 0.0311	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01	0.114 0.11 0.0838 0.0719 0.0411	0 6 14 22 28
E19RP083-03 Hungary H-3360 Heves Europe, North F, 2019	Pear Vilmos	1) 05.05.2015 2) 17.06.2019 - 24.06.2019 3) 05.08.2019 - 19.08.2019 4) 14.03.2016	75.3 *30.1 g/(ha*m)*	502 *201 L/(ha*m)*	15.0	22.07.2019/0	81	fruit	81 85 87 87 89	0.173 0.0932 0.0875 0.0515 0.0593	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01	0.183 0.1032 0.0975 0.0615 0.0693	0 7 14 21 28
E19RP083-04 Hungary H-3985 Alsoberecki Europe, North F, 2019	Pear Vilmos	1) 20.04.2001 2) 14.06.2019 - 21.06.2019 3) 05.08.2019 - 19.08.2019 4) 25.03.2002	90.4 *30.1 g/(ha*m)*	603 *201 L/(ha*m)*	15.0	23.07.2019/0	81	fruit	81 85 87 87 89	0.200 0.121 0.0822 0.0490 0.0546	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01	0.21 0.131 0.0922 0.059 0.0646	0 6 13 20 27

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method information which metabolites are included
(g) Study reference
* prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
\# no data available

Southern zone

Data Point:	KCA 6.3.4/03
Report Author:	[redacted]
Report Year:	2020
Report Title:	Determination of the residues of AE C656948 in/on apple after a spray application of fluopyram SC 250 in Italy, southern France and Portugal
Report No:	E19RP105
Document No:	M-757080-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and methods

Four residue trials on apples were conducted in southern Europe (in Italy, France and Portugal) during the 2019 season.

One spray application was conducted with Fluopyram SC 250, a SC formulation containing 250 g/L of fluopyram.

The target application rate was 0.03 kg as/ha x m height with a pre harvest interval of 14 days.

As the canopies height ranges between 2.8 and 3.2 m, these applications rates corresponds to a range of 0.087 – 0.096 kg as/ha.

At harvest, each field sample was placed in doubled labelled bags and stored deep-frozen within 24 hours after sampling and until dispatch. All field samples were shipped deep-frozen under monitored conditions during shipment and arrived in good condition. The field samples were stored in a freezer at ≤- 18 °C until preparation of the examination samples.

For the preparation of examination samples, the deep-frozen field samples were shredded and homogenized with dry ice in a cutter. Representative parts of the shredded samples were transferred into polystyrene boxes and stored at ≤ -18 °C.

Samples were analysed according to the analytical method 00984 ([redacted], 05/02/2007, M-283301-01-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA-4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Briefly, residues were extracted from 5 g of sample material by two successive extractions using a high speed blender with a mixture of acetonitrile:water (80:20; *v:v*). After centrifugation the extract volume was adjusted. Then, the extracts were diluted ten times by adding the internal standards:

- dilution performed under acidic conditions and measured in positive electrospray ionization for the determination of FLU-PCA and negative ion mode for FLU-methyl-sulfoxide.
- dilution performed under basic conditions and measured in positive electrospray ionization for the determination of fluopyram, FLU-benzamide, FLU-PAA and FLU-7-hydroxy.

*Due to its instability, the analytical standard of fluopyram-pyridyl-acetic acid (BCS-AA10139) was made available under its sodium salt form (BCS-AA10189) which was used as reference item.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels. For each calibration curve, the correlation coefficient R was above 0.99.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.01 mg/kg.

The examination samples were kept deep-frozen until their analysis. The quantity necessary for analysis was weighed while the sample was still deep-frozen, and the remaining sample was immediately returned to the freezer. All final extracts were analysed within 24 hours.

Findings

In order to check the performance of the method, recovery determinations were included in each set of analyses. Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study.

The apparent residues in the control sample used for the determination of recoveries were below 30 % of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these determinations.

The mean recoveries per fortification level were within the range of 70 % – 110 % and the RSD values were below 20 %. The overall mean concurrent recoveries were also in the acceptable range of 70 % and 110 %, with the RSD < 20 %.

The storage period of deep-frozen samples intended for the analysis of fluopyram and its metabolites ranged between 220 and 287 days.

The detailed results obtained for apple samples in northern Europe are summarized in the Table 6.3.4- 8

Conclusion

Four supervised residue trials on apples were conducted in southern Europe with the application of Fluopyram SC 250 at the required rate corresponding to the supported GAP (1x75 g as/ha, PHI=14 days), and according to GLP. Samples of apples were analysed for the residues of fluopyram and its metabolite fluopyram-benzamide (AEF148815, alias FLU-benzamide), fluopyram-pyridyl-acetic-acid (BCS-AA10139, alias FLU-PAA), fluopyram-pyridyl-carboxylic acid (AE C657188, alias FLU-PCA), fluopyram-7-hydroxy (BCS-AA10065, alias FLU-7-OH) and fluopyram-methyl-sulfoxide (AE1344122, alias FLU-methyl-sulfoxide). The results of the trials presented above show the following residues 14 days after last application.

- Fluopyram residues range between 0.03 and 0.10 mg/kg.
- Fluopyram-benzamide residues were <0.01 mg/kg
- FLU-7-OH residues were <0.01 mg/kg
- FLU-PAA residues were <0.01 mg/kg
- FLU-PCA residues were <0.01 mg/kg

- FLU-methyl-sulfoxide residues were <0.01 mg/kg
- The total residue of fluopyram (sum of FLU + FLU-benzamide, expressed as fluopyram) range between 0.04 and 0.11 mg/kg.

Assessment and conclusion by applicant:

The study is acceptable.

Table 6.3.4- 7: Concurrent recovery data for Fluopyram (study E19RP105)

Portion analysed	Fortification level (mg/kg)	Recovery (%)				
		Individual recoveries	Min	Max	Mean	RSD
Fluopyram (AE C656948)						
apple / fruit	0.01	99, 106, 107, 110	99	110	106	4.4
	0.10	93, 98, 99	93	99	97	3.3
	0.40	101, 103, 104, 109	101	109	104	3.3
		Overall recovery (n=11)			**103**	**5.1**
Fluopyram-benzamide						
apple / fruit	0.01	99, 99, 104, 109	99	109	103	4.7
	0.10	99, 100, 102	99	102	100	1.5
	0.40	98, 99, 101, 105	98	105	101	3.1
		Overall recovery (n=11)			**101**	**3.3**
Fluopyram-7-OH (BCS-AA10065)						
apple / fruit	0.01	98, 101, 105, 106	98	106	103	3.6
	0.10	96, 98, 99	96	99	98	1.6
	0.40	99, 102, 103, 106	99	106	103	2.8
		Overall recovery (n=11)			**101**	**3.4**
Fluopyram-PAA (BCS-AA10039)						
apple / fruit	0.01	85, 92, 98, 101	85	101	94	7.5
	0.10	80, 84, 91	80	91	85	6.6
	0.40	90, 93, 94, 95	90	95	93	2.3
		Overall recovery (n=11)			**91**	**6.8**
Fluopyram-PCA (AE C657188)						
apple / fruit	0.01	91, 101, 104, 107	91	107	101	6.9
	0.10	98, 101, 101	98	101	100	1.5
	0.40	95, 97, 99, 100	95	100	98	2.3
		Overall recovery (n=11)			**99**	**4.3**
Fluopyram-methylsulfoxide (AE1344122)						
apple / fruit	0.01	102, 103, 107, 107	102	107	105	2.5
	0.10	99, 99, 101	99	101	100	1.2
	0.40	92, 97, 100, 108	92	108	99	6.8
		Overall recovery (n=11)			**101**	**4.7**

RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with fluopyram, determined as fluopyram and calculated as fluopyram
Fortified with fluopyram-benzamide, determined as fluopyram-benzamide and calculated as fluopyram
Fortified with fluopyram-7-OH, determined as fluopyram-7-OH and calculated as fluopyram
Fortified with fluopyram-PAA, determined as fluopyram-PAA and calculated as fluopyram
Fortified with fluopyram-PCA, determined as fluopyram-PCA and calculated as fluopyram
Fortified with fluopyram-methulsulfoxide, determined as fluopyram-methulsulfoxide and calculated as fluopyram
These recoveries were performed during the conduct of the study E19RP105

Table 6.3.4- 8: Summary of the E19RP105 (M-757080-01-1) study on apple

Total residue calculated : sum of fluopyram+FLU-benzamide

Trial No. / Location / EU zone / Year	Commodity / Variety (a)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting (b)	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment (d)	Portion analyzed	Growth stage at sampling (d)	Residues (mg/kg)							PHI (days) €
			g a.s./ha	Water (L/ha)	g a.s./hl					fluopyram as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-PAA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residues calc.	
E19RP105-01 Italy 44124 San Martino FE Europe, South F, 2019	Apple Fuji	1) 22.01.2006 2) 18.04.2019 - 30.04.2019 3) 09.09.2019 - 30.09.2019	87.4 *31.2 g/(ha*m)*	962 *344 L/(ha*m)*	9.09	28.08.2019/ 0	81	fruit	81 85 87 87 87	0.104 0.0635 0.0392 0.0264 0.0492	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01	0.114 0.0735 0.0492 0.0364 0.0592	0 7 14 21 28
E19RP105-02 France, south 31340 Vacquiers Europe, South F, 2019	Apple Golden delicious	1) 2000 2) 05.04.2019 - 20.04.2019 3) 20.09.2019 - 01.10.2019	95.8 *29.9 g/(ha*m)*	1120 *349 L/(ha*m)*	9.55	06.09.2019/ 0	85	fruit	85 87 87 89 89	0.0624 0.0258 0.0258 0.0264 0.0230	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01	0.0724 0.0358 0.0358 0.0364 0.033	0 7 14 21 28
E19RP105-03 Portugal 2540-558 Braçais Europe, South F, 2019	Apple Brock Field	1) 06.04.2004 2) 07.05.2019 - 15.05.2019 3) 02.08.2019 - 23.08.2019	90.4 *30.1 g/(ha*m)*	1060 *352 L/(ha*m)*	8.53	30.07.2019/ 0	77	fruit	77 78 81 81 81	0.173 0.110 0.0988 0.0804 0.0870	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01	<0.01 <0.01 <0.01 <0.01 <0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 0.12 < 0.01 < 0.01 < 0.01	0.183 0.12 0.1088 0.0904 0.097	0 7 14 21 28

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)							PHI (days) €
			g a.s./ha	Water (L/ha)	g a.s./hL					fluopyram as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-PAA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residues calc.	
	(a)	(b)					(d)		(d)								
E19RP105-04 Italy 85024 Lavello (PZ) Europe, South F, 2019	Apple Golden	1) 2011 2) 15.04.2019 - 30.04.2019 3) 01.09.2019 - 30.09.2019	93.7 *31.2 g/(ha*m)*	780 *260 L/(ha*m)*	12.0	20.08.2019/ 0	79	fruit	79	0.0798	< 0.01	<0.01	<0.01	< 0.01	< 0.01	0.0898	0
									79	0.0447	< 0.01	<0.01	<0.01	< 0.01	< 0.01	0.0547	7
									81	0.0306	< 0.01	<0.01	<0.01	< 0.01	< 0.01	0.0406	14
									87	0.0254	< 0.01	<0.01	< 0.01	< 0.01	< 0.01	0.0354	21
									87	0.0236	< 0.01	<0.01	< 0.01	< 0.01	< 0.01	0.0336	28

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(f) Days after last application (Label pre-harvest interval, PHI, underline)
(g) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g)* Study reference prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Data Point:	KCA 6.3.4/04
Report Author:	[redacted].
Report Year:	2020
Report Title:	Determination of the residues of AE C656948 in/on pear after spray application of fluopyram SC 250 in France (South), Spain and Portugal
Report No:	E19RP106
Document No:	M-755520-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and methods

Four residue trials on pear were conducted in southern Europe (in France, Spain and Portugal) during the 2019 season.

One spray application was conducted with Fluopyram SC 250, a SC formulation containing 250 g/L of fluopyram.

The target application rate was 0.03 kg as/ha x m height with a pre harvest interval of 14 days.

As the canopies height ranges between 2.5 and 2.7 m, these applications rates corresponds to a range of 0.075 – 0.080 kg as/ha.

At harvest, each field sample was placed in doubled labelled bags and stored deep-frozen within 24 hours after sampling and until dispatch. All field samples were shipped deep-frozen under monitored conditions during shipment and arrived in good condition. The field samples were stored in a freezer at ≤- 18 °C until preparation of the examination samples.

For the preparation of examination samples, the deep-frozen field samples were shredded and homogenized with dry ice in a cutter. Representative parts of the shredded samples were transferred into polystyrene boxes and stored at ≤ -18 °C.

Samples were analysed according to the analytical method 00984 ([redacted], 05/02/2007, M-283301-01-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA-4 which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Briefly, residues were extracted from 5 g of sample material by two successive extractions using a high speed blender with a mixture of acetonitrile:water (80:20; *v:v*). After centrifugation the extract volume was adjusted. Then, the extracts were diluted ten times by adding the internal standards:

- dilution performed under acidic conditions and measured in positive electrospray ionization for the determination of FLU-PCA.
- dilution performed under basic conditions and measured in positive electrospray ionization for the determination of fluopyram, FLU-benzamide and FLU-PAA.

*Due to its instability, the analytical standard of fluopyram-pyridyl-acetic acid (BCS-AA10139) was made available under its sodium salt form (BCS-AA10189) which was used as reference item.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels. For each calibration curve, the correlation coefficient R was above 0.99.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.01 mg/kg.

The examination samples were kept deep-frozen until their analysis. The quantity necessary for analysis was weighed while the sample was still deep-frozen, and the remaining sample was immediately returned to the freezer. All final extracts were analysed within 24 hours.

Findings

In order to check the performance of the method, recovery determinations were included in each set of analyses. Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study.

The apparent residues in the control sample used for the determination of recoveries were below 30 % of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these determinations.

The mean recoveries per fortification level were within the range of 70 % – 110 % and the RSD values were below 20 %. The overall mean concurrent recoveries were also in the acceptable range of 70 % and 110 %, with the RSD < 20 %.

The storage period of deep-frozen samples intended for the analysis of fluopyram and its metabolites ranged between 314 and 403 days.

The detailed results obtained for apple samples in northern Europe are summarized in the Table 6.3.4- 10.

Conclusion

Four supervised residue trials on apples were conducted in southern Europe with the application of Fluopyram SC 250 at the required rate corresponding to the supported GAP (1x75 g as/ha, PHI=14 days), and according to GLP. Samples of apples were analysed for the residues of fluopyram and its metabolite fluopyram-benzamide (AEF148815, alias FLU-benzamide), fluopyram-pyridyl-acetic-acid (BCS-AA10139, alias FLU-PAA) and fluopyram-pyridyl-carboxylic acid (AE C657188, alias FLU-PCA). The results of the trials presented above show the following residues 14 days after last application.

- Fluopyram residues range between 0.08 and 0.21 mg/kg.
- Fluopyram-benzamide residues were <0.01 mg/kg
- FLU-PAA residues range between <0.01 and 0.03 mg/kg.
- FLU-PCA residues were <0.01 mg/kg
- The total residue of fluopyram (sum of FLU + FLU-benzamide, expressed as fluopyram) range between 0.09 and 0.22 mg/kg.

Assessment and conclusion by applicant:

The study is acceptable.

Table 6.3.4- 9: Concurrent recovery data for Fluopyram (study E19RP106)

Portion analysed	Fortification level (mg/kg)	Recovery (%)				
		Individual recoveries	Min	Max	Mean	RSD
Fluopyram (AE C656948)						
pear / fruit	0.01	91, 93, 96	91	96	93	2.7
	0.10	93, 94, 98	93	98	95	2.8
		Overall recovery (n=6)			**94**	**2.6**
Fluopyram-benzamide						
pear / fruit	0.01	101, 104, 105	101	105	103	2.0
	0.10	90, 93, 93	90	93	92	1.9
		Overall recovery (n=6)			**98**	**6.6**
Fluopyram-PAA (BCS-AA10139)						
pear / fruit	0.01	91, 95, 102	91	102	96	5.8
	0.10	92, 95, 96	92	96	94	2.2
		Overall recovery (n=6)			**95**	**4.1**
Fluopyram-PCA (AE C657188)						
apple / fruit	0.01	92, 102, 105	92	105	99	6.7
	0.10	93, 97, 99	92	99	96	3.8
		Overall recovery (n=6)			**98**	**5.2**

RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with fluopyram, determined as fluopyram and calculated as fluopyram
Fortified with fluopyram-benzamide, determined as fluopyram-benzamide and calculated as fluopyram
Fortified with fluopyram-PAA, determined as fluopyram-PAA and calculated as fluopyram
Fortified with fluopyram-PCA, determined as fluopyram-PCA and calculated as fluopyram
These recoveries were performed during the conduct of the study E19RP106

Table 6.3.4- 10: Summary of the E19RP106 (M-755520-01-1) study on pear

Total residue calculated : sum of fluopyram+FLU-benzamide

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)					PHI (days)
			g a.s./ha	Water (L/ha)	g a.s./hL				(d)	fluopyram as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-PAA as AE C656948	Total residues calc	(e)
	(a)	(b)					(d)								
E19RP106-01 France, south 13570 Barbentane Europe, South F, 2019	Pear Williams	1) 1959 2) 20.05.2019 - 01.06.2019 3) 01.08.2019 - 30.08.2019	75.4 *30.2* *g/(ha*m)*	603 *241* *L/(ha*m)*	12.5	33.07.2019/0	79	fruit	79 85 87 87 87	0.168 0.131 <u>0.0771</u> 0.0617 0.0672	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01	0.168 0.141 <u>0.0871</u> 0.0717 0.0772	0 8 <u>14</u> 22 28
E19RP106-02 Spain 46370 Chiva Europe, South F, 2019	Pear Castell	1) 2001 2) 15.05.2019 - 31.05.2019 3) 15.06.2019 - 30.06.2019	75 *30.0* *g/(ha*m)*	626 *250* *L/(ha*m)*	12	07.06.2019/0	85	fruit	85 87 89 89 89	0.347 0.252 <u>0.205</u> 0.137 0.105	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 0.0123 0.0192 0.0262	0.357 0.262 <u>0.215</u> 0.187 0.115	0 6 <u>14</u> 21 27
E19RP106-03 Spain 30520 Jumilla Europe, South F, 2019	Pear Ercolini	1) 1991 2) 10.05.2019 - 31.05.2019 3) 15.07.2019 - 25.07.2019	78.6 *30.2* *g/(ha*m)*	655 *252* *L/(ha*m)*	12.0	20.06.2019/0	77	fruit	77 87 87 89 89	0.150 0.0984 <u>0.0752</u> 0.0519 0.0412	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 0.0100 0.0239 0.0288 0.0303	0.16 0.1084 <u>0.0852</u> 0.0619 0.0512	0 7 <u>14</u> 21 27
E19RP106-04 Portugal 2500 Caldas da Rainha Europe, South F, 2019	Pear Rocha	1) 2002 2) 15.03.2019 - 15.04.2019 3) 15.08.2019 - 09.09.2019	79.5 *29.4* *g/(ha*m)*	671 *248* *L/(ha*m)*	11.9	07.08.2019/0	85	fruit	85 85 89 89 89	0.104 0.120 <u>0.0751</u> 0.0133 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01	0.114 0.13 <u>0.0851</u> 0.0233 <0.02	0 7 <u>14</u> 21 27

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) Study reference
*prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Based on the residue definition for risk assessment, the sum of fluopyram and fluopyram-benzamide expressed as fluopyram, the total residues for pome fruits are summarized in the Table 6.3.4- 11

Table 6.3.4- 11: Summary of fluopyram total residue data for pome fruits trials to be supported

Crop	Region/Indoor (a)	Trial results relevant to the critical GAP (mg/kg)	STMR (b)	HR (c)
Apple/Pear	NEU	0.036, 0.038, 0.038, 0.054, 0.056, 0.084, 0.092 0.098	0.055	0.098
	SEU	0.036, 0.041, 0.059, 0.085, 0.085, 0.087, 0.11, 0.22	0.085	0.22
	NEU/SEU	2x0.036, 2x0.038, 0.041, 0.054, 0.056, 0.059, 0.084, 2x0.085, 0.087, 0.092, 0.098, 0.11, 0.22	0.072	0.22

(a) **NEU** or **SEU** for northern or southern outdoor trials in EU member states,
(b) **STMR**: Supervised Trials Median Residue
(c) **HR**: Highest residue

Table 6.3.4- 12: Summary of fluopyram metabolites data for pome fruits trials to be supported

Crop	Region/Indoor (a)	Trial results relevant to the critical GAP (mg/kg)			
		FLU-PAA	FLU-PCA	FLU-7OH	FLU-methylsulfoxide
Apple/Pear	NEU	8x<0.01	8x<0.01	4x<0.01	4x<0.01
	SEU	7x<0.01, 0.03	8x<0.01	4x<0.01	4x<0.01
	NEU/SEU	15x<0.01, 0.03	16x<0.01	8x<0.01	8x<0.01

(a) **NEU** or **SEU** for northern or southern outdoor trials in EU member states,
(b) **STMR**: Supervised Trials Median Residue
(c) **HR**: Highest residue

CA 6.3.6 Barley

Information on the intended use pattern (GAP) is summarised in Table 6.3.5- 1.

Table 6.3.5- 1: Use patterns (critical GAP) for the spray application of Fluopyram in/on barley in European fields (northern and southern residue regions)

GAP number	Formulation	F/ GH	No. of appl.	Growth stage at application (BBCH Code)	Application rate per treatment (g a.s./ha)	Water volume (L/ha)	Interval (days)	PHI (days)

1	BIX+FLU+PTZ EC260	F	1	30-61	78	200-400	-	
2	BIX+FLU+PTZ EC260	F	1	30-61	39	200-400	-	

F field

Residue trials to support GAP 1 were conducted in Europe (north and south) at a similar GAP.

Table 6.3.5- 2: Overview of European residue trials conducted in barley per geographical "residue region" and vegetation period

Crop	Region	No. of independent trials					Report No.	Formulation	Document number	Reference
		Vegetation period				Σ				
		2012	2013	2017	2018					
Barley	NEU	11	-	4	-	15	12-2130	BIX+FLU+PTZ EC260	M-475081-03-1	KCA 6.3.5/01
							12-2163	FLU+PTZ SE250	M-472556-02-1	KCA 6.3.5/02
							17-2017	FLU+ISY+PTZ EC234	M-655225-01-1	KCA 6.3.5/03
	SEU	7	1	4	4	16	12-2132	BIX+FLU+PTZ EC260	M-474260-04-1	KCA 6.3.5/04
							13-2004	BIX+FLU+PTZ EC260	M-479739-01-1	KCA 6.3.5/05
							17-2018	FLU+ISY+PTZ EC234	M-656993-01-1	KCA 6.3.5/06
							18-2101	BIX+FLU SC200	M-668264-01-1	KCA 6.3.5/07

NEU = northern EU field SEU = southern EU field

In the Northern zone, three trials were conducted according to the GAP in 2012. Twelve residue trials were conducted in the 2012 and 2017 growing seasons with a higher dose rate compared to the supported GAP. As all other GAP parameter respect the GAP, a downscaling factor is applied to the residue results. According to EFSA Technical Report (EFSA supporting publication 2018-EN-1503), *the proportionality concept can be applied to residue data from field trials conducted within a dose rate range of between 0.3x and 4x the GAP dose rate*. Thus, a proportionality factor is applied to the results of the northern residue studies (12-2163 and 17-2017) to fit with the intended GAP.

Table 6.3.5- 3: Scaling factors to be applied on barley total residues of fluopyram (northern residue regions)

Study number	Trial number	Dose rate (g ai/ha)	BBCH at application	Scaling factor
12-2163	12-2163-01	125	61	0.62
	12-2163-02	125	61	0.62
	12-2163-03	125	61	0.62
	12-2163-04	125	61	0.62
	12-2163-05	125	61	0.62
	12-2163-06	125	61	0.62
	12-2163-07	125	61	0.62
	12-2163-08	125	61	0.62

Study number	Trial number	Dose rate (g ai/ha)	BBCH at application	Scaling factor
17-2017	17-2017-01	101	61	0.77
	17-2017-02	101	61	0.77
	17-2017-03	101	61	0.77
	17-2017-04	101	61	0.77

In the Southern zone, eight trials were conducted according to the GAP in 2012 and 2013. Eight residue trials were conducted in the 2017 and 2018 growing seasons with a higher dose rate compared to the supported GAP. As all other GAP parameter are respected, a downscaling factor is applied to the residue results. According to EFSA Technical Report (EFSA supporting publication 2018-EN-1503), *the proportionality concept can be applied to residue data from field trials conducted within a dose rate range of between 0.3x and 4x the GAP dose rate*. Thus, a proportionality factor is applied to the results of the northern residue studies (17-2018 and 18-2101) to fit with the intended GAP.

Table 6.3.5- 4: Scaling factors to be applied on barley total residues of fluopyram (southern residue regions)

Study number	Trial number	Dose rate (g ai/ha)	BBCH at application	Scaling factor
17-2018	17-2018-01	101	61	0.77
	17-2018-02	101	61	0.77
	17-2018-03	101	61	0.77
	17-2018-04	101	61	0.77
18-2101	18-2101-01	103	61	0.75
	18-2101-02	100	61	0.78
	18-2101-03	101	59	0.77
	18-2101-04	101	61	0.77

GAP 1 northern zone

Data Point:	KCA 6.3.5/01
Report Author:	[redacted]
Report Year:	2016
Report Title:	Amendment No. 2 to Final Report No: 12-2130 - Determination of the residues of AE C656948, BYF 00587 and prothioconazole in/on spring barley and winter barley after spray application of bixafen & fluopyram & prothioconazole EC 260 in the field in northern France, the United Kingdom, Belgium and Germany
Report No:	12-2130
Document No:	M-475081-03-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC EC Guidance working document 7029/VI/95 rev.5 (1997-07-22) OECD 509 Adopted 2009-09-07, OECD GUIDELINE FOR THE TESTING OF CHEMICALS, Crop Field Trial US EPA OCSPP Guideline No. 860.1500
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted Study was not found in DAR/RAR and the Addenda
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and Methods

Four supervised residue trials on barley were conducted in northern Europe (in northern France, Belgium, the United Kingdom, in Germany) during the 2012 season.

One-spray applications was conducted with BIX+FLU+PTZ EC 260, an EC (emulsifiable concentrate) formulation containing 130 g/L prothioconazole, 65 g/L of fluopyram and 65 g/L of bixafen. For the purpose of this renewal, only results for fluopyram and its metabolite fluopyram-benzamide will be discussed.

The applications were conducted at the BBCH of 61 and at the application rates of 1.2 L/ha corresponding to the single application 0.078 kg a.s./ha. Water application rates were at 200 – 300 L/ha.

Samples of barley green material were collected at BBCH 61, 65, 71, 83, and barley grain and straw at BBCH 89. Due to a bird attack, no sample of grain and straw was taken from trial 12-2130-03.

Each field sample was placed in doubled labelled bags and stored deep-frozen within 24 hours after sampling and until dispatch. All field samples were shipped deep-frozen (at -18 °C or lower temperatures) under monitored conditions during shipment and arrived in good condition, except for the shipment of some samples from the trial 12-2130-01 which were shipped in two shipments at higher average shipment temperatures. Nevertheless, also these samples arrived in good and frozen condition. A short term storage stability study was conducted to show that this deviation has no impact on the stability of the residues (see CA 6.1.1, KCA 6.1/10, M-480441-06-1).

The field samples were stored in a freezer at ≤-18 °C until preparation of the examination samples. For the preparation of examination samples, the deep-frozen field samples were shredded and homogenized with dry ice in a cutter. Representative parts of the shredded samples were transferred into polystyrene boxes

and stored at ≤-18°C. For each field sample, one or several examination samples were prepared for analysis and one examination sample was prepared as a reserve sample.

Samples were analysed according to the analytical method 00984/M003 ([redacted], 2015, M-467323-03-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues were extracted from 5 g of sample material by extraction using a shaker (15 min) with a mixture of acetonitrile:water (4:1, *v:v*). After filtration the extracts of the solutions were made up to volume. The solutions were centrifuged, and the extract volume was adjusted. The extracts were diluted by adding the internal standard. An aliquot of the extracts was injected into a HPLC-(MS/MS).

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.01 mg/kg.

All final extracts were analysed within 2 days after extraction. Fluopyram and fluopyram-benzamide were found to be stable in extracts of matrices of plant origin for at least four weeks at 4 ± 3 °C which was tested within the validation of method 00984/M003.

Findings

In order to check the performance of the method, recovery determinations were included in each set of analyses. Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study. The apparent residues in the control sample used for the determination of recoveries were below 30 % of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these determinations.

The mean recoveries per fortification level were within the acceptable range of 70 % – 110 % and with the RSDs < 20 %. The overall mean concurrent recoveries were also in the acceptable range of 70 % and 110 %, with the RSD < 20 %.

The storage period of deep-frozen samples intended for the analysis of fluopyram and its metabolite fluopyram-benzamide ranged between 322 and 429 days.

The detailed results obtained for barley samples in northern Europe are summarized in the Table 6.3.5- 6

Conclusion

Four supervised residue trials on barley were conducted in northern Europe with the application of BIX+FLU+PTZ EC 260 at the required rates and according to GLP. Samples of barley were analyzed for the residues of fluopyram and its metabolite fluopyram-benzamide. The results of the trials presented above show the following residues in grain at harvest:

- Fluopyram residues range between <0.01and 0.02 mg/kg.
- Fluopyram-benzamide residues were <0.01 mg/kg
- The total residue of fluopyram (sum of FLU + FLU-benzamide, expressed as fluopyram) range between <0.02 and 0.035 mg/kg.

Residues in straw at harvest :

- Fluopyram residues range between 0.03 and 0.06 mg/kg.
- Fluopyram-benzamide residues were <0.01 mg/kg

- The total residue of fluopyram (sum of FLU + FLU-benzamide, expressed as fluopyram) range between 0.04 and 0.07 mg/kg.

Assessment and conclusion by applicant:

The study is acceptable.

Table 6.3.5- 5: Concurrent recovery data for Fluopyram (study 12-2130).

Portion analysed	Fortification level (mg/kg)	Recovery (%)				
		Individual recoveries	Min	Max	Mean	RSD
Fluopyram (AE C656948)						
Barley / grain	0.01	86; 90; 94; 94; 99	86	99	93	4.1
	0.10	102; 103; 103; 104	102	104	103	0.8
	0.80	90; 99; 100	90	100	96	6
		Overall recovery (n= 12)			**97**	**6.2**
Barley / green material	0.01	88; 88; 93; 98; 100; 100	88	100	95	6.0
	0.10	96; 96; 98; 102	96	102	98	2.9
	0.80	95; 97; 99; 100; 101	95	101	98	2.4
	4.0	93; 93; 94	93	94	93	0.6
		Overall recovery (n= 18)			**96**	**4.3**
Barley / straw	0.01	73; 95; 96; 96	73	96	90	12.6
	0.10	94; 96; 97	94	97	96	1.6
	15	94; 97; 100	94	100	97	3.1
		Overall recovery (n= 10)			**94**	**8.0**
Fluopyram-benzamide						
Barley / grain	0.01	85; 87; 96; 97; 99	85	99	93	6.8
	0.10	98; 99; 102; 103	98	103	101	2.4
	0.80	88; 97; 98	88	98	94	5.8
		Overall recovery (n = 12)			**96**	**6.1**
Barley / green material	0.01	91; 92; 97; 101; 102; 108	91	108	99	6.6
	0.10	90; 98; 99; 100	90	100	97	4.7
	0.80	96; 99; 101; 105; 108	96	108	102	4.7
	4.0	86; 87; 90	86	90	88	2.4
		Overall recovery (n = 18)			**97**	**6.9**
Barley / straw	0.01	92; 95; 97; 98	92	98	96	2.8
	0.10	89; 92; 94	89	94	92	2.7
	15	104; 108; 110	104	110	107	2.8
		Overall recovery (n = 10)			**98**	**7.3**

RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with fluopyram, determined as fluopyram and calculated as fluopyram

Fortified with fluopyram-benzamide, determined as fluopyram-benzamide and calculated as fluopyram
These recoveries were performed during the conduct of the study12-2130
Note: The shown mean and RSD values values may differ from corresponding values stated in the Analytical Phase Report due to the number of decimals used for calculation of these mean and RSD values.

Table 6.3.5- 6: Summary of the 12-2130 (M-475081-03-1) study on barley

Total residue calculated : sum of fluopyram+FLU-benzamide

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)			PHI (days)
			g a.s./ha	Water (L/ha)	g a.s./hL					fluopyram as AE C656948	FLU-benzamide as AE C656948	Total residue calc.	
	(a)	(b)				(c)	(d)		(d)				(e)
12-2130-01 France, north 95710 Chaussy Europe, North F 2012	Barley, winter Volume; Barley winter	1) 04.10.2011 2) 14.05.2012 - 25.05.2012 3) 17.07.2012 - 20.07.2012	78	300	26	14.05.2012/0	61	green material	61 65 71 83	1.0 0.23 0.06 0.035	<0.01 <0.01 <0.01 <0.01	1.0 0.34 0.17 0.045	0 7 14 28
								grain	89	0.015	<0.01	<u>0.025</u>	64
								straw	89	0.054	<0.01	0.064	64
12-2130-02 Belgium 1495 Marbais Europe, North F 2012	Barley, spring Quench; Malting variety	1) 15.03.2012 2) 22.06.2012 - 26.06.2012 3) 08.08.2012 - 17.08.2012	78	250	31	22.06.2012/0	61	green material	61	1.3	<0.01	1.31	0
								grain	89	0.025	<0.01	<u>0.035</u>	47
								straw	89	0.058	<0.01	0.068	47
12-2130-03 United Kingdom CB22 5EU Cambridge Europe, North F, 2012	Barley, winter Carrat; Winter barley	1) 07.09.2011 2) 21.05.2012 3) 15.07.2012 - 16.08.2012	78	200	39	23.05.2012/0	61	green material	61 69 71 83	1.7 0.86 0.28 0.080	<0.01 0.029 0.016 <0.01	1.7 0.89 0.30 0.090	0 7 14 27
12-2130-04 Germany 51399 Burscheid Europe, North F 2012	Barley, spring Simba; typical of the region	1) 16.03.2012 2) 05.06.2012 - 11.06.2012 3) 10.08.2012 - 24.08.2012	78	300	26	05.06.2012/0	61	green material	61	1.8	<0.01	1.8	0
								grain	89	<0.01	<0.01	<u><0.02</u>	69
								straw	89	0.024	0.017	0.041	69

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method information which metabolites are included
(g) Study reference
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date

G greenhouse F field * prior to last treatment ** residue in control # no data available

Data Point:	KCA 6.3.5/02
Report Author:	[redacted]
Report Year:	2014
Report Title:	Determination of the residues of AE C656948 and prothioconazole in/on spring barley and winter barley after spray application of AEC656948 & JAU 6476 SE 250 in the field in northern France, Germany, the Netherlands, Belgium and United Kingdom
Report No:	12-2163
Document No:	M-472556-02-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC EC Guidance working document 7029/VI/95 rev.5 (1997-07-22) OECD 509 Adopted 2009-09-07, OECD GUIDELINE FOR THE TESTING OF CHEMICALS, Crop Field Trial US EPA OCSPP Guideline No. 860.1500
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted Study was not found in DAR/RAR and the Addenda
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and Methods

Eight supervised residue trials on barley were conducted in northern Europe (in northern France, in Germany, in the Netherlands, in Belgium, in the United Kingdom) during the 2012 season.

One-spray applications was conducted with FLU+PTZ SE 250 a suspo-emulsion formulation containing 125 g/L of fluopyram and 125 g/L of prothioconazole. For the purpose of this renewal, only results for fluopyram and its metabolite fluopyram-benzamide will be discussed.

The applications were conducted at the BBCH of 61 and at the application rates of 1.0 L/ha corresponding to the single application 0.125 kg a.s./ha. Water application rates were at 200 – 300 L/ha. As the dose rate is higher than the intended GAP (1x125g as/ha Vs 1x78g), the residues are downscaled according to the scaling factors calculated and presented in Table 6.3.5- 3.

Samples of barley green material were collected at BBCH 61, 65, 71, 73, 75, 83, barley grain and straw at BBCH of 89.

Each field sample was placed in doubled labelled bags and stored deep-frozen within 24 hours after sampling and until dispatch. All field samples were shipped deep-frozen under monitored conditions during shipment and arrived in good condition. Some temperature problems occurred during the transport of the samples from the trials 12-2163-01 and 12-2163-02. A short term storage stability study was conducted to show that this deviation has no impact on the stability of the residues (see MCA 6.1.2, M-480441-06-1).

The field samples were stored in a freezer at ≤-18 °C until preparation of the examination samples.

For the preparation of examination samples, the deep-frozen field samples were shredded and homogenized with dry ice in a cutter. Representative parts of the shredded samples were transferred into polystyrene boxes and stored at ≤-18 °C.

Samples were analysed according to the analytical method 00984/M003 ([redacted], 2015, M-467323-03-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues were extracted from 5 g of sample material by extraction using a shaker (15 min) with a mixture of acetonitrile:water (4:1, *v:v*). After filtration the extracts of the solutions were made up to volume. The solutions were centrifuged, and the extract volume was adjusted. The extracts were diluted by adding the internal standard. An aliquot of the extracts was injected into a HPLC-(MS/MS).

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.01 mg/kg.

All final extracts were analysed within 2 days after extraction. Fluopyram and fluopyram-benzamide were found to be stable in extracts of matrices of plant origin for at least four weeks at 4 ± 3 °C which was tested within the validation of method 00984/M003.

Findings

In order to check the performance of the method, recovery determinations were included in each set of analyses. Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study.

The apparent residues in the control sample used for the determination of recoveries were below 30 % of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these determinations.

The mean recoveries per fortification level were within the acceptable range of 70 % – 110 % and with the RSDs < 20 %. The overall mean concurrent recoveries were also in the acceptable range of 70 % and 110 %, with the RSD < 20 %.

The storage period of deep-frozen samples intended for the analysis of fluopyram and its metabolite fluopyram-benzamide ranged between 246 and 331 days.

The detailed results obtained for barley samples in northern Europe are summarized in the Table 6.3.5- 8

Conclusion

Eight supervised residue trials on barley were conducted in northern Europe with the application of FLU+PTZ SE 250 at a higher rate than required in the supported GAP (1x125g as/ha Vs 1x78g), and according to GLP. Samples of barley were analyzed for the residues of fluopyram and its metabolite fluopyram-benzamide. The results of the trials presented above show the following residues after donwscaling in grain at harvest.

- Fluopyram residues range between <0.01 and 0.02 mg/kg.
- Fluopyram-benzamide residues were <0.01 mg/kg
- The total residue of fluopyram (sum of FLU + FLU-benzamide, expressed as fluopyram) after downscaling to the right dose (1x125g as/ha Vs 1x78g), range between <0.02 and 0.03 mg/kg.

Residues after downscaling in straw at harvest :

- Fluopyram residues range between 0.01 and 0.11 mg/kg.
- Fluopyram-benzamide residues were between <0.01 mg/kg and 0.02 mg/kg
- The total residue of fluopyram (sum of FLU + FLU-benzamide, expressed as fluopyram) after downscaling to the right dose (1x125g as/ha Vs 1x78g), range between 0.02 and 0.11 mg/kg.

Assessment and conclusion by applicant:

The study is acceptable.
The residue level in these GLP trials was corrected with a proportionality factor according to EFSA Technical Report (EFSA supporting publication 2018-EN-1503).

Table 6.3.5- 7: Concurrent recovery data for Fluopyram (study 12-2163)

Portion analysed	Fortification level (mg/kg)	Recovery (%)				
		Individual recoveries	Min	Max	Mean	RSD
Fluopyram (AE C656948)						
Barley / green material	0.01	98; 105; 108	98	108	104	5.0
	1.0	91; 92; 93	91	93	92	1.1
	3.0	98; 99	98	99	99	-
		Overall recovery (n=8)			**98**	**6.2**
Barley / grain	0.01	100; 105; 106	100	106	104	3.1
	0.10	101; 102; 102	101	102	102	0.6
		Overall recovery (n=6)			**103**	**2.3**
Barley / straw	0.01	105; 110; 113	105	113	109	3.7
	1.0	89; 91; 95	89	95	92	3.3
		Overall recovery (n=6)			**101**	**10.1**
Fluopyram-benzamide						
Barley / green material	0.01	78; 89; 91	78	91	86	8.1
	1.0	93; 95; 97	93	97	95	2.1
	3.0	93; 94	93	94	94	-
		Overall recovery (n= 8)			**91**	**6.4**
Barley / grain	0.01	96; 103; 108	96	108	102	5.9
	0.10	98; 99; 105	98	105	101	3.8
		Overall recovery (n =6)			**102**	**4.5**
Barley / straw	0.01	74; 80; 101	74	101	85	16.7
	1.0	85; 92; 97	85	97	91	6.6
		Overall recovery (n = 6)			**88**	**11.7**

RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with fluopyram, determined as fluopyram and calculated as fluopyram
Fortified with fluopyram-benzamide, determined as fluopyram-benzamide and calculated as fluopyram
These recoveries were performed during the conduct of the study12-2163

Table 6.3.5- 8: Summary of the 12-2163 (M-472556-02-1) study on barley

Total residue calculated : sum of fluopyram+FLU-benzamide

Trial No. / Location / EU zone / Year	Commodity / Variety (a)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting (b)	Application rate per treatment			Dates of treatment / Application interval (c)	Growth stage at last treatment (d)	Portion analyzed	Growth stage at sampling (d)	Residues (mg/kg)					PHI (days) (e)
			g a.s./ha	Water (L/ha)	g a.s./hL					fluopyram as AE C656948	FLU-benzamide as AE C656948	Total residue calc.	Scaling factor	Scaled residue	
12-2163-01 France, north 95710 Chaussy Europe, North F 2012	Barley, winter Volume	1) 04.10.2011 2) 14.05.2012 - 25.05.2012 3) 16.07.2012 - 19.07.2012	125	300	42	14.05.2012/0	61	green material	61 65 71 83	1.8 0.26 0.19 0.043	<0.01 <0.01 <0.01 <0.01	1.8 0.27 0.20 0.053	0.62	1.1 0.17 0.12 0.033	0 7 14 28
								grain	89	0.018	<0.01	0.028		0.017	64
								straw	89	0.14	0.013	0.15		0.093	64
12-2163-02 Germany 49377 Vechta - Langförden Europe, North F 2012	Barley, winter Meridian; multiline, high harvest	1) 30.09.2011 2) 21.05.2012 - 04.06.2012 3) 05.07.2012 - 25.07.2012	125	300	42	22.05.2012/0	61	green material	61 73 75 83	1.8 0.95 0.31 0.14	<0.01 <0.01 <0.01 <0.01	1.8 0.96 0.32 0.15	0.62	1.1 0.60 0.20 0.093	0 7 14 28
								grain	92	0.025	<0.01	0.035		0.022	62
								straw	92	0.066	<0.01	0.076		0.047	62
12-2163-03 Netherlands 1774 Slootdorp Europe, North F 2012	Barley, winter Winter Malt	1) 14.10.2011 2) 01.06.2012 - 15.06.2012 3) 22.07.2012 - 02.08.2012	125	300	42	01.06.2012/0	61	green material	61 65 65 83	2.6 0.62 0.30 0.062	<0.01 <0.01 <0.01 <0.01	2.6 0.63 0.31 0.072	0.62	1.6 0.39 0.19 0.045	0 7 14 28
								grain	89	0.027	<0.01	0.037		0.023	54
								straw	89	0.057	<0.01	0.067		0.042	54

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)					PHI (days)
			g a.s./ha	Water (L/ha)	g a.s./hL					fluopyram as AE C656948	Fluopyram benzamide as AE C656948	Total residue calc	Scaling factor	Scaled residue	
	(a)	(b)				(c)	(d)		(d)						(e)
12-2163-04 Netherlands 9076 PP Sint Annaparochie Europe, North F 2012	Barley, winter Winter Malt	1) 10.10.2011 2) 30.05.2012 - 18.06.2012 3) 15.07.2012 - 01.08.2012	125	300	42	30.05.2012/0	61	green material	61	2.1	<0.01	2.1	0.62	1.3	0
									65	0.25	<0.01	0.26		0.16	7
									65	0.088	<0.01	0.098		0.061	14
									83	0.030	<0.01	0.040		0.025	28
								grain	89	0.014	<0.01	0.024		<0.02	56
								straw	89	0.025	<0.01	0.035		0.022	56
12-2163-05 France, north 37310 Chambourg sur Indre Europe, North F, 2012	Barley, spring Sebastian	1) 02.03.2012 2) 01.06.2012 - 08.06.2012 3) 20.07.2012 - 25.07.2012	125	300	42	01.06.2012/0	61	green material	61	1.9	<0.01	1.9	0.62	1.2	0
								grain	89	0.018	<0.01	0.028		0.017	53
								straw	89	0.014	0.020	0.034		0.021	53
12-2163-06 Belgium 1495 Marbais Europe, North F 2012	Barley, spring Quench; Malting variety	1) 15.03.2012 2) 22.06.2012 - 26.06.2012 3) 08.08.2012 - 17.08.2012	125	200	63	22.06.2012/0	61	green material	61	2.2	<0.01	2.2	0.62	1.4	0
								grain	89	0.026	<0.01	0.036		0.022	47
								straw	89	0.081	<0.01	0.091		0.056	47

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)					PHI (days)
			g a.s./ha	Water (L/ha)	g a.s./hL					fluopyram as AE C656948	Fluopyram benzamide as AE C656948	Total residue calc.	Scaling factor	Scaled residue	
	(a)	(b)				(c)	(d)		(d)						(e)
12-2163-07 United Kingdom CB22 5EU Little Shelford Farm Europe, North F, 2012	Barley, spring Propino	1) 23.03.2012 2) 25.06.2012 - 09.07.2012 3) 06.08.2012 - 20.08.2012	125	200	63	25.06.2012/0 - 61	61	green material	61	2.4	<0.01	2.4	0.62	1.5	0
								grain	89	0.033	0.013	0.046		0.029	46
								straw	89	0.13	0.037	0.17		0.11	46
12-2163-08 Germany 51399 Burscheid Europe, North F, 2012	Barley, spring Simba	1) 16.03.2012 2) 05.06.2012 - 11.06.2012 3) 10.08.2012 - 24.08.2012	125	300	42	05.06.2012/0 - 61	61	green material	61	2.6	<0.01	2.6	0.62	1.6	0
								grain	89	0.016	<0.01	0.017		<0.02	69
								straw	89	0.11	0.029	0.14		0.087	69

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (label: pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) Study reference
* prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Data Point:	KCA 6.3.5/03
Report Author:	[redacted]
Report Year:	2019
Report Title:	Determination of the residues of BCS-CN88460, prothioconazole and AE C656948 in/on spring barley after spray application of FLU & BCS-CN88460 & PTZ EC 234 in Germany, Denmark, the United Kingdom and northern France
Report No:	17-2017
Document No:	M-655225-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted Study was not found in DAR/RAR and the Addenda
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and Methods

Four supervised residue trials on barley were conducted in northern Europe (Germany, Denmark, in the United Kingdom, northern France) during the 2017 season.

One-spray applications were conducted with FLU+ISY+PTZ EC 234 an emulsfiable concentrate formulation containing 67 g/L of fluopyram, 42 g/L of isoflucypram, and 125 g/L of prothioconazole. For the purpose of this renewal, only results for fluopyram and its metabolite fluopyram-benzamide will be discussed.

The applications were conducted at the BBCH of 61 and at the application rates of 1.5 L/ha corresponding to the single application rate of 0.101 kg a.s./ha. Water application rates were at 200 – 250 L/ha. As the dose rate is higher than the intended GAP (1x101g as/ha Vs 1x78g), the residues are downscaled according to the scaling factors calculated and presented in Table 6.3.5- 3.

Samples of barley green material were collected at BBCH 61, 65, 73, 75, 77, 83, 85, 87, barley grain and straw at BBCH of 89.

Each field sample was placed in double labelled bags and stored deep-frozen at ≤-18 °C or below, within 24 hours after sampling and until dispatch. All field samples were shipped deep-frozen under monitored conditions during shipment and arrived in good condition. The field samples were stored in a freezer at ≤-18°C until preparation of the examination samples, except for trial 17-2017-02 and trial 17-2017-03 where temperature deviated over a short period during the field storage (trial 17-2017-03) or during shipment (trial 17-2017-02). However, these deviations were considered acceptable.

For the preparation of examination samples, the deep-frozen field samples were shredded and homogenized with dry ice in a cutter. Representative parts of the shredded samples were transferred into polystyrene boxes and stored at ≤-18°C.

Samples were analysed according to the analytical method 00984/M003 ([redacted], 2015, M-467323-03-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues were extracted from 5 g of sample material (additionally 2.5 g for the sample material straw) by extraction using a shaker (15 min) with a mixture of acetonitrile:water (4:1, *v:v*).

The filtering procedure under low vacuum was replaced by a centrifugation step. Then 0.5 mL of internal standard solution (1 mg/L) was added to the extract followed by 5 min centrifugation at 4000 rpm at 10 °C.

An 0.1 mL aliquot of this extract was diluted with 0.9 mL of Milli-Q-water and then proceeded to HPLC-MS/MS analysis.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.01 mg/kg.

All final extracts were analysed within 7 days after extraction. Fluopyram and fluopyram-benzamide were found to be stable in extracts of matrices of plant origin for at least four weeks at 4 ± 3 °C which was tested within the validation of method 00984/M003.

Findings

In order to check the performance of the method, recovery determinations were included in each set of analyses. Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study.

The apparent residues in the control sample used for the determination of recoveries were below 30 % of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these determinations.

The mean recoveries of fluopyram and its metabolite fluopyram-benzamide per fortification level were within the acceptable range of 70 % - 110 % and with the RSDs < 20 % (when applicable). The overall mean concurrent recoveries were also in the acceptable range of 70 % and 110 %, with the RSD < 20 %.

The storage period of deep-frozen samples intended for the analysis of fluopyram and its metabolite fluopyram-benzamide ranged between 432 and 541 days.

The detailed results obtained for barley samples in northern Europe are summarized in the Table 6.3.5- 10.

Conclusion

Four supervised residue trials on barley were conducted in northern Europe with the application of FLU+ISY+PTZ EC 234 at a higher rate than required in the supported GAP (1x101g as/ha Vs 1x78g), and according to GLP. Samples of barley were analyzed for the residues of fluopyram and its metabolite fluopyram-benzamide. The results of the trials presented above show the following residues after donwscaling in grain at harvest.

- Fluopyram residues range between 0.02 and 0.08 mg/kg.
- Fluopyram-benzamide residues were <0.01 mg/kg

- The total residue of fluopyram (sum of FLU + FLU-benzamide, expressed as fluopyram) after downscaling to the right dose (1x78 g ai/ha Vs 1x101 g ai/ha), range between 0.03 and 0.08 mg/kg.

Residues after downscaling in straw at harvest :

- Fluopyram residues range between 0.04 and 0.28 mg/kg.
- Fluopyram-benzamide residues were between <0.01 mg/kg and 0.01 mg/kg
- The total residue of fluopyram (sum of FLU + FLU-benzamide, expressed as fluopyram) after downscaling to the right dose (1x101g as/ha Vs 1x78g ha), range between 0.05 and 0.29 mg/kg.

Assessment and conclusion by applicant:

The study is acceptable.
The residue level in these GLP trials was corrected with a proportionality factor according to EFSA Technical Report (EFSA supporting publication 2018-EN-1503).

Table 6.3.5- 9: Concurrent recovery data for Fluopyram (study 17-2017)

Portion analysed	Fortification level (mg/kg)	Recovery (%)				
		Individual recoveries	Min	Max	Mean	RSD
Fluopyram (AE C656948)						
Spring barley / grain	0.01	87; 92; 95	87	95	91	4.4
	1.0	95; 99; 101	95	101	98	3.1
		Overall recovery (n=6)			**95**	**5.3**
Spring barley / green material	0.01	82; 85; 90	82	90	86	4.7
	5.0	104; 106; 107	104	107	106	1.4
	80	86; 91; 94; 95; 102; 104	86	104	95	7.1
		Overall recovery (n=12)			**96**	**9.3**
Spring barley / straw	0.01	95; 96; 98	95	98	96	1.6
	0.10	96; 96; 102	96	102	98	3.5
	0.40	93; 94; 100	93	100	96	4.0
		Overall recovery (n=9)			**97**	**3.0**
Fluopyram-benzamide						
Spring barley / grain	0.01	87; 92; 93	87	93	91	3.5
	0.10	94; 96; 98	94	98	96	2.1
		Overall recovery (n=6)			**93**	**4.0**
Spring barley / green material	0.01	81; 83; 89	81	89	84	4.9
	5.0	99; 101; 103	99	103	101	2.0
	80	81; 89; 94; 96; 97; 115	81	115	96	11.7
		Overall recovery (n =12)			**94**	**10.6**
Spring barley / straw	0.01	89; 97; 101	89	101	96	6.4
	0.10	92; 96; 100	92	100	96	4.2
	0.40	92; 94; 96	92	96	94	2.1
		Overall recovery (n = 9)			**95**	**4.1**

RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with fluopyram, determined as fluopyram and calculated as fluopyram
Fortified with fluopyram-benzamide, determined as fluopyram-benzamide and calculated as fluopyram

Table 6.3.5- 10: Summary of the 17-2017 (M-655225-01-1) study on barley

Total residue calculated : sum of fluopyram+FLU-benzamide

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)					PHI (days)
			g a.s./ha	Water (L/ha)	g a.s./hL					fluopyram as AE C656948	FLU-benzamide as AE C656948	Total residue calc.	Scaling factor	Scaled residue	
	(a)	(b)				(c)	(d)		(d)						(f)
17-2017-01 Germany 16818 Kränzlin Europe, North F 2017	Barley, spring Simba	1) 30.03.2017 2) 14.06.2017 - 17.06.2017 3) 09.08.2017 - 09.08.2017	100.5	200	50.25	14.06.2017/0	61	green material	61	3.9	0.01	3.9	0.77	3.0	0
									73	2.2	0.052	2.3		1.8	7
									75	1.1	0.016	1.1		0.85	14
									77	0.19	0.010	0.20		0.15	22
									83	<0.01	<0.01	<0.02		<0.02	30
								grain	89	0.098	<0.01	0.10		0.077	55
								straw	89	0.12	0.012	0.13		0.10	55
17-2017-02 Denmark 6360 Tinglev Europe, North F 2017	Barley, spring OVERTURE	1) 31.03.2017 2) 26.06.2017 - 28.06.2017 3) 15.08.2017 - 15.08.2017	100.5	200	50.25	26.06.2017/0	61	green material	61	1.8	<0.01	1.8	0.77	1.4	0
									73	0.59	<0.01	0.60		0.46	7
									75	0.23	<0.01	0.24		0.19	9
									77	0.19	<0.01	0.20		0.15	15
									83	0.11	<0.01	0.12		0.092	21
									85	0.077	<0.01	0.087		0.067	28
								grain	89	0.040	<0.01	0.041		0.032	50
								straw	89	0.065	<0.01	0.066		0.051	50

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)				PHI (days)
			g a.s./ha	Water (L/ha)	g a.s./hL					fluopyram as AE C656948	FLU-benzamide as AE C656948	Total residue calc.	Scaling factor	Scaled residue	
	(a)	(b)				(c)	(d)		(d)						(f)
17-2017-03 United Kingdom OX15 6EP Banbury, Oxfordshire Europe, North F, 2017	Barley, spring Octavia	1) 27.04.2017 2) 27.04.2017 - 04.07.2017 3) 28.08.2017 - 10.09.2017	100.5	250	40.2	06.07.2017/0	61	green material	61 65 73 73 73	3.9 1.5 0.76 0.23 0.15	<0.01 0.029 0.035 <0.01 <0.01	3.9 1.5 0.80 0.23 0.16	0.77	3.0 1.2 0.62 0.18 0.12	0 7 13 21 27
								grain	89	0.024	<0.01	0.034		0.026	57
								straw	89	0.096	0.011	0.11		0.085	57
17-2017-04 France, north 02190 Juvincourt et Dammary Europe, North F, 2017	Barley, spring IRINA	1) 01.04.2017 2) 20.06.2017 - 30.06.2017 3) 09.08.2017 - 09.08.2017	100.5	200	50.25	29.06.2017/0	61	green material	61 71 83 87 75	3.2 1.1 0.52 0.22 0.51	<0.01 0.018 0.020 0.011 0.013	3.2 1.1 0.54 0.23 0.52	0.77	2.5 0.85 0.42 0.18 0.40	0 7 20 28 14
								grain	89	0.049	<0.01	0.059		0.045	41
								straw	89	0.34	0.025	0.37		0.29	41

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) Study reference
* prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

GAP 1 southern zone

Data Point:	KCA 6.3.5/04
Report Author:	[redacted]
Report Year:	2016
Report Title:	Amendment no. 3 to report no: 12-2132 - Determination of the residues of AE C656948, BYF 00587 and prothioconazole in/on barleyafter spray application of bixafen & fluopyram & prothioconazole EC 260 in the field in Southern France, Spain, Italy, Portugal and Greece
Report No:	12-2132
Document No:	M-474260-04-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC, EC Guidance working document 7029/VI/95 rev.5 (1997-07-22), OECD 509 Adopted 2009-09-07, OECD GUIDELINE FOR THE TESTING OF CHEMICALS, Crop Field Trial, US EPA OCSPP Guideline No. 860.1500
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted Study was not found in DAR/RAR and the Addenda
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and Methods

Seven supervised residue trials on barley were conducted in southern Europe (in southern France, Spain, Italy, Portugal, and Greece) during the 2012 season.

One-spray applications was conducted with with BIX+FLU+PTZ EC 260, an EC (emulsifiable concentrate) formulation containing 130 g/L prothioconazole, 65 g/L of fluopyram and 65 g/L of bixafen. For the purpose of this renewal, only results for fluopyram and its metabolite fluopyram-benzamide will be discussed.

The applications were conducted at the BBCH of 61 (BBCH 65 for the trial 12-2132-08) and at the application rates of 1.2 L/ha corresponding to the single application 0.078 kg a.s./ha. Water application rates were at 282 – 400 L/ha.

Samples of barley green material were collected at BBCH 61, 69, 73, 77, 83, 85, 87, barley grain and straw at BBCH of 89.

Each field sample was placed in doubled labelled bags and stored deep-frozen within 24 hours after sampling and until dispatch. All field samples were shipped deep-frozen under monitored conditions during shipment and arrived in good condition. The field samples were stored in a freezer at ≤- 18 °C until preparation of the examination samples. Temperature above -18°C were recorded during shipment in 4 trials. A short term storage stability study was conducted to show that this deviation has no impact on the stability of the residues (see MCA 6.1.2, M-480441-06-1).

For the preparation of examination samples, the deep-frozen field samples were shredded and homogenized with dry ice in a cutter. Representative parts of the shredded samples were transferred into polystyrene boxes and stored at ≤-18 °C. All examination samples for analysis were stored deep-frozen until analysis or further shipment.

Samples were analysed according to the analytical method 00984/M003 ([redacted], 2015, M-467323-03-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues were extracted from 5 g of sample material by using a shaker (15 min) with a mixture of acetonitrile/water (4:1; *v:v*). After filtration the extracts of the solutions were made up to volume. The solutions were centrifuged, and the extract volume was adjusted. The extracts were diluted by adding the internal standard. An aliquot of the extracts was injected into a HPLC-(MS/MS).

The linearity was demonstrated in each analytical batch with a 1/x-weighted calibration curve established with at least 5 concentration levels.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.01 mg/kg.

All final extracts were analysed within 2 days after extraction. Fluopyram and fluopyram-benzamide were found to be stable in extracts of matrices of plant origin for at least four weeks at 4 ± 3 °C which was tested within the validation of method 00984/M003.

Findings

In order to check the performance of the method recovery determinations were included in each set of analyses. Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study.

The apparent residues in the control sample used for the determination of recoveries were below 30 % of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these determinations.

The mean recoveries of fluopyram and its metabolite fluopyram-benzamide per fortification level were within the acceptable range of 70 % – 110 % and with the RSDs < 20 % (when applicable). The overall mean concurrent recoveries were also in the acceptable range of 70 % and 110 %, with the RSD < 20 %.

The storage period of deep-frozen samples intended for the analysis of fluopyram and its metabolite fluopyram-benzamide ranged between 369 and 440 days.

The detailed results obtained for barley samples in southern Europe are summarized in the Table 6.3.5- 12

Conclusion

Seven supervised residue trials on barley were conducted in southern Europe with the application of BIX+FLU+PTZ EC 260 at the required rates and according to GLP. Samples of barley were analyzed for the residues of fluopyram and its metabolite fluopyram-benzamide. The results of the trials presented above show the following residues in grain at harvest.

- Fluopyram residues range between <0.01 and 0.11 mg/kg.
- Fluopyram-benzamide residues between <0.01 mg/kg and 0.01 mg/kg.
- The total residue of fluopyram (sum of FLU + FLU-benzamide, expressed as fluopyram) range between <0.02 and 0.12 mg/kg.

Residues in straw at harvest :

- Fluopyram residues range between 0.03 and 1.1 mg/kg.
- Fluopyram-benzamide residues were from <0.01 mg/kg to 0.06 mg/kg
- The total residue of fluopyram (sum of FLU + FLU-benzamide, expressed as fluopyram) range between 0.04 and 1.2 mg/kg.

Assessment and conclusion by applicant:

The study is acceptable.

Table 6.3.5- 11: Concurrent recovery data for Fluopyram (study 12-2132)

Portion analysed	Fortification level (mg/kg)	Recovery (%)				
		Individual recoveries	Min	Max	Mean	RSD
Fluopyram (AE C656948)						
Barley / grain	0.01	93; 94; 93; 97; 107	93	107	97	5.8
	0.10	90; 90; 90			90	0.0
	0.80	99; 100; 102	99	102	100	1.5
		Overall recovery (n=11)			**96**	**5.7**
Barley / green material	0.01	80; 80; 84; 89; 92; 93; 100	80	100	88	8.4
	0.10	90; 91; 92	90	92	91	1.1
	0.80	98; 98; 99; 100	98	100	99	1.0
	15	89; 91; 93	89	93	91	2.2
		Overall recovery (n=17)			**92**	**6.8**
Barley / straw	0.01	89; 91; 93; 97; 98	89	98	94	4.1
	0.10	86; 94; 99	86	99	93	7.1
	1.5	98; 99; 102	98	102	100	2.1
	15	92; 95; 99	92	99	95	3.7
		Overall recovery (n=14)			**95**	**4.8**
Fluopyram-benzamide						
Barley / grain	0.01	92; 94; 100; 107; 110	92	110	101	7.1
	0.10	90; 91; 96	90	96	92	3.5
	0.80	101; 103; 108	101	108	104	3.5
		Overall recovery (n=11)			**100**	**7.0**
Barley / green material	0.01	84; 90; 97; 99; 101; 102; 106	84	106	97	7.8
	0.10	90; 91; 94	90	94	92	2.3
	0.80	102; 104; 105; 108	102	108	105	2.4
	15	94; 97; 101	94	101	97	3.6
		Overall recovery (n=17)			**98**	**6.8**
Barley / straw	0.01	82; 92; 95; 108; 108	82	108	97	11.5

Portion analysed	Fortification level (mg/kg)	Recovery (%)				
		Individual recoveries	Min	Max	Mean	RSD
	0.10	92; 101; 103	92	103	99	5.9
	1.5	93; 94; 97	93	97	95	2.2
	15	86; 90; 94	86	94	90	4.4
		Overall recovery (n=14)			**95**	**7.9**

RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with fluopyram, determined as fluopyram and calculated as fluopyram
Fortified with fluopyram-benzamide, determined as fluopyram-benzamide and calculated as fluopyram
These recoveries were performed during the conduct of the study 12-2132

Table 6.3.5- 12: Summary of the 12-2132 (M-474260-04-1) study on barley
Total residue calculated : sum of fluopyram+FLU-benzamide

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)			PHI (days)
			g a.s./ha	Water (L/ha)	g a.s./hL					fluopyram as AE C656948	FLU-benzamide as AE C656948	Total residue calc.	
	(a)	(b)				(c)	(d)		(d)				(e)
12-2132-01 France, south 31620 Bouloc Europe, South F 2012	Barley Queen; Winter Barley	1) 14.10.2011 2) 07.05.2012 - 14.05.2012 3) 20.06.2012 - 01.07.2012	78	300	26	07.05.2012/0	61	green material	61	1.3	<0.01	1.3	0
									69	0.32	<0.01	0.34	7
									83	0.11	<0.01	0.12	14
									85	0.042	<0.01	0.052	28
								grain	89	0.012	<0.01	0.022	49
								straw	89	0.093	0.019	0.12	49
12-2132-02 France, south 86200 Pouant Europe, South F 2012	Barley Cervoise; Winter variety	1) 14.10.2011 2) 03.05.2012 - 10.05.2012 3) 25.06.2012 - 30.06.2012	78	300	26	03.05.2012/0	61	green material	61	1.5	<0.01	1.5	0
								grain	89	0.01	<0.01	<0.02	57
								straw	89	0.025	<0.01	0.035	57
12-2132-03 Spain 17184 Salitja Europe, South F 2012	Barley Gomera; winter barley	1) 23.01.2012 2) 15.05.2012 - 25.05.2012 3) 15.06.2012 - 07.07.2012	73.3	282.01	26.0	16.05.2012/0	61	green material	61	2.0	<0.01	2.0	0
									73	0.58	0.010	0.59	7
									77	0.17	0.010	0.18	15
									85	0.10	<0.01	0.11	28
								grain	89	0.028	<0.01	0.038	42
								straw	89	0.18	0.015	0.20	42
12-2132-04 Spain 08520 Les Franqueses del Valles - Marata Europe, South F 2012	Barley Graphic; summer barley	1) 01.02.2012 2) 14.05.2012 - 21.05.2012 3) 15.06.2012 - 07.07.2012	78	300	26	14.05.2012/0	61	green material	61	2.0	<0.01	2.0	0
									77	0.71	0.010	0.72	7
									83	0.55	0.016	0.57	14
									87	0.32	0.011	0.33	28
								grain	89	0.034	<0.01	0.044	42
								straw	89	0.77	0.028	0.80	42

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)			PHI (days)
			g a.s./ha	Water (L/ha)	g a.s./hL					Fluopyram as AE C656948	FLU-benzamide as AE C656948	Total residue calc.	
	(a)	(b)				(c)	(d)		(d)				(e)
12-2132-05 Italy 01016 Tarquinia Europe, South F 2012	Barley Quench; Distich	1) 21.12.2011 2) 24.04.2012 - 04.05.2012 3) 15.06.2012 - 15.07.2012	78	300	26	26.04.2012/0	61	green material	61	2.0	<0.01	2.0	0
									69	0.89	<0.01	0.90	7
									73	0.58	0.019	0.60	14
									85	0.62	0.019	0.64	28
								grain	89	0.079	0.011	0.090	56
								straw	89	1.1	0.061	1.2	56
12-2132-07 Portugal 2580-230- Meca Alenquer Europe, South F 2012	Barley scarlet; Cevada	1) 03.01.2012 2) 24.04.2012 - 27.04.2012 3) 15.06.2012 - 21.06.2012	78	400	19	24.04.2012/0	61	green material	61	2.1	<0.01	2.1	0
								grain	89	<0.01	<0.01	<0.02	55
								straw	89	0.09	0.021	0.12	55
12-2132-08 Greece GR-61100 Kastanies, Kilkis Europe, South F 2012	Barley Lutes; Six lines ear	1) 20.12.2011 2) 03.05.2012 - 09.12.2012 3) 13.07.2012 - 13.07.2012	78	300	26	09.05.2012/0	65	green material	65	2.0	<0.01	2.0	0
								grain	89	0.11	<0.01	0.12	35
								straw	89	0.40	0.020	0.42	35

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) Study reference
* prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Data Point:	KCA 6.3.5/05
Report Author:	[redacted]
Report Year:	2014
Report Title:	Determination of the residues of AE C656948, BYF 00557 and prothioconazole in/on barley after spray application of bixafen & fluopyram & prothioconazole EC 260 in Italy
Report No:	13-2004
Document No:	M-479739-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC EC Guidance working document 7029/VI/95 rev.5 (1997-07-22) OECD 509 Adopted 2009-09-07, OECD GUIDELINE FOR THE TESTING OF CHEMICALS, Crop Field Trial US EPA OCSPP Guideline No. 860.1500
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted Study was not found in DAR/RAR and the Addenda
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and Methods

One supervised residue trial on barley were conducted in southern Europe (Italy) during the 2013 season.

One-spray applications was conducted with BIX+FLU+PTZ EC 260, an EC (emulsifiable concentrate) formulation containing 130 g/L prothioconazole, 65 g/L of fluopyram and 65 g/L of bixafen. For the purpose of this renewal, only results for fluopyram and its metabolite fluopyram-benzamide will be discussed.

The applications were conducted at the BBCH of 61 and at the application rates of 1.2 L/ha corresponding to the single application 0.078 kg a.s./ha. Water application rate was at 300 L/ha.

Samples of barley green material were collected at BBCH 83, samples of barley grain and straw at BBCH 89.

Each field sample was placed in doubled labelled bags and stored deep-frozen within 24 hours after sampling and until dispatch. All field samples were shipped deep-frozen under monitored conditions during shipment and arrived in good condition. The field samples were stored in a freezer at ≤- 18 °C until preparation of the examination samples.

For the preparation of examination samples, the deep-frozen field samples were shredded and homogenized with dry ice in a cutter. Representative parts of the shredded samples were transferred into polystyrene boxes and stored at ≤-18 °C. For each field sample, one or several examination samples were prepared for analysis and one examination sample was prepared as a reserve sample.

Samples were analysed according to the analytical method 00984/M003 ([redacted], 2015, M-467323-03-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues were extracted from 5 g of sample material with a mixture of acetonitrile:water (4:1, *v:v*) using a shaker (15 min). After filtration, the internal standards were added, and the extracts made up to volume. An aliquot of the extract was injected into an HPLC-(MS/MS).

Slight modifications on the extraction procedure were made during analysis of fluopyram and fluopyram-benzamide recovery samples (for barley grain at the fortification level of 0.1 mg/kg). After shaking, the sample was centrifuged, an 0.1 mL aliquot of the sample was filled up with 0.9 mL water and subjected to HPLC-(MS/MS) analysis. These modifications have no impact on the quality of the analytical method.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.01 mg/kg.

All final extracts were analysed within 4 days after extraction. Fluopyram and fluopyram-benzamide were found to be stable in extracts of matrices of plant origin for at least four weeks at 4 ± 3 °C which was tested within the validation of method 00984/M003.

Findings

In order to check the performance of the method, recovery determinations were included in each set of analyses. Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study. The apparent residues in the control sample used for the determination of recoveries were below 30 % of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these recoveries with one exception: One recovery of fluopyram-benzamide at LOQ in barley / green material was corrected by the inherent amount of residues in the corresponding control sample (22.4 % of LOQ; 0.00224 mg/kg).
The overall mean concurrent recoveries were also in the acceptable range of 70 % and 110 %, with the RSD < 20 % (when applicable).
The storage period of deep-frozen samples intended for the analysis of fluopyram and its metabolite fluopyram-benzamide ranged between 224 and 249 days.
The detailed results obtained for barley samples in southern Europe are summarized in the Table 6.3.5- 14

Conclusion

One supervised residue trials on barley were conducted in southern Europe with the application of BIX+FLU+PTZ EC 260 at the required rates and according to GLP. Samples of barley were analyzed for the residues of fluopyram and its metabolite fluopyram-benzamide. The results of the trials presented above show the following residues in grain at harvest.

- Fluopyram residues were 0.02 mg/kg.
- Fluopyram-benzamide residues were <0.01 mg/kg
- The total residue of fluopyram (sum of FLU + FLU-benzamide, expressed as fluopyram) were 0.03 mg/kg.

Residues in straw at harvest :

- Fluopyram residues were 0.81 mg/kg.

- Fluopyram-benzamide residues were 0.04 mg/kg
- The total residue of fluopyram (sum of FLU + FLU-benzamide, expressed as fluopyram) were 0.85 mg/kg.

Assessment and conclusion by applicant:

The study is acceptable.

Table 6.3.5- 13: Concurrent recovery data for Fluopyram (study 13-2004)

Portion analysed	Fortification level (mg/kg)	Recovery (%)				
		Individual recoveries	Min	Max	Mean	RSD
Fluopyram (AE C656948)						
Barley / green material	0.01	102	-	-	102	-
	2.0	99	-	-	99	-
		Overall recovery (n=2)	**-**	**-**	**101**	**-**
Barley / grain	0.01	113	-	-	113	-
	0.10	100*; 103*	100	103	102	-
		Overall recovery (n=3)			**105**	**6.5**
Barley / straw	0.01	100			100	-
	2.0	98		-	98	-
		Overall recovery (n=2)			**99**	**-**
Fluopyram-benzamide						
Barley / green material	0.01	83* (130*)		-	83* (130*)	-
	2.0	95	-	-	95	-
		Overall recovery (n=2)			**89**	**-**
Barley / grain	0.01	108	-	-	108	-
	0.10	99*; 100*	99	100	100	-
		Overall recovery (n=3)		**-**	**102**	**4.8**
Barley / straw	0.01	100	-	-	100	-
	2.0	95	-	-	95	-
		Overall recovery (n=2)			**98**	**-**

RSD = Relative standard deviation; LOQ = Practical limit of quantification
Fortified with fluopyram, determined as fluopyram and calculated as fluopyram
Fortified with fluopyram-benzamide, determined as fluopyram-benzamide and calculated as fluopyram
*For the extraction of these samples, the analytical method 00984/M003 was slightly modified

Table 6.3.5- 14: Summary of the 13-2004 (M-479739-01-1) study on barley

Total residue calculated : sum of fluopyram+FLU-benzamide

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)			PHI (days)
			g a.s./ha	Water (L/ha)	g a.s./hl					fluopyram as AE C656948	FLU-benzamide as AE C656948	Total residue calc.	
	(a)	(b)				(c)	(d)		(d)				(e)
13-2004-01 Italy 00053 Civitavecchia (RM) Europe, South F 2013	Barley Quench; Distichous barley	1) 08.01.2013 2) 02.05.2013 - 13.05.2013 3) 15.06.2013 - 15.07.2013	78	300	26	02.05.2013/0	61	green material	83	0.27	<0.01	0.28	28
								grain	89	0.017	<0.01	0.027	53
								straw	89	0.81	0.037	0.85	53

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
(e) Days after last application (Label/pre-harvest interval) PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

(g) Study reference
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
(l) Method validation
(m) Storage (max): ! based on date of analysis, P based on production date

- G greenhouse F field
- * prior to last treatment
- ** residue in control
- # no data available

Data Point:	KCA 6.3.5/06
Report Author:	[redacted]
Report Year:	2019
Report Title:	Determination of the residues of BCS-CN88460, prothioconazole and AE C656948 in/on barley after spray application of FLU & BCS-CN88460 & PTZ EC 234 in southern France, Italy, Spain and Greece
Report No:	17-2018
Document No:	M-656993-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500 Crop Field Trial
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted Study was not found in DAR/RAR and the Addenda
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and Methods

Four supervised residue trials on barley were conducted in southern Europe (Germany, Denmark, in the United Kingdom, northern France) during the 2017 season.

One-spray applications were conducted with FLU+ISY+PTZ EC 234 an emulsfiable concentrate formulation containing 67 g/L of fluopyram, 42 g/L of isoflucypram and 125 g/L of prothioconazole. For the purpose of this renewal, only results for fluopyram and its metabolite fluopyram-benzamide will be discussed.

The applications were conducted at the BBCH of 61 and at the application rates of 1.5 L/ha corresponding to the single application rate of 0.101 kg a.s./ha. Water application rates were at 250 – 400 L/ha. As the dose rate is higher than the intended GAP (1x101g as/ha Vs 1x78g as/ha), the residues are downscaled according to the scaling factors calculated and presented in Table 6.3.5- 4.

Samples of barley green material were collected at several growth stages, barley grain and straw at BBCH of 89.

Each field sample was placed in double labelled bags and stored deep-frozen at ≤-18 °C or below, within 24 hours after sampling and until dispatch. All field samples were shipped deep-frozen under monitored conditions during shipment and arrived in good condition. The field samples were stored in a freezer at ≤-18°C until preparation of the examination samples.

For the preparation of examination samples, the deep-frozen field samples were shredded and homogenized with dry ice in a cutter. Representative parts of the shredded samples were transferred into polystyrene boxes and stored at ≤-18 °C.

Samples were analysed according to the analytical method 00984/M003 ([redacted], 2015, M-467323-03-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented

within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues were extracted from 5 g of sample material (additionally 2.5 g for the sample material straw) by extraction using a shaker (15 min) with a mixture of acetonitrile:water (4:1, *v:v*).

The filtering procedure under low vacuum was replaced by a centrifugation step. Then 0.5 mL of internal standard solution (1 mg/L) was added to the extract followed by 5 min centrifugation at 4000 rpm at 10 °C.

An 0.1 mL aliquot of this extract was diluted with 0.9 mL of Milli-Q-water and then proceeded to HPLC-MS/MS analysis.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.01 mg/kg.

All final extracts were analysed within 12 days after extraction. Fluopyram and fluopyram-benzamide were found to be stable in extracts of matrices of plant origin for at least four weeks at 4 ± 3 °C which was tested within the validation of method 00984/M003.

Findings

In order to check the performance of the method, recovery determinations were included in each set of analyses. Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study.

The apparent residues in the control sample used for the determination of recoveries were below 30 % of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these determinations.

The mean recoveries of fluopyram and its metabolite fluopyram-benzamide per fortification level were within the acceptable range of 70 % – 110 % (with few exceptions, see Table 6.3.5- 15) and with the RSDs < 20 % (when applicable). The overall mean concurrent recoveries were also in the acceptable range of 70 % and 110 %, with the RSD < 20 %.

The storage period of deep-frozen samples intended for the analysis of fluopyram and its metabolite fluopyram-benzamide ranged between 457 and 663 days.

The detailed results obtained for barley samples in northern Europe are summarized in the Table 6.3.5- 16

Conclusion

Four supervised residue trials on barley were conducted in northern Europe with the application of FLU+ISY+PTZ EC 234 at a higher rate than required in the supported GAP (1x101g as/ha Vs 1x78g as/ha), and according to GLP. Samples of barley were analyzed for the residues of fluopyram and its metabolite fluopyram-benzamide. The results of the trials presented above show the following residues after donwscaling in grain at harvest:

- Fluopyram residues were between <0.01 mg/kg and 0.03 mg/kg
- Fluopyram-benzamide residues were <0.01 mg/kg
- The total residue of fluopyram (sum of FLU + FLU-benzamide, expressed as fluopyram) after downscaling to the right dose (1x101g as/ha Vs 1x78g as/ha), range between <0.02 and 0.04 mg/kg.

Residues after downscaling in straw at harvest :

- Fluopyram residues were between <0.01 mg/kg and 0.92 mg/kg

- Fluopyram-benzamide residues were between <0.01 mg/kg and 0.02 mg/kg
- The total residue of fluopyram (sum of FLU + FLU-benzamide, expressed as fluopyram) after downscaling to the right dose (1x101g as/ha Vs 1x78g as/ha), range between 0.02 and 0.92 mg/kg.

Assessment and conclusion by applicant:

The study is acceptable.
The residue level in these GLP trials was corrected with a proportionality factor according to EFSA Technical Report (EFSA supporting publication 2018-EN-1503).

Table 6.3.5- 15: Concurrent recovery data for Fluopyram (study 17-2018)

Portion analysed	Fortification level (mg/kg)	Recovery (%)				
		Individual recoveries	Min	Max	Mean	RSD
Fluopyram (AE C656948)						
Spring barley / grain	0.01	91; 91; 98	91	98	93	4.2
	0.10	94; 94; 96; 98	94	98	96	2.0
	0.50	117*	-	-	-	-
		Overall recovery (n = 8)			**97**	**8.6**
Spring barley / green material	0.01	94; 96; 99	94	99	96	2.6
	0.10	96; 97; 98	96	98	97	1.0
	4.0	110; 111; 112	110	112	111**	0.9
	10	95; 95; 97	95	97	96	1.2
	45	109	-	-	-	-
		Overall recovery (n = 13)			**101**	**6.9**
Spring barley / straw	0.01	108; 111; 120	108	120	113***	5.5
	0.10	93; 96; 96; 100	93	100	96	3.0
	0.30	100; 100; 101	100	101	100	0.6
	0.50	91	-	-	-	-
	1.0	97; 103; 104	97	104	101	3.7
		Overall recovery (n = 14)			**101**	**7.5**
Fluopyram-benzamide						
Spring barley / grain	0.01	85; 91; 93	85	93	90	4.6
	0.10	87; 92; 95; 98*	87	98	93	5.0
	0.50	107	-	-	-	-
		Overall recovery (n = 8)			**94**	**7.3**
Spring barley / green material	0.01	97; 102; 103	97	103	101	3.2
	0.10	96; 100; 100	96	100	99	2.3

Portion analysed	Fortification level (mg/kg)	Recovery (%)				
		Individual recoveries	Min	Max	Mean	RSD
	4.0	105; 106; 107	105	107	106	0.9
	10	93; 94; 96	93	96	94	1.6
	15	104	-	-	-	-
		Overall recovery (n = 13)			**100**	**4.7**
Spring barley / straw	0.01	83; 89; 95	83	95	89	6.7
	0.10	82*; 90; 92; 92	82	92	89	5.3
	0.20	95; 97; 99	95	99	97	2.1
	0.50	94			-	-
	1.0	93; 98; 101	93	101	97	4.2
		Overall recovery (n = 14)			**93**	**6.0**

FL = Fortification level, RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with fluopyram, determined as fluopyram and calculated as fluopyram
Fortified with fluopyram-benzamide, determined as fluopyram-benzamide and calculated as fluopyram

* This value at the fortification level at 0.50 mg/kg was accepted, because this fortification level is not the most relevant to the residue levels for fluopyram found in the study samples (the highest residue for fluopyram is 0.041 mg/kg. It is covered by the recoveries at the fortification level 0.10 mg/kg)

** This value at the fortification level of 1.0 mg/kg was accepted since it only slightly exceeds the guideline range of 70 – 110 % and because the average recoveries at fortification levels of 0.01 mg/kg, 0.10 mg/kg and 10 mg/kg are within 70 - 110%).

*** This value at the fortification level of 0.01 mg/kg was accepted with reference to the criteria of the OECD guidance on pesticide residue analytical methods ENV/JM/MONO(2007)17.

Table 6.3.5- 16: Summary of the 17-2017 (M-655225-01-1) study on barley

Total residue calculated : sum of fluopyram+FLU-benzamide

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)					PHI (days)
			g a.s./ha	Water (L/ha)	g a.s./hL					fluopyram as AE C656948	FLU-benzamide as AE C656948	Total residue calc	Scaling factor	Scaled residue	
	(a)	(b)				(c)	(d)		(d)						(f)
17-2018-01 France, south 26120 Upie Europe, South F 2017	Barley Maltesse, winter	1) 17.11.2016 2) 14.05.2017 - 30.05.2017 3) 23.06.2017	101	300	33.5	15.05.2017/0	61	green material	61	2.7	<0.01	2.7	0.77	2.1	0
									65	0.58	<0.01	0.59		0.45	7
									69	0.58	0.015	0.60		0.46	14
									73	0.37	0.011	0.38		0.29	22
									75	0.31	0.011	0.32		0.25	28
								grain	89	0.036/0.035**	<0.01	<0.02		<0.02	38
								straw	89	0.46/0.43**	0.029/0.025**	0.03		0.023	38
17-2018-02 Italy 20090 Settala Europe, South F 2017	Barley Concerto	1) 21.03.2017 2) 28.06.2017 - 04.07.2017 3) 11.08.2017	101	400	25.2	28.06.2017/0	61	green material	61	4.1	0.010	4.1	0.77	3.2	0
									65	0.63	0.017	0.65		0.50	7
									73	0.30	<0.01	0.31		0.24	14
									75	0.19	0.010	0.20		0.15	22
									83	0.19	0.014	0.20		0.15	28
								grain	89	<0.01	<0.01	<0.02		<0.02	44
								straw	89	<0.01	<0.01	<0.02		<0.02	44

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)					PHI (days)
			g a.s./ha	Water (L/ha)	g a.s./hL					fluopyram as AE C656948	FLU-benzamide as AE C656948	Total residue calc.	Scaling factor	Scaled residue	
	(a)	(b)				(c)	(d)		(d)						(f)
17-2018-03 Spain 08717 Montmaneu Europe, South F 2017	Barley Meseta; Winter	1) 21.11.2016 2) 04.05.2017 - 12.05.2017 3) 21.06.2017	101	250	40	04.05.2017/0	61	green material	61	2.4	<0.01	2.4	0.77	1.8	0
									69	1.5	<0.01	1.5		1.2	8
									73	1.1	0.020	1.1		0.85	14
									75	0.94	0.022	0.96		0.74	20
									83	0.88	0.029	0.89		0.69	27
									75	1.1	0.021	1.1		0.85	18
								grain	89	0.041	<0.01	0.05		0.038	48
								straw	89	1.2	0.034	1.2		0.92	48
17-2018-04 Greece GR 54500 Drymos Europe, South F 2017	Barley Hyvito	1) 07.11.2016 2) 25.04.2017 - 05.05.2017 3) 01.06.2017 - 10.06.2017	101	300	33.5	27.04.2017/0	61	green material	61	6.8	<0.01	6.8	0.77	5.2	0
									71	2.4	0.31	2.7		2.1	7
									73	2.2	0.040	2.2		1.7	14
									73	1.7	0.016	1.7		1.3	21
									75	1.8	0.017	1.8		1.4	23
									77	1.6	0.011	1.6		1.2	27
								grain	89	0.019	<0.01	0.029		0.022	43
								straw	89	0.38	<0.01	0.39		0.30	43.

(a) According to OECD Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) Study reference
* prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Data Point:	KCA 6.3.5/07
Report Author:	[redacted].
Report Year:	2019
Report Title:	Determination of the residues of BYF 00587 and AE C656948 in/on barley after spraying of bixafen & fluopyram EC 200 in the field in France (south), Italy and Greece
Report No:	18-2101
Document No:	M-668264-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and Methods

Four supervised residue trials on barley were conducted in southern Europe (Germany, Denmark, in the United Kingdom, northern France) during the 2017 season.

One-spray applications were conducted with BIX+FLU EC200 an emulsifiable concentrate formulation containing 100 g/L of bixafen and 100 g/L of fluopyram. For the purpose of this renewal, only results for fluopyram and its metabolite fluopyram-benzamide will be discussed.

The applications were conducted at the BBCH of 59-61 and at the application rates of 1. L/ha corresponding to the single application rate of 0.101 kg a.s./ha. Water application rates were at 300 L/ha. As the dose rate is higher than the intended GAP (1x101g as/ha Vs 1x78g as/ha), the residues are downscaled according to the scaling factors calculated and presented in Table 6.3.5- 4.

Samples of barley green material were collected at several growth stages, barley grain and straw at BBCH of 89.

Each field sample was placed in double labelled bags and stored deep-frozen at ≤-18 °C or below, within 24 hours after sampling and until dispatch. All field samples were shipped deep-frozen under monitored conditions during shipment and arrived in good condition. The field samples were stored in a freezer at ≤-18°C until preparation of the examination samples.

For the preparation of examination samples, the deep-frozen field samples were shredded and homogenized with dry ice in a cutter. Representative parts of the shredded samples were transferred into polystyrene boxes and stored at ≤-18°C.

Samples were analysed according to the analytical method 00984/M003 ([redacted], 2015, M-467323-03-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues were extracted from 5 g of sample material (additionally 2.5 g for the sample material straw) by extraction using a shaker (15 min) with a mixture of acetonitrile:water (4:1, *v:v*).

The filtering procedure under low vacuum was replaced by a centrifugation step. Then 0.5 mL of internal standard solution (1 mg/L) was added to the extract followed by 5 min centrifugation at 4000 rpm at 10 °C.

An 0.1 mL aliquot of this extract was diluted with 0.9 mL of Milli-Q-water and then proceeded to HPLC-MS/MS analysis.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.01 mg/kg.

All final extracts were analysed within 4 days after extraction. Fluopyram and fluopyram-benzamide were found to be stable in extracts of matrices of plant origin for at least four weeks at 4 ± 3 °C which was tested within the validation of method 00984/M003.

Findings

In order to check the performance of the method, recovery determinations were included in each set of analyses. Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study.

The apparent residues in the control sample used for the determination of recoveries were below 30 % of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these determinations.

The mean recoveries of fluopyram and its metabolite fluopyram-benzamide per fortification level were within the acceptable range of 70 % – 110 % (with few exceptions, see Table 6.3.5- 15) and with the RSDs < 20 % (when applicable). The overall mean concurrent recoveries were also in the acceptable range of 70 % and 110 %, with the RSD < 20 %.

The storage period of deep-frozen samples intended for the analysis of fluopyram and its metabolite fluopyram-benzamide ranged between 457 and 663 days.

The detailed results obtained for barley samples in northern Europe are summarized in the Table 6.3.5- 18

Conclusion

Four supervised residue trials on barley were conducted in northern Europe with the application of BIX+FLU EC 200 at a higher rate than required in the supported GAP (1x101g as/ha Vs 1x78g as/ha), and according to GLP. Samples of barley were analyzed for the residues of fluopyram and its metabolite fluopyram-benzamide. The results of the trials presented above show the following residues after donwscaling in grain at harvest.

- Fluopyram residues were between 0.01 mg/kg and 0.03 mg/kg
- Fluopyram-benzamide residues were <0.01 mg/kg
- The total residue of fluopyram (sum of FLU + FLU-benzamide, expressed as fluopyram) after downscaling to the right dose (1x101g as/ha Vs 1x78g as/ha), range between <0.02 and 0.04 mg/kg.

Residues after downscaling in straw at harvest :

- Fluopyram residues were between 0.02 mg/kg and 0.11 mg/kg
- Fluopyram-benzamide residues were between <0.01 mg/kg and 0.07 mg/kg

- The total residue of fluopyram (sum of FLU + FLU-benzamide, expressed as fluopyram) after downscaling to the right dose (1x101g as/ha Vs 1x78g as/ha), range between 0.04 and 0.17 mg/kg.

Assessment and conclusion by applicant:

The study is acceptable.
The residue level in these GLP trials was corrected with a proportionality factor according to EFSA Technical Report (EFSA supporting publication 2018-EN-1503).

Table 6.3.5- 17: Concurrent recovery data for Fluopyram (study 18-2101)

Portion analysed	Fortification level (mg/kg)	Recovery (%)				
		Individual recoveries	Min	Max	Mean	RSD
Fluopyram (AE C656948)						
Spring barley / green material	0.01	92; 98; 100	92	100	97	4.3
	0.10	95; 96; 98	95	98	96	1.6
	5.0	102; 102; 105	102	105	103	1.7
		Overall recovery (n = 9)			**99**	**4.1**
Spring barley / grain	0.01	100; 101; 103	100	103	101	1.5
	0.10	98; 100; 100	98	100	99	1.2
		Overall recovery (n = 6)			**100**	**1.6**
Spring barley / straw	0.01	91 (116)*; 97 (122)*; 100 (128)*	91	100	96	4.8
	0.10	96 (99)*; 96 (99)*; 97 (100)*	96	97	96	0.6
	0.50	106; 114; 112	106	112	110	2.9
		Overall recovery (n = 9)			**101**	**7.3**
Fluopyram-benzamide						
Spring barley / green material	0.01	98; 101; 105	98	105	101	3.5
	0.10	101; 103; 107	101	107	104	2.9
	5.0	103; 103; 106	103	106	104	1.7
		Overall recovery (n = 9)			**103**	**2.7**
Spring barley / grain	0.01	102; 111; 113	102	113	109	5.4
	0.10	106; 109; 111	106	111	109	2.3
		Overall recovery (n = 6)			**109**	**3.7**
Spring barley / straw	0.01	103; 108; 113	103	113	108	4.6
	0.10	103; 103; 109	103	109	105	3.3
	0.50	104; 111; 111	104	111	109	3.7
		Overall recovery (n = 9)			**107**	**3.8**

FL = Fortification level, RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with fluopyram, determined as fluopyram and calculated as fluopyram
Fortified with fluopyram-benzamide, determined as fluopyram-benzamide and calculated as fluopyram
* These recoveries were background corrected since the control sample used for spiking (18-2101-01-0006E) was found to contain (apparent) residues at a level of 0.00254 mg/kg. The uncorrected recovery is shown in brackets.

Table 6.3.5- 18: Summary of the 18-2101 (M-668264-01-1) study on barley

Total residue calculated : sum of fluopyram+FLU-benzamide

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)			Scaling factor	Scaled residue	PHI (days)
			g a.s./ha	Water (L/ha)	g a.s./hL					fluopyram as AE C656948	FLU-benzamide as AE C656948	Total residue calc.			
	(a)	(b)				(c)	(d)		(d)						(f)
18-2101-01 18-2101-01-T France, south 31330 St Caprais Europe, South F, 2018	Barley Cassia	1) 25.10.2017 2) 01.05.2018 - 10.05.2018 3) 25.06.2018 - 05.07.2018	103	308	33.3	02.05.2018-0	61	Grain	89	0.032	0.014	0.046	0.75	<u>0.035</u>	57
								straw	89	0.14	0.091	0.23		0.17	57
18-2101-02 18-2101-02-T France, south 84170 Monteux Europe, South F 2018	Barley Augusta	1) 11.12.2017 2) 01.05.2018 - 06.05.2018 3) 18.06.2018 - 22.06.2018	100	300	33.4	02.05.2018-0	61	green material	61	3.6	<0.01	3.6	0.78	2.8	0
									69	1.2	0.011	1.2		0.94	7
									75	0.35	<0.01	0.36		0.28	14
									77	0.16	<0.01	0.17		0.13	21
									83	0.074	<0.01	0.084		0.066	28
								grain	89	0.028	<0.01	0.038		<u>0.030</u>	48
								straw	89	0.046	<0.01	0.056		0.044	48

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)			Scaling factor	Scaled residue	PHI (days)
			g a.s./ha	Water (L/ha)	g a.s./hL					Fluopyram as AE C656948	FLU-benzamide as AE C656948	Total residue calc.			
	(a)	(b)				(c)	(d)		(d)						(f)
18-2101-03 18-2101-03-T Italy 44123 Boara Ferrara Europe, South F, 2018	Barley Monroe	1) 19.10.2017 2) 02.05.2018 - 16.05.2018 3) 11.06.2018 - 25.06.2018	101	302	33.3	30.04.2018/0	59	Grain	89	0.038	<0.01	0.048	0.77	0.037	44
								straw	89	0.14	0.017	0.16		0.12	44
18-2101-04 18-2101-04-T Greece 50100 Thymaria, Kozanis Europe, South F 2018	Barley Explorer	1) 09.12.2017 2) 03.05.2018 - 07.05.2018 3) 14.06.2018 - 14.06.2018	101	305	33.2	03.05.2018/0	61	green material	61	1.3	<0.01	1.3	0.77	1.0	0
									71	0.41	<0.01	0.42		0.32	7
									73	0.16	<0.01	0.17		0.13	14
									75	0.047	<0.01	0.057		0.044	21
									85	0.027	<0.01	0.037		0.028	28
								grain	89	0.014	<0.01	0.024		0.018	42
								straw	89	0.038	<0.01	0.048		0.037	42

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) Study reference
* prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Based on the residue definition for risk assessment, the sum of fluopyram and fluopyram-benzamide expressed as fluopyram, the total residues for barley are summarized in the Table 6.3.5- 19

Table 6.3.5- 19: Summary of fluopyram total residue data for barley trials supporting GAP 1 (after results scaling)

Crop	Commodity	Region/Indoor (a)	Trial results relevant to the critical GAP (mg/kg)	STMR (b)	HR (c)
Barley	Grain	NEU	<0.02 ; 0.011 ; 0.015 ; 2x 0.017 ; 2x 0.022 ; 0.023 ; 0.025 ; 0.026 ; 0.029 ; 0.032 ; 0.035 ; 0.045 ; 0.077	0.023	0.077
		SEU	4x <0.02 ; 0.012 ; 0.018 ; 2x 0.022 0.027 ; 0.030 ; 0.035 ; 0.037 ; 2x 0.038 ; 0.044 ; 0.090	0.025	0.090
		NEU/SEU	5x <0.02 ; 0.011 ; 0.012 ; 0.015 ; 2x 0.017 ; 0.018 ; 4x 0.022 ; 0.023 ; 0.025 ; 0.026 ; 0.027 ; 0.029 ; 0.030 ; 0.032 ; 2x 0.035 ; 0.037 ; 2x 0.038 ; 0.044 ; 0.045 ; 0.077 ; 0.090	0.023	0.090
	Straw	NEU	0.021 ; 0.022 ; 0.041 ; 0.042 ; 0.047 ; 0.051 ; 0.056 ; 0.064 ; 0.068 ; 0.085 ; 0.087 ; 0.093 ; 0.11 ; 0.19 ; 0.29	0.064	0.29
		SEU	0.02 ; 0.023 ; 0.035 ; 0.037 ; 0.042 ; 3x 0.12 ; 0.17 ; 0.20 ; 0.30 ; 0.42 ; 0.42 ; 0.80 ; 0.85 ; 0.92 ; 1.2	0.145	1.2
		NEU/SEU	0.02 ; 0.021 ; 0.022 ; 0.023 ; 0.035 ; 0.037 ; 0.041 ; 0.042 ; 0.044 ; 0.047 ; 0.051 ; 0.056 ; 0.064 ; 0.068 ; 0.085 ; 0.087 ; 0.093 ; 0.11 ; 3x 0.12 ; 0.17 ; 0.19 ; 0.20 ; 0.29 ; 0.30 ; 0.42 ; 0.80 ; 0.85 ; 0.92 ; 1.2	0.087	1.2

(a) **NEU** or **SEU** for northern or southern outdoor trials in EU member states,
(b) **STMR**: Supervised Trials Median Residue
(c) **HR**: Highest residue

Table 6.3.5- 20: : Overview of European residue trials conducted on barley per geographical "residue region" and vegetation period to support GAP 2

Crop	Region	No. of independent trials		Report No. (Formulation)	Document number	Reference
		Vegetation period	Σ			
		2019				
Barley	NEU	4	4	E19RP074, (FLU SC250)	M-761575-01-1	KCA 6.3.5/08
	SEU	4	4	E19RP109, (FLU SC250)	M-761576-01-1	KCA 6.3.5/09

NEU = northern EU field SEU = southern EU field

GAP 2 northern zone

Data Point:	KCA 6.3.5/08
Report Author:	[redacted]
Report Year:	2021
Report Title:	Determination of the residues of prothioconazole, BYF 00587 and AE C656948 in/on barley after a spray application of bixafen & fluopyram & prothioconazole EC 260 in Germany, northern France and the Netherlands
Report No:	E19RP074
Document No:	M-761575-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and Methods

Four supervised residue trials on barley were conducted in northern Europe (in northern France, Germany and the Netherlands) during the 2019 season.

One spray applications was conducted with BIX+FLU+PTZ EC 260, an EC (emulsifiable concentrate) formulation containing 130 g/L prothioconazole, 65 g/L of fluopyram and 65 g/L of bixafen. For the purpose of this renewal, only results for fluopyram and its metabolites fluopyram-benzamide, fluopyram-

pyridyl-carboxylic-acid, fluopyram-pyridyl-acetic-acid),fluopyram-7-hydroxy and fluopyram-methyl sulfoxide will be discussed.

The applications were conducted at the BBCH of 61 and at the application rates of 0.6 L/ha corresponding to the single application 0.039 kg a.s./ha. Water application rates were at 300 L/ha.

Samples of barley green material were collected at BBCH 61 and barley grain and straw at harvest BBCH 89.

Each field sample was placed in doubled labelled bags and stored deep-frozen within 24 hours after sampling and until dispatch. All field samples were shipped deep-frozen (at -18 °C or lower temperatures) under monitored conditions during shipment and arrived in good condition.

The field samples were stored in a freezer at ≤-18 °C until preparation of the examination samples. For the preparation of examination samples, the deep-frozen field samples were shredded and homogenized with dry ice in a cutter. Representative parts of the shredded samples were transferred into polystyrene boxes and stored at ≤-18°C. For each field sample, one or several examination samples were prepared for analysis and one examination sample was prepared as a reserve sample.

Residues of fluopyram and its metabolites (FLU-benzamide, FLU-PCA, FLU-PAA, FLU-7-OH, FLU-methyl-sulfoxide), were determined by LC-MS/MS according to method 00984 ([redacted], 05/02/2007, M-283301-01-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues were extracted from 10 g of sample material by two successive extractions using a high-speed blender with a mixture of acetonitrile:water (80:20; *v:v*). After centrifugation the extract volume was adjusted. Then, the extracts were diluted ten times by adding the internal standards:

- One dilution was performed under acidic conditions: this extract allowed the determination of FLU-PCA and FLU-methyl-sulfoxide.
- In parallel, another dilution was performed under basic conditions: this extract allowed the determination of fluopyram, FLU-benzamide and FLU-7-OH.

An aliquot of the extracts was injected into LC-MS/MS operated in Electrospray Ionization mode:

- One injection in positive electrospray ionization for the determination of fluopyram, FLU-benzamide and FLU-7-OH.
- Another injection in negative electrospray ionization for the determination of FLU-PCA and FLU-methyl-sulfoxide under different conditions.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels. For each calibration curve, the correlation coefficient R was above 0.99.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.05 mg/kg for wheat straw and 0.01 mg/kg for all other matrices.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Conditions used in this study: The method was used as described in the original method with some minor modifications, which have no impact on the validity of the study:

Residues were extracted from 5 g of sample material of grain and green material and from 2.5 g of straw. Extraction was done with only 30 mL (straw 40 mL) acetonitrile/water (80/20 v/v). The blender is washed with 5 mL acetonitrile/water (80/20 v/v). The internal standard concentrations were changed from 0.01 mg/L to 10 mg/L and the dilution step after filtration was omitted. The adjustment to pH= 8 was done with

ammonium chloride/ammonia buffer followed by the dilution with acetonitrile/water (4/1, v/v) to get Extract A. The determination was done from an aliquot of Extract A in the positive ion mode.

The time between the beginning of the sample preparation and the sample analysis did not exceed 24 hours for grain and straw and 4 days for samples of the matrix green material. The stability of analytes in extracts of green material, grain and straw of barley were investigated in this study. Extracts of green material, grain and straw were found to be stable for at least 5 days when stored at < 6 °C.

Findings

In order to check the performance of the method, recovery determinations were included in each set of analyses. Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study. The apparent residues in the control sample used for the determination of recoveries were below 30 % of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these determinations.

The mean recoveries per fortification level were within the acceptable range of 70 % - 110 % and with the RSDs < 20 %. The overall mean concurrent recoveries were also in the acceptable range of 70 % and 110 %, with the RSD < 20 %.

The storage period of deep-frozen samples intended for the analysis of fluopyram and its metabolites ranged between 311 and 375 days.

The detailed results obtained for barley samples in northern Europe are summarized in the Table 6.3.5- 21

Conclusion

Four supervised residue trials on barley were conducted in northern Europe with the application of BIX+FLU+PTZ EC 260 at the required rates and according to GLP. Samples of barley were analyzed for the residues of fluopyram and its metabolite fluopyram-benzamide. The results of the trials presented above show the following residues in grain at harvest.

- Fluopyram residues range between <0.01and 0.11 mg/kg. According to Dixon's test the highest value can be considered as an outlier.
- Fluopyram-benzamide residues were <0.01 mg/kg
- The total residue of fluopyram (sum of FLU + FLU-benzamide, expressed as fluopyram) range between <0.02 and 0.12 mg/kg.
- Fluopyram-pyridyl-carboxylic-acid residues range between <0.01and 0.01 mg/kg
- Fluopyram-pyridyl-acetic-acid residues were <0.01 mg/kg
- Fluopyram-7-hydroxy residues were <0.01 mg/kg
- Fluopyram-methylsulfoxide residues were <0.01 mg/kg

Residues in straw at harvest :

- Fluopyram residues range between <0.05 and 0.808 mg/kg.
- Fluopyram-benzamide residues were <0.05 mg/kg
- The total residue of fluopyram (sum of FLU + FLU-benzamide, expressed as fluopyram) range between <0.1 and 0.858 mg/kg.
- Fluopyram-pyridyl-carboxylic-acid residues were <0.05 mg/kg
- Fluopyram-pyridyl-acetic-acid residues were <0.05 mg/kg
- Fluopyram-7-hydroxy residues between <0.05 and 0.068 mg/kg.
- Fluopyram-methylsulfoxide residues were <0.05 mg/kg

Assessment and conclusion by applicant:

The study is acceptable.
According to Dixon's test the highest value of fluopyram in grain can be considered as an outlier.

Table 6.3.5- 21: Concurrent recovery data for Fluopyram (study E19RP074)

Portion analysed	Fortification level (mg/kg)	Recovery (%)				
		Individual recoveries	Min	Max	Mean	RSD
Fluopyram (AE C656948)						
Barley / grain	0.01	97; 102; 102; 102; 106	97	106	102	3.1
	0.2	95; 95; 97; 99; 102	95	102	98	3.0
		Overall recovery (n= 10)			**100**	**3.7**
Barley / green material	0.01	95; 96; 96; 101; 102	95	102	98	3.3
	1.20	96; 96; 98; 100; 100	96	100	98	1.9
		Overall recovery (n= 10)			**98**	**2.6**
Barley / straw	0.05	94; 95; 96; 98; 98	94	98	96	1.6
	0.9	85; 89; 96; 97; 99	85	99	93	5.4
		Overall recovery (n= 10)			**95**	**4.6**
Fluopyram-benzamide						
Barley / grain	0.01	92; 93; 98; 98; 103	92	103	97	4.6
	0.2	91; 93; 94; 98; 101	91	101	95	4.2
		Overall recovery (n= 10)			**96**	**4.2**
Barley / green material	0.01	84; 90; 90; 94; 96; 97; 98; 101	84	101	94	5.8
	1.20	96; 96; 96; 98; 100	96	100	97	1.8
		Overall recovery (n= 13)			**95**	**4.9**
Barley / straw	0.05	77; 80; 80; 80; 87	77	87	81	4.6
	0.9	78; 79; 80; 82; 83	78	83	80	2.6
		Overall recovery (n= 10)			**81**	**3.5**
Fluopyram-pyridyl-carboxilic-acid						
Barley / grain	0.01	92; 99; 103; 103; 114	92	114	102	7.8
	0.2	96; 97; 98; 100; 101	96	101	98	2.1
		Overall recovery (n= 10)			**100**	**5.9**
Barley / green material	0.01	90; 95; 97; 100; 100; 102; 102; 106	90	106	99	5.0
	1.20	99; 100; 100; 100; 101	99	101	100	0.7
		Overall recovery (n = 13)			**99**	**3.8**
Barley / straw	0.05	98; 99; 105; 105; 111	98	111	104	5.1
	0.9	91; 95; 96; 96; 100	91	100	96	3.4
		Overall recovery (n= 10)			**100**	**5.9**
Fluopyram-pyridyl-acetic-acid						
Barley / grain	0.01	93; 97; 100; 102; 113	93	113	101	7.4

Portion analysed	Fortification level (mg/kg)	Recovery (%)				
		Individual recoveries	Min	Max	Mean	RSD
	0.2	96; 97; 100; 101; 111	96	111	101	5.9
		Overall recovery (n = 10)			**101**	**6.3**
Barley / green material	0.01	92; 96; 98; 99; 101; 103; 106; 114	92	114	101	6.7
	1.20	92; 93; 95; 98; 102	92	102	96	4.2
		Overall recovery (n = 13)			**99**	**6.3**
Barley / straw	0.05	99; 100; 101; 102; 110	99	110	102	4.3
	0.9	91; 93; 97; 99; 105	91	105	97	5.6
		Overall recovery (n = 10)			**100**	**5.5**
Fluopyram-7-hydroxy						
Barley / grain	0.01	95; 96; 100; 103; 103	95	103	99	3.8
	0.2	92; 95; 95; 101; 102	92	102	97	4.4
		Overall recovery (n = 10)			**98**	**4.1**
Barley / green material	0.01	89; 89; 92; 94; 95; 99; 100; 101	89	101	95	5.0
	1.20	86; 89; 93; 95; 95	86	95	92	4.3
		Overall recovery (n = 13)			**94**	**4.9**
Barley / straw	0.05	87; 87; 92; 92; 94	87	94	90	3.6
	0.9	82; 87; 88; 91; 95	82	95	89	5.4
		Overall recovery (n = 10)			**90**	**4.4**
Fluopyram-methyl-sulfoxide						
Barley / grain	0.01	90; 94; 95; 97; 98	90	98	95	3.3
	0.2	90; 96; 97; 98; 105	90	105	97	5.5
		Overall recovery (n = 10)			**96**	**4.5**
Barley / green material	0.01	87; 92; 98; 106; 109	87	109	98	9.4
	1.2	96; 102; 103; 105; 112	96	112	104	5.6
		Overall recovery (n = 10)			**101**	**7.7**
Barley / straw	0.05	87; 87; 88; 89; 90	87	90	88	1.5
	0.9	86; 92; 92; 93; 97	86	97	92	4.3
		Overall recovery (n = 10)			**90**	**3.8**

RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with fluopyram, determined as fluopyram and calculated as fluopyram
Fortified with fluopyram-benzamide, determined as fluopyram-benzamide and calculated as fluopyram
Fortified with fluopyram-pyridyl-carboxilic-acid, determined as fluopyram- pyridyl-carboxilic-acid and calculated as fluopyram
Fortified with fluopyram- pyridyl-acetic-acid, determined as fluopyram- pyridyl-acetic-acid and calculated as fluopyram
Fortified with fluopyram-7-hydroxy, determined as fluopyram-7-hydroxy and calculated as fluopyram
Fortified with fluopyram-methylsulfoxide, determined as fluopyram-methylsulfoxide and calculated as fluopyram

Table 6.3.5- 22: Summary of the E19RP074 (M-761575-01-1) study on barley

Total residue calculated : sum of fluopyram+FLU-benzamide

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)							PHI (days)
			g a.s./ha	Water (L/ha)	g a.s./hL					AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-PAA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residue calc.	
	(a)	(b)				(c)	(d)		(d)								(e)
E19RP074-01 Germany 51399 Burscheid Europe, North F,2019	Barley, spring Avalon	1) 01.04.2019 2) 11.06.2019 - 15.06.2019 3) 01.08.2019 - 31.08.2019	38.1	294	13	11.06.2019/ 0	61	green material	61	1.21	<0.01	<0.01	<0.01	<0.01	<0.01	1.12	0
								grain	89	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	57
								straw	89	0.058	<0.05	<0.05	<0.05	0.068	<0.05	0.108	57
E19RP074-02 France, north 37270 Athée sur Cher Europe, North F,2019	Barley, spring Sebastian	1) 18.02.2019 2) 26.05.2019 - 01.06.2019 3) 05.07.2019 - 15.07.2019	39.5	304	13	27.05.2019/ 0	61	green material	61	0.872	<0.01	<0.01	<0.01	<0.01	<0.01	0.88	0
								grain	89	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	39
								straw	89	0.224	<0.05	<0.05	<0.05	0.050	<0.05	0.274	39

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)							PHI (days)
			g a.s./ha	Water (L/ha)	g a.s./hL					AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-PAA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residue calc.	
	(a)	(b)				(c)	(d)		(d)								(e)
E19RP074-03 Germany 78176 Blumberg OT Kommingen Europe, North F,2019	Barley, spring Avalon	1) 30.03.2019 2) 26.06.2019 - 10.07.2019 3) 24.07.2019 - 07.08.2019	39.4	303	13	04.07.2019/ 0	61	green material	61	1.00	<0.01	<0.01	<0.01	<0.01	<0.01	1.01	0
								grain	89	0.11	<0.01	<0.01	<0.01	<0.01	<0.01	0.12	27
								straw	89	0.808	<0.05	<0.05	<0.05	0.0542	<0.05	0.858	27
E19RP074-04 Netherlands 1681 ND Zwaagdijk Europe, North F,2019	Barley, spring Plant	1) 11.04.2019 2) 28.06.2019 - 08.07.2019 3) 01.08.2019 - 15.08.2019	40.6	313	13	28.06.2019/ 0	61	green material	61	0.992	<0.01	<0.01	<0.01	<0.01	0.01	1.00	0
								grain	89	0.011	<0.01	0.011	<0.01	<0.01	<0.01	0.021	36
								straw	89	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.1	36

(a) According to CODEX Classification Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method
information which metabolites are included
(g) Study reference
prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

GAP 2 southern zone

Data Point:	KCA 6.3.5/09
Report Author:	[redacted]
Report Year:	2021
Report Title:	Determination of the residues of prothioconazole, BYF 00587 and AE C656948 in/on barley after a spray application of bixafen & fluopyram & prothioconazole EC 260 in southern France and Italy
Report No:	E19RP109
Document No:	M-761576-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509, published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and Methods

Four supervised residue trials on barley were conducted in southern Europe (in southern France, and Italy) during the 2019 season.

One-spray applications was conducted with BIX+FLU+PTZ EC 260, an EC (emulsifiable concentrate) formulation containing 130 g/L prothioconazole, 65 g/L of fluopyram and 65 g/L of bixafen. For the purpose of this renewal, only results for fluopyram and its metabolites fluopyram-benzamide, fluopyram-pyridyl-carboxylic-acid, fluopyram-pyridyl-acetic-acid, fluopyram-7-hydroxy and fluopyram-methyl sulfoxide will be discussed.

The applications were conducted at the BBCH of 59-61 and at the application rates of 0.6 L/ha corresponding to the single application 0.039 kg a.s./ha. Water application rates were at 300-350 L/ha.

Samples of barley green material were collected at BBCH 61 and barley grain and straw at harvest BBCH 89.

Each field sample was placed in doubled labelled bags and stored deep-frozen within 24 hours after sampling and until dispatch. All field samples were shipped deep-frozen (at -18 °C or lower temperatures) under monitored conditions during shipment and arrived in good condition except for the shipment of samples from the trial E19RP109-03 and -04 which were shipped in two shipments at higher average shipment temperatures. Nevertheless, also these samples arrived in good and frozen condition. A short term storage stability study was conducted to show that this deviation has no impact on the stability of the residues (see CA 6.1.1, KCA 6.1/10, M-480441-06-1).

The field samples were stored in a freezer at ≤-18 °C until preparation of the examination samples. For the preparation of examination samples, the deep-frozen field samples were shredded and homogenized with dry ice in a cutter. Representative parts of the shredded samples were transferred into polystyrene boxes and stored at ≤-18°C. For each field sample, one or several examination samples were prepared for analysis and one examination sample was prepared as a reserve sample.

Residues of fluopyram and its metabolites (FLU-benzamide, FLU-PCA, FLU-PAA, FLU-7-OH, FLU-methyl-sulfoxide), were determined by LC-MS/MS according to method 00984 ([redacted] 05/02/2007, M-283301-01-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues were extracted from 10 g of sample material by two successive extractions using a high-speed blender with a mixture of acetonitrile:water (80:20; v/v). After centrifugation the extract volume was adjusted. Then, the extracts were diluted ten times by adding the internal standards.

- One dilution was performed under acidic conditions: this extract allowed the determination of FLU-PCA and FLU-methyl-sulfoxide.
- In parallel, another dilution was performed under basic conditions: this extract allowed the determination of fluopyram, FLU-benzamide and FLU-7-OH.

An aliquot of the extracts was injected into LC-MS/MS operated in Electrospray Ionization mode:

- One injection in positive electrospray ionization for the determination of fluopyram, FLU-benzamide and FLU-7-OH.
- Another injection in negative electrospray ionization for the determination of FLU-PCA and FLU-methyl-sulfoxide under different conditions.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels. For each calibration curve the correlation coefficient R was above 0.99.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.05 mg/kg for wheat straw and 0.01 mg/kg for all other matrices.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Conditions used in this study: The method was used as described in the original method with some minor modifications which have no impact on the validity of the study:

Residues were extracted from 5 g of sample material of grain and green material and from 2.5 g of straw. Extraction was done with only 30 mL (straw 40 mL) acetonitrile/water (80/20 v/v). The blender is washed with 5 mL acetonitrile/water (80/20 v/v). The internal standard concentrations were changed from 0.01 mg/L to 10 mg/L and the dilution step after filtration was omitted. The adjustment to pH= 8 was done with ammonium chloride/ammonia buffer followed by the dilution with acetonitrile/water (4/1, v/v) to get Extract A. The determination was done from an aliquot of Extract A in the positive ion mode.

The time between the beginning of the sample preparation and the sample analysis did not exceed 24 hours for grain. For green material and straw longer delays were observed. The stability of analytes in extracts of green material and straw of barley were investigated in this study. Extracts were found to be stable for at least 11 days when stored at < 6°C.

Findings

In order to check the performance of the method, recovery determinations were included in each set of analyses. Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study. The apparent residues in the control sample used for the determination of recoveries were below 30 % of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these determinations.
The mean recoveries per fortification level were within the acceptable range of 70 % – 110 % and with the RSDs < 20 %. The overall mean concurrent recoveries were also in the acceptable range of 70 % and 110 %, with the RSD < 20 %.
The storage period of deep-frozen samples intended for the analysis of fluopyram and its metabolites ranged between 319 and 409 days.
The detailed results obtained for barley samples in northern Europe are summarized in the Table 6.3.5- 21

Conclusion

Four supervised residue trials on barley were conducted in northern Europe with the application of BIX+FLU+PTZ EC 260 at the required rates and according to GLP. Samples of barley were analyzed for the residues of fluopyram and its metabolite fluopyram-benzamide. The results of the trials presented above show the following residues in grain at harvest.

- Fluopyram residues range between <0.01 and 0.012 mg/kg
- Fluopyram-benzamide residues were <0.01 mg/kg
- The total residue of fluopyram (sum of FLU + FLU-benzamide, expressed as fluopyram) range between <0.02 and 0.022 mg/kg.
- Fluopyram-pyridyl-carboxylic-acid residues were <0.01 mg/kg
- Fluopyram-pyridyl-acetic-acid residues were <0.01 mg/kg
- Fluopyram-7-hydroxy residues were <0.01 mg/kg
- Fluopyram-methylsulfoxide residues were <0.01 mg/kg

Residues in straw at harvest :

- Fluopyram residues range between <0.05 and 0.185 mg/kg.
- Fluopyram-benzamide residues were <0.05 mg/kg
- The total residue of fluopyram (sum of FLU + FLU-benzamide, expressed as fluopyram) range between <0.1 and 0.235 mg/kg.
- Fluopyram-pyridyl-carboxylic-acid residues were <0.05 mg/kg
- Fluopyram-pyridyl-acetic-acid residues were <0.05 mg/kg
- Fluopyram-7-hydroxy residues between <0.05 and 0.078 mg/kg.
- Fluopyram-methylsulfoxide residues were <0.05 mg/kg

Assessment and conclusion by applicant:

The study is acceptable.

Table 6.3.5- 23: Concurrent recovery data for Fluopyram (study E19RP074)

Portion analysed	Fortification level (mg/kg)	Recovery (%)				
		Individual recoveries	Min	Max	Mean	RSD
Fluopyram (AE C656948)						
Barley / grain	0.01	90; 93; 96; 99; 106	90	106	97	6.3
	0.4	94; 98; 99; 100; 102	94	102	99	3.0
		Overall recovery (n = 10)			**98**	**4.8**
Barley / green material	0.01	88; 94; 95; 100; 102	88	102	96	5.7
	0.4	93; 94; 95; 97; 97	93	97	95	1.9
	2.0	90; 93; 95; 98; 101	90	101	95	4.5
		Overall recovery (n = 15)			**95**	**4.0**
Barley / straw	0.05	78; 79; 79; 80; 81	78	81	79	1.4
	0.4	73; 76; 79; 83; 86	73	86	79	6.6
		Overall recovery (n = 10)			**79**	**4.5**
Fluopyram-benzamide						
Barley / grain	0.01	96; 98; 99; 102; 104	96	104	100	3.2
	0.4	89; 91; 92; 93; 93	89	93	92	1.8
		Overall recovery (n = 10)			**96**	**5.2**
Barley / green material	0.01	92; 92; 92; 94; 101	92	101	94	4.1
	0.4	88; 89; 90; 90; 94	88	94	90	2.5
		Overall recovery (n = 10)			**92**	**4.0**
Barley / straw	0.05	70; 72; 74; 75; 78	70	78	74	4.1
	0.4	72; 72; 74; 81; 83	72	83	76	6.8
		Overall recovery (n = 10)			**75**	**5.7**
Fluopyram-pyridyl-carboxylic-acid						
Barley / grain	0.01	79; 82; 89; 90; 115	79	117	91	16.5
	0.4	88; 92; 94; 95; 103	88	103	94	5.8
		Overall recovery (n = 10)			**93**	**11.6**
Barley / green material	0.01	89; 100; 100; 102; 102	89	102	99	5.5
	0.4	92; 93; 95; 96; 97	92	97	95	2.2
		Overall recovery (n = 10)			**97**	**4.6**
Barley / straw	0.05	86; 87; 91; 92; 96	86	96	90	4.5
	0.4	70; 75; 79; 81; 91	70	91	79	9.9
		Overall recovery (n = 10)			**85**	**9.8**
Fluopyram-pyridyl-acetic-acid						
Barley / grain	0.01	93; 103; 104; 105; 112	93	112	103	6.6
	0.4	95; 98; 98; 100; 101	95	101	98	2.3
		Overall recovery (n = 10)			**101**	**5.4**
Barley / green material	0.01	99; 101; 101; 102; 103	99	103	101	1.5
	0.4	94; 96; 98; 98; 103	94	103	98	3.4

Portion analysed	Fortification level (mg/kg)	Recovery (%)				
		Individual recoveries	Min	Max	Mean	RSD
		Overall recovery (n = 10)			**100**	**3.0**
Barley / straw	0.05	82; 82; 82; 85; 91	82	91	84	4.6
	0.4	78; 79; 85; 87; 93	78	93	84	7.3
		Overall recovery (n = 10)			**84**	**5.8**
Fluopyram-7-hydroxy						
Barley / grain	0.01	90; 90; 90; 94; 97	90	97	92	3.5
	0.4	89; 90; 90; 90; 95	89	95	91	2.6
		Overall recovery (n = 10)			**92**	**3.0**
Barley / green material	0.01	84; 88; 91; 92; 100	84	100	91	6.5
	0.4	88; 90; 91; 91; 93	88	93	91	2.0
		Overall recovery (n = 10)			**91**	**4.5**
Barley / straw	0.05	70; 71; 72; 76; 82	70	82	74	6.6
	0.4	69; 72; 73; 79; 88	69	88	76	9.9
		Overall recovery (n = 10)			**75**	**8.1**
Fluopyram-methyl-sulfoxide						
Barley / grain	0.01	84; 91; 93; 100; 101	84	101	94	7.4
	0.4	92; 92; 93; 94; 96	92	96	93	1.8
		Overall recovery (n = 10)			**94**	**5.1**
Barley / green material	0.01	92; 96; 104; 104; 106	92	106	100	6.0
	0.4	90; 93; 93; 97; 98	90	98	94	3.5
		Overall recovery (n = 10)			**97**	**5.8**
Barley / straw	0.05	75; 77; 80; 80; 88	75	88	80	6.2
	0.4	75; 76; 82; 85; 85	75	85	81	6.0
		Overall recovery (n = 10)			**80**	**5.8**

RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with fluopyram, determined as fluopyram and calculated as fluopyram
Fortified with fluopyram-benzamide, determined as fluopyram-benzamide and calculated as fluopyram
Fortified with fluopyram-pyridyl-carboxilic-acid, determined as fluopyram- pyridyl-carboxilic-acid and calculated as fluopyram
Fortified with fluopyram- pyridyl-acetic-acid, determined as fluopyram- pyridyl-acetic-acid and calculated as fluopyram
Fortified with fluopyram-7-hydroxy, determined as fluopyram-7-hydroxy and calculated as fluopyram
Fortified with fluopyram-methylsulfoxide, determined as fluopyram-methylsulfoxide and calculated as fluopyram

Table 6.3.5- 24: Summary of the E19RP109 (M-761576-01-1) study on barley

Total residue calculated : sum of fluopyram+FLU-benzamide

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)							PHI (days)
			g a.s./ha	Water (L/ha)	g a.s./hL					AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-PAA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residue calc.	
	(a)	(b)				(c)	(d)		(d)								(e)
E19RP109-01 France, south 31330 ST Caprais Europe, South F, 2019	Barley Irina	1) 20.02.2019 2) 25.05.2019 - 05.06.2019 3) 10.07.2019 - 25.07.2019	37.7	290	13	27.05.2019/ 0	61	green material	64	0.784	<0.01	<0.01	<0.01	<0.01	<0.01	0.794	0
								grain	89	0.012	<0.01	<0.01	<0.01	<0.01	<0.01	0.022	46
								straw	89	0.185	<0.05	<0.05	<0.05	0.078	<0.05	0.235	46
E19RP109-02 France, south 13103 St Etienne du Gres Europe, South F, 2019	Barley Rafaela	1) 24.10.2018 2) 15.04.2019 - 25.04.2019 3) 17.06.2019 - 30.06.2019	39	300	13	15.04.2019/ 0	61	green material	61	0.573	<0.01	<0.01	<0.01	<0.01	<0.01	0.583	0
								grain	89	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	64
								straw	89	0.0913	<0.05	<0.05	<0.05	0.068	<0.05	0.141	64

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)							PHI (days)
			g a.s./ha	Water (L/ha)	g a.s./hL					AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-PAA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residue calc.	
	(a)	(b)				(c)	(d)		(d)								(e)
E19RP109-03 Italy 44123 Boara Ferrara Europe, South F, 2019	Barley Sidney	1) 25.02.2019 2) 06.05.2019 - 16.05.2019 3) 17.06.2019 - 15.07.2019	39.2	353	11.1	14.05.2019/0	59	green material	61	0.965	<0.01	<0.01	<0.01	<0.01	<0.01	0.975	0
								grain	89	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<u><0.02</u>	50
								straw	89	<0.05	<0.05	<0.05	<0.05	0.065	<0.05	<0.1	50
E19RP109-04 Italy 76014 Spinazzola Europe, South F, 2019	Barley Pandora	1) 03.11.2018 2) 01.05.2019 - 15.05.2019 3) 01.06.2019 - 30.06.2019	40	308	13	03.05.2019/0	61	green material	61	1.14	<0.01	<0.01	<0.01	<0.01	0.01	1.15	0
								grain	89	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<u><0.02</u>	57
								straw	89	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.1	57

(a) According to CODEX classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions, Reference to analytical method information which metabolites are included
(g) Study reference
* prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Based on the residue definition for risk assessment, the sum of fluopyram and fluopyram-benzamide expressed as fluopyram, the total residues for barley are summarized in the Table 6.3.5- 25

Table 6.3.5- 25: Summary of fluopyram total residue data for barley trials supporting GAP 2

Crop	Commodity	Region/Indoor (a)	Trial results relevant to the critical GAP (mg/kg)	STMR (b)	HR (c)
Barley	Grain	NEU	2x<0.02, 0.02, 0.12	0.02	0.12
		SEU	3x<0.02, 0.02	0.02	0.02
		NEU/SEU	5x<0.02, 2x0.02, 0.12	0.02	0.12
	Straw	NEU	<0.1, 0.11, 0.27, 0.86	0.19	0.86
		SEU	2x<0.1, 0.14, 0.24	0.12	0.24
		NEU/SEU	3x<0.1, 0.11, 0.14, 0.24, 0.27, 0.86	0.125	0.86

(a) **NEU** or **SEU** for northern or southern outdoor trials in EU member states,
(b) **STMR**: Supervised Trials Median Residue
(c) **HR**: Highest residue

According to Dixon's test the highest value of fluopyram in grain can be considered as an outlier.

CA 6.3.7 Lettuce

Information on the intended use pattern (GAP) is summarised in Table 6.3.6- 1.

Table 6.3.6- 1: Use patterns (critical GAP) for the spray application of Fluopyram in/on lettuce in European greenhouses

Formulation	F/ GH	No. of appl.	Growth stage at application (BBCH Code)	Application rate per treatment (g a.s./ha)	Water volume (L/ha)	Interval (days)	PHI (days)
FLU+TFS SC500	GH	2	12-49	200	500-1000	7	7

GH : greenhouse

Residue trials to support GAP were conducted in greenhouse in Europe at a similar GAP.

Table 6.3.6- 2: Overview of European residue trials conducted in greenhouse with lettuce per vegetation period

Crop	Region	No. of independent trials				Report No.	Formulation	Document number	Reference
		Vegetation period			Σ				
		2007	2014	2018					
Lettuce	GH	4	6	3	13	RA-2620/07 14-2028 18-2048	FLU+TFS SC500	M-308622-01-1 M-534623-01-1 M-675904-01-1	KCA 6.3.6/01 KCA 6.3.6/02 KCA 6.3.6/03

GH : greenhouse

Data Point:	KCA 6.3.6/01
Report Author:	[redacted]
Report Year:	2008
Report Title:	Determination of the residues of AE C656948 and trifloxystrobin in/on head lettuce and lettuce after spraying of AE C656948 & CGA279202 (500 SC) in the greenhouse in Germany, Netherlands and Southern France
Report No:	RA-2620/07
Document No:	M-308622-01-1
Guideline(s) followed in study:	91/414/EEC of July 15, 1991, 7029/VI/95 rev. 5 (1997-07-22)
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and Methods

Four supervised residue trials on lettuce were conducted in greenhouse (in southern France, the Netherlands and Germany) during the 2007 season.

Two-spray applications were conducted with FLU+TFS SC500, a SC (soluble concentrate) formulation containing 250 g/L of fluopyram and 250 g/L of trifloxystrobin. For the purpose of this renewal, only results for fluopyram and its metabolite fluopyram-benzamide will be discussed.

The applications were conducted at the BBCH of 41 to 49 and at the application rates of 0.8 L/ha corresponding to the single application 0.20 kg a.s./ha. Water application rates were at 300 - 1000 L/ha.

Samples of lettuce (head) were collected at BBCH 45 to 49 (51).

Each field sample was placed in doubled labelled bags and stored deep-frozen within 24 hours after sampling and until dispatch. All field samples were shipped deep-frozen (at -18 °C or lower temperatures) under monitored conditions during shipment and arrived in good condition.

The field samples were stored in a freezer at ≤ -18 °C until preparation of the examination samples. For the preparation of examination samples, the deep-frozen field samples were shredded and homogenized with dry ice in a cutter. Representative parts of the shredded samples were transferred into polystyrene boxes and stored at ≤-18°C. For each field sample, one or several examination samples were prepared for analysis and one examination sample was prepared as a reserve sample.

Residues of fluopyram and its metabolites were determined by LC-MS/MS according to method 00984/M001 ([redacted], 2007, M-295145-03-1, see MCA section 4.1.21. Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues were extracted from 10 g of sample material by two successive extractions using a high speed blender with a mixture of acetonitrile/water (80:20; v:v).

Subsequently, the raw extracts were diluted 10-fold by adding internal standard solutions:

- One dilution performed under acidic conditions (for determination of fluopyram-PCA)
- Another dilution performed under basic conditions (for determination of fluopyram, FLU-benzamide and FLU-PAA).

Residues were quantified by reversed-phase chromatography coupled with tandem mass spectrometry (MS/MS) with electrospray ionisation. One injection in positive electrospray ionisation allowed the determination of fluopyram, FLU-benzamide and FLU-PAA. Another injection in negative electrospray ionisation allowed the determination of FLU-PCA under different conditions.

For analysis fast LC-conditions were used (0.4 to 0.8 mL on a 2 mm i.d., column with 2.5 µm material). Differing to the original method the following transitions were used:

compound	mode	Q1-mass [m/z]	Q3-mass [m/z]
FLU-benzamide MRM 1	ESI-pos	190	170
FLU-benzamide ISTD	ESI-pos	196	136
FLU-PAA MRM 1	ESI-pos	240	212
FLU-PAA ISTD	ESI-pos	244	197
FLU-PCA MRM 1	ESI-neg	224	198

The quantitation was carried out by internal standardization using internal stable labelled standards. The Limit of Quantitation (LOQ, calculated and expressed as fluopyram parent equivalents for fluopyram and its metabolites), defined as the lowest validated fortification level, was 0.01 mg/kg in all matrices for all analytes. All residues are calculated as parent fluopyram.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

Findings

In order to check the performance of the method, recovery determinations were included in each set of analyses. Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study. The apparent residues in the control sample were below the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these determinations.
The mean recoveries per fortification level were within the acceptable range of 70 % – 110 % and with the RSDs < 20 %. The overall mean concurrent recoveries were also in the acceptable range of 70 % and 110 %, with the RSD < 20 %.
The storage period of deep-frozen samples intended for the analysis of fluopyram and its metabolites ranged between 419 and 520 days.
The detailed results obtained for lettuce samples in greenhouse are summarized in the Table 6.3.6- 3

Conclusion

Four supervised residue trials on lettuce were conducted in greenhouse with the application of FLU+TFS SC500 at the required rates and according to GEP. Samples of lettuce were analyzed for the residues of fluopyram and its metabolite fluopyram-benzamide, fluopyram-pyridyl-acetic-acid and fluopyram-pyridyl-carboxylic-acid. The results of the trials presented above show the following residues in lettuce (head) at harvest following a pre harvest interval (PHI) of 7 days.

- Fluopyram residues range between 0.23 and 2.1 mg/kg
- Fluopyram-benzamide range between <0.01 and 0.02 mg/kg
- Fluopyram-PAA and Fluopyram-PCA were <0.01 mg/kg
- The total residue of fluopyram (sum of FLU + FLU-benzamide, expressed as fluopyram) range between 0.24 and 2.1 mg/kg.

Assessment and conclusion by applicant:

The study is acceptable.

Table 6.3.6- 3: Concurrent recovery data for Fluopyram (study RA-2620/07)

Portion analysed	Fortification level (mg/kg)	Recovery (%)				
		Individual recoveries	Min	Max	Mean	RSD
Fluopyram (AE C656948)						
Lettuce / head	0.01	80; 69; 91; 83	69	91	81	11.3
	1.0	71; 71; 76; 72; 72	71	76	72	2.9
	5.0	77; 78	77	78	78	--
		Overall recovery (n= 12)			76	8.5
Fluopyram-benzamide(AEF148815)						

Portion analysed	Fortification level (mg/kg)	Recovery (%)				
		Individual recoveries	Min	Max	Mean	RSD
Lettuce / head	0.01	77; 75; 75; 68	68	77	74	5.4
	1.0	70; 74; 77; 74; 73	70	77	74	3.4
		Overall recovery (n= 12)			74	4.1
Fluopyram-pyridyl-acetic-acid (BCS-AA10139)						
Lettuce / head	0.01	66; 84; 79; 85	66	85	79	11.1
	1.0	69; 75; 75; 75; 78	69	78	74	4.4
		Overall recovery (n= 12)			76	8.2
Fluopyram-pyridyl-carboxilic-acid (AE C657188)						
Lettuce / head	0.01	79; 63; 77	63	79	73	11.9
	1.0	73; 77 77; 73	73	77	75	3.1
		Overall recovery (n = 12)			74	7.3

RSD = Relative standard deviation,
Final determination as: fluopyram Residues calculated as: fluopyram
Final determination as: FLU-benzamide Residues calculated as: fluopyram
Final determination as: FLU-PAA Residues calculated as: fluopyram
Final determination as: FLU-PCA Residues calculated as: fluopyram
These recoveries were performed during the conduct of the study RA-2620/07

Table 6.3.6- 4: Summary of the RA-2620/07 (M-308622-01-1) study on lettuce

Total residue calculated : sum of fluopyram+FLU-benzamide

Trial No. / Location / EU zone / Year	Commodity / Variety (a)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting (b)	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment (d)	Portion analyzed	Growth stage at sampling (d)	Residues (mg/kg)					PHI (days) (e)
			g a.s./ha	Water (L/ha)	g a.s./hL					Fluopyram as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-PAA as AE C656948	Total residues calc	
R 2007 0266/3(0266-07) Germany D-42799 Leichlingen (Nordrhein-Westfalen) Europe, North G, 2007	Lettuce, head Alexandria	1) 10.02.2007 3) 03.04.2007 - 15.04.2007	200.0 200.0	600 600	33.25 33.25	20.03.2007/0 27.03.2007/7	45	head	45 45 47 49 49 49	1.5 5.8 4.7 2.1 0.73 0.51	<0.01 0.01 0.01 0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	1.51 5.81 4.71 2.11 0.74 0.52	0* 0 3 7 14 21
R 2007 0642/1(0642-07) Netherlands NL-1693 NR Wervershoof Europe, North G, 2007	Lettuce Lolo Rosso	1) 17.04.2007 3) 14.05.2007 - 31.05.2007	200.0 200.0	1000 1000	20.00 20.00	01.05.2007/0 08.05.2007/7	47	head	47 47 48 49 49 49	0.81 5.5 1.2 0.92 0.44 0.24	0.02 0.02 0.02 0.02 0.01 0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	0.83 5.52 1.22 0.94 0.45 0.25	0* 0 3 7 14 21
R 2007 0644/8(0644-07) France, south F-86380 Ouzilly (Poitou-Charentes) Europe, South G, 2007	Lettuce, head Santoro	1) 16.04.2007 3) 21.05.2007 - 05.06.2007	200.0 200.0	600 600	33.25 33.25	09.05.2007/0 16.05.2007/7	45	head	45 45 47 49 49 51	0.39 3.4 2.3 1.4 0.37 0.07	<0.01 <0.01 0.01 0.02 0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	0.40 3.41 2.31 1.42 0.38 0.08	0* 0 3 7 14 21
R 2007 0645/6(0645-07) Germany D-88074 Meckenbeuren (Baden-Württemberg) Europe, North G, 2007	Lettuce Alexandria; Butterhead	1) 30.01.2007 1) 09.03.2007 3) 12.03.2007 - 18.05.2007	200.0 200.0	300 300	66.75 66.75	18.04.2007/0 25.04.2007/7	45	head	45 45 45 47 49 49	0.69 3.5 0.63 0.23 0.19 0.07	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	0.70 3.51 0.64 0.24 0.20 0.08	0* 0 3 7 13 21

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method information which metabolites are included
(g) Study reference
prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Data Point:	KCA 6.3.6/02
Report Author:	[redacted]
Report Year:	2015
Report Title:	Determination of the residues of fluopyram and trifloxystrobin in/on lettuce after spray application of fluopyram & trifloxystrobin SC 500 in the greenhouse in Germany, the Netherlands, Belgium and France
Report No:	14-2028
Document No:	M-534623-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP Guideline No. 860.1500 on Crop Field Trial
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and Methods

Six supervised residue trials on lettuce were conducted in greenhouse (France, the Netherlands, Belgium and Germany) during the 2014 season.

Two-spray applications were conducted with FLU+TFS SC500, a SC (soluble concentrate) formulation containing 250 g/L of fluopyram and 250 g/L of trifloxystrobin. For the purpose of this renewal, only results for fluopyram and its metabolite fluopyram-benzamide will be discussed.

The applications were conducted at the BBCH of 19-48 and at the application rates of 0.8 L/ha corresponding to the single application 0.20 kg a.s./ha. Water application rates were at 300 - 1000 L/ha.

Samples were collected in a manner designed to obtain representative samples. Samples of lettuce (head) were collected at BBCH 45 to 49.

Each field sample was placed in doubled labelled bags and stored deep-frozen within 24 hours after sampling and until dispatch. All field samples were shipped deep-frozen (at -18 °C or lower temperatures) under monitored conditions during shipment and arrived in good condition.

The field samples were stored in a freezer at ≤-18 °C until preparation of the examination samples. For the preparation of examination samples the deep-frozen field samples were shredded and homogenized with dry ice in a cutter. Representative parts of the shredded samples were transferred into polystyrene boxes and stored at ≤-18 °C. For each field sample, one or several examination samples were prepared for analysis and one examination sample was prepared as a reserve sample.

Samples were analysed according to the analytical method 00984/M003 ([redacted], 2015, M-467323-03-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues were extracted from 5 g of sample material by extraction using a shaker (15 min) with a mixture of acetonitrile:water (4:1, *v:v*). After filtration the extracts of the solutions were made up to volume. The solutions were centrifuged, and the extract volume was adjusted. The extracts were diluted by adding the internal standard. An aliquot of the extracts was injected into a HPLC-(MS/MS).

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.01 mg/kg.

All final extracts were analysed within 20 days after extraction. Fluopyram and fluopyram-benzamide were found to be stable in extracts of matrices of plant origin for at least four weeks at 4 ± 3°C which was tested within the validation of method 00984/M003.

Findings

In order to check the performance of the method, recovery determinations were included in each set of analyses. Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study. The apparent residues in the control sample used for recovery experiments were below 30% of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these determinations. The mean recoveries per fortification level were within the acceptable range of 70 % – 110 % except for fluopyram (114% at LOQ level) and with the RSDs < 20 %. The overall mean concurrent recoveries were also in the acceptable range of 70% and 110 % with the RSD < 20 %.
The storage period of deep-frozen samples intended for the analysis of fluopyram and its metabolites ranged between 127 and 369 days.
The detailed results obtained for lettuce samples in greenhouse are summarized in the Table 6.3.6- 6

Conclusion

Four supervised residue trials on lettuce were conducted in greenhouse with the application of FLU+TFS SC500 at the required rates and according to GLP. Samples of lettuce were analyzed for the residues of fluopyram and its metabolite fluopyram-benzamide. The results of the trials presented above show the following residues in lettuce (head) at harvest following a pre harvest interval (PHI) of 7 days.

- Fluopyram residues range between 0.82 and 10 mg/kg
- Fluopyram-benzamide range between <0.01 and 0.03 mg/kg
- The total residue of fluopyram (sum of FLU + FLU-benzamide, expressed as fluopyram) range between 0.84 and 10 mg/kg.

Assessment and conclusion by applicant:

The study is acceptable.

Table 6.3.6- 5: Concurrent recovery data for Fluopyram (study 14-2028)

Portion analysed	Fortification level (mg/kg)	Recovery (%)				
		Individual recoveries	Min	Max	Mean	RSD
Fluopyram (AE C656948)						
Lettuce / head	0.01	109; 119	109	119	114	
	0.10	97; 97	97	97	97	
	1.0	98	-	-		
	10	73	-	-		
		Overall recovery (n= 12)			**99**	**15.6**
Fluopyram-benzamide(AEF148815)						
Lettuce / head	0.01	64; 78	64	78	71	-
	0.10	95; 98	95	98	97	-
	1.0	96	-	-	-	
	10	82	-	-		-
		Overall recovery (n= 12)			**86**	**15.6**

RSD = Relative standard deviation,
Fortified with fluopyram, determined as fluopyram and calculated as fluopyram
Fortified with fluopyram-benzamide, determined as fluopyram-benzamide and calculated as fluopyram
These recoveries were performed during the conduct of the study 14-2028

Table 6.3.6- 6: Summary of the 14-2028 (M-534623-01-1) study on lettuce

Total residue calculated : sum of fluopyram+FLU-benzamide

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)			PHI (days)
			g a.s./ha	Water (L/ha)	g a.s./hL					fluopyram as AE C656948	FLU-benzamide as AE C656948	Total residues calc.	
	(a)	(b)				(c)	(d)	(d)					(e)
14-2028-01 Germany 42799Leichlingen Europe, North G 2014	Lettuce Lugano; Lollo bionda (loose leaf variety)	1) 05.02.2014 3) 01.04.2014 - 15.04.2014	200 200	300 300	66.7 66.7	21.03.2014/0 28.03.2014/7	47	head	47 47 48 49 49	1.6 5.8 2.5 1.6 0.84	0.01 0.014 <0.01 0.011 <0.01	1.61 5.81 2.51 <u>1.61</u> 0.85	0* 0 3 <u>7</u> 14
14-2028-02 Netherlands 2988DA Ridderkerk Europe, North G 2014	Lettuce Satine; Lollo Rosso, (loose leaf variety)	1) 15.05.2014 3) 18.06.2014 - 23.06.2014	200 200	1000 1000	20 20	06.06.2014/0 13.06.2014/7	47	head	47 47 48 49 49	2.0 12 4.8 3.6 1.1	0.015 0.039 0.027 0.028 0.011	2.015 12.039 4.827 <u>3.628</u> 1.111	0* 0 3 <u>7</u> 14
14-2028-03 Belgium 6221Saint-Amand Europe, North G 2014	Lettuce Sansula; Oakleaf variety; (loose leaf variety)	1) 08.04.2014 3) 10.05.2014 - 05.06.2014	200 200	900 900	22 22	25.04.2014/0 02.05.2014/7	45	head	45 45 46 47 49	0.42 5.3 1.5 0.83 0.47	<0.01 0.014 <0.01 <0.01 <0.01	0.43 5.314 1.51 <u>0.84</u> 0.48	0* 0 3 <u>7</u> 14

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)			PHI (days)
			g a.s./ha	Water (L/ha)	g a.s./hL	(c)	(d)	(d)	(d)	fluopyram as AE C656948	FLU-benzamide as AE C656948	Total residues calc.	(e)
	(a)	(b)											
14-2028-04 France, south 82100Castelsarrasin Europe, South G 2014	Lettuce Parinice; oak leaf variety (loose leaf variety)	1) 12.05.2014 3) 09.06.2014 - 16.06.2014	200 200	800 800	25 25	26.05.2014/0 02.06.2014/3	42	head	42 42 42 49 49	1.2 7.6 5.5 0.94 0.17	<0.01 0.030 0.016 0.010 <0.01	1.21 7.63 5.516 <u>0.95</u> 0.18	0* 0 3 <u>7</u> 14
14-2028-05 Netherlands 2988DA Ridderkerk Europe, North G 2014	Lettuce Korentina; (loose leaf variety)	1) 17.09.2014 3) 15.11.2014 - 04.12.2014	200 200	600 600	33 33	04.11.2014/0 11.11.2014/7	46	head	46 46 47 49 49	6.0 13 12 10 7.1	<0.01 0.025 0.014 0.013 <0.01	6.01 13.025 12.014 <u>10.013</u> 7.11	0* 0 3 <u>7</u> 14
14-2028-06 France, north 49160Longué Europe, North G 2014	Lettuce Kimpala; oakleaf variety; (loose leaf variety)	1) 11.09.2014 3) 20.10.2014 - 10.11.2014	200 200	600 600	33 33	13.10.2014/0 20.10.2014/7	48	head	48 48 49 49 49	1.1 5.7 5.4 3.9 0.85	<0.01 0.017 0.011 0.010 <0.01	1.11 5.717 5.411 <u>3.91</u> 0.86	0* 0 3 <u>7</u> 14

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) Study reference
* prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Data Point:	KCA 6.3.6/03
Report Author:	[redacted]
Report Year:	2019
Report Title:	Determination of the residues of trifloxystrobin and AE C656948 in/on lettuce after spray application of AE C656948 & CGA279202 SC 500 in the greenhouse in Germany, the Netherlands and southern France
Report No:	18-2048
Document No:	M-675904-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP Guideline No. 860.1500 on Crop Field Trial
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and Methods

Three supervised residue trials on lettuce were conducted in greenhouse (France, the Netherlands and Germany) during the 2014 season.

Two-spray applications were conducted with FLUXTFS SC500, a SC (soluble concentrate) formulation containing 250 g/L of fluopyram and 250 g/L of trifloxystrobin. For the purpose of this renewal, only results for fluopyram and its metabolite fluopyram-benzamide will be discussed.

The applications were conducted at the BBCH of 44-48 and at the application rates of 0.8 L/ha corresponding to the single application 0.20 kg a.s./ha. Water application rates were at 480 - 825 L/ha.

Samples were collected in a manner designed to obtain representative samples. Samples of lettuce (head) were collected at BBCH 46 to 49.

Each field sample was placed in doubled labelled bags and stored deep-frozen within 24 hours after sampling and until dispatch. All field samples were shipped deep-frozen (at -18 °C or lower temperatures) under monitored conditions during shipment and arrived in good condition.

The field samples were stored in a freezer at ≤-18 °C until preparation of the examination samples. For the preparation of examination samples, the deep-frozen field samples were shredded and homogenized with dry ice in a cutter. Representative parts of the shredded samples were transferred into polystyrene boxes and stored at ≤-18°C. For each field sample, one or several examination samples were prepared for analysis and one examination sample was prepared as a reserve sample.

Samples were analysed according to the analytical method 00984/M003 ([redacted], 2015, M-467323-03-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented

within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

However, slight adaptions were made to the extraction procedure described within the analytical method modification 00984/M003 which are as follows: residues were extracted from 5 g of sample material by extraction using a shaker (15 min) with a mixture of acetonitrile:water (4:1, v:v).: The filtering procedure under low vacuum was replaced by a centrifugation step. Then, 0.5 mL of internal standard solution (1 mg/L) were added to the extract followed by 5 min centrifugation at 4750 rpm at 10 °C and further proceeded to the HPLC-MS/MS analysis

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.01 mg/kg.

All final extracts were analysed within one day after extraction. Fluopyram and fluopyram-benzamide were found to be stable in extracts of matrices of plant origin for at least four weeks at 4 ± 3 °C which was tested within the validation of method 00984/M003.

Findings

In order to check the performance of the method, recovery determinations were included in each set of analyses. Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study. The apparent residues in the control sample used for recovery experiments were below 30% of the LOQ. Recoveries were not corrected for apparent residues in the control samples used for these determinations. The mean recoveries per fortification level were within the acceptable range of 70 % – 110 % and with the RSDs < 20 %. The overall mean concurrent recoveries were also in the acceptable range of 70 % and 110 %, with the RSD < 20 %.
The storage period of deep-frozen samples intended for the analyses of fluopyram and its metabolites ranged between 143 and 326 days.
The detailed results obtained for lettuce samples in greenhouse are summarized in the Table 6.3.6- 8

Conclusion

Four supervised residue trials on lettuce were conducted in greenhouse with the application of FLU+TFS SC500 at the required rates and according to GLP. Samples of lettuce were analyzed for the residues of fluopyram and its metabolite fluopyram-benzamide.. The results of the trials presented above show the following residues in lettuce (head) at harvest following a pre harvest interval (PHI) of 7 days.

- Fluopyram residues range between 1.3 and 5.5 mg/kg
- Fluopyram-benzamide range between <0.01 and 0.01 mg/kg
- The total residue of fluopyram (sum of FLU + FLU-benzamide, expressed as fluopyram) range between 1.31 and 5.51 mg/kg

Assessment and conclusion by applicant:

The study is acceptable.

Table 6.3.6- 7: Concurrent recovery data for Fluopyram (study 18-2048)

Portion analysed	Fortification level (mg/kg)	Recovery (%)				
		Individual recoveries	Min	Max	Mean	RSD
Fluopyram (AE C656948)						
Lettuce / head	0.01	81; 95; 103	81	103	93	12.0
	0.10	72; 91; 97	72	97	87	15.0
	1.0	92; 97; 98	92	98	96	3.4
	10	69; 90; 93	69	93	84	15.6
		Overall recovery (n= 12)			**90**	**11.7**
Fluopyram-benzamide(AEF148815)						
Lettuce / head	0.01	69; 85; 96	69	96	83	16.7
	0.10	69; 88; 94	69	94	84	15.6
	1.0	91; 95; 98	91	98	95	3.7
		Overall recovery (n= 12)			**87**	**12.7**

RSD = Relative standard deviation,
Fortified with fluopyram, determined as fluopyram and calculated as fluopyram
Fortified with fluopyram-benzamide, determined as fluopyram-benzamide and calculated as fluopyram
These recoveries were performed during the conduct of the study 18-2048

Table 6.3.6- 8 Summary of the 18-2048 (M-675904-01-1) study on lettuce

Total residue calculated : sum of fluopyram+FLU-benzamide

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)			PHI (days)
			g a.s./ha	Water (L/ha)	g a.s./hL					fluopyram as AE C656948	FLU-benzamide as AE C656948	Total residues calc.	
	(a)	(b)				(c)	(d)		(d)				(e)
18-2048-01 Germany 42799 Leichlingen Europe, North G 2018	Lettuce Macai RZ; typical of region	1) 28.03.2018 3) 04.05.2018 - 15.05.2018	193 208	579 623	33.3 33.3	23.04.2018/0 30.04.2018/7	47	head	47 47 48 48 49	2.9 9.1 6.7 4.5 1.3	<0.01 <0.01 <0.01 <0.01 <0.01	2.91 9.11 6.71 <u>5.51</u> 1.31	0* 0 3 <u>7</u> 14
18-2048-02 Netherlands 1822 AD Alkmaar Europe, North G 2018	Lettuce Lollo Rossa Satine	1) 05.09.2018 3) 21.10.2018 - 26.10.2018	200 192	500 480	40.0 40.0	09.10.2018/0 16.10.2018/7	48	head	48 48 48 49 49	2.2 6.6 7.6 5.0 4.6	<0.01 <0.01 <0.01 <0.01 <0.01	2.21 6.61 7.61 <u>5.01</u> 4.61	0* 0 3 <u>7</u> 14
18-2048-03 France, south 31200 Toulouse Europe, South G 2018	Lettuce Sumitie RZ; Batavia Blonde	1) 08.04.2018 3) 22.05.2018 - 30.05.2018	206 193	825 788	25.0 25.0	04.05.2018/0 11.05.2018/7	46	head	46 46 47 49 49	0.97 5.4 2.3 1.3 0.46	<0.01 <0.01 0.010 0.011 0.014	0.98 5.41 2.31 <u>1.31</u> 0.47	0* 0 3 <u>7</u> 14

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) Study reference
* prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Based on the residue definition for risk assessment, the sum of fluopyram and fluopyram-benzamide expressed as fluopyram, the total residues for lettuce (head) are summarized in the Table 6.3.6- 9

Table 6.3.6- 9: Summary of fluopyram total residue data for lettuce trials to be supported

Crop	Region/Indoor	Trial results relevant to the critical GAP (mg/kg)	STMR (b)	HR (c)
Lettuce / head	Greenhouse	0.24, 0.84, 0.94, 0.95, 1.31, 1.42, 1.61, 2.11, 3.63, 3.91, 5.01, 5.51, 10.01	1.61	10

(b) **STMR**: Supervised Trials Median Residue
(c) **HR**: Highest residue

In four of the submitted greenhouse residue trials on lettuce, residues of FLU-pyridyl-acetic-acid and FLU-pyridyl-carboxylic acid were shown to be below the LOQ of 0.01 mg/kg.

CA 6.4 Feeding studies

European dietary burden calculations

Fluopyram is sought for use on cereals and apple which parts of this crops might be fed to livestock (grain, straw and by-products of grain for barley and apple pomace)

The dietary burdens were therefore calculated for different groups of livestock as described in the OECD Guidance Document on Residues in Livestock (ENV/JM/MONO(2013)8 dated of 04-Sep-2013) and using the Excel spreadsheet dated of 2017 available in the EU Commission website (pesticides_mrl_guidelines_animal_model_2017.xls).

Based on the proposed plant residue definition for risk assessment (sum of fluopyram and fluopyram - benzamide expressed as fluopyram), input values were derived from:

- the residue data from representative uses as summarized in MCA 6.3.4 and 6.3.5 in the Table 6.3.4-11 and Table 6.3.5- 19.
- the residues data from succeeding crops summarized in MCA 6.6.2 in the Table 6.6.2- 3.

These input data are summarized Table 6.4- 1.

Table 6.4- 1: Input residue data for livestock dietary burden calculations

Feed commodity	Median dietary burden		Maximum dietary burden	
	(mg/kg)	Comment	(mg/kg)	Comment
Barley straw	0.15	STMR from N+SEU	1.2	HR from N+SEU
Oat straw				
Barley grain	0.025	STMR from N+SEU	0.025	STMR from N+SEU
Oat grain				
Brewer's grain	0.08	STMRxPF (0.025 x 3.3 [a])	0.08	STMRxPF (0.025 x 3.3 [a])
Apple, wet pomace	0.16	STMRxPF (0.07 x 2.3 [a])	0.16	STMRxPF (0.07 x 2.3 [a])
Carrot, root	0.05	STMR rotational	0.06	HR rotational
Potato, tuber	0.03	STMR rotational	0.03	HR rotational
Rape, seed	0.02	STMR rotational	0.02	HR rotational
Wheat, grain	0.02	STMR rotational	0.02	HR rotational

NEU: northern Europe SEU southern Europe N+SEU northern and southern Europe
HR highest residues
STMR supervised trials median residue
[a] Default processing factor (PF)

Table 6.4- 2: Anticipated dietary burden for fluopyram residues based on EU residue data and OECD guideline

Animals	Dietary burden expressed in mg/kg bw/d		Above 0.004 mg /kg bw	Dietary burden expressed in mg/kg DM		Highest contributing commodities
	Median	Maximum		Median	Maximum	
Cattle (Beef)	0.0508	0.060	Yes	2.12	2.48	Potato process waste
Cattle (Dairy)	0.0624	0.076	Yes	1.62	1.99	Potato process waste
Sheep (Ram/Ewe)	0.0717	0.094	Yes	2.15	2.8	Potato process waste
Sheep (Lamb)	0.0503	0.081	Yes	1.18	1.91	Potato process waste
Swine (Breeding)	0.026	0.026	Yes	1.12	1.14	Potato process waste
Swine (Finishing)	0.011	0.012	Yes	0.38	0.40	Potato dried pulp
Poultry (Broiler)	0.023	0.024	Yes	0.32	0.33	Potato dried pulp
Poultry (Layer)	0.018	0.023	Yes	0.27	0.34	Barley straw
Poultry (Turkey)	0.005	0.006	Yes	0.07	0.08	Carrot culls

Livestock feeding studies were conducted on dairy cow and poultry hens. Multi-region livestock diet calculations were conducted in order to conduct the studies in a manner appropriate to the entire scope of fluopyram uses, allowing data to be generated in a fashion such that, for animal welfare considerations, a low number of animals will be used, while yielding valid data to evaluate expected residue levels in all key animal tissues and products.

The test substance used in the feeding studies should be representative of the residue in the feedstuffs. In the case of fluopyram, by far the major part of the residue in plants is formed by parent compound fluopyram.

Based on the results of the metabolism studies several compounds were measured in the feeding studies:

- In milk, eggs and all tissues free residues of fluopyram (AE C656948) and its metabolites, fluopyram-benzamide and fluopyram-olefins (sum of BCS-AA10627 (E-isomer) and BCS-AA10650 (Z-isomer)) were determined.
- Additionally, the total residue of fluopyram (sum of parent fluopyram + fluopyram-benzamide + fluopyram-olefins) was calculated.

CA 6.4.1 Poultry

Data Point:	KCA 6.4.1/01
Report Author:	[redacted]
Report Year:	2008
Report Title:	Fluopyram: Feeding Study Laying Hens (Gallus gallus domesticus)
Report No:	MR-07/234
Document No:	M-297674-01-1
Guideline(s) followed in study:	US: EPA Residue Chemistry Test Guidelines OPPTS 860.1000 "Background" OPPTS 860.1480 "Meat, milk, poultry and eggs"; EU: 7001/VI/95rev. 4 "Livestock feeding studies"; EU Directive EC 91/414, Appendix A; OECD: Guidelines for the Testing of Chemicals, 505, 2007-01-08
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted, rev. 1 to Vol 3 of DAR B7, August 2012 (reference relied on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and Methods

The purpose of this study was to determine the magnitude of the residues of fluopyram (AE C656948) and its metabolites, fluopyram-benzamide and total of fluopyram-olefins, which may be expected in meat and eggs from poultry that have been fed with feedstuffs containing residues of fluopyram.

Test system, dosing

After an acclimatization phase of about 28 days, 72 laying hens (Gallus gallus domesticus) were dosed orally via commercially available ground layer hen diet ad libitum which was mixed with the test item in appropriate amounts corresponding to the feeding levels. Laying hens were fed for 28 consecutive days with fluopyram at target dose rates of either:

- 0 mg/kg feed (0X, control, 1 group A of 9 hens),
- 0.05 mg/kg feed (0.1X, 3 subgroups B, 12 hens),
- 0.50 mg/kg feed (1X; 3 subgroups C, 12 hens),
- 1.5 mg/kg feed (3X, 3 subgroups D, 12 hens),
- 5.0 mg/kg feed (10X, 3 subgroups E, 12 hens).

These levels were based on field residue data and were approx. 0X (control), 0.1X, 1X, 3X and 10X the anticipated maximum dietary burden arising from the use of fluopyram in the US and EU at the time of the study conduct.

The 1X group in this study is around 40% higher than the maximum calculated dietary burden for the present renewal (0.024 mg/kg bw/day for Poultry/Broiler see Table 6.4- 2). As a worse case the residue results from this 1X group will be uses as input data for the risk assessment.

The actual dose levels per feed item were 0 mg fluopyram/kg feed (dose level 0X), 0.05 mg a.s./kg feed (actual dose level 0.1X), 0.49 mg a.s./kg feed (actual dose level 1X), 1.6 mg a.s./kg feed (actual dose level 3X) and 4.8 mg a.s./kg feed (actual dose level 10X).

The actual dose rates per body weight (bw) were:

- 0 µg/kg bw/day (dose group A, 0X),
- 3.4 µg/kg bw/day (dose group B, 0.1X),
- 35 µg/kg bw/day (dose group C, 1X),
- 110 µg/kg bw/day (dose group D, 3X),
- 320 µg/kg bw/day (dose group E, 10X),
- 330 µg/kg bw/day (dose group F, 10X).

Additionally, three sub-groups of laying hens (5 hens/group) were fed at the 10X feeding level for 28 consecutive days followed by untreated feed for another 8 days (dose group F1), 13 days (F2) and 21 days (F3) in order to investigate the depuration of fluopyram and its metabolites in eggs and tissues.

Table 6.4.1- 1: Summary of actual fluopyram dose administration

Dose groups	Sub-groups	Number of hens	Dose levels	
			Per animal (µg/kg bw/day)*	In feed (mg/kg DM)*
0X Control	A	9	0	0
0.1X	B1, B2, B3	12	3.4	0.05
1X	C1, C2, C3	12	35	0.49
3X	D1, D2, D3	12	110	1.6
10X	E1, E2, E3	12	320	4.8
10X depuration	F1, F2, F3	15	330	4.8

DM: dry matter
*: Actual dose based on average feed consumption data collected from the study

The hens were fed ad libitum with a commercially available ground layer hen diet ad libitum (type: "V6220-000 ssniff Hühner-Z 1, Mehl e.v"., "ssniff Spezialdiäten GmbH", D-59494 Soest, Germany) which was mixed with the test item in appropriate amounts corresponding to the feeding levels.

The diet for the exposure period was prepared at the food preparation centre of Bayer HealthCare AG in Wuppertal, Germany under responsibility of Dr. A. Folkens by incorporation of technical grade fluopyram into feeding material. Content and homogeneity of the batches were also tested.

The hens were allowed ad libitum access to tap water. Dose levels used in this study were based on residues found in the magnitude of the field residue trials.

Representative samples of the treated diet were checked to verify the amount of technical fluopyram and to determine the stability of the test item during storage. A representative number of samples from each batch was analyzed before first dosing. The storage stability was verified and confirmed by reanalysis after 7 days (storage at ambient temperature) and 4 weeks (deep-freezer storage).

The actual dose rates were calculated based on the actual fluopyram concentration in feed, average body weights of hens and average feed consumption during the dosing phase.

For each individual, a weekly amount of feed was portioned and stored under deep-freezer conditions until use.

Actual feed consumption was recorded once a week, individually for each hen. In case the weekly ration of feed seemed to be not sufficient, additional feed was supplied. During the dosing phase, feed hoppers were controlled daily in the morning and filled if necessary, together with morning inspection, watering and egg collection, and once per week all refused feed was emptied out, re-weighed and replaced with fresh food.

The birds did not have access to other feedstuffs during the dosing period.

After the end of the dosing period (groups A, B, C, D and E), animals of the F (depuration phase) group were fed with untreated feedstuff until necropsy for approx. one (F1), two (F2) or three weeks (F3)..

Sampling

For the purpose of this study, a Study Day period was defined from morning inspection, feeding, watering etc… to sampling of eggs on the following morning. The changeover to the following study day took place after sampling of eggs. The eggs were sampled beginning with the control A and continuing with the lowest dose group B to the highest dose groups. Clean disposable gloves were worn for egg collection and contact with surfaces that may be contaminated with feed residues was avoided. The number of eggs from each sub-group was recorded. The eggs were placed on a labelled egg tray in a refrigerator (about 4 °C) for up to 4 days and transferred then to the Test Facility for storage.

For the dose groups A, B, C, D and E, eggs for analysis were collected on days -13, -6, -1, 0, 1, 2, 5, 7, 9, 12, 14, 16, 21, 23, 26, and 28. For the depuration group F, eggs for analysis were collected on day 21, 23, 26, 28, 30, 33, 36, 37 (only F2 and F3), 40 (only F2 and F3), 41 (only F2 and F3), and for the depuration group F3 additionally on day 44, 48 and 49. A note was made if broken eggs were found. Broken eggs were removed from the pen and they were not retained for analysis or part of any calculation. The eggs were pooled for each sub-group and deep-frozen on the same day.

The laying hens were withdrawn from their pens on day 28 (dose groups A (control), B, C, D and E), day 36 (subgroup F1), day 41 (subgroup F2), and on day 49 (subgroup F3) approximately 3 to 7 hours after the last morning inspection (= time between last dosing and sacrifice). The birds were weighed, anaesthetized by a neck beat and sacrificed by immediate exsanguination by decapitation. The following tissues were collected from each bird and weighed:

- muscle, approx. equal sized-pieces of leg and breast (approximately 100 g in total), trimmed of adherent connective tissue and fat,
- skin with subcutaneous fat and abdominal fat
- liver (entire organ).

During necropsy, the cross-contamination between tissues of different dosing groups was prevented as far as possible by using fresh instruments after treating a distinct sub-group. Tissue samples were transferred as soon as possible for deep-freezing at ≤ -18 °C and sample preparation.

Analysis

Egg and tissue samples were analysed for the parent compound fluopyram and its major metabolites fluopyram-benzamide, fluopyram-olefins (BCS-AA10627 (E-isomer) and BCS-AA10650 (Z-isomer)). The residues were analysed according to the analytical method 01061 ([redacted], 2007, M-295705-02-1, see MCA Section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA-4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Briefly, the residues were extracted from the animal tissues and eggs by a mixture of acetonitrile/water. After filtration the extracts are cleaned-up on a Mega Bond Elut-C18 cartridges. Aliquots of the extracts are further diluted with a mixture of methanol/water (containing the corresponding internal standards). The

residues are analysed by reversed phase HPLC with electrospray and MS/MS-detection. To compensate the matrix effect, the quantification was done using external calibration with matrix matched standards.

The limit of quantitation (LOQ) was 0.01 mg/kg for fluopyram and the FLU-benzamide metabolite, expressed as parent equivalents. In case of FLU-olefins, the total residue of FLU-olefins was calculated as sum of the two individual FLU-olefin isomers, also expressed as parent equivalent. For this study the LOQ of the Total residue of FLU-olefins was set to 0.02 mg/kg for all matrices under actual use conditions of the study. For the total residue of fluopyram (each expressed as parent equivalents) 0.04 mg/kg was set for all investigated animal matrices.

The limit of detection (LOD) was estimated to be 0.003 mg/kg for fluopyram, 0.001 mg/kg for benzamide, 0.004 mg/kg for the total residue of FLU-olefins, and 0.008 mg/kg for the total residue of fluopyram (each expressed as parent equivalents) for all investigated animal matrices.

In order to check the analytical method performance, concurrent recovery experiments were conducted along with the analysis of samples. The concurrent recovery levels for all matrices tested (egg, liver, muscle, skin with fat) were between 0.01 mg/kg (LOQ level) and 2.0 mg/kg (200-fold LOQ level) for fluopyram and the FLU-benzamide metabolite, expressed as parent equivalents, and between 0.02 mg/kg (LOQ level) and 4.0 mg/kg (200-fold LOQ level) for the total residue of FLU-olefins, expressed as parent equivalents. All concurrent recovery values are presented in the Table 6.4.1- 6.

II. Findings

Dose verification and storage stability

The dosing solutions for the preparation of the fortified feedstuffs of the different batches were analyzed for fluopyram in order to determine the content and homogeneity as well as the storage stability of fluopyram for the duration of use in the in-life phase of the study. The dose preparation was accurate, and the feedstuffs were shown to be stable over the course of the study.

Analysis of feedstuff

No residues of fluopyram were detected in the untreated feedstuff.

In-life observations

Feed consumption, body weights, and egg production were not adversely affected by treatment with fluopyram. The appearance and the behavior of all birds were observed once daily throughout the study. Nothing special was observed. Hens were healthy during the whole study.

Analysis of eggs and tissues

The mean values of the concurrent recovery rates per compound, sample material, and spiking level were in the range of 70 – 110 %, with relative standard deviations < 20 %. In few cases the recovery means were slightly above 110 %, or the RSD was slightly above 20 %. Nevertheless, the data demonstrate acceptable method performance during sample analysis. Details of recovery data are shown in Table 6.4.1- 6.

The tissue and egg samples in this study were analysed within 30 days after collection; therefore, freezer storage stability studies on poultry tissue and egg matrices were not required.

The control samples of eggs and tissues were analysed concurrently with the treated samples. The residues of fluopyram and its metabolites were below the relevant LOQ in all the control samples.
As an overview, the mean residue levels in eggs are presented in

Table 6.4.1- 2. More details in Table 6.4.1- 7.

During the course of the study, the residues of fluopyram and the total residue of the two olefin metabolites in eggs remained in all dose groups below the LOQ (most of the time < LOD), with some exception for the total olefins mean in eggs on days 16, 21, 23, 26, and 28 in the group 10X in which the mean values were at the LOQ (0.02 mg/kg).

Therefore, the plateau in eggs was:

- below the LOQ (0.01 mg/kg for Fluopyram; most of the time below the LOD)
- at the LOQ (0.02 mg/kg for total residue of olefins).

During the course of the study, the residues of the FLU-benzamide metabolite in eggs in the nominal 1X dose group C increased from < LOD mg/kg on day 0 to 0.08 mg/kg on day 21, to 0.07 mg/kg on day 23 and 26, and 0.08 mg/kg on day 28.

Thus, the plateau of FLU-benzamide in eggs was:

- 0.08 mg/kg (mean of day 21, 23, 26 and 28) for the 1X dose group (C)
- 0.21 mg/kg (mean of day 21, 23, 26 and 28) for the 3X hens
- 0.71 mg/kg (mean of day 21, 23, 26 and 28) for the 10X hens

No residues of fluopyram or FLU-olefins above the LOD were found in any of the egg samples from the 0.1X group (with one exception < LOQ for fluopyram on the day of sampling 12), whereas residues of FLU-benzamide in the eggs from the 0.1X group were always either <LOD (for the sampling days -13, -6, -1, 0, 1) or < LOQ (for the sampling days from day 2 to 28).

The residues found in the individual egg samples as well as the calculated values for the total residue of FLU-olefins are summarized in the tables below.

Since no residues of fluopyram were determined in the individual egg matrices (egg yolks and egg white), the distribution could not be investigated. Nevertheless, the residues of FLU-benzamide and the total olefins in egg yolks and whites are shown in the tables below

Table 6.4.1- 2: Residues (mg/kg) of fluopyram and its metabolites, FLU-benzamide, total of FLU-olefins and the total residue of fluopyram in eggs

Day	Residues (mg parent equivalents/kg) in Eggs							
	Dose Group B (0.1X)				Dose Group C (1X)			
	fluopyram	FLU-Benzamide	FLU-Olefins	Total residue	fluopyram	FLU-Benzamide	FLU-Olefins	Total residue
0	<LOD	<LOD	<LOD	<LOD	<LOD	< LOD	<LOD	<LOD
1	<LOD	<LOD	<LOD	<LOD	<LOD	LOQ	<LOD	<0.04
2	<LOD	<LOQ	<LOD	<LOD	<LOD	0.01	<LOD	<0.04
5	<LOD	<LOD	<LOD	<0.04	<LOD	0.04	<LOD	0.05
7	<LOD	<LOD	<LOD	<LOD	<LOD	0.05	<LOD	0.06
9	<LOD	<LOD	<LOD	<LOD	<LOD	0.06	<LOD	0.07
12	<LOQ	<LOD	<LOD	<0.04	<LOQ	0.07	<LOD	0.07
14	<LOD	<LOD	<LOD	<LOD	<LOD	0.06	<LOD	0.07
16	<LOD	<LOD	<LOD	<LOD	<LOD	0.06	<LOD	0.07
21	<LOD	<LOD	<LOD	<0.04	<LOD	0.08	<LOD	0.09
23	<LOD	<LOD	<LOD	<LOD	<LOD	0.07	<LOD	0.08
26	<LOD	<LOD	<LOD	<0.04	<LOD	0.07	<LOD	0.08
28	<LOD	<LOD	<LOD	<0.04	<LOD	0.08	<LOD	0.09
Day	**Dose Group D (3X)**				**Dose Group E (10X)**			
	fluopyram	**FLU-Benzamide**	**FLU-Olefins**	**Total residue**	**fluopyram**	**FLU-Benzamide**	**FLU-Olefins**	**Total residue**
0	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
1	<LOD	0.01	<LOD	0.04	<LOD	0.03	<LOD	0.04
2	<LOD	0.03	<LOD	0.04	<LOD	0.09	<LOD	0.10
5	<LOD	0.10	<LOD	0.11	<LOD	0.36	<LOD	0.37
7	<LOD	0.13	<LOD	0.14	<LOD	0.45	<LOQ	0.46
9	<LOD	0.15	<LOD	0.16	<LOD	0.50	<LOQ	0.51
12	<LOQ	0.17	<LOD	0.18	<LOD	0.59	<LOQ	0.60
14	<LOD	0.17	<LOD	0.18	<LOD	0.56	<LOQ	0.57
16	<LOD	0.18	<LOD	0.19	<LOQ	0.62	<LOQ	0.64
21	<LOD	0.22	<LOD	0.23	<LOD	0.72	<LOQ	0.73
23	<LOD	0.20	<LOD	0.21	<LOD	0.70	0.02	0.71
26	<LOD	0.20	<LOQ	0.22	<LOD	0.70	0.02	0.71
28	<LOD	0.22	<LOQ	0.24	<LOD	0.72	0.02	0.73

In case we have 3 levels of which at least one is <LOD and others <LOQ, it was legitimate to set the mean value as <LOQ;
When one or two individual values are >LOQ and the others <LOQ, residues <LOQ mg/kg are considered equal to LOQ
LOQ = 0.01 mg/kg for fluopyram and for the benzamide metabolite, expressed as parent equivalents.
LOQ = 0.02 mg/kg for the total residue of FLU-olefins was calculated as sum of the two individual olefin isomers.
LOQ = 0.04 mg/kg for total residue of fluopyram
LOD = 0.003 mg/kg for fluopyram, LOD = 0.001 mg/kg for FLU-benzamide, LOD = 0.004 mg/kg for the total residue of olefins.
LOD = 0.008 mg/kg for the total residue of fluopyram (each expressed as parent equivalents)

Table 6.4.1- 3: Residue levels in whole egg, egg yolk and egg white

Group Dose	Sample material	Eggs Sampling Time (Day)*	Fluopyram [mg/kg]**	FLU-Benzamide [mg/kg]	Total residues of Olefins [mg/kg]	Total residue [mg/kg]
10X 320 µg/kg bw/day 4.8 mg/kg DM **Sub-groups**: E1, E2, E3	Whole Egg	22	-	0.69	0.02	0.71
		25	-	0.71	0.02	0.73
		27	-	0.77	0.03	0.80
	Egg Yolk	22	-	0.97	0.06	1.03
		25	-	0.94	0.04	0.98
		27	-	0.97	0.07	1.04
	Egg White	22	-	0.61	< LOD	0.61
		25	-	0.65	< 0.02	0.67
		27	-	0.68	< LOD	0.68

*Overall study day
**No residues of fluopyram were determined, therefore distribution could not be investigated.
All metabolite residues expressed in parent compound equivalents
LOQ = 0.01 mg/kg for fluopyram and the benzamide metabolite, expressed as parent equivalents.
LOQ = 0.02 mg/kg for the total residue of olefins was calculated as sum of the two individual olefin isomers.
LOQ = 0.04 mg/kg for the total residue of fluopyram (each expressed as parent equivalents).
LOD = 0.003 mg/kg for fluopyram
LOD = 0.001 mg/kg for benzamide
LOD = 0.004 mg/kg for the total residue of olefins.
LOD = 0.008 mg/kg for the total residue of fluopyram (each expressed as parent equivalents)

As an overview, the mean residue levels in tissues are presented in

Table 6.4.1- 4.

Residues of fluopyram were always below the LOD (with one exception for the mean level of fluopyram <LOQ in skin fat in the group 10X.

FLU-benzamide was found in most of the tissue samples. The fluopyram-benzamide residues showed a clear dose-response.

The mean total residues of olefins were mostly below the LOQ level.

Table 6.4.1- 4: Mean residues (mg/kg) of fluopyram and its metabolite in animal tissues.

Residues (mg parent equivalents/kg)				
Dose Group B (0.1X)				
Tissue	fluopyram	FLU-benzamide	FLU-olefins	Total residue
Skin with Fat	< LOD	< LOQ	< LOD	<0.04
Liver	< LOD	0.01	< LOD	0.04
Muscle	< LOD	< LOQ	< LOD	<0.04
Dose Group C (1X)				
Tissue	fluopyram	FLU-benzamide	FLU-olefins	Total residue
Skin with Fat	< LOD	0.04	< LOQ	0.06
Liver	< LOD	0.16	< LOD	0.18
Muscle	< LOD	0.03	< LOD	0.04
Dose Group D (3X)				
Tissue	fluopyram	FLU-benzamide	FLU-olefins	Total residue
Skin with Fat	< LOD	0.1	0.02	0.12
Liver	< LOD	0.41	< LOD	0.42
Muscle	< LOD	0.09	< LOD	0.09
Dose Group E (10X)				
Tissue	fluopyram	FLU-benzamide	FLU-olefins	Total residue
Skin with Fat	< LOQ	0.41	0.05	0.47
Liver	< LOD	1.4	< LOD	1.42
Muscle	< LOD	0.29	0.03	0.32

For the calculation of the mean residues, in case we have 5 levels of which at least one is <LOD and others <LOQ, it was legitimate to set the mean value as <LOQ;
For the calculation of the mean residues, in case one or two individual values are <LOQ and the others < LOQ, it was deemed appropriate to consider residues <LOQ mg/kg as being equal to LOQ mg/kg. This approach represents the worst-case scenario.
In tissue samples, if among three values there was at least one value > LOQ, for the calculation of the mean all the other values <LOQ and <LOD were set equal to LOQ and LOD, respectively; this approach represents the worst-case scenario.

The specimens were stored at temperatures of ≤ 18 °C until analysis. They were stored for a maximum of 23 days between sampling and extraction. Therefore, no special storage stability studies were necessary.

The calculated transfer factors, i.e. the ratio of residue level in the tissue to the residue level in the feed, are summarized in the

Table 6.4.1- 5. In most of the case, only transfer factor for fluopyram-benzamide could be calculated.

Table 6.4.1- 5: Calculated transfer factors (TF) in poultry

Feeding level	0.05 mg/kg dry feed (0.1X group)		0.49 mg/kg dry feed (1X group)		1.6 mg/kg dry feed (3X group)		4.8 mg/kg dry feed (10X group)	
fluopyram								
Commodity	**mg/kg**	**TF**	**mg/kg**	**TF**	**mg/kg**	**TF**	**mg/kg**	**TF**
eggs	< LOD	nd	< LOD	nd	< LOD	nd	< LOQ	nd
Skin with Fat	< LOD	nd	< LOD	nd	< LOD	nd	< LOD	nd
Liver	< LOD	nd	< LOD	nd	< LOD	nd	< LOD	nd
Muscle	< LOD	nd	< LOD	nd	< LOD	nd	< LOD	nd
Fluopyram-benzamide								
Commodity	**mg/kg**	**TF**	**mg/kg**	**TF**	**mg/kg**	**TF**	**mg/kg**	**TF**
eggs	< LOQ	nd	0.08	**0.16**	0.22	**0.14**	0.72	**0.15**
Skin with Fat	< LOQ	nd	0.04	**0.08**	0.1	**0.06**	0.41	**0.09**
Liver	0.01	nd	0.16	**0.33**	0.41	**0.26**	1.4	**0.29**
Muscle	< LOQ	nd	0.03	**0.06**	0.09	**0.06**	0.29	**0.06**
fluopyram-olefins								
Commodity	**mg/kg**	**TF**	**mg/kg**	**TF**	**mg/kg**	**TF**	**mg/kg**	**TF**
eggs	< LOD	nd	< LOD	**nd**	< LOD	**nd**	0.02	**0.004**
Skin with Fat	< LOD	nd	< LOQ	**nd**	0.02	**0.013**	0.05	**0.01**
Liver	< LOD	nd	< LOD	**nd**	< LOQ	**nd**	< LOQ	**nd**
Muscle	< LOD	nd	< LOD	**nd**	< LOD	**nd**	0.02	**0.004**

Depuration phase:

Three subgroups of hens were fed at the 10X dose level for 28 consecutive days, followed by untreated feeding period:

- 8 days (dose group F1)
- 13 days (F2)
- 21 days (F3)

Residues were determined at sacrifice, on day 36 (F1), 41 (F2) and 49 (F3).

In eggs, residues of FLU-benzamide decreased from 0.83 mg/kg (day 28, mean value for eggs from group F) to 0.30 mg/kg (day 36, eggs from all hens of group F1), 0.11 mg/kg (day 41, eggs from F2 and F3) and 0.03 mg/kg (day 49, eggs from subgroup F3).

In tissues, fluopyram was found to be <LOD in the three subgroups F1, F2 and F3.

Residues of FLU-benzamide were:

- In skin with fat, 0.41 mg/kg on average on day 28 (dose group E) and declined to 0.12 mg/kg, 0.05 mg/kg and 0.02 mg/kg on day 36, 41 and 49 (F1, F2 and F3), respectively.
- In liver, 1.4 mg/kg on average on day 28 (dose group E) and declined to 0.49 mg/kg, 0.19 mg/kg and 0.05 mg/kg on day 36, 41 and 49 (F1, F2 and F3), respectively.
- In muscle, 0.29 mg/kg on average on day 28 (dose group E) and declined to 0.21 mg/kg, 0.08 mg/kg and 0.02 mg/kg on day 36, 41 and 49 (F1, F2 and F3), respectively.

The total residues of FLU-olefins, in skin with fat, the residues were:

- In skin with fat, 0.05 mg/kg on average on day 28 (dose group E), 0.06 mg/kg, 0.03 mg/kg and <LOD on day 36, 41 and 49 (F1, F2 and F3), respectively.

- In liver, 0.02 mg/kg on average on day 28 (dose group E) and declined to <0.02 mg/kg, <0.02 mg/kg, and <LOD on day 36, 41 and 49 (F1, F2 and F3), respectively.
- In muscle, the total residues of olefins were 0.03 mg/kg on average on day 28 (dose group E) and declined to <0.02 mg/kg, <LOD, and <LOD on day 36, 41 and 49 (F1, F2 and F3), respectively.

A graphical presentation of the depuration curves for the major metabolite, fluopyram-benzamide, is presented in the Figure 6.1.1- 1 and Figure 6.1.1- 2.

Figure 6.1.1- 1: Depuration curve of residue of Fluopyram-benzamide in eggs

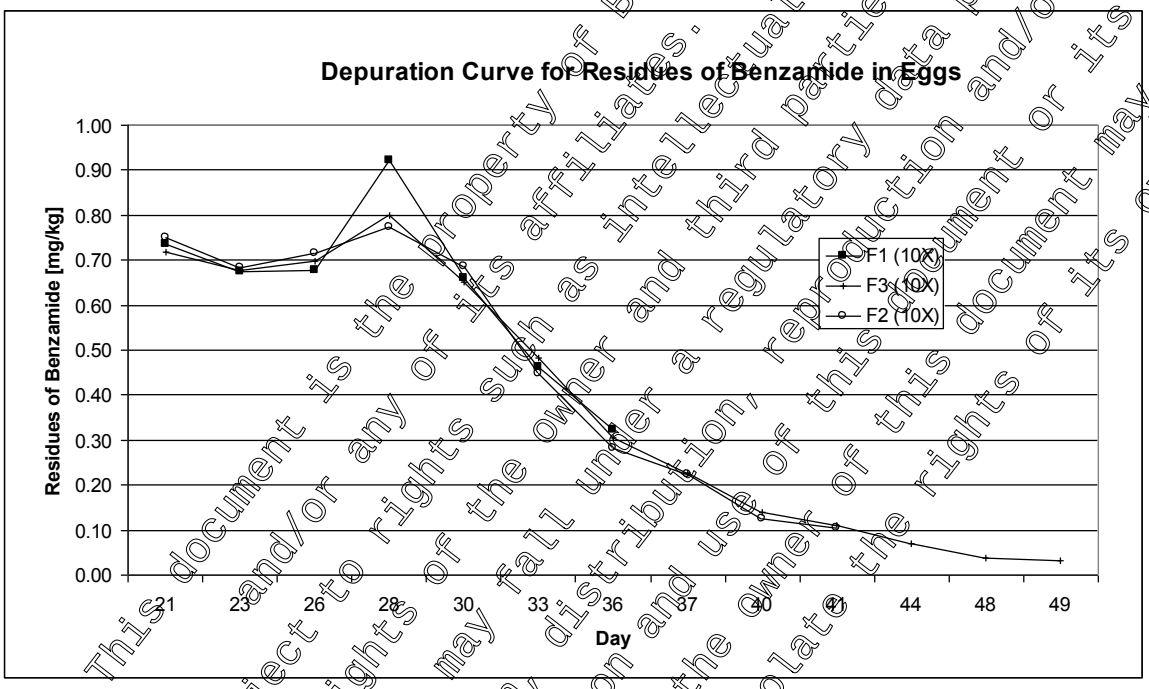
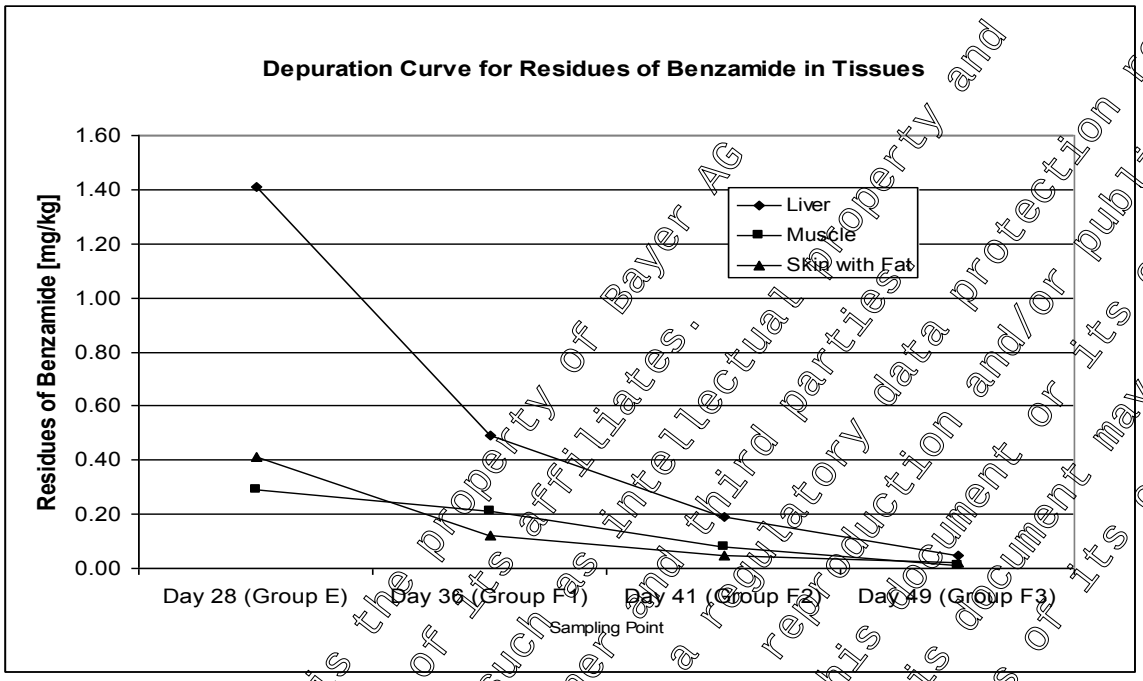


Figure 6.1.1- 2: Depuration curve of residue of Fluopyram-benzamide in tissues.



According to the observed linear correlation between dose and residue levels in animal commodities after dosing, we assume that the same behavior applies during the depuration phase in the 0.1X, 1X and 3X doses.

III. Conclusions

A feeding study was conducted with fluopyram on poultry in order to elucidate the levels of relevant residues in poultry tissues and in eggs.

Fluopyram was administered orally via feed to laying hens for 28 consecutive days at the actual dose rates of :

- 0 µg/kg bw/day (dose group A, 0X)
- 3.4 µg/kg bw/day (dose group B, 0.1X)
- 35 µg/kg bw/day (dose group C, 1X)
- 110 µg/kg bw/day (dose group D, 3X)
- 320 µg/kg bw/day (dose groups E, 10X)
- 330 µg/kg bw/day (dose group F, 10X)

Feed consumption, body weights, and egg production were not adversely affected by compound administration.

Prior to sacrifice, residues in eggs were measured at various intervals. After the final dose, the animals were sacrificed and the key edible tissues were analysed for the residues of fluopyram and its metabolites fluopyram-benzamide, and total residues of fluopyram-olefins in all matrices. In addition, the total residues of fluopyram was determined in all animal matrices.

Overall, most of the time residues of fluopyram, and the total residues fluopyram-olefins in eggs and tissues were below the respective LOQs.

Fluopyram-benzamide was found in most of the tissue samples and this metabolite showed a clear dose-response. During the depuration phase, the residues of fluopyram-benzamide decreased from 0.83 mg/kg (day 28) to 0.30 mg/kg (day 36), 0.11 mg/kg (day 41) and 0.03 mg/kg (day 49).

In general, the residue data provided in this study are suitable for regulatory purposes.

Table 6.4.1- 6: Concurrent recovery data for fluopyram and its metabolites in poultry matrices

Crop / Sample material	FL [mg/kg]	Single values [%]	Mean value [%]	RSD [%]	LOQ [mg/kg]
Fluopyram (AE C656948) (1)					
Egg*	0.01	91; 96; 102; 94; 101; 97; 97; 88; 91; 97; 99	96	4.6	0.01
	0.10	91; 94	93	--	
	0.50	93; 97; 108; 101; 97; 99	99	5.1	
	1.0	94; 91; 88; 84; 101; 99; 100; 104; 98; 96; 98; 102	96	6.2	
	2.0	93	93	--	
		Overall recovery (n=32)	**96**	**5.3**	
Liver	0.01	95; 97	96	--	0.01
	2.0	91; 98; 102	97	6.2	
		Overall recovery (n=5)	**97**	**4.5**	
Muscle	0.01	101; 99; 114; 80; 93	97	13.7	0.01
	2.0	104; 107; 107	106	1.6	
		Overall recovery (n=8)	**101**	**10.3**	
Skin with fat	0.01	96; 95; 117	103	12.0	0.01
	2.0	100; 97; 92	96	4.2	
		Overall recovery (n=6)	**100**	**9.0**	
FLU-Benzamide (AE F148815) (2)					
Egg*	0.01	100; 98; 97; 97; 98; 90; 93; 91; 92; 99; 96	96	3.6	0.01
	0.10	97; 99	98	--	
	0.50	96; 94; 94; 94; 92; 95	94	1.4	
	1.0	91; 94; 105; 95; 98; 99; 101; 99; 96; 97; 96; 97	97	3.6	
	2.0	86	--	--	
		Overall recovery (n=32)	**96**	**3.8**	
Liver	0.01	106; 105; 102	104	2.0	0.01
	2.0	88; 91; 91	90	1.9	
		Overall recovery (n=6)	**97**	**8.3**	
Muscle	0.01	100; 97; 93; 79; 87	91	9.2	0.01
	2.0	93; 93; 93	93	0.0	
		Overall recovery (n=8)	**92**	**7.0**	
Skin with fat	0.01	105; 94	99	--	0.01
	2.0	99; 89; 94	94	5.3	
		Overall recovery (n=5)	**94**	**8.7**	
Total residues of FLU-Olefins (3)					
Egg	0.02	99; 105; 112; 104; 101; 110; 107; 97; 97; 102; 99	103	5.0	0.02
	0.20	119; 127	123**	--	
	1.0	108; 108; 110; 112; 106; 112	109	2.1	
	2.0	123; 118; 112; 106; 121; 122; 120; 123; 125; 122; 94; 92	115	10.1	
	4.0	79	--	--	

Crop / Sample material	FL [mg/kg]	Single values [%]	Mean value [%]	RSD [%]	LOQ [mg/kg]
		Overall recovery (n=32)	**109**	**10.3**	
Liver	0.02	101; 106; 112	106	5.2	0.02
	4.0	84; 93; 94	90	6.1	
		Overall recovery (n=6)	**98**	**10.1**	
Muscle	0.02	113; 95; 93; 72; 103	95	15.9	0.02
	4.0	92; 96; 95	94	2.2	
		Overall recovery (n=8)	**95**	**12.2**	
Skin with fat	0.02	82; 97; 95	91	8.6	0.02
	4.0	87; 82; 84	84	3.0	
		Overall recovery (n=6)	**87**	**7.0**	

FL = Fortification level, RSD = Relative standard deviation, LOQ = Practical limit of quantification

(1) Final determination as: Fluopyram Residues calculated as: Fluopyram

(2) Final determination as: FLU-benzamide Residues calculated as: Fluopyram

(3) Final determination as: FLU-olefins (E- and Z-isomer) Residues calculated as: Total Residue of Olefins (expressed as parent equivalents). Both isomers were fortified with a ratio of 1/1.

* Egg white and egg yolk are covered by egg (= whole egg without shell).

Table 6.4.1- 7: Residue levels in eggs (mean of 3 sub-groups)

Group Dose	Sampling date*	Residue levels of individual analytes (mg/kg) Mean of 3 sub-groups (individual values) 5*							
		Fluopyram		FLU-benzamide		Total residues of FLU-olefins		Total Residue of Fluopyram	
		Indiv.	mean	Indiv.	mean	Indiv.	mean	Indiv.	mean
0.1X 3.4 µg/kg bw/day 0.05 mg/kg DM **Sub-groups:** B1, B2, B3	-13	3x<LOD	<LOD	3x<LOD	<LOD	3x<LOD	<LOD	3x<LOD	<LOD
	-6	3x<LOD	<LOD	3x<LOD	<LOD	3x<LOD	<LOD	3x<LOD	<LOD
	-1	3x<LOD	<LOD	3x<LOD	<LOD	3x<LOD	<LOD	3x<LOD	<LOD
	0[b]	3x<LOD	<LOD	3x<LOD	<LOD	3x<LOD	<LOD	3x<LOD	<LOD
	1	3x<LOD	<LOD	3x<LOD	<LOD	3x<LOD	<LOD	3x<LOD	<LOD
	2	< LOD	<LOD	<LOD; 2x<LOQ	<LOQ	3x<LOD	<LOD	3x<LOD	<LOD
	5	3x<LOD	<LOD	3x<LOQ	<LOD	3x<LOD	<LOD	2x<LOD; <0.04	<0.04
	7	3x<LOD	<LOD	3x<LOQ	<LOD	3x<LOD	<LOD	3x<LOD	<LOD
	9	3x<LOD	<LOD	3x<LOQ	<LOD	3x<LOD	<LOD	3x<LOD	<LOD
	12	2x<LOD; <LOQ	<LOQ	3x<LOQ	<LOD	3x<LOD	<LOD	2x<LOD; <0.04	<0.04
	14	3x<LOD	<LOD	3x<LOQ	<LOD	3x<LOD	<LOD	3x<LOD	<LOD
	16	3x<LOD	<LOD	3x<LOQ	<LOD	3x<LOD	<LOD	3x<LOD	<LOD
	21	3x<LOD	<LOD	3x<LOQ	<LOD	3x<LOD	<LOD	3x<LOQ	<0.04
	23	3x<LOD	<LOD	3x<LOQ	<LOD	3x<LOD	<LOD	3x<LOD	<LOD
	26	3x<LOD	<LOD	3x<LOQ	<LOD	3x<LOD	<LOD	2x<LOD; <0.04	<0.04
	28	3x<LOD	<LOD	3x<LOQ	<LOD	3x<LOD	<LOD	2x<LOD; <0.04	<0.04

In case we have 3 levels of which at least one is <LOD and others <LOQ, it was legitimate to set the mean value as <LOQ;
When one or two individual values are >LOQ and the others <LOQ or <LOD, residues <LOQ or <LOD are considered equal to LOQ or LOD
LOQ = 0.01 mg/kg for fluopyram and the benzamide metabolite, expressed as parent equivalents.
LOQ = 0.02 mg/kg for the total residue of FLU-olefins was calculated as sum of the two individual olefin isomers.
LOQ = 0.04 mg/kg for total residue of fluopyram
LOD = 0.003 mg/kg for fluopyram, LOD = 0.001 mg/kg for FLU-benzamide, LOD = 0.004 mg/kg for the total residue of olefins

Group Dose	Sampling date*	Residue levels of individual analytes (mg/kg)							
		Fluopyram		FLU-benzamide		Total residues of FLU-olefins		Total Residue of Fluopyram	
		Indiv.	mean	Indiv.	mean	Indiv.	mean	Indiv.	mean
1X 35 µg/kg bw/day 0.49 mg/kg DM **Sub-groups**: C1, C2, C3	-13	3x<LOD	<LOD	3x<LOD	<LOD	3x<LOD	<LOD	3x<LOD	<LOD
	-6	3x<LOD	<LOD	3x<LOD	<LOD	3x<LOD	<LOD	3x<LOD	<LOD
	-1	3x<LOD	<LOD	3x<LOD	<LOD	3x<LOD	<LOD	3x<LOD	<LOD
	0	3x<LOD	<LOD	3x<LOD	<LOD	3x<LOD	<LOD	3x<LOD	<LOD
	1	3x<LOD	<LOD	<LOD; 2x<LOQ	LOQ	3x<LOD	<LOD	3x<LOD	<LOQ
	2	3x<LOD	<LOD	3x0.01	0.01	3x<LOD	<LOD	3x<LOQ	<LOQ
	5	3x<LOD	<LOD	2x0.04; 0.03	0.04	3x<LOD	<LOD	2x0.05; 0.04	0.05
	7	3x<LOD	<LOD	2x0.05; 0.04	0.05	3x<LOD	<LOD	2x0.06; 0.05	0.06
	9	3x<LOD	<LOD	2x0.06; 0.05	0.06	3x<LOD	<LOD	2x0.07; 0.06	0.07
	12	2x<LOD; <LOQ	<LOQ	2x0.07; 0.06	0.07	3x<LOD	<LOD	2x0.07; 0.08	0.07
	14	3x<LOD	<LOD	3x0.06	0.06	3x<LOD	<LOD	3x0.07	0.07
	16	3x<LOD	<LOD	3x0.06	0.06	3x<LOD	<LOD	3x0.07	0.07
	21	3x<LOD	<LOD	2x0.08; 0.09	0.08	3x<LOD	<LOD	2x0.09; 0.10	0.09
	23	3x<LOD	<LOD	2x0.07; 0.08	0.07	3x<LOD	<LOD	2x0.08; 0.09	0.08
	26	3x<LOD	<LOD	3x0.07	0.07	3x<LOD	<LOD	3x0.08	0.08
	28	3x<LOD	<LOD	2x0.08; 0.09	0.08	3x<LOD	<LOD	2x0.09; 0.10	0.09

In case we have 3 levels of which at least one is <LOD and others <LOQ, it was legitimate to set the mean value as <LOQ;
When one or two individual values are >LOQ and the others <LOQ or <LOD, residues <LOQ or <LOD are considered equal to LOQ or LOD
LOQ = 0.01 mg/kg for fluopyram and the benzamide metabolite, expressed as parent equivalents.
LOQ = 0.02 mg/kg for the total residue of FLU-olefins was calculated as sum of the two individual olefin isomers.
LOQ = 0.04 mg/kg for total residue of fluopyram
LOD = 0.003 mg/kg for fluopyram, LOD = 0.001 mg/kg for FLU-benzamide, LOD = 0.004 mg/kg for the total residue of olefins.

Group Dose	Sampling date	Residue levels of individual analytes (mg/kg) Mean of 3 sub-groups (individual values) **							
		Fluopyram		FLU-benzamide		Total residues of FLU-olefins		Total Residue of Fluopyram	
		Indiv.	mean	Indiv.	mean	Indiv.	mean	Indiv.	mean
3X 110 µg/kg bw/day 1.6 mg/kg DM **Sub-groups**: D1, D2, D3	-13	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	-6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	-1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	0	3x<LOD	<LOD	3x<LOD	LOD	3x<LOD	<LOD	3x<LOD	<LOD
	1	3x<LOD	<LOD	3x0.01	0.01	3x<LOD	<LOD	3x0.02	< 0.04
	2	3x<LOD	<LOD	2x0.03; 0.02	0.03	3x<LOD	<LOD	2x0.04; 0.03	0.04
	5	3x<LOD	<LOD	3x0.10	0.10	3x<LOD	<LOD	3x0.11	0.11
	7	3x<LOD	<LOD	3x0.13	0.13	3x<LOD	<LOD	3x0.14	0.14
	9	3x<LOD	<LOD	3x0.15	0.15	3x<LOD	<LOD	3x0.16	0.16
	12	2x<LOD; <LOQ	<LOQ	2x0.18; 0.18	0.17	3x<LOD	<LOD	2x0.17; 0.19	0.18
	14	3x<LOD	<LOD	3x0.17	0.17	3x<LOD	<LOD	3x0.18	0.18
	16	3x<LOD	<LOD	3x0.18	0.18	3x<LOD	<LOD	3x0.19	0.19
	21	3x<LOD	<LOD	0.20; 0.22; 0.23	0.22	3x<LOD	<LOD	0.21, 0.23, 0.24	0.23
	23	3x<LOD	<LOD	2x0.20; 0.21	0.20	3x<LOD	<LOD	2x0.21, 0.22	0.21
	26	3x<LOD	<LOD	2x0.20; 0.21	0.20	2x<LOD; <LOQ	<LOQ	2x0.22; 0.21	0.22
	28	3x<LOD	<LOD	0.20; 0.22; 0.23	0.22	2x<LOD; <LOQ	<LOQ	0.22; 0.24; 0.25	0.24

In case we have 3 levels of which at least one is <LOD and others <LOQ, it was legitimate to set the mean value as <LOQ;
When one or two individual values are >LOD and the others < LOQ or <LOD, residues <LOQ or <LOD are considered equal to LOQ or LOD
LOQ = 0.01 mg/kg for fluopyram and the benzamide metabolite, expressed as parent equivalents
LOQ = 0.02 mg/kg for the total residue of FLU-olefins was calculated as sum of the two individual olefin isomers.
LOQ = 0.04 mg/kg for total residue of fluopyram
LOD = 0.003 mg/kg for fluopyram, LOD = 0.001 mg/kg for FLU-benzamide, LOD = 0.004 mg/kg for the total residue of olefins.

Group Dose	Sampling date	Residue levels of individual analytes (mg/kg) Mean of 3 sub-groups (individual values) **							
		Fluopyram		FLU-benzamide		Total residues of FLU-olefins		Total Residue of Fluopyram	
		Indiv.	mean	Indiv.	mean	Indiv.	mean	Indiv.	mean
10X 320 µg/kg bw/day 4.8 mg/kg DM **Sub-groups:** E1, E2, E3	-13	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	-6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	-1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	0	3x<LOD	<LOD	3x<LOD	<LOD	3x<LOD	<LOD	< LOD	<LOD
	1	3x<LOD	<LOD	2x0.03; 0.02	0.03	3x<LOD	<LOD	2x0.04; 0.03	0.04
	2	3x<LOD	<LOD	2x0.09; 0.10	0.09	3x<LOD	<LOD	2x0.10; 0.11	0.10
	5	3x<LOD	<LOD	2x0.35; 0.38	0.36	3x<LOD	<LOD	2x0.36; 0.39	0.37
	7	3x<LOD	<LOD	2x0.46; 0.43	0.45	2x<LOQ; <LOD	<LOQ	2x0.47; 0.44	0.46
	9	3x<LOD	<LOD	0.50; 0.54; 0.45	0.50	3x<LOQ	<LOQ	0.51;0.55; 0.46	0.51
	12	3x<LOD	<LOD	2x0.56; 0.64	0.59	3x<LOQ	<LOQ	0.57; 0.65; 0.57	0.60
	14	3x<LOD	<LOD	0.57; 0.62; 0.56	0.56	3x<LOQ	<LOQ	0.58; 0.63; 0.51	0.57
	16	2x<LOQ; <LOD	<LOQ	0.63; 0.57; 0.57	0.62	3x<LOQ	<LOQ	0.64; 0.68; 0.58	0.64
	21	3x<LOD	<LOD	0.70; 0.76; 0.71	0.72	3x<LOQ	<LOQ	0.71; 0.77; 0.72	0.73
	23	3x<LOD	<LOD	0.76; 0.71; 0.64	0.70	0.02; 2x<LOQ	0.02	0.77; 0.72; 0.65	0.71
	26	3x<LOD	<LOD	0.69; 0.67; 0.75	0.70	0.02; 2x<LOQ	0.02	0.70; 0.68; 0.76	0.71
	28	3x<LOD	<LOD	0.76; 0.74; 0.65	0.72	2x0.02; LOQ	0.02	0.77; 0.75; 0.66	0.73

In case we have 3 levels of which at least one is <LOD and others <LOQ, it was legitimate to set the mean value as <LOQ;
When one or two individual values are >LOQ and the others < LOQ or <LOD, residues <LOQ or <LOD are considered equal to LOQ or LOD
LOQ = 0.01 mg/kg for fluopyram and the benzamide metabolite, expressed as parent equivalents
LOQ = 0.02 mg/kg for the total residue of FLU-olefins was calculated as sum of the two individual olefin isomers.
LOQ = 0.04 mg/kg for total residue of fluopyram
LOD = 0.003 mg/kg for fluopyram, LOD = 0.001 mg/kg for FLU-benzamide, LOD = 0.004 mg/kg for the total residue of olefins

Group Dose	Sampling date*	Residue levels of individual analytes (mg/kg) Mean of 3 sub-groups (individual values) **							
		Fluopyram		FLU-benzamide		Total residues of FLU-olefins		Total Residue of Fluopyram	
		Indiv.	mean	Indiv.	mean	Indiv.	mean	Indiv.	mean
10X depuration 330 µg/kg bw/day 4.8 mg/kg DM **Sub-groups:** F1, F2, F3	-13	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	-6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	-1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	21	2x<LOQ; <LOD	<LOQ	0.74; 0.75; 0.72	0.74	2x<LOQ; 0.02	0.02	0.77; 0.78; 0.75	0.77
	23	3x<LOD	<LOD	2x0.68; 0.67	0.68	3x<LOQ	<LOQ	0.69; 0.70; 0.70	0.70
	26	3x<LOD	<LOD	0.68; 0.72; 0.70	0.70	3x<LOQ	<LOQ	0.70; 0.74; 0.72	0.72
	28	3x<LOD	<LOD	0.92; 0.77; 0.80	0.83	2x0.02; 0.03	0.02	0.95; 0.79; 0.82	0.85
	30	2x<LOD; <LOQ	<LOQ	0.66; 0.69; 0.65	0.67	3x<LOQ	<LOQ	2x0.68; 0.71	0.69
	33	3x<LOD	<LOD	0.46; 0.45; 0.48	0.46	3x<LOQ	<LOQ	0.48; 0.47; 0.50	0.48
	36	3x<LOQ	<LOQ	0.32; 0.28; 0.30	0.30	3x<LOQ	<LOQ	0.35; 0.29; 0.33	0.32
	37	2x<LOQ	<LOQ	0.22; 0.23	0.23	2x<LOQ	<LOQ	0.25; 0.26	0.25
	40	<LOD; <LOQ	<LOQ	0.12; 0.14	0.13	2x<LOQ	<LOQ	0.14; 0.17	0.15
	41	2x<LOD	<LOD	0.11; 0.11	0.11	2x<LOQ	<LOQ	2x0.13	0.13
	44	<LOD	<LOD	0.070	0.07	<LOQ	<LOQ	0.09	0.09
	48	<LOD	<LOD	0.04	0.04	<LOQ	<LOQ	0.06	0.06
	49	<LOD	<LOD	0.03	0.03	<LOQ	<LOQ	0.05	0.05

In case we have 3 levels of which at least one is <LOD and others <LOQ, it was legitimate to set the mean value as <LOQ;
When one or two individual values are >LOQ and the others < LOQ or <LOD, residues <LOQ or <LOD are considered equal to LOQ or LOD
LOQ = 0.01 mg/kg for fluopyram and the benzamide metabolite, expressed as parent equivalents.
LOQ = 0.02 mg/kg for the total residue of FLU-olefins was calculated as sum of the two individual olefin isomers.
LOQ = 0.04 mg/kg for total residue of fluopyram
LOD = 0.003 mg/kg for fluopyram, LOD = 0.001 mg/kg for FLU-benzamide, LOD = 0.004 mg/kg for the total residue of olefinsLOD = 0.008 mg/kg for the total residue of fluopyram (each expressed as parent equivalents).

Table 6.4.1- 8: Residue levels in poultry tissues

Group	Dose (µg/kg bw/day)	Dose (mg/kg DM)	Sub-group	Sampling Time (Day)	Residue levels (mg/kg)			
					Fluopyram	FLU-benzamide	Total residues of FLU-olefins	Total Residue of Fluopyram
Skin with fat								
0.1X	3.4	0.05	B1	**28**	< LOD	0.01	< LOD	< LOQ
			B2	28	< LOD	< 0.01	< LOD	< LOQ
			B3	28	< LOD	< 0.01	< LOD	< LOQ
				Mean	**< LOD**	**< 0.01**	**< LOD**	**< LOQ**
1X	35	0.49	C1	28	< LOD	0.03	< 0.02	0.05
			C2	28	< LOD	0.04	< 0.02	0.06
			C3	28	< LOD	0.04	< 0.02	0.06
				Mean	**< LOD**	**0.04**	**< 0.02**	**0.06**
3X	110	1.6	D1	28	< LOD	0.10	0.03	0.13
			D2	28	< LOD	0.11	0.02	0.13
			D3	28	< LOD	0.10	0.02	0.12
				Mean	**< LOD**	**0.10**	**0.02**	**0.12**
10X	320	4.8	E1	28	< 0.01	0.33	0.08	0.41
			E2	28	< LOD	0.63	< LOD	0.64
			E3	28	< LOD	0.26	0.06	0.32
				Mean	**< 0.01**	**0.41**	**0.05**	**0.47**
10X depuration	320	4.8	F1	36	< LOD	0.12	0.06	0.18
			F2	41	< LOD	0.05	0.03	0.08
			F3	49	< LOD	0.02	< LOD	0.04
				Mean	**< LOD**	**0.06**	**0.03**	**0.10**

In case we have 3 levels of which at least one is <LOD and others ≥LOQ, it was legitimate to set the mean value as <LOQ;
When one or two individual values are >LOQ and the others ≤LOQ or <LOD, residues <LOQ or <LOD are considered equal to LOQ or LOD
LOQ = 0.01 mg/kg for fluopyram and the benzamide metabolite, expressed as parent equivalents.
LOQ = 0.02 mg/kg for the total residue of FLU-olefins was calculated as sum of the two individual olefin isomers.
LOQ = 0.04 mg/kg for total residue of fluopyram
LOD = 0.003 mg/kg for fluopyram, LOD = 0.001 mg/kg for FLU-benzamide, LOD = 0.004 mg/kg for the total residue of olefinsLOD = 0.008 mg/kg for the total residue of fluopyram (each expressed as parent equivalents).

Group	Dose (µg/kg bw/day)	Dose (mg/kg DM)	Sub-group	Sampling Time (Day)*	Residue levels (mg/kg) **			
					Fluopyram	FLU-benzamide	Total residues of FLU-olefins	Total Residue of Fluopyram
Liver								
0.1X	3.4	0.05	B1	28	< LOD	0.02	< LOD	0.04
			B2	28	< LOD	0.02	< LOD	0.04
			B3	28	< LOD	0.01	< LOD	0.04
				Mean	**< LOD**	**0.02**	**< LOD**	**0.04**
1X	35	0.49	C1	28	< LOD	0.16	< LOD	0.16
			C2	28	< LOD	0.16	< 0.02	0.18
			C3	28	< LOD	0.16	0.02	0.18
				Mean	**< LOD**	**0.16**	**< 0.02**	**0.18**
3X	110	1.6	D1	28	< LOD	0.42	< LOD	0.43
			D2	28	< LOD	0.39	< LOD	0.40
			D3	28	< LOD	0.43	< LOD	0.44
				Mean	**< LOD**	**0.41**	**< LOD**	**0.42**
10X	320	4.8	E1	28	< LOD	1.6	<0.02	1.62
			E2	28	< LOD	1.4	<0.02	1.42
			E3	28	< LOD	1.3	0.02	1.32
				Mean	**< LOD**	**1.4**	**0.02**	**1.42**
10X depuration	330	4.8	F1	36	< LOD	0.49	< 0.02	0.51
			F2	41	< LOD	0.19	< 0.02	0.22
			F3	49	< LOD	0.05	< LOD	0.05
				Mean	**< LOD**	**0.24**	**< 0.02**	**0.26**

In case we have 3 levels of which at least one is <LOD and others <LOQ, it was legitimate to set the mean value as <LOQ;
When one or two individual values are ≥LOQ and the others < LOQ or <LOD, residues <LOQ or <LOD are considered equal to LOQ or LOD
LOQ = 0.01 mg/kg for fluopyram and the benzamide metabolite, expressed as parent equivalents.
LOQ = 0.02 mg/kg for the total residue of FLU-olefins was calculated as sum of the two individual olefin isomers.
LOQ = 0.04 mg/kg for total residue of fluopyram
LOD = 0.003 mg/kg for fluopyram, LOD = 0.001 mg/kg for FLU-benzamide, LOD = 0.004 mg/kg for the total residue of olefinsLOD = 0.008 mg/kg for the total residue of fluopyram (each expressed as parent equivalents)

Group	Dose (µg/kg bw/day)	Dose (mg/kg DM)	Sub-group	Sampling Time (Day)*	Residue levels (mg/kg) **			
					Fluopyram	FLU-benzamide	Total residues of FLU-olefins	Total Residue of Fluopyram
Muscle								
0.1X	3.4	0.05	B1	28	< LOD	< 0.01	< LOD	< LOD
			B2	28	< LOD	< 0.01	< LOD	< LOD
			B3	28	< LOD	< 0.01	< LOD	< LOD
				Mean	**< LOD**	**< 0.01**	**< LOD**	**< LOD**
1X	35	0.49	C1	28	< LOD	0.04	< LOD	0.04
			C2	28	< LOD	0.03	< LOD	0.04
			C3	28	< LOD	0.04	< LOD	0.04
				Mean	**< LOD**	**0.04**	**< LOD**	**0.04**
3X	110	1.6	D1	28	< LOD	0.09	< LOD	0.09
			D2	28	< LOD	0.09	< LOD	0.09
			D3	28	< LOD	0.10	< LOD	0.10
				Mean	**< LOD**	**0.09**	**< LOD**	**0.09**
10X	320	4.8	E1	28	< LOD	0.33	< LOD	0.33
			E2	28	< LOD	0.24	0.06	0.30
			E3	28	< LOD	0.29	< 0.02	0.31
				Mean	**< LOD**	**0.29**	**0.03**	**0.32**
10X depuration	330	4.8	F1	36	< LOD	0.21	< 0.02	0.23
			F2	41	< LOD	0.08	< LOD	0.08
			F3	49	< LOD	0.02	< LOD	0.04
				Mean	**< LOD**	**0.10**	**< 0.02**	**0.12**

In case we have 3 levels of which at least one is <LOD and others <LOQ, it was legitimate to set the mean value as <LOQ;
When one or two individual values are ≥LOQ and the others < LOQ or <LOD, residues <LOQ or <LOD are considered equal to LOQ or LOD
LOQ = 0.01 mg/kg for fluopyram and the benzamide metabolite, expressed as parent equivalents.
LOQ = 0.02 mg/kg for the total residue of FLU-olefins was calculated as sum of the two individual olefin isomers.
LOQ = 0.04 mg/kg for total residue of fluopyram
LOD = 0.003 mg/kg for fluopyram, LOD = 0.001 mg/kg for FLU-benzamide, LOD = 0.004 mg/kg for the total residue of olefinsLOD = 0.008 mg/kg for the total residue of fluopyram (each expressed as parent equivalents)

CA 6.4.2 Ruminants

Data Point:	KCA 6.4.2/01
Report Author:	[redacted]; [redacted]
Report Year:	2008
Report Title:	Fluopyram: Feeding study with dairy cows
Report No:	MR-07/367
Document No:	M-298635-01-1
Guideline(s) followed in study:	US: EPA Residue Chemistry Test Guidelines OPPTS 860.1000 "Background", OPPTS 860.1480 "Meat, milk, poultry and eggs"; EC: 7031/VI/95 rev. 4 "Livestock feeding studies"; EU Directive EC 91/414, Appendix G; OECD: Guidelines for the Testing of Chemicals, 505, 2002-01-08
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted rev. 1 to Vol. 3 of DAR B7 August 2012 (references added on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP, officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and Methods

The purpose of this study was to determine the magnitude of the residues of fluopyram and its metabolites Fluopyram-benzamide, and total residue of fluopyram-olefines (BCS-AA10627 and BCS-AA10650) that may be expected in milk, muscle, liver, kidney and fat following oral administration of fluopyram to dairy cows.

Test system, dosing

Fifteen clinically healthy, not-pregnant in their 1st to 4th lactation period dairy cows were selected for the study. Fourteen days before the first application (-14), the animals were taken up into the experimental housing facilities for the allocation and adaptation. The cows were divided into five experimental groups and one control animal (according to random group mapping) on the day they were introduced into the experimental stall. During the acclimatisation period, the animals of the depuration group (10XE) served as reserve animals for the experimental groups 0X, 0.1X, 1X, 3X and 10X.

During the acclimatisation period (at the day -5), one cow had to be excluded from the study due to the abomasal dislocation – this cow was replaced by another one (animal number 8) and the data set were recorded only as of study day -5 of the acclimatisation period. Further, due to the low average food intake during the dosing period, one cow (animal number 12) had to be excluded from the study.

After the acclimatisation period the dairy cows were dosed orally *via* double-coated gelatine capsules with fluopyram for 29 consecutive days. The actual daily dose rates were 0.04 mg/kg bw/day (dose group 0.1X), 0.44 mg/kg bw/day (dose group 1X), 1.21 mg/kg bw/day (dose group 3X), 4.05 mg/kg bw/day (dose group 10X) and 4.38 mg/kg bw/day (dose group 10XE, during feeding phase), corresponding to actual daily residue intake in diet of 1.5 mg/kg dry feed/day (dose group 0.1X), 14.4 mg/kg dry feed/day (dose group 1X), 44.1 mg/kg dry feed/day (dose group 3X), 133.1 mg/kg dry feed/day (dose group 10X) and 145.9

mg/kg dry feed/day (dose group 10XE, during feeding phase), respectively. Details of the doses for corresponding groups are given in the Table 6.4.2- 1.

Table 6.4.2- 1: Summary of fluopyram (AE C656948) dose administration

Dose group	Number of animals	Animal number	Actual dose level (mg/kg bw/day)	Dose levels in feed (mg/kg DM)
0X	1	1	0	0
0.1X	3	2, 3, 4	0.04	1.5
1X	3	5, 6, 7	0.44	14.4
3X	3	8, 9, 10	1.21	44.1
10X	2	11, 13	4.05	133.1
10XE	3	14, 15, 16	4.38	145.9

DM – feed, dry matter
bw – body weight

The doses were calculated based on the average body weight of dairy cows, an average feed ration, and the worst-case residue derived from field studies and were approximately 0.1X, 1X, 3X and 10X of the anticipated maximum dietary burden arising from the use of fluopyram in the US.

The 1X group in this study is around 4 / 5 times higher than the calculated maximum dietary burden for the present renewal (0.094 mg/kg bw/day for Ram/Ewe see Table 6.4-2).

- In tissues, as a worse case the residue results from this 1X group will be used as input data to calculate the risk assessment.
- For milk, the dietary burden of 0.076 mg/kg bw/day in dairy cattle is close to the 0.1 X dose value of 0.04 mg/kg bw/day. Values in milk from the 0.1 X dose group are considered to calculate the dietary risk assessment.

The dose capsules were prepared prior to the dosing phase by weighing the test item into capsules. Capsules were analysed for fluopyram in order to determine the content and homogeneity as well as the storage stability of fluopyram in capsules for the duration of the in-life phase of the study. A representative number of samples from dose groups 0.1X and 10X were analysed immediately after preparation.

The gelatine capsules with different dosages were stored separately in labelled plastic boxes at room temperature. The oral administration was performed via bolus dispenser. After the cows swallowed the gelatine capsules, the personnel waited for possible retching reaction. In case the capsule was disgorged, the test item was registered once again.

Every day, the animal in the control group (0X) received a gelatine capsule without the test item. The animals in the groups 0.1X, 1X, and 3X received one capsule per day and the animals in the groups 10X and 10XE two capsules per day. The capsules were administered in the order of sequence of the dosage groups 0X, 0.1X, 1X, 3X, 10X and 10XE. The first application was performed on day 0; and for 29 consecutive days, the application was performed after the evening milking and evening feeding.

General health status of animals was observed and inspected daily by veterinarian.

The animals had ad libitum access to drinking water via automatic drinking troughs providing water from the municipal drinking water supply.

The animals were fed twice daily via their throughs. The daily food consumption of the animals was recorded daily and for this purpose, the expected daily need for each cow was weighed individually. The weights of the feed leftovers were determined individually for each animal on the day after the feeding in the morning before the new morning feeding. For this purpose, the feed leftovers were taken from the

troughs and weighed. Since each cow had their individual through area, it was always possible clearly to assign the respective feed leftovers to the individual cow. The composition of the species-adequate mixed feed (with cereal-based dietary supplement improving milk yield and vitamin-mineral mixtures covering the daily requirements) is presented in the Table 6.4.2- 2.

Table 6.4.2- 2: Feed ration composition

Ingredient	%
Corn silage	54.6
Gras silage	31.2
Corn pellets	4.9
Soy pellets	2.9
Rape seed pellets	4.9
Feed lime	0.1
Salt	0.1
Molasses cutlets	1.0
QS- Mineral Feed Blatin 205 ADE SM for cattle (Percentage of ingredients: 13.1% Calcium, 6.0% Phosphorus, 10.2% Sodium, 10.0% Magnesium	0.2

Sampling

The cows were milked twice daily (in the morning and in the evening) with a mobile milking unit according to a good professional and hygienic practice common in dairy farming. On the day of slaughtering the cows were milked in the morning. Milk yields were recorded twice daily.

On day -12 during the acclimatisation period, approximately 2 kg milk were taken from animal no. 1. Until its transfer to Sample Logistics Lab, this sample was deep-frozen at -18 °C.

For the residue analysis, duplicate milk samples of the animals of the groups 0X, 0.1X, 1X, 3X, and 10X were taken before the 1st application as well as on the 1st, 2nd, 4th, 8th, 10th, 13th, 17th, 21st, 24th, 26th and 29th day after the 1st dosing. Milk samples of the three animals from the depuration group 10XE were taken accordingly from the 21st day of the experiment until the 29th day. Additionally, milk samples were taken from the animals of the group 10XE three times per week from the 30th day of the experiment until the day of the respective diagnostic slaughtering.

Milk samples were taken directly after milking after thorough mixing of the milk yield of one individual animal. If mastitis was diagnosed, the milk from the affected udder quarters was not mixed into the milk for the taking of milk samples. The morning and the evening milk samples were always taken in proportion to the respective milk yields, so that the proportions of evening and morning milk in one milk sample corresponded to the respective weight proportions of the milk yield. The evening milk sample was deep-frozen at below – 18 °C. After the morning milking, the necessary amount of morning milk was added to the frozen evening milk. These mixed samples were deep-frozen at below -18 °C until their transfer to Sample Logistics Lab for the residue analysis.

In the evening of the 20th day of the experiment and in the morning of the 21st day of the experiment, approximately 1 kg milk of each animal from the group 10X was taken for analytical evaluation of the accumulation of active items in the milk fat. These milk samples were stored cooled at 4 – 8 °C, and transferred to Sample Logistics Lab, cooled at > 0 °C, on the 21st day of the experiment.

The labelling of the sample containers indicated study number, group membership, animal or stall position number and time of the milking (date and indication of morning or evening milking).

The deep-frozen milk samples were transferred on dry ice between facilities and upon consultation. The sample duplicates (reserve samples) remained at one facility until the second confirmed complete and intact arrival of the samples.

Skim milk and cream was prepared by centrifugation of milk taken from the dose group 10X.

Terminal procedure

The diagnostic slaughtering was performed at the slaughterhouse in compliance with valid legal requirements and controlled by a public veterinary officer.

A dissection with pathological-anatomical examination was performed. Relevant findings that deviated from the physiological status were recorded in the dissection and sampling protocol. The diagnostic slaughtering of the animals of the study group 0.1X, 1X, 3X, and 10X as well as of the control animals of the 0X group took place on the day after the final dosing. Every day, the dosing occurred after the evening milking. Thus, it was ensured that the time point of the diagnostic slaughtering of the animals of the groups 0X, 0.1X, 1X, 3X and 10X was less than 24 hours after the final application. The animals were slaughtered diagnostically in groups, beginning with the control animal and subsequently with increasing dosage groups. The animals of the group 10XE were slaughtered diagnostically 7, 14 or 21 days after the final application (36, 43, and 50 days counting from the 1st day of this experiment).

The absolute organ weights of the liver and both kidneys were determined and recorded in the dissection and sampling protocol.

At least the following duplicate samples were taken from each animal:

- Kidney fat (approx. 2 × 250 g)
- Intestinal fat (approx. 2 × 400 g)
- Subcutaneous fat (approx. 2 × 400 g)
- Liver (without bile, approx. 2 × 250 g)
- Kidneys (approx. 2 × 250 g)
- Muscular tissue from sirloin, round muscle and skirt muscle (approx. 2 x 100 g each). The muscles samples were mixed into a joint muscle sample.

On the day of sampling, the organ and tissue samples were cut into pieces of approx.5 cm side lengths and frozen in layers in a sampling bag. The sampling bags were labelled with the study number, the group membership, the animal or stall position number, the type of sample and the day of the sampling. The samples were stored at below – 18 °C until their transfer to Sample Logistics Lab.

The deep-frozen organ and tissue samples were transferred on dry ice between facilities and upon consultation. The sample duplicates (reserve samples) remained at one facility until the second confirmed complete and intact arrival of the samples.

Analysis

The tissue samples except for fat were chopped together with dry ice by means of a meat chopper. Two 100-g portions of each sample were transferred into labelled containers. One container was handed over to the analytical laboratory. The second portion was retained and stored at – 18 °C.

The fat samples were chopped together with dry ice and divided into three 100-g portions. One container was handed over to the analytical laboratory. The second portion was retained and stored at – 18 °C. The third container was sent frozen to SGS Germany GmbH, Laboratory Services Hamburg, Weidenbaumsweg 137, D-21035 Hamburg to determine the actual fat content.

Residue of fluopyram and its metabolites (Fluopyram-benzamide and the total residue of Fluopyram-olefins) in milk and tissues were determined using the analytical method 01061 ([redacted], 2007, M-295705-02-1; see MCA Section 4.1.2). Full details and acceptable validation data to support this

method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

The residues of fluopyram and its metabolites Fluopyram-benzamide and total residue of Fluopyram-olefines were extracted from milk, fat, liver, muscle, and kidney using a mixture of acetonitrile/water (4/1). After filtration, the extracts were cleaned-up on Mega Bond Elut-C18 cartridges. Aliquots of the extracts were diluted with a mixture of methanol/water (containing the corresponding internal standards).

The residues were determined by LC-MS/MS equipped with electrospray detection. (external calibration, matrix-matched standards + internal standards).

The limit of quantification (LOQ) is 0.01 mg/kg for all analytes (expressed as parent equivalents) for all matrices. (whereas the LOQ for the total residues of olefins is 0.02 mg/kg for all matrices). Any residue measured or calculated to be less than the LOQ was reported as "< LOQ".

The Limit of Detection (LOD) was set to 0.003 mg/kg for fluopyram, 0.001 mg/kg for FLU-benzamide and 0.002 mg/kg for the two FLU-olefins isomers (expressed as parent equivalents each). The LOD for the total residues of olefines was set to 0.004 mg/kg. Any residue value that was below the LOD is given as "< LOD"

In order to check the performance of the analytical method concurrent recovery experiments were conducted along with the analysis of the samples.

The fortification levels for the measured matrices were between 0.01 mg/kg (LOQ level) 40 mg/kg for fluopyram and FLU-benzamide (expressed as parent equivalents). The fortification levels for the measured matrices were between 0.02 mg/kg (LOQ level) and 80 mg/kg for total residues of FLU-olefins (expressed as parent equivalents). Recoveries for the total residues of olefins were measured by spiking both isomers at fortification levels of 0.01 mg/kg each, separate analyses of both isomers, summing up residues and subsequent calculation of the recovery.

Concurrent recoveries are presented in Table 6.4.2- 3.

II. Findings

Dose verification and storage stability

Capsules were prepared prior to the dosing phase by weighing the test item into capsules. Upon analysis by HPLC-MS/MS, fluopyram concentration in capsules ranged from 94 % to 110 % of nominal values (mean per level: 22.75 mg/capsule, n = 5 for dose group 0.1X; mean per level: 1145 mg/capsule, n = 5 for dose group 10X) immediately after application. After the final dosing, fluopyram concentration in capsules ranged from 92 % to 114 % of nominal values (mean per level: 23.65 mg/capsule, n = 5 for dose group 0.1X; mean per level: 1111 mg/capsule, n = 5 for dose group 10X) indicating the capsules to be stable over the course of the study.

In-life observations

All 15 animals of the groups 0, 0.1X, 1X, 3X, 10X and 10XE tolerated the 29-fold oral application of the test material without any clinical complications. During the dosing period and also during the depuration period, the daily clinical control of the health status of none of the animals resulted in any findings deviating from the physiological normal values that related to the application of the test item. Further, no negative influence of the test item on food consumption was determined with the study conditions.

Analysis of milk, milk products and tissues

Recoveries of fluopyram and its metabolites were measured concurrently with each set of samples to verify method performance. The concurrent recovery data are summarized in Table 6.4.2- 3. The data demonstrate acceptable method performance during sample analysis.

No apparent residues of fluopyram and its metabolites above 30 % of LOQ were found in any of the control samples used for recovery experiments. Procedural recoveries from the fortified control samples were not corrected for any apparent residue in the associated unfortified control.

The tissue and milk samples in this study were analysed within 30 days of collection; therefore, freezer storage stability studies on dairy cattle tissue and milk matrices were not required.

Table 6.4.2- 3: Concurrent recovery data for fluopyram and its metabolites in milk and bovine tissues

Sample material	FL [mg/kg]*	Single values [%]	Mean value [%]	RSD [%]	LOQ [mg/kg]*
Fluopyram					
Cattle/milk	0.01	89; 82; 112; 102; 92; 94; 109; 117; 82	98	13.2	0.01
	0.10	97; 98; 103; 102	100	2.9	
	1.0	73; 70; 71	71	2.1	
	5.0	113; 115; 103; 113; 111; 107	110	4.1	
	20	83; 91	87		
		Overall recovery (n = 24)	**97**	**14.9**	
Cattle/cream	0.01	95; 92	94	--	0.01
	0.10	90; 91	91	--	
	20	96	96	--	
		Overall recovery (n = 5)	**93**	**2.8**	
Cattle/skim milk	0.01	103; 106	105	--	0.01
	0.10	100; 103	102	--	
	20	100	100	--	
		Overall recovery (n = 5)	**102**	**2.5**	
Cattle/muscle	0.01	83	83	--	0.01
	0.10	88	88	--	
	20	72	72	--	
		Overall recovery (n = 3)	**81**	**10.1**	
Cattle/kidney	0.01	64; 88	76	--	0.01
	0.10	88	88	--	
	20	70	70	--	
		Overall recovery (n = 4)	**78**	**16.0**	
Cattle/liver	0.01	104; 91	98	--	0.01
	0.10	80	80	--	
	40	108	108	--	
		Overall recovery (n = 4)	**96**	**13.3**	
Cattle/fat	0.01	88; 93; 101; 112; 94	98	**9.5**	0.01
	0.10	92	92	--	
	10	83; 79	81	--	
		Overall recovery (n = 8)	**93**	**11.1**	
Sample material	**FL [mg/kg]***	**Single values [%]**	**Mean value [%]**	**RSD [%]**	**LOQ [mg/kg]***
Fluopyram-benzamide					
Cattle/milk	0.01	86; 87; 97; 98; 100; 97; 115; 118; 90	99	11.4	0.01
	0.10	87; 88; 99; 98	93	6.9	

Sample material	FL [mg/kg]*	Single values [%]	Mean value [%]	RSD [%]	LOQ [mg/kg]*
	1.0	92; 89; 92	91	1.9	
	5.0	110; 112; 108; 99; 106; 100	106	5.0	
	20	79; 86	83	--	
		Overall recovery (n = 24)	**97**	**10.5**	
Cattle/cream	0.01	100; 96	98	--	0.01
	0.10	95; 94	95	--	
	20	95	95	--	
		Overall recovery (n = 5)	**96**	**2.4**	
Cattle/skim milk	0.01	99; 99	99	--	0.01
	0.10	90; 93	92	--	
	20	95	95	--	
		Overall recovery (n = 5)	**95**	**4.1**	
Cattle/muscle	0.01	79	79	--	0.01
	0.10	78	78	--	
	20	67	67	--	
		Overall recovery (n = 3)	**75**	**8.9**	
Cattle/kidney	0.01	58; 101	80	--	0.01
	0.10	74	74	--	
	20	69	69	--	
		Overall recovery (n = 4)	**76**	**24.2**	
Cattle/liver	0.01	99; 96	98	--	0.01
	0.10	71	71	--	
	40	104	104	--	
		Overall recovery (n = 4)	**93**	**15.9**	
Cattle/fat	0.01	87; 94; 99; 101; 101	96	6.2	0.01
	0.10	86	86	--	
	10	75; 83	79	--	
		Overall recovery (n = 8)	**91**	**10.5**	
Sample material	**FL* [mg/kg]**	**Single values [%]**	**Mean value [%]**	**RSD [%]**	**LOQ [mg/kg]***
Total Residue of Fluopyram-olefins**					
Cattle/milk	0.01	100; 112; 94; 100; 96; 90; 125; 100; 101	103	10.5	0.02
	0.10	90; 93; 101; 97	95	5.0	
	1.0	97; 95; 98	97	1.6	
	5.0	140; 146; 130; 132; 131; 133	135	4.7	
	20	79; 85	82	--	
		Overall recovery (n = 24)	**107**	**17.7**	
Cattle/cream	0.01	97; 96	97	--	0.02
	0.10	100; 95	98	--	
	20	92	92	--	
		Overall recovery (n = 5)	**96**	**3.0**	
Cattle/skim milk	0.01	99; 95	97	--	0.02
	0.10	93; 93	93	--	
	20	94	94	--	
		Overall recovery (n =5)	**95**	**2.6**	
Cattle/muscle	0.01	91	91	--	0.02
	0.10	94	94	--	
	20	76	76	--	

Sample material	FL [mg/kg]*	Single values [%]	Mean value [%]	RSD [%]	LOQ [mg/kg]*
		Overall recovery (n = 3)	**87**	**11.1**	
Cattle/kidney	0.01	72; 103	88	--	0.02
	0.10	108	108	--	
	20	81	81	--	
		Overall recovery (n = 4)	**91**	**19.0**	
Cattle/liver	0.01	103, 92	98	--	0.02
	0.10	102	102	--	
	40	99	99	--	
		Overall recovery (n = 4)	**99**	**5.0**	
Cattle/fat	0.01	82; 94; 87; 93; 101	91	7.9	0.02
	0.10	100	100	--	
	10	81; 92	87	--	
		Overall recovery (n = 8)	**91**	**8.2**	

FL = Fortification level, RSD = Relative standard deviation, LOQ = Practical limit of quantification
* : Expressed as parent equivalents.
** : Both isomers (E- and Z-isomer) were fortified with a ratio of 1/1.
Final determination as: Fluopyram Residues calculated as: Fluopyram
Final determination as: FLU-benzamide Residues calculated as: Fluopyram
Final determination as: FLU-olefins (E- and Z-isomer) Residues calculated as: Total Residue of Olefins (expressed as parent equivalents)

No residues of fluopyram and its metabolites above the respective LOQs were detected in any of the untreated samples of milk and tissues (control samples).

Residues of fluopyram and its metabolites in bovine milk and tissues samples are summarized in the Table 6.4.2- 4 and Table 6.4.2- 6 respectively.

All residues further discussed are calculated and expressed as fluopyram parent equivalents.

The mean residues of fluopyram in milk :

- residues remained below the LOD in the dose group B (0.1 X)
- between <LOD and 0.02 mg/kg in the dose group C (1X) until residue plateau at 0.01 mg/kg.
- between 0.02 and 0.05 mg/kg group D (3X), until residue plateau around 0.3 mg/kg.
- between 0.08 and 0.16 mg/kg, group E (10X), (0.16 mg/kg occurring on day 4). Until residue plateau around 0.10-0.12 mg/kg

Mean residues of FLU-benzamide in milk :

- between <LOQ and 0.04 mg/kg group B (0.1X), until residue plateau at 0.02 mg/kg
- between 0.01 mg/kg and 0.25 mg/kg group C (1X), until residue plateau around 0.23-0.25 mg/kg
- between 0.03 mg/kg and 0.65 mg/kg group D (3X), (0.65 mg/kg occurring on day 4) until residue plateau around 0.52-0.65 mg/kg
- between 0.08 mg/kg and 1.8 mg/kg group E (10X), (1.8 mg/kg occurring on day 8) until residue plateau around 1.2-1.4 mg/kg

The mean values of the total residues of FLU-olefins in milk:

- residues remained below the LOD in the group B (0.1X)
- between <LOD and 0.02 mg/kg in the dose group C (1X) until residue plateau at 0.02 mg/kg.
- between <LOD and 0.03 mg/kg group D (3X)g until residue plateau around 0.02-0.03 mg/kg
- between <LOD and 0.12 mg/kg group E (10X), (0.12 mg/kg occurring on day 26) until residue plateau around 0.1 mg/kg

The results are summarized in the table Table 6.4.2- 4 and detailed in the Table 6.4.2- 8.

Table 6.4.2- 4: Residues (mg/kg) of AE C656948 and its metabolites in milk

Day	Residues (mg parent equivalents/kg) in Milk							
	Dose Group B (0.1X)				Dose Group C (1X)			
	fluopyram	FLU-benzamide	FLU-olefins	Total residue	fluopyram	FLU-benzamide	FLU-olefins	Total residue
-7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
1	<LOD	<LOD	<LOD	<0.04	<LOQ	0.02	<LOD	<0.04
2	<LOD	<LOD	<LOD	<0.04	0.02	0.05	<LOD	0.07
4	<LOD	0.01	<LOD	<0.04	0.01	0.12	< LOQ	0.15
8	<LOD	0.02	<LOD	<0.04	0.01	0.19	0.02	0.22
10	<LOD	0.02	<LOD	<0.04	0.01	0.19	< LOQ	0.22
13	<LOD	0.02	<LOD	<0.04	0.01	0.21	0.02	0.24
17	<LOD	0.02	<LOD	<0.04	0.01	0.22	0.02	0.25
21	<LOD	0.04	<LOD	<0.04	0.01	0.24	0.02	0.27
24	<LOD	0.02	<LOD	<0.04	0.01	0.25	0.02	0.32
26	<LOD	0.02	<LOD	<0.04	0.01	0.23	0.02	0.25
29	<LOD	0.02	<LOD	<0.04	0.01	0.25	0.02	0.28
Day	Dose Group D (3X)				Dose Group E (10X)			
	fluopyram	FLU-benzamide	FLU-olefins	Total residue	fluopyram	FLU-benzamide	FLU-olefins	Total residue
-7	<LOD	<LOD	<LOD	<LOD	< LOD	< LOD	< LOD	<LOD
1	0.02	0.03	< LOQ	0.06	0.09	0.08	0.02	0.18
2	0.05	0.13	< LOQ	0.20	0.13	0.34	0.03	0.50
4	0.05	0.33	0.02	0.37	0.16	0.81	0.05	1.01
8	0.04	0.55	0.02	0.61	0.11	1.8	0.09	1.95
10	0.03	0.54	0.02	0.59	0.09	1.5	0.08	1.61
13	0.03	0.53	0.02	0.58	0.08	1.6	0.09	1.76
17	0.02	0.53	0.03	0.57	0.08	1.7	0.10	1.88
21	0.02	0.45	0.02	0.49	0.10	1.2	0.10	1.39
24	0.05	0.52	0.03	0.60	0.12	1.3	0.10	1.52
26	0.03	0.65	0.03	0.70	0.11	1.3	0.12	1.47
29	0.03	0.65	0.02	0.61	0.10	1.4	0.10	1.60

For the calculation of the mean residues, in case we have 3 levels of which at least one is <LOD and others <LOQ, it was legitimate to set the mean value as <LOQ;
For the calculation of the mean residues, in case one or two individual values are >LOQ and the others < LOQ, it was deemed appropriate to consider residues <LOQ mg/kg as being equal to LOQ mg/kg. This approach represents the worst-case scenario.
In milk samples, if among three values there was at least one value > LOQ, for the calculation of the mean all the other values <LOQ and <LOD were set equal to LOQ and LOD, respectively, this approach represents the worst-case scenario.

Mean total residues of fluopyram (sum of FLU+FLU-benzamide+FLU-olefins) in milk :

- between <LOQ and <0.04 mg/kg group B (0.1X),
- between <0.04 mg/kg and 0.32 mg/kg group C (1X), until residue plateau around 0.3 mg/kg
- between 0.06 mg/kg and 0.70 mg/kg group D (3X), until residue plateau around 0.6 mg/kg
- between 0.18 mg/kg and 1.95 mg/kg group E (10X), until residue plateau around 1.5 mg/kg

Milk taken from dose group E (10X) was separated by centrifugation into skim milk and cream. The mean residues of fluopyram in skim milk and cream were 0.02 mg/kg and 1.3 mg/kg, respectively. The mean

residues of FLU-benzamide in skim milk and cream were 1.5 mg/kg and 0.85 mg/kg, respectively. The mean value of the total residues of olefins in skim milk and cream were below the LOD and 1.0 mg/kg, respectively. All the residue values are presented in the Table 6.4.2- 5.

Table 6.4.2- 5: Residues (mg/kg) of fluopyram and its metabolites in skim milk and cream

Group Dose	Sample	Residue levels of individual analytes (mg/kg) in skim milk and cream Mean of 3 sub-groups (individual values) *			
		Fluopyram	FLU-benzamide	Total residues of Olefins	Total residue
10X 4.05 mg/kg bw/day 133.1 mg/kg DM **Sub-groups:** E1, E2, E3	Skim milk (milk whey)	0.02 *(0.02; n.a.; 0.02)*	1.5 *(1.5; n.a.; 1.4)*	< LOD *(< LOD; n.a.; < LOD)*	1.47 (1.42; 1.42)
	Cream (milk fat)	1.3 *(1.1; n.a.; 1.4)*	0.85 *(0.98; n.a.; 0.72)*	1.0 *(0.78; n.a.; 1.3)*	3.14 (2.86; 3.42)

* Mean of the 2 sub-groups calculated based on the unrounded residue results.
n.a. not applicable
LOQ = 0.01 mg/kg for fluopyram and the benzamide metabolite, expressed as parent equivalents
LOQ = 0.02 mg/kg for the total residue of olefins was calculated as sum of the two individual olefin isomers.
LOD = 0.003 mg/kg for fluopyram
LOD = 0.001 mg/kg for FLU-benzamide
LOD = 0.004 mg/kg for the total residue of olefins.

In animal tissues, fluopyram residues showed a clear dose response and were mainly found as follows:

- in liver, mean residues were between 0.25 mg/kg (0.1X group) and 4.0 mg/kg (10X group).
- in fat between 0.03 and 0.69 mg/kg (mesenteric fat), between 0.04 and 0.57 mg/kg (subcutaneous fat) and between 0.04 and 0.49 mg/kg (perirenal fat) residues were measured.
- in kidney values from <LOD (0.1X group) to 0.07 mg/kg (10X group)
- in muscle values from <LOD (0.1X group) to 0.03 mg/kg (10X group).

Fluopyram-benzamide residues showed a clear dose response and were mainly found as follows :

- in liver, mean residues were between 0.1 mg/kg (0.1X group) and 6.9 mg/kg (10X group).
- In fat between <0. 01 (0.1X group) and 0.72 mg/kg (10X group) (mesenteric fat), between 0.01 (0.1X group) and 1.0 mg/kg (10X group) (subcutaneous fat) and between 0.18 (1X group) and 0.85 mg/kg (10X group) (perirenal fat) were measured.
- In kidney between 0.03 (0.1X group) and 1.6 mg/kg (10X group).
- In muscle between 0.02 (0.1X group) and 1.4 mg/kg (10X group). The FLU-benzamide

Fluopyram-olefins residues showed a clear dose response and were mainly found as follows

- in liver ranging from 0.04 to 0.50 mg/kg
- in fat, residues between 0.09 (1X group) and 0.85 mg/kg (10X group) (perirenal fat), between 0.07 (1X group) and 0.90 mg/kg (10X group) (mesenteric fat) and between 0.06 (1X group) and 0.55 mg/kg (10X group) (subcutaneous fat) were measured.
- in kidney values from <LOD (0.1X group) to 0.13 mg/kg (10X group)
- in muscle values from <LOD (0.1 X group) to 0.04 mg/kg (10X group)

The results are summarized in the Table 6.4.2- 6 and detailed in the

Table 6.4.2- 9.

Table 6.4.2- 6: Residues (mg/kg) of Fluopyram and its metabolites in animal tissues.

Residues (mg parent equivalents/kg)				
Dose Group B (0.1X)				
Tissue	**fluopyram**	**FLU-benzamide**	**FLU-olefins**	**Total residue**
Perirenal Fat	< LOQ	< LOQ	< LOQ	<0.04
Mesenteric Fat	< LOQ	< LOQ	< LOQ	<0.04
Subcutaneous Fat	< LOQ	0.01	< LOQ	0.04
Liver	0.25	0.10	< LOD	0.35
Muscle	< LOD	0.02	< LOD	<0.04
Kidney	< LOD	0.03	< LOD	0.04
Dose Group C (1X)				
Tissue	**fluopyram**	**FLU-benzamide**	**FLU-olefins**	**Total residue**
Perirenal Fat	0.04	0.18	0.09	0.31
Mesenteric Fat	0.03	0.16	0.07	0.26
Subcutaneous Fat	0.04	0.18	0.06	0.28
Liver	0.74	12	0.04	1.95
Muscle	< LOQ	0.29	< LOQ	0.32
Kidney	< LOQ	0.28	< LOQ	0.31
Dose Group D (3X)				
Tissue	**fluopyram**	**FLU-benzamide**	**FLU-olefins**	**Total residue**
Perirenal Fat	0.25	0.27	0.28	0.80
Mesenteric Fat	0.25	0.26	0.29	0.80
Subcutaneous Fat	0.24	0.37	0.18	0.79
Liver	2.1	2.8	0.12	5.02
Muscle	0.02	0.6	0.02	0.64
Kidney	0.03	0.72	0.04	0.79
Dose Group E (10X)				
Tissue	**fluopyram**	**FLU-benzamide**	**FLU-olefins**	**Total residue**
Perirenal Fat	0.49	0.85	0.85	2.19
Mesenteric Fat	0.69	0.72	0.9	2.31
Subcutaneous Fat	0.57	1.0	0.55	2.12
Milk Fat (Cream)	1.3	0.85	1.0	
Milk Whey (Skim Milk)	0.02	1.45	< LOD	
Liver	4.0	6.9	0.5	11.4
Muscle	0.03	1.4	0.04	1.47
Kidney	0.07	1.6	0.13	1.80

In case we have 3 levels of which at least one is <LOD and others <LOQ, it was legitimate to set the mean value as <LOQ;
When one or two individual values are >LOQ and the others < LOQ or <LOD, residues <LOQ or <LOD are considered equal to LOQ or LOD.
LOQ = 0.04 mg/kg for fluopyram and the benzamide metabolite, expressed as parent equivalents.
LOQ =0.02 mg/kg for the total residue of FLU-olefins was calculated as sum of the two individual olefin isomers.
LOQ=0.04 mg/kg for the total residue of Fluopyram calculated as sum of the parent + FLU-benzamide + the two olefin isomers
LOD = 0.002 mg/kg for fluopyram, LOD = 0.001 mg/kg for FLU-benzamide, LOD = 0.004 mg/kg for the total residue of olefins.

Mean total residues of fluopyram (sum of FLU+FLU-benzamide+FLU-olefins) in tissues :

- in liver, mean residues were between 0.35 mg/kg (0.1X group) and 11.4 mg/kg (10X group).
- In fat between <0. 04 (0.1X group) and 2.31 mg/kg (10X group) (mesenteric fat), between 0.04 (0.1X group) and 2.12 mg/kg (10X group) (subcutaneous fat) and between <0.04 (0.1X group) and 2.19 mg/kg (10X group) (perirenal fat) were measured.
- In kidney between 0.03 (0.1X group) and 1.6 mg/kg (10X group).
- In muscle between 0.02 (0.1X group) and 1.4 mg/kg (10X group). The FLU-benzamide

The calculated transfer factors, i.e. the ratio of residue level in milk and tissue to the residue level in the feed, are summarised in the Table 6.4.2- 7.

Table 6.4.2- 7: Calculated transfer factors in cattle

Feeding level	1.5 mg/kg DM feed (0.1X group)		14.4 mg/kg DM feed (1X group)		44.1 mg/kg DM feed (3X group)		145.9 mg/kg DM feed (10X group)	
Fluopyram								
Commodity	**mg/kg**	**TF**	**mg/kg**	**TF**	**mg/kg**	**TF**	**mg/kg**	**TF**
Milk	< LOD	**nd**	0.01	**0.0007**	0.05	**0.001**	0.12	**0.0008**
Perirenal Fat	< LOQ	**nd**	0.04	**0.003**	0.25	**0.006**	0.49	**0.003**
Mesenteric Fat	< LOQ	**nd**	0.04	**0.003**	0.25	**0.006**	0.69	**0.005**
Subcutaneous Fat	< LOQ	**nd**	0.04	**0.003**	0.25	**0.006**	0.57	**0.004**
Liver	0.25	**0.167**	0.71	**0.05**	2.1	**0.05**	4	**0.03**
Muscle	< LOD	**nd**	< LOQ	**nd**	0.02	**0.0005**	0.03	**0.0002**
Kidney	< LOD	**nd**	< LOQ	**nd**	0.03	**0.0007**	0.07	**0.0005**
Fluopyram-benzamide								
Commodity	**mg/kg**	**TF**	**mg/kg**	**Tf**	**mg/kg**	**TF**	**mg/kg**	**Tf**
Milk	0.04	**0.03**	0.25	**0.02**	0.65	**0.01**	1.7	**0.01**
Perirenal Fat	< LOQ	**nd**	0.18	**0.01**	0.27	**0.006**	0.85	**0.006**
Mesenteric Fat	< LOQ	**nd**	0.16	**0.01**	0.26	**0.006**	0.72	**0.005**
Subcutaneous Fat	< LOQ	**nd**	0.18	**0.01**	0.37	**0.008**	1	**0.007**
Liver	0.1	**0.07**	1.2	**0.08**	2.8	**0.06**	6.9	**0.05**
Muscle	0.02	**0.01**	0.29	**0.02**	0.6	**0.01**	1.4	**0.01**
Kidney	0.03	**0.02**	0.28	**0.02**	0.72	**0.02**	1.6	**0.01**
Fluopyram-olefins								
Commodity	**mg/kg**	**TF**	**mg/kg**	**TF**	**mg/kg**	**Tf**	**mg/kg**	**TF**
Milk	< LOD	**nd**	< LOQ	**nd**	0.03	**0.0007**	0.12	**0.0008**
Perirenal Fat	< LOQ	**nd**	0.08	**0.006**	0.28	**0.006**	0.85	**0.006**
Mesenteric Fat	< LOQ	**nd**	0.07	**0.005**	0.29	**0.007**	0.9	**0.006**
Subcutaneous Fat	< LOQ	**nd**	0.06	**0.004**	0.18	**0.004**	0.55	**0.004**
Liver	< LOD	**nd**	0.04	**0.003**	0.12	**0.003**	0.5	**0.003**
Muscle	< LOD	**nd**	< LOQ	**nd**	0.02	**0.0005**	0.04	**0.0003**
Kidney	< LOD	**nd**	< LOQ	**nd**	0.04	**0.0009**	0.13	**0.0009**

Depuration phase

For the depuration phase of the study, an additional dosing group of three animals were fed at the 10X dose level simultaneously with the animals from dose group E (10X) followed by untreated feeding for another 7 days (animal No. 16), 14 days (animal No. 15) and 21 days (animal No. 14).

Milk was collected from the additional dosing group on day 21 to 36 after the first dosing and from the remaining cows additionally on 38 to 50. Residues in tissues were determined at sacrifice, on Day 36 (animal No. 16), 43 (animal No. 15) and 50 (animal No. 14).

During the depuration phase (study conducted for the dose group 10XE), the mean residues of fluopyram in milk was 0.11 mg/kg at the end of the dosing period (day 29) for the three animals. The mean residues of fluopyram decreased to mean values below the LOQ for all animals on day 31 and afterwards remained below the LOD until the end of the study.

The mean residue of FLU-benzamide in milk was 2.7 mg/kg at the end of the dosing period (day 29) for the three animals and decreased to mean values of 0.02 mg/kg on day 43, and after for one anima (cow No. 14) values below the LOQ were measured from Day 47 until the end of the study.

The mean value of the total residues of olefins in milk was 0.13 mg/kg at the end of the dosing period (day 29) for the three animals and decreased to values between 0.04 mg/kg and 0.02 from day 36 till 45, and for one animal (cow no. 14) values below the LOQ were measured from Day 47 until the end of the study.

In tissues, residues of fluopyram were below the LOD with the exception of the liver sample taken from animal No. 16 (day 36), where 0.06 mg/kg of fluopyram were measured.

Residues of FLU-benzamide

- in liver decreased from 2.8 mg/kg (animal No. 16, day 36) to 0.42 mg/kg measured on day 50 (animal No. 14)
- in muscle decreased from 0.77 mg/kg (animal No. 16, day 36) to 0.15 mg/kg (animal No. 15, day 43) and 0.19 mg/kg (animal No. 14, day 50)
- in perirenal, mesenteric and subcutaneous fat on day 36 (animal No. 16) ranged between 0.45 and 0.58 mg/kg and decreased to values below the LOQ or LOD on day 43 and 50.

The total residues of FLU-olefins

- in liver decreased from 0.08 mg/kg (on day 36, animal No. 16) to 0.04 mg/kg on day 50 (animal No. 14)
- in subcutaneous fat increased from 0.04 mg/kg (animal No. 16, day 36) to 0.28 mg/kg (animal No. 14, day 50).
- in perirenal and mesenteric fat (0.12 and 0.13 mg/kg, respectively) (animal No. 16, day 36) increased to 0.21 and 0.26 mg/kg on day 43 (animal No. 15) and reached a total residue level of 0.11 mg/kg on day 50 (animal No. 14)
- in muscle were below the LOQ.

A graphical presentation of the depuration curves for the major metabolite, FLU-benzamide, is presented from 0 to Figure 6.1.1- 5 for milk, fat and other tissues, respectively.

Figure 6.1.1- 3: Depuration curve of residue of Fluopyram-benzamide in milk.

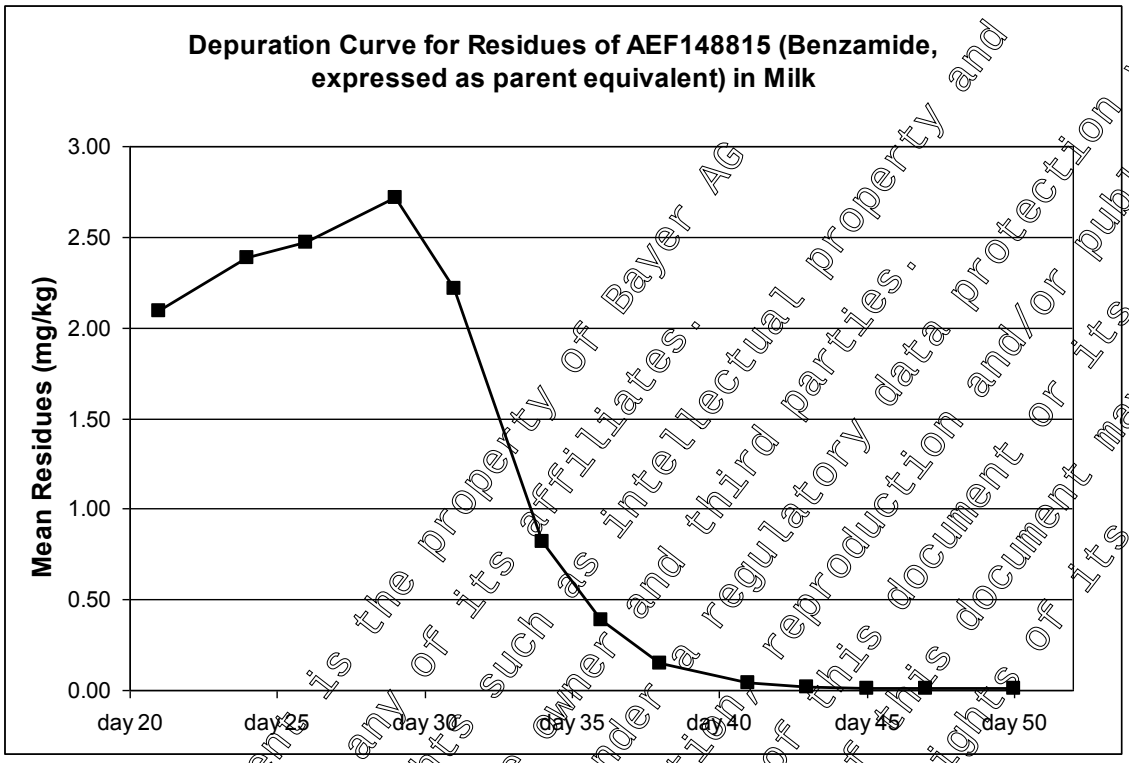


Figure 6.1.1- 4: **Depuration curve of residue of Fluopyram-benzamide in tissues.**

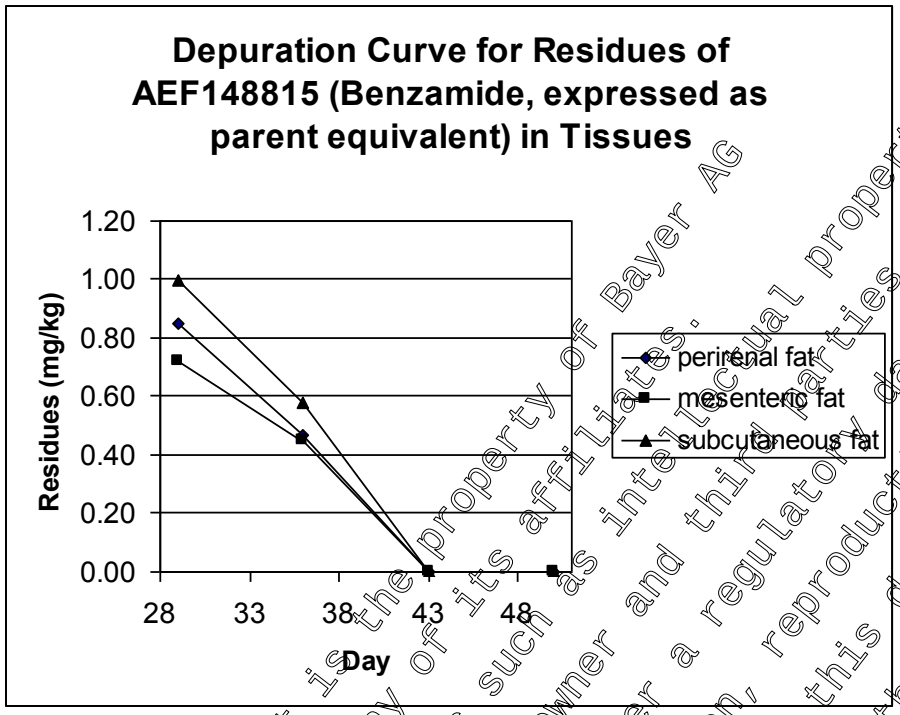
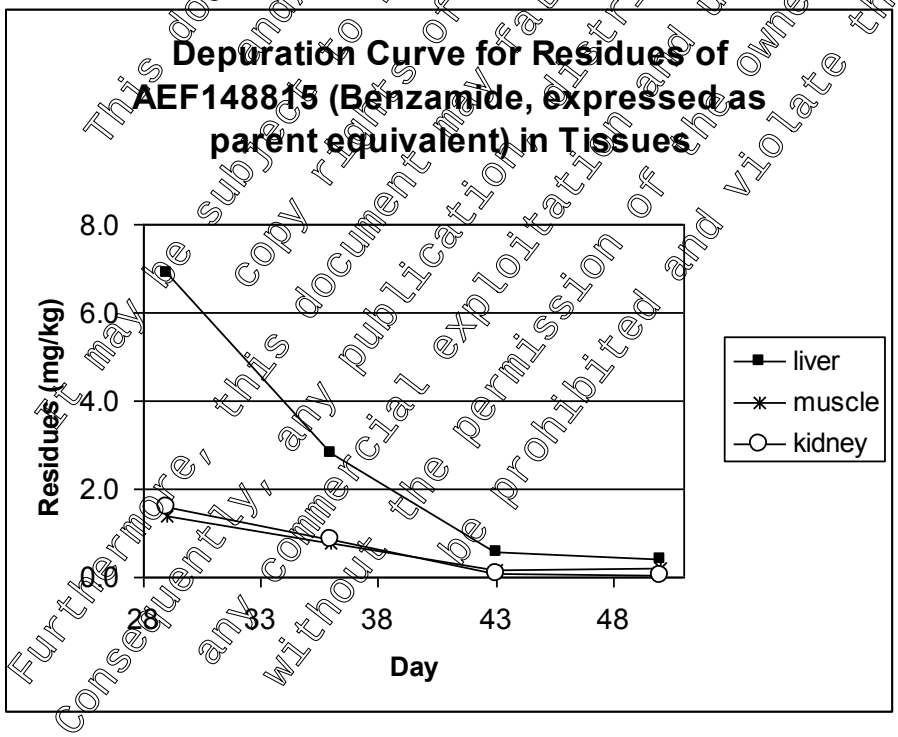


Figure 6.1.1- 5: **Depuration curve of residue of Fluopyram-benzamide in tissues.**



According to the observed linear correlation between dose and residue levels in animal commodities after dosing, we assume that the same behaviour applies during the depuration phase in the 0.1X, 1X and 3X doses.

III. Conclusions

A feeding study was conducted with fluopyram on dairy cows in order to elucidate the levels of relevant residues in cow tissues and in milk.

Fluopyram was administered orally *via* double-coated gelatine capsules to dairy cows for 29 consecutive days at the actual dose rates of 0.04 mg/kg bw/day (0.1X), 0.44 mg/kg bw/day (1X), 1.21 mg/kg bw/day (3X), 4.05 mg/kg bw/day (10X), and 4.38 mg/kg bw/day (10XE feeding + depuration phase).

Feed consumption, body weights, and milk production were not adversely affected by compound administration.

Prior to sacrifice, residues in milk were measured at various intervals. After the final dose, the animals were sacrificed and the key edible tissues were analysed for the residues of fluopyram and its metabolites fluopyram-benzamide, and total residues of fluopyram-olefins in all matrices. Additionally, the residues were determined in skim milk and cream produced from the milk obtained from the dose group E (10X).

Overall, most of the time residues of fluopyram, and its metabolites (FLU-benzamide and total residues of FLU-olefins) were found in milk and tissues and showed a clear dose-response; therefore, the residue data set provided in this study is suitable for the regulatory purposes.

Table 6.4.2- 8: Summary of residues of fluopyram and its metabolites in bovine milk, skimmed milk and cream (mg/kg)

Group Dose	Sampling date*	Residue levels of individual analytes (mg/kg)							
		fluopyram		FLU-benzamide		Total residue of FLU-olefins		Total residue	
		Indiv.	mean	Indiv.	mean	Indiv.	mean	Indiv.	mean
0.1X 0.04 mg/kg bw/day 1.5 mg/kg DM **Sub-groups:** B1, B2, B3	Pre-dosing -7	3x<LOD	<LOD	3x<LOD	<LOD	3x<LOD	<LOD	3x<LOD	<LOD
	1	3x<LOD	<LOD	3x<LOQ	<LOD	3x<LOD	<LOD	3x<0.04	<0.04
	2	3x<LOD	<LOD	3x<LOQ	<LOD	3x<LOD	<LOD	3x<0.04	<0.04
	4	3x<LOD	<LOD	<LOQ; 0.01; 0.02	0.01	3x<LOD	<LOD	3x<0.04	<0.04
	8	3x<LOD	<LOD	0.02; 0.02; 0.02	0.02	3x<LOD	<LOD	3x<0.04	<0.04
	10	3x<LOD	<LOD	0.01; 0.01; 0.02	0.02	3x<LOD	<LOD	3x<0.04	<0.04
	13	3x<LOD	<LOD	0.01; 0.02; 0.03	0.02	3x<LOD	<LOD	3x<0.04	<0.04
	17	3x<LOD	<LOD	0.02; 0.02; 0.02	0.02	3x<LOD	<LOD	3x<0.04	<0.04
	21	3x<LOD	<LOD	0.09; 0.02; 0.02	0.04	3x<LOD	<LOD	2x<0.04; 0.05	<0.04
	24	3x<LOD	<LOD	0.02; 0.02; 0.02	0.02	3x<LOD	<LOD	3x<0.04	<0.04
	26	3x<LOD	<LOD	0.02; 0.01; 0.02	0.02	3x<LOD	<LOD	3x<0.04	<0.04
	29	3x<LOD	<LOD	0.03; 0.01; 0.02	0.02	3x<LOD	<LOD	3x<0.04	<0.04
1X 0.44 mg/kg bw/day 14.4 mg/kg DM **Sub-groups:** C1, C2, C3	Pre-dosing -7	3x<LOD	<LOD	3x<LOD	<LOD	3x<LOD	<LOD	3x<LOD	<LOD
	1	2x<LOQ; 0.01	<LOQ	0.01; 0.02; 0.02	0.02	3x<LOD	<LOD	3x<0.04	<0.04
	2	<LOQ; 0.02; 0.02	0.02	0.05; 0.06; 0.04	0.05	3x<LOD	<LOD	0.06; 0.08; 0.08	0.07
	4	<LOQ; 0.01; 0.02	0.01	0.12; 0.12; 0.12	0.12	<LOD; 2x<LOQ	< LOQ	0.13; 0.15; 0.16	0.15
	8	<LOQ; 0.01; 0.02	0.01	0.17; 0.18; 0.22	0.19	2x<LOQ; 0.02	0.02	0.20; 0.21; 0.24	0.22
	10	<LOQ; 0.01; 0.02	0.01	0.20; 0.17; 0.19	0.19	3x<LOQ	< LOQ	0.23; 0.20; 0.23	0.22
	13	<LOQ; 0.01; 0.02	0.01	0.23; 0.21; 0.19	0.21	2x<LOQ; 0.02	0.02	0.26; 0.24; 0.23	0.24
	17	2x<LOQ; 0.01	0.01	0.20; 0.20; 0.25	0.22	2x<LOQ; 0.02	0.02	0.23; 0.23; 0.28	0.25
	21	<LOQ; 0.01; 0.01	0.01	0.18; 0.26; 0.27	0.24	2x<LOQ; 0.02	0.02	0.21; 0.31; 0.30	0.27
	24	<LOQ; 0.01; 0.01	0.01	0.17; 0.26; 0.31	0.25	2x<LOQ; 0.02	0.02	0.30; 0.32; 0.33	0.32
	26	<LOQ; 0.01; 0.01	0.01	0.15; 0.22; 0.31	0.23	2x<LOQ; 0.02	0.02	0.15; 0.25; 0.34	0.25
	29	2x<LOQ; 0.02	0.01	0.16; 0.21; 0.37	0.25	2x<LOQ; 0.02	0.02	0.19; 0.24; 0.41	0.28

* overall study day, n.a. not applicable
In case we have 3 levels of which at least one is <LOD and others <LOQ, it was legitimate to set the mean value as <LOQ;
When one or two individual values are ≥LOQ and the others < LOQ or <LOD, residues <LOQ or <LOD are considered equal to LOQ or LOD

LOQ = 0.01 mg/kg for fluopyram and the benzamide metabolite, expressed as parent equivalents.
LOQ = 0.02 mg/kg for the total residue of FLU-olefins was calculated as sum of the two individual olefin isomers.
LOD = 0.003 mg/kg for fluopyram, LOD = 0.001 mg/kg for FLU-benzamide, LOD = 0.004 mg/kg for the total residue of olefins.

Table 6.4.2-8 (contd): Summary of residues of fluopyram and its metabolites in bovine milk, skimmed milk and cream (mg/kg)

Group Dose	Sampling date*	Residue levels of individual analytes (mg/kg)							
		fluopyram		FLU-benzamide		Total residue of FLU-olefins		Total residue	
		Indiv.	mean	Indiv.	mean	Indiv.	mean	Indiv.	mean
3X 1.21 mg/kg bw/day 44.1 mg/kg DM **Sub-groups:** D1, D2, D3	Pre-dosing -7	3x<LOD	<LOD	3x<LOD	<LOD	3x<LOD	<LOD	3x<LOD	<LOD
	1	0.03; 0.02; 0.02	0.02	0.03; 0.02; 0.03	0.03	2x<LOD; <LOQ	< LOQ	0.06; 0.06; 0.05	0.06
	2	0.06; 0.03; 0.06	0.05	0.12; 0.13; 0.13	0.13	3x<LOQ	<LOQ	0.20; 0.18; 0.23	0.20
	4	0.06; 0.03; 0.07	0.05	0.26; 0.29; 0.44	0.33	2x<LOQ; 0.02	0.02	0.34; 0.34; 0.43	0.37
	8	0.04; 0.03; 0.04	0.04	0.63; 0.50; 0.53	0.55	0.02; 0.03; 0.02	0.02	0.69; 0.56; 0.59	061
	10	0.04; 0.02; 0.02	0.03	0.44; 0.44; 0.75	0.54	3x 0.02	0.02	0.50; 0.48; 0.79	0.59
	13	0.04; 0.02; 0.02	0.03	0.68; 0.42; 0.49	0.53	<LOQ; 0.02; 0.02	0.02	0.72; 0.46; 0.55	0.58
	17	3x 0.02	0.02	0.55; 0.54; 0.50	0.53	3x 0.02	0.02	0.59; 0.58; 0.54	0.57
	21	3x 0.02	0.02	0.47; 0.48; 0.40	0.45	3x 0.02	0.02	0.51; 0.52; 0.44	0.49
	24	0.09; 0.03; 0.02	0.05	0.66; 0.50; 0.41	0.52	0.05; 0.02; 0.02	0.03	0.80; 0.55; 0.45	0.60
	26	0.03; 0.02; n.a	0.03	0.77; 0.52; n.a	0.65	0.03; 0.03; n.a	0.03	0.83; 0.57; -	0.70
	29	0.02; 0.02; 0.02	0.03	0.72; 0.53; 0.43	0.65	3x 0.02	0.02	0.78; 0.57; 0.47	0.61
10X 4.05 mg/kg bw/day 133.1 mg/kg DM **Sub-groups:** E1, E2, E3	Pre-dosing -7	2x<LOD; n.a	< LOD	2x<LOD; n.a	< LOD	2x<LOD; n.a	< LOD	2x<LOD	<LOD
	1	0.06; n.a.; 0.11	0.09	0.07; n.a.; 0.08	0.08	< LOQ; n.a.; 0.02	0.02	0.15; 0.21	0.18
	2	0.11; n.a.; 0.14	0.13	0.33; n.a.; 0.35	0.34	0.03; n.a.; 0.03	0.03	0.47; 0.52	0.50
	4	0.14; n.a.; 0.17	0.16	0.80; n.a.; 0.81	0.81	0.04; n.a.; 0.05	0.05	0.98; 1.03	1.01
	8	0.11; n.a.; 0.11	0.11	1.7; n.a; 1.8	1.8	0.11; n.a.; 0.07	0.09	1.92; 1.98	1.95
	10	0.08; n.a.; 0.09	0.09	1.4; n.a.; 1.8	1.5	0.07; n.a.; 0.08	0.08	1.25; 1.97	1.61
	13	0.07; n.a.; 0.08	0.08	1.3; n.a.; 1.9	1.6	0.07; n.a.; 0.10	0.09	1.44; 2.08	1.76
	17	0.07; n.a.; 0.09	0.08	1.7; n.a.; 1.7	1.7	0.08; n.a.; 0.12	0.10	1.85; 1.91	1.88
	21	0.08; n.a.; 0.11	0.10	1.3; n.a.; 1.1	1.2	0.08; n.a.; 0.11	0.10	1.46; 1.32	1.39
	24	0.10; n.a; 0.13	0.12	1.3; n.a.; 1.3	1.3	0.09; n.a.; 0.11	0.10	1.49; 1.54	1.52
	26	0.06; n.a; 0.15	0.11	1.2; n.a.; 1.3	1.3	0.09; n.a.; 0.14	0.12	1.35; 1.59	1.47
	29	0.08; n.a.; 0.11	0.10	1.4; n.a.; 1.4	1.4	0.09; n.a.; 0.11	0.10	1.57; 1.62	1.60

* overall study day, n.a. not applicable
In case we have 3 levels of which at least one is <LOD and others <LOQ, it was legitimate to set the mean value as <LOQ;
When one or two individual values are ≥ LOQ and the others < LOQ or <LOD, residues <LOQ or <LOD are considered equal to LOQ or LOD
LOQ = 0.01 mg/kg for fluopyram and the benzamide metabolite, expressed as parent equivalents.
LOQ = 0.02 mg/kg for the total residue of FLU-olefins was calculated as sum of the two individual olefin isomers.
LOD = 0.003 mg/kg for fluopyram, LOD = 0.001 mg/kg for FLU-benzamide, LOD = 0.004 mg/kg for the total residue of olefins.

Table 6.4.2-8 (contd): Summary of residues of fluopyram and its metabolites in bovine milk, skimmed milk and cream (mg/kg)

Group Dose	Sampling date*	Residue levels of individual analytes (mg/kg)							
		fluopyram		FLU-benzamide		Total residue of FLU-olefins		Total residue	
		Indiv.	mean	Indiv.	mean	Indiv.	mean	Indiv.	mean
10XE 4.38 mg/kg bw/day 145.9 mg/kg DM **Sub-groups:** F1, F2, F3	Pre-dosing - 7	2x<LOD; <LOQ	<LOD	3x<LOD	<LOD	3x<LOD	<LOD	3x<LOD	<LOD
	21	0.08; 0.06; 0.03	0.06	3.6; 0.94; 1.8	2.1	0.10; 0.09; 0.07	0.09	3.78; 1.09; 4.9	2.26
	24	0.10; 0.09; 0.11	0.10	3.4; 0.98; 2.7	2.4	0.09; 0.08; 0.22	0.13	3.59; 1.17; 3.03	2.60
	26	0.12; 0.08; 0.16	0.12	3.2; 1.1; 3.1	2.5	0.08; 0.11; 0.30	0.18	3.44; 1.29; 3.52	2.75
	29	0.09; 0.13; 0.12	0.11	3.1; 1.1; 4.0	2.7	0.09; 0.10; 0.20	0.13	3.28; 1.33; 3.82	2.98
	31	<LOD; 2x<LOQ	<LOQ	2.1; 0.91; 3.6	2.2	0.06; 0.07; 0.08	0.07	2.16; 0.99; 3.69	2.28
	34	3x<LOD	<LOD	0.84; 0.28; 1.3	0.81	3x 0.05	0.05	0.89; 0.33; 1.35	0.86
	36	3x<LOD	<LOD	0.37; 0.11; 0.69	0.39	3x 0.04	0.04	0.41; 0.15; 0.73	0.43
	38	2x <LOD; n.a.	<LOD	0.22; 0.07; n.a.	0.15	0.03; 0.04; n.a.	0.04	0.25; 0.11	0.18
	41	2x <LOD; n.a.	<LOD	0.06; 0.02; n.a.	0.04	0.03; 0.02; n.a.	0.03	0.09; 0.04	0.07
	43	2x <LOD; n.a.	<LOD	0.02; 0.01; n.a.	0.02	0.02; 0.03; n.a.	0.03	0.04; 0.04	0.04
	45	<LOD; 2x n.a	<LOD	0.01; 2x n.a.	0.01	0.02; n.a.; n.a.	0.02	0.03	<0.04
	47	<LOD; 2x n.a	<LOD	<LOQ; 2x n.a	<LOQ	<LOQ; 2x n.a	<LOQ	<0.04	<0.04
	50	<LOD; 2x n.a	<LOD	<LOQ; 2x n.a	<LOQ	<LOQ; 2x n.a	<LOQ	<0.04	<0.04

* overall study day, n.a. not applicable
In case we have 3 levels of which at least one is <LOD and others <LOQ, it was legitimate to set the mean value as <LOQ
When one or two individual values are >LOQ and the others < LOQ residues <LOQ mg/kg are considered equal to LOQ
LOQ = 0.01 mg/kg for fluopyram and the benzamide metabolite expressed as parent equivalents.
LOQ = 0.02 mg/kg for the total residue of FLU-olefins was calculated as sum of the two individual olefin isomers.
LOD = 0.003 mg/kg for fluopyram, LOD = 0.001 mg/kg for FLU-benzamide, LOD = 0.004 mg/kg for the total residue of olefins.

Table 6.4.2- 9: Summary of residues of Fluopyram and its metabolites in bovine tissues (mg/kg)

Group	Dose (mg/kg bw/day)	Dose (mg/kg DM)	Sub-group	Sampling Time (Day)*	Residue levels (mg/kg)			
					fluopyram	FLU-benzamide	Total residue of FLU-olefins	Total residue
Perirenal fat								
0.1X	0.04	1.5	B1	30	< LOQ	< LOQ	< LOQ	<0.04
			B2	30	< LOQ	< LOQ	< LOQ	<0.04
			B3	30	< LOQ	< LOQ	< LOQ	<0.04
				Mean	**< LOQ**	**< LOQ**	**< LOQ**	**<0.04**
1X	0.44	14.4	C1	30	0.06	0.08	0.08	0.22
			C2	30	0.01	0.14	0.06	0.21
			C3	30	0.04	0.33	0.12	0.49
				Mean	**0.04**	**0.18**	**0.09**	**0.31**
3X	1.21	44.1	D1	30	0.33	0.34	0.28	0.95
			D2	30	0.18	0.28	0.26	0.72
			D3	30	0.23	0.18	0.29	0.70
				Mean	**0.25**	**0.27**	**0.28**	**0.80**
10X	4.05	133.1	E1	30	0.37	0.76	0.86	1.99
			E2	30	*n.a.*	*n.a.*	*n.a.*	-
			E3	30	0.60	0.94	0.83	2.37
				Mean	**0.49**	**0.85**	**0.85**	**2.19**
10XE depuration	4.38	145.9	F3	36	< LOD	0.47	0.12	0.59
			F2	43	LOD	< LOQ	0.21	0.22
			F1	30	< LOD	< LOQ	0.11	0.12
				Mean	**< LOD**	**0.16**	**0.15**	**0.31**

In case we have 3 levels of which at least one is <LOD and others <LOQ, it was legitimate to set the mean value as <LOQ;
When one or two individual values are >LOQ and the others <LOQ or <LOD, residues <LOQ or <LOD are considered equal to LOQ or LOD
LOQ = 0.01 mg/kg for fluopyram and the benzamide metabolite, expressed as parent equivalents.
LOQ = 0.02 mg/kg for the total residue of FLU-olefins was calculated as sum of the two individual olefin isomers.
LOQ=0.04 mg/kg for the total residue of Fluopyram calculated as sum of the parent + FLU-benzamide + the two olefin isomers
LOD = 0.003 mg/kg for fluopyram, LOD = 0.001 mg/kg for FLU-benzamide, LOD = 0.004 mg/kg for the total residue of olefins.

Table 6.4.2-9 (contd): Summary of residues of Fluopyram and its metabolites in bovine tissues (mg/kg)

Group	Dose (mg/kg bw/day)	Dose (mg/kg DM)	Sub-group	Sampling Time (Day)*	Residue levels (mg/kg)			
					fluopyram	FLU-benzamide	Total residue of FLU-olefins	Total residue
Mesenteric fat								
0.1X	0.04	1.5	B1	30	< LOQ	< LOQ	< LOQ	<0.04
			B2	30	< LOQ	< LOQ	< LOQ	<0.04
			B3	30	< LOQ	< LOQ	< LOQ	<0.04
				Mean	**< LOQ**	**< LOQ**	**< LOQ**	**<0.04**
1X	0.44	14.4	C1	30	0.07	0.08	0.09	0.24
			C2	30	0.01	0.10	0.06	0.17
			C3	30	0.02	0.29	0.07	0.38
				Mean	**0.03**	**0.16**	**0.07**	**0.26**
3X	1.21	44.1	D1	30	0.33	0.31	0.30	0.94
			D2	30	0.16	0.27	0.26	0.69
			D3	30	0.27	0.19	0.32	0.78
				Mean	**0.25**	**0.26**	**0.29**	**0.80**
10X	4.05	133.1	E1	30	0.67	0.64	0.86	2.17
			E2	30	*n.a.*	*n.a.*	*n.a.*	-
			E3	30	0.71	0.80	0.94	2.45
				Mean	**0.69**	**0.72**	**0.90**	**2.31**
10XE depuration	4.38	145.9	F3	36	< LOD	0.45	0.13	0.58
			F2	43	< LOD	< LOQ	0.26	0.27
			F1	50	< LOD	< LOD	0.11	0.12
				Mean	**< LOD**	**0.15**	**0.17**	**0.22**

In case we have 3 levels of which at least one is <LOD and others <LOQ, it was legitimate to set the mean value as <LOQ;
When one or two individual values are >LOQ and the others <LOQ or <LOD, residues <LOQ or <LOD are considered equal to LOQ or LOD
LOQ = 0.01 mg/kg for fluopyram and the benzamide metabolite, expressed as parent equivalents.
LOQ = 0.02 mg/kg for the total residue of FLU-olefins was calculated as sum of the two individual olefin isomers.
LOD = 0.003 mg/kg for fluopyram, LOD = 0.001 mg/kg for FLU-benzamide, LOD = 0.004 mg/kg for the total residue of olefins.

Table 6.4.2-9 (contd): Summary of residues of Fluopyram and its metabolites in bovine tissues (mg/kg)

Group	Dose (mg/kg bw/day)	Dose (mg/kg DM)	Sub-group	Sampling Time (Day)*	Residue levels (mg/kg)			
					fluopyram	FLU-benzamide	Total residue of FLU-olefins	Total residue
Subcutaneous fat								
0.1X	0.04	1.5	B1	30	< LOQ	0.01	< LOQ	0.04
			B2	30	< LOQ	< LOQ	< LOQ	<0.04
			B3	30	< LOQ	< LOQ	< LOQ	<0.04
				Mean	**< LOQ**	**0.01**	**< LOQ**	**0.04**
1X	0.44	14.4	C1	30	0.07	0.09	0.06	0.22
			C2	30	0.01	0.13	0.04	0.18
			C3	30	0.03	0.31	0.08	0.42
				Mean	**0.04**	**0.18**	**0.06**	**0.28**
3X	1.21	44.1	D1	30	0.43	0.45	0.18	0.96
			D2	30	0.13	0.44	0.14	0.71
			D3	30	0.27	0.22	0.23	0.72
				Mean	**0.24**	**0.37**	**0.18**	**0.79**
10X	4.05	133.1	E1	30	0.65	0.93	0.52	2.10
			E2	30	*n.a.*	*n.a.*	*n.a.*	-
			E3	30	0.48	1.1	0.57	2.15
				Mean	**0.57**	**1.0**	**0.55**	**2.12**
10XE depuration	4.38	145.9	F3	36	< LOD	0.58	0.04	0.62
			F2	43	< LOD	< LOQ	0.13	0.14
			F1	50	< LOD	< LOQ	0.28	0.29
				Mean	**< LOD**	**0.20**	**0.15**	**0.35**

In case we have 3 levels of which at least one is <LOD and others <LOQ, it was legitimate to set the mean value as <LOQ;
When one or two individual values are >LOQ and the others <LOQ or <LOD, residues <LOQ or <LOD are considered equal to LOQ or LOD
LOQ = 0.01 mg/kg for fluopyram and the benzamide metabolite, expressed as parent equivalents.
LOQ = 0.02 mg/kg for the total residues of FLU-olefins was calculated as sum of the two individual olefin isomers.
LOD = 0.003 mg/kg for fluopyram, LOD = 0.001 mg/kg for FLU-benzamide, LOD = 0.004 mg/kg for the total residue of olefins.

Table 6.4.2-9 (contd): Summary of residues of Fluopyram and its metabolites in bovine tissues (mg/kg)

Group	Dose (mg/kg bw/day)	Dose (mg/kg DM)	Sub-group	Sampling Time (Day)*	Residue levels (mg/kg)			
					fluopyram	FLU-benzamide	Total residue of FLU-olefins	Total residue
Liver								
0.1X	0.04	1.5	B1	30	0.26	0.10	LOD	0.36
			B2	30	0.24	0.10	< LOD	0.34
			B3	30	0.24	0.09	< LOD	0.33
				Mean	**0.25**	**0.10**	**< LOD**	**0.35**
1X	0.44	14.4	C1	30	0.98	0.84	0.03	1.85
			C2	30	0.80	0.90	0.03	1.73
			C3	30	0.35	1.90	0.06	2.31
				Mean	**0.71**	**1.2**	**0.04**	**1.95**
3X	1.21	44.1	D1	30	1.6	3.2	0.12	4.92
			D2	30	1.8	2.7	0.10	4.60
			D3	30	2.8	2.5	0.13	5.43
				Mean	**2.1**	**2.8**	**0.12**	**5.02**
10X	4.05	133.1	E1	30	4.0	6.7	0.41	11.11
			E2	30	*n.a.*	*n.a.*	*n.a.*	-
			E3	30	3.9	7.0	0.58	11.48
				Mean	**4.0**	**6.9**	**0.50**	**11.40**
10XE depuration	4.38	145.9	F3	36	0.06	2.8	0.08	2.94
			F2	43	LOQ	0.59	0.08	0.67
			F1	50	< LOD	0.42	0.04	0.46
				Mean	**0.02**	**1.3**	**0.07**	**1.39**

In case we have 3 levels of which at least one is <LOD and others <LOQ, it was legitimate to set the mean value as <LOQ;
When one or two individual values are >LOQ and the others <LOQ or <LOD, residues <LOQ or <LOD are considered equal to LOQ or LOD
LOQ = 0.01 mg/kg for fluopyram and the benzamide metabolite, expressed as parent equivalents.
LOQ = 0.02 mg/kg for the total residues of FLU-olefins was calculated as sum of the two individual olefin isomers.
LOD = 0.003 mg/kg for fluopyram, LOD = 0.001 mg/kg for FLU-benzamide, LOD = 0.004 mg/kg for the total residue of olefins.

Table 6.4.2-9 (contd): Summary of residues of Fluopyram and its metabolites in bovine tissues (mg/kg)

Group	Dose (mg/kg bw/day)	Dose (mg/kg DM)	Sub-group	Sampling Time (Day)*	Residue levels (mg/kg)			
					fluopyram	FLU-benzamide	Total residue of FLU-olefins	Total residue
Muscle								
0.1X	0.04	1.5	B1	30	< LOD	0.02	< LOD	<0.04
			B2	30	< LOD	0.02	< LOD	<0.04
			B3	30	< LOD	0.02	< LOD	<0.04
				Mean	**< LOD**	**0.02**	**< LOD**	**<0.04**
1X	0.44	14.4	C1	30	< LOQ	0.22	< LOQ	0.25
			C2	30	< LOD	0.22	< LOQ	0.54
			C3	30	< LOQ	0.44	< LOQ	0.47
				Mean	**< LOQ**	**0.29**	**< LOQ**	**0.32**
3X	1.21	44.1	D1	30	0.04	0.79	0.03	0.86
			D2	30	< LOQ	0.62	< LOQ	0.65
			D3	30	0.01	0.39	0.01	0.41
				Mean	**0.02**	**0.60**	**0.02**	**0.64**
10X	4.05	133.1	E1	30	0.03	1.3	0.04	1.37
			E2	30	*n.a.*	*n.a.*	*n.a.*	-
			E3	30	0.03	1.5	0.03	1.56
				Mean	**0.03**	**1.4**	**0.04**	**1.47**
10XE depuration	4.38	145.9	F3	36	< LOD	0.77	< LOQ	0.79
			F2	43	< LOD	0.15	< LOQ	0.17
			F1	50	< LOD	0.19	< LOQ	0.21
				Mean	**< LOD**	**0.37**	**< LOQ**	**0.39**

In case we have 3 levels of which at least one is <LOD and others <LOQ, it was legitimate to set the mean value as <LOQ;
When one or two individual values are >LOQ and the others <LOQ or <LOD, residues <LOQ or <LOD are considered equal to LOQ or LOD
LOQ = 0.01 mg/kg for fluopyram and the benzamide metabolite, expressed as parent equivalents.
LOQ = 0.02 mg/kg for the total residue of FLU-olefins was calculated as sum of the two individual olefin isomers.
LOD = 0.003 mg/kg for fluopyram, LOD = 0.001 mg/kg for FLU-benzamide, LOD = 0.004 mg/kg for the total residue of olefins.

Table 6.4.2-9 (contd): Summary of residues of Fluopyram and its metabolites in bovine tissues (mg/kg)

Group	Dose (mg/kg bw/day)	Dose (mg/kg DM)	Sub-group	Sampling Time (Day)*	Residue levels (mg/kg)			
					fluopyram	FLU-benzamide	Total residue of FLU-olefins	Total residue
Kidney								
0.1X	0.04	1.5	B1	30	< LOD	0.03	< LOD	0.04
			B2	30	< LOD	0.02	< LOD	0.04
			B3	30	< LOD	0.03	< LOD	0.04
				Mean	**< LOD**	**0.03**	**< LOD**	**0.04**
1X	0.44	14.4	C1	30	< LOQ	0.20	< LOQ	0.23
			C2	30	< LOD	0.26	< LOQ	0.28
			C3	30	< LOQ	0.38	< LOQ	0.41
				Mean	**< LOQ**	**0.28**	**< LOQ**	**0.31**
3X	1.21	44.1	D1	30	0.05	0.88	0.04	0.97
			D2	30	0.02	0.72	0.03	0.77
			D3	30	0.02	0.55	0.04	0.61
				Mean	**0.03**	**0.72**	**0.04**	**0.79**
10X	4.05	133.1	E1	30	0.05	1.5	0.10	1.65
			E2	30	*n.a.*	*n.a.*	*n.a.*	-
			E3	30	0.08	1.6	0.15	1.83
				Mean	**0.07**	**1.6**	**0.13**	**1.80**
10XE depuration	4.38	145.9	F3	36	< LOD	0.86	0.03	0.89
			F2	43	< LOD	0.07	0.04	0.11
			F1	50	< LOD	0.05	< LOQ	0.07
				Mean	**< LOD**	**0.33**	**0.03**	**0.36**

In case we have 3 levels of which at least one is <LOD and others <LOQ, it was legitimate to set the mean value as <LOQ;
When one or two individual values are >LOQ and the others <LOQ or <LOD, residues <LOQ or <LOD are considered equal to LOQ or LOD
LOQ = 0.01 mg/kg for fluopyram and the benzamide metabolite, expressed as parent equivalents.
LOQ = 0.02 mg/kg for the total residues of FLU-olefins was calculated as sum of the two individual olefin isomers.
LOD = 0.003 mg/kg for fluopyram, LOD = 0.001 mg/kg for FLU-benzamide, LOD = 0.004 mg/kg for the total residue of olefins.

CA 6.4.3 Pigs

The maximum dietary burden for pigs remains <0.004 mg/kg bw/day. Besides, the metabolic pathways do not differ significantly in the rat as compared to ruminants (cf CA 6.2.4). Therefore, a feeding study in pigs is not required.

CA 6.4.4 Fish

No residue study in fish was conducted. Currently, no test method or Guidance document is available for conducting such study. In these cases, waiving of this particular data requirement is considered acceptable according to the "Guidance document for applicants on preparing dossiers for the approval of a chemical new active substance and the renewal of approval of the chemical active substance according to Regulation (EU) No. 283/2013 and Regulation (EU) No. 284/2013" (SANCO/10081/2013-rev.2 of 2-May-2013).

CA 6.5 Effects of processing

Parent fluopyram and the metabolites fluopyram-7-hydroxy, fluopyram-benzamide and fluopyram-pyridyl-carboxylic acid (PCA) were proved to be stable under the test conditions representative for pasteurization, baking, brewing, boiling and sterilization. However, fluopyram-pyridyl-acetic acid can be transformed to fluopyram-picoline.

Processing studies for grape fractions have been previously reviewed at the EU level (DAR and adendum). Additional processing studies on grape are submitted to calculate processing factors in grape fractions. The results of the study indicate that residues of fluopyram remain to a large extent in wet pomace after pressing with processing factor at 3.2. Residues of fluopyram (mean processing factor = 0.4) are very low in wine (see Table 6.5.3- 42).

Processing studies on apple are submitted to calculate processing factors in apple fractions. The results of the study indicate that residues of fluopyram remain to a large extent in peel and pomace after pressing but residues of fluopyram are very low in juice (mean processing factor = 0.2) (see Table 6.5.3- 54).

CA 6.5.1 Nature of the residue

Data to address this point were presented in the dossier submitted for first inclusion in Annex and were deemed acceptable following evaluation and peer review at EU level (2013).

For details of data submitted previously please refer also to the Baseline dossier CA 6.2. For completeness, a summary of these previously submitted studies are included below.

Data already evaluated during the first EU review process for inclusion on Annex I.(no new studies)

High temperature hydrolysis of parent compound

Data Point:	KCA 6.5.1/01
Report Author:	[redacted]
Report Year:	2006
Report Title:	[Phenyl-UL-14C]AE C656948 and [pyridyl-2,6-14C]AE C656948: Aqueous hydrolyses under conditions of processing studies
Report No:	MEF-06/170
Document No:	M-278527-01-1
Guideline(s) followed in study:	EU: 91/414/EEC amended by 96/68/EC Section 6.5.1, subsection 6.5.1. Guideline 7035/VI/95 Revision 5, Appendix C (July 1997)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted rev. 1 to Vol.3 of DAR BZ, August 2012 (reference relied on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The degradation behaviour of [phenyl-UL-^{14}C]fluopyram and [pyridyl-2,6-^{14}C]fluopyram was investigated in buffered local drinking water, at a projected dose of 1.0 mg/L. The hydrolysis conditions are mimicking pasteurization (90 °C at pH 4 for 20 min), baking, brewing, boiling (100 °C at pH 5 for 60 min) and sterilization (120 °C at pH 6 for 20 min).

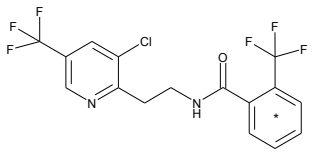
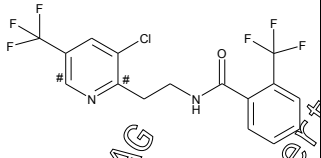
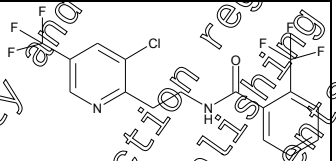
The material balances in the samples after incubation ranged between 100.4% and 102.2% of the applied dose for [phenyl-UL-^{14}C]fluopyram and between 99.2% and 102.3% of the applied dose for [pyridyl-2,6-^{14}C]fluopyram. The complete material balances demonstrate that no radioactive material dissipated from the test systems.

Chromatography analysis of the solutions demonstrated that parent fluopyram (AE C656948) is resistant to hydrolysis under all of the above mentioned conditions.

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	 *positions of radiolabel	 # positions of radiolabel	
Compound	AE C656948		
IUPAC name	*N*-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]ethyl}-2-(trifluoromethyl)benzamide		
CAS name	Benzamide, *N*-[2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-(trifluoromethyl)-(9Cl)		
CAS no.	658066-35-4		
Radiolabel position	Phenyl-UL-^{14}C	Pyridyl-2,6-^{14}C	Unlabelled
Batch no.	BECH 1910	BECH 1905	RFC633-04
Specific radioactivity	3.85 MBq/mg (104.15 µCi/mg, 23121.3000 dpm/mg)		
Radiochemical Purity	> 99% (HPLC and TLC)	> 98% (HPLC), > 99% (TLC)	-
Chemical Purity	> 98% (HPLC)	> 99% (HPLC)	99.8%

2. Water: Aqueous buffer solutions prepared from tap water from the local provider "Verbandswasserwerk Langenfeld-Monheim"

B. Study Design

1. Experimental conditions:

Solutions of fluopyram (AE C656948) in buffered drinking water were subjected to three different pH / temperature scenarios, to mimic pasteurization, baking/brewing/boiling and sterilization, respectively: pH 4/90 °C, pH 5/100 °C (in a water bath) and pH 6/120 °C (in an autoclave). The durations of the treatments were 20, 60 and 20 minutes for the three scenarios, respectively. Closed 10-mL crimp-cap glass vials were used as test vessels, which contained 5 mL of test solution each.

A generic target application rate for fluopyram (AE C656948) of 1.0 mg /L buffered drinking water containing 1% acetonitrile was selected for this test.

For preparation of the stock solutions the total amounts of the radiolabelled test items were dissolved in 2 mL acetonitrile. The content of radioactive residues was determined by LSC, and resulted in 3.8 MBq/mL, corresponding to 0.99 mg/mL, for the phenyl-label and 4.1 MBq/mL, corresponding to 1.06 mg/mL, for the pyridyl-label.

The application solutions were prepared by diluting 500 µL of the respective stock solution with 4500 µL acetonitrile, and aliquots were used for the determination of the homogeneity and the radioactivity content by LSC and an aliquot was used for HPLC-MS/MS confirmation of the identity of the test item.

Dosing was carried out by pipetting aliquots of 50 µL of the application solutions into two vials (each containing 4950 µL of the respective buffer solution) per scenario each.

Two vials per scenario (i.e. one per radiolabel) were incubated, another two vials per scenario served as time-zero samples and were not incubated. Additionally, control vessels containing 5 mL of the corresponding blank buffers were also subjected to incubation for monitoring the actual incubation temperature and the pH value.

2. Sampling

Before opening the radioactive test vessels, 2 mL of acetonitrile were added to each sample using a syringe, and the vessels were shaken for homogeneity.

The radioactive residue content of each vessel was determined by LSC, both for the time zero samples and for the incubated solutions directly after termination of the test.

C. Analytical Procedures

Aliquots of each sample were analysed by HPLC within a day. Samples from the pH 6/120 °C test series were used for exemplary confirmative TLC analysis and co-elution checks with mixtures of unlabelled reference compounds.

The radioactive residues of liquid samples were determined by liquid scintillation counting (LSC) using the scintillator Quicksafe A containing 5% of water.

II. Results and Discussion

The degradation behaviour of [phenyl-UL-^{14}C]fluopyram and [pyridyl-2,6-^{14}C]fluopyram was investigated in buffered local drinking water at a projected dose of 1.0 mg a.s./L. The hydrolysis conditions were chosen to mimic pasteurization (90 °C at pH 4 for 20 min), baking, brewing, boiling (100 °C at pH 5 for 60 min) and sterilization (120 °C at pH 6 for 20 min).

The applied dose was defined as the amount of radioactive residues measured in the samples at zero time. The total amount of applied active substance was 1.0 mg/L for [phenyl-UL-^{14}C]fluopyram and 1.1 mg/L for [pyridyl-2,6-^{14}C]fluopyram. Based on the results of LSC measurements immediately after test termination, a balance of radioactive residues was established for each experiment. The material balances ranged between 100.4% and 103.2% of the applied dose for [phenyl-UL-^{14}C]fluopyram and between 99.2% and 102.3% of the applied dose for [pyridyl-2,6-^{14}C]fluopyram (Table 6.5.1-1).

The complete material balances demonstrate that no radioactive material dissipated from the test systems.

Table 6.5.1-1: TRR values in water samples after application of [phenyl-UL-^{14}C]fluopyram and [pyridyl-2,6-^{14}C]fluopyram

Conditions	Conditions of hydrolysis	% of applied dose after incubation*	mg a.s./L
[Phenyl-UL-^{14}C]			
Pasteurization	pH=4, 90 °C, 20 min	100.4	1.0
Baking, brewing, boiling	pH=5, 100 °C, 60 min	100.9	1.1
Sterilization	pH=6, 120 °C, 60 min	102.2	1.1
[Pyridyl-2,6-^{14}C]			
Pasteurization	pH=4, 90 °C, 20 min	99.4	1.1
Baking, brewing, boiling	pH=5, 100 °C, 60 min	99.2	1.1
Sterilization	pH=6, 120 °C, 60 min	102.3	1.1

* The corresponding zero-incubation control was set as 100%

Quantitative analysis of all test samples was performed by reversed phase HPLC with radio-detection. Confirmative TLC analysis was performed exemplarily for the pH 6/120 °C test series.

In all processing scenarios the parent active substance represented the only residue, and no degradation products were detected. The peak of fluopyram represented 100% of the injected radioactive dose in the samples. Thus, no degradation of the active substance occurred in the processing scenarios.

III. Conclusions

In all processing scenarios (pasteurization, baking, brewing, boiling and sterilization) the parent active substance fluopyram (AE C656948) represented the only residue, and no degradation products were detected.

Assessment and conclusion by applicant:

The study is valid and acceptable.

High temperature hydrolysis of fluopyram-benzamide

Data Point:	KCA 6.5.1/02
Report Author:	[redacted]
Report Year:	2006
Report Title:	[Phenyl-UL-14C]AE C656948-benzamide: Aqueous hydrolysis under conditions of processing studies
Report No:	MEF-06/273
Document No:	M-278640-01-1
Guideline(s) followed in study:	EU: 91/414/EEC amended by 96/68/EC Section 6.5, Subsection 6.5.1, Guideline 7035/VI/95 Revision 5, Appendix E (July 1997)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted rev. 1 to Vol.3 of FAR B7 August 2012 (references relied on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The degradation behaviour of the metabolite [phenyl-UL-^{14}C]fluopyram-benzamide was investigated in buffered local drinking water, at a projected dose of 1.0 mg/L. The hydrolysis conditions are mimicking pasteurization (90 °C at pH 4 for 20 min), baking, brewing, boiling (100 °C at pH 5 for 60 min) and sterilization (120 °C at pH 6 for 20 min).

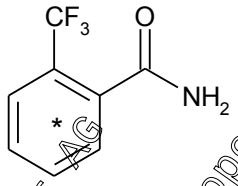
The material balances in the samples after incubation ranged between 97.3% and 107.8% of the applied dose. The complete material balances demonstrate that no radioactive material dissipated from the test systems.

Chromatography analysis of the solutions demonstrated that [phenyl-UL-^{14}C]fluopyram-benzamide is resistant to hydrolysis under all of the above mentioned conditions.

I. Materials and Methods

A. Materials

1 Test Material:

Chemical structure	 * position of the ^{14}C-radiolabel
Compound	AE C656948-benzamide
IUPAC Name	2-(trifluoromethyl) benzamide
CAS Name	Benzamide, 2-(trifluoromethyl)-
CAS Number	360-64-5
Empirical formula	$C_8H_6F_3NO$
Molar mass	189.14 g/mol
Position of radiolabel	[Phenyl-U-^{14}C]
Batch number	BECH 1958
Specific radioactivity	4.07 MBq/mg
Radiochemical purity	> 99% (HPLC and TLC)
Chemical purity	> 99% (HPLC)

2. Water: Aqueous buffer solutions prepared from tap water from the local provider "Verbandswasserwerk Langenfeld-Monheim"

B. Study Design

1. Experimental conditions:

Solutions of fluopyram-benzamide in buffered drinking water were subjected to three different pH / temperature scenarios to mimic pasteurization, baking/brewing/boiling and sterilization, respectively: pH 4/90 °C, pH 5/100 °C (in a water bath) and pH 6/120 °C (in an autoclave). The durations of the treatments were 20, 60 and 20 minutes for the three scenarios, respectively. Closed 10-mL crimp-cap glass vials were used as test vessels, which contained approximately 5 mL of test solution each.

A generic target application rate for fluopyram-benzamide of 1.0 mg /L buffered drinking water containing 1% acetonitrile was selected for this test.

For preparation of the stock solutions the total amounts of the radiolabelled test item were dissolved in 2 mL acetonitrile.

The application solutions were prepared by diluting 500 µL of the respective stock solution with 4500 µL acetonitrile, and aliquots were used for the determination of the homogeneity and the radioactivity content by LSC and resulted in 0.26 MBq/mL, corresponding to 0.10 mg/mL. An aliquot was used for HPLC-MS/MS confirmation of the identity of the test item.

Dosing was carried out by pipetting aliquots of 50 µL of the application solutions into four vials (each containing 4950 µL of the respective buffer solution) per scenario each.

Two vials per scenario (i.e. one per radiolabel) were incubated, another two vials per scenario served as time-zero samples and were not incubated. Additionally, control vessels containing 5 mL of the corresponding blank buffers were also subjected to incubation for monitoring the actual incubation temperature and the pH value.

2. Sampling

Before sampling, 2 mL of acetonitrile were added to each sample and the vessels were shaken for homogeneity.

The radioactive residue content of each vessel was determined by LSC, both for the time zero samples and for the incubated solutions directly after termination of the test.

C. Analytical Procedures

Aliquots of each sample were analysed by HPLC. Samples from the pH 6/120 °C test series were used for exemplary confirmative TLC analysis and co-elution checks with mixtures of unlabelled reference compounds.

The radioactive residues of liquid samples were determined by liquid scintillation counting (LSC) using the scintillator Quicksafe A containing 5% of water.

II. Results and Discussion

The degradation behaviour of [phenyl-UL-^{14}C]fluopyram-benzamide was investigated in buffered local drinking water, at a projected dose of 1.0 mg a.s./L. The hydrolysis conditions were chosen to mimic pasteurization (90 °C at pH 4 for 20 min), baking, brewing, boiling (100 °C at pH 5 for 60 min) and sterilization (120 °C at pH 6 for 20 min).

The applied dose was defined as the amount of radioactive residues measured in the samples at zero time. The total amount of applied active substance was 1.07 mg/L to 1.10 mg/L. Based on the results of LSC measurements immediately after test termination, a balance of radioactive residues was established for each experiment. The material balances ranged between 97.3% and 107.8% of the applied dose (Table 6.5.1-2).

The complete material balances demonstrate that no radioactive material dissipated from the test systems.

Table 6.5.1-2: TRR values in water samples after application of [phenyl-UL-^{14}C]fluopyram-benzamide

Conditions	Conditions of hydrolysis	% of applied dose after incubation*	mg a.s./L
Pasteurization	pH=4, 90 °C, 20 min	99.8	1.09
Baking, brewing, boiling	pH=5, 100 °C, 60 min	97.3	1.08
Sterilization	pH=6, 120 °C, 60 min	107.8	1.15

* The corresponding zero-incubation control was set as 100%

Quantitative analysis of all test samples was performed by reversed phase HPLC with radio-detection. Confirmative TLC analysis was performed exemplarily for the pH 6/120 °C test series.

In all processing scenarios the parent active substance ([phenyl-UL-^{14}C]fluopyram-benzamide) represented the only residue, and no degradation products were detected. The peak of fluopyram-benzamide represented 100% of the injected radioactive dose in the samples. Thus, no degradation of the active substance occurred in the processing scenarios.

III. Conclusions

In all processing scenarios (pasteurization, baking, brewing, boiling and sterilization) the test item/metabolite fluopyram-benzamide represented the only residue, and no degradation products were detected. Therefore, the tested processing conditions do not affect the nature of fluopyram-benzamide in raw agricultural commodities.

Assessment and conclusion by applicant
The study is valid and acceptable.

High temperature hydrolysis of fluopyram-7-hydroxy

Data Point:	KCA 6.5.1/03
Report Author:	[redacted]
Report Year:	2006
Report Title:	[Pyridine-2,6-14C]AE C656948-7-hydroxy: Aqueous hydrolysis under conditions of processing studies
Report No:	MEF-06/349
Document No:	M-278554-01-1
Guideline(s) followed in study:	EU: 91/414/EEC amended by 96/68/EC Section 6.5, Subsection 6.5.1, Guideline 7035/VI/95 Revision 5, Appendix E (July 1997)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted rev. 1 to Vol.3 of FAR B, August 2012 (references relied on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The degradation behaviour of the metabolite test item [pyridyl-2,6-^{14}C]fluopyram-7-hydroxy was investigated in buffered local drinking water, at a projected dose of 1.0 mg/L. The hydrolysis conditions are mimicking pasteurization (90 °C at pH 4 for 20 min), baking, brewing, boiling (100 °C at pH 5 for 60 min) and sterilization (120 °C at pH 6 for 20 min).

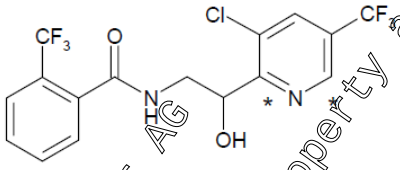
The material balances in the samples after incubation ranged between 100.7% and 102.1% of the applied dose. The complete material balances demonstrate that no radioactive material dissipated from the test systems.

Chromatography analysis of the solutions demonstrated that [pyridyl-2,6-^{14}C]fluopyram-7-hydroxy is resistant to hydrolysis under all of the above mentioned conditions.

I. Materials and Methods

A. Materials

1 Test Material:

Chemical structure	 * position of the ^{14}C-radiolabel
Compound	fluopyram-7-hydroxy
IUPAC Name	N-{2-[3-chloro-5-(trifluoromethyl)pyridine-2-yl]-2-hydroxyethyl}-2-(trifluoromethyl)-7-hydroxy
CAS Name	n.a.
CAS Number	n.a.
Empirical formula	$C_{16}H_{11}ClF_6N_2O_2$
Molar mass	412.72 g/mol
Position of radiolabel	[Pyridyl-2,6-^{14}C]
Batch number	BECH-1980 (radiolabelled)
Specific radioactivity	3.16 MBq/mg = 85.43 µCi/mg
Radiochemical purity	> 98% (HPLC)
Chemical purity	> 99% (HPLC)

n.a. not yet available

2. Water: Aqueous buffer solutions prepared from tap water from the local provider "Verbandswasserwerk Langenfeld-Monheim"

B. Study Design

1. Experimental conditions:

Solutions of fluopyram-7-hydroxy in buffered drinking water were subjected to three different pH / temperature scenarios, to mimic pasteurization, baking/brewing/boiling and sterilization, respectively: pH 4/90 °C, pH 5/100 °C (in a water bath) and pH 6/120 °C (in an autoclave). The durations of the treatments were 20, 60 and 20 minutes for the three scenarios, respectively. Closed 10-mL crimp-cap glass vials were used as test vessels, which contained approximately 5 mL of test solution each.

A generic target application rate for fluopyram-7-hydroxy of 1.0 mg /L buffered drinking water containing less than 1% acetonitrile was selected for this test.

For preparation of the stock solutions, the total amounts of the radiolabelled test item were dissolved in 2 mL acetonitrile.

The application solutions were prepared by diluting 500 µL of the respective stock solution with 4500 µL acetonitrile, and aliquots were used for the determination of the homogeneity and the radioactivity content by LSC and resulted in 0.43 MBq/mL, corresponding to 0.14 mg/mL. An aliquot was used for HPLC-MS/MS confirmation of the identity of the test item.

Dosing was carried out by pipetting aliquots of 38 µL of the application solutions into four vials (each containing 4960 µL of the respective buffer solution) per scenario each.

Two vials per scenario (i.e. one per radiolabel) were incubated, another two vials per scenario served as time-zero samples and were not incubated. Additionally, control vessels containing 5 mL of the corresponding blank buffers were also subjected to incubation for monitoring the actual incubation temperature and the pH value.

2. Sampling

Before sampling, 2 mL of acetonitrile were added to each sample and the vessels were shaken for homogeneity.

The radioactive residue content of each vessel was determined by LSC, both for the time zero samples and for the incubated solutions directly after termination of the test.

C. Analytical Procedures

Aliquots of each sample were analysed by HPLC. Samples from the pH 6/120 °C test series were used for exemplary confirmative TLC analysis and co-elution checks with mixtures of unlabelled reference compounds.

The radioactive residues of liquid samples were determined by liquid scintillation counting (LSC) using the scintillator Quicksafe A containing 5% of water.

II. Results and Discussion

The degradation behaviour of [phenyl-UL-^{14}C]fluopyram was investigated in buffered local drinking water, at a projected dose of 1.0 mg a.s./L. The hydrolysis conditions were chosen to mimic pasteurization (90 °C at pH 4 for 20 min), baking, brewing, boiling (100 °C at pH 5 for 60 min) and sterilization (120 °C at pH 6 for 20 min).

The applied dose was defined as the amount of radioactive residues measured in the samples at zero time. The total amount of applied active substance was 1.0 mg/L to 1.1 mg/L. Based on the results of LSC measurements immediately after test termination, a balance of radioactive residues was established for each experiment. The material balances ranged between 100.7% and 102.1% of the applied dose (Table 6.5.1-3).

The complete material balances demonstrate that no radioactive material dissipated from the test systems.

Table 6.5.1-3: TRR values in water samples after application of [Pyridyl-2,6-^{14}C]fluopyram-7-hydroxy

Conditions	Conditions of hydrolysis	% of applied dose after incubation*	mg a.s./L
Pasteurization	pH=4, 90 °C, 20 min	100.7	1.1
Baking, brewing, boiling	pH=5, 100 °C, 60 min	102.0	1.1
Sterilization	pH=6, 120 °C, 60 min	102.1	1.1

* The corresponding zero-incubation control was set as 100%

Quantitative analysis of all test samples was performed by reversed phase HPLC with radio-detection. Confirmative TLC analysis was performed exemplarily for the pH 6/120 °C test series.

In all processing scenarios the parent metabolite ([pyridyl-2,6-^{14}C]fluopyram-7-hydroxy) represented the only residue, and no degradation products were detected. The peak of fluopyram-7-hydroxy represented 100% of the injected radioactive dose in the samples. Thus, no degradation of the test item occurred in the processing scenarios.

III. Conclusions

In all processing scenarios (pasteurization, baking, brewing, boiling and sterilization) the metabolite fluopyram-7-hydroxy represented the only residue, and no degradation products were detected. Therefore, the tested processing conditions will not affect the nature of fluopyram-7-hydroxy residues in raw agricultural commodities.

Assessment and conclusion by applicant:

The study is valid and acceptable.

High temperature hydrolysis of fluopyram-Pyridyl-Carboxylic Acid

Data Point:	KCA 6.5.1/04
Report Author:	[redacted]
Report Year:	2006
Report Title:	[Pyridyl-2,6-14C]AE C656948-pyridyl-carboxylic acid: Aqueous hydrolysis under conditions of processing studies
Report No:	MEF-06/358
Document No:	M-278803-02-1
Guideline(s) followed in study:	EU: 91/414/EEC amended by 96/68/EC Section 5, Subsection 5.1, Guideline 7035/VI/95 Revision 5, Appendix E (July 1997)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted rev. 1 to Vol.3 of DAR 27 August 2012 (references cited on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The degradation behaviour of [pyridyl-2,6-^{14}C]fluopyram-pyridyl-carboxylic acid was investigated in buffered local drinking water, at a projected dose of 1.0 mg/L. The hydrolysis conditions are mimicking pasteurization (90 °C at pH 4 for 20 min), baking, brewing, boiling (100 °C at pH 5 for 60 min) and sterilization (120 °C at pH 6 for 20 min).

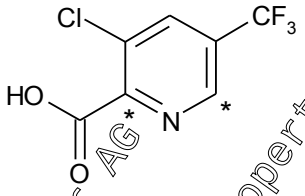
The material balances ranged between 93.1% and 106.1% of the applied dose (AR). The complete material balances demonstrate that no radioactive material dissipated from the test systems.

Chromatography analysis of the solutions demonstrated that [pyridyl-2,6-^{14}C]fluopyram-pyridyl-carboxylic acid is resistant to hydrolysis under all of the above mentioned conditions.

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	 * position of the ^{14}C-radiolabel
Compound	fluopyram-pyridyl-carboxylic acid
IUPAC Name	3-chloro-5-(trifluoromethyl)pyridine-2-carboxylic acid
CAS Name	2-Pyridinecarboxylic acid, 3-chloro-5-(trifluoromethyl)- (9CI)
CAS Number	80194-68-9
Empirical formula	$C_7H_3ClF_3NO_2$
Molar mass	225.26 g/mol
Position of radiolabel	[Pyridyl-2,6-^{14}C]
Batch number	BECH 1972 (labeled), AE C657188 00 1B97 0001 (unlabeled)
Specific radioactivity	4.33 MBq/mg
Radiochemical purity	> 98% (HPLC)
Chemical purity	>98% (HPLC)

2. Water: Aqueous buffer solutions prepared from tap water from the local provider "Verbandswasserwerk Langenfeld-Monheim"

B. Study Design

1. Experimental conditions:

Solutions of fluopyram-pyridyl-carboxylic acid in buffered drinking water were subjected to three different pH / temperature scenarios to mimic pasteurization, baking/brewing/boiling and sterilization, respectively: pH 4/90 °C, pH 5/100 °C (in a water bath) and pH 6/120 °C (in an autoclave). The durations of the treatments were 20, 60 and 20 minutes for the three scenarios, respectively. Closed 10-mL crimp-cap glass vials were used as test vessels, which contained 5 mL of test solution each.

Citrate buffers (20 mM) prepared from drinking water were used as test matrices. A generic target application rate for fluopyram-pyridyl-acetic acid of 1.0 mg /L buffered drinking water containing 1% acetonitrile was selected for this test.

For preparation of the stock solutions the total amount of the radiolabelled test item was dissolved in 2 mL acetonitrile.

The application solutions were prepared by diluting 500 µL of the respective stock solution with 4500 µL acetonitrile, and aliquots were used for the determination of the homogeneity and the radioactivity content by LSC and an aliquot was used for HPLC-MS/MS confirmation of the identity of the test item. The content of radioactive residues was determined by LSC, and resulted in 0.469 MBq/mL, corresponding to 0.11 mg/mL. Before and after treatment of all test systems, the radiochemical purity of the ^{14}C-labeled test item within its application solution was determined by HPLC, and found to be 99.9% and 99.8%, respectively.

Dosing was carried out by pipetting aliquots of 50 µL of the application solutions into two vials (each containing 4950 µL of the respective citrate buffer solution) per scenario each.

Two vials per scenario were incubated and another two vials per scenario served as time-zero samples and were not incubated. Additionally, control vessels containing 5 mL of the corresponding blank buffers were also subjected to incubation for monitoring the actual incubation temperature and the pH value.

2. Sampling

After processing and cooling to room temperature, 2 mL of acetonitrile were added to each test vessel, the vials were shaken for homogeneity and aliquots were taken for LSC and HPLC analysis.

The radioactive residue content of each vessel was determined by LSC, both for the time zero samples and for the incubated solutions directly after termination of the test.

C. Analytical Procedures

Aliquots of each sample were analysed by HPLC. Samples from the pH 6/120 °C test series were used for exemplary confirmative TLC analysis. Co-elution checks with mixtures of unlabelled reference item and samples from one of the test series were conducted in both chromatography systems.

The radioactive residues of liquid samples were determined by liquid scintillation counting (LSC) using the scintillator Quicksafe A containing 5% of water.

II. Results and Discussion

The degradation behaviour of [pyridyl-2,6-^{14}C] fluopyram-pyridyl-carboxylic acid was investigated in buffered local drinking water, at a projected dose of 1.0 mg a.s./L. The hydrolysis conditions were chosen to mimic pasteurization (90 °C at pH 4 for 20 min), baking, brewing, boiling (100 °C at pH 5 for 60 min) and sterilization (120 °C at pH 6 for 20 min).

The applied dose was defined as the amount of radioactive residues measured in the samples at zero time. The total amount of applied active substance was 1.06 mg/L–1.19 mg/L. Based on the results of LSC measurements immediately after test termination, a balance of radioactive residues was established for each experiment. The material balances ranged between 93.1% and 106.1% of the AR (Table 6.5.1-4).

The complete material balances demonstrate that no radioactive material dissipated from the test systems.

Table 6.5.1-4: TRR values in water samples after application of [pyridyl-2,6-^{14}C] fluopyram- pyridyl-carboxylic acid

Conditions	Conditions of hydrolysis	% of applied dose after incubation*	mg a.s./L
Pasteurization	pH=4, 90 °C, 20 min	93.1	1.11
Baking, brewing, boiling	pH=5, 100 °C, 60 min	106.1	1.12
Sterilization	pH=6, 120 °C, 60 min	99.5	1.07

* The corresponding zero-incubation control was set as 100%

Quantitative analysis of all test samples was performed by reversed phase HPLC with radio-detection. Confirmative TLC analysis was performed exemplarily for the pH 6/120 °C test series.

In all processing scenarios, fluopyram-pyridyl-carboxylic acid represented the only residue, and no degradation products were detected. The peak of fluopyram-pyridyl-carboxylic acid represented 99.8–99.9% of the applied radioactivity in the time zero samples, and 93.0–106.0% of the AR in the processed samples. Thus, no degradation of the active substance occurred in the tested processing scenarios.

III. Conclusions

fluopyram-pyridyl-carboxylic acid was found to be resistant against hydrolysis under conditions being representative for pasteurization, baking, brewing, boiling and sterilization. Therefore, it can be concluded that the above processing operations will not affect the nature of fluopyram-pyridyl-carboxylic acid residues in raw agricultural commodities.

Assessment and conclusion by applicant:

The study is valid and acceptable.

High temperature hydrolysis of fluopyram-pyridyl-acetic acid

Data Point:	KCA 6.5.1/05
Report Author:	[redacted]
Report Year:	2006
Report Title:	[Pyridine-2,6-14C]AE C656948 pyridyl-acetic acid: Aqueous hydrolysis under conditions of processing studies
Report No:	MEF-06/354
Document No:	M-278760-01-1
Guideline(s) followed in study:	EU: 91/414/EEC amended by 96/68/EC Section 6.5, Subsection 6.5.1, Guideline 7035/VI/95 Revision 5, Appendix E (July 1997)
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted rev. 1 to Vol.3 of DAR 07 August 2012 (references cited on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The degradation behaviour of the metabolite [pyridyl-2,6-^{14}C]fluopyram-pyridyl-acetic was investigated in buffered local drinking water at a projected dose of 1.0 mg/L. The hydrolysis conditions are mimicking pasteurization (90 °C at pH 4 for 20 min), baking, brewing, boiling (100 °C at pH 5 for 60 min) and sterilization (120 °C at pH 6 for 20 min).

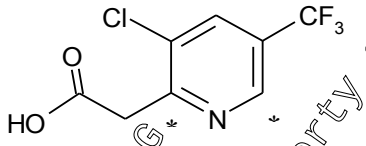
The material balances ranged between 90.2% and 93.1% of the applied dose for the unshaken samples and between 95.2% and 97.1% of the applied dose for the shaken samples. The complete material balances demonstrate that no radioactive material dissipated from the test systems, and that small amounts of the test substance were present as volatiles in the gaseous phase of the samples.

Chromatography analysis of the solutions demonstrated that fluopyram-pyridyl-acetic acid is not resistant to hydrolysis under any of the above mentioned conditions. Under these conditions the transformation product fluopyram-picoline was formed up to 96.7% of the applied radioactivity (AR).

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	 * position of the ^{14}C-radiolabel
Compound	AE C656948-pyridyl-acetic acid
IUPAC Name	[3-chloro-5-(trifluoromethyl)pyridine-2-yl]-acetic acid
CAS Name	n.a.
CAS Number	n.a.
Empirical formula	$C_8H_5ClF_3NO_2$
Molar mass	239.58 g/mol (free acid); 261.56 g/mol (sodium salt)
Position of radiolabel	[Pyridine-2,6-^{14}C]
Batch number	BECH 1981 (labeled), BCS-AA10089-PU-01 (unlabeled)
Specific radioactivity	4.68 MBq/mg (126.42 µCi/mg)
Radiochemical purity	> 98% (HPLC)
Chemical purity	> 98% (HPLC)

2. Water: Aqueous buffer solutions prepared from tap water from the local provider "Verbandswasserwerk Langenfeld-Monheim"

B. Study Design

1. Experimental conditions:

Solutions of fluopyram-pyridyl-acetic acid in buffered drinking water were subjected to three different pH / temperature scenarios, to mimic pasteurization, baking/brewing/boiling and sterilization, respectively: pH 4/90 °C, pH 5/100 °C (in a water bath) and pH 6/120 °C (in an autoclave). The durations of the treatments were 20, 60 and 20 minutes for the three scenarios, respectively. Closed 10-mL crimp-cap glass vials were used as test vessels, which contained 5 mL of test solution each.

Citrate buffers (20 mM) prepared from drinking water were used as test matrices. A generic target application rate for fluopyram-pyridyl-acetic acid of 1.0 mg /L buffered drinking water, containing less than 1% acetonitrile, was selected for this test.

For preparation of the stock solutions the total amount of the radiolabelled test item was dissolved in 1.9 mL water and 100 µL 0.4 M aqueous sodium hydroxide.

The application solutions were prepared by diluting 500 µL of the respective stock solution with 4400 µL pure water and 100 µL 0.4 M aqueous sodium hydroxide, and aliquots thereof were used for the determination of the homogeneity, the radioactivity content by LSC and the confirmation of the identity of the test item by HPLC-MS/MS. The content of radioactive residues accounted for 0.586 MBq/mL, corresponding to 0.125 mg/mL.

For each of the three test scenarios, dosing was carried out by pipetting aliquots of 42 µL of the application solutions into six vials, each containing 4960 µL of the respective citrate buffer solution.

Four vials per scenario were incubated and another two vials per scenario served as time-zero samples and were not incubated. Additionally, control vessels containing 5 mL of the corresponding blank buffers were also subjected to incubation for monitoring the actual incubation temperature and the pH value.

2. Sampling

After processing and cooling to room temperature, 5 mL of acetonitrile were pipetted into the test solutions, the solutions were shaken gently and aliquots were taken for LSC and HPLC analysis. Two vials per scenario were further processed in order to dissolve possible volatiles into the organic phase (denoted as shaken samples): After incubation and cooling to room temperature, 5 mL of acetonitrile were injected through the septa into the test solutions. The vials were shaken vigorously for approximately 2 minutes prior to LSC and HPLC analysis.

The radioactive residue content of each vessel was determined by LSC, both for the time zero samples and for the incubated solutions directly after termination of the test.

C. Analytical Procedures

Aliquots of each sample were analysed by HPLC. Samples from the pH 4/90 °C test series were used for exemplary confirmative HPLC-MS/MS analysis and co-elution checks with mixtures of unlabelled and labelled reference compounds.

The radioactive residues of liquid samples were determined by liquid scintillation counting (LSC) using the scintillator Quicksafe A containing 5% of water.

II. Results and Discussion

The degradation behaviour of [pyridyl-2,6-^{14}C] fluopyram-pyridyl-acetic acid was investigated in buffered local drinking water at a projected dose of 1.0 mg a.s./L. The hydrolysis conditions were chosen to mimic pasteurization (90 °C at pH 4 for 20 min), baking, brewing, boiling (100 °C at pH 5 for 60 min) and sterilization (120 °C at pH 6 for 20 min).

The applied dose was defined as the amount of radioactive residues measured in the samples at zero time. The total amount of applied active substance was 0.99 mg/L. Based on the results of LSC measurements immediately after test termination, a balance of radioactive residues was established for each experiment. The material balances ranged between 90.2% and 93.1% of the AR the unshaken samples and between 95.2% and 97.1% of the AR for the shaken samples (Table 6.5.1-5).

The complete material balances demonstrate that no radioactive material dissipated from the test systems, and that small amounts of the test substance were present as volatiles in the gaseous phase of the samples.

Table 6.5.1-5: TRR values in water samples after application of [pyridyl-2,6-^{14}C] fluopyram-pyridyl-acetic acid

Conditions	Conditions of hydrolysis	% of applied dose after incubation*	mg a.s./L	% of applied dose after incubation* (shaken samples#)	mg a.s./L (shaken samples#)
Pasteurization	pH=4, 90 °C, 20 min	91.1	0.90	97.1	0.96
Baking, brewing, boiling	pH=5, 100 °C, 60 min	90.2	0.90	95.4	0.95
Sterilization	pH=6, 120 °C, 60 min	93.1	0.92	95.2	0.95

* The corresponding zero-incubation control was set as 100%.
\# shaken samples: 5 mL acetonitrile were injected through the septa of the test vessels; then the vessels were shaken vigorously to solve volatiles into the liquid phase.

Quantitative analysis of all test samples was performed by reversed phase HPLC with radio-detection. Confirmative HPLC-MS/MS analysis was performed exemplarily for the pH 4/90 °C test series.

The peak of fluopyram-pyridyl-acetic acid represented 95.1% to 99.0% of the AR in the time zero samples, and 0%, 0% and 18.8% of the AR in the pasteurization, baking and sterilization samples, respectively. The formation of the transformation product fluopyram-picoline was depended on the processing scenario. fluopyram-picoline was formed to 96.7%, 94.5% and 76.0% of the AR in the pasteurization, baking and sterilization samples, respectively (Table 6.5.1-6 and Figure 6.5.1-1).

Table 6.5.1-6: Quantification of fluopyram-pyridyl-acetic acid and transformation products, expressed as % of applied radioactivity (% AR)

Compound	Rep. No.	pH 4 0 h	pH 4 20 min 90 °C	pH 4 20 min 90 °C, shaken	pH 5 0 h	pH 5 60 min 100 °C	pH 5 60 min 100 °C, shaken	pH 6 0 h	pH 6 20 min 120 °C	pH 6 20 min 120 °C, shaken
fluopyram-pyridyl-acetic acid (M40)	Mean	95.1	0.0	0.0	99.0	0.0	0.0	99.0	19.0	18.8
fluopyram-picoline (M46)	Mean	3.7	89.8	96.7	0.0	89.4	94.5	0.0	73.9	76.0
Unknown compounds	Mean	1.2	1.3	0.4	1.0	0.7	0.9	1.0	0.2	0.4
Total % recovery	Mean	100.0	91.1	97.1	100.0	90.2	95.4	100.0	93.1	95.2
% AR in gaseous phase	Mean	6.0			5.2			2.1		

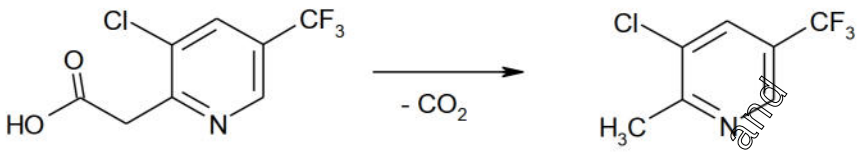


Figure 6.5.1-1: **Proposed metabolic pathway of [pyridyl-2,6-^{14}C]fluopyram-pyridyl-acetic acid under conditions of pasteurization, baking and sterilization**

III. Conclusions

Fluopyram-pyridyl-acetic acid was found to be unstable under conditions being representative for pasteurization, baking, brewing, boiling and sterilization. Under these conditions the transformation product fluopyram-picoline was formed up to 96.7% of the AR. Therefore, it can be concluded that the above processing operations will affect the nature of fluopyram-pyridyl-acetic acid residues in raw agricultural commodities.

Assessment and conclusion by applicant:
The study is valid and acceptable.

CA 6.5.2 Distribution of the residue in incdible peel and pulp

Not relevant for the representative uses.

CA 6.5.3 Magnitude of residues in processed commodities

Data on residues in strawberry and tomato after processing were submitted for the first inclusion of fluopyram under Regulation (EC) N° 1107/2009.

- For strawberry, 4 European processing trials (2 from Northern Europe, 2 from Southern Europe) from 2006 to 2007 were available to support these representative uses.
- For tomato, a total of 4 Southern European processing trials and 1 US processing trial performed from 2006 to 2007 were available to support these representative uses.

As strawberry and tomato are not representative uses for the current active substance renewal dossier, these below listed trials will not be summarized for a sake of clarity.

First inclusion fluopyram processing trials conducted on strawberry and tomato

Residue trials				Dossier reference
Zone	Crop	References Report No. Authors, year	Total number of trials	
NEU	strawberry	M-295535-01-1 [redacted] 2007	2	KCA 6.3.5/06
SEU	strawberry	M-295517-01-1 [redacted] 2007	2	KCA 6.5.3/05

SEU	tomato	M-295818-02-1 [redacted] 2007	3	KCA 6.3.5/07
	tomato	M-298072-01-1 [redacted] 2007	1	KCA 6.5.3/08
US	tomato	M-299429-01-1 [redacted] 2008	1	KCA 6.5.3/09

Data Point:	KCA 6.5.3/01
Report Author:	[redacted]
Report Year:	2008
Report Title:	Determination of the residues of AE C656948 in/on grape (bunch of grapes) and bunch of grapes for wine prod. and the processed fractions (juice; raw juice; washings; pomace, dried; pomace, wet; berry, washed; retentate; pomace, grape; must; wine at 1st taste test; wine) after low-volume spraying of AE C656948 (400 SC) in the field in Northern France
Report No:	RA-3611/06
Document No:	M-296826-01-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC Guidance Working Document 7029/VI/95 rev. 5 (1997-07-22); Equivalent to US EPA OPPTS Guideline No. 860.1500 (SUPP)
Deviations from current test guideline:	none
Previous evaluation:	yet evaluated and accepted rev. 1 to Vol.3 of DAR D7 August 2012 (references relied on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/officially recognised testing facilities
Acceptability/Reliability:	Yes

Data Point:	KCA 6.5.3/02
Report Author:	[redacted]
Report Year:	2008
Report Title:	Determination of the residues of AE C656948 in/on grape (bunch of grapes) and bunch of grapes for wine proc. and the processed fractions (juice; raw juice; washings; pomace, dried; pomace, wet; berry, washed; retentate; pomace, grape; must;
Report No:	RA-3647/06
Document No:	M-296549-01-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC Guidance Working Document 7029/VI/95 rev. 5 (1997-07-22); US-EPA OPPTS Guideline No. 860.1520 Supplemental
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted rev. 1 to Vol.3 of DAR, B7 August 2013 (references refind on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Balance studies on processing of grapes into grape wine were conducted to determine the transfer of fluopyram and its metabolites Fluopyram-benzamide, fluopyram-pyridyl-acetic-acid and fluopyram-pyridyl-carboxylic-acid from bunch of grapes into processed fractions.

Materials and Methods

Wine was produced from red grapes obtained from 4 different trials located in Southern and Northern France in the 2006 season in order to determine the magnitude of the residues of fluopyram (AE C656948) and its metabolites fluopyram-PAA (BCS-AI0139), fluopyram-benzamide and fluopyram-PCA (AEC 657188) in grape processed commodities (juice; raw juice; washings; pomace, dried; pomace, wet; berry, washed; retentate; pomace, grape; must; wine at 1st taste test; wine) following low-volume spraying of fluopyram (500 SC).

Field part

In the field trials (study reference RA-2611/06, KCA 6.3.1/01 and RA-2647/06, KCA 6.3.1/02), two foliar spray applications with Fluopyram SC500 - a suspension concentrate formulation containing 500 g/L fluopyram - were conducted at a single application rate of 250 g a.s./ha/application and water rate at 200 L/ha. The achieved total seasonal rate was 0.500 kg a.s./ha. The applications were conducted at BBCH of 81 (for the first application) and BBCH of 85-89 (for the second application).

For residue analysis, grape samples (bunches of grapes) were taken from the treated and the control plot on day 3 after the last application. Grape samples (bunches of grapes) for processing were taken from the treated plots and the control plots on the same day.

The field sub-samples from both trials were stored deep-frozen within 24 hours after sampling and until dispatch to the laboratory for logistics and preparation. All field sub samples were shipped by deep-freeze lorry and arrived in good condition. The samples for residue analysis were stored in a freezer at ≤-18°C until preparation of the examination samples.

The samples for processing were stored in a freezer at ≤-18°C or below until processing procedures.

The samples from processing were deep-frozen again immediately after the respective processing step. The processed samples were stored at ≤-18°C until preparation of the examination samples. For the preparation of examination samples of raw agricultural commodity and processed commodities, the deep-frozen samples were shredded with dry ice in a cutter. Representative parts of the shredded samples were transferred into polystyrene boxes and stored at ≤-18°C until analysis.

Processing procedures

The processing of bunch of grapes into berries, washed; washing water; pomace, wet; pomace, dried; retentate; raw juice and juice simulated the industrial practice at a laboratory scale.

The processing of bunches of grapes into must, pomace, grape, wine at 1st test taste and wine was performed using industrial practice.

All processing procedures are outlined on the flow charts (

Diagram 6.5.3- 1 and Diagram 6.5.3- 2) below.

Processing of Bunches of Grapes into Berry, washed; Washing Water; Retentate; Pomace, wet; Pomace, dried; Raw Juice and Juice

The processing started in deep-frozen state. The bunches of grapes were destemmed into berries, stalks and stems. The waste of stalks and stems was disposed. The destemmed berries were washed for approx. 2 min in luke-warm standing water (ratio water/fruits = 2/1 (w/w)) by moving them around slowly. After washing, the berries were let to drain in a sieve. An aliquot of the obtained laboratory sample of berry, washed was filled into a plastic bag. The obtained laboratory sample of washing water was prepared by filling a portion into polystyrene boxes.

The washed berries remaining for further processing were crushed in a cutter to get mash. During mashing in two trials (R 2006 0650/8 TA and R 2006 0415/7 TA) the cutter was leaky, and portions of about > 1 kg were lost. The mash was pressed in a high-pressure press into raw juice and pomace, wet. The obtained laboratory sample of pomace, wet was prepared by filling an aliquot into a plastic bag. The laboratory sample of raw juice was prepared by filling an aliquot into polystyrene boxes. In addition, a small amount of the raw juice was taken apart for the determination of the pH-value and titratable acid. The remaining portion of pomace, wet was dried in a fan-assisted oven at about 100°C until the water content was nearly 10%. The obtained laboratory sample of pomace, dried was filled into a plastic bag.

Table 6.5.3- 1: Water content of pomace, wet and pomace, dried

trials	Région	% Water content	
		Pomace, wet	Pomace, dried
R 2006 0415/7 CA	NEU	49	11
R 2006 0415/7 TA		54	11
R 2006 0650/8 TA		53	14
R 2006 0623/0 CA	SEU	55	11
R 2006 0623/0 TA		52	11
R 2006 0622/2 TA		51	8

The portion of raw juice remaining for further processing was depectinised by heating for approx. 30 sec to 80 – 85°C. The raw juice was cooled down to 45 – 50°C using icewater. The pectolytic enzyme Novo Pectinex 3XL (200 µL/kg juice) was added and the raw juice was left to stand for one hour at room temperature. Then the juice was stored for about 20 hours (overnight) at 4°C, except in trial R 2006 0622/2 TA, where the raw juice was stored for 2 hours at room temperature until the following processing. After the cold storage, the stated clear juice was lifted up by a soup ladle. The restless juice with lees was separated by centrifugation for 10 min at 7000 rpm. The waste of lees was disposed. The Brix value of the coarsely fined grape juice was determined. The coarsely fined juice was filtrated using a laboratory ultra-filtration set-up to obtain clear juice and retentate. The Brix value of clear juice and retentate was determined. An aliquot of the obtained laboratory sample of retentate was filled into polystyrene boxes. The filtrated juice was pasteurised in a plate-heat-exchanger. After pasteurisation, the juice was collected in fractions. The Brix value of each fraction was determined to differentiate and combine the right fractions of grape juice without rates of water. The pasteurisation value is calculated (pasteurisation time and temperature) and should be in a range of 2 to 5 (according to apple juice).

Table 6.5.3- 2: Pasteurisation values

trials	Région	Pasteurisation values
		Juice
R 2006 0415/7 CA	NEU	2.1
R 2006 0415/7 TA		1.9
R 2006 0650/8 TA		4
R 2006 0623/0 CA	SEU	3
R 2006 0623/0 TA		4.7
R 2006 0622/2 TA		3.8

CA : control sample
TA : treated sample

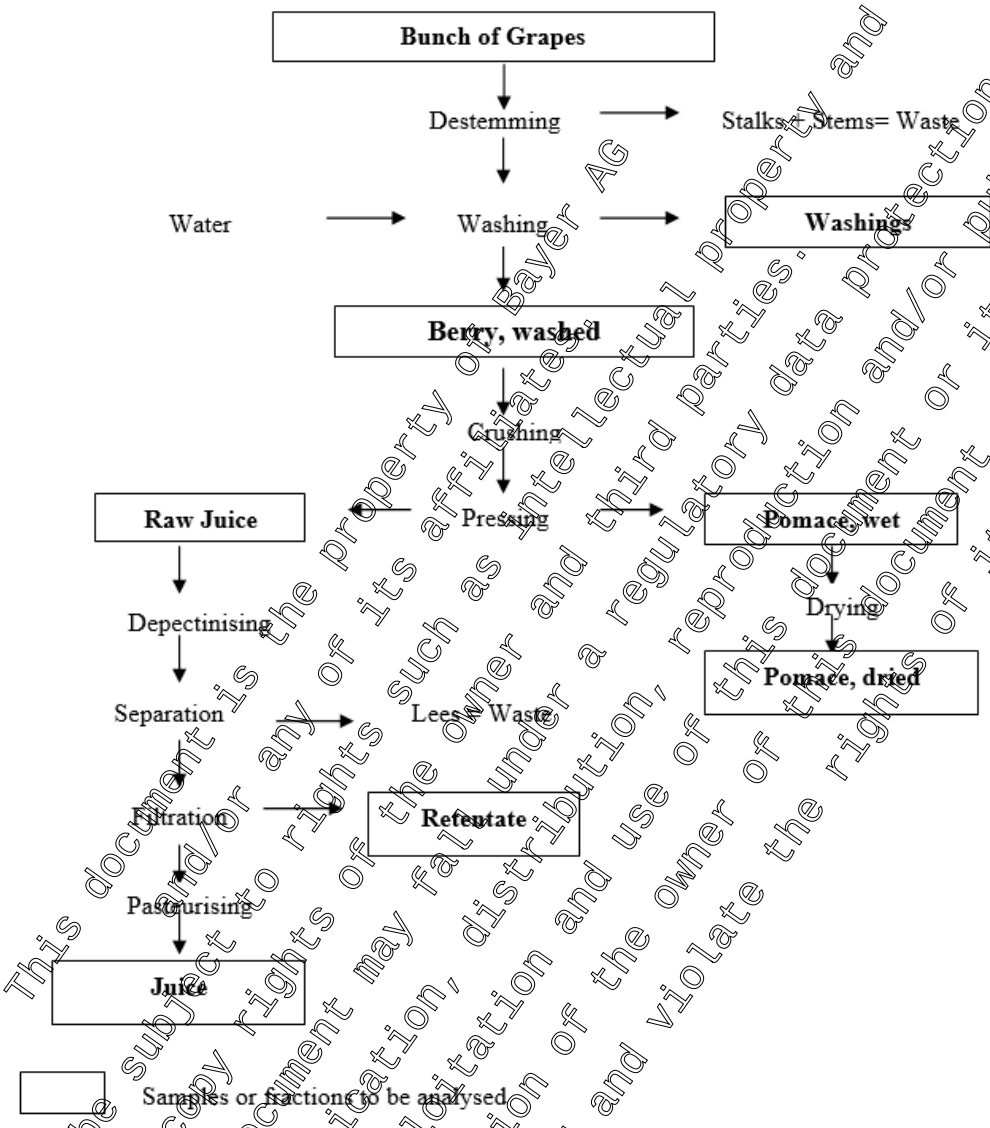
An aliquot of the obtained laboratory sample of juice was filled into polystyrene boxes. In addition, a small amount of the juice was taken apart for the determination of the pH-value and titratable acid.

All aliquots of laboratory samples were stored deep-frozen at ≤-18°C for 1 to 14 days until handover to preparation of the examination samples.

Table 6.5.3- 3: Determination of Brix Value, pH-value and titratable acid

Trial no.	Région	Sample material	Brix value	pH- value	Total Acid (calculated as tartaric acid) ρ in g/L
R 2006 0415/7 CA	NEU	Raw juice	n.d.	3.5	5.0
		Juice	15.9 °	3.6	4.3
R 2006 0415/7 TA		Raw juice	n.d.	3.5	4.8
		Juice	14.4 °	3.5	4.3
R 2006 0650/8 TA		Raw juice	n.d.	3.6	4.8
		Juice	15.2 °	3.6	4.5
R 2006 0623/0 CA	SEU	Raw juice	n.d.	3.9	5.3
		Juice	16.6°	3.9	5.1
R 2006 0623/0 TA		Raw juice	n.d.	3.8	4.9
		Juice	14.6	3.8	4.6
R 2006 0622/2 TA		Raw juice	n.d.	4.0	2.1
		Juice	24.4°	4.1	1.8

Diagram 6.5.3- 1: Flow chart for the preparation of bunches of grapes into berries, washed; washing water; retentate; pomace, wet; pomace, dried; raw juice and juice



Processing of Bunches of Grapes into Must, Pomace, Grape; Wine at 1st Test Taste and Wine

The vinification started on 2007-03-28. The grapes were allowed to thaw. The partly thawn grape samples were weighed and subsequently crushed and destemmed in a grape crusher. A mash heating was performed by heating up the obtained mash with steam to a temperature of T = 82°C for 3 min while stirring. The mash was allowed to cool down. The mash was pressed in a cloth press, while increasing the pressure step by step from 0.2 to 2.0 bar. The obtained laboratory sample of pomace, grape was prepared by filling an aliquot into polystyrene boxes. The obtained must was filled into vessels and a hyposulfite solution (100 mg hyposulfite per litre must) was added. To prevent a later protein haze in the wine, 2 g/L of the fining agent bentonite was added to the wine. The must was allowed to clarify. After clarifying overnight, the must was decanted from the lees. An aliquot of the obtained laboratory sample of freshly decanted must was filled into polystyrene boxes on 2007-03-29. The density of the obtained must was determined. No sugar was added prior to fermentation.

Table 6.5.3- 4: Density of Must

trials	Région	Must Density in °Oe
R 2006 0415/7 CA	NEU	77
R 2006 0415/7 TA		75
R 2006 0650/8 TA		75
R 2006 0623/0 CA	SEU	76
R 2006 0623/0 TA		74
R 2006 0622/2 TA		115

The fermentation was started by addition of 20 g/100 L of pure-culture yeast to the must. During the fermentation, the loss of weight due to the conversion of sugar into CO_2 was monitored. The first racking was done 29 days after start of fermentation on 2007-03-29. After removal of the yeast sediment, 50 mg/L of potassium hyposulfite was added to the young wine and the finishing of the wine was started. A second racking was not done. The wine was filtered on 2007-05-15. A portion of the obtained laboratory sample of wine (wine 1) was filled in polystyrene boxes and stored deep-frozen at ≤-18 °C until shipment to Monheim. For the preparation of wine at 1st taste test (wine 2), a portion of the laboratory sample wine 1 was filled into bottles and stored at a temperature of approx. 12 °C. The laboratory sample of wine 1 and the sample for preparation of wine 2 were delivered in Monheim on 2007-05-30. The maturing of wine 2 was ongoing until 2007-09-24 and lasted for 132 days overall. The obtained laboratory sample of wine at 1st taste test (wine 2) was filled in polystyrene boxes and deep-frozen at a temperature of ≤-18 °C until the preparation into examination samples followed on the same day.

Diagram 6.5.3- 2: Flow chart of the preparation of bunches of grapes into pomace, grape; must; wine and wine at 1st taste test

Residue analysis

Residues of fluopyram and its metabolites were determined by LC-MS/MS according to method 00984/M001 ([redacted], 2007, M-295145-03-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues were extracted from 10 g of sample material (pomace, wet; pomace, dried and pomace, grape: 5 g) by two successive extractions using a high speed blender with a mixture of acetonitrile:water (80:20; v:v).

Subsequently, the raw extracts were diluted 10-fold by adding internal standard solutions:

- One dilution and an additional clean-up step performed under acidic conditions (for determination of FLU-PCA)
- Another dilution performed under basic conditions (for determination of fluopyram, FLU-benzamide and FLU-PAA)

Residues were quantified by reversed-phase chromatography coupled with tandem mass spectrometry (MS/MS) with electrospray ionisation. One injection in positive electrospray ionisation allowed the determination of fluopyram, FLU-benzamide and FLU-PAA. Another injection in negative electrospray ionisation allowed the determination of FLU-PCA under different conditions.

The Limit of Quantitation (LOQ, calculated and expressed as fluopyram parent equivalents for fluopyram and its metabolites), defined as the lowest validated fortification level, was 0.01 mg/kg in all matrices for all analytes. All residues are calculated as parent fluopyram.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

Findings

No apparent residues above the LOQ were present in the control samples. In order to check the performance of the method, recovery determinations were concurrently performed to the analyses of control and treated samples in each set of analyses. Concurrent recoveries were obtained during the conduct of the residue studies RA-2611/06, RA-2612/06 and RA-2647/06 (KCA 6.3.1/01 to 03) and corresponding processing studies (RA-3611/06, RA-3612/06 and RA-3647/06). All data of the method performance and recoveries are shown in the tables below.

The mean concurrent recoveries were within the acceptable range of 70 % – 110% in all tested matrices. The RSD values (although not all could be calculated) were ≤ 20 %.

The storage period of deep-frozen processed samples ranged between 2 and 125 days.

Table 6.5.3-5: Concurrent recoveries of Fluopyram and its metabolites

Portion analysed	Matrices covered	n	Fortification level (mg/kg) *	Recovery (%)				
				Individual recoveries	Min	Max	Mean	RSD
Fluopyram (AE C656948)								
Bunch of grapes	bunch of grapes; berry, washed	3	0.01	108; 111; 102	102	111	107	4.3
		4	0.10	91; 105; 100; 92	91	105	97	6.9
		2	1.0	90; 93	90	93	92	--
			Overall Recovery (n = 9)				**99**	**8.0**
Juice	juice; raw juice; washings; retentate	3	0.01	96; 92; 95	92	96	94	2.2
		3	0.10	99; 95; 87	87	99	94	6.5
			Overall Recovery (n = 6)				**94**	**4.4**
Pomace, dried	pomace, dried; pomace, wet; pomace, grape; raisin;	2	0.01	95; 105	95	105	100	--
		2	1.0	96; 98	96	98	97	--
		1	2.0	97	--	--	97	--
		1	10	86	--	--	86	--

Portion analysed	Matrices covered	n	Fortification level (mg/kg) *	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
	raisin waste		**Overall Recovery (n = 6)**				**96**	**6.4**
Must	must	1	0.01	100	--	--	100	--
		1	0.10	97	--	--	97	--
		1	0.50	94	--	--	94	--
			Overall Recovery (n = 3)				**97**	**3.1**
Wine at 1st taste test	wine at 1st taste test; grape wine	2	0.01	97; 87	87	97	92	--
		2	0.10	98; 96	96	98	97	--
			Overall Recovery (n = 4)				**95**	**5.4**
Fluopyram-pyridyl-acetic-acid (BCS-AA10139)								
Bunch of grapes	bunch of grapes; berry, washed	3	0.01	95; 83; 86	83	95	88	7.1
		4	0.10	101; 80; 80; 83	80	101	86	11.7
		2	1.0	111; 80	80	111	96	--
			Overall Recovery (n = 9)				**89**	**12.5**
Juice	juice; raw juice; washings; retentate	3	0.01	97; 92; 85	85	97	91	--
		3	0.10	91; 91; 90	90	91	91	--
			Overall Recovery (n = 6)				**91**	**4.2**
Pomace, dried	pomace, dried; pomace, wet; pomace, grape; raisin; raisin waste	2	0.01	85; 92	85	92	89	--
		2	1.0	83; 87	83	87	85	--
		1	2.0	96	--	--	--	--
		1	10	85	--	--	--	--
			Overall Recovery (n = 6)				**88**	**5.7**
Must	must	3	0.01	87	--	--	87	--
		1	0.10	92	--	--	92	--
		1	0.50	95	--	--	95	--
			Overall Recovery (n = 3)				**91**	**4.4**
Wine at 1st taste test	wine at 1st taste test; grape wine	2	0.01	90; 77	77	90	84	--
		2	0.10	89; 95	89	95	92	--
			Overall Recovery (n = 4)				**88**	**8.7**
Fluopyram-benzamide (AE F148815)								
Bunch of grapes	bunch of grapes; berry, washed	3	0.01	113; 100; 92	92	113	102	10.4
		4	0.10	94; 87; 91; 90	87	94	91	3.2
		2	1.0	101; 93	93	101	97	--
			Overall Recovery (n = 9)				**96**	**8.3**
Juice	juice; raw juice; washings; retentate	3	0.01	91; 103; 104	91	104	99	7.3
		3	0.10	91; 106; 95	91	106	97	8.0
			Overall Recovery (n = 6)				**98**	**6.9**
Pomace, dried	pomace, dried; pomace, wet; pomace, grape; raisin; raisin waste	2	0.01	85; 99	85	99	92	--
		2	1.0	91; 93	91	93	92	--
		1	2.0	103	--	--	103	--
		1	10	93	--	--	93	--
			Overall Recovery (n = 6)				**94**	**6.7**
Must	must	1	0.01	95	--	--	95	--
		1	0.10	89	--	--	89	--
		1	0.50	94	--	--	94	--
			Overall Recovery (n = 3)				**93**	**3.5**
Wine at 1st taste test	wine at 1st taste test; grape wine	2	0.01	87; 105	87	105	96	--
		2	0.10	95; 111	95	111	103	--
			Overall Recovery (n = 4)				**100**	**10.7**
Fluopyram-pyridyl-carboxylic-acid (AEC657188)								
Bunch of grapes	bunch of grapes; berry, washed	3	0.01	83; 103; 86	83	103	91	11.9
		4	0.10	79; 96; 85; 89	79	96	87	8.2
		2	1.0	70; 79	70	79	75	--

Portion analysed	Matrices covered	n	Fortification level (mg/kg) *	Recovery (%)				
				Individual recoveries	Min	Max	Mean	RSD
			Overall Recovery (n = 9)				86	11.4
Juice	juice; raw juice; washings; retentate	3	0.01	97; 86; 95	86	97	93	6.3
		3	0.10	102; 102; 106	102	106	103	2.2
			Overall Recovery (n = 6)				98	7.2
Pomace, dried	pomace, dried; pomace, wet; pomace, grape; raisin; raisin waste	2	0.01	91; 111	91	111	101	--
		2	1.0	97; 105	97	105	101	--
		1	2.0	99	--	--	99	--
		1	10	85	--	--	85	--
			Overall Recovery (n = 6)				98	9.6
Must	must	1	0.01	96	--	--	96	--
		1	0.10	100	--	--	100	--
		1	0.50	101	--	--	101	--
			Overall Recovery (n = 3)				99	2.7
Wine at 1st taste test	wine at 1st taste test; grape wine	2	0.01	103; 107	103	107	105	--
		2	0.10	100; 104	100	104	102	--
			Overall Recovery (n = 4)				104	2.8

FL: fortification level, RSD - Relative Standard Deviation
* some RSDs were not calculated as there were only two individual recoveries given
Final determination as: fluopyram Residues calculated as: fluopyram
Final determination as: FLU-benzamide Residues calculated as: fluopyram
Final determination as: FLU-PCA Residues calculated as: fluopyram
Final determination as: FLU-PAA Residues calculated as: fluopyram

In bunch of grapes used for juice and wine production residues of fluopyram were between 0.36 and 0.58 mg/kg. Residues of fluopyram in the final products juice and wine were < 0.01 mg/kg and 0.06 – 0.08 mg/kg respectively. Residue values of fluopyram in juice, wine and the processed by-products are summarised in Table 6.5.3- 9 and Table 6.5.3- 10.

During processing for juice production a mean of 77% of the absolute residue was recovered after destemming in berries, washed and the washings. On further processing, a major part counting for 35% of the absolute residue remained in the pomace, whereas the residue in raw juice only accounted for 8% of the initial absolute residue. After filtration and pasteurisation no residues above the LOQ were detected in the final product juice.

With wine processing a mean of 43% of the initial absolute residue remained in the grape pomace, and 16% were recovered in must. Fermenting and filtration further reduced the residue concentration of fluopyram so that the final product wine only contained 8% and wine at first taint test 10% of the initial absolute residue.

Processing factors (PF) were calculated by dividing the fluopyram residue in grape processed commodities by the fluopyram residue in the raw agricultural commodity (RAC) bunch of grapes. Processing factors calculated for the individual grape processed commodity samples are summarized in the tables below.

- Concentration of fluopyram residues was observed only in dried pomace, wet pomace and grape pomace and its mean processing factors calculated based on four residue trials results were 6.4, 3.2 and 3.4, respectively. For the other matrices, no concentration of fluopyram was observed (PF <1.0).
- Concentration of fluopyram-benzamide residues was observed only in dried pomace, wet pomace and grape pomace and its mean processing factors calculated based on four residue trials results were 2.75, 1.25 and 1.25, respectively. For the other matrices, no concentration of fluopyram-benzamide was observed (PF <1.0),
- Concentration of fluopyram-pyridyl-carboxylic-acid residues was observed only in dried pomace, wet pomace and grape pomace and its mean processing factors calculated based on

four residue trials results were 2, 1 and 1, respectively. For the other matrices, no concentration of fluopyram-PCA was observed (PF <1.0),

- Concentration of fluopyram-pyridyl-acetic-acid was not observed.

Table 6.5.3- 6: Processing factors for fluopyram

Sample material	R 2006 0650/8 France North		R 2006 0415/7 France North		R 2006 0622/2 France South		R 2006 0623/0 France South		Mean PF
	Residues (mg/kg)	PF*	Residues (mg/kg)	PF*	Residues (mg/kg)	PF*	Residues (mg/kg)	PF*	
Bunch of grapes (RAC)	**0.51**	--	**0.58**	--	**0.36**	--	**0.58**	--	-
Juice	<0.01**	0.02	<0.01**	0.02	<0.01**	0.03	<0.01**	0.02	0.02
Raw Juice	0.06	0.1	0.06	0.1	0.05	0.1	0.09	0.2	0.1
Washings	0.05	0.1	0.06	0.1	0.03	0.1	0.02	0.03	0.1
Pomace, dried	3.0	6.0	2.8	4.8	2.7	7.5	4.2	7.2	6.4
Pomace, wet	1.6	3.1	1.3	2.2	1.4	3.9	2.1	3.6	3.2
Berry, washed	0.30	0.6	0.38	0.6	0.18	0.5	0.43	0.7	0.6
Retentate	0.05	0.1	0.05	0.1	0.03	0.1	0.08	0.1	0.1
Pomace, grape	2.0	4.0	1.6	2.7	1.2	3.3	2.1	3.6	3.4
Must	0.11	0.2	0.12	0.2	0.11	0.3	0.13	0.2	0.2
Wine at 1st taste test (wine 2)	0.10	0.2	0.08	0.1	0.07	0.2	0.10	0.1	0.2
Wine (wine 1)	0.08	0.2	0.06	0.1	0.08	0.2	0.08	0.2	0.2.

*Processing factor calculated according to the following equation:

$$PF = \frac{Residue\ concentration\ in\ the\ processed\ product\ [\frac{mg}{kg}]}{Residue\ concentration\ in\ the\ RAC\ [\frac{mg}{kg}]}$$

Remark: Procesing factors were calculated from the corresponding residue concentrations using unrounded values. For this reason, deviations can occur when the rounded values given in column for levels of residues of fluopyram in relevant matrices are used for recalculation.
RAC: Raw Agriculture Commodity
**: Values below LOQ which were set to the LOQ for the calculation of procesing factors

Table 6.5.3- 7: Processing factors for fluopyram-benzamide

Sample material	R 2006 0650/8 France North		R 2006 0415/7 France North		R 2006 0622/2 France South		R 2006 0623/0 France South		Mean PF
	Residues (mg/kg)	PF*	Residues (mg/kg)	PF*	Residues (mg/kg)	PF*	Residues (mg/kg)	PF*	
Bunch of grapes (RAC)	**<0.01**	-	**0.02**	--	**<0.01**	-	**0.02**	--	-
Juice	<0.01	-	0.01	0.5	<0.01	-	0.01	0.5	0.5
Raw Juice	<0.01	-	0.01	0.5	<0.01	-	0.02	1	0.75
Washings	<0.01	-	<0.01**	0.5	<0.01	-	<0.01**	0.5	0.5
Pomace, dried	<0.01	-	0.05	2.5	0.02	-	0.06	3	2.75
Pomace, wet	<0.01	-	0.02	1	<0.01	-	0.03	1.5	1.25
Berry, washed	<0.01	-	0.02	1	<0.01	-	0.02	1	1
Retentate	<0.01	-	0.01	0.5	<0.01	-	0.01	0.5	0.5
Pomace, grape	<0.01	-	0.02	1	<0.01	-	0.03	1.5	1.25
Must	<0.01	-	0.02	1	<0.01	-	0.02	1	1
Wine at 1st taste test (wine 2)	<0.01	-	0.02	1	<0.01	-	0.02	1	1
Wine (wine 1)	<0.01	-	0.01	0.5	<0.01	-	0.01	0.5	0.5

*Processing factor calculated according to the following equation:

$$PF = \frac{Residue\ concentration\ in\ the\ processed\ product\ [\frac{mg}{kg}]}{Residue\ concentration\ in\ the\ RAC\ [\frac{mg}{kg}]}$$

Remark: Procesing factors were calculated from the corresponding residue concentrations using unrounded values. For this reason, deviations can occur when the rounded values given in column for levels of residues of fluopyram-benzamide in relevant matrices are used for recalculation.
RAC: Raw Agriculture Commodity

**: Values below LOQ which were set to the LOQ for the calculation of procesing factors.

Table 6.5.3- 8: Processing factors for fluopyram-pyridyl-carboxylic-acid

Sample material	R 2006 0650/8 France North		R 2006 0415/7 France North		R 2006 0622/2 France South		R 2006 0623/0 France South		Mean PF
	Residues (mg/kg)	PF*	Residues (mg/kg)	PF*	Residues (mg/kg)	PF*	Residues (mg/kg)	PF*	
Bunch of grapes (RAC)	**<0.01**	-	**0.02**	-	**<0.01**	-	**0.04**	-	-
Juice	<0.01	-	<0.01**	0.5	<0.01	-	0.01	0.25	0.4
Raw Juice	<0.01	-	<0.01**	0.5	<0.01	-	0.02	0.5	0.5
Washings	<0.01	-	<0.01**	0.5	<0.01	-	<0.01**	0.25	0.4
Pomace, dried	<0.01	-	0.04	2	<0.01	-	0.08	2	2
Pomace, wet	<0.01	-	0.02	1	<0.01	-	0.04	1	1
Berry, washed	<0.01	-	0.01	0.5	<0.01	-	0.02	0.5	0.5
Retentate	<0.01	-	0.01	0.5	<0.01	-	0.02	0.5	0.5
Pomace, grape	<0.01	-	0.02	1	<0.01	-	0.04	1	1
Must	<0.01	-	0.02	1	<0.01	-	0.03	0.75	0.9
Wine at 1st taste test (wine 2)	<0.01	-	0.02	1	<0.01	-	0.04	1	1
Wine (wine 1)	<0.01	-	0.01	0.5	<0.01	-	0.02	0.5	0.5

*Processing factor calculated according to the following equation:

$$PF = \frac{Residue\ concentration\ in\ the\ processed\ product\ [\frac{mg}{kg}]}{Residue\ concentration\ in\ the\ RAC\ [\frac{mg}{kg}]}$$

Remark: Procesing factors were calculated from the corresponding residue concentrations using unrounded values. For this reason, deviations can occur when the rounded values given in column for levels of residues of fluopyram-PCAin relevant matrices are used for recalculation.
RAC: Raw Agriculture Commodity
**: Values below LOQ which were set to the LOQ for the calculation of procesing factors.

As no residue were detected for fluopyram-pyridyl-acetic-acid (BCS AA10139), no processing factors were calculated.

Conclusion

The study was conducted according to the relevant guidelines. The results of control samples, and recovery samples were in the expected range.

Mean processing factors between 3.2 and 6.4 were calculated for fluopyram in pomace (wet, dried and grape). Procesing factors below 1 were calculated for fluopyram in the other processed commodities.

Mean processing factors between 1.25 and 2.75 were calculated for fluopyram-benzamide in pomace (wet, dried and grape). Processing factors below 1 were calculated fluopyram-benzamide in the other processed commodities.

Mean processing factors between 1 and 2 were calculated for fluopyram-PCA in pomace (wet, dried and grape). Procesing factors below 1 were calculated for fluopyram-PCA in the other processed commodities.

Assessment and conclusion by applicant:

The study is acceptable.

Table 6.5.3- 9: Results of processing trials conducted with fluopyram on grape

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treat-ment	Portion analysed	Growth stage at sampling	Residues (mg/kg)				PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					fluopyram	FLU-PCA	FLU-benzamide	FLU-PAA		
	(a)	(b)				(c)	(d)		(d)					(e)	(f)
R 2006 0650/8 0650-06 France, north F-71850 Charnay les Macon (Rhone-Alpes) Europe, North F 2006	Grape Pinot noir /PG161.49; red variety	1) 01.04.2002 2) 07.06.2006 - 15.06.2006 3) 10.09.2006 - 15.09.2006	250.0 250.0	200 200	125.00 125.00	11.08.2006/0 08.09.2006/28	89	bunch of grapes	89 89 89 89 91 92	0.22 0.51 0.51 0.48 0.41 0.36 0.30	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	0* 0 3 7 14 21 28	(g) RA-2611/06 (h) Fluopyram 500 SC (i) Spraying (j) Analytical method: 00984/M001 (k) LOQ: 0.01 mg/kg (l) Method Validation Data: 00984/M001, (m) Storage: wine at first taste test: 380 days wine: 323 days, washings: 379 days, retentate: 379 days, raw juice: 379 days, pomace, wet: 379 days, pomace, grape: 323 days, pomace, dried: 379 days, must: 323 days, juice: 379 days, bunch of grapes: 200 days, berry, washed: 379 days
								juice	89	<0.01	<0.01	<0.01	<0.01	3	
								raw juice	89	0.06	<0.01	<0.01	<0.01	3	
								washings	89	0.05	<0.01	<0.01	<0.01	3	
								pomace, dried	89	3.0	<0.01	<0.01	<0.01	3	
								pomace, wet	89	1.6	<0.01	<0.01	<0.01	3	
								berry, washed	89	0.30	<0.01	<0.01	<0.01	3	
								retentate	89	0.05	<0.01	<0.01	<0.01	3	
								pomace, grape	89	2.0	<0.01	<0.01	<0.01	3	
								must	89	0.11	<0.01	<0.01	<0.01	3	
								wine at first taste test	89	0.10	<0.01	<0.01	<0.01	3	
								wine	89	0.08	<0.01	<0.01	<0.01	3	

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treat-ment	Portion analysed	Growth stage at sampling	Residues (mg/kg)				PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					fluopyram	FLU-PCA	FLU-benzamide	PLU-PAA		
	(a)	(b)				(c)	(d)		(d)					(e)	(f)
R 2006 0415/7 0415-06 France, north F-37270 Athée sur Cher (Centre) Europe, North F 2006	Grape Gamay; red variety	1) 01.01.1995 2) 08.06.2006 - 15.06.2006 3) 18.09.2006	250.0 250.0	200 200	125.00 125.00	08.08.2006/0 12.09.2006/35	85	bunch of grapes	85 85 89 89 89 91	0.88 0.66 0.58 0.50 0.40 0.50	0.02 0.02 0.02 0.02 0.02 0.03	0.02 0.02 0.02 0.02 0.02 0.03	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	0 0 3 7 14 21 28	(g) RA-2611/06 (h) Fluopyram 500 SC (i) Spraying (j) Analytical method: 00984/M001 (k) LOQ: 0.01 mg/kg (l) Method Validation Data: 00984/M001 (m) Storage: wine at first taste test: 376 days 4 wine: 319 days washings: 375 days retentate: 375 days raw juice: 375 days pomace, wet: 375 days pomace, grape: 319 days pomace, dried: 375 days must: 319 days juice: 375 days bunch of grapes: 188 days berry, washed: 375 days
								juice	89	<0.01	<0.01	0.01	<0.01	3	
								raw juice	89	0.06	0.01	0.01	<0.01	3	
								washings	89	0.06	<0.01	<0.01	<0.01	3	
								pomace, dried	89	0.8	0.04	0.05	<0.01	3	
								pomace, wet	89	1.3	0.02	0.02	<0.01	3	
								berry, washed	89	0.38	0.01	0.02	<0.01	3	
								retentate	89	0.05	0.01	0.01	<0.01	3	
								pomace, grape	89	1.6	0.02	0.02	<0.01	3	
								must	89	0.12	0.02	0.02	<0.01	3	
								wine at first taste test	89	0.08	0.02	0.02	<0.01	3	
								wine	89	0.06	0.01	0.01	<0.01	3	

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) study reference
* prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Table 6.5.3- 10: Results of processing trials conducted with fluopyram on grape

Analyte 1: Fluopyram AE C656948 (determined as AE C656948, calculated as AE C656948), Analyte 2: AE C657188 (determined as AE C657188, calculated as AE C656948), Analyte 3: AE C656948-benzamide/ AE F148815 (determined as AE C656948-benzamide/ AE F148815, calculated as AE C656948), Analyte 4: AE C656948-pyridyl-acetic acid/BCS-AA10139 (determined as AE C656948-pyridyl-acetic acid, calculated as AE C656948)

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treat-ment	Portion analysed	Growth stage at sampling	Residues (mg/kg)				PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					fluopyram	FLU-PCA	FLU-benzamide	FLU-PAA		
	(a)	(b)				(c)	(d)		(d)					(e)	(f)
R 2006 0622/2 0622-06 France, south F-30290 Laudun (Languedoc-Roussillon) Europe, South F 2006	Grape Grenache Noir; Red variety	1) 01.04.1985 2) 28.05.2006 - 05.06.2006 3) 15.09.2006 - 16.09.2006	250.0 250.0	200 200	125.0 125.0	01.08.2006/0 12.09.2006/4 2	85	bunch of grapes	85	0.17	<0.01	<0.01	<0.01	0*	(g) RA-2647/06 (h) Fluopyram 500 SC (i) Application method: Spraying, low-volume (j) Analytical method: 00984/M001 (k) LOQ: 0.01 mg/kg (l) Method Validation Data: 00984/M001 (m) Storage: wine at first taste test: 376 days wine: 319 days washings: 376 days retentate: 376 days raw juice: 376 days pomace, wet: 376 days pomace, grape: 319 days pomace, dried: 376 days must: 319 days juice: 376 days bunch of grapes: 175 days berry, washed: 376 days
									85	0.44	<0.01	<0.01	<0.01	0	
									89	0.16	<0.01	<0.01	<0.01	3	
									89	0.29	<0.01	<0.01	<0.01	7	
									89	0.23	<0.01	<0.01	<0.01	14	
									89	0.27	<0.01	<0.01	<0.01	21	
									89	0.35	<0.01	<0.01	<0.01	28	
								juice	89	<0.01	<0.01	<0.01	<0.01	3	
								raw juice	89	0.05	<0.01	<0.01	<0.01	3	
								washings	89	0.03	<0.01	<0.01	<0.01	3	
								pomace, dried	89	2.7	<0.01	0.02	<0.01	3	
								pomace, wet	89	1.4	<0.01	<0.01	<0.01	3	
								berry, washed	89	0.18	<0.01	<0.01	<0.01	3	
								retentate	89	0.03	<0.01	<0.01	<0.01	3	
								pomace, grape	89	1.2	<0.01	<0.01	<0.01	3	
								must	89	0.11	<0.01	<0.01	<0.01	3	
								wine at first taste test	89	0.07	<0.01	<0.01	<0.01	3	
								wine	89	0.08	<0.01	<0.01	<0.01	3	

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treat-ment	Portion analysed	Growth stage at sampling	Residues (mg/kg)				PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					Fluopyram	FLU-PCA	FLU-benzamide	FLU-PAA		
	(a)	(b)				(c)	(d)		(d)					(e)	(f)
R 2006 0623/0 0623-06 France, south F-31620 Fronton (Midi-Pyrenees) Europe, South F 2006	Grape Négrette; red variety	1) 01.01.1990 2) 01.06.2006 - 15.06.2006 3) 10.09.2006 - 29.09.2006	250.0 250.0	200 200	125.00 125.00	09.08.2006/0 01.09.2006/2 3	89	bunch of grapes	89	0.13	0.02	0.01	<0.01	0	(g) RA-2647/06 (h) Fluopyram 500 SC (i) Application method: Spraying, low-volume (j) Analytical method: 00984/M001 (k) LOQ: 0.01 mg/kg (l) Method Validation Data: 00984/M001 (m) Storage: wine at first taste test: 387 days wine: 330 days washings: 387 days retentate: 387 days raw juice: 387 days pomace, wet: 387 days pomace, grape: 330 days pomace, dried: 387 days must: 330 days juice: 387 days bunch of grapes: 186 days berry, washed: 387 days
									89	0.39	0.02	0.01	<0.01	0	
									89	0.58	0.04	0.02	<0.01	3	
									89	0.37	0.05	0.02	<0.01	7	
									89	0.25	0.06	0.02	<0.01	14	
									89	0.26	0.05	0.02	<0.01	21	
									89	0.24	0.06	0.02	<0.01	28	
								juice	89	<0.01	0.01	0.01	<0.01	3	
								raw juice	89	0.09	0.02	0.02	<0.01	3	
								washings	89	0.02	<0.01	<0.01	<0.01	3	
								pomace, dried	89	4.2	0.08	0.06	<0.01	3	
								pomace, wet	89	2.1	0.04	0.03	<0.01	3	
								berry, washed	89	0.43	0.02	0.02	<0.01	3	
								retentate	89	0.08	0.02	0.01	<0.01	3	
								pomace, grape	89	2.1	0.04	0.03	<0.01	3	
								must	89	0.13	0.03	0.02	<0.01	3	
								wine at first taste test	89	0.10	0.04	0.02	<0.01	3	
								wine	89	0.08	0.02	0.01	<0.01	3	

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) Study reference
prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Data Point:	KCA 6.5.3/03
Report Author:	[redacted]
Report Year:	2008
Report Title:	Determination of the residues of AE C656948 in/on table grape (bunch of grapes) and the processed fractions (raisin; raisin waste; washings) after spraying of AE C656948 (500 SC) in the field in Spain, Portugal, Italy and Greece
Report No:	RA-3612/06
Document No:	M-296512-01-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC Guidance Working Document 7029/VI/95 rev. 5 (1997-07-22); US-EPA OPPTS Guideline No. 860.1520 Supplemental
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted rev. 1 to Vol.3 of DAR 07 August 2012 (references used on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/officially recognised testing facilities
Acceptability/Reliability:	Yes

The study included four supervised field residue trials with grape conducted in Southern Europe (Spain, Portugal, Italy, and Greece) in the 2006 season in order to determine the magnitude of the residues of fluopyram and its metabolites Fluopyram-benzamide, fluopyram-pyridylacetic-acid and fluopyram-pyridyl-carboxylic-acid in table grapes and in processed fractions and processing by-products (raisin; raisin waste; washings) following spraying of fluopyram (500 SC).

I. Materials and Methods

Field part

In the field trials (KCA 6.3.1/03), two foliar spray applications with fluopyram (500 SC) - a suspension concentrate formulation containing 500 g/L fluopyram - were conducted at a single application rate of 250 g a.s./ha/application and water rate at 600-1000 L/ha. The achieved total seasonal rate was 0.500 kg a.s./ha. The applications were conducted at BBCH of 81(for the first application) and BBCH of 85-89 (for the second application).

For residue analysis, table grape samples (bunches of grapes) were taken on day 3 after the last application. Table grape samples (bunches of grapes) for processing were taken on the same day. A table grape sample (bunches of grapes) for processing of control material was taken from trial R 2006 0417/3. The field sub samples from all trials were stored deep-frozen within 24 hours after sampling. The samples for residue analysis and for processing were stored in a freezer at ≤-18°C until preparation of the examination samples and processing procedures.

The samples for processing were stored in a freezer at ≤-18°C or below until processing procedures.

The samples from processing were deep-frozen again immediately after the respective processing step. The processed samples were stored at ≤-18°C until preparation of the examination samples. For the preparation of examination samples of raw agricultural commodity and processed commodities, the deep-frozen samples were shredded with dry ice in a cutter. Representative parts of the shredded samples were transferred into polystyrene boxes separately for analysis (UP samples) and archiving (UR samples) and stored at ≤-18°C until analysis.

Processing procedures

The processing of the samples of bunches of grapes into raisins was performed in the Food Processing Laboratory (FPL) of BCS-D-ROCS in Monheim and simulated the industrial practice at a laboratory scale.
Processing procedures are outlined on the flow chart (Diagram 6.5.3- 3) below.

Processing of Bunches of Grapes into Raisins

Damaged and unripe berries were sorted out. The bunches of grapes were destemmed before drying.

In trial R 2006 0651/6 the whole berries were already removed from the stalks. In trial R 2006 0417/3 about 90 % of the whole berries were already removed from the stalks. The destemmed berries and the sample of raisin waste were dried at a temperature of 60 to 65 °C in a fan-assisted oven until a water content of approx. 10 – 15 % was achieved.

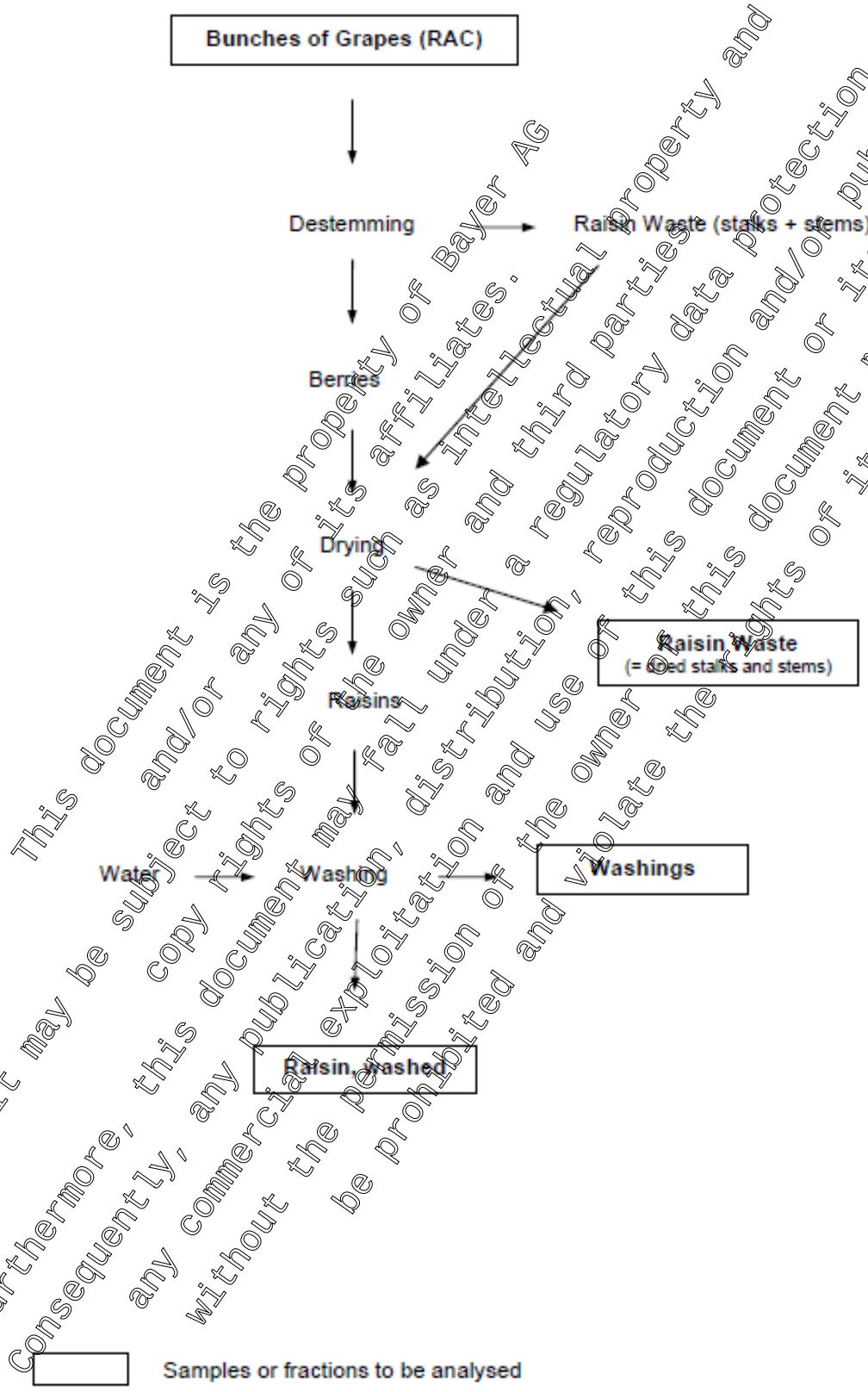
The dried berries were washed in lukewarm standing water by moving them around slowly (ratio dried berries / water = 1/2 (w/w)). During washing the dried berries absorb water. After washing the water content should be in a range between 9 and 15 %. Depending on the sugar content, the raisins absorb more water than desired (up to 23 %).

The washed raisins, the raisin waste and the washing water were sampled.

Table 6.5.3- 11: Water content of raisins during the processing

Trial	Water Content after Drying	Time of Drying	Water Content after Washing
R 2006 0417/3 C	14 %	27 h	16 %
R 2006 0417/3 T	16 %	37 h	22 %
R 2006 0624/9 T	13 %	26 h	19 %
R 2006 0651/6 T	12 %	32 h	23 %
R 2006 0652/4 T	10 %	45 h	16 %

Diagram 6.5.3- 3: Flow chart for the preparation of bunches of grapes into raisin and raisin by-products



Residue analysis

Residues of fluopyram and its metabolites were determined by LC-MS/MS according to method 00984/M001 ([redacted], 2007, M-295145-03-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA-4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues were extracted from 10 g of sample material (raisin and raisin waste: 5 g) by two successive extractions using a high speed blender with a mixture of acetonitrile:water (80:20; v:v).

Subsequently, the raw extracts were diluted 10-fold by adding internal standard solutions:

- One dilution and an additional clean-up step performed under acidic conditions (for determination of fluopyram-PCA)
- Another dilution performed under basic conditions (for determination of fluopyram, FLU-benzamide and FLU-PAA)

Residues were quantified by reversed-phase chromatography coupled with tandem mass spectrometry (MS/MS) with electrospray ionisation. One injection in positive electrospray ionisation allowed the determination of fluopyram, FLU-benzamide and FLU-PAA. Another injection in negative electrospray ionisation allowed the determination of FLU-PCA under different conditions.

The quantitation was carried out by internal standardization using internal stable labelled standards. The Limit of Quantitation (LOQ, calculated and expressed as fluopyram parent equivalents for fluopyram and its metabolites), defined as the lowest validated fortification level, was 0.01 mg/kg in all matrices for all analytes. All residues are calculated as parent fluopyram.

II. Findings

No apparent residues above the LOQ were present in the control samples. In order to check the performance of the method, recovery determinations were concurrently performed to the analyses of control and treated samples in each set of analyses. Concurrent recoveries were obtained during the conduct of the residue studies RA-2611/06, RA-2612/06 and RA-2647/06 (KCA 6.3.1/01 to 03) and corresponding processing studies (RA-3611/06, RA-3612/06 and RA-3647/06, KCA 6.5.3/01 to 03).
All data of the method performance and recoveries are shown in the Table 6.5.3- 12.
The mean concurrent recoveries were within the acceptable range of 70 % – 110% in all tested matrices.
The RSD values (although not all could be calculated) were ≤ 20 %.
The storage period of deep-frozen processed samples is displayed in Table 6.5.3- 14.

Table 6.5.3- 12: Concurrent recoveries of Fluopyram and its metabolites

Portion analysed	Matrices covered	n	Fortification level (mg/kg) *	Individual recoveries	Recovery (%) Min	Max	Mean	RSD
Fluopyram (AE C656948)								
Bunch of grapes	bunch of grapes; berry, washed	3	0.01	108; 111; 102	102	111	107	4.3
		4	0.10	91; 105; 100; 92	91	105	97	6.9
		2	1.0	90; 93	90	93	92	--
			Overall Recovery (n = 9)				**99**	**8.0**
Juice	juice; raw juice; washings; retentate	3	0.01	96; 92; 95	92	96	94	2.2
		3	0.10	99; 95; 87	87	99	94	6.5
			Overall Recovery (n = 6)				**94**	**4.4**
Pomace, dried	pomace, dried; pomace, wet; pomace, grape; raisin; raisin waste	2	0.01	95; 105	95	105	100	--
		2	1.0	96; 98	96	98	97	--
		1	2.0	97	--	--	97	--
		1	10	86	--	--	86	--
			Overall Recovery (n = 6)				**96**	**6.4**
Must	must	1	0.01	100	--	--	100	--
		1	0.10	97	--	--	97	--
		1	0.50	94	--	--	94	--
			Overall Recovery (n = 3)				**97**	**3.1**
Wine at 1st taste test	wine at 1st taste test; grape wine	2	0.01	97; 87	87	97	92	--
		2	0.10	98; 96	96	98	97	--
			Overall Recovery (n = 4)				**95**	**5.4**
Fluopyram-pyridyl-aceticacid (BCS-AA10139)								
Bunch of grapes	bunch of grapes; berry, washed	3	0.01	95; 83; 86	83	95	88	7.1
		4	0.10	101; 80; 80; 83	80	101	86	11.7
		2	1.0	111; 80	80	111	96	--
			Overall Recovery (n = 9)				**89**	**12.5**
Juice	juice; raw juice; washings; retentate	3	0.01	97; 92; 85	85	97	91	--
		3	0.10	91; 91; 90	90	91	91	--
			Overall Recovery (n = 6)				**91**	**4.2**
Pomace, dried	pomace, dried; pomace, wet; pomace, grape; raisin; raisin waste	2	0.01	85; 92	85	92	89	--
		2	1.0	83; 87	83	87	85	--
		1	2.0	96	--	--	--	--
		1	10	85	--	--	--	--
			Overall Recovery (n = 6)				**88**	**5.7**
Must	must	1	0.01	87	--	--	87	--
		1	0.10	92	--	--	92	--
		1	0.50	95	--	--	95	--
			Overall Recovery (n = 3)				**91**	**4.4**
Wine at 1st taste test	wine at 1st taste test; grape wine	2	0.01	90; 77	77	90	84	--
		2	0.10	89; 95	89	95	92	--
			Overall Recovery (n = 4)				**88**	**8.7**

Fluopyram-benzamide (AE F148815)								
Bunch of grapes	bunch of grapes; berry, washed	3	0.01	113; 100; 92	92	113	102	10.4
		4	0.10	94; 87; 91; 90	87	94	91	3.2
		2	1.0	101; 93	93	101	97	--
			Overall Recovery (n = 9)				**96**	**8.3**
Juice	juice; raw juice; washings; retentate	3	0.01	91; 103; 104	91	104	99	7.3
		3	0.10	91; 106; 95	91	106	97	8.0
			Overall Recovery (n = 6)				**98**	**6.9**
Pomace, dried	pomace, dried; pomace, wet; pomace, grape; raisin; raisin waste	2	0.01	85; 99	85	99	92	--
		2	1.0	91; 93	91	93	92	--
		1	2.0	103	--	--	103	--
		1	10	93	--	--	93	--
			Overall Recovery (n = 6)				**94**	**6.7**
Must	must	1	0.01	95	--	--	95	--
		1	0.10	89	--	--	89	--
		1	0.50	94	--	--	94	--
			Overall Recovery (n = 3)				**93**	**3.5**
Wine at 1st taste test	wine at 1st taste test; grape wine	2	0.01	87; 105	87	105	96	--
		2	0.10	95; 111	95	111	103	--
			Overall Recovery (n = 4)				**100**	**10.7**
Fluopyram-pyridyl-carboxylic acid (AEC 657188)								
Bunch of grapes	bunch of grapes; berry, washed	3	0.01	83; 103; 86	83	103	91	11.9
		4	0.10	79; 96; 85; 89	79	96	87	8.2
		2	1.0	70; 79	70	79	75	--
			Overall Recovery (n = 9)				**86**	**11.4**
Juice	juice; raw juice; washings; retentate	3	0.01	97; 86; 95	86	97	93	6.3
		3	0.10	102; 102; 106	102	106	103	2.2
			Overall Recovery (n = 6)				**98**	**7.2**
Pomace, dried	pomace, dried; pomace, wet; pomace, grape; raisin; raisin waste	2	0.01	91; 111	91	111	101	--
		2	1.0	97; 105	97	105	101	--
		1	2.0	99	--	--	99	--
		1	10	85	--	--	85	--
			Overall Recovery (n = 6)				**98**	**9.6**
Must	must	1	0.01	96	--	--	96	--
		1	0.10	100	--	--	100	--
		1	0.50	101	--	--	101	--
			Overall Recovery (n = 3)				**99**	**2.7**
Wine at 1st taste test	wine at 1st taste test; grape wine	2	0.01	103; 107	103	107	105	--
		2	0.10	100; 104	100	104	102	--
			Overall Recovery (n = 4)				**104**	**2.8**

FL: fortification level, RSD - Relative Standard Deviation
* some RSDs were not calculated as there were only two individual recoveries given
Final determination as: fluopyram Residues calculated as: fluopyram
Final determination as: FLU-benzamide Residues calculated as: fluopyram
Final determination as: FLU-PCA Residues calculated as: fluopyram
Final determination as: FLU-PAA Residues calculated as: fluopyram

In bunch of grapes used for raisin production residues of fluopyram were between 0.30 and 0.66 mg/kg. During processing for raisin production a mean of 7% of the absolute residue was recovered with raisin waste. A major part counting for 59% of the initial absolute residue remained in the dried and washed raisin leading to values between 0.96 mg/kg and 2.1 mg/kg in the final product. Residue values of fluopyram in bunch of grapes, raisin, raisin waste and washings are summarised in

Table 6.5.3- 13.

Processing factors (PF, processing factor) were calculated by dividing the fluopyram residue and fluopyram metabolites in grape processed commodities by the fluopyram residue and its metabolites in the bunch of grapes (raw agricultural commodity: RAC). Since residues of fluopyram-PAA were <0.01 mg/kg in all tested matrices, processing factors could not be calculated for this analyte. Processing factors calculated for the individual grape processed commodity samples are summarized in the

Table 6.5.3- 13.

Table 6.5.3- 13: Summary of residues in grape matrices and processing factors for fluopyram and its metabolites

trial	R 2006 0417/3		R 2006 0624/9		R 2006 0651/6		R 2006 0652/4		
Sample material	**Residues (mg/kg)**	**PF***	**Residues (mg/kg)**	**PF**	**Residues (mg/kg)**	**PF**	**Residues (mg/kg)**	**PF**	**Mean PF**
Fluopyram (AEC656948)									
Bunch of grapes	**0.55**	--	**0.32**	--	**0.66**	--	**0.30**	--	-
Raisin	1.1	2.1	2.1	6.5	1.9	2.8	0.96	3.2	3.7
Raisin waste	4.6	8.3	5.3	17	6.1	9.1	3.3	11	11.4
Washings	0.02	0.04	0.03	0.1	0.01**	0.02	0.02	0.1	0.1
Fluopyram-benzamide (AEF148815)									
Bunch of grapes	**0.03**	--	**<0.01**	--	**0.02**	--	**0.01**	--	-
Raisin	0.07	2.8	0.03	2.6	0.07	3.7	0.04	3.5	3.0
Raisin waste	0.29	11	0.06	5.8	0.28	16	0.11	11	11.0
Washings	<0.01**	0.4	<0.01**	1.0	<0.01**	0.6	<0.01**	1.0	0.8
Fluopyram-pyridyl-carcoxylic-acid (AEC657188)									
Bunch of grapes	**0.07**	--	**<0.01**	--	**0.02**	--	**0.01**	--	-
Raisin	0.17	2.4	0.02	6.9	0.07	2.8	0.03	2.5	4.0
Raisin waste	1.9	27	0.10	29	0.59	25	0.39	33	27.0
Washings	0.01	0.1	<0.01**	1.0	<0.01**	0.4	<0.01**	0.8	0.6
Fluopyram-pyridyl-acetic-acid (BCS-AA10139)									
Bunch of grapes	<0.01	--	<0.01	--	<0.01	--	<0.01	--	-
Raisin	<0.01	NR	<0.01	NR	<0.01	NR	<0.01	NR	-
Raisin waste	<0.01	NR	<0.01	NR	<0.01	NR	<0.01	NR	-
Washings	<0.01	NR	<0.01	NR	<0.01	NR	<0.01	NR	-

*Processing factor calculated according to the following equation: $PF = \frac{Residue\ concentration\ in\ the\ processed\ product\ [\frac{mg}{kg}]}{Residue\ concentration\ in\ the\ RAC\ [\frac{mg}{kg}]}$

Remark: Processing factors were calculated from the corresponding residue concentrations using unrounded values. For this reason, deviations can occur when the rounded values given in column for levels of residues of fluopyram and its metabolites in relevant matrices are used for recalculation.
RAC: Raw Agriculture Commodity
**: Values below LOQ which were set to the LOQ for the calculation of processing factors. NR = Not Relevant.

Processing factors between 2.1 and 6.5 (raisin), between 8.3 and 17 (raisin waste) and between 0.02 and 0.1 (washings) were calculated for AE C656948.
Processing factors between 2.6 and 3.7 (raisin), between 5.8 and 16 (raisin waste) and between 0.4 and 1.0 (washings) were calculated for FLU-benzamide.
Processing factors between 2.4 and 6.9 (raisin), between 25 and 33 (raisin waste) and between 0.1 and 1.0 (washings) were calculated for FLU-PCA.

III. Conclusion

The study was conducted according to the relevant guidelines. The results of control samples, and recovery samples were in the expected range.
Concentration of fluopyram and its two metabolites (FLU-benzamide and FLU-PCA) was observed in most of the tested matrices (PF>1), except for washing water.

Assessment and conclusion by applicant:
The study is acceptable.

Table 6.5.3- 14: Results of processing trials conducted with fluopyram on grape

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analysed	Growth stage at sampling	Residues (mg/kg)				PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					fluopyram	FLU-PCA	FLU-benzamide	FLU-PAA		
	(a)	(b)				(c)	(d)		(d)					(e)	(f)
R 2006 0417/3 0417-06 Spain E-03688 Hondón de las Nieves (Comunidad Valenciana) Europe, South F 2006	Table grape Italia	1) 02.02.1995 2) 10.05.2006 - 30.05.2006 3) 01.09.2006 - 30.09.2006	250.0 250.0	1000 1000	25.00 25.00	08.08.2006/0 08.09.2006/31	89	bunch of grapes	89 89 89 89 89 89	0.21 0.90 0.58 0.19 0.16 0.12	0.06 0.06 0.07 0.04 0.05 0.03	0.02 0.02 0.03 0.02 0.02 0.02	0.01 <0.01 <0.01 <0.01 <0.01 <0.01	0* 0 3 7 14 21	(g) RA-2612/06 (h) SC (fluopyram 500 g/L) (i) Application method: Spraying (j) Analytical method: 00984/M001 (k) LOQ: 0.01 mg/kg (l) Method Validation Data: 00984/M001 (m) Storage: washings: 322 days raisin waste: 322 days raisin: 322 days bunch of grapes: 207 days
									89	0.11	0.04	0.02	<0.01	28	
								raisin	89	1.1	0.17	0.07	<0.01	3	
								raisin waste	89	4.6	1.9	0.29	<0.01	3	
								washings	89	0.02	0.01	<0.01	<0.01	3	
R 2006 0624/9 0624-06 Portugal P-2580-347 Passinha-Alenquer (Ribatejo e Oeste) Europe, South F	Table grape Cardinal	1) 15.03.2006 2) 10.05.2006 - 22.05.2006 3) 15.07.2006 - 30.07.2006	250.0 250.0	600 600	41.50 41.50	03.07.2006/0 11.07.2006/8	85	bunch of grapes	85 85 85 85 89 89 89	0.06 0.35 0.32 0.22 0.34 0.28 0.18	<0.01 <0.01 <0.01 <0.01 0.02 0.02 0.02	<0.01 <0.01 <0.01 <0.01 0.01 0.01 0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	0* 0 3 7 14 21 28	(g) RA-2612/06 (h) SC (fluopyram 500 g/L) (i) Application method: Spraying (j) Analytical method: 00984/M001 (k) LOQ: 0.01 mg/kg (l) Method Validation Data: 00984/M001
								raisin	85	2.1	0.02	0.03	<0.01	3	
								raisin waste	85	5.3	0.10	0.06	<0.01	3	

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analysed	Growth stage at sampling	Residues (mg/kg)				PHI (days)	Details on trial
	(a)	(b)	g a.s./ha	Water (L/ha)	g a.s./hL	(c)	(d)		(d)	fluopyram	FLU-PCA	FLU-benzamide	FLU-PAA	(e)	(f)
2006								washings	88	0.03	<0.01	<0.01	<0.01	1	(m) Storage: washings: 381 days raisin waste: 381 days raisin: 381 days bunch of grapes: 266 days

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analysed	Growth stage at sampling	Residues (mg/kg)				PHI (days)	Details on trial
	(a)	(b)	g a.s./ha	Water (L/ha)	g a.s./hL	(c)	(d)		(d)	fluopyram	FLU-PCA	FLU-benzamide	FLU-PAA	(e)	(f)
R 2006 0651/6 0651-06 Italy I-71049 Trinitapoli (FG) (Puglia) Europe, South F 2006	Table grape Italia	1) 03.03.1988 2) 15.05.2006 - 25.05.2006 3) 10.08.2006 - 10.09.2006	250.0 250.0	1000 1000	25.00 25.00	26.07.2006/0 11.08.2006/16	NA	bunch of grapes	85 85 85 89 89 89 89 89	0.22 0.43 0.66 0.56 0.43 0.33 0.41	0.02 0.01 0.02 0.02 0.03 0.03 0.04	<0.01 <0.01 0.02 0.02 0.02 0.02 0.02	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	0* 0 3 7 14 21 28	(g) RA-2612/06 (h) SC (fluopyram 500 g/L) (i) Application method: Spraying (j) Analytical method: 00984/M001 (k) LOQ: 0.01 mg/kg (l) Method Validation Data: 00984/M001 (m) Storage: washings: 350 days raisin waste: 350 days raisin: 350 days bunch of grapes: 255 days
								raisin	85	1.9	0.07	0.07	<0.01	3	
								raisin waste	85	6.1	0.59	0.28	<0.01	3	
								washings	85	<0.01	<0.01	<0.01	<0.01	3	

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analysed	Growth stage at sampling	Residues (mg/kg)				PHI (days)	Details on trial
	(a)	(b)	g a.s./ha	Water (L/ha)	g a.s./hL	(c)	(d)		(d)	fluopyram	FLU-PCA	FLU-benzamide	FLU-PAA	(e)	(f)
R 2006 0652/4 0652-06 Greece GR-20006 Assos (Peloponnesos) Europe, South F 2006	Table grape Soultanina	1) 10.03.1993 2) 20.05.2006 - 30.05.2006 3) 20.08.2006 - 20.09.2006	250.0 250.0	1000 1000	25.00 25.00	25.07.2006/0 25.08.2006/31	88	bunch of grapes	89	0.43	0.01	0.01	<0.01	0	(g) RA-2612/06 (h) SC (fluopyram 500 g/L) (i) Application method: Spraying (j) Analytical method: 00984/M001 (k) LOQ: 0.01 mg/kg (l) Method Validation Data: 00984/M001 (m) Storage: washings: 336 days raisin waste: 336 days raisin: 336 days bunch of grapes: 242 days
									89	0.51	0.01	0.01	<0.01	0	
									89	0.30	0.01	0.01	<0.01	3	
									89	0.18	0.01	0.01	<0.01	7	
									89	0.09	0.01	0.01	<0.01	14	
									89	0.22	0.01	0.01	<0.01	21	
									89	0.22	0.02	0.01	<0.01	28	
								raisin	89	0.96	0.03	0.04	<0.01	3	
								raisin waste	89	3.3	0.39	0.11	<0.01	3	
								washings	89	0.02	<0.01	<0.01	<0.01	3	

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions, Reference to analytical method and information which metabolites are included
(g) Study reference
* prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Data Point:	KCA 6.5.3/04
Report Author:	[redacted]
Report Year:	2008
Report Title:	AE C656948 500 SC - Magnitude of the residue on grape processed commodities
Report No:	RAGMP042
Document No:	M-298571-01-1
Guideline(s) followed in study:	EPA Ref.: OPPTS 860.1520; PMRA Ref.: DACO 7.4.5
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted rev. 1 to Vol.3 of DAR B7 August 2012 (reference relied on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The study included one supervised residue trial with grape conducted in North America (USA) in the 2006 season in order to determine the magnitude of the residues of fluopyram (AE C656948) in bunch of grapes and the processed fractions (washed fruits, heated juice, jelly, and raisin) following two spray applications of fluopyram (500 SC).

I. Materials and methods

Field part

In the trial, two air blast applications with fluopyram (500 SC) - a suspension concentrate formulation containing 500 g/L fluopyram - were conducted at a single application rate of 1.25 kg a.s./ha/application and water rate at 519 – 523 L/ha. The achieved total seasonal rate was 2.50 kg a.s./ha. The applications were conducted at BBCH of 85 (for the first application) and BBCH of 89 (for the second application), with a 14-day interval between the two applications. This application rate was equivalent to five times (5X) the total maximum proposed label rate for a single growing season

One control and one treated bulk grape samples were collected at a 7-day PHI. Bulk grape samples were harvested by hand first from the control plot followed by the treated plot. Grapes were sampled from the adjacent inner facing half of the two plot rows. Each bulk sample consisted of 200 lbs (91 kg) of grapes. The samples were put into labelled containers in the field, placed in a pick-up truck, surrounded with frozen plastic bottles of water, and hand delivered to the processing facility the same day.

Processing procedures

The samples were harvested and hand delivered at ambient temperatures to The National Food Laboratory, Inc. for sub-sampling and processing. The samples arrived in good condition and were placed in a cool storage room at 18 ± 5 °C Prior to processing, a random sub-sample of the control and treated bulk grapes were collected for analysis, and the remainder of the grape samples were used to generate the required processed commodities of raisins and grape juice. In addition, samples of washed fruit and jelly were generated for use in the dietary risk assessment for fluopyram. Processing was performed using procedures which simulated commercial processing practices.
Details of processing of the control and treated grapes are presented on flow chart diagrams and material balance summaries. The sub-samples were shipped to Bayer CropScience via freezer truck for analysis.

Food processing involves the use of water to start up and shut down certain unit processes. Water is mixed with the food product when it is introduced into the running machine and again when the food product is exhausted. To minimize water dilution of the food product in this study, some of the product may be discarded at each end of the process. Therefore, weight losses may result when diluted product is discarded. Or weight losses may occur if product is spilled or not fully recovered from processing equipment. Weight gains may result if the sample contains additional water. This is a normal part of food processing, especially small batch processing. The term "material balance" generally refers to showing a balance of a constant, measurable quantity, such as total solids, through a unit process or a series of unit processes. Since a total solids assay was not performed on all samples, a mass balance is not an accurate term. Therefore, this report presents weight distributions as measured throughout the processing operations. These weight distributions are presented on the flow charts below on Diagram 6.5.3- 4 to Diagram 6.5.3- 7.

Processing of the Control and Treated grapes began on August 30, 2006. The initial weight of the Control and Treated grapes was determined. Fraction samples of raw unwashed grapes from Control and Treated were collected. The fraction samples were assembled by taking grapes from each of the bags delivered for each sample. The fraction samples were bagged, weighed, labelled, and placed in the freezer. Grapes for sun drying of Control and Treated raisins were removed and were taken to sun drying. Control and Treated grapes for wash as in the home were washed under running water, the samples were bagged, weighed, labelled, and placed in the freezer.

Crushing/Destemming

Grapes for processing into grape juice from Control and Treated samples were initially passed through the crusher/destemmer. The crusher/destemmer crushes the berries and separates the stems from the crush (crushed berries and juice). The crush was collected, weighed and transported to the depectinization step. Stems were collected, weighed and discarded.

Depectinization

Each crush was transferred to a steam-jacketed kettle for the depectinization step. Novozyme Pectinex 3XL pectinase enzyme was added to each crush at a level of approximately 0.06 % by weight. The enzyme-inoculated crush was then heated and held for approximately 2 to 3 hours. The target temperature for the enzyme treatment was approximately 49 – 60 °C. The Crushed Control grapes were heated to 57 – 60 °C for a period of 146 minutes during the enzymatic depectinization process. The Crushed Treated grapes were heated to 60 - 58 °C for a period of 121 minutes during the enzymatic depectinization process.

Extraction

The 2 depectinized grape crush slurries were passed through a screw press at the depectinization temperature. For each slurry, the extracted, unclarified juice was collected, weighed, and transferred to a steam-jacketed kettle. The wet pomace was collected, weighed, and discarded.

Juice Clarification and Argol Settling

Each unclarified juice batch was placed into a steam-jacketed kettle for heating. The unclarified juice was heated to inactivate the pectinase enzyme and cooled prior to placement into refrigerated storage for argol settling. Control juice was heated to 87 °C and cooled to 24 °C. The Treated juice was heated to 88 °C and then cooled to 24 °C. Cooled, unfiltered juice samples from Control and Treated were collected, weighed, and placed in refrigerated storage for argol settling.

Juice Filtration

Argol settling for the Control and Treated juice samples took place under refrigeration at -1 °C to 2 °C ; settling time was 26 days for Control juice and 25 days for Treated juice. Upon completion of the argol settling process, the argol-settled juice was filtered to prepare clarified juice for canning, concentrating, and jelly making. Upon completion of the argol settling process, the argol-settled juice was filtered to prepare clarified juice for canning, concentrating, and jelly making. The Millipore filter was set up using

filter paper and was precoated with approximately 2 % of diatomaceous earth filter aid (Celite 545), based on the weight of juice filtered. The juice was filtered through the filter using air over pressure. A portion of the filtered juice was transferred to Juice Canning and a portion was transferred to Jelly Making.

Single Strength Juice Canning
A portion (4.5 kg for Control and 3.9 kg for Treated) of the filtered juice was weighed, placed in a steam-jacketed kettle and heated to canning temperature. Hot juice was filled into plastic jars and sealed. Hot-filled plastic jars were held inverted for 7 minutes before cooling. When cool, the jars were dried, weighed, labelled, bagged, and placed in the freezer. Control filtered and clarified single strength juice was heated to 92 °C, filled into plastic jars, and held for 7 minutes prior to cooling. Treated filtered and clarified single strength juice was heated to 92 °C, filled into plastic jars, and held for 7 minutes prior to cooling.

Grape Jelly Preparation
A portion of the filtered juice (1.3 kg for Control and 1.3 kg for Treated) was weighed and placed into a steam-jacketed kettle. The pH of the juice was adjusted to about 3.0 with citric acid. Control juice required 36.0 g of citric acid, and Treated juice required 42.6 g. Sugar (2.9 kg) and pectin (34.4 g) were added to the Control filtered juice. The mixture was heated to boiling and then placed into plastic jars and sealed. The fill temperature was 104 °C. The jars were held inverted for 5 minutes and then cooled in running water. The jars were then labelled and placed into the freezer.
Sugar (2.7 kg) and pectin (32.2 g) were added to the Treated filtered juice. The mixture was heated to boiling and then placed into plastic jars. The fill temperature was 105 °C. The jars were held inverted for 6 minutes and then cooled in running water. The jars were then labelled and placed into the freezer.

Sun Drying of Grapes
For sun drying, grape bunches were spread out on stainless steel drying trays (on the pilot plant/low bay roof). Each sample of grapes was physically separated to avoid cross contamination. Drying started on August 30, 2006. On October 31, 2006 the moisture of the sun-dried grapes was 12.8 % for the Control and 14.6 % for the Treated; this was within the specified moisture range of 12 to 16 %, so the Control and Treated raisins were collected and transferred to sweat box storage.

"Sweat Box" Moisture Equilibration
The Control and Treated dried grapes were placed into separate plastic bags (acting as a "sweat box"), weighed, and placed in the 19 – 21 °C storage room until they were removed for destemming and further processing. Control and Treated dried grapes were placed into the 19 – 21 °C storage room on October 31, 2006 and were both removed on November 2, 2006.

Destemming and Cap Stem Removal
The destemming and cap stem removal consists of separating the dried grapes from the stems and then removing the residual cap stems by hand. This process was performed only on enough dried grapes to obtain approximately 2.7 – 3.6 kg of sample for the Control and Treated samples. The remaining dried grapes and the stems were discarded. The Control sample was frozen from November 2, 2006 to November 3, 2006 to make the cap stems more fragile and easy to remove. The Treated sample was frozen from November 2, 2006 to November 6, 2006 to make the cap stems more fragile and easy to remove.

Dried Grape Washing and Rehydration to Raisins
Washing and rehydrating of dried grapes was accomplished by placing destemmed dried grapes into a stainless steel mesh basket and immersing them into fresh water for 1 to 2 seconds. After this immersion, excess surface water on the rehydrated raisins was drained before weighing the sample. The moisture for the Control and Treated samples was determined to be 18.1 % and 18.7 %, respectively. Samples of

raisins were bagged, weighed, labelled, and placed in the freezer on November 8, 2006 for both the Control and the Treated sample.

Residue analysis

To prepare samples for analysis, the grape raw agricultural commodity sub-samples and the grape processed commodities of raisins and washed fruit were homogenized with dry ice. All samples were returned to frozen storage immediately following homogenization, and the samples always remained in frozen storage except during sub-sampling for analysis. Grape juice and jelly were analysed as received from the processor.

The residue data for grape raw agricultural commodity and grape processed commodities were obtained using the analytical method for determining the fluopyram residue in plant 00984 [redacted], 05/02/2007, M-283301-01-1, see MCA section 4.1.2) with modifications ([redacted] 2007, No. GM-001-P07-01, M-297568-01-2)

Briefly, a 5-g aliquot of the crop matrix was extracted by blending twice, each time with a mixture of acetonitrile/water (4/1; *v/v*). Each extract was filtered, the filtrates were combined, and an isotopically labelled internal standard was added. An aliquot of the mixture was loaded on a small C-18 solid phase extraction (SPE) cartridge and eluted into an HPLC vial with 0.1 % (aq.) acetic acid for analysis by LC/MS/MS.

The quantitation was carried out using isotopically labelled internal standards. The Limit of Quantitation (LOQ), defined as the lowest validated fortification level, was 0.01 mg/kg in all tested matrices.

Diagram 6.5.3- 4: Flow chart of grape processing and weight distribution – untreated control – juice and raisins

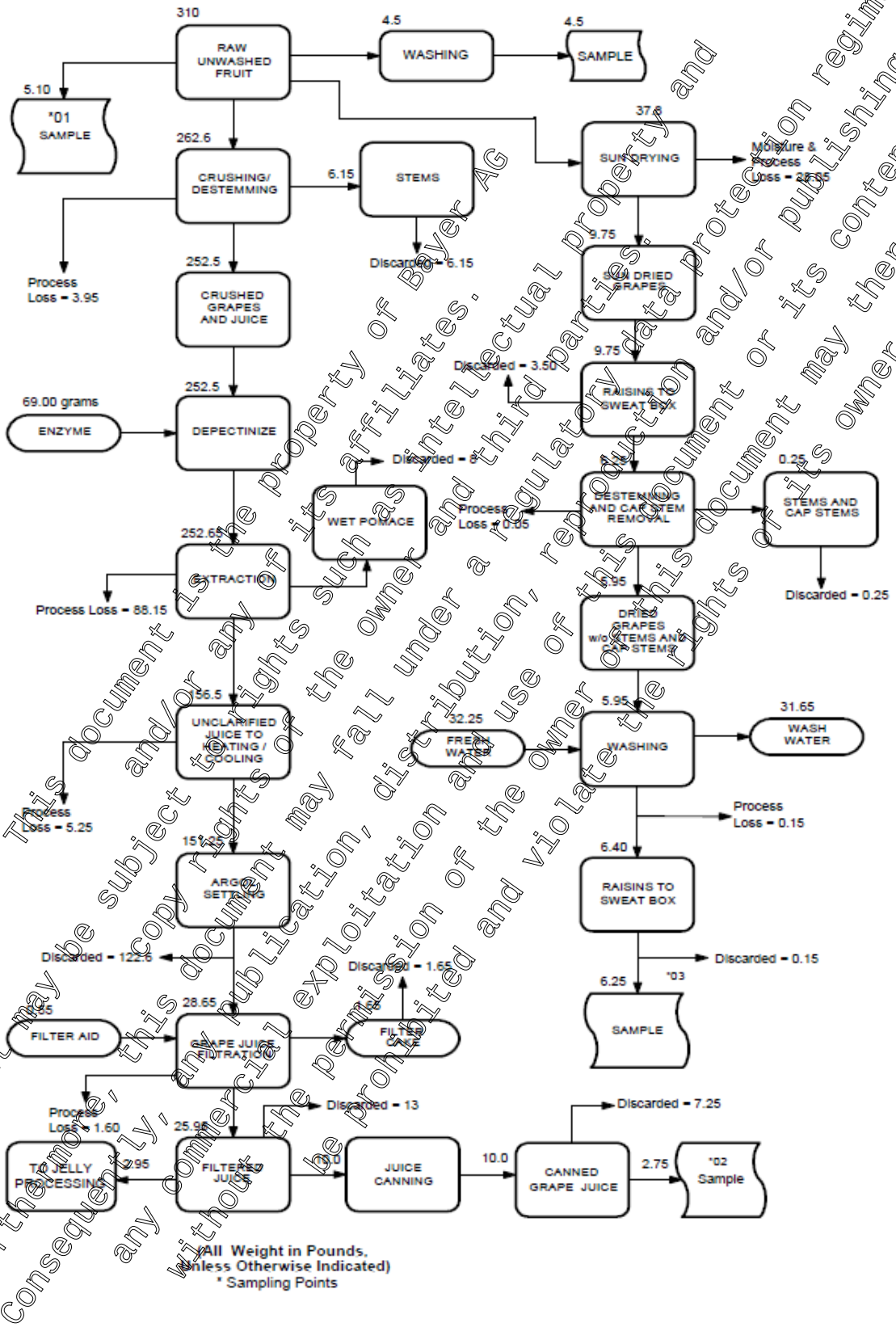


Diagram 6.5.3- 5: Flow chart of grape processing and weight distribution – untreated control – jelly

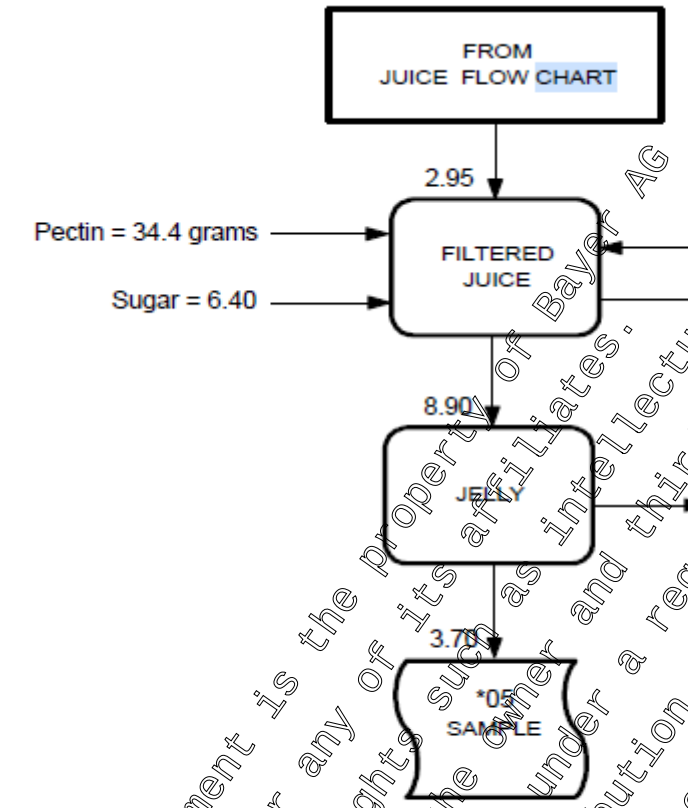


Diagram 6.5.3- 6: Flow chart of grape processing and weight distribution – treated – juice and raisins

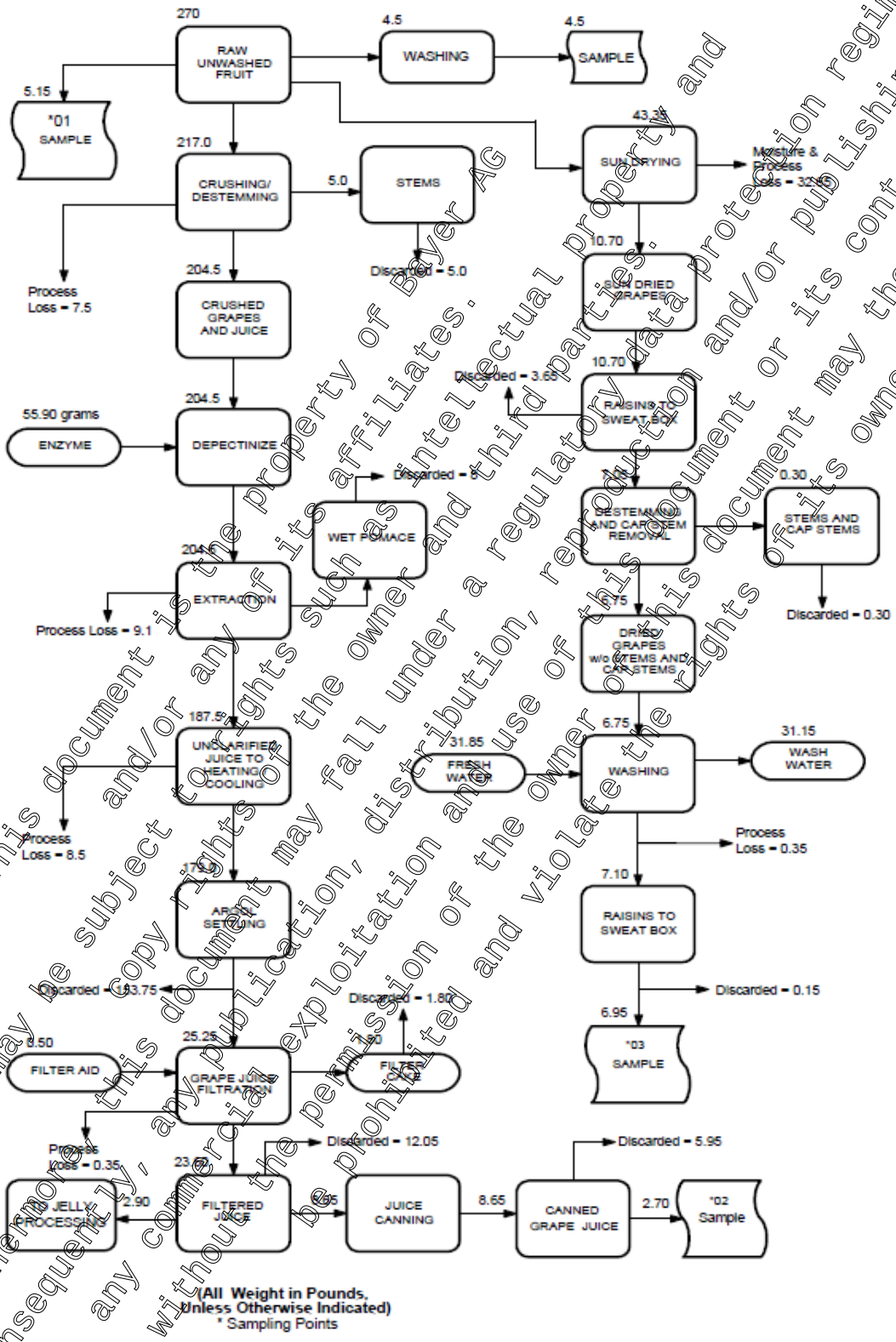
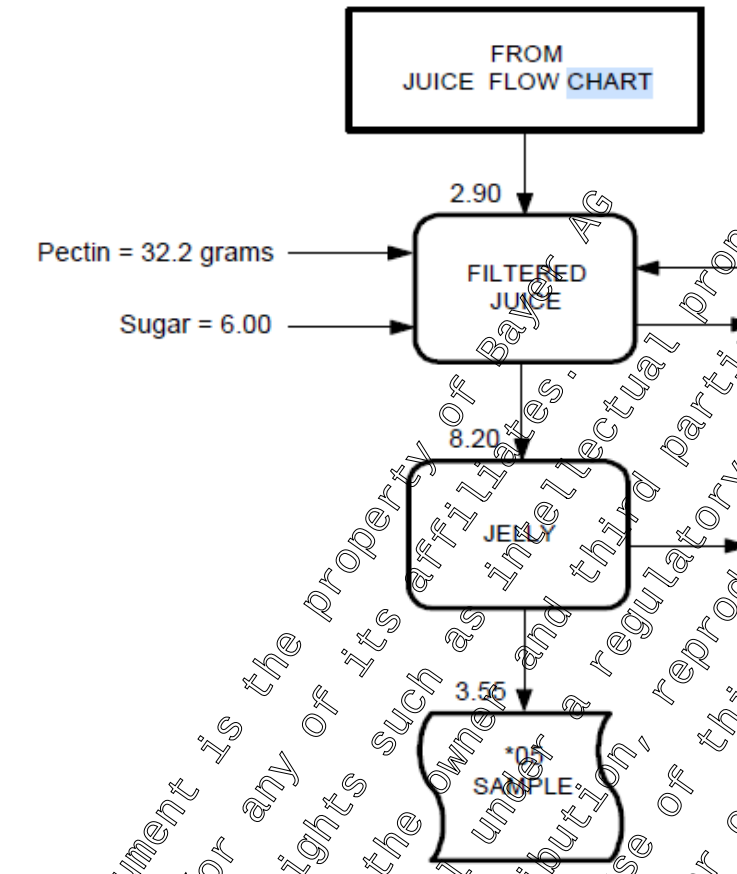


Diagram 6.5.3- 7: Flow chart of grape processing and weight distribution – treated - jelly



II. Findings

No apparent residues above the LOQ were present in the control samples. In order to check the performance of the method, recovery determinations were concurrently performed to the analyses of control and treated samples in each set of analyses. Concurrent recoveries were obtained during the conduct of this study. All data of the method performance and recoveries are shown in the tables below.
The mean concurrent recoveries were within the acceptable range of 70 % – 110% in all tested matrices.
The RSD values (although not all could be calculated) were ≤ 20 %.
The grape RAC samples analyzed in this study were held in frozen storage for a maximum of 10 months (300 days) prior to extraction. The grape processed commodities in this study were also analyzed within 10 months (299 days) of generation
The mean fluopyram residue level in fresh grapes collected at PHI 7 was 1.25 mg/kg, in grape raisin 3.05 mg/kg, in grape juice 0.67 mg/kg, in washed fruit 0.96 mg/kg, in jelly 0.18 mg/kg.
Processing factors (PFs) were calculated by dividing the fluopyram residue in grape processed commodities by the fluopyram residue in grape fruit (RAC).
Processing factors calculated for the individual grape processed commodity samples are summarized in the tables below.
Processing factors for fluopyram were as follows: 0.1 (for jelly), 0.5 (for grape juice), 0.8 (for washed fruit) and 2.4 (for raisin).

III. Conclusion

The study was conducted according to the relevant guidelines. The results of control samples, and recovery samples were in the expected range. Concentration of fluopyram was observed in raisin (PF>1).

Assessment and conclusion by applicant:
The study is acceptable.

Table 6.5.3- 15: Concurrent recoveries of fluopyram

Portion analysed	n	Fortification level (mg/kg)*	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
Fluopyram (AE C656948)							
Fresh fruit	7	0.01	114; 109; 89; 92; 94; 102; 115	89	115	102	10.5
	3	2.00	99; 101; 99	99	101	100	1.2
Juice	3	0.01	104; 93; 107	93	107	101	7.3
	3	2.00	101; 99; 101	99	101	100	1.2
Jelly	3	0.01	95; 103; 95	95	103	98	4.7
	3	2.00	96; 97; 96	96	97	96	0.6
Raisins	3	0.01	88; 98; 84	84	98	90	8.0
	3	2.00	91; 93; 90	90	93	91	1.7
	3	5.00	103; 95; 96	95	103	98	4.5

FL: fortification level, RSD - Relative Standard Deviation
Final determination as: fluopyram Residues calculated as: fluopyram

Table 6.5.3- 16: Results of processing trials conducted with fluopyram on grape

Trial No. / Location / EU zone / Year	Commodity / Variety (a)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting (b)	Application rate per treatment			Dates of treatment / Application interval (c)	Growth stage at last treatment (d)	Portion analysed	Growth stage at sampling (d)	Residues (mg/kg) AE C656948 (e)	PHI (days) (e)	Details on trial (f)
			g a.s./ha	Water (L/ha)	g a.s./hL							
GM105-06PA USA Fresno America, North F 2006	Table grape Thompson Seedllees	1) 05.02.1992	1252 1253	523 519	239 241	08.08.2006/0 22.08.2006/14	89	bunch of grapes		1.25 1.27 1.22 **Mean: 1.25**	7	(g) RAGMP042 (h) SC (fluopyram 500 g/L) (i) Application method: Spraying (j) Analytical method: GM-001-P-07-01, for details see residue report (k) LOQ: 0.01 mg/kg (l) Method Validation Data:study RAGMP042 (M-298571-01-1) (m) Storage: raisin: 303! days juice, heated: 308! days jelly: 308! days fruit, washed: 303! days bunch of grapes: 303! days application x5 overdosed for processing
								fruit, washed		3.01 2.91 3.22 **Mean: 3.05**	7	
								juice, heated		0.60 0.71 0.69 **Mean: 0.67**	7	
								jelly		0.18 0.18 0.18 **Mean: 0.18**	7	
								raisin		3.01 2.91 3.22 **Mean: 3.05**	7	

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) Study reference
* prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Table 6.5.3- 17: Summary of residues in grape matrices and processing factors for fluopyram

Sample material	RAGMP042	
	Residues (mg/kg)	PF*
Fluopyram (AE C656948)		
Grape (RAC)	1.25 1.27 1.22 **Mean: 1.25**	--
Raisins	3.01 2.91 3.22 **Mean: 3.05**	2.4
Grape juice	0.60 0.71 0.69 **Mean: 0.67**	0.5
Fruit, washed	0.94 0.97 0.97 **Mean: 0.96**	0.8
Jelly	0.18 0.18 0.18 **Mean: 0.18**	0.1

*Processing factor calculated according to the following equation: $PF = \frac{Residue\ concentration\ in\ the\ processed\ product\ [\frac{mg}{kg}]}{Residue\ concentration\ in\ the\ RAC\ [\frac{mg}{kg}]}$

RAC: Raw Agriculture Commodity

New studies

Data Point:	KCA 6.5.3/10
Report Author:	[redacted]
Report Year:	2009
Report Title:	Determination of residues of AE C656948 in/on grape and processed fractions after spraying of fluopyram SC 500 in the field in Germany
Report No:	08-3077
Document No:	M-352682-01-1
Guideline(s) followed in study:	EU: Council Directive 91/414/EEC, Annex II, part A, section 6 and Annex III, part A, section 8; EC guidance working document 7029/VI/95 rev. 5 (1997-07-22)
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Data Point:	KCA 6.5.3/11
Report Author:	[redacted]
Report Year:	2016
Report Title:	Amendment no. 1 to report No: 08-3081- Determination of residues of AE C656948 in/on grape and processed fractions after spraying of fluopyram SC 500 in the field in France (South)
Report No:	08-3081
Document No:	M-352686-02-1
Guideline(s) followed in study:	EU: Council Directive 91/414/EEC, Annex II, part A, section 6 and Annex III, part A, section 8; EC guidance working document 7029/VI/95 rev. 5 (1997-07-22)
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Test system:

Balance studies on processing of grapes into grape wine were conducted to determine the transfer of fluopyram and its metabolites fluopyram-pyridyl-acetic acid, fluopyram-benzamide and fluopyram-pyridyl-carboxylic acid from bunch of grapes into processed fractions.

Wine was produced from red grapes obtained from two different trials located in Germany and Southern France. The processing of bunch of grapes into washed berries, wet pomace, lees, must, bottled wine and wine at 1st taste test was performed at Weinstr. Nord 27, D-67487 Maikammer/Germany. The processing simulated the industrial practice on a laboratory scale. A flow chart to describe the production of grape juice is presented in Diagram 6.5.3- 8.

Diagram 6.5.3- 8: Flow chart for the preparation of bunches of grapes into berries, washed; pomace; lees; must; bottled wine; and wine at first taste test

Samples were analysed according to the analytical method 00984 ([redacted], 05/02/2007, M-283301-01-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues were extracted from 10 g of sample material (raisin and raisin waste 5 g) by two successive extractions using a high speed blender with a mixture of acetonitrile:water (80:20; v:v).

Subsequently, the raw extracts were diluted 10-fold by adding internal standard solutions:

- One dilution performed under acidic conditions (for determination of fluopyram-PCA)
- Another dilution performed under basic conditions (for determination of fluopyram, FLU-benzamide and FLU-PAA)

Residues were quantified by reversed-phase chromatography coupled with tandem mass spectrometry (MS/MS) with electrospray ionisation. One injection in positive electrospray ionisation allowed the determination of fluopyram, FLU-benzamide and FLU-PAA. Another injection in negative electrospray ionisation allowed the determination of FLU-PCA under different conditions.

The quantitation was carried out by internal standardization using internal stable labelled standards. The Limit of Quantitation (LOQ, calculated and expressed as fluopyram parent equivalents for fluopyram and its metabolites), defined as the lowest validated fortification level, was 0.01 mg/kg in all matrices for all analytes. All residues are calculated as parent fluopyram.
The storage period of deep frozen processed samples ranged between 1 and 264 days.

Table 6.5.3- 18: Recovery results for fluopyram in bunch of grapes and processed materials of wine production

Report No.	Sample Material	Matrices covered	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
Analyte: fluopyram							
08-3077 & 08-3081	Pomace	Pomace, lees, must	0.01	102, 104, 107, 106, 104, 109	105	2.4	0.01
			0.10	98, 101, 99, 103, 102, 104	101	2.3	
			Overall Recovery (n = 12)		**103**	**3.1**	
	wine at 1st taste test	Wine at 1st taste test, grape wine	0.01	113, 111, 111, 102, 104, 106, 104	107	4.0	0.01
			0.10	106, 104, 105	105	1.0	
			1.0	93	93	-	
			5.0	117	117	-	
			Overall Recovery (n = 12)		**106**	**5.8**	

FL: fortification level, RSD - Relative Standard Deviation
* some RSDs were not calculated as there were only one individual recoveries given
Final determination as: fluopyram Residues calculated as: fluopyram

Table 6.5.3- 19: Recovery results for fluopyram-pyridyl-acetic acid in bunch of grapes and processed materials of wine production

Report No.	Sample Material	Matrices covered	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
Analyte: fluopyram-pyridyl-acetic acid							
08-3077	Pomace	Pomace, lees, must	0.01	78, 84, 78, 81, 78, 79	80	3.0	0.01
			0.10	70, 72, 72, 77, 78, 80	75	5.4	
			Overall Recovery (n = 12)		**77**	**5.0**	
	wine at 1st taste test	Wine at 1st taste test; grape wine	0.01	76, 82, 72, 72, 79, 86, 71	77	7.0	0.01
			0.10	78, 74, 74	75	3.1	
			1.0	71	71	-	
			5.0	89	89		
			Overall Recovery (n = 12)		**77**	**7.6**	

FL: fortification level, RSD - Relative Standard Deviation
* some RSDs were not calculated as there were only one individual recoveries given
Final determination as: FLU-PAA Residues calculated as: fluopyram

Table 6.5.3- 20: Recovery results for fluopyram-benzamide in bunch of grapes and processed materials of wine production

Report No.	Sample Material	Matrices covered	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
Analyte: fluopyram-benzamide							
08-3077	Pomace	Pomace, lees, must	0.01	107, 108, 104, 96, 102, 103	103	4.1	0.01
			0.10	98, 96, 99, 97, 96, 96	97	1.3	
			Overall Recovery (n = 12)		**100**	**4.5**	
	wine at 1st taste test	Wine at 1st taste test; grape wine	0.01	103, 99, 98, 100, 102, 100, 109	102	3.6	0.01
			0.10	108, 103, 108	106	2.7	
			1.0	101	101	-	
			5.0	119	119	-	
			Overall Recovery (n = 12)		**104**	**5.7**	

FL: fortification level, RSD - Relative Standard Deviation
* some RSDs were not calculated as there were only one individual recoveries given
Final determination as: FLU-benzamide Residues calculated as: fluopyram

Table 6.5.3- 21: Recovery results for fluopyram-pyridyl-carboxylic acid in bunch of grapes and processed materials of wine production

Report No.	Sample Material	Matrices covered	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
Analyte: fluopyram -pyridyl-carboxylic acid							
08-3077	Pomace	Pomace, lees, must	0.01	104, 96, 82, 91, 94, 85	92	8.6	0.01
			0.10	87, 82, 74, 91, 94, 90	86	8.4	
			Overall Recovery (n = 12)		**89**	**8.8**	
	wine at 1st taste test	Wine at 1st taste test; grape wine	0.01	89, 93, 104, 81, 95, 98, 90	93	7.9	0.01
			0.10	100, 102, 105	102	2.5	
			1.0	96	96	-	
			5.0	107	107	-	
			Overall Recovery (n = 12)		**97**	**7.9**	

FL: fortification level, RSD - Relative Standard Deviation
* some RSDs were not calculated as there were only one individual recoveries given
Final determination as: FLU-PCA Residues calculated as: fluopyram

Findings:

In bunch of grapes used for juice and wine production residues of fluopyram were at 0.25 and 0.88 mg/kg. Residues of fluopyram in the final product wine at 1st taste test were 0.17 mg/kg and 0.71 mg/kg respectively. Residue values of fluopyram in wine and the processed by-products are summarised in Table 6.5.3- 22.

With wine processing, 56 - 70% of the initial residue remained in the grape pomace, and 49 - 74% was recovered in must. Fermenting and filtration further reduced the residue concentration of fluopyram so that the final product wine contained 32 - 39% and wine at first taint test 35 - 42% of the initial residue.

Table 6.5.3- 22: Residues of fluopyram and metabolites in bunch of grapes and processed commodities of wine production.

Country Study No. Trial No.	Crop Portion analysed	DALT (days)	Residues (mg/kg) expressed as AE C656948 equivalents			
			fluopyram	FLU-pyridyl-acetic acid	FLU-benzamide	FLU-pyridyl-carboxylic acid
Germany 08-3077 08-3077-01	bunch of grapes	3	0.88	< 0.01	0.01	0.03
	pomace	3	2.29	< 0.01	0.03	0.07
	lees	3	2.22	< 0.01	0.03	0.14
	must	3	0.95	< 0.01	0.03	0.03
	Wine bottled	3	0.65	< 0.01	0.02	0.02
	wine at 1st taste test	3	0.71	< 0.01	0.02	0.03
Southern France 08-3081 08-3081-01	bunch of grapes	3	0.25	< 0.01	< 0.01	< 0.01
	pomace	3	0.84	< 0.01	0.01	< 0.01
	lees	3	0.29	< 0.01	< 0.01	< 0.01
	must	3	0.17	< 0.01	< 0.01	< 0.01
	Wine bottled	3	0.16	< 0.01	< 0.01	< 0.01
	wine at 1st taste test	3	0.17	< 0.01	< 0.01	< 0.01

DALT = days after last application

An average processing factor of 0.7 was calculated for the transfer of fluopyram from bunch of grapes into wine. The transfer factors of fluopyram into wine and the processed by-products are summarised in Table 6.5.3- 23.

Table 6.5.3- 23: Transfer factors for the residue of fluopyram in processed commodities of wine production

Sample material	Transfer factors for residues of fluopyram		
	08-3077-01 Germany	08-3081-01 France South	Mean
pomace wet	2.6	3.4	3.2
lees	2.5	1.2	1.9
must	1.1	0.7	0.9
Wine bottled	0.7	0.6	0.7
wine at 1st taste test	0.8	0.7	0.8

* Values below LOQ which were set at LOQ for the calculation of transfer factors

Conclusion:

Residues of fluopyram concentrate in wet pomace but are reduced in must and wine. This result is comparable to the data presented in EFSAs "Conclusion on the peer review of the pesticide risk assessment of the active substance fluopyram (EFSA Journal 2013;11(4):3052)".

Assessment and conclusion by applicant:

The study is acceptable.

Data Point:	KCA 6.5.3/12
Report Author:	[redacted]
Report Year:	2010
Report Title:	Determination of the residues of AE C656948 and trifloxystrobin in/on grape and the processed fractions (must; pomace, grape; wine at bottling and wine at first taste test) after spraying of AE C656948 & CGA279202 SC 500 in the field in France (South) and Italy
Report No:	08-3204
Document No:	M-384844-01-1
Guideline(s) followed in study:	EU: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8; EC guidance working document 7029/VI/95 rev. 5 (1997-07-22)
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and Methods

The study included two supervised residue trials with grape, conducted in the field in southern Europe (southern France and Italy) in the 2008 season. The purpose of this study was to determine the magnitude of the residues of fluopyram and its metabolites fluopyram-benzamide (AE F148815, alias FLU-benzamide), fluopyram-pyridyl-acetic acid (BCS-AA10139, alias FLU-PAA) and fluopyram-pyridyl-carboxylic acid (AE C657188, alias FLU-PCA) in/on grape (bunch of grapes) and its processed fractions of wine (must, pomace, wine at bottling and wine at first taste test).

Field part

In each field trial from study 08-2204, the treated plot was sprayed twice at BBCH growth stages from 81 (beginning of ripening: berries begin to develop variety-specific colour) to 85 (softening of berries) with AEC656948 & CGA279202 SC 500, a suspension concentrate (SC) formulation containing 250 g/L fluopyram (AEC656948) and 250 g/L trifloxystrobin (CGA279202). The application rate was 50 g a.s. fluopyram/ha using a spray volume of 200-500 L/ha. The interval between applications was 14-15 days and the pre-harvest interval was 14 days. All treatments were made at the scheduled rates.

Bunch of grape samples were taken 14 days after the last treatment at BBCH 89. The sample 08-2204-03-0003F for processing for trial 08-3204-02 was not receipt at the processing laboratory and a new sample was taken at DALT21. Samples for processing were shipped to processing laboratory in fresh conditions and then were stored in a cold room (5-10°C) until processing on the next day.

The grapes were crushed and after taking a laboratory sample of the must, the crushed grapes (=must) were transported by car at ambient temperature for the flash vacuum-expansion (CIRAD, Montpellier, France). The must and liquors obtained after vacuum-expansion were grouped together in a stainless steel tank and transported by car at ambient temperature to the processing laboratory to start the alcoholic fermentation.

Directly after processing the laboratory sample wine at first taste test was stored in a cold room (approx. 5-10°C) until shipment by courier at ambient temperature to the laboratory for preparation of the examination samples. The other laboratory samples were stored deep-frozen after sampling at ≤-18°C until deep-frozen shipment in boxes with dry ice. The laboratory sample wine at first taste test was stored

for approximately 6 months at 12°C and then stored deep-frozen until preparation of the examination samples. The other laboratory samples were stored in a freezer at ≤-18°C until preparation of the examination samples where the deep-frozen lab samples were shredded with dry ice in a cutter. Representative parts of the shredded lab samples were transferred into polystyrene boxes separately for analysis (examination samples) and archiving (retain samples) and stored for analysis or archiving at ≤-18°C until analysis.

Processing procedures

The processing procedures followed industry procedures adapted to residue studies in small volumes.
The red wine grape specimens were processed into red wine with flash vacuum-expansion of the must.

Crushing-stemming
The grape specimens were crushed and stemmed with an electric crusher/stemmer. The crushed grapes (= must) were recovered in a stainless steel tank and weight. Two 0.1 kg must sub-specimens were collected into plastic bottles, labelled and frozen (≤-18°C).

Flash vacuum-expansion
With regards to the oxidation, the must was sulphited by addition of potassium metabisulphite at a level of 0.04 g/L. The must volume was estimated by division of the weight of the grapes with a coefficient depending on grape variety: 1.3 (for the two trials).
The crushed grapes were transported in car at ambient temperature to CIRAD, Montpellier, France for the flash vacuum-expansion.
The crushed grapes were heated by portions of about 2 kg with water steam at approx. 90°C for 5 min in a steam heating chamber, then decanted quickly in a vacuum vessel. The first vacuum expanded portion (between 1.649 kg and 1.914 kg) was discarded.
As the flash vacuum-expansion equipment is a single exemplar, for the trial No 08-3204-01, the untreated specimen was processed before the treated specimen with a great cleaning between each specimen to avoid all contamination.
The obtained amount of vacuum expanded must is dependent on different parameters, notably, the lost during the manipulation and the addition of the injected water steam until 10% of weight in supplement.
When all amount of grapes was vacuum expanded, the obtained must and liquors were grouped together in a stainless steel tank and transported in a car at ambient temperature at the processing laboratory to start the alcoholic fermentation.

Alcoholic fermentation
The necessary quantity of potassium metabisulphite was weight and diluted in approx. 10 times its weight in distilled water. The necessary quantity of enzymes was weight and rehydrated in a volume of must equal to 10 times its weight. The necessary quantity of yeast was weight and rehydrated in a sugared water (the mixture equalled 10 times the weight of the yeasts used).
Potassium metabisulphite (0.06 g/L), pectolytic enzymes (0.02 g/L) and yeasts (0.10 g/L) were added to the must in the tank and mixed. The pH of the must was measured using a pH-meter.
For the trial No 08-3204-01, as the alcohol content estimated from the refractometric degree was judged insufficient to produce normal quality wine, white crystallised sugar was added. For the control sample (8.2%) and treated sample (8.0%) from 08-3204-01, 34 g/L of sugar were added to the must during alcoholic fermentation, in order to increase the probable alcohol content of each specimen to 2%. The progress of the alcoholic fermentation (AF) was followed each working day by measuring the density and temperature of the must. The density and temperature were measured using a mustimeter, which was plunged into a 250-mL measuring cylinder containing the must.
The alcoholic fermentation was considered to be completed when the density of the must was stabilized under the value 1000 (998 for control and treated samples from trial 08-3204-01 and 996 for treated sample from trial 08-3204-02).

Pressing
The stainless steel tanks were weighed after alcoholic fermentation. The wine was run off to the tank (free-run wine) end the solid part was pressed with a water press to recover the maximum quantity of wine. The pressed wine was added to the free-run wine and all the wine obtained was weighed. The wet pomace obtained was weighed and two 0.1 kg wet pomace sub-specimens were taken and packaged into plastic bags, labelled and frozen (s -180C). The remaining pomace was discarded.

Malolactic fermentation
The malolactic fermentation was carried out in absence of air into demijohns, at ambient temperature with a direct inoculation of lactic bacteria: *leuconostoc oenos* (0.01 g/L) to accelerate this process. The wine unused was weighed and discarded.
The progress of the malolactic fermentation was followed-up one or two times each week by chromatography on paper.
After the malolactic fermentation was complete, 0.10 g/L of potassium metabisulphite was added to wine.

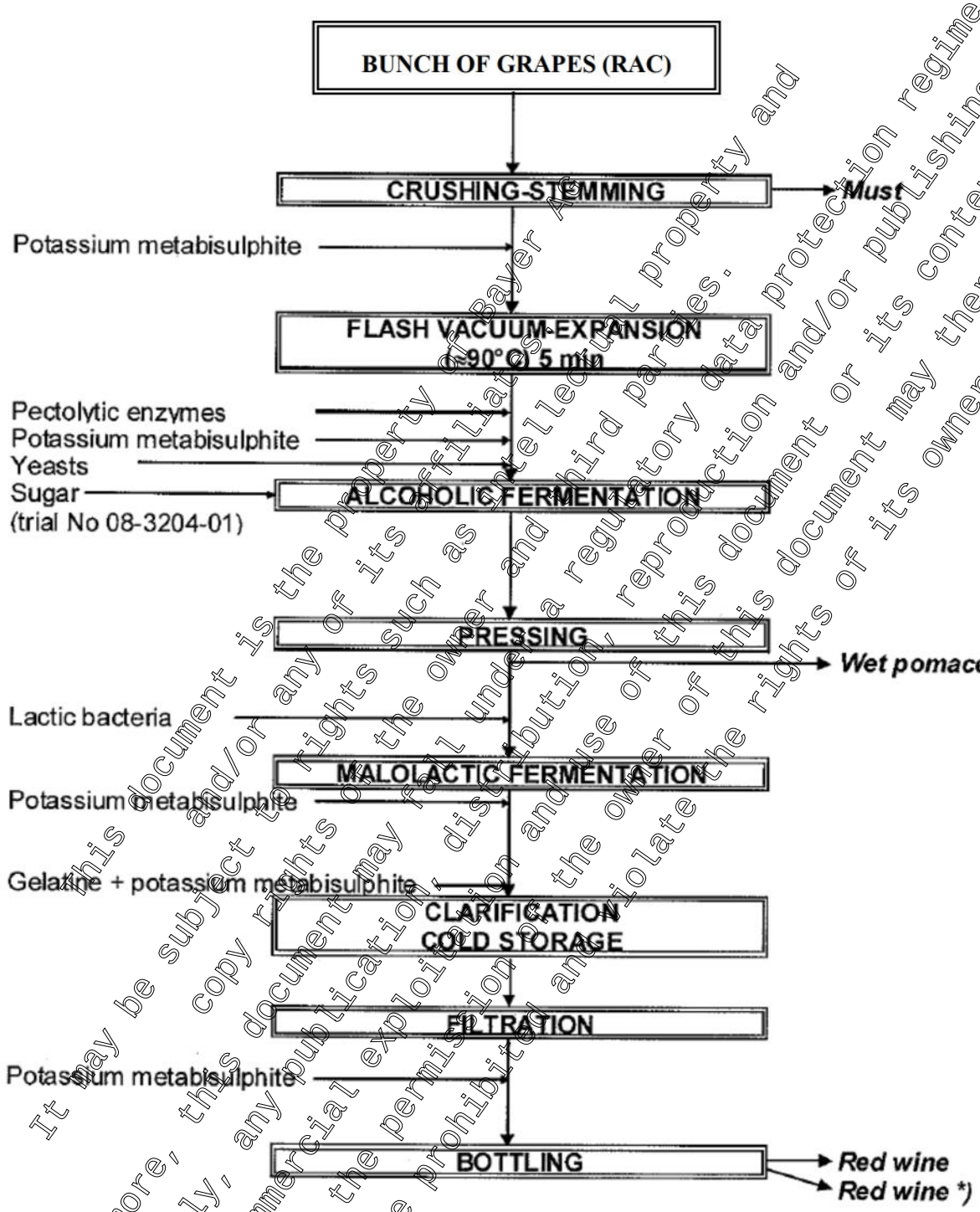
Clarification-Cold storage
The natural clarification lasted four days for all specimens. The wine was racked. The obtained malolactic fermentation wine and lees were weighed. Lees were discarded. 0.10 g/L of dry gelatine and 0.04 g/L of potassium metabisulphite were added to obtained wine, to improve the clarification.
The wine was kept into demijohns and stored in a cold room (5-10°C) to be stabilized with regard to tartaric deposits and so that clarification could be achieved.
To remove impurities (solid material), the wine was racked. The racked wine and sediments were weighed. Sediments were discarded.

Filtration
The racked wine was filtered using a stainless steel filtration unit with a 10 liter capacity under pressure using nitrogen (3 bars maximum).
The filtration was carried out over cellulose filter plate of 90 mm diameter and 2.5 µm of porosity. After this operation, the specimens received 0.40 g/L of potassium metabisulphite, which protects the wine from oxidation.

Bottling
On the same day, two 0.1 kg red wine sub-specimens were collected into plastic bottles, labelled and frozen (≤-18 °C) and two 0.75 L red wine sub-specimens were collected into glass bottles, labelled and stored in a cold room (approx. 5-10 °C).

The processes are illustrated in Diagram 6.5.3-9.

Diagram 6.5.3- 9: Flow chart of the red wine processing with flash vacuum-expansion of the must



Samples or fractions to be analysed:
- Must
- Wet pomace = Pomace, grape
- Red wine = Wine at bottling
- Red wine *) = Wine at first taste test (kept after bottling at standard wine storage temperature (between ca. 5 and 10°C)

RAC = Raw Agricultural Commodity

Residue analysis

Residues of fluopyram and its metabolites were determined by LC-MS/MS according to method 00984/M001 ([redacted], 2007, M-295145-03-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4 which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues were extracted from 10 g of sample material by two successive extractions using a high speed blender with a mixture of acetonitrile:water (4:1; v:v).

Subsequently, the raw extracts were diluted 10-fold by adding internal standard solutions:

- One dilution and an additional clean-up step were performed under acidic conditions for determination of FLU-PCA
- Another dilution was performed under basic conditions for determination of fluopyram, FLU-benzamide and FLU-PAA

Residues were quantified by reversed-phase chromatography coupled with tandem mass spectrometry (MS/MS) with electrospray ionisation. One injection in positive electrospray ionisation allowed the determination of fluopyram, FLU-benzamide and FLU-PAA. Another injection in negative electrospray ionisation allowed the determination of FLU-PCA under different conditions.

The quantitation was carried out by internal standardization using stable-labelled internal standards in pure solvent.

The Limit of Quantification (LOQ) was 0.01 mg/kg for fluopyram and its metabolites, all calculated as parent equivalents and for all matrices.

I. Findings

During the course of this study, the method performance was checked by concurrent recoveries. Details on concurrent recovery data are shown in Table 6.5.3- 24 to Table 6.5.3- 27. The average recoveries were within the acceptable range of 70 – 110%. If applicable, the RSD values were always below 20%. The overall RSD values where at 22.9% and 28.1% for must and pomace, grape, respectively.
The levels of residues of fluopyram and its metabolites in the processed samples are summarized in Table 6.5.3- 29. No residues above the LOQ were found in the control samples. The results were not corrected for concurrent recoveries.

The residue level of fluopyram in bunch of grape (RAC for the calculation of the processing factors) was at 0.04 mg/kg in trial 08-2204-02 (processing trial 08-3204-01). The sample 08-2204-03-0003F for processing of trial 08-3204-02 was not receipt at the processing laboratory and a new sample was taken at DALT21. Unfortunately no RAC sample taken on DALT 21 is available, and therefore for calculation of the transfer factors, the residue value of the must was used for the RAC value. The must was obtained after crushing and stemming of the bunches of grapes, and therefore residue levels are not expected to differ significantly from those in the RAC. This is also confirmed by the results of trial 08-3204-01, where for all analytes for must the same residue value as for the RAC was found. The residue level of fluopyram in must (RAC for the calculation of the processing factors) was at 0.07 mg/kg in trial 08-3204-02. Residues values of fluopyram ranged from 0.01 to 0.28 mg/kg in processed commodities.

For both metabolites fluopyram-benzamide and fluopyram-pyridyl-acetic-acid, residue levels were always <0.01 mg/kg with one exception for pomace, grape where residues of fluopyram-benzamide were at 0.02 mg/kg in trial 08-3204-02.

For fluopyram-pyridyl-carboxylic-acid, residues ranged from <0.01 mg/kg to 0.03 mg/kg.

The processing factors (PF) were calculated based on the residue level in the treated processed commodity and residue level in the RAC specimens (bunch of grape for trial 08-3204-01 and must for trial 08-3204-02). When residues in the RAC and in the processed fractions were both <0.01 mg/kg a processing factor could not be calculated. The proposed processing factors are summarised below in

Table 6.5.3- **28**.

The analyses were done after a maximum frozen storage period of 361 days for bunch of grapes and processed commodities.

III. Conclusions

Two residue trials were conducted in southern Europe in 2008. Grapes were treated twice at a growth stage from BBCH 81 to BBCH 85 (pre-harvest interval of 14 days) with a dose rate of 2 x 50 g a.s./ha. All applications were at the required rates.

Bunch of grape samples from red grape varieties were processed in order to obtain red wine. The samples (RAC and processed fraction) were analysed for the residues of fluopyram parent compound and its metabolites FLU-benzamide, FLU-PAA and FLU-PCA. The processing study was conducted according to GLP.

The results of the study indicate that residues of fluopyram and its metabolite FLU-benzamide remain to a large extent in wet pomace after pressing with processing factor at 3.4 and >2.0, respectively. PF at 1.0 was found in must for fluopyram and FLU-PCA and in wet pomace for FLU-PCA. Residues of fluopyram (PF at 0.34 at bottling and at 1st taste test) and FLU-PCA (PF <0.84 at bottling and at 0.84 at 1st taste test) are very low in wine.

Assessment and conclusion by applicant:

The study is acceptable.

Table 6.5.3- 24: Recovery results for fluopyram in processed commodities into wine

Study	Portion analysed	n	Fortification level* (mg/kg)	Recovery (%)				
				Individual recoveries	Min	Max	Mean	RSD
08-3204	Grape / must	2	0.01	101; 102	101	102	102	-
		1	1.0	84	84	84		
		3	Overall		84	102	96	10.6
	Grape / pomace, grape	2	0.01	96; 98	96	98	97	-
		1	1.0	83	83	83		
		3	Overall		83	98	92	8.8
	Grape / wine at first taste test	2	0.01	102; 105	102	105	104	-
		1	0.10	103	103	103		
		3	Overall		102	105	103	1.5

FL: fortification level, RSD - Relative Standard Deviation
* some RSDs were not calculated as there were only two individual recoveries given
Final determination as: fluopyram Residues calculated as: fluopyram

Table 6.5.3- 25: Recovery results for fluopyram-benzamide in processed commodities into wine

Study	Portion analysed	n	Fortification level* (mg/kg)	Recovery (%)				
				Individual recoveries	Min	Max	Mean	RSD
08-3204	Grape / must	2	0.005	67; 72	67	72	70	-
		1	0.50	101	101	101	-	-
		3	Overall		67	101	80	22.9
	Grape, pomace, grape	2	0.005	67; 67	67	67	67	-
		1	0.50	106	106	106	-	-
		3	Overall		67	106	80	28.1
	Grape / wine at first taste test	2	0.005	77; 87	77	87	82	-
		1	0.50	100	100	100	-	-
		3	Overall		77	100	88	13.1

FL: fortification level, RSD - Relative Standard Deviation
* some RSDs were not calculated as there were only two individual recoveries given
Final determination as: fluopyram-benzamide Residues calculated as: fluopyram

Table 6.5.3- 26: Recovery results for fluopyram-pyridyl-acetic acid in processed commodities into wine

Study	Portion analysed	n	Fortification level* (mg/kg)	Recovery (%)				
				Individual recoveries	Min	Max	Mean	RSD
08-3204	Grape / must	2	0.007	93; 93	93	93	93	-
		1	0.70	105	105	105	-	-
		3	Overall		93	105	97	7.1
	Grape / pomace, grape	2	0.007	83; 97	83	97	90	-
		1	0.70	93	93	93	-	-
		3	Overall		83	97	91	7.9
	Grape / wine at first taste test	2	0.007	88; 109	88	109	99	-
		1	0.70	110	110	110	-	-
		3	Overall		88	110	102	12.1

FL: fortification level, RSD - Relative Standard Deviation
* some RSDs were not calculated as there were only two individual recoveries given
Final determination as: FLU-PAA Residues calculated as: fluopyram

Table 6.5.3- 27: Recovery results for fluopyram-pyridyl-carboxylic acid in processed commodities into wine

Study	Portion analysed	n	Fortification level* (mg/kg)	Recovery (%)				
				Individual recoveries	Min	Max	Mean	RSD
08-3204	Grape / must	2	0.006	84; 103	84	103	94	-
		1	0.60	108	108	108	-	-
		3	Overall		84	108	98	12.9
	Grape / pomace, grape	2	0.006	98; 106	98	106	102	-
		1	0.60	113	113	113	-	-
		3	Overall		98	113	106	7.1
	Grape / wine at first taste test	2	0.006	98; 103	98	103	101	-
		1	0.60	103	103	103	-	-
		3	Overall		98	103	101	2.8

FL: fortification level, RSD - Relative Standard Deviation
* some RSDs were not calculated as there were only two individual recoveries given
Final determination as: FLU-PCA Residues calculated as: fluopyram

Table 6.5.3- 28: Summary of residues in grape and proposed processing factors for fluopyram and its metabolites for grape (Tested GAP: 2 × 50 g a.s./ha at BBCH from 81 to 85)

Trial	08-3204-01		08-3204-02		Proposed PF (mean, if applicable)
Sample material	Residues (mg/kg)	PF	Residues (mg/kg)	PF	
Fluopyram					
Bunch of grapes (RAC)	**0.04**	--	**0.09**	--	
Must	**0.04**	1.0	**0.07**	1.0	1.0
Pomace, grape	**0.11**	2.8	**0.28**	4.0	3.4
Wine at bottling	**0.01**	0.25	**0.03**	0.43	0.34
Wine at 1st taste test	**0.01**	0.25	**0.03**	0.43	0.34
Fluopyram-benzamide					
Bunch of grapes (RAC)	<0.01	--	<0.01	--	--
Must	<0.01	n.c	<0.01	n.c	n.c
Pomace, grape	<0.01	n.c	**0.02**	2.0	2.0
Wine at bottling	<0.01	n.c	<0.01	n.c	n.c
Wine at 1st taste test	<0.01	n.c	<0.01	n.c	n.c
Fluopyram-pyridyl-carboxylic-acid					
Bunch of grapes (RAC)	**0.01**	--	**0.02**	--	--
Must	**0.01**	1.0	**0.03**	1.0	1.0
Pomace, grape	**0.01**	1.0	**0.03**	1.0	1.0
Wine at bottling	<0.01	<1.0	**0.02**	0.67	<0.84
Wine at 1st taste test	**0.01**	1.0	**0.02**	0.67	<0.84
Fluopyram-pyridyl-acetic-acid					
Bunch of grapes (RAC)	<0.01	--	<0.01	--	--
Must	<0.01	n.c	<0.01	n.c	n.c
Pomace, grape	<0.01	n.c	<0.01	n.c	n.c
Wine at bottling	<0.01	n.c	<0.01	n.c	n.c
Wine at 1st taste test	<0.01	n.c	<0.01	n.c	n.c

Notes: for trial 08-3204-02, since no RAC (Raw Agricultural Commodity) sample with DALT 21 is available, the residue value of the must was taken as the RAC value for calculation of the transfer factor.

PF (processing factor) = residue concentration in processed commodity (mg/kg) / residue concentration in the RAC (mg/kg)

n.c: no processing factor can be calculated (residues <LOQ in RAC and in processed commodity)

< value: in case the residue level in the RAC is ≥ LOQ but residues in the processed commodity are < LOQ, the processing factor is calculated as to be below the worst case value calculated with the LOQ of the processed commodity.

> value: in case the residue level in the RAC is < LOQ but residues in the processed commodity are ≥ LOQ, the processing factor is calculated as to be above the value calculated with the LOQ of the RAC.

Table 6.5.3- 29: Detailed results of the grape processing study 08-3204

GAP Summary of the 08-2204 trials

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL			
	(a)	(b)				(c)	(d)	(f)
08-2204-02 France, south 31620 Fronton Europe, South F 2008	Grape Negrette	1) 01.01.1990 2) 10.06.2008 - 20.06.2008 3) 11.09.2008 - 30.09.2008	50 50	200 200	25 25	13.08.2008/0 28.08.2008/15	85	(g) 08-2204 (h) SC (fluopyram 250 g/L ,trifloxystrobin 250 g/L) (i) Application method: Spraying
08-2204-03 Italy 70031 Andria (BA) Europe, South F 2008	Grape Montepulciano	1) 13.03.2000 2) 10.05.2008 - 25.05.2008 3) 20.09.2008 - 10.10.2008	50 50	500 500	10 10	01.09.2008/0 15.09.2008/14	85	(g) 08-2204 (h) SC (fluopyram 250 g/L ,trifloxystrobin 250 g/L) (i) Application method: Spraying

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
(f) Remarks may include: Climatic conditions, Reference to analytical method and information which metabolites are included.

Trial No. / Location / EU zone / Year (a)	Commodity / Variety (a)	Portion analyzed	Growth stage at sampling (d)	Residues (mg/kg)				PHI (days) (e)	Details on trial (f)
				fluopyram as fluopyram	FLU-benzamide as fluopyram	FLU-pyridyl-carboxylic-acid as fluopyram	FLU-pyridyl-acetic acid as fluopyram		
08-2204-02 France, south 31620 Fronton Europe, South F 2008	Grape Negrette	bunch of grapes	89	0.04	<0.01	0.01	<0.01	14	(g) 08-3204 (j) Analytical method: 00984/M001 (k) LOQ: 0.01 mg/kg (l) Method Validation Data: 00984/M001 (m) Storage: wine, bottled: 268 days wine at first taste test: 77 days pomace, grape: 330 days must: 301 days bunch of grapes: 361 days
		Processing into wine (Trial 08-3204-01)							
		must	89	0.04	<0.01	0.01	<0.01	14	
		pomace, grape	89	0.11	<0.01	0.01	<0.01	14	
		wine, bottled	89	0.01	<0.01	<0.01	<0.01	14	
		wine at first taste test	89	0.01	<0.01	0.01	<0.01	14	

Trial No. / Location / EU zone / Year	Commodity / Variety (a)	Portion analyzed	Growth stage at sampling (d)	Residues (mg/kg)				PHI (days) (e)	Details on trial (f)
				fluopyram as fluopyram	FLU-benzamide as fluopyram	FLU-pyridyl-carboxylic acid as fluopyram	FLU-pyridyl-acetic acid as fluopyram		
08-2204-03 Italy 70031 Andria (BA) Europe, South F 2008	Grape Montepulciano	bunch of grapes	89	0.09	<0.01	0.02	<0.01	21	(g) 08-3204 (j) Analytical method: 00984/M001 (k) LOQ: 0.01 mg/kg (l) Method Validation Data: : 00984/M001 (m) Storage: wine, bottled: 287 days, wine at first taste test: 98 days, pomace, grape: 325 days, must: 335 days bunch of grapes: 343 days * sample for processing 08-2204-03-0003F for trial 08-3204-02 was not receipt at the processing laboratory and a new sample was taken at DALT21
		Processing into wine (Trial 08-3204-02)							
		must	89	0.07	<0.01	0.03	<0.01	21*	
		pomace, grape	89	0.28	0.02	0.03	<0.01	21*	
		wine, bottled	89	0.03	<0.01	0.02	<0.01	21*	
		wine at first taste test	89	0.03	<0.01	0.02	<0.01	21*	

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) Study reference
* prior to last treatment
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
Bunch of grapes: between deep-freezing (in the field) and date of last extraction
Processed commodities: between their deep-freezing and date of last extraction

Data Point:	KCA 6.5.3/13
Report Author:	[redacted].
Report Year:	2012
Report Title:	Processing Study - Determination of the residues of AE C656948 and fenhexamid in/on grape and the processed fractions (must; pomace, grape; wine at bottling and wine at first taste test) after low-volume spraying of Fluopyram SC 500 and Fenhexamid WG 50 in the field in France (south)
Report No:	09-3240
Document No:	M-440382-01-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91414/EEC of July 15, 1991, Annex II, part A, point 6 and Annex III, part A, section 8; Residues in or on Treated Products, Food and Feed; EC Guidance working document 7029/VI/95 rev.5 (1997-07-22); EC Guidance working document 7035/VI/95 rev.5 (1997-07-22)
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and Methods

The study included two supervised residue trial with grape, conducted in the field in southern Europe (southern France) in the 2009 season. The purpose of this study was to determine the magnitude of the residues of fluopyram and its metabolites fluopyram-benzamide (AE F148815, alias FLU-benzamide), fluopyram-pyridyl-acetic acid (BCS-AA10139, alias FLU-PAA) and fluopyram-pyridyl-carboxylic acid (AE C657188, alias FLU-PCA) in/on grape (bunch of grapes) and its processed fractions of wine (must, pomace, wine at bottling and wine at first taste test).

Field part

In field trial 09-2240-01-T1, the treated plot was sprayed once at BBCH growth stage 67 (70% of flowerhoods fallen) with fenhexamid WG 50, a water-dispersible granules formulation and 60 days later once at BBCH 81 (beginning of ripening: berries begin to develop variety-specific colour) with fluopyram SC 500, a suspension concentrate (SC) formulation containing 500 g/L fluopyram. In field trial 09-2240-01-T2, the treated plot was sprayed once at BBCH growth stage 67 with fluopyram SC 500 and 60 days later once at BBCH 81 with fenhexamid WG 50. The application rates were 300 g a.s. fluopyram/ha using a spray volume of 200 L/ha. All treatments were made at the scheduled rates.

Bunch of grape samples were taken 49 days after the last treatment at BBCH 89 (last treatment was done with fluopyram on trial -T1 and with fenhexamid on trial -T2).

Samples for processing were shipped to processing laboratory in fresh conditions and then were stored deep frozen until processing.

After processing, the processed samples (except “wine at first taste test”) were stored at ≤ -18ºC in a freezer at the processing laboratory until shipment to laboratory logistics in Lyon (France) either deep-frozen in polystyrene boxes with dry ice or by courier at ambient temperature (wine at first taste test). Then, samples were transported deep-frozen or fresh (wine at first taste test) to the laboratory for preparation of the examination samples.

Processing procedures

The processing study simulated industrial practice at a laboratory scale. The red wine grape specimens were processed into red wine with flash vacuum-expansion of the must according to the standard operating procedures in use in the processing laboratory.

Crushing-stemming
The grape specimens were crushed and stemmed with an electric crusher/stemmer. The crushed grapes (= must) were recovered in a stainless steel tank and weight. Two 0.1 kg must sub-specimens were collected into plastic bottles, labelled and frozen (≤-18°C).

Flash vacuum-expansion
With regards to the oxidation, the must was sulphited by addition of potassium metabisulphite at a level of 0.04 g/L. The must volume was estimated by division of the weight of the grapes with a coefficient depending on grape variety: 1.4.
The crushed grapes were transported in car at ambient temperature to CIRAD, Montpellier, France for the flash vacuum-expansion.
The crushed grapes were heated by portions of about 2 kg with water steam at approx. 90°C for 5 min in a steam heating chamber, then decanted quickly in a vacuum vessel. As the flash vacuum-expansion equipment is a single exemplar, the untreated specimen was processed before the treated specimens with a great cleaning between each specimen to avoid all contamination and for each specimen, approximately 1 kg of crushed grapes was vacuum expanded and discarded. The obtained amount of vacuum expanded must is dependent on different parameters, notably, the lost during the manipulation and the addition of the injected water steam until 10% of weight in supplement. When all amount of grapes was vacuum expanded, the obtained must and liquors were grouped together in a stainless steel tank and transported in a car at ambient temperature at the processing laboratory to start the alcoholic fermentation.

Alcoholic fermentation
The necessary quantity of potassium metabisulphite was weight and diluted in approx. 10 times its weight in distilled water. The necessary quantity of enzymes was weight and rehydrated in a volume of must equal to 10 times its weight. The necessary quantity of yeast was weight and rehydrated in a sugared water (the mixture equalled 10 times the weight of the yeasts used).
Potassium metabisulphite (0.06 g/L), pectolytic enzymes (0.02 g/L) and yeasts (0.10 g/L) were added to the must in the tank and mixed. The pH of the must was measured using a pH-meter.
The progress of the alcoholic fermentation (AF) was followed each working day by measuring the density and temperature of the must. The density and temperature were measured using a mustimeter, which was plunged into a 250-mL measuring cylinder containing the must.
The alcoholic fermentation was considered to be completed when the density of the must was stabilized under the value 1000 (993 for control sample and 994 for treated samples -T1 and -T2).

Pressing
The stainless steel tanks were weighed after alcoholic fermentation. The wine was run off to the tank (free-run wine) and the solid part was pressed with a water press to recover the maximum quantity of wine. The pressed wine was added to the free-run wine and all the wine obtained was weighed. The wet pomace obtained was weighed and two 0.1 kg wet pomace sub-specimens were taken and packaged into plastic bags, labelled and frozen (≤-18°C). The remaining pomace was discarded.

Malolactic fermentation
The malolactic fermentation was carried out in absence of air into demijohns, at ambient temperature with a direct inoculation of lactic bacteria: *leuconostoc oenos* (0.01 g/L) to accelerate this process. The wine unused was weighed and discarded.
The progress of the malolactic fermentation was followed-up one or two times each week by chromatography on paper.
After the malolactic fermentation was complete, 0.10 g/L of potassium metabisulphite was added to wine.

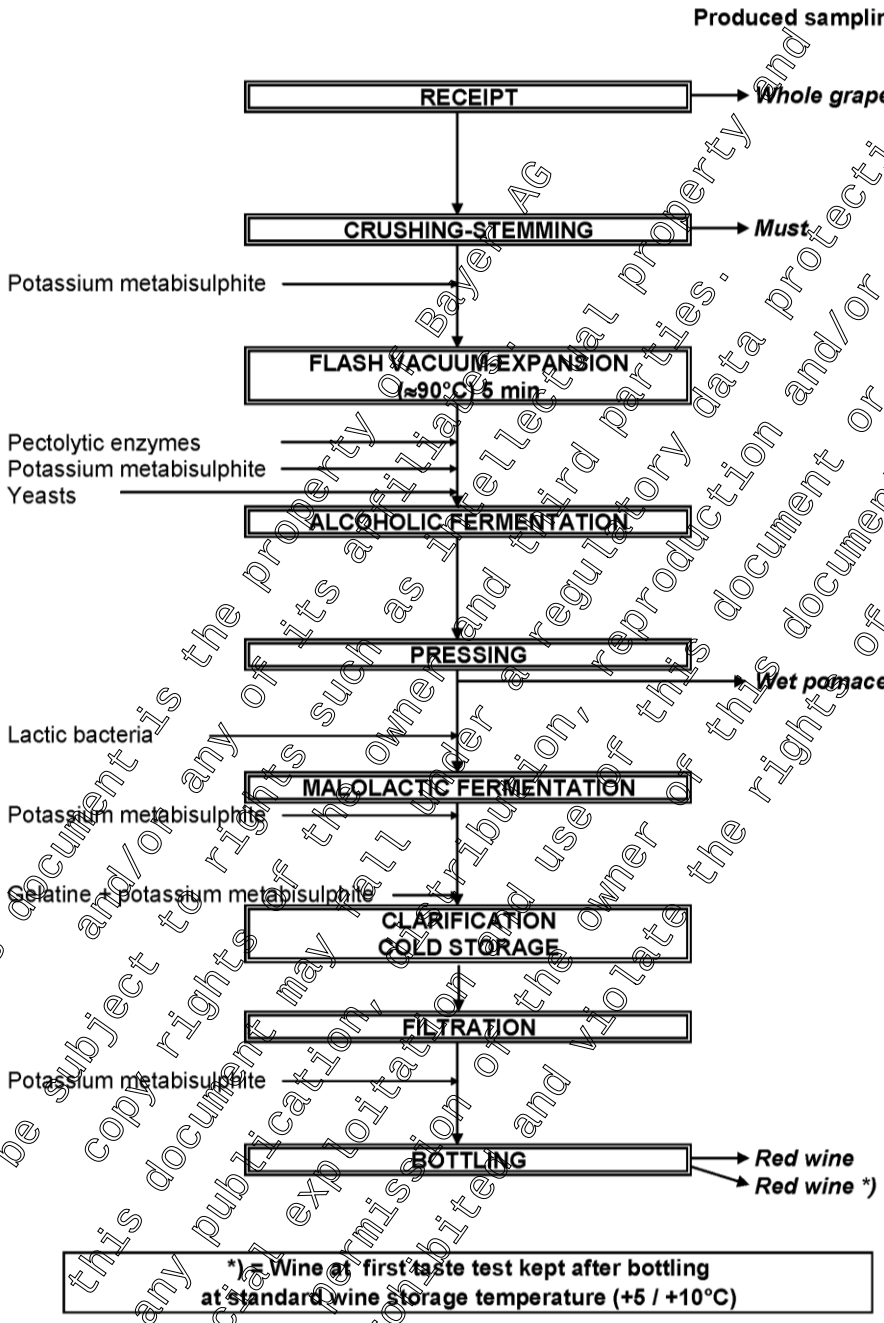
Clarification-Cold storage
The natural clarification lasted four days for control sample and treated sample -T1 and five days for treated sample -T2. The wine was racked. The obtained malolactic fermentation wine and lees were weighed. Lees were discarded. 0.10 g/L of dry gelatine and 0.04 g/L of potassium metabisulphite were added to obtained wine, to improve the clarification.
The wine was kept into demijohns and stored in a cold room (5-10°C) to be stabilized with regard to tartaric deposits and so that clarification could be achieved.
To remove impurities (solid material), the wine was racked. The racked wine and sediments were weighed. Sediments were discarded.

Filtration
The racked wine was filtered using a stainless steel filtration unit with a 10 liter capacity under pressure using nitrogen (3 bars maximum). Filter equipment was used for control and treated samples.
The filtration was carried out over cellulose filter plate of 90 mm diameter and 2.5 µm of porosity. After this operation, the specimens received 0.10 g/L of potassium metabisulphite, which protects the wine from oxidation.

Bottling
On the same day, two 0.1 kg red wine sub-specimens were collected into plastic bottles, labelled and frozen (≤-18 °C) and two 0.75 L red wine sub-specimens were collected into glass bottles, labelled and stored in a cold room (approx. 5-10 °C).

The processes are illustrated in Diagram 6.5.3-10.

Diagram 6.5.3- 10: Flow chart of the red wine processing with flash vacuum-expansion of the must



Residue analysis

Residues of fluopyram in/on plant material were determined by HPLC-MS/MS according to BCS method 01207 based on the QuEChERS multi-residue method ([redacted] S., 11/12/2013, M-424756-02-1, see section MCA 4.1.2).

Analytical method BCS 01207 is based on the QuEChERS multi-residue method. Extraction of residues was done with acetonitrile/water (4/1, v/v) by shaking. The residues of fluopyram, FLU-benzamide and FLU-PCA were extracted together but separately to FLU-PAA.
An aliquot of the extracts was taken, and stable-labelled internal standards were added excepted for FLU-PAA where the stable-labelled internal standard was added at the beginning of the extraction procedure.
The final extracts were subjected to LC-MS/MS. The quantification was performed by internal standardization using stable-labelled internal standards in pure solvent for fluopyram, FLU-benzamide and FLU-PCA. The quantification was performed with matrix matched standards with internal standard for FLU-PAA.

The Limit of Quantification (LOQ) was 0.01 mg/kg for fluopyram and its metabolites, all calculated as parent equivalents and for all matrices.

I. Findings

During the course of this study the method performance was checked by concurrent recoveries. Details on concurrent recovery data are shown in Table 6.5.3- 30 to Table 6.5.3- 33.
No average recovery or RSD value can be calculated. The overall average recoveries ($2 \leq n \leq 3$) were within the acceptable range of 70 – 110% with one overall RSD value below 20%.
The levels of residues of fluopyram and its metabolites in the processed samples are summarized in Table 6.5.3- 35. No residues above the LOQ were found in the control samples. The results were not corrected for concurrent recoveries.

The residue level of fluopyram in bunch of grape (RAC for the calculation of the processing factors) ranged from 0.02 mg/kg (trial 09-2240-01-T2) to 0.16 mg/kg (trial 09-2240-01-T1). Residues values of fluopyram ranged from <0.01 to 0.46 mg/kg in processed commodities.

For all metabolites, residues levels were always <0.01 mg/kg with one exception for wet pomace where residues of FLU-pyridyl-carboxylic-acid were at the LOQ level (0.01 mg/kg) in trial 09-3240-01-T1.

The processing factors (PF) were calculated based on the residue level in the treated processed commodity and residue level in the RAC specimens (bunch of grape). When residues in the RAC and in the processed fractions were both <0.01 mg/kg a processing factor could not be calculated. The proposed processing factors are summarised below in Table 6.5.3- 34.

The analyses were done after a maximum frozen storage period of 527 days for bunch of grapes and 818 days for processed commodities.

III. Conclusions

Two residue trials were conducted in southern Europe in 2009. Grapes were treated once with fluopyram SC 500 at a growth stage BBCH 67 or BBCH 81 with a dose rate at 1 x 300 g a.s./ha. All applications were at the required rates.

Bunch of grape samples from red grape varieties were processed in order to obtain red wine. The samples (RAC and processed fraction) were analysed for the residues of fluopyram parent compound and its metabolites FLU-benzamide, FLU-PAA and FLU-PCA. The processing study was conducted according to GLP.

The results of the study indicate that residues of fluopyram remain to a large extent in wet pomace after pressing with processing factor at 4.5. PF at 1.0 was found in must for fluopyram and in wet pomace for FLU-PCA.

Assessment and conclusion by applicant:

The study is acceptable.

Table 6.5.3- 30: Recovery results for fluopyram in processed commodities into wine

Study	Portion analysed	n	Fortification level* (mg/kg)	Recovery (%)				
				Individual recoveries	Min	Max	Mean	RSD
09-3240	Grape / must, wine, wine at 1st taste test	1	0.01	110	110	110	-	-
		1	0.1	86	86	86	-	-
		2	Overall		86	110	98	
	Grape / pomace	1	0.01	109	109	109	-	-
		1	0.1	100	100	100	-	-
		1	0.5	99	99	99		
		3	Overall		99	109	103	5.4

FL: fortification level, RSD - Relative Standard Deviation
* some RSDs were not calculated as there were only one individual recoveries given
Final determination as: fluopyram Residues calculated as: fluopyram

Table 6.5.3- 31: Recovery results for fluopyram-benzamide in processed commodities into wine

Study	Portion analysed	n	Fortification level* (mg/kg)	Recovery (%)				
				Individual recoveries	Min	Max	Mean	RSD
09-3240	Grape / must, wine, wine at 1st taste test	1	0.01	102	102	102	-	-
		1	0.1	76	76	76	-	-
		2	Overall		76	102	89	
	Grape / pomace	1	0.01	92	92	92	-	-
		1	0.1	74	74	74	-	-
		2	Overall		74	92	83	-

FL: fortification level, RSD - Relative Standard Deviation
* some RSDs were not calculated as there were only one individual recoveries given
Final determination as: FLU-benzamide Residues calculated as: fluopyram

Table 6.5.3- 32: Recovery results for fluopyram-pyridyl-acetic acid in processed commodities into wine

Study	Portion analysed	n	Fortification level (mg/kg)	Recovery (%)				
				Individual recoveries	Min	Max	Mean	RSD
09-3240	Grape / must, wine, wine at 1st taste test	1	0.01	81	81	81	-	-
		1	0.1	73	73	73	-	-
		2	Overall		73	81	77	
	Grape / pomace	1	0.01	70	70	70	-	-
		1	0.1	70	70	70	-	-
		2	Overall		70	70	70	-

FL: fortification level, RSD - Relative Standard Deviation
* some RSDs were not calculated as there were only one individual recoveries given
Final determination as: FLU-PAA Residues calculated as: fluopyram

Table 6.5.3- 33: Recovery results for fluopyram-pyridyl-carboxylic acid in processed commodities into wine

Study	Portion analysed	n	Fortification level (mg/kg)	Recovery (%)				
				Individual recoveries	Min	Max	Mean	RSD
09-3240	Grape / must, wine, wine at 1st taste test	1	0.01	81	81	81	-	-
		1	0.1	83	83	83	-	-
		2	Overall		81	83	82	
	Grape pomace	1	0.01	83	82	83	-	-
		1	0.1	84	84	84	-	-
		2	Overall		83	84	84	-

FL: fortification level, RSD - Relative Standard Deviation
* some RSDs were not calculated as there were only one individual recoveries given
Final determination as: FLU-PCA Residues calculated as: fluopyram

Table 6.5.3- 34: Summary of residues in grape and proposed processing factors for fluopyram and its metabolites for grape

Tested GAP: 1 × 300 g a.s./ha at BBCH 67 (-T2) or 81 (-T1)

Trial	09-3240-01-T1		09-3240-01-T2		Proposed PF (mean if applicable)
Sample material	Residues (mg/kg)	PF	Residues (mg/kg)	PF	
Fluopyram					
Bunch of grapes (RAC)	**0.16**	--	**0.02**	--	--
Must	**0.09**	0.56	**0.03**	1.5	1.0
Pomace, grape	**0.46**	2.9	**0.12**	6.0	4.5
Wine at bottling	**0.05**	0.31	**0.01**	0.50	0.41
Wine at 1st taste test	**0.03**	0.19	<0.01	<0.50	<0.35
Fluopyram-benzamide*					
Bunch of grapes (RAC)	<0.01	--	<0.01	--	--
Must	0.01	n.c	<0.01	n.c	n.c
Pomace, grape	<0.01	n.c	<0.01	n.c	n.c
Wine at bottling	<0.01	n.c	<0.01	n.c	n.c
Wine at 1st taste test	<0.01	n.c	<0.01	n.c	n.c
Fluopyram-pyridyl-carboxylic-acid*					
Bunch of grapes (RAC)	**0.01**	--	<0.01	--	--
Must	<0.01	<1.0	<0.01	n.c	<1.0

Trial	09-3240-01-T1		09-3240-01-T2		Proposed PF (mean if applicable)
Sample material	Residues (mg/kg)	PF	Residues (mg/kg)	PF	
Pomace, grape	**0.01**	1.0	<0.01	n.c	1.0
Wine at bottling	<0.01	<1.0	<0.01	n.c	<1.0
Wine at 1st taste test	<0.01	<1.0	<0.01	n.c	<1.0
Fluopyram-pyridyl-acetic-acid*					
Bunch of grapes (RAC)	<0.01	--	<0.01	--	--
Must	<0.01	n.c	<0.01	n.c	n.c
Pomace, grape	<0.01	n.c	<0.01	n.c	n.c
Wine at bottling	<0.01	n.c	<0.01	n.c	n.c
Wine at 1st taste test	<0.01	n.c	<0.01	n.c	n.c

*expressed as parent compound

PF (processing factor) = residue concentration in processed commodity (mg/kg) / residue concentration in the RAC (mg/kg)

n.c: no processing factor can be calculated (residues <LOQ in RAC and in processed commodity)

< value: in case the residue level in the RAC is ≥ LOQ but residues in the processed commodity are < LOQ, the processing factor is calculated as to be below the worst case value calculated with the LOQ of the processed commodity.

Table 6.5.3- 35: Detailed results of the grape processing study 09-3240

GAP Summary of the 09-3240 trials

Trial No. / Location / EU zone / Year (a)	Commodity / Variety (a)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting (b)	Application rate per treatment			Dates of treatment / Application interval (c)	Growth stage at last treatment (d)	Details on trial (f)
			g a.s./ha	Water (L/ha)	g a.s./hL			
09-3240-01 09-3240-01-T1 France, south 30290 Laudun Europe, South F 2009	Grape Grenache noir, red variety	1) 01.04.1985 2) 23.05.2009 - 04.06.2009 3) 15.09.2009 - 16.09.2009	300	200	150	28.07.2009/60	81	(g) 09-3240 (h) SC (fluopyram 500 g/L) (i) Application method: Spraying, low-volume Other a.s. in product sequence trial: fenhexamid 50 %
09-3240-01 09-3240-01-T2 France, south 30290 Laudun Europe, South F 2009	Grape Grenache noir, red variety	1) 01.04.1985 2) 23.05.2009 - 04.06.2009 3) 15.09.2009 - 16.09.2009	300	200	150	29.05.2009/0	81	(g) 09-3240 (h) SC (fluopyram 500 g/L) (i) Application method: Spraying, low-volume Other a.s. in product sequence trial: fenhexamid 50 %

(a) According to CODEX Classification Guide (b) Only if relevant (c) Year must be indicated
(d) Either growth stage description or BBCH Code (f) Remarks may include: Climatic conditions; reference to analytical method and information which metabolites are included

Trial No. / Location / EU zone / Year	Commodity /Variety (a)	Portion analyzed	Growth stage at sampling (d)	Residues (mg/kg) fluopyram as fluopyram	FLU-benzamide as fluopyram	FLU-pyridyl-carboxylic-acid as fluopyram	FLU-pyridyl-acetic acid as fluopyram	PHI (days) (e)	Details on trial (f)
09-3240-01 09-3240-01-T1 France, south 30290 Laudun Europe, South F 2009	Grape Grenache noir, red variety	bunch of grapes	89	0.16	<0.01	0.01	<0.01	49	(g) 09-3240 (j) Analytical method: 01207 (k) LOQ: 0.01 mg/kg (l) Method Validation Data: 01207 (m) Storage: wine at first taste test: 580 days wine at bottling: 756 days pomace, grape: 809 days must: 818 days bunch of grapes: 478 days
		must	89	0.09	<0.01	<0.01	<0.01	49	
		pomace, grape	89	0.46	<0.01	0.01	<0.01	49	
		wine at bottling	89	0.05	<0.01	<0.01	<0.01	49	
		wine at first taste test	89	0.03	<0.01	<0.01	<0.01	49	
09-3240-01 09-3240-01-T2 France, south 30290 Laudun Europe, South F 2009	Grape Grenache noir, red variety	bunch of grapes	89	0.02	<0.01	<0.01	<0.01	49	(g) 09-3240 (j) Analytical method: 01207 (k) LOQ: 0.01 mg/kg (l) Method Validation Data: 01207 (m) Storage: wine at first taste test: 580 days wine at bottling: 756 days pomace, grape: 805 days must: 818 days bunch of grapes: 478 days
		must	89	0.03	<0.01	<0.01	<0.01	49	
		pomace, grape	89	0.12	<0.01	<0.01	<0.01	49	
		wine at bottling	89	0.01	<0.01	<0.01	<0.01	49	
		wine at first taste test	89	<0.01	<0.01	<0.01	<0.01	49	

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include climatic conditions; Reference to analytical method and
(g) Study reference
prior to last treatment)
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
Bunch of grapes: between deep-freezing (in the field) and date of last extraction
Processed commodities: between their deep-freezing and date of last extraction

Data Point:	KCA 6.5.3/14
Report Author:	[redacted]
Report Year:	2014
Report Title:	Determination of the residues of AE C656948 and fenhexamid in/on grape and the processed fractions (champagne) after praying of fluopyram SC 500 and fenhexamid WG 50 in the field in France (north)
Report No:	09-3242
Document No:	M-485016-01-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC guidance working document 7029/VI/95 rev. 5 (1997-07-22)
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and Methods

The study included two supervised residue trial with grape, conducted in the field in northern Europe (northern France) in the 2009 season. The purpose of this study was to determine the magnitude of the residues of fluopyram and its metabolites fluopyram-benzamide (AE F148815, alias FLU-benzamide), fluopyram-pyridyl-acetic acid (BCS-AA10139, alias FLU-PAA) and fluopyram-pyridyl-carboxylic acid (AE C657188, alias FLU-PCA) in/on grape (bunch of grapes) and its processed fractions of champagne (after botting and after maturation).

Field part

In field trial 09-2242-01-T1, the treated plot was sprayed once at BBCH growth stage 67 (70% of flowerhoods fallen) with fenhexamid WG 50, a water-dispersible granules formulation and 63 days later once at BBCH 81 (beginning of ripening: berries begin to develop variety-specific colour) with fluopyram SC 500, a suspension concentrate (SC) formulation containing 500 g/L fluopyram. In field trial 09-2240-01-T2, the treated plot was sprayed once at BBCH growth stage 67 with fluopyram SC 500 and 63 days later once at BBCH 81 with fenhexamid WG 50. The application rates were 300 g a.s. fluopyram/ha using a spray volume of 200 L/ha. All treatments were made at the scheduled rates.

Bunch of grape samples were taken 28 days after the last treatment at BBCH 89 (last treatment was done with fluopyram on trial -T1 and with fenhexamid on trial -T2).

Samples for processing were transported to processing laboratory in fresh conditions.

After processing, the processed samples of champagne after bottling were shipped to laboratory logistics in Lyon (France) and then transported at ambient temperature to the laboratory for preparation of the examination samples. The laboratory samples were stored in a freezer at -18°C until preparation of the examination samples.

Processing procedures

The processing study simulated industrial practice at a laboratory scale. The white grape specimens were processed into champagne according to the standard operating procedures in use in the processing laboratory.

The bunches of grapes were pressed to extract “cuvee” and “taille” (must). The obtained must was filled into a steel tank of 50L. After sulfiting with 6 g/hL, the must was racked for 12-16 hours and thereafter clarified.

Alcoholic Fermentation (AF):

The clarified must was vinified in 30L glass carboys with chaptalization of RCM and addition of yeast (Saccharomyces cerevisiae, Trade name: LSA Levuline CHP; 10 g LSA / hL must). Alcoholic fermentation was carried out at 16-18°C for approx. a month under monitoring density and temperature measurements over time.

Malolactic Fermentation (MLF):

At the end of alcoholic fermentation, the wines were racked into 20-L carboys and immediately inoculated with malolactic bacteria (*Oenococcus oeni*, Trade name: Inobacter). At the end of MLF, the wines were racked and 20 mg/L of SO2 was added to each lot, after sampling for oenological analysis and degustation test.

The remaining wine was bottled for residue analysis (champagne, after bottling) or used for “champagnization”.

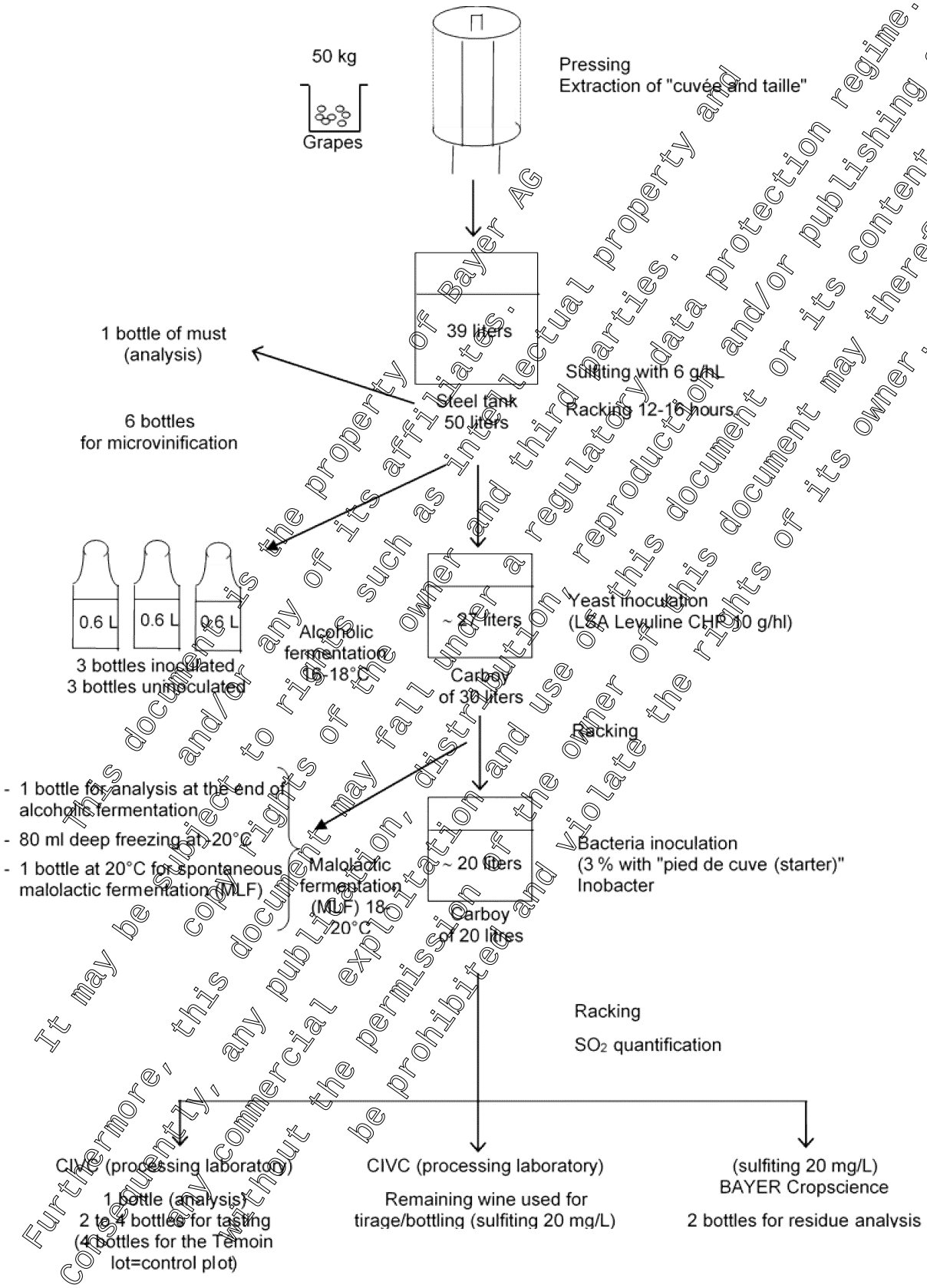
Processing to Champagne:

The processing to champagne was carried out after MLF by racking of wines in 10L carboys and addition of 20 mg/L SO2. The wine in carboys was stored at -4°C for 10 days. Then it was filtrated through 1.2 µm membrane filter. After filtration, the wine was bottled / tiraged with 20 g/L sugar in form of RCM (natural alcoholic content at the end of AF = 12% vol.), 60 mL/hL of riddling adjuvant and 4% of yeast (strain Levuline CHP).

Second fermentation (formation of carbon dioxide; bubbles) was done at 18-20°C under monitoring and analysis at the end of fermentation. Finally, the wines were stored in a wine cellar at 14°C for 12 months. Then riddling, disgorging and tasting were carried out, yielding in samples for residue analysis (champagne, after maturation).

The processes are illustrated in Diagram 6.5.3- 1d.

Diagram 6.5.3- 11: Flow chart of champagne processing



Residue analysis

Residues of fluopyram in/on plant material were determined by HPLC-MS/MS according to BCS method 01207 based on the QuEChERS multi-residue method ([redacted] S., 11/12/2013, M-424756-02-1, see section MCA 4.1.2).

Analytical method BCS 01207 is based on the QuEChERS multi-residue method. Extraction of residues was done with acetonitrile/water (4/1, v/v) by shaking. The residues of fluopyram, FLU-benzamide and FLU-PCA were extracted together but separately to FLU-PAA.
An aliquot of the extracts was taken, and stable-labelled internal standards were added excepted for FLU-PAA where the stable-labelled internal standard was added at the beginning of the extraction procedure.
The final extracts were subjected to LC-MS/MS. The quantification was performed by internal standardization using stable-labelled internal standards in pure solvent for fluopyram, FLU-benzamide and FLU-PCA. The quantification was performed with matrix matched standards with internal standard for FLU-PAA.

The Limit of Quantification (LOQ) was 0.01 mg/kg for fluopyram and its metabolites, all calculated as parent equivalents and for all matrices.

I. Findings

During the course of this study the method performance was checked by concurrent recoveries. Details on concurrent recovery data are shown in Table 6.5.3- 36. No average recovery or RSD value can be calculated. The overall average recoveries (n=2) were within the acceptable range of 70 – 110%.
The levels of residues of fluopyram and its metabolites in the processed samples are summarized in Table 6.5.3- 38. No residues above the LOQ were found in the control samples. The results were not corrected for concurrent recoveries.

The residue level of fluopyram in bunch of grape (RAC for the calculation of the processing factors) ranged from 0.03 mg/kg (trial 09-2242-01-T2) to 0.22 mg/kg (trial 09-2242-01-T1). Residues values of fluopyram ranged from < 0.01 to 0.04 mg/kg in processed commodities.

For all metabolites, residues levels were always 0.01 mg/kg in the RAC and in all processed commodities.

The processing factors (PF) were calculated based on the residue level in the treated processed commodity and residue level in the RAC specimens (bunch of grape). When residues in the RAC and in the processed fractions were both < 0.01 mg/kg a processing factor could not be calculated. The proposed processing factors are summarised below in Table 6.5.3- 37.

The analyses were done after a maximum frozen storage period of 520 days for bunch of grapes and 278 days for processed commodities.

III. Conclusions

Two residue trials were conducted in northern Europe in 2009. Grapes were treated once with fluopyram SC 500 at a growth stage BBCH 67 or BBCH 81 with a dose rate at 1 x 300 g a.s./ha. All applications were at the required rates.

Bunch of grape samples from white grape varieties were processed in order to obtain champagne. The samples (RAC and processed fraction) were analysed for the residues of fluopyram parent compound and its metabolites FLU-benzamide, FLU-PAA and FLU-PCA. The processing study was conducted according to GLP.

The results of the study indicate that residues of fluopyram can decreased from bunch of grape with processing procedures resulting in very low residues in champagne (PF < 0.24 after botting and < 0.26 after maturation).

Assessment and conclusion by applicant:

The study is acceptable.

Table 6.5.3- 36: Recovery results for fluopyram and its metabolite in processed commodities into champagne

Study	Portion analysed	n	Fortification level* (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
09-3242	**Fluopyram**							
	Grape / champagne (after bottling)	1	0.01	88	88	88	-	-
		1	0.10	78	78	78	-	-
		2	Overall		78	88	83	-
	Fluopyram-benzamide							
	Grape / champagne (after bottling)	1	0.01	73	73	73	-	-
		1	0.10	78	78	78	-	-
		2	Overall		73	78	76	-
	Fluopyram- pyridyl-acetic acid							
	Grape / champagne (after bottling)	1	0.01	104	104	104	-	-
		1	0.10	86	86	86	-	-
		2	Overall		86	104	95	-
	Fluopyram-pyridyl-carboxylic acid							
	Grape / champagne (after bottling)	1	0.01	81	81	81	-	-
		1	0.10	83	83	83	-	-
		2	Overall		81	83	82	-

FL: fortification level, RSD = Relative Standard Deviation
Final determination as: fluopyram Residues calculated as: fluopyram

Table 6.5.3- 37: Summary of residues in grape and proposed processing factors for fluopyram and its metabolites for grape. Tested GAP: 1 × 300 g a.s./ha at BBCH 67 (-T2) or 81 (-T1)

Trial	09-3242-01-T1		09-3242-01-T2		Proposed PF (mean if applicable)
Sample material	Residues (mg/kg)	PF	Residues (mg/kg)	PF	
Fluopyram					
Bunch of grapes (RAC)	**0.22**	--	**0.03**	--	--
Champagne after bottling	**0.03**	0.14	<0.01	<0.33	<0.24
Champagne after maturation	**0.04**	0.18	<0.01	<0.33	<0.26
Fluopyram-benzamide*					
Bunch of grapes (RAC)	<0.01	--	<0.01	--	--
Champagne after bottling	<0.01	n.c	<0.01	n.c	n.c
Champagne after maturation	<0.01	n.c	<0.01	n.c	n.c
Fluopyram-pyridyl-carboxylic-acid*					
Bunch of grapes (RAC)	<0.01	--	<0.01	--	--
Champagne after bottling	<0.01	n.c	<0.01	n.c	n.c
Champagne after maturation	<0.01	n.c	<0.01	n.c	n.c
Fluopyram-pyridyl-acetic-acid*					
Bunch of grapes (RAC)	<0.01	--	<0.01	--	--
Champagne after bottling	<0.01	n.c	<0.01	n.c	n.c

Trial	09-3242-01-T1		09-3242-01-T2		Proposed PF (mean if applicable)
Sample material	Residues (mg/kg)	PF	Residues (mg/kg)	PF	
Champagne after maturation	<0.01	n.c	<0.01	n.c	n.c

*expressed as parent compound

PF (processing factor) = residue concentration in processed commodity (mg/kg) / residue concentration in the RAC (mg/kg)

n.c: no processing factor can be calculated (residues <LOQ in RAC and in processed commodity)

< value: in case the residue level in the RAC is ≥ LOQ but residues in the processed commodity are < LOQ, the processing factor is calculated as to be below the worst case value calculated with the LOQ of the processed commodity.

Table 6.5.3- 38: Detailed results of the grape processing study 09-3242

GAP Summary of the 09-3242 trials

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL	(c)	(d)	(f)
	(a)	(b)						
09-3242-01 09-3242-01-T1 France, north 71850 Charnay les Macon/ Bourgogne Europe, North F 2009	Grape Chardonnay; white variety	1) 01.03.2006 2) 28.05.2009 - 06.06.2009 3) 01.09.2009 - 10.09.2009	300	200	150	05.08.2009/63	81	(g) 09-3242 (h) SC (fluopyram 500 g/L) (i) Application method: Spraying, low-volume Other a.s. in product sequence trial: fenhexamid 50 %
09-3242-01 09-3242-01-T2 France, north 71850 Charnay les Macon/ Bourgogne Europe, North F 2009	Grape Chardonnay; white variety	1) 01.03.2006 2) 28.05.2009 - 06.06.2009 3) 01.09.2009 - 10.09.2009	300	200	150	03.06.2009/0	81	(g) 09-3242 (h) SC (fluopyram 500 g/L) (i) Application method: Spraying, low-volume Other a.s. in product sequence trial: fenhexamid 50 %

(a) According to CODEX Classification / Guide (b) Only if relevant (c) Year must be indicated
(d) Either growth stage description or BBCH Code (f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Trial No. / Location / EU zone / Year	Commodity / Variety (a)	Portion analyzed	Growth stage at sampling (d)	Residues (mg/kg) AE C656948 as AE C656948	AE C656948-benzamide as AE C656948	AE C657188 as AE C656948	AE C656948-pyridyl-acetic acid as AE C656948	PHI (days) (e)	Details on trial (f)
09-3242-01 09-3242-01-T1 France, north 71850 Charnay les Macon/ Bourgogne Europe, North F 2009	Grape Chardonnay; white variety	bunch of grapes	89	0.22	<0.01	<0.01	<0.01	28	(g) 09-3242 (j) Analytical method: 01207 (k) LOQ: 0.01 mg/kg (l) Method Validation Data: 01207 (m) Storage: champagne (after bottling and after maturation): 278 days bunch of grapes: 520 days
		champagne after bottling	89	0.03	<0.01	<0.01	<0.01	28	
		champagne after maturation	89	0.04	<0.01	<0.01	<0.01	28	
09-3242-01 09-3242-01-T2 France, north 71850 Charnay les Macon/ Bourgogne Europe, North F 2009	Grape Chardonnay; white variety	bunch of grapes	89	0.03	<0.01	<0.01	<0.01	28	(g) 09-3242 (j) Analytical method: 01207 (k) LOQ: 0.01 mg/kg (l) Method Validation Data: 01207 (m) Storage: champagne (after bottling and after maturation): 278 days bunch of grapes: 520 days
		champagne after bottling	89	<0.01	<0.01	<0.01	<0.01	28	
		champagne after maturation	89	<0.01	<0.01	<0.01	<0.01	28	

(a) According to CODEX Classification Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) Study reference
* prior to last treatment # no data available
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
Bunch of grapes: between deep-freezing (in the field) and date of last extraction
Processed commodities: between their deep-freezing and date of last extraction

Data Point:	KCA 6.5.3/15
Report Author:	[redacted]
Report Year:	2016
Report Title:	Amendment no.1 to final report BCS-0497 - Determination of residues of fluopyram in wine processed from grapes treated with two applications of Luna Privilege 500 SC at rates of 7.5 or 15 g a.i./100 L
Report No:	BCS-0497
Document No:	M-558007-02-1
Guideline(s) followed in study:	APVMA Residue Guideline No. 11 US EPA OCSPP Guideline Number: 860.SUPP
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and Methods

The study included 13 supervised residue trial with grape, conducted in the field in Australia in the 2014 season. The purpose of this study was to determine the magnitude of the residues of fluopyram in/on grape (berries) and wine.

Field part

Two residue studies BCS-0481 and BCS-0482 were conducted in wine grapes in 2014 in Australia to assess residues of fluopyram at six test sites following a number of different treatments of Luna Privilege 500 SC. To enable the transfer of residues from grapes to wine to be assessed, two replicate samples were taken. One was used to determine fluopyram residues in wine grapes and the other was used to process to wine and analyse for fluopyram as part of this study BCS-0497.
Field and application data are reported in reports BCS-0481 and BCS-0482.
For study BCS-0481, residue trials were conducted by Bayer CropScience in wine grapes at two test sites: B481-1 at Yering and B481-2 at Wahgunyah, both in Victoria (Australia).
For study BCS-0482, residue trials were conducted by Eurofins Agrisearch in wine grapes at four test sites: A482-1 at Barossa in South Australia (Merlot wine grapes), A482-2 at Southern Vales in South Australia (Shiraz wine grapes), A482-3 at Riverland in South Australia (Cabernet Sauvignon wine grapes) and A482-4 at Orange in New South Wales (Sauvignon Blanc wine grapes).
Two applications of Luna Privilege 500 SC, a suspension concentrate (SC) formulation containing 500 g/L fluopyram were made at various timings at rates of 7.5 or 15 g/hL fluopyram corresponding to 48-72 or 48-173 g/ha fluopyram, respectively and using a spray volume of 323-971 L/ha.
Treated plot were sprayed twice from 15 days before growth stage BBCH 57 (12 leaves separated, inflorescence well developed, single flowers separated) to growth stage BBCH 61 (10% cap fall). The interval between applications was 5-21 days and the pre-harvest interval was 87-126 days. All treatments were made at the scheduled rates.

Frozen samples of wine grapes were received at the processing laboratory and were stored frozen prior to commencement of the processing phase.

All samples of wine were transported to the analytical test site at ambient temperature.

Processing into wine:

Grape study samples were analysed as whole commodity without caps and stems. Samples were partially defrosted and prepared. An approximately 800 g subsample of grapes was separated for vinification. These samples were processed. Vinification subsamples were thawed overnight then manually crushed and the must added to a one litre glass fermentation vessel to which approximately 50 mg/L sulphur dioxide, as potassium metabisulphite, and 200 mg/L diammonium phosphate solution was added. The must was then inoculated with rehydrated active dried wine yeast, and fermented on skins at 25°C, with daily mixing of the skins and liquid. After seven days, the ferment was pressed twice, each time at approximately 19 Nm for two minutes, with mixing of the pomace between pressings.
The wine was returned to the original vessel and allowed to ferment to dryness (< 1 g/L residual sugar) at 25°C. Once fermentation was established as complete using *Clinistix* strips a further 100 mg/L potassium metabisulphite solution was added and the wines stored at approximately 4°C to cold settle and clarify. After approximately seven days the wines were racked from the gross lees and 2 x 155 mL subsamples were taken and transported to the analytical test site.

Residue analysis

The samples were analysed for fluopyram using multi-residue analytical method ATM-0038-04 (Bayer CropScience Australia, 2011) which was validated on grape, almond (kernel, hull and shell), tomato and banana.

Analytical method ATM-0038-04 was developed for the quantitative determination of fluopyram, tebuconazole, triadimenol, trifloxystrobin and trifloxystrobin acid residues in or on plant matrices from the high protein, high starch, high oil, high water and high acid raw agricultural commodity groups by LC MS/MS.

Extraction of residues was done with acetonitrile:water (4/1, v/v) followed by high-speed blending. After filtration, dilution and addition of internal standard, final extracts were subjected to LC-MS/MS. The quantification for fluopyram was performed by internal standardization using a stable-labelled internal standard in pure solvent.

The Limit of Quantification (LOQ) was 0.01 mg/kg for fluopyram and for all matrices.

This method was locally developed and validated in Australia. As analyses during a processing study are performed to calculate transfer factor and not absolute residue levels this method is considered as fit for purpose. The results are fully comparable and the processing factors for wine are in the same range than the other processing studies conducted on grapes.

I. Findings

During the course of this study, the method performance was validated by concurrent recoveries. Details on concurrent recovery data are shown in Table 6.5.3- 39. The average recoveries were within the acceptable range of 70 – 110%. The RSD values were below 20%.

The levels of residues of fluopyram in the processed samples are summarized in Table 6.5.3- 41. No residues above the LOQ were found in the control samples. The results were not corrected for concurrent recoveries.

The residue level of fluopyram in berry (RAC for the calculation of the processing factors) ranged from <0.01 to 0.12 mg/kg. Residues values of fluopyram ranged from <0.01 to 0.05 mg/kg in wine.

The processing factors (PF) were calculated based on the residue level in the treated processed commodity and residue level in the RAC specimens (berry). When residues in the RAC and in the processed fractions were both <0.01 mg/kg a processing factor could not be calculated. The proposed processing factor is summarised below in Table 6.5.3- 40.

The analyses were done after a maximum frozen storage period of 75 days for berries and 203 days for wine.

III. Conclusions

Thirteen residue trials were conducted in Australia in 2014. Grapes were treated twice with fluopyram SC 500 (Luna Privilege) at a growth stage 15 days before BBCH 57 to BBCH 61 with a dose rate at 2 x 7.5 or 15 g/hL fluopyram. All applications were at the required rates.

Berries samples from grape varieties (red and white) were processed in order to obtain wine. The samples (RAC and processed fraction) were analysed for the residues of fluopyram. The processing study was conducted according to GLP.

The results of the study indicate that residues of fluopyram can decreased from berries with processing procedure resulting in low residues in wine (PF < 1.0). The results are fully comparable and the processing factors for wine are in the same range than the other processing studies conducted on grapes.

Assessment and conclusion by applicant
The study is acceptable.

Table 6.5.3- 39: Recovery results for fluopyram in wine

Study	Portion analysed	n	Fortification level* (mg/kg)	Recovery (%)			
				Individual recoveries	Min	Max	Mean	RSD
BCS-0497	Grape / wine	3	0.01	86, 89, 90	86	90	88	2.4
		3	0.1	99, 100, 100	99	100	100	0.58
		6	Overall		86	100	94	6.8

RSD = Relative Standard Deviation
*Fortified and calculated as fluopyram

Table 6.5.3- 40: Summary of residues in grape and proposed processing factor for grape for fluopyram

Tested GAP: 2 x 7.5 or 2 x 15 g/hL fluopyram from 15 days before BBCH 57 to BBCH 61

Sample material	Berry	Wine	PF	Mean PF
Trial	Residues (mg/kg)	Residues (mg/kg)		
Fluopyram				
A482-1-T3	<0.01	<0.01	n.c	0.37
A482-1-T7	0.01	<0.01	<1.0	
A482-2-T2	0.02	<0.01	<0.50	
A482-2-T3	0.05	0.01	**0.20**	
A482-2-T6	0.05	0.01	**0.33**	
A482-2-T7	0.05	0.03	**0.60**	
A482-3-T6	0.02	<0.01	<0.50	
A482-3-T7	0.03	<0.01	<0.33	
A482-4-T3	0.02	<0.01	<0.50	
A482-4-T6	0.07	0.02	**0.29**	
A482-4-T7	0.12	0.05	**0.42**	
B481-1-T7	<0.01	<0.01	n.c	

Sample material	Berry	Wine	PF	Mean PF
Trial	Residues (mg/kg)	Residues (mg/kg)		
B481-2-T7	<0.01	<0.01	n.c	

PF (processing factor) = residue concentration in processed commodity (mg/kg) / residue concentration in the RAC (mg/kg)
n.c: no processing factor can be calculated (residues <LOQ in RAC and in processed commodity)
< value: in case the residue level in the RAC is ≥ LOQ but residues in the processed commodity are < LOQ, the processing factor is estimated with the LOQ as residue level in the processed commodity.
The mean value is calculated for residues >0.01 mg/kg in wine samples.

Table 6.5.3- 41: Detailed results of the grape processing study BCS-0497

Trial No. / Location / EU zone / Year (a)	Commodity / Variety (a)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting (b)	Application rate per treatment: g a.s./ha	Application rate per treatment: Water (L/ha)	Application rate per treatment: g a.s./hL	Dates of treatment / Application interval (c)	Growth stage at last treatment (d)	Portion analyzed (d)	Growth stage at sampling (d)	Residues (mg/kg) fluopyram as fluopyram (e)	PHI (days) (e)	Details on trial (f)
A482-1 A482-1-T3 Australia Barossa Oceania F 2014	Grape Merlot; red variety	1) 2003	94.21 112.5	634 757	14.86 14.86	22.10.2014/0 06.11.2014/15	55-57	berry wine	commercial harvest	<0.01 <0.01	126 126	(g) BCS-0497 (h) SC (fluopyram 500 g/L) (i) Application method: Spraying (j) Analytical method: ATM-0038.04 (k) LOQ: 0.01 mg/kg (l) Method Validation Data: BCS-0497 (M-558007-02-1) (m) Storage: wine: 175 days berry: 51 days Field and application data taken from report BCS-0482
A482-1 A482-1-T7 Australia Barossa Oceania F 2014	Grape Merlot; red variety	1) 2003	109.8 109.4	739 736	14.86 14.86	06.11.2014/0 20.11.2014/14	61	berry wine	commercial harvest	0.01 <0.01	112 112	(g) BCS-0497 (h) SC (fluopyram 500 g/L) (i) Application method: Spraying (j) Analytical method: ATM-0038.04 (k) LOQ: 0.01 mg/kg (l) Method Validation Data:, BCS-0497 (M-558007-02-1) (m) Storage: wine: 175 days berry: 51 days Field and application data taken from report BCS-0482

Trial No. / Location / EU zone / Year (a)	Commodity / Variety (a)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting (b)	Application rate per treatment: g a.s./ha	Application rate per treatment: Water (L/ha)	Application rate per treatment: g a.s./hL	Dates of treatment / Application interval (c)	Growth stage at last treatment (d)	Portion analyzed (d)	Growth stage at sampling (d)	Residues (mg/kg) fluopyram as fluopyram (e)	PHI (days) (e)	Details on trial (f)
A482-2 A482-2-T2 Australia Southern Vales Oceania F 2014	Grape Shiraz; red variety	1) 1985	48.0 54.2	646 730	7.43 7.43	22.10.2014/0 06.11.2014/15	57	berry wine	commercial harvest	0.02 <0.01	102 102	(g) BCS-0497 (h) SC (fluopyram 500 g/L) (i) Application method: Spraying (j) Analytical method: ATM-0038.04 (k) LOQ: 0.01 mg/kg (l) Method Validation Data: BCS-0497 (M-558007-02-1) (m) Storage: wine: 199 days berry: 75 days
A482-2 A482-2-T3 Australia Southern Vales Oceania F 2014	Grape Shiraz; red variety	1) 1985	96.44 110.7	649 745	14.86 14.86	22.10.2014/0 06.11.2014/15	87	berry wine	commercial harvest	0.05 0.01	102 102	(g) BCS-0497 (h) SC (fluopyram 500 g/L) (i) Application method: Spraying (j) Analytical method: ATM-0038.04 (k) LOQ: 0.01 mg/kg (l) Method Validation Data: BCS-0497 (M-558007-02-1) (m) Storage: wine: 199 days berry: 75 days
A482-2 A482-2-T6 Australia Southern Vales Oceania F 2014	Grape Shiraz; red variety	1) 1985	55.6 62.5	748 836	7.43 7.43	06.11.2014/0 21.11.2014/15	61	berry wine	commercial harvest	0.03 0.01	87 87	(g) BCS-0497 (h) SC (fluopyram 500 g/L) (i) Application method: Spraying (j) Analytical method: ATM-0038.04 (k) LOQ: 0.01 mg/kg (l) Method Validation Data: BCS-0497 (M-558007-02-1) (m) Storage: wine: 199 days berry: 75 days

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg) fluopyram as fluopyram	PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL							
	(a)	(b)				(c)	(d)		(d)	(e)		(f)
A482-2 A482-2-T7 Australia Southern Vales Oceania F 2014	Grape Shiraz; red variety	1) 1985	112.0 104.3	754 702	14.86 14.86	06.11.2014/0 21.11.2014/15	69	berry wine	commercial harvest	0.05 0.03	87 87	(g) BCS-0497 (h) SC (fluopyram 500 g/L) (i) Application method: Spraying (j) Analytical method: ATM-0038.04 (k) LOQ: 0.01 mg/kg (l) Method Validation Data: BCS-0497 (M-558007-02-1) (m) Storage: wine: 199 days berry: 75 days
A482-3 A482-3-T6 Australia Riverland Oceania F 2014	Grape Cabernet Sauvignon; red variety	1) 1990	66.0 72.2	888 971	7.43 7.43	24.10.2014/0 07.11.2014/14	61	berry wine	commercial harvest	0.02 <0.01	102 102	(g) BCS-0497 (h) SC (fluopyram 500 g/L) (i) Application method: Spraying (j) Analytical method: ATM-0038.04 (k) LOQ: 0.01 mg/kg (l) Method Validation Data: BCS-0497 (M-558007-02-1) (m) Storage: wine: 198 days berry: 74 days
A482-3 A482-3-T7 Australia Riverland Oceania F 2014	Grape Cabernet Sauvignon; red variety	1) 1990	134.6 145.3	906 964	14.86 14.86	24.10.2014/0 07.11.2014/14	61	berry wine	commercial harvest	0.03 <0.01	102 102	(g) BCS-0497 (h) SC (fluopyram 500 g/L) (i) Application method: Spraying (j) Analytical method: ATM-0038.04 (k) LOQ: 0.01 mg/kg (l) Method Validation Data: BCS-0497 (M-558007-02-1) (m) Storage: wine: 198 days berry: 74 days

Trial No. / Location / EU zone / Year (a)	Commodity / Variety (a)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting (b)	Application rate per treatment: g a.s./ha	Application rate per treatment: Water (L/ha)	Application rate per treatment: g a.s./hL	Dates of treatment / Application interval (c)	Growth stage at last treatment (d)	Portion analyzed (d)	Growth stage at sampling (d)	Residues (mg/kg) fluopyram as fluopyram (e)	PHI (days) (e)	Details on trial (f)
A482-4 A482-4-T3 Australia 2800 Orange Oceania F 2014	Grape Sauvignon blanc; white variety	1) 2003	74.30 117.7	500 792	14.86 14.86	05.11.2014/0 26.11.2014/21	57	berry wine	commercial harvest	0.02 <0.01	97 97	(g) BCS-0497 (h) SC (fluopyram 500 g/L) (i) Application method: Spraying (j) Analytical method: ATM-0038.04 (k) LOQ: 0.01 mg/kg (l) Method Validation Data: BCS-0497 (M-558007-02-1) (m) Storage: wine: 184 days berry: 60 days
A482-4 A482-4-T6 Australia 2800 Orange Oceania F 2014	Grape Sauvignon blanc; white variety	1) 2003	59.4 60.0	800 808	7.43 7.43	26.11.2014/0 06.12.2014/10	61	berry wine	commercial harvest	0.07 0.02	87 87	(g) BCS-0497 (h) SC (fluopyram 500 g/L) (i) Application method: Spraying (j) Analytical method: ATM-0038.04 (k) LOQ: 0.01 mg/kg (l) Method Validation Data: BCS-0497 (M-558007-02-1) (m) Storage: wine: 184 days berry: 60 days
A482-4 A482-4-T7 Australia 2800 Orange Oceania F 2014	Grape Sauvignon blanc; white variety	1) 2003	118.9 120.1	800 808	14.86 14.86	26.11.2014/0 06.12.2014/10	61	berry wine	commercial harvest	0.12 0.05	87 87	(g) BCS-0497 (h) SC (fluopyram 500 g/L) (i) Application method: Spraying (j) Analytical method: ATM-0038.04 (k) LOQ: 0.01 mg/kg (l) Method Validation Data: BCS-0497 (M-558007-02-1) (m) Storage: wine: 184 days berry: 60 days

Trial No. / Location / EU zone / Year (a)	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting (b)	Application rate per treatment: g a.s./ha	Water (L/ha)	g a.s./hL	Dates of treatment / Application interval (c)	Growth stage at last treatment (d)	Portion analyzed	Growth stage at sampling (d)	Residues (mg/kg) fluopyram as fluopyram (e)	PHI (days) (e)	Details on trial
B481-1 B481-1-T7 Australia 3770 Yering Oceania F 2014	Grape Cabernet Sauvignon	1) 1999	126.6 173.7	852 1169	14.86 14.86	13.11.2014/0 27.11.2014/14	61	berry wine	89	<0.01 <0.01	110 110	(g) BCS-0497 (h) SC (fluopyram 500 g/L) (i) Application method: Spraying (j) Analytical method: ATM-0038.04 (k) LOQ: 0.01 mg/kg (l) Method Validation Data: BCS-0497 (M-558007-02-1) (m) Storage: wine: 170 days berry: 6 days
B481-2 B481-2-T7 Australia 3687 Wahgunyah Oceania F 2014	Grape Chardonnay	1) 1960's	48.00 57.36	323 386	14.86 14.86	26.10.2014/0 31.10.2014/5	81	berry wine	89	<0.01 <0.01	104 104	(g) BCS-0497 (h) SC (fluopyram 500 g/L) (i) Application method: Spraying (j) Analytical method: ATM-0038.04 (k) LOQ: 0.01 mg/kg (l) Method Validation Data: BCS-0497 (M-558007-02-1) (m) Storage: wine: 203 days berry: 13 days

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse
F field
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) Study reference
* prior to last treatment
# no data available
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
Berries: between deep-freezing (in the field) and date of analysis (last samples analysed
Processed commodities: between their deep-freezing and date of analysis (last samples analysed)

Overall summary of calculated processing factors in grape commodities for all submitted studies.

Table 6.5.3- 42: Overall processing factors for fluopyram

Sample material	Trial nb	Mean PF	Studies		Sample material	Overall trial nb	Overall mean PF
Juice	4	0.02	KCA 6.5.3.1-01 & 02		Juice	5	0.1
Pomace, dried	4	6.4			Pomace, dried	4	6.4
Pomace, wet	4	3.2			Pomace, wet	6	3.2
Berry, washed	4	0.6			Pomace, grape	8	3.4
Pomace, grape	4	3.4			Berry, washed	5	0.6
Must	4	0.2			Must	10	0.2
wine at 1st taste test	4	0.2			lees	2	1.19
Wine (wine 1)	4	0.2.			Wine at 1st taste test	8	0.4
Raisin	4	3.7	KCA 6.5.3.1-03		Wine	15	0.4
Raisin waste	4	11.4			Raisin	5	3.4
Raisins	1	2.4			Raisin waste	4	11.4
Grape juice	1	0.5	KCA 6.5.3.1-04		Jelly	1	0.1
Fruit, washed	1	0.8			-	-	-
Jelly	1	0.1			-	-	-
pomace wet	2	3.2	KCA 6.5.3.1-10 & 11		-	-	-
lees	2	1.9			-	-	-
must	2	0.9			-	-	-
Wine bottled	2	0.7			-	-	-
wine at 1st taste test	2	0.8			-	-	-
Must	2	1.0	KCA 6.5.3.1-12		-	-	-
Pomace, grape	2	3.4			-	-	-
Wine at bottling	2	0.34			-	-	-
Wine at 1st taste test	2	0.34			-	-	-
Must	2	1.0	KCA 6.5.3.1-13		-	-	-
Pomace, grape	2	4.5			-	-	-
Wine at bottling	2	0.41			-	-	-
Wine at 1st taste test	2	<0.35			-	-	-
Champagne after bottling	2	<0.24	KCA 6.5.3.1-14		-	-	-
Champagne after maturation	2	<0.26			-	-	-
Wine	5	0.37	KCA 6.5.3.1-15		-	-	-

*Processing factor calculated according to the following equation:

$$PF = \frac{Residue\ concentration\ in\ the\ processed\ product\ [\frac{mg}{kg}]}{Residue\ concentration\ in\ the\ RAC\ [\frac{mg}{kg}]}$$

Estimated values "<xxx" are not considered in overall mean calculation

Data Point:	KCA 6.5.3/16
Report Author:	[redacted]
Report Year:	2007
Report Title:	Determination of the residues of AE C656948 in/on apple fruit and the processed fractions (fruit, washed; raw sauce; sauce; washings; strain rest; juice; pomace, wet; pomace, dried; raw juice; fruit, dried, peel rest; fruit, peeled) after s
Report No:	RA-3605/06
Document No:	M-295672-01-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed Pre-Registration: SANCO/3029/99 Rev. 4, 2000-07-11
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Data Point:	KCA 6.5.3/17
Report Author:	[redacted]
Report Year:	2007
Report Title:	Determination of the residues of AE C656948 in/on apple fruit and the processed fractions (fruit, washed; raw sauce; sauce; washings; strain rest; juice; pomace, wet; pomace, dried; raw juice; fruit, dried, peel rest; fruit, peeled).....
Report No:	RA-3606/06
Document No:	M-295719-01-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed Pre-Registration: SANCO/3029/99 Rev. 4, 2000-07-11
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Test System

Balance studies on processing of apple into juice, sauce and dried fruit were conducted to determine the transfer of fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815, FLU-benzamide), fluopyram-pyridyl-carboxylic-acid (AE C657188, FLU-PCA), fluopyram-pyridyl-acetic-acid (BCS-AA 10139, FLU-PAA), from apple fruits into processed fractions. Trials summary is presented in Table 6.5.3-49.

Juice, sauce and fruit dried were produced from apples obtained from 4 different trials located in Southern and Northern Europe. The processing of apple fruits into fruit (washed), raw sauce, sauce,

washings, strain rest, juice, pomace (wet and dried), raw juice, fruit (dried), peel rest and fruit (peeled) was performed in the Food Processing Laboratory (FPL) of BCS-D-ROCS in Monheim. The washing of apples simulated household processing whereas the apple preparation of juice, sauce, and fruit dried simulated the industrial practice at a laboratory scale.

Sauce production: The apples were washed in lukewarm standing water. The washed apples were cut with a knife into small pieces and were heated after addition of water to 98 - 100°C for 40 min. After blanching the apple pulp was passed through a strainer to separate raw sauce and strain rest. The raw sauce was mixed with sugar, filled in preserving cans and afterwards pasteurized in an autoclave.

Juice production: The deep-frozen apples were washed in lukewarm standing water. The washed fruits were crushed into mash in a cutter and afterwards warmed up to 40°C for 30 - 40 min. The warm apple mash was then pressed in a high-pressure press into raw juice and pomace, wet. The wet pomace was dried in a fan-assisted oven at about 100°C. The raw juice shortly was heated to 80 - 90°C and then cooled down to 45-50°C. The sample was enzymated by adding the enzymes Pectinex XXL and Amylase AG300L. After 20 h cooled storage, the sample was centrifuged and the supernatand was cleared by ultra-filtration. Thereupon, the filtrated juice was pasteurised in a plate heat-exchanger.

Dried fruit production:

The lightly defrosted apples were peeled with a knife and the apple cores were removed. Subsequently the fruits were cut into 5 to 7 mm slices. To prevent enzymatic reactions, oxidation and to save the vitamins a treatment with sulphite solution (w = 0.01) and citric acid solution (w = 0.01) followed. The treated apple slices were thoroughly washed in standing lukewarm water and the washed apple slices were dried in a fan-assisted oven at 80°C.

The processes are described in detail in Diagram 6.5.3- 12 to Diagram 6.5.3- 14.

Diagram 6.5.3- 12: Processing of Apple Fruits into Fruit, washed; Washing Water; Raw Sauce; Sauce and Strain Rest, wet

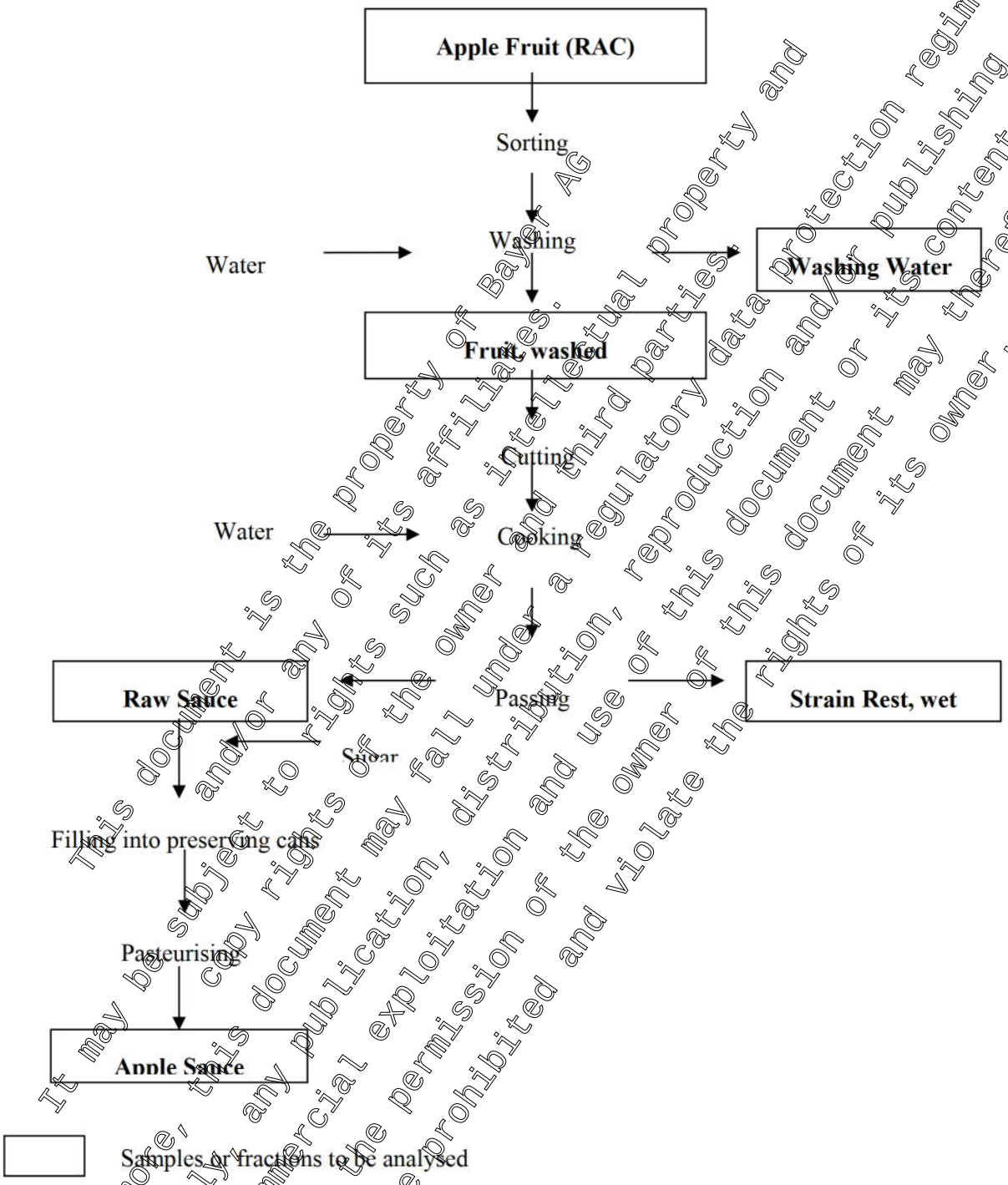


Diagram 6.5.3- 13: Processing of Apple Fruits into Raw Juice, Juice, Pomace, wet and Pomace, dried

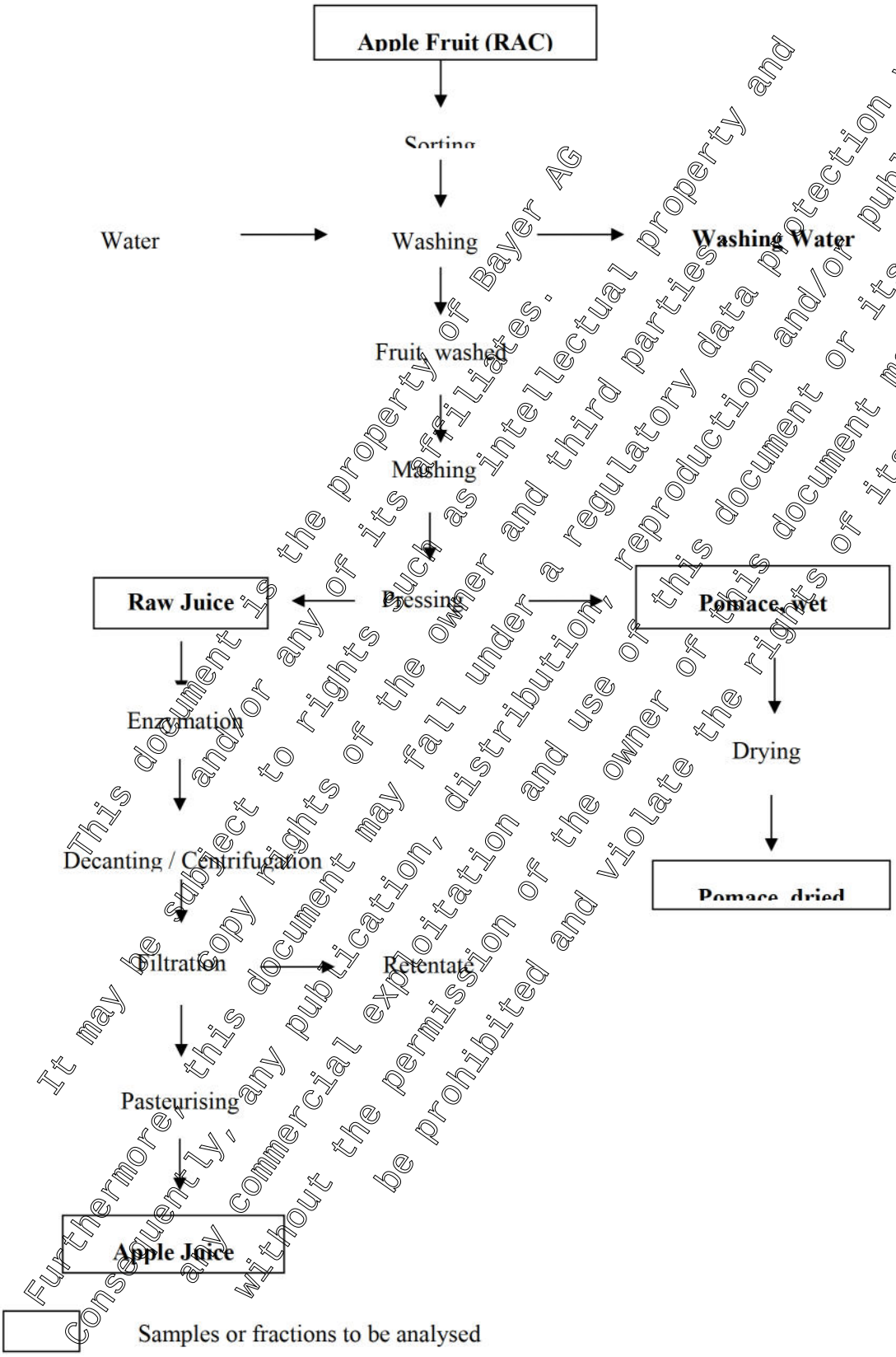
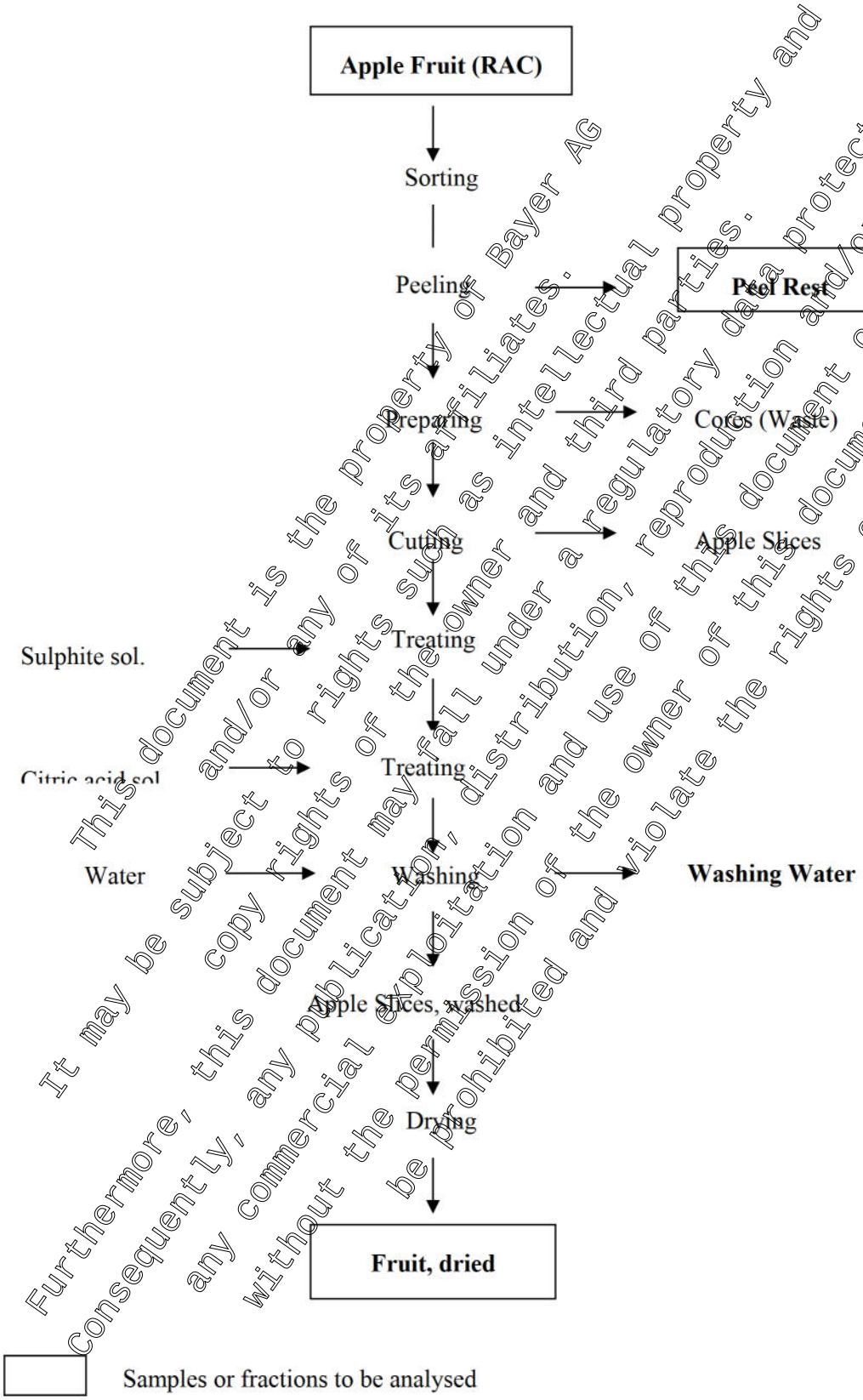


Diagram 6.5.3- 14: Processing of Apple Fruits into Fruit, peeled; Peel Rest and Fruit, dried



Residue analysis

Residues of fluopyram and its metabolites were determined by LC-MS/MS according to method 00984/M001 ([redacted], 2007, M-295145-03-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues were extracted from 10 g of sample material by two successive extractions using a high-speed blender with a mixture of acetonitrile:water (4:1; v:v).

Subsequently, the raw extracts were diluted 10-fold by adding internal standard solutions:

- One dilution and an additional clean-up step were performed under acidic conditions for determination of FLU-PCA
- Another dilution was performed under basic conditions for determination of fluopyram, FLU-benzamide and FLU-PAA

Residues were quantified by reversed-phase chromatography coupled with tandem mass spectrometry (MS/MS) with electrospray ionisation. One injection in positive electrospray ionisation allowed the determination of fluopyram, FLU-benzamide and FLU-PAA. Another injection in negative electrospray ionisation allowed the determination of FLU-PCA under different conditions.

The quantitation was carried out by internal standardization using stable-labelled internal standards in pure solvent.

The Limit of Quantification (LOQ) was 0.01 mg/kg for fluopyram and its metabolites, all calculated as parent equivalents and for all matrices.

Recovery findings in apple fruits and in processed commodities were within guideline requirements (70-110%, RSD <20%). Details of the recovery results are summarised in

Table 6.5.3- **43** to Table 6.5.3- 46. No residues above the LOQ of 0.01 mg/kg could be detected in any of the corresponding control samples. The storage period of deep frozen processed samples ranged between 215 and 250 days.

Table 6.5.3- 43: Recovery results for fluopyram in apple fruits and processed commodities of apple sauce, apple juice and fruit dried production

Report No.	Sample Material	Matrices covered	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
Analyte: fluopyram (AE C656948)							
RA-2605/06 RA-2606/06 RA-3605/06 RA-3606/06	apple fruit	apple fruit apple fruit, washed apple fruit, peeled	0.01	102; 95; 117; 117	108	10.3	0.01
			0.10	103; 96	100	-	
			1.0	93; 93	93	-	
			Overall Recovery (n = 8)		**102**	**9.8**	
	apple sauce	apple raw sauce apple sauce	0.01	113	-	-	0.01
			0.10	99	-	-	
			Overall Recovery (n = 2)		**106**	**-**	
	apple juice	apple washings apple juice apple raw juice	0.01	101	-	-	0.01
			1.0	103	-	-	
			Overall Recovery (n = 2)		**102**	**-**	
	pomace, dried	apple strain rest apple pomace, wet apple pomace, dried apple peel rest	0.01	109	-	-	0.01
			1.0	103	-	-	
			Overall Recovery (n = 2)		**105**	**-**	
	apple fruit dried	apple fruit, dried	0.01	115	-	-	0.01
			1.0	95	-	-	
			Overall Recovery (n = 2)		**105**	**-**	

FL: fortification level, RSD: Relative Standard Deviation
* some RSDs were not calculated as there were only two individual recoveries given
Final determination as: fluopyram Residues calculated as: fluopyram

Table 6.5.3- 44: Recovery results for fluopyram-pyridyl-acetic acid in apple fruits and processed commodities of apple sauce, apple juice and fruit dried production

Report No.	Sample Material	Matrices covered	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
Analyte: fluopyram-pyridyl-acetic acid (BCS AA10139)							
RA-2605/06 RA-2606/06 RA-3605/06 RA-3606/06	apple fruit	apple fruit apple fruit, washed apple fruit, peeled	0.01	82; 83; 99; 98	91	10.2	0.01
			0.10	94; 90	92	-	
			1.0	90; 80	85	-	
			Overall Recovery (n = 8)		**90**	**8.1**	
	apple sauce	apple raw sauce apple sauce	0.01	84	-	-	0.01
			0.10	84	-	-	
			Overall Recovery (n = 2)		**84**	**-**	
	apple juice	apple washings apple juice apple raw juice	0.01	86	-	-	0.01
			1.0	83	-	-	
			Overall Recovery (n = 2)		**85**	**-**	
	pomace, dried	apple strain rest apple pomace, wet apple pomace, dried apple peel rest	0.01	116	-	-	0.01
			1.0	84	-	-	
			Overall Recovery (n = 2)		**100**	**-**	
	apple fruit dried	apple fruit, dried	0.01	112	-	-	0.01
			1.0	90	-	-	
			Overall Recovery (n = 2)		**101**	**-**	

FL: fortification level, RSD = Relative Standard Deviation
* some RSDs were not calculated as there were only two individual recoveries given
Final determination as: FLU-PAA Residues calculated as: fluopyram

Table 6.5.3- 45: Recovery results for fluopyram-benzamide in apple fruits and processed commodities of apple sauce, apple juice and fruit dried production

Report No.	Sample Material	Matrices covered	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
Analyte: fluopyram-benzamide (AE F148815)							
RA-2605/06 RA-2606/06 RA-3605/06 RA-3606/06	apple fruit	apple fruit apple fruit, washed apple fruit, peeled	0.01	94; 82; 97; 99	93	8.2	0.01
			0.10	89; 88	89	0.8	
			1.0	100; 87	94	-	
			Overall Recovery (n = 8)		**92**	**6.9**	
	apple sauce	apple raw sauce apple sauce	0.01	90	-	-	0.01
			0.10	87	-	-	
			Overall Recovery (n = 2)		**89**	**-**	
	apple juice	apple washings apple juice apple raw juice	0.01	100	-	-	0.01
			1.0	98	-	-	
			Overall Recovery (n = 2)		**99**	**-**	
	pomace, dried	apple strain rest apple pomace, wet apple pomace, dried apple peel rest	0.01	96	-	-	0.01
			1.0	96	-	-	
			Overall Recovery (n = 2)		**96**	**-**	
	apple fruit dried	apple fruit, dried	0.01	97	-	-	0.01
			1.0	93	-	-	
			Overall Recovery (n = 2)		**95**	**-**	

FL: fortification level, RSD: Relative Standard Deviation
* some RSDs were not calculated as there were only two individual recoveries given
Final determination as: FDU-benzamide Residues calculated as: fluopyram

Table 6.5.3- 46: Recovery results for fluopyram-pyridyl-carboxylic acid in apple fruits and processed commodities of apple sauce, apple juice and fruit dried production

Report No.	Sample Material	Matrices covered	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
Analyte: fluopyram-pyridyl-carboxylic acid (AE C657188)							
RA-2605/06	apple fruit	apple fruit apple fruit, washed apple fruit, peeled	0.01	89; 81; 92; 101	93	13.6	0.01
			0.10	100; 107	102	-	
			1.0	92; 98	95	-	
			Overall Recovery (n = 8)		**95**	**9.9**	
	apple sauce	apple raw sauce apple sauce	0.01	99	-	-	0.01
			0.10	93	-	-	
			Overall Recovery (n = 3)		**96**	-	
RA-2606/06 RA-3605/06	apple juice	apple washings apple juice apple raw juice	0.01	94	-	-	0.01
			1.0	103	-	-	
			Overall Recovery (n = 2)		**99**	-	
RA-3606/06	pomace, dried	apple strain rest apple pomace, wet apple pomace, dried apple peel rest	0.01	106	-	-	0.01
			1.0	103	-	-	
			Overall Recovery (n = 2)		**105**	-	
	apple fruit dried	apple fruit dried	0.01	87	-	-	0.01
			1.0	96	-	-	
			Overall Recovery (n = 2)		**92**	-	

FL: fortification level, RSD - Relative Standard Deviation
* some RSDs were not calculated as there were only two individual recoveries given
Final determination as FLU-PCA Residues calculated as: fluopyram

Findings:
In apple fruits used for processing, residues of fluopyram were between 0.08 and 0.21 mg/kg. A mean of 34 % of the absolute residue was recovered in the washing water. A major part counting for 69 % of the initial absolute residue remained in the washed fruit.
During apple sauce production 28 % of the absolute residue retained in the strain rest whereas 55 % were transferred into raw sauce, leading to values between 0.04 mg/kg and 0.05 mg/kg in the final product apple sauce.
With apple juice production a mean of 61 % and 51 % of the absolute residue was recovered in wet pomace and dried pomace respectively. A minor part counting for only 16 % of the initial absolute residue could be found in raw juice, leading to values below the LOQ of 0.01 mg/kg in the final product juice.
During apple dried fruit production a mean of 67 % of the absolute residue was recovered in the peel rest whereas 20 % of the initial absolute residue remained in the peeled fruit leading to values between 0.07 mg/kg and 0.13 mg/kg in the final product dried apple fruit.

Conclusion

Residue values of fluopyram in apple fruits and processed commodities of juice, sauce and dried fruit production are summarized in Table 6.5.3- 47.

Assessment and conclusion by applicant:

The study is acceptable.

Table 6.5.3- 47: Residues of fluopyram and metabolites in apple fruits and processed commodities of apple sauce, apple juice and fruit dried production

Country Study No. Trial No.	Crop Portion analysed	DALT (days)	Residues (mg/kg) expressed as fluopyram equivalents			
			fluopyram	pyridyl-acetic acid	benzamide	pyridyl-carboxylic acid
Belgium RA-3605/06 R 2006 0396/7	fruit	3	0.11	< 0.01	< 0.01	< 0.01
	fruit, washed	3	0.06	< 0.01	< 0.01	< 0.01
	raw sauce	3	0.06	< 0.01	< 0.01	< 0.01
	sauce	3	0.04	< 0.01	< 0.01	< 0.01
	washings	3	0.01	< 0.01	< 0.01	< 0.01
	strain rest	3	0.70	< 0.01	< 0.01	< 0.01
	juice	3	0.01	< 0.01	< 0.01	< 0.01
	pomace, wet	3	0.19	< 0.01	< 0.01	< 0.01
	pomace, dried	3	0.60	< 0.01	< 0.01	< 0.01
	raw juice	3	0.02	< 0.01	< 0.01	< 0.01
	fruit, dried	3	0.07	< 0.01	< 0.01	< 0.01
	peel rest	3	0.60	< 0.01	< 0.01	< 0.01
	fruit, peeled	3	0.02	< 0.01	< 0.01	< 0.01
United Kingdom RA-3605/06 R 2006 0413/0	fruit	3	0.21	< 0.01	< 0.01	< 0.01
	fruit, washed	3	0.09	< 0.01	< 0.01	< 0.01
	raw sauce	3	0.08	< 0.01	< 0.01	< 0.01
	sauce	3	0.05	< 0.01	< 0.01	< 0.01
	washings	3	0.04	< 0.01	< 0.01	< 0.01
	strain rest	3	1.0	< 0.01	< 0.01	< 0.01
	juice	3	0.01	< 0.01	< 0.01	< 0.01
	pomace, wet	3	0.26	< 0.01	< 0.01	< 0.01
	pomace, dried	3	1.2	< 0.01	< 0.01	< 0.01
	raw juice	3	0.03	< 0.01	< 0.01	< 0.01
	fruit, dried	3	0.13	< 0.01	< 0.01	< 0.01
	peel rest	3	1.6	< 0.01	< 0.01	< 0.01
	fruit, peeled	3	0.05	< 0.01	< 0.01	< 0.01
Southern France RA-3606/06 R 2006 0399/1	fruit	3	0.08	< 0.01	< 0.01	< 0.01
	fruit, washed	3	0.11	< 0.01	0.03	< 0.01
	raw sauce	3	0.07	< 0.01	0.02	< 0.01
	sauce	3	0.05	< 0.01	0.02	< 0.01
	washings	3	< 0.01	< 0.01	< 0.01	< 0.01
	strain rest	3	0.75	< 0.01	0.03	< 0.01
	juice	3	< 0.01	< 0.01	0.01	< 0.01
	pomace, wet	3	0.33	< 0.01	0.02	< 0.01
	pomace, dried	3	0.95	< 0.01	0.09	< 0.01
	raw juice	3	0.03	< 0.01	0.02	< 0.01
	fruit, dried	3	0.07	< 0.01	0.02	< 0.01
	peel rest	3	0.64	< 0.01	0.09	< 0.01
	fruit, peeled	3	0.02	< 0.01	0.01	< 0.01

Country Study No. Trial No.	Crop Portion analysed	DALT (days)	Residues (mg/kg) expressed as fluopyram equivalents			
			fluopyram	pyridyl-acetic acid	benzamide	pyridyl-carboxylic acid
Italy RA-3606/06 R 2006 0414/9	fruit	3	0.11	< 0.01	< 0.01	< 0.01
	fruit, washed	3	0.04	< 0.01	< 0.01	< 0.01
	raw sauce	3	0.05	< 0.01	< 0.01	< 0.01
	sauce	3	0.04	< 0.01	< 0.01	< 0.01
	washings	3	0.03	< 0.01	< 0.01	< 0.01
	strain rest	3	0.75	< 0.01	< 0.01	< 0.01
	juice	3	< 0.01	< 0.01	< 0.01	< 0.01
	pomace, wet	3	0.27	< 0.01	< 0.01	< 0.01
	pomace, dried	3	0.84	< 0.01	< 0.01	< 0.01
	raw juice	3	0.03	< 0.01	< 0.01	< 0.01
	fruit, dried	3	0.10	< 0.01	< 0.01	< 0.01
	peel rest	3	0.63	< 0.01	< 0.01	< 0.01
	fruit, peeled	3	0.03	< 0.01	< 0.01	< 0.01

DALT = days after last application

Average factors of 0.1, 0.43 and 0.75 were calculated for the transfer of fluopyram from apple fruits into juice, sauce and dried fruit respectively. The transfer factors of fluopyram from apple raw agricultural commodity into juice, sauce, dried fruit and the processed by-products are summarised in Table 6.5.3-48.

Table 6.5.3- 48: Transfer factors for the residue of fluopyram in processed commodities of apple juice, sauce and dried fruit production

Sample material	Transfer factors for residues of fluopyram				
	RA-3605/06		RA-3606/06		Mean
	R 2006 0396/7 Belgium	R 2006 0413/0 United Kingdom	R 2006 0399/1 France South	R 2006 0414/9 Italy	
fruit, washed	0.5	0.4	1.5	0.4	0.70
washing water	0.1	0.2	0.1	0.2	0.15
raw sauce	0.5	0.4	0.9	0.4	0.55
sauce	0.4	0.3	0.7	0.3	0.43
strain rest, wet	6.7	4.8	10	6.6	7.03
raw juice	0.2	0.1	0.4	0.2	0.23
juice	0.1*	0.1*	0.1*	0.1*	0.10
pomace, wet	1.7	1.2	4.3	2.3	2.38
pomace, dried	5.3	5.4	13	7.4	7.78
fruit peeled	0.2	0.2	0.3	0.2	0.23
peel rest	5.3	7.4	8.6	5.5	6.70
fruit, dried	0.7	0.6	0.9	0.8	0.75

Mean values calculated based on rounded results

* For calculation of the transfer factor the residue in the processed product was set at the LOQ (0.01 mg/kg)

Table 6.5.3- 49: Results of processing trials conducted with fluopyram on apple

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)				PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					fluopyram as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-PAA as AE C656948		
	(a)	(b)				(c)								(d)	(f)
R 2006 0396/7 0396-06 Belgium B-6220 Fleurus (Hainaut) Europe, North F 2006	Apple Jonagored	1) 01.03.1991 2) 25.04.2006 - 30.05.2006 3) 01.09.2006 - 30.09.2006	125.0 125.0 125.0 125.0	1000 1000 1000 1000	12.50 12.50 12.50 12.50	09.08.2006/0 16.08.2006/7 23.08.2006/7 30.08.2006/7	87	Fruit	87 87 89 89	0.06 0.14 0.11 0.11	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01	0* 0 1 3	(g) RA-2605/06 (h) SC 500 (i) Application method: Spraying (j) Analytical method: 00984/M001 (k) LOQ:0.01 mg/kg (m) Storage: whole fruit, washed: 249 days washings: 249 days strain rest: 249 days sauce: 249 days raw sauce: 249 days raw juice: 249 days pomace, wet: 249 days pomace, dried: 249 days peel rest: 249 days juice: 249 days fruit, peeled: 249 days fruit, dried: 249 days fruit: 247 days
									89	0.10	<0.01	<0.01	<0.01	7	
									89	0.09	<0.01	<0.01	<0.01	10	
								whole fruit, washed	89	0.06	<0.01	<0.01	<0.01	3	
								raw sauce	89	0.06	<0.01	<0.01	<0.01	3	
								sauce	89	0.04	<0.01	<0.01	<0.01	3	
								washings	89	0.01	<0.01	<0.01	<0.01	3	
								strain rest	89	0.76	<0.01	<0.01	<0.01	3	
								juice	89	<0.01	<0.01	<0.01	<0.01	3	
								pomace, wet	89	0.19	<0.01	<0.01	<0.01	3	
								pomace, dried	89	0.60	0.01	<0.01	<0.01	3	
								raw juice	89	0.02	<0.01	0.01	0.01	3	
								fruit, dried	89	0.07	<0.01	<0.01	<0.01	3	
								peel rest	89	0.60	0.01	<0.01	<0.01	3	

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)				PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					fluopyram as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-PAA as AE C656948		
	(a)	(b)				(c)								(d)	(f)
								fruit peeled	89	0.02	<0.01	0.01	0.01	3	
R 2006 0413/0 0413-06 United Kingdom GB-SG8 8SS Royston (Herefordshire) Europe, North F, 2006	Apple Jonathon	1) 01.12.1994 2) 05.04.2006 - 30.04.2006 3) 01.10.2006 - 25.10.2006	125.0 125.0 125.0 125.0	500 500 500 500	25.00 25.00 25.00 25.00	11.09.2006/0 18.09.2006/7 26.09.2006/8 03.10.2006/7	87	fruit	87	0.09	<0.01	<0.01	<0.01	0*	(g) RA-2605/06 (h) SC 500 (i) Application method: Spraying (j) Analytical method: 00984/M001 (k) LOQ:0.01 mg/kg (m) Storage: whole fruit, washed: 215 days washings: 215 days strain rest: 215 days sauce: 215 days raw sauce: 215 days raw juice: 215 days pomace, wet: 215 days pomace, dried: 215 days peel rest: 215 days juice: 215 days fruit, peeled: 215 days fruit, dried: 215 days fruit: 213 days
									87	0.05	<0.01	<0.01	<0.01	0	
									87	0.24	<0.01	<0.01	<0.01	1	
									87	0.21	<0.01	<0.01	<0.01	3	
									87	0.13	<0.01	<0.01	<0.01	8	
									87	0.10	<0.01	<0.01	<0.01	10	
								whole fruit, washed	87	0.09	<0.01	<0.01	<0.01	3	
								raw sauce	87	0.08	<0.01	<0.01	<0.01	3	
								sauce	87	0.05	<0.01	<0.01	<0.01	3	
								washings	87	0.04	<0.01	<0.01	<0.01	3	
								strain rest	87	1.0	<0.01	<0.01	<0.01	3	
								juice	87	<0.01	<0.01	<0.01	<0.01	3	
								pomace, wet	87	0.26	<0.01	<0.01	<0.01	3	
								pomace, dried	87	1.2	<0.01	<0.01	<0.01	3	
								raw juice	87	0.03	<0.01	<0.01	<0.01	3	
								fruit, dried	87	0.13	<0.01	<0.01	<0.01	3	
								peel rest	87	1.6	<0.01	<0.01	<0.01	3	

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)				PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					fluopyram as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-PAA as AE C656948		
	(a)	(b)				(c)								(d)	(f)
								fruit peeled	87	0.05	<0.01	<0.01	<0.01	3	
R 2006 0399/1 0399-06 France, south F-82370 Reyniès (Midi-Pyrenees) Europe, South F 2006	Apple Granny	1) 01.01.2003 2) 01.04.2006 - 25.04.2006 3) 05.10.2006 - 10.10.2006	125.0 125.0 125.0 125.0	1500 1500 1500 1500	8.50 8.50 8.50 8.50	19.09.2006/0 26.09.2006/7 03.10.2006/7 10.10.2006/7	87	Fruit	87 87 87 89 89 89	0.06 0.03 0.13 0.08 0.07 0.06	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	0* 0 1 3 7 10	(g) RA-2605/06 (h) SC 500 (i) Application method: Spraying (j) Analytical method: 00984/M001 (k) LOQ:0.01 mg/kg (m) Storage: whole fruit, washed: 214 days washings: 214 days strain rest: 214 days sauce: 214 days raw sauce: 214 days raw juice: 214 days pomace, wet: 214 days pomace, dried: 214 days peel rest: 214 days juice: 214 days fruit, peeled: 214 days fruit, dried: 214 days fruit: 258 days
								whole fruit, washed	89	0.11	0.03	<0.01	<0.01	3	
								raw sauce	89	0.07	0.02	<0.01	<0.01	3	
								sauce	89	0.05	0.02	<0.01	<0.01	3	
								washings	89	<0.01	<0.01	<0.01	<0.01	3	
								strain rest	89	0.75	0.03	<0.01	<0.01	3	
								juice	89	<0.01	0.01	<0.01	<0.01	3	
								pomace, wet	89	0.33	0.02	<0.01	<0.01	3	
								pomace, dried	89	0.95	0.09	<0.01	<0.01	3	
								raw juice	89	0.03	0.02	<0.01	<0.01	3	
								fruit, dried	89	0.07	0.02	<0.01	<0.01	3	
								peel rest	89	0.64	0.09	<0.01	<0.01	3	

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)				PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					fluopyram as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-PAA as AE C656948		
	(a)	(b)				(c)								(d)	(f)
								fruit peeled	89	0.02	0.01	<0.01	<0.01	3	
R 2006 0414/9 0414-06 Italy I-37059 Zevio (Veneto) Europe, South F, 2006	Apple Golden Rainders	1) 1998 2) 11.04.2006 - 25.04.2006 3) 10.09.2006 - 30.09.2006	125.0 125.0 125.0 125.0	1250 1250 1250 1250	10.00 10.00 10.00 10.00	18.08.2006/0 25.08.2006/7 01.09.2006/7 08.09.2006/7	87	Fruit	87	0.09	<0.01	<0.01	<0.01	0*	(g) RA-2605/06 (h) SC 500 (i) Application method: Spraying (j) Analytical method: 00984/M001 (k) LOQ:0.01 mg/kg (m) Storage: whole fruit, washed: 246 days washings: 246 days strain rest: 246 days sauce: 246 days raw sauce: 246 days raw juice: 246 days pomace, wet: 246 days pomace, dried: 246 days peel rest: 246 days juice: 246 days fruit, peeled: 246 days fruit, dried: 246 days fruit: 290 days
									87	0.20	0.01	<0.01	<0.01	0	
									87	0.10	<0.01	<0.01	<0.01	1	
									87	0.11	<0.01	<0.01	<0.01	3	
									87	0.06	<0.01	<0.01	<0.01	7	
									87	0.05	0.01	<0.01	<0.01	10	
								whole fruit washed	87	0.04	<0.01	<0.01	<0.01	3	
								raw sauce	87	0.05	<0.01	<0.01	<0.01	3	
								sauce	87	0.04	<0.01	<0.01	<0.01	3	
								washings	87	0.03	<0.01	<0.01	<0.01	3	
								strain rest	87	0.75	<0.01	<0.01	<0.01	3	
								juice	87	<0.01	<0.01	<0.01	<0.01	3	
								pomace, wet	87	0.27	<0.01	<0.01	<0.01	3	
								pomace, dried	87	0.84	0.01	<0.01	<0.01	3	
								raw juice	87	0.03	<0.01	<0.01	<0.01	3	
								fruit, dried	87	0.10	<0.01	<0.01	<0.01	3	
								peel rest	87	0.63	0.01	0.01	<0.01	3	

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)				PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL					fluopyram as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-PAA as AE C656948		
	(a)	(b)				(c)								(d)	(f)
								fruit peeled	87	0.03	<0.01	<0.01	<0.01	3	

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI, undefine)
(f) Remarks may include: Climatic conditions, reference to analytical method and information which metabolites are included
(g) Study reference
* prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Data Point:	KCA 6.5.3/18
Report Author:	[redacted]
Report Year:	2007
Report Title:	AE C656948 500 SC - Magnitude of the residue on apple processed commodities
Report No:	RAGMP033
Document No:	M-299547-01-1
Guideline(s) followed in study:	EPA Ref.: OPPTS 860.1520; PMRA Ref.: DACO 7.4.5
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Material and Methods

An apple processing trial was conducted to measure the magnitude of fluopyram [N [2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-(trifluoromethyl)benzamide] residue in apples and apple processed commodities following exaggerated rate applications of AE C656948 500 SC to tomatoes. The test substance, fluoipyram 500 SC, is a suspension concentrate formulation containing 500 g ai/L.

Information on the trial site and the actual use pattern is provided in Table 6.5.3- 50. Two airblast applications were made to apple trees in the treated plot using equipment customarily used to apply pesticides in this manner to apples. The first application was made to the apples at the beginning of ripening (BBCH 81), with a 6-day interval between the two applications. The test substance applications were applied at a target rate of 1.115 lb ai/A/application (1250 g ai/ha/application) in a target spray volume of 35-70 GPA (327-655 L/ha). The achieved total seasonal rate was 2.24 lb ai/A (2511 g ai/ha). This rate is equivalent to five times (5X) the total maximum proposed label rate for a single growing season.

Table 6.5.3- 50: Study Use Pattern for AE C656948 on Apples.

Location: City, State, NAFTA Region	Trial No.	Year	End-Use Product	Application							Tank Mix Adjuvant
				Method	Timing[a]	Plot Name	Rate lb ai/A (kg ai/ha)	RTI[b] (days)	Spray Volume GPA (L/ha)	Total Rate lb ai/A (kg ai/ha)	
North Rose, New York Region 1	GM01-06P	2006	500 SC	Airblast	Beginning of ripening	TRT 5X	1.125 (1.261)	0	65.3 (610)	2.24 (2.511)	None
				Airblast	Advanced Ripening		1.115 (1.250)	6	65.2 (610)		

[a] Timing = First Application occurred 11 days prior to harvest
[b] RTI = Retreatment Interval

One control bulk apple sample and one treated bulk apple sample were collected at a 5-day pre-harvest interval (PHI) and shipped via overnight carrier to the processing facility. Prior to processing, random sub-samples of the control and treated bulk apple samples were collected for analysis, and the remainder of the apple samples were used to generate the required processed commodities of apple wet pomace and juice. In addition, samples of washed fruit, peeled fruit, applesauce, and dried fruit were generated for use in the dietary risk assessment for fluopyram. Processing was performed using procedures which simulated commercial processing practices. The resultant apple samples and processed commodities were analyzed to determine fluopyram residue.

The residue data for grape raw agricultural commodity and grape processed commodities were obtained using the analytical method for determining the fluopyram residue in plant 00984 ([redacted] 05/02/2007, M-283301-01-1, see MCA section 4.1.2) with modifications ([redacted] 2007, No. GM-001-P07-01, M-297568-01-2)

Briefly, a 5-g aliquot of the crop matrix was extracted by blending twice, each time with a mixture of acetonitrile/water (4/1; *v/v*). Each extract was filtered, the filtrates were combined, and an isotopically labelled internal standard was added. An aliquot of the mixture was loaded on a small C-18 solid phase extraction (SPE) cartridge and eluted into an HPLC vial with 0.1 % (aq.) acetic acid for analysis by LC/MS/MS.

The quantitation was carried out using isotopically labelled internal standards. The Limit of Quantitation (LOQ), defined as the lowest validated fortification level, was 0.01 mg/kg in all tested matrices.

Recovery of fluopyram from apples and apple processed commodities was measured concurrently with the set of samples to verify method performance. The validation and concurrent recovery data for fluopyram residue are summarized in Table 6.5.3- 51. The data demonstrate acceptable method performance during sample analysis.

Table 6.5.3- 51: Summary of concurrent recoveries of fluopyram from apple fresh fruit and apple processed commodities

Matrix [a]	Fortification Level (mg/kg)	Sample Size (n)	Recoveries (%)	Mean Recovery ± Standard Deviation
Fresh Fruit	0.01	7	95, 98, 104, 87, 92, 91, 93	94 ± 5
	2.00	3	110, 108, 106	108 ± 2
Wet Pomace	0.01	4	92, 90, 82, 78	86 ± 7
	4.00	3	96, 99, 97	97 ± 2
Juice	0.01	3	95, 92, 89	92 ± 3
	1.00	3	107, 110, 108	108 ± 2
Washed Fruit	0.01	5	99, 99, 94, 79, 77	90 ± 11
Peeled Fruit	0.01	4	95, 98, 74, 70	84 ± 14
Apple Sauce	0.01	3	90, 87, 91	89 ± 2
	0.10	3	103, 92, 101	99 ± 6
Dried Fruit	0.01	3	93, 82, 88	88 ± 6
	0.10	3	87, 90, 94	90 ± 4

Final determination as: fluopyram Residues calculated as: fluopyram

The apple fresh fruit and the apple processed commodities analyzed in this study were held in frozen

storage for a maximum of 11 months prior to extraction, see Table 6.5.3- 52.

Table 6.5.3- 52: Summary of Storage Conditions for apple RAC and Apple Processed Commodities

Residue Component(s)	Matrix (RAC)	Storage Temperature (°C)[a]	Actual Study Duration (days)[b]	Limit of Demonstrated Storage Stability (days)[c]
AE C656948	Apple Fruit	<-15˚C	245	See Section IIA Point 6.1
AE C656948	Processed Commodities	<-15˚C	232-234	

[a] Temperature is the sample storage temperature from sample receipt at the analytical facility through the last extraction..

[b] Actual storage duration = number of days between harvest and first extract date for RAC sample or number of days between processing and first extract date for processed commodity.

[c] Demonstrated freezer stability in diverse crops is representative of the freezer stability of AE C656948 residues to be expected for the apple and apple processed commodities from this study

Findings

All control interferences were less than the LOQ (<0.01 mg/kg) for apple fresh fruit and all the apple processed commodities.

The fluopyram residue data for apple fresh fruit and the apple processed commodities of apple wet pomace, juice, washed fruit, peeled fruit, and dried fruit are summarized in Table 6.5.3- 53.

Table 6.5.3- 53: Apple fruit and apple processed commodities residue data from the processing study with fluopyram.

RAC	Processed Commodity	Total Rate lb ai/A (kg ai/ha)	fluopyram Residue (mg/kg)	Processing Factor[a]
Apple	NA[b]	2.24 (2.51)	0.95 0.92 0.92 Avg: 0.93	NA
	Wet Pomace		2.22 2.11 2.09 Avg: 2.14	2.3 X
	Juice		0.43 0.37 0.44 Avg: 0.41	<1.0 (0.4X)
	Washed Fruit		0.69 0.63 0.62 Avg: 0.65	< 1.0 (0.7X)
	Peeled Fruit		0.03 0.02 0.03 Avg: 0.03	<1.0 (0.03X)

RAC	Processed Commodity	Total Rate lb ai/A (kg ai/ha)	fluopyram Residue (mg/kg)	Processing Factor[a]
	Applesauce		0.01 0.01 0.01 Avg: 0.01	<1.0 (0.01X)
	Dried Fruit		0.03 0.03 0.03 Avg: 0.03	<1.0 (0.03X)

[a] Processing Factor is the ratio of average residue in the processed samples divided by the average residue in the unprocessed samples.
[b] NA = Not applicable.

The average fluopyram residue in apple RAC was 0.93 mg/kg. The average fluopyram residue for the required processed commodities was 2.14 mg/kg in apple wet pomace and 0.41 mg/kg in apple juice. The average fluopyram residue data for additional risk assessment samples was 0.65 mg/kg in washed fruit, 0.03 mg/kg in peeled fruit, 0.01 mg/kg in applesauce, and 0.03 mg/kg in dried fruit.

Processing factors were calculated by dividing the average fluopyram residue in the apple processed commodities by the average fluopyram residue in the unwashed apple RAC. The fluopyram residue was found to concentrate in apple wet pomace (2.3X). No concentration (<1X) of fluopyram residue was found in apple juice (0.4X). In the additional samples generated for dietary risk assessment, no concentration (<1X) of fluopyram residue was found in washed fruit (0.7X), peeled fruit (0.03X), applesauce (0.01X), or dried fruit (0.03X).

Conclusions:
Following the exaggerated (5X) use of fluopyram 500 SC apple trees and subsequent processing of apples into the required commodities of apple wet pomace and apple juice, fluopyram 500 SC residue did concentrate (processing factor >1X) in apple wet pomace (2.3 X), but did not concentrate (processing factor < 1X) in apple juice (0.4X). In the additional samples generated for dietary risk assessment, AE C656948 residue did not concentrate in washed fruit (0.7X), peeled fruit (0.03X), applesauce (0.01X) or dried fruit (0.03X)

The processing factors determined for fluopyram residue in this study are less than the maximum observed (experimental) concentration factors cited in the EPA Residue Chemistry Test Guideline OPPTS 860.1520.

Assessment and conclusion by applicant:
The study is acceptable.

Overall summary of calculated processing factors for apple

Table 6.5.3- 54: Overall processing factors for fluopyram for apple commodities

Sample material	Trial nb	Mean PF	Studies		Sample material	Overall trial nb	Overall mean PF
fruit, washed	4	0.70	KCA 6.5.3.1-16 & 17		fruit, washed	5	0.7
washing water	4	0.15			washing water	4	0.15
raw sauce	4	0.55			raw sauce	4	0.55
sauce	4	0.43			sauce	5	0.35
strain rest, wet	4	7.03			strain rest, wet	4	7.03
raw juice	4	0.23			raw juice	4	0.23
juice	4	0.10			juice	5	0.2
pomace, wet	4	2.38			pomace, wet	5	2.35
pomace, dried	4	7.78			pomace, dried	4	7.78
fruit peeled	4	0.23			fruit peeled	5	0.25
peel rest	4	6.70			peel rest	4	6.70
fruit, dried	4	0.75			fruit, dried	5	0.6
Wet Pomace	1	2.3	KCA 6.5.3.1-18				-
Juice	1	0.4			-	-	-
Washed Fruit	1	0.7			-	-	-
Peeled Fruit	1	0.3			-	-	-
Applesauce	1	0.01			-	-	-
Dried Fruit	1	0.63			-	-	-

*Processing factor calculated according to the following equation:

$$PF = \frac{Residue\ concentration\ in\ the\ processed\ product\ [\frac{mg}{kg}]}{Residue\ concentration\ in\ the\ RAC\ [\frac{mg}{kg}]}$$

Estimated values "<xxx" are not considered in overall mean calculation.

Data Point:	KCA 6.5.3/19
Report Author:	[redacted]
Report Year:	2020
Report Title:	Selected food processing techniques as a factor for pesticide residue removal in apple fruit
Report No:	M-763588-01-1
Document No:	M-763588-01-1
Guideline(s) followed in study:	not applicable
Deviations from current test guideline:	--
Previous evaluation:	
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	

Additional study from public literature

Executive summary

This article from Environmental Science and Pollution Research is a study to measure the influence of different processing techniques on the residue level of fluopyram and other active substances in apple. For a sake of clarity, only fluopyram results are summarized in this renewal dossier.Washing, peeling, pasteurization and boiling show a reduction on the incurted residues in the raw agricultural commodity. The processing factors calculated are at the same levels of those calculated in the GLP studies presented above.

Material and method

The orchard of a land area of six hectares was located in Rzeszów (south-eastern Poland). Two separate treatments were performed using the following preparations recommended in Poland for apple protection: combination of Score 250 EC (dose 0.2 L/ha, active substance—difenoconazole) and Switch 62.5 WG (dose 0.75 kg/ha, active substances—cyprodinil, fludioxonil) and the second treatment with Luna Experience 400 SC (dose 0.75 L/ha, active substances— tebuconazole, fluopyram (150 g ai/ha)). Apple samples (variety Gala) were collected the next day after application of formulations. Each sample consisted of apples randomly chosen from a row of apple trees. The weight of collected samples of ripe apples was ≥ 1 kg, as required by the national regulation (Regulation 2013). Samples were packed in polyethylene bags and transported to a laboratory.

Analytical samples, respectively collected after each treatment, were divided into a few parts (subsample, depending on the number of processing techniques. One of them was not subjected to any process, but it was used to assess the initial concentrations of pesticide residues in the samples. Each sample was analysed in three replicates.

Processing techniques used :

Washing with tap water

In total, 250 g of apple samples was washed in running water for 1 min. Water temperature was 21 °C with hardness of 260 mg CaCO3/l and a flow rate of 5 l/min.

Ultrasonic washing

In total, 250 g of apple samples was washed in an Ultrasonic cleaner (Bandelin Sonorex RK 52, Germany) in 1 l of distilled water for 1, 5, and 15 min. Water temperature was 21 °C, ultrasonic frequency 35,000 Hz, and ultrasonic power 240W.

Boiling

In total, 250 g of apple samples was immersed in 0.5 l of distilled water for 1, 5, and 15 min at the temperature of 100 °C.

Pasteurization and sterilization

In total, 250 g of apple samples was put into an incubator in a twisted glass jar (Binder GmbH, Germany) for 1, 5, and 15 min at the temperature of 120 °C.

Peeling

In total, 250 g of apple samples was peeled to a depth of 1–1.5 mm with a knife. Both peeled parts and skins were subjected to further testing.

Juicing

In total, 250 g of apple sample was subjected to pressing in order to obtain juice (Hurom, model HH-2G, rotational speed 40 rpm, power 150 W; South Korea).

Preparation of apple sample was based on the QuEChERS procedure (Lehotay et al. 2010; PN-EN 15662 2008). However, in order to allow the final determination of pesticide residues with the use of selective detectors such as μECD and NPD, the procedure was modified by solvent exchanged to petroleum ether directly before GC analysis (Słowik-Borowiec and Szpyrka 2018).

Before the experiments, a number of quality control tests were carried out to ensure the reliability and robustness of the pesticide residue determination process. The following parameters were assessed: trueness, precision, selectivity, limits of quantification (LOQs), and measurement of uncertainty. The results of validation were interpreted according to the criteria adopted in Europe, recommended by the European Commission and published in document SANTE/11813/2017 (2017).

In validation experiments, blank apple samples were spiked at two fortification levels (in at least five repetitions, n = 5).

The limits of quantification (LOQs) for each pesticide were set at the lowest spiking concentrations for which validation criteria in the term of recovery and precision were fulfilled. LOQs were within the range of 0.009–0.021 mg/kg.

Precision was calculated from the recovery experiments, and it was expressed in terms of relative standard deviation (RSD) at each spiking level. For all pesticides, range percentage of mean recovery and RSD was acceptable and amounted to 83–110% and 2–11%, respectively.

Selectivity of the method for all pesticides was assessed considering the lack of interfering peaks from co-extractives. For this purpose, extracts without pesticide were analyzed. In this study, there were no extracted matrix interferences, so the method could be considered selective

Linearity of calibration curves was studied over the concentration range between 0.009 and 2.096 μg/ml by the GC/ μECD/NPD analysis of matrix-matched calibration standards (at five levels prepared in apple extract). For all tasted pesticides, over the studied concentration range, the linearity was highly satisfactory with coefficients of determination (R^2) higher than 0.99.

The values of characteristic parameters obtained in validation process confirmed that the method meets the requirements of the European Commission and it is suitable for the determination of pesticide residue in apples.

Findings

The fluopyram residue data for apple fresh fruit and the apple processed commodities are summarized in Table 6.5.3- 55.

Table 6.5.3- 55: Apple fruit and apple processed commodities residue data from the processing study with fluopyram.

RAC	Processed Commodity	fluopyram Residue (mg/kg)	Processing Factor[a]
Apple	NA[b]	0.288	NA
	Washed apple	0.123	0.44
	Apple without skin	0.056	0.20
	skin	1.228	4.36
	juice	0.105	0.37
	extrudate	0.410	1.45
	Pasteurization and sterilization 120°C	5 min 0.309	1.10
		15 min 0.251	0.89
		30 mon 0.257	0.91
		60 min 0.278	0.99
	Ultrasonic washing	1 min 0.104	0.37
		5 min 0.060	0.21
		15 mon 0.044	0.16
	Boiling at 100°C	1 min 0.143	0.5
		5 min 0.062	0.22
		15 mon 0.078	0.28

[a] Processing Factor is the ratio of average residue in the processed samples divided by the residue in the unprocessed samples.
[b] NA = Not applicable.

Conclusion

The calculated processing factors show a decrease of the residue level in Juice and also in fruit after washing, peeling and cooking.

These results are in line with those measured in the GLP residue trials submitted by the applicant for the fluopyram renewal.

Assessment and conclusion by applicant:

The results from this non OECD public study are not more conservative than the results obtain from the GLP trials submitted by the applicant.

CA 6.6 Residues in rotational crops

CA 6.6.1 Metabolism in rotational crops

Data to address this point were presented in the dossier submitted for first inclusion in Annex and were deemed acceptable following evaluation and peer review at EU level (2013).

For details of data submitted previously please refer also to the Baseline dossier CA 6.2. For completeness, a summary of these previously submitted studies are included below.

Data already evaluated during the first EU review process for inclusion on Annex I.(no new studies)

Metabolism in rotational crops (soil application)

Metabolism studies in rotational crops were conducted with [phenyl-UL-^{14}C]fluopyram:

Data Point:	KCA 6.6.1/01
Report Author:	[redacted]
Report Year:	2008
Report Title:	Metabolism of [phenyl-UL-14C]AZ C65048 in confined rotational crops
Report No:	MEF-03/412
Document No:	M-297921-01-1
Guideline(s) followed in study:	US EPA OPPTS 860.1850
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted rev. 1 to Vol.3 of DAR B7 August 2012 (references relied on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The metabolism of [phenyl-UL-^{14}C]fluopyram was investigated in rotational crops covering the three crop types (cereal, leafy and root): wheat, Swiss chard and turnips for three consecutive rotations.

[Phenyl-UL-^{14}C]fluopyram was applied uniformly onto to the bare soil of a planting container (area approximately 1 m^2) by spray application (day 0). The application rate amounted to 534 g a.s./ha based on the highest recommended annual field rate of 500 g a.s./ha. Crops of the 1st, 2nd and 3rd rotation were sown 30 days, 139 days and 280 days after soil application, respectively.

Wheat forage was harvested at BBCH 29–31, wheat hay was harvested at 77–83 BBCH and dried. The remaining RACs were harvested at maturity.

The total radioactive residue (TRR) of the single rotations in wheat RACs amounted to 0.100–0.785 mg eq/kg in forage, 1.120–1.783 mg eq/kg in hay, 1.032–6.156 mg eq/kg in straw and 0.167–0.054 mg eq/kg in grains. In Swiss chard the TRR decreased from 0.540 mg eq/kg to 0.164 mg eq/kg, in turnip, the TRR decreased from 0.884 to 0.103 mg eq/kg in leaves and 0.065–0.009 mg eq/kg in roots.

Conventional extraction of the RACs using acetonitrile/water released > 96% of the TRR in Swiss chard and turnip leaves and roots, 87–95% of the TRR in wheat forage, hay and straw and 78–85% of the TRR in wheat grains.

The post extraction solids (PES) of wheat hay (all rotations) and wheat straw (1st and 2nd rotation) after conventional extraction were extracted using acetonitrile/water in a microwave with increased temperature. Microwave extraction released further 3–5% of the TRR in wheat hay and straw. Conventional and microwave extracts of wheat hay and straw showed identical metabolite patterns.

Diastase treatment of the post extraction solids of wheat grain released further 9% of the TRR from solids of the 1st rotation, and 15% of the solids of the 2nd rotation. The solutions after diastase treatment were further characterised as polar and probably natural compounds by partitioning (1st rotation).

Parent fluopyram and 26 metabolites were detected in the various samples of the three rotations. Of these, the active substance and 10 metabolites were identified by LC-MS and LC-MS/MS. The other 16 metabolites were characterised by their extraction and retention in radio-HPLC; 15 of them were < 0.03 mg eq/kg. One very polar region was characterised in wheat grain accounting for up to 21.3% of the TRR at a low residue level of only 0.01 mg eq/kg in the second rotation. This could be assimilated $^{14}CO_2$, which was incorporated in the starch matrix.

Parent fluopyram accounted for the major part of the residues in all RACs of all rotations and covered 56–84% of the TRR in the RACs of the 1st rotation, 33–78% of the TRR in the RACs of the 2nd rotation and 28–59% of the TRR in the RACs in the 3rd rotation.

Fluopyram-7-hydroxy and its various conjugates with glucose, malonic acid (2 isomers) and sulphuric acid were relevant metabolites mainly in Swiss chard, where the fluopyram-7-hydroxy yielded 21% of the TRR in the 1st rotation increasing to about 35% of the TRR in the following rotations. In the other RACs, the amount of fluopyram-7-hydroxy was distinctively lower (2–13%). The sulphuric acid conjugate of fluopyram-7-hydroxy, fluopyram-7-OH-SA, was also a prominent metabolite in Swiss chard increasing from 7% of TRR in the 1st rotation to 16% and 12% of the TRR in the 2nd and 3rd rotation.

Two label specific metabolites were identified: fluopyram-benzamide and fluopyram-benzoic acid.

fluopyram-8-hydroxy and its conjugate were only of minor importance. At least one of them were detected in all RACs but at very low levels of < 2.7% of the TRR in sum.

fluopyram-phenol-glc was detected in turnip leaves only, where it amounted to 10%, 16% and 10% of the TRRs of the 1st, 2nd and 3rd rotation.

Identification rate was very high and ranged from 86–97% of the TRR in all RACs apart from wheat grains. In grains the identification rate was lower (50.7–79.4% of the TRR) but up to 76.6–84.7% of the TRR was at least characterised and further 8–15% of the TRR were assigned to natural compounds after enzyme treatment of the extraction solids with diastase followed by partitioning with organic solvents.

The metabolic transformations detected in rotational crops were:

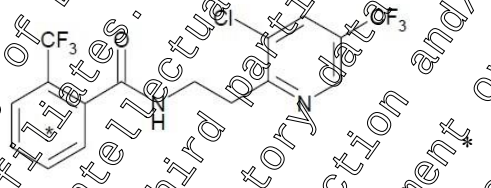
- hydroxylation of the ethyl linking group of the parent compound forming fluopyram-7-hydroxy and -8-hydroxy metabolites
- hydroxylation of the phenyl ring and subsequent conjugation with glucose

- conjugation of the hydroxylated metabolites with glucose, malonic acid and sulphuric acid
- hydrolytic cleavage and subsequent oxidation to fluopyram-benzamide and fluopyram-benzoic acid
- formation of natural compounds by assimilation of $^{14}CO_2$ from mineralization of fluopyram residues in soil

I. Materials and Methods

A. Materials

1. Test Material:

Chemical structure	 * position of the radiolabel
Compound	AE C656948
IUPAC name	*N*-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]ethyl}-2-(trifluoromethyl)benzamide
CAS name	Benzamide, *N*-[2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-(trifluoromethyl)-(9Cl)
CAS #	658066-35-4
Radiolabel position	Phenyl-UL-^{14}C
Specific radioactivity	3.85 MBq/mg (104.2 µCi/mg)
Purity Chemical Purity	≥ 99% (HPLC), ≥ 99% (TLC) ≥ 99% (HPLC)

The active substance was not formulated, but the application solution was prepared directly from stock solution (test item dissolved in acetonitrile) by dilution with water.

2. Soil: "Monheim 3" (sandy loam from Germany), pH ($CaCl_2$) = 6.4, 60.7% sand, 26.3% silt and 13.0% clay, 2.3% organic carbon, cation exchange capacity (CEC) of 5.9 meq/100 g.

3. Plant: see Table 6.1-1

Table 6.6.1-1: Details on rotational crops

Crop	Variety	Plant back intervals [days]	Growth stage at harvest	Harvested RAC
Spring wheat	Thasos	30 (1st rotation)	BBCH 29–31	forage
		139 (2nd rotation)	BBCH 77–83	hay
		280 (3rd rotation)	BBCH 89	straw and grain
Swiss chard	Luckullus	30 (1st rotation)	BBCH 45–48	leaves
		139 (2nd rotation)		
		280 (3rd rotation)		
Turnips	Rondo	30 (1st rotation)	BBCH 49	roots and leaves
		139 (2nd rotation)		
		280 (3rd rotation)		

B. Study Design

1. Experimental conditions

fluopyram was applied once on bare soil at 534 g/ha with a track sprayer and a flat fan nozzle. Wheat, Swiss chard and turnips of the 1st, 2nd and 3rd rotation were sown 30 days, 139 days and 280 days after the application, respectively.

The study was conducted in the vegetation area (building 6682) and in the greenhouse (building 6681) of Bayer CropScience AG, Metabolism / Environmental Fate, Monheim, Germany. The vegetation hall allows plant growth under natural sunlight and temperatures; however, a glass roof was automatically closed at the beginning of rainfall or at bad weather conditions. If necessary, the soil was watered in order to maintain adequate moisture contents during the ageing period. Application, ageing period and first rotation were conducted in the vegetation hall, second and third rotation were conducted in the greenhouse. Plants were cultivated in a planting container with a surface area of 1.0 m^2, filled with a sandy loam soil. The plants were irrigated by pouring to maintain optimal growing conditions.

2. Sampling

Wheat: At BBCH growth stage 29–31 about 20% of the wheat plants were cut shortly above the ground as forage sample. At BBCH 77–83 ("early milk stage") again 20% of the wheat plants were cut as hay sample and dried at room temperature for four days. At BBCH 89–92 (maturity) the remaining wheat plants were cut and grains were separated by hand. The remaining ears and chaffs were combined with the straw.

Swiss chard: At BBCH 45–48, the Swiss chard plants were cut above the roots.

Turnip: At BBCH 49 (maturity) the whole turnips plants were removed from soil and separated into leaves and roots.

Directly after harvest, all RACs from wheat, Swiss chard and turnips were cut into pieces before homogenisation with liquid nitrogen using a Polytron homogenizer. Residual plant material was stored at approximately -18 °C.

C. Analytical Procedures

The homogenized samples were extracted immediately after harvest and the extracts were analysed by HPLC. The identification of parent compound and metabolites was based on HPLC-MS, HPLC-MS/MS and co-chromatography.

1. Extraction

An aliquot of each homogenized RAC was extracted three or four times with acetonitrile/water (4/1; v/v). Turnip roots from the third rotation were not extracted, since the TRR obtained from combustion/LSC was < 0.01 mg eq/kg.

The extracts were combined, purified by SPE and the volume was determined. The extracts were separated from the solids by filtration and radioactivity was determined by volume measurement and LSC. The remaining solids after extraction were air-dried and subjected to combustion for determination of radioactivity by LSC. If needed, post extraction solids were further extracted, using microwave conditions with acetonitrile/water (1/1; v/v) at 120°C. Post extraction solids form grain were further extracted using the starch-cleaving enzyme diastase.

In general, ^{14}C-radioactivity of liquid samples was determined by liquid scintillation counting (LSC) using Quicksafe A containing 5% of water. The radioactivity in solid samples was measured by combustion. The released $^{14}CO_2$ was absorbed in an alkaline scintillation cocktail and radioassayed by LSC.

The actual TRR value of each RAC was determined after extraction by summing up the radioactivity measured in the extracts and in the remaining solids. The residue levels are expressed as parent compound equivalents per weight. The concentrated acetonitrile/water extracts were analysed by reversed phase HPLC coupled to a radioactivity detector with glass scintillation cell.

2. Identification and characterisation:

Active ingredient and metabolites were identified by LC-MS, LC-MS/MS, LC-MS/MS/MS and FT-MS. Conjugates with glucose, malonic acid and sulphuric acid were identified before and additionally after hydrolysis to obtain further information on the skeletal structure (e.g. position of hydroxy groups). Identification of metabolites was further supported by co-chromatography (HPLC, TLC) with isolated metabolites and reference items, by comparison of the metabolic profiles of individual RACs within one rotation and with the ones from the other rotations and by comparison with selected profiles from the confined rotational crop study conducted with [pyridyl-2,6-^{14}C]fluopyram.

Hydrolysis experiments with the enzyme ß-glucosidase were performed to characterise metabolites and to support identification of metabolites. Since experiments with hydrochloric acid and the enzyme ß-glucosidase did not provide clear HPLC results, hydrolysis experiments were mainly performed with isolated metabolites.

3. Storage stability:

All samples were extracted and quantified within 19 days after sampling the latest. Only for wheat forage (1st rotation), the metabolic profile of the extract was first analysed with the preliminary profiling method directly after extraction. This profile was repeated with the final profiling method later yielding in the same qualitative and quantitative profile as before with the preliminary method. Biological samples, reference and test items were stored in a freezer at about ≤-18 °C. Extracts and fractions were stored in a refrigerator at about 5 °C, and solids at room temperature. The metabolite pattern remained stable in reanalysed extracts *e.g.* for co-chromatography or identification purposes. No significant degradation or transformation of metabolites in extracts were detected.

II. Results and Discussion

The metabolism of [phenyl-UL-^{14}C]fluopyram was investigated rotational crops after a single application of 534 g/ha on bare soil.

The TRRs in the RACs of all rotations were determined by summing the TRR in the extracts and extracted solids. The TRRs from all RACs and all rotations are shown in Table 6.6.1-2. Apart from wheat forage the TRRs declined from the 1st rotation to the 3rd rotation by a factor of 1.2–9.

The TRR amounted to 0.100–0.197 mg eq/kg in wheat forage, 1.783–1.527 mg eq/kg in wheat hay, 6.156–1.032 mg eq/kg in wheat straw and 0.167–0.023 mg eq/kg in wheat grain (each 1st - 3rd rotation). In Swiss chard the TRR decreased from 0.540 mg eq/kg to 0.164 mg eq/kg and in turnip the TRR accounted for 0.884–0.103 mg eq/kg in leaves and 0.065–0.009 mg eq/kg in roots (each 1st to 3rd rotation).

Table 6.6.1-2: TRR values in wheat, Swiss chard and turnip RACs after soil application of [phenyl-UL-^{14}C]fluopyram

TRR [mg eq/kg]	Wheat				Swiss chard	Turnip	
	forage	hay	straw	grain		leaves	roots
1st rotation	0.100	1.783	6.156	0.167	0.540	0.884	0.065
2nd rotation	0.785	1.120	3.450	0.054	0.377	0.113	0.013
3rd rotation	0.197	1.527	1.032	0.023	0.164	0.103	0.009*

* no extraction performed, TRR calculated from combustion values

The RACs were extracted with acetonitrile/water (4/1; v/v), the extracts were analysed by HPLC and parent compound and metabolites were identified.

Conventional extraction of the RACs using acetonitrile/water released > 96% of the TRR in Swiss chard and turnip leaves and roots, 87–95% of the TRR in wheat forage, hay and straw and 78–85% of the TRR in wheat grains (Table 6.6.1-3).

Exhaustive extraction of post extraction solids of wheat hay (all rotations) and wheat straw (1st and 2nd rotation) using acetonitrile/water (1/1; v/v) and microwave conditions released further 3–5% of the TRR. The radioactivity in the solids of wheat grain after conventional extraction amounted to 15%–24% of the TRR but at very low absolute levels (0.026 mg eq/kg, 0.013 mg eq/kg and 0.005 mg eq/kg). Diastase treatment released additional 9% of the TRR from solids of the 1st rotation and 15% of the solids of the 2nd rotation. Solids from the 3rd rotation were not further subjected to diastase treatment due to their very low absolute residue value (0.005 mg/kg). The diastase extracts were further characterised as natural compounds by partitioning (1st rotation), and not further analysed for the 2nd rotation because they were < 0.01 mg eq/kg. The residues remaining after diastase treatment from grain solids from the 1st and the 2nd rotation were at very low absolute levels (0.012 mg eq/kg and 0.005 mg eq/kg, respectively).

Table 6.6.1-3: Distribution of radioactivity in the extracts of wheat, Swiss chard and turnip RACs after soil application of [phenyl-UL-^{14}C]fluopyram

	1st rotation		2nd rotation		3rd rotation	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Wheat forage						
Conventionally extracted Solids	94.4 5.6	0.094 0.006	94.7 5.3	0.743 0.041	92.4 7.6	0.182 0.015
TRR	100.0	0.100	100.0	0.785	100.0	0.197
Wheat hay						
Conventionally extracted Microwave extracted Solids	94.0 3.3 2.8	1.675 0.059 0.049	86.7 5.2 8.0	0.971 0.059 0.090	92.1 3.6 4.3	1.406 0.055 0.066
TRR	100.0	1.783	100.0	1.120	100.0	1.527
Wheat straw						
Conventionally extracted Microwave extracted Solids	92.6 3.3 4.1	5.701 0.201 0.254	90.4 4.2 5.4	3.118 0.144 0.188	90.8 - 9.2	0.937 - 0.095
TRR	100.0	6.156	100.0	3.450	100.0	1.032
Wheat grain						
Conventionally extracted Diastase extracted Solids	84.7 8.5 6.8	0.142 0.014 0.012	76.6 15.3 8.1	0.041 0.008 0.005	78.1 - 21.9	0.018 - 0.005
TRR	100.0	0.167	100.0	0.054	100.0	0.023
Swiss chard						
Conventionally extracted Solids	99.4 0.6	0.536 0.003	98.8 1.2	0.373 0.004	98.4 1.6	0.161 0.003
TRR	100.0	0.540	100.0	0.377	100.0	0.164
Turnip leaves						
Conventionally extracted Solids	99.5 0.5	0.880 0.004	98.7 1.3	0.112 0.001	99.0 1.0	0.102 0.001
TRR	100.0	0.884	100.0	0.113	100.0	0.103
Turnip roots						
Conventionally extracted Solids	98.7 1.3	0.064 0.001	96.7 3.3	0.013 < 0.001	- -	- -
TRR	100.0	0.065	100.0	0.013		0.009*

* no extraction performed, TRR calculated from combustion values

For the elucidation of metabolism, the solvent extracts (acetonitrile/water) were analysed by HPLC. Metabolites were identified by LC-MS and LC-MS/MS and additionally by co-chromatography (HPLC).

The identification rate was very high and ranged from 86–97% of the TRR in all RACs apart from wheat grains. In grains the identification rate was lower (50.7–79.4% of the TRR) but up to 76.6–84.7% of the TRR was at least characterised and further 8–15% of the TRR were assigned to natural compounds after enzyme treatment of the extraction solids with diastase followed by partitioning with organic solvents.

Parent fluopyram and 26 metabolites were detected in the various samples of the three rotations. Of these the parent compound and 10 metabolites were identified by LC-MS and LC-MS/MS. The other 16 metabolites were characterised by their extraction and retention in radio-HPLC. The TRR of all those samples amounted to < 0.03 mg eq/kg. Additionally, a very polar region was characterised in wheat grain

accounting for a maximum of 21.3% of the TRR at a low residue level of only 0.01 mg eq/kg in the second rotation which could be due to assimilated $^{14}CO_2$ which was incorporated in the starch matrix. The amounts of active substance and metabolites in all RACs as well as additional quantitative information are shown below in Table 6.6.1-4: to Table 6.6.1-6 for the three rotations.

Wheat: The parent compound was the main compound and declined from the 1st to 3rd rotation, from *ca.* 74–77% of the TRR to *ca.* 50–59% of the TRR in wheat forage, hay and straw and from *ca.* 62% to *ca.* 28% of the TRR in grain. Fluopyram-7-hydroxy and its various conjugates with glucose, malonic acid and sulphuric acid were detected in all samples and accounted for *ca.* 8–14% of the TRR in wheat forage, hay and straw of the 1st rotation, and increased up to *ca.* 28% of the TRR in these RACs of the 3rd rotation. Furthermore, they accounted for *ca.* 1–6% of the TRR in grain. Two label specific metabolites represented by fluopyram-benzamide and fluopyram-benzoic acid were further important metabolites: the value for the label specific metabolites increased in wheat grain from *ca.* 11% of the TRR in the 1st rotation to *ca.* 17% in the next ones. The values for the label specific metabolites were lower in wheat forage, hay and straw and ranged from *ca.* 3–7% of the TRR in the first rotation up to *ca.* 6–8% of the TRR in later rotations. fluopyram-benzoic acid was the most prominent label specific metabolite in wheat grain. fluopyram-benzamide was the prominent component of this metabolite group in wheat forage, hay and straw. fluopyram-8-hydroxy and its conjugate were only of minor importance and accounted for < 3% TRR in wheat RACs.

Swiss chard: The parent compound was the main compound and declined from the 1st to 3rd rotation, from *ca.* 56% to *ca.* 33%. fluopyram-7-hydroxy and its various conjugates with glucose, malonic acid and sulphuric acid were detected in all samples and accounted in total for *ca.* 29–53% of the TRR with a strong increase from the 1st to the 2nd rotation. fluopyram-7-hydroxy was the prominent metabolite of this metabolite group in Swiss chard amounting to *ca.* 21% of the TRR in the 1st rotation and increasing to about 35% of the TRR in the following rotations. The sulphuric acid conjugate of fluopyram-7-hydroxy, fluopyram-7-OH-SA, increased in Swiss chard from *ca.* 7% of TRR in the 1st rotation to *ca.* 16% and 12% of the TRR in the 2nd and 3rd rotation. The values for the label specific metabolites (fluopyram-benzamide and fluopyram-benzoic acid) in Swiss chard decreased from *ca.* 11% of the TRR to *ca.* 10% of the TRR. fluopyram-benzamide was the prominent component of this metabolite group in Swiss chard. fluopyram-8-hydroxy and its conjugate were only of minor importance and accounted for < 2% TRR in Swiss chard.

Turnip: The parent compound was the main compound and declined from the 1st to 3rd rotation, from *ca.* 68–84% to *ca.* 59% in turnips. fluopyram-7-hydroxy and its various conjugates with glucose, malonic acid and sulphuric acid accounted in total for *ca.* 11% of the TRR in turnips. fluopyram-7-hydroxy itself increased from *ca.* 3–4% in the 1st rotation to 8.2% in the 3rd rotation.

The various conjugates of fluopyram-7-hydroxy were less prominent and represented each < 3% of the TRR in turnips. Two label specific metabolites represented by fluopyram-benzamide and fluopyram-benzoic acid were further important metabolites: the values for the label specific metabolites ranged from *ca.* 8–10% of the TRR and increased to *ca.* 12% of the TRR. fluopyram-benzamide was the prominent component of this metabolite group in turnips. fluopyram-phenol-glc was detected in turnip leaves only, where it amounted to *ca.* 10%, 16% and 10% of the TRRs of the 1st, 2nd and 3rd rotation, respectively. fluopyram-8-hydroxy and its conjugate were only of minor importance and accounted for < 3% TRR in turnip RACs.

Table 6.6.1-4: Residues in RACs after a 30 day plant back interval (1st rotation, phenyl-label)

1. Rotation	Wheat forage		Wheat hay		Wheat straw		Wheat grain	
TRR [mg eq/kg] =	0.100		1.783		6.156		0.167	
Compound	% TRR	mg eq/ kg	% TRR	mg eq/ kg	% TRR	mg eq/ kg	% TRR	mg eq/ kg
AE C656948, a.s., fluopyram	74.8	0.075	76.9	1.370	74.1	4.557	61.9	0.104
fluopyram-benzoic acid (M33)	1.7	0.002	0.6	0.011	-	-	6.9	0.012
fluopyram-benzamide (M25)	5.6	0.006	4.3	0.076	2.8	0.169	4.1	0.007
fluopyram-7-hydroxy-glc-MA (M12), isomer 1	3.2	0.003	5.1	0.090	4.5	0.278	1.7	0.003
fluopyram-7-hydroxy-glc-MA (M12), isomer 2	-	-	1.0	0.017	1.0	0.062	-	-
fluopyram-8-hydroxy-glc-MA (M19)	1.0	0.001	0.8	0.014	0.7	0.046	-	-
fluopyram-7-OH-SA (M10)	-	-	-	-	-	-	-	-
fluopyram-7-hydroxy-glc (M11)	2.2	0.002	1.4	0.026	2.8	0.169	1.2	0.002
fluopyram-phenol-glc (M06)	-	-	-	-	-	-	-	-
fluopyram-7-hydroxy (M08)	2.4	0.002	4.3	0.077	5.6	0.346	2.7	0.005
fluopyram-8-hydroxy (M18)	0.4	0.001	0.6	0.010	1.4	0.087	0.9	0.001
Total identified	91.3	0.091	94.9	1.691	92.9	5.715	79.4	0.133
Characterized by HPLC retention*	3.1	0.003	2.0	0.036	2.4	0.147	5.3	0.009
Characterized by diastase treatment	-	-	-	-	-	-	8.5	0.014
Total extractable	94.4	0.094	97.3	1.734	95.9	5.901	84.7	0.142
Total bound residues (PES)	5.6	0.006	2.8	0.049	4.1	0.254	6.8	0.012
Not analysed	-	-	0.4	0.007	0.7	0.040	-	-
Accountability	100.0	0.100	100.0	1.783	100.0	6.156	100.0	0.167

1. Rotation	Swiss chard		Turnip leaves		Turnip roots	
TRR [mg eq/kg] =	0.539		0.884		0.065	
Compound	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg
AE C656948, a.s., fluopyram	56.0	0.302	68.4	0.605	83.5	0.054
fluopyram-benzoic acid (M33)	-	-	0.6	0.005	3.7	0.002
fluopyram-benzamide (M25)	11.1	0.060	9.7	0.086	4.0	0.003
fluopyram-7-hydroxy-glc-MA (M12), isomer 1	0.2	0.001	1.3	0.011	-	-
fluopyram-7-hydroxy-glc-MA (M12), isomer 2	-	-	0.9	0.008	-	-
fluopyram-8-hydroxy-glc-MA (M19)	-	-	-	-	-	-
fluopyram-7-OH-SA (M10)	7.0	0.038	0.7	0.006	-	-
fluopyram-7-hydroxy-glc (M11)	1.0	0.005	0.4	0.004	-	-
fluopyram-phenol-glc (M06)	-	-	10.4	0.092	-	-
fluopyram-7-hydroxy (M08)	20.9	0.113	3.3	0.029	3.6	0.002
fluopyram-8-hydroxy (M18)	0.7	0.004	0.3	0.003	0.9	0.001
Total identified	97.5	0.526	96.7	0.855	97.8	0.063
Characterized by HPLC retention*	1.7	0.009	2.8	0.025	0.9	0.001
Characterized by diastase treatment	-	-	-	-	-	-
Total extractable	99.4	0.536	99.5	0.880	98.7	0.064
Total bound residues (PES)	0.6	0.003	0.5	0.004	1.3	0.001
Not analysed	0.3	0.001	-	-	-	-
Accountability	100.0	0.539	100.0	0.884	100.0	0.065

* up to 16 minor metabolites characterised by extraction and chromatographic behaviour, all of them ≤5.1% of the TRR and <0.03 mg eq/kg,

Table 6.6.1-5: Residues in RACs after a 139 day plant back interval (2nd rotation, phenyl-label)

2. Rotation	Wheat forage		Wheat hay		Wheat straw		Wheat grain	
TRR [mg eq/kg] =	0.785		1.120		3.450		0.054	
Compound	% TRR	mg eq/ kg	% TRR	mg eq/ kg	% TRR	mg eq/ kg	% TRR	mg eq/ kg
AE C656948, a.s., fluopyram	74.9	0.588	66.7	0.748	67.8	2.338	37.1	0.020
fluopyram-benzoic acid (M33)	0.4	0.003	0.3	0.004	-	-	13.6	0.007
fluopyram-benzamide (M25)	3.5	0.028	3.7	0.041	3.2	0.111	3.3	0.002
fluopyram-7-hydroxy-glc-MA (M12), isomer 1	4.9	0.039	6.1	0.069	6.4	0.219	-	-
fluopyram-7-hydroxy-glc-MA (M12), isomer 2	1.3	0.010	0.7	0.008	1.3	0.046	-	-
fluopyram-8-hydroxy-glc-MA (M19)	1.2	0.009	1.4	0.016	1.2	0.043	-	-
fluopyram-7-OH-SA (M10)	-	-	-	-	-	-	-	-
fluopyram-7-hydroxy-glc (M11)	2.0	0.016	2.0	0.023	2.8	0.097	-	-
fluopyram-phenol-glc (M06)	-	-	-	-	-	-	-	-
fluopyram-7-hydroxy (M08)	3.8	0.030	5.2	0.059	6.0	0.208	1.3	0.001
fluopyram-8-hydroxy (M18)	0.5	0.004	0.8	0.009	1.4	0.050	-	-
Total identified	92.4	0.726	87.2	0.977	90.2	3.111	55.3	0.030
Characterized by HPLC retention*	2.3	0.018	3.8	0.043	3.6	0.124	21.3	0.011
Characterized by diastase treatment	-	-	-	-	-	-	15.3	0.008
Total extractable	94.7	0.743	91.9	1.030	94.6	3.262	76.6	0.041
Total bound residues (PES)	5.3	0.041	8.0	0.090	5.4	0.188	8.1	0.005
Not analysed	-		0.9	0.010	0.8	0.027	-	-
Accountability	100.0	0.785	100.0	1.120	100.0	3.450	100.0	0.054

2. Rotation	Swiss chard		Turnip leaves		Turnip roots	
TRR [mg eq/kg] =	0.377		0.113		0.013	
Compound	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg
AE C656948, a.s.	32.7	0.123	54.3	0.062	77.7	0.010
fluopyram benzoic acid (M33)	-	-	-	-	-	-
fluopyram benzamide (M25)	7.4	0.028	5.6	0.006	5.8	0.001
fluopyram 7-hydroxy-glc-MA (M12), isomer 1	-	-	2.1	0.002	-	-
fluopyram 7-hydroxy-glc-MA (M12), isomer 2	-	-	0.8	0.001	-	-
fluopyram 8-hydroxy-glc-MA (M19)	-	-	-	-	-	-
fluopyram-7-OH-SA (M10)	15.6	0.059	1.0	0.001	-	-
fluopyram 7-hydroxy-glc (M11)	1.8	0.007	0.9	0.001	-	-
fluopyram phenol-glc (M06)	-	-	16.2	0.018	-	-
fluopyram 7-hydroxy (M08)	35.4	0.134	6.3	0.007	4.1	0.001
fluopyram 8-hydroxy (M18)	0.9	0.003	0.4	0.000	2.3	0.000
Total identified	93.9	0.354	87.6	0.099	89.9	0.012
Characterized by HPLC retention*	4.9	0.019	11.1	0.013	6.8	0.001
Characterized by diastase treatment		-	-	-	-	-
Total extractable	98.8	0.373	98.7	0.112	96.7	0.013
Total bound residues (PES)	1.2	0.004	1.3	0.001	3.3	<0.001
Not analysed	-	-	-	-	-	-
Accountability	100.0	0.377	100.0	0.113	100.0	0.013

* up to 14 metabolites, 13 of them ≤1.3% of the TRR and <0.03 mg eq /kg; and one of them from 0.9 to 21.3% of the TRR and from 0.001 to 0.019 mg eq /kg

Table 6.6.1-6: Residues in RACs after a 280 day plant back interval (3rd rotation, phenyl-label)

3. Rotation	Wheat forage		Wheat hay		Wheat straw		Wheat grain	
TRR [mg eq/kg] =	0.197		1.527		1.032		0.023	
Compound	% TRR	mg eq/ kg	% TRR	mg eq/ kg	% TRR	mg eq/ kg	% TRR	mg eq/ kg
AE C656948, a.s., fluopyram	59.2	0.117	57.5	0.878	50.1	0.516	28.4	0.007
fluopyram benzoic acid (M33)	-	-	-	-	-	-	13.0	0.003
fluopyram benzamide (M25)	8.0	0.016	6.2	0.095	6.6	0.068	5.9	0.001
fluopyram 7-hydroxy-glc-MA (M12), isomer 1	8.5	0.017	7.1	0.108	6.3	0.065	-	-
fluopyram 7-hydroxy-glc-MA (M12), isomer 2	1.2	0.002	4.0	0.062	2.9	0.030	-	-
fluopyram 8-hydroxy-glc-MA (M19)	2.2	0.004	1.4	0.021	-	-	-	-
fluopyram 7-OH-SA (M10)	-	-	-	-	-	-	-	-
fluopyram 7-hydroxy-glc (M11)	3.4	0.007	3.4	0.052	6.9	0.071	-	-
fluopyram phenol-glc (M06)	-	-	-	-	-	-	-	-
fluopyram 7-hydroxy (M08)	6.3	0.012	12.6	0.193	12.3	0.127	3.4	0.001
fluopyram 8-hydroxy (M18)	0.5	0.001	1.1	0.016	1.3	0.014	-	-
Total identified	89.3	0.176	93.3	1.424	86.4	0.891	50.7	0.012
Characterized by HPLC retention*	3.1	0.006	1.5	0.023	3.3	0.034	27.4	0.006
Characterized by diastase treatment	-	-	-	-	-	-	-	-
Total extractable	92.4	0.182	95.7	1.461	90.8	0.937	78.1	0.018
Total bound residues (PES)	7.6	0.015	4.3	0.066	9.2	0.095	21.9	0.005
Not analysed	-	-	0.9	0.014	1.1	0.011	-	-
Accountability	100.0	0.197	100.0	1.527	100.0	1.032	100.0	0.023

3. Rotation	Swiss chard		Turnip leaves		Turnip roots	
TRR [mg eq/kg] =	0.164		0.103		0.009	
Compound	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg
AE C656948, a.s., fluopyram	33.7	0.055	58.6	0.060	Not extracted due to residues < 0.01 mg eq/kg	
fluopyram benzoic acid (M33)	-	-	-	-		
fluopyram benzamide (M25)	10.3	0.017	11.7	0.012		
fluopyram 7-hydroxy-glc-MA (M12), isomer 1	-	-	1.2	0.001		
fluopyram 7-hydroxy-glc-MA (M12), isomer 2	-	-	0.8	0.001		
fluopyram 8-hydroxy-glc-MA (M19)	-	-	-	-		
fluopyram 7-OH-SA (M10)	12.1	0.020	-	-		
fluopyram 7-hydroxy-glc (M11)	1.3	0.002	-	-		
fluopyram phenol-glc (M06)	-	-	10.1	0.010		
fluopyram 7-hydroxy (M08)	34.9	0.057	8.2	0.008		
fluopyram 8-hydroxy (M18)	1.4	0.002	0.4	< 0.001		
Total identified	93.6	0.153	91.0	0.094		
Characterized by HPLC retention*	4.8	0.008	8.0	0.008		
Characterized by diastase treatment	-	-	-	-		
Total extractable	98.4	0.161	99.0	0.102		
Total bound residues (PES)	1.6	0.003	1.0	0.001		
Not analysed	-	-	-	-		
Accountability	100.0	0.164	100.0	0.103		

* up to 8 metabolites, 7 of them ≤7.6% of the TRR and <0.01 mg eq /kg; and one of them from 0.4 to 19.9% of the TRR and from 0.004 to 0.034 mg eq /kg

III. Conclusions

After application of [phenyl-UL^{14}C]fluopyram on rotational crops (wheat, Swiss chard, turnip, 3 rotations), most of the recovered radioactivity was detected in the dry leaf RACs wheat straw (1.032–6.156 mg eq/kg) and hay (1.120–1.783 mg eq/kg). Medium amounts of radioactivity were found in the fresh leaf RACs wheat forage (0.100–0.785 mg eq/kg), Swiss chard (0.164–0.540 mg eq/kg) and turnip leaves (0.103–0.884 mg eq/kg). Minor residues were detected in grain (0.023–0.167 mg eq/kg) and turnip roots (0.009–0.065 mg eq/kg).

Over the study period of three rotations, a significant decline (factor of 3 to 9) of the TRR was observed for all RACs except wheat forage and hay, where the residues remained at a similar level.

In total, 29 components were detected in the various samples of this study, and 12 of them, including parent and major metabolites, were identified.

Parent fluopyram accounted for the major part of the residues in all RACs of all rotations and covered 56–84% of the TRR in the RACs of the 1st rotation, 33–78% of the TRR in the RACs of the 2nd rotation and 28–59% of the TRR in the RACs in the 3rd rotation.

fluopyram-7-hydroxy and its various conjugates with glucose, malonic acid (2 isomers) and sulphuric acid were important metabolites mainly in Swiss chard, where the fluopyram-7-hydroxy yielded 21% of the TRR in the 1st rotation increasing to about 35% of the TRR in the following rotations. In the other RACs, the amount of fluopyram-7-hydroxy was distinctively lower than in Swiss chard, only 2–6% of the TRR in the 1st rotation but also increasing from rotation to rotation up to 6–13% of the TRR.

The sulphuric acid conjugate of fluopyram-7-hydroxy, fluopyram-7-OH-SA, was also a prominent metabolite in Swiss chard increasing from 7% of TRR in the 1st rotation to 16% and 12% of the TRR in the 2nd and 3rd rotation. The sum of the various 7-hydroxy conjugates were less prominent but increased also in most of the RACs from the 1st to the 3rd rotation, except wheat grains, where the sum of fluopyram-7-hydroxy and one conjugate ranged from 1.3% to 5.6% of the TRR.

The main metabolic transformations detected were:

- hydroxylation of the ethyl-linking group of the parent compound forming fluopyram-7-hydroxy and -8-hydroxy metabolites
- hydroxylation of the phenyl ring and subsequent conjugation with glucose leading to fluopyram-phenol-glc (turnip leaves only)
- conjugation of the hydroxylated metabolites with glucose, malonic acid and sulphuric acid
- hydrolytic cleavage and subsequent oxidation to fluopyram-benzamide and fluopyram-benzoic acid
- formation of polar natural compounds which were incorporated into the starch matrix of grains

The proposed metabolic pathway for [phenyl-UL-^{14}C14C]fluopyram in rotational crops is given in Figure 6.6.1-1.

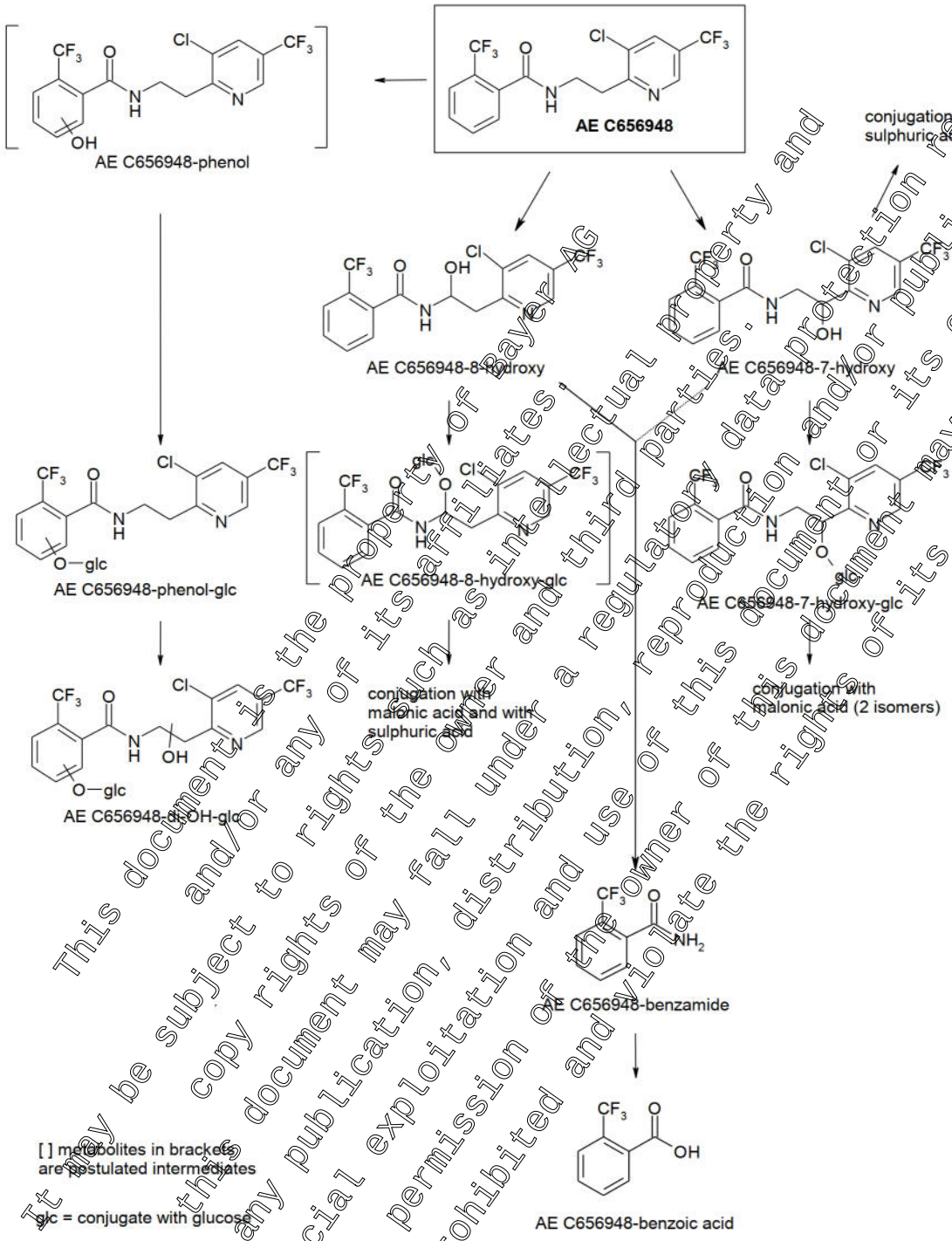


Figure 6.6.1-1: Proposed metabolic pathway of [phenyl-UL-^{14}C]fluopyram in confined rotational crops (CRC)

Assessment and conclusion by applicant:

The study is valid and acceptable.

Metabolism studies in rotational crops were conducted with [pyridyl-2,6-^{14}C]AE C656948:

Data Point:	KCA 6.6.1/02
Report Author:	[redacted]
Report Year:	2008
Report Title:	Metabolism of [pyridyl-2,6-14C]AE C656948 in confined rotational crops
Report No:	MEF-07/413
Document No:	M-298035-01-1
Guideline(s) followed in study:	US EPA OPPTS 860.1850, Canadian PMRA Ref. DACO 7.4.3
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted rev. 1 to Vol.3 of DAR, B.7 August 2013 (references refer to)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The metabolism of [pyridyl-2,6-^{14}C]AE C656948 was investigated in the rotational crops representing the three crop groups (cereal, leafy, root): wheat, Swiss chard and turnips for three consecutive rotations.

[Pyridyl-2,6-^{14}C]AE C656948 was applied uniformly onto to the bare soil of a planting container (area approximately 1 m^2) by spray application (day 0). The application rate amounted to 514 g a.s./ha based on the highest recommended annual field rate of 500 g a.s./ha. Crops of the 1st, 2nd and 3rd rotation were sown 30 days, 139 days and 280 days after soil application, respectively.

Wheat forage was harvested at BBCH 29–31, wheat hay was harvested at 77–83 BBCH and dried. The remaining RACs were harvested at maturity.

The total radioactive residue (TRR) of all wheat rotations amounted to 0.157–0.568 mg eq/kg in forage, 0.709–1.802 mg eq/kg in hay, 1.622–6.663 mg eq/kg in straw and 0.037–0.412 mg eq/kg in grain. In Swiss chard the TRR decreased from 0.570 mg eq/kg to 0.211 mg eq/kg and in turnip TRR decreased from 0.565–0.095 mg eq/kg in leaves and 0.036–0.012 mg eq/kg in roots.

Conventional extraction of the RACs using acetonitrile/water released > 97% of the radioactive residues in Swiss chard and turnip leaves and roots, 85–96% of the radioactive residues in wheat forage, hay and straw and 82–92% of the radioactive residues in wheat grains.

The post extraction solids of wheat hay (2nd and 3rd rotation) and wheat straw (1st and 2nd rotation) after conventional extraction were exhaustively extracted using acetonitrile/water in a microwave with

increased temperature. Microwave extraction released further 3–6% of the TRR in wheat hay and straw. Conventional and microwave extracts of wheat hay and straw showed identical metabolite pattern.

Diastase treatment of the post extraction solids of wheat grain released further 4% of the TRR from solids of the 1st rotation, and 9% of the solids of the 2nd rotation. The solutions after diastase treatment were further characterised as polar and probably natural compounds by partitioning (1st rotation).

Parent fluopyram and 26 metabolites were detected in the various samples of the three rotations. Of these the active substance and 10 metabolites were identified by LC-MS, LC-MS/MS and co-chromatography. The other 17 metabolites were characterised by their extraction and retention in radio-HPLC. All of them were ≤0.011 mg eq/kg representing 0.1–2.0% of the respective TRR, except the unknown metabolites in wheat straw, which were in the range of 0.4–1.3% of the TRR and equivalent to 0.011–0.042 mg eq/kg.

Apart from wheat grain, the parent compound was the main compound in all RACs of all rotations and covered 57–86% of the TRR in the RACs of the 1st rotation, 37–95% of the TRR in the RACs of the 2nd rotation and 39–92% of the TRR in the RACs in the 3rd rotation. In wheat grain it accounted for 20.4–33.4% of the TRR in the three rotations.

Fluopyram-7-hydroxy (M08) and its various conjugates with glucose, malonic acid (two isomers) and sulphuric acid were important metabolites mainly in Swiss chard, where the fluopyram-7-hydroxy yielded 28% of the TRR in the 1st rotation increasing to about 39% of the TRR in the following rotations. In the other RACs, the amount of fluopyram-7-hydroxy was distinctively lower. The sulphuric acid conjugate of fluopyram-7-hydroxy, fluopyram-7-OH-SA, was also a prominent metabolite in Swiss chard increasing from 8% of TRR in the 1st rotation to 17% and 14% of the TRR in the 2nd and 3rd rotation.

Two label specific metabolites were identified, fluopyram-pyridyl-carboxylic acid (M43) and fluopyram-methyl-sulfoxide (M45). They formed the major part of the residues in wheat grain (in sum 48.9–65.4% of the TRR of the three rotations). In analogy to the behaviour of other organic acids in higher plants, the presence of significant amounts of fluopyram-pyridyl-carboxylic acid in wheat grains was considered to be the result of the phloem transport into the grains (as a phloem sink) rather than the results of metabolism of the parent compound in this compartment.

Fluopyram-8-hydroxy (M18) and its conjugate M19 were only of minor importance. Both or at least one of them were detected in all RACs but at very low levels of < 2.9% of the TRR in sum.

Fluopyram-phenol-glc (M06) was detected in turnip leaves only, where it amounted to *ca.* 12%, 18% and 15% of the TRRs of the 1st, 2nd and 3rd rotation.
Identification rate was very high and ranged from 82–98% of the TRR in all RACs.

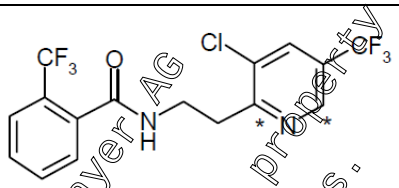
The metabolic transformations detected were:

- hydroxylation of the ethyl linking group of the parent compound forming fluopyram-7 hydroxy and -8-hydroxy metabolites
- hydroxylation of the phenyl ring and subsequent conjugation with glucose
- conjugation of the hydroxylated metabolites with glucose, malonic acid and sulphuric acid
- hydrolytic cleavage and subsequent oxidation to fluopyram-pyridyl carboxylic acid and fluopyram-methyl sulfoxide. These metabolites were possibly formed at low proportions in the soil or by enzymes located in the roots of the plants, and selectively translocated into grains following phloem transport.
- formation of polar, probably natural compounds which were incorporated into the starch matrix of grains

I. Materials and Methods

A. Materials

2. Test Material

Chemical structure	 * positions of the radiolabel
Compound	AE C656948
IUPAC name	*N*-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]ethyl}-2-(trifluoromethyl)benzamide
CAS name	Benzamide, *N*-[2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-(trifluoromethyl)-(9Cl)
CAS #	658066-35-4
Radiolabel position	Pyridyl-2,6-^{14}C
Specific radioactivity	3.85 MBq/mg (104.2 µCi/mg)
Purity Chemical Purity	≥ 99% (HPLC) > 98% (TLC) ≥ 99% (HPLC)

The active substance was not formulated, but the application solution was prepared directly from stock solution (test item dissolved in acetonitrile) by dilution with water.

2. Soil: "Monheim 3" (sandy loam from Germany), pH ($CaCl_2$) = 6.4, 60.7% sand, 26.3% silt and 13.0% clay, 2.3% organic carbon, cation exchange capacity (CEC) of 5.9 meq/100 g

3. Plant: see Table 6.6.1-7

Table 6.6.1-7: Details on rotational crops

Crop	Variety	Plant back intervals [days]	Growth stage at harvest	Harvested RAC
Spring wheat	Thasos	30 (1st rotation)	BBCH 29–31	forage
		139 (2nd rotation)	BBCH 77–83	hay
		280 (3rd rotation)	BBCH 89	straw and grain
Swiss chard	Luckullus	30 (1st rotation)	BBCH 45–48	leaves
		139 (2nd rotation)		
		280 (3rd rotation)		
Turnips	Rondo	30 (1st rotation)	BBCH 49	roots and leaves
		139 (2nd rotation)		
		280 (3rd rotation)		

B. Study Design

1. Experimental conditions

fluopyram was applied on bare soil at 534 g/ha with a track sprayer and a flat fan nozzle 30, 139 or 280 days prior to sowing of the first, second or third of Swiss chard, turnips and wheat, respectively.

Plants were grown in the vegetation area (building 6682) and in the greenhouse (building 6681) of Bayer CropScience AG, Metabolism / Environmental Fate, Monheim, Germany. The vegetation hall allows plant growth under natural sunlight and temperatures; however, a glass roof was automatically closed at the beginning of rainfall or at bad weather conditions. A metal net construction around the facility kept animals and birds out. If necessary, the soil was watered in order to maintain adequate moisture contents during the ageing period. The plants were irrigated by pouring to maintain optimal growing conditions. Application, ageing period and first rotation were conducted in the vegetation hall, second and third rotation were conducted in the greenhouse. Plants were cultivated in a planting container with a surface area of 1.0 m2, filled with a sandy loam soil.

2. Sampling

Wheat: At growth stage BBCH 29–31 about 20% of the wheat plants were cut shortly above the ground as forage sample. At BBCH 77–83 ("early milk stage") again 20% of the wheat plants were dried at room temperature for four days. At BBCH 89–92 (maturity) the remaining wheat plants were cut and grains were separated by hand. The remaining ears and chaffs were combined with the straw.

Swiss chard: At BBCH 45–48, the Swiss chard plants were cut above the roots.

Turnip: At BBCH 49 (maturity) the whole turnips plants were removed from soil and separated into leaves and roots.

Directly after harvest, the samples were cut into pieces before homogenization with liquid nitrogen using

a Polytron homogenizer. At approx. -18 °C residual plant material was stored.

C. Analytical Procedures

The homogenized samples were extracted and the extracts were analysed by HPLC for metabolite profiling. The identification of parent compound and metabolites was based on HPLC-MS, HPLC-MS/MS and co-chromatography.

1. Extraction

An aliquot of each homogenized RAC was extracted three or four times with acetonitrile/water (4/1, v/v). The extracts were combined, purified by SPE and the volume was determined. The extracts were separated from the solids by filtration and radioactivity was determined by volume measurement and LSC. The remaining solids were air-dried and subjected to combustion for determination of radioactivity by LSC.

If needed post extraction of solids were further extracted exhaustively, using microwave conditions with ACN/water (1/1; v/v) at 120 °C. Grain solids (1st and 2nd rotation) from the conventional extraction were further extracted using the starch-cleaving enzyme diastase.

The ^{14}C-radioactivity of liquid samples was determined by LSC using Quicksafe A containing 5% of water. The radioactivity in solid samples was measured by combustion. The released $^{14}CO_2$ was absorbed in an alkaline scintillation cocktail and radio assayed by LSC.

The actual TRR value of each RAC was determined after extraction by summing up the radioactivity measured in the extracts and in the remaining solids. The residue levels are expressed as parent compound equivalents per weight. The concentrated acetonitrile/water extracts were analysed by reversed phase HPLC coupled to a radioactivity detector with glass scintillation cell.

2. Identification and characterisation:

Active ingredient and metabolites were identified by HPLC-MS and HPLC-MS/MS. Conjugates with glucose, malonic acid and sulphuric acid were identified before and additionally after hydrolysis to obtain further information on the skeletal structure (e.g. position of hydroxy groups).

The identification of metabolites was further supported by co-chromatography (HPLC, TLC) with isolated metabolites and reference items, by comparing individual RAC metabolic profiles of one rotation with those of the other rotations, and by comparison with selected profiles from the confined rotational crop study conducted with [phenyl-UL-^{14}C]fluopyram.

3. Storage stability:

All samples were extracted and quantified within 20 days after sampling the latest. Only for wheat forage (1st rotation), the metabolic profile of the extract was first analysed with the preliminary profiling method directly after extraction. This profile was repeated with the final profiling method later yielding in the same qualitative and quantitative profile as before with the preliminary method. Biological samples, reference and test items were stored in a freezer at about ≤-18°C. Extracts and fractions were stored in a refrigerator at about 5°C, and solids at room temperature. The metabolite pattern remained stable in reanalysed extracts e.g. for co-chromatography or identification purposes. It was concluded that the

results of the present study were not impacted by the storage of the samples and that no further storage stability investigations are required.

II. Results and Discussion

The metabolism of [pyridyl-2,6-^{14}C]fluopyram was investigated rotational crops after a single application of 534 g/ha on bare soil.

The total radioactive residues (TRRs) in the RACs of all rotations were determined by summing the radioactive residues in the extracts and extracted solids. The TRRs from all RACs and all rotations are shown in Table 6.6.1-8. Apart from wheat forage the TRRs declined from the 1st rotation to the 3rd rotation by a factor of 2 to 6 and in wheat grain from 2 to 11.

The TRR amounted to 0.157–0.568–0.167 mg eq/kg in wheat forage (1st–2nd–3rd rotation). The TRR in wheat hay decreased from 1.802 mg eq/kg to 0.709 mg eq/kg, from 6.663 mg eq/kg to 1.622 mg eq/kg in wheat straw and from 0.412 mg eq/kg to 0.037 mg eq/kg in wheat grain (each 1st to 3rd rotation). In Swiss chard the TRR decreased from 0.570 mg eq/kg to 0.211 mg eq/kg and in turnip from 0.565 mg eq/kg to 0.095 mg eq/kg in leaves and from 0.036 mg eq/kg to 0.012 mg eq/kg in roots (each 1st to 3rd rotation).

Table 6.6.1-8: TRR values in wheat, Swiss chard and turnip RACs after application of [pyridyl-2,6-^{14}C]fluopyram

TRR [mg eq/kg]	Wheat				Swiss chard	Turnip	
	forage	hay	straw	grain		leaves	roots
1st rotation	0.157	1.802	6.663	0.412	0.570	0.565	0.036
2nd rotation	0.568	0.971	2.562	0.072	0.343	0.103	0.010
3rd rotation	0.167	0.709	1.622	0.037	0.211	0.095	0.012

The RACs were extracted with acetonitrile/water (4/1, v/v), the extracts were analysed by HPLC and parent compound and metabolites were identified.

Conventional extraction of the RACs using acetonitrile/water released > 97% of the radioactive residues in Swiss chard and turnip leaves and roots, 85–96% of the radioactive residues in wheat forage, hay and straw and 82–92% of the radioactive residues in wheat grain (Table 6.6.1-9).

Exhaustive extraction of post extraction solids of wheat hay (2nd and 3rd rotation) and wheat straw (1st and 2nd rotation) using acetonitrile/water mixtures and microwave conditions released further 3–6% of the TRR. The radioactivity in the solids of wheat grain after conventional extraction amounted to 8%–18% of the TRR but at very low absolute levels (0.032 mg eq/kg, 0.010 mg eq/kg and 0.007 mg eq/kg). Diastase treatment released additional 4% of the TRR from solids of the 1st rotation and 9% of the solids of the 2nd rotation. Solids from the 3rd rotation were not further subjected to diastase treatment due to their very low absolute residue value (0.007 mg eq/kg). The diastase extracts of grain were further characterised as natural compounds by partitioning (1st rotation), and not further analysed for the 2nd rotation because they were < 0.02 mg eq/kg. The residues remaining after diastase treatment from grain solids from the 1st and the 2nd rotation were at very low absolute levels (0.015 mg eq/kg and 0.003 mg eq/kg, respectively).

Table 6.6.1-9: Distribution of radioactivity in the extracts of wheat, Swiss chard and turnip RACs after application of [pyridyl-2,6-^{14}C]AE 656948

	1st rotation		2nd rotation		3rd rotation	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Wheat forage						
Conventionally extracted	95.7	0.151	95.2	0.540	92.4	0.155
Solids	4.3	0.007	4.8	0.027	7.6	0.013
TRR	100.0	0.157	100.0	0.568	100.0	0.167
Wheat hay						
Conventionally extracted	96.7	1.742	85.3	0.829	91.2	0.646
Microwave extracted	-	-	6.3	0.061	4.7	0.033
Solids	3.3	0.059	8.4	0.081	4.1	0.029
TRR	100.0	1.802	100.0	0.971	100.0	0.709
Wheat straw						
Conventionally extracted	93.5	6.229	87.5	2.241	91.0	1.477
Microwave extracted	2.5	0.169	5.2	0.132	-	-
Solids	4.0	0.265	7.4	0.189	9.0	0.146
TRR	100.0	6.663	100.0	2.562	100.0	1.622
Wheat grain						
Conventionally extracted	92.3	0.380	86.1	0.062	82.2	0.031
Diastase extracted	4.1	0.017	9.4	0.007	-	-
Solids	3.6	0.015	4.4	0.003	17.8	0.007
TRR	100.0	0.412	100.0	0.072	100.0	0.037
Swiss chard						
Conventionally extracted	99.8	0.569	99.2	0.341	98.2	0.207
Solids	0.2	0.001	0.8	0.003	1.8	0.004
TRR	100.0	0.570	100.0	0.343	100.0	0.211
Turnip leaves						
Conventionally extracted	99.5	0.562	97.4	0.100	98.7	0.094
Solids	0.5	0.003	2.6	0.003	1.3	0.001
TRR	100.0	0.565	100.0	0.103	100.0	0.095
Turnip roots						
Conventionally extracted	98.7	0.036	98.1	0.010	97.8	0.011
Solids	1.3	< 0.001	1.9	< 0.001	2.2	< 0.001
TRR	100.0	0.036	100.0	0.010	100.0	0.012

For the elucidation of metabolism, the solvent extracts (acetonitrile/water) were analysed by HPLC. Metabolites were identified by LC-MS and LC-MS/MS and additionally by co-chromatography (HPLC).

The identification rate was very high and ranged from 82–98% of the TRR in all RACs. In total, 27 components were detected in the various samples of the three rotations. Of these, the active substance and 10 metabolites were identified by LC-MS, LC-MS/MS and co-chromatography. The remaining 16 metabolites were characterised by their extraction and retention in radio-HPLC. All of them were ≤ 0.011 mg eq/kg representing 0.1–2.0% of the respective TRR, except the two unknown metabolites in wheat straw, which were in the range of 0.3–1.3% of the TRR and equivalent to 0.008–0.042 mg eq/kg, each. The amounts of active substance and metabolites in all RACs as well as additional quantitative information for the three rotations are shown below in Table 6.6.1-10 to Table 6.6.1-12.

Wheat: The parent compound was the main compound and declined from the 1st to 3rd rotation, from *ca.* 72–79% to *ca.* 58–70% of the TRR in wheat forage, hay and straw. In wheat grain, the parent compound declined from *ca.* 33% of the TRR in the 1st rotation to 20.4% of the TRR in the 2nd rotation to *ca.* 31% of the TRR in the 3rd rotation. fluopyram-7-hydroxy and its various conjugates with glucose, malonic acid and sulphuric acid accounted in total for *ca.* 7–17% of the TRR in wheat forage, hay and straw of the 1st rotation, and increased up to *ca.* 26% of the TRR in these RACs of the 3rd rotation. In grain, fluopyram-7-hydroxy and its conjugates are of little importance, since they only accounted for *ca.* 1–2% of the TRR.

Two label specific metabolites represented by fluopyram-methyl-sulfoxide (M45) and fluopyram-pyridyl carboxylic acid (M43) were further important metabolites.

In forage and hay, fluopyram-pyridyl carboxylic acid was a prominent metabolite, but only in the 1st rotation accounting for up to *ca.* 17% of the TRR (0.026 mg eq/kg). In the following rotations, this metabolite was ≤ 1% of the TRR. Fluopyram-methyl-sulfoxide was detected only in the 2nd and 3rd rotation and accounted for up to *ca.* 5% of the TRR (0.046 mg eq/kg).

In wheat straw, the two label specific metabolites covered only about 1% of the TRR in all rotations. AE C656949-pyridyl-carboxylic acid was detected only in the 1st rotation at an absolute amount of 0.06 mg eq/kg. fluopyram-methyl-sulfoxide was detected only in the 2nd and 3rd rotation at absolute amounts of 0.031 mg eq/kg and 0.019 mg eq/kg, respectively.

In wheat grain, the amount for fluopyram-pyridyl-carboxylic acid decreased from *ca.* 56% of the TRR (0.23 mg eq/kg) in the 1st rotation down to *ca.* 16% of the TRR (0.01 mg eq/kg) and 29% of the TRR (0.01 mg eq/kg) in the 2nd and 3rd rotation, respectively. The absolute value of 0.23 mg eq/kg for fluopyram-pyridyl carboxylic acid in grain of the 1st rotation was high, but very low in the 2nd and 3rd rotation (0.01 mg eq/kg). The relative high amount of fluopyram-pyridyl-carboxylic acid in wheat grains (*ca.* 56% of the TRR in the 1st rotation equivalent to 0.23 mg eq/kg) is not considered to be the result of metabolic conversion of the parent compound in this compartment. It is more likely that this metabolite was taken up from soil and then selectively transported into the grain.

Only *ca.* 1% of the TRR was covered by fluopyram-methyl-sulfoxide in grains of the 1st rotation and increased to about 49% of the TRR (0.035 mg eq/kg) and 20% of the TRR (0.008 mg eq/kg) in the 2nd and 3rd rotation, respectively. The absolute amounts for fluopyram-methyl-sulfoxide in grains were significantly lower than for fluopyram-pyridyl-carboxylic acid and amounted to 0.005 mg eq/kg (1st rotation), 0.035 mg eq/kg (2nd rotation) and 0.008 mg eq/kg (3rd rotation).

fluopyram-8-hydroxy and its conjugate were only of minor importance and accounted for < 3% TRR in wheat RACs.

Swiss chard: The parent compound was the main compound amounting to *ca.* 57% of the TRR (0.323 mg eq/kg), *ca.* 37% of the TRR (0.128 mg eq/kg) and *ca.* 39% of the TRR (0.081 mg eq/kg) in the 1st, 2nd and 3rd rotation, respectively. fluopyram-7-hydroxy and its various conjugates with glucose, malonic acid and sulphuric acid accounted in total for *ca.* 37–55% of the TRR. fluopyram-7-hydroxy was the prominent metabolite of this metabolite group amounting to *ca.* 28% of the TRR in the 1st rotation and increasing to *ca.* 39% of the TRR in the following rotations. The sulphuric acid conjugate of fluopyram-7-hydroxy, fluopyram-7-OH-SA, increased in Swiss chard from *ca.* 8% of TRR in the 1st rotation to *ca.* 17% and 14% of the TRR in the 2nd and 3rd rotation, respectively.

The values for the label specific metabolites (fluopyram-methyl-sulfoxide and fluopyram-pyridyl carboxylic acid) remained at a comparable level accounting for ≤ 6% of the TRR. fluopyram-8-hydroxy and its conjugate were only of minor importance and accounted for < 3% TRR in Swiss chard.

Turnip: The parent compound was the main compound in turnip roots and leaves. In turnips roots, it amounted to *ca.* 86% of the TRR (0.031 mg eq/kg), 95% of the TRR (0.010 mg eq/kg) and *ca.* 92% of the TRR (0.010 mg eq/kg) in the 1st, 2nd and 3rd rotation, respectively. In turnips leaves, the parent compound accounted for *ca.* 70% of the TRR (0.399 mg eq/kg), 60% of the TRR (0.061 mg eq/kg) and *ca.* 64% of the TRR (0.061 mg eq/kg) in the 1st, 2nd and 3rd rotation, respectively.

Besides the parent compound, fluopyram-phenol-glc was a prominent metabolite found exclusively in turnip leaves where it amounted to ca. 12% of the TRR (0.068 mg eq/kg), 18% of the TRR (0.019 mg eq/kg) and 15% of the TRR (0.014 mg eq/kg) in the 1st, 2nd and 3rd rotation, respectively.

fluopyram-7-hydroxy increased from *ca.* 3–4% in the 1st rotation to 6–8% in the 3rd rotation. The various conjugates of fluopyram-7-hydroxy were less prominent and represented each < 3% of the TRR in turnips RACs.

The amounts of the label specific metabolites (fluopyram-methyl-sulfoxide and fluopyram-pyridyl carboxylic acid) remained at a comparable level, accounting for ≤ 6% of the TRR.

fluopyram-8-hydroxy and its conjugate were only of minor importance and accounted for < 3% TRR in turnip RACs.

Table 6.6.1-10: Residues in RACs after a 30 day plant back interval (1st rotation, pyridyl-label)

1. Rotation	Wheat forage		Wheat hay		Wheat straw		Wheat grains	
TRR [mg eq/kg] =	0.157		1.802		6.663		0.412	
Compound	% TRR	mg eq/ kg	% TRR	mg eq/ kg	% TRR	mg eq/ kg	% TRR	mg eq/ kg
AE C656948, a.s., fluopyram	71.7	0.113	78.9	1.421	73.9	4.926	33.4	0.137
fluopyram-methyl-sulfoxide (M45)	-	-	-	-	-	-	1.2	0.005
fluopyram-pyridyl-carboxylic acid (M43)	16.5	0.026	4.9	0.088	0.9	0.060	55.9	0.230
fluopyram-7-hydroxy-glc-MA (M12), isomer 1	3.3	0.005	4.2	0.076	5.6	0.376	-	-
fluopyram-7-hydroxy-glc-MA (M12), isomer 2	-	-	1.2	0.021	1.1	0.072	-	-
fluopyram-8-hydroxy-glc-MA (M19)	-	-	0.6	0.010	0.4	0.028	-	-
fluopyram-7-OH-SA (M10)	-	-	-	-	-	-	-	-
fluopyram-7-hydroxy-glc (M11)	1.3	0.002	0.9	0.016	3.1	0.203	-	-
fluopyram-phenol-glc (M06)	-	-	-	-	-	-	-	-
fluopyram-7-hydroxy (M08)	2.1	0.003	3.8	0.068	7.4	0.494	1.8	0.008
fluopyram-8-hydroxy (M18)	-	-	0.4	0.006	1.3	0.087	-	-
Total identified	95.0	0.149	94.7	1.707	93.7	6.248	92.3	0.380
Characterized by HPLC retention*	0.7	0.001	1.8	0.032	1.1	0.074	-	-
Characterized by diastase treatment	-	-	-	-	-	-	4.1	0.017
Total extractable	95.7	0.151	96.7	1.742	96.0	6.398	92.3	0.380
Total bound residues (PES)	4.3	0.007	3.3	0.059	4.0	0.265	3.6	0.015
Not analysed	-	-	0.3	0.004	1.2	0.076	-	-
Accountability	100.0	0.157	100.0	1.802	100.0	6.663	100.0	0.412

1. Rotation	Swiss chard		Turnip leaves		Turnip roots	
TRR [mg eq/kg] =	0.570		0.565		0.036	
Compound	% TRR	mg eq/ kg	% TRR	mg eq/ kg	% TRR	mg eq/ kg
AE C656948, a.s., fluopyram	56.7	0.323	70.6	0.399	86.1	0.031
fluopyram-methyl-sulfoxide (M45)	0.4	0.003	-	-	-	-
fluopyram-pyridyl-carboxylic acid (M43)	1.8	0.010	4.3	0.024	6.0	0.002
fluopyram-7-hydroxy-glc-MA (M12), isomer 1	-	-	1.9	0.011	-	-
fluopyram-7-hydroxy-glc-MA (M12), isomer 2	-	-	0.6	0.003	-	-
fluopyram-8-hydroxy-glc-MA (M19)	-	-	-	-	-	-
fluopyram-7-OH-SA (M10)	7.9	0.045	0.7	0.004	-	-
fluopyram-7-hydroxy-glc (M11)	0.9	0.005	0.3	0.002	-	-
fluopyram-phenol-glc (M06)	-	-	12.0	0.068	-	-
fluopyram-7-hydroxy (M08)	28.0	0.160	3.5	0.020	3.1	0.001
fluopyram-8-hydroxy (M18)	0.9	0.005	0.3	0.002	1.5	0.001
Total identified	96.6	0.551	94.3	0.532	96.7	0.035
Characterized by HPLC retention*	3.2	0.018	5.2	0.030	2.0	0.001
Characterized by diastase treatment	-	-	-	-	-	-
Total extractable	99.8	0.569	99.5	0.562	98.7	0.036
Total bound residues (PES)	0.2	0.001	0.5	0.003	1.3	< 0.001
Not analysed	-	-	-	-	-	-
Accountability	100.0	0.570	100.0	0.565	100.0	0.035

* up to 17 minor metabolites characterised by extraction and chromatographic behaviour, all of them ≤2.0% of the TRR and ≤ 0.043 mg eq/kg

Table 6.6.1-11: Residues in RACs after a 139 day plant back interval (2nd rotation, pyridyl-label)

2. Rotation	Wheat forage		Wheat hay		Wheat straw		Wheat grains	
TRR [mg eq/kg] =	0.568		0.971		2.562		0.072	
Compound	% TRR	mg eq/ kg	% TRR	mg eq/ kg	% TRR	mg eq/ kg	% TRR	mg eq/ kg
AE C656948, a.s., fluopyram	77.9	0.442	66.9	0.650	73.5	1.881	20.4	0.015
fluopyram-methyl-sulfoxide (M45)	2.2	0.012	4.7	0.046	1.2	0.031	49.0	0.035
fluopyram-pyridyl-carboxylic acid (M43)	1.0	0.006	0.6	0.006	-	-	16.4	0.012
fluopyram-7-hydroxy-glc-MA (M12), isomer 1	5.6	0.032	7.0	0.068	3.5	0.089	-	-
fluopyram-7-hydroxy-glc-MA (M12), isomer 2	0.7	0.004	1.4	0.013	2.5	0.064	-	-
fluopyram-8-hydroxy-glc-MA (M19)	1.2	0.007	1.4	0.014	0.9	0.022	-	-
fluopyram-7-OH-SA (M10)	-	-	-	-	-	-	-	-
fluopyram-7-hydroxy-glc (M11)	1.0	0.005	2.5	0.024	2.3	0.059	-	-
fluopyram-phenol-glc (M06)	-	-	-	-	-	-	-	-
fluopyram-7-hydroxy (M08)	3.1	0.018	5.9	0.057	6.1	0.156	0.8	0.001
fluopyram-8-hydroxy (M18)	0.7	0.004	0.7	0.007	1.2	0.030	-	-
Total identified	93.2	0.530	91.1	0.886	91.0	2.331	86.5	0.062
Characterized by HPLC retention*	2.0	0.011	0.5	0.004	0.8	0.020	-	-
Characterized by diastase treatment	-	-	-	-	-	-	9.1	0.007
Total extractable	95.2	0.540	91.6	0.890	92.7	2.373	86.5	0.062
Total bound residues (PES)	4.8	0.027	8.4	0.081	7.4	0.189	4.4	0.003
Not analysed	-	-	-	-	0.9	0.022	-	-
Accountability	100.0	0.568	100.0	0.971	100.0	2.562	100.0	0.072

2. Rotation	Swiss chard		Turnip leaves		Turnip roots	
TRR [mg eq/kg] =	0.343		0.103		0.010	
Compound	% TRR	mg eq/ kg	% TRR	mg eq/ kg	% TRR	mg eq/ kg
AE C656948, a.s., fluopyram	37.3	0.128	59.9	0.061	94.6	0.010
fluopyram-methyl-sulfoxide (M45)	0.4	0.001	-	-	-	-
fluopyram-pyridyl-carboxylic acid (M43)	0.2	0.001	1.5	0.001	-	-
fluopyram-7-hydroxy-glc-MA (M12), isomer 1	-	-	2.8	0.003	-	-
fluopyram-7-hydroxy-glc-MA (M12), isomer 2	-	-	1.2	0.001	-	-
fluopyram-8-hydroxy-glc-MA (M19)	-	-	-	-	-	-
fluopyram-7-OH-SA (M10)	16.8	0.058	0.7	0.001	-	-
fluopyram-7-hydroxy-glc (M11)	1.8	0.006	1.4	0.001	-	-
fluopyram-phenol-glc (M06)	-	-	18.4	0.019	-	-
fluopyram-7-hydroxy (M08)	36.5	0.125	5.3	0.005	3.5	< 0.001
fluopyram-8-hydroxy (M18)	1.1	0.004	0.5	0.001	-	-
Total identified	94.0	0.323	91.7	0.094	98.1	0.010
Characterized by HPLC retention*	5.2	0.018	5.7	0.006	-	-
Characterized by diastase treatment	-	-	-	-	-	-
Total extractable	99.2	0.341	97.4	0.100	98.1	0.010
Total bound residues (PES)	0.8	0.003	2.6	0.003	1.9	< 0.001
Not analysed	-	-	-	-	-	-
Accountability	100.0	0.344	100.0	0.103	100.0	0.010

* up to 14 minor metabolites characterised by extraction and chromatographic behaviour, all of them ≤ 1.7% of the TRR and < 0.012 mg eq/kg

Table 6.6.1-12: Residues in RACs after a 280 day plant back interval (3rd rotation, pyridyl-label)

3. Rotation	Wheat forage		Wheat hay		Wheat straw		Wheat grains	
TRR [mg eq/kg] =	0.167		0.709		1.622		0.037	
Compound	% TRR	mg eq/ kg	% TRR	mg eq/ kg	% TRR	mg eq/ kg	% TRR	mg eq/ kg
AE C656948, a.s. fluopyram	70.2	0.118	66.8	0.473	58.3	0.945	31.0	0.012
fluopyram-methyl-sulfoxide (M45)	2.0	0.003	3.5	0.025	1.2	0.019	20.3	0.008
fluopyram-pyridyl-carboxylic acid (M43)	0.9	0.002	0.5	0.004	-	-	28.6	0.011
fluopyram-7-hydroxy-glc-MA (M12)	7.5	0.013	9.5	0.067	7.3	0.118	-	-
fluopyram-7-hydroxy-glc-MA (M12)	1.1	0.002	1.2	0.008	4.5	0.072	-	-
fluopyram-8-hydroxy-glc-MA (M19)	1.7	0.003	1.7	0.012	1.2	0.019	-	-
fluopyram-7-OH-SA (M10)	-	-	-	-	-	-	-	-
fluopyram-7-hydroxy-glc (M11)	3.0	0.005	3.0	0.021	3.7	0.060	-	-
fluopyram-phenol-glc (M06)	-	-	-	-	-	-	-	-
fluopyram-7-hydroxy (M08)	5.6	0.009	8.6	0.061	10.1	0.164	2.3	0.001
fluopyram-8-hydroxy (M18)	-	-	-	-	1.7	0.028	-	-
Total identified	92.0	0.154	95.4	0.671	87.9	1.425	82.2	0.031
Characterized by HPLC retention*	0.4	0.001	0.6	0.004	2.1	<0.001	-	-
Characterized by diastase treatment	-	-	-	-	-	-	-	-
Total extractable	92.4	0.155	95.9	0.679	91.0	1.477	82.2	0.031
Total bound residues (PES)	7.6	0.013	4.1	0.029	9.0	0.146	17.8	0.007
Not analysed	-	-	0.5	0.004	1.0	0.016	-	-
Accountability	100.0	0.167	100.0	0.709	100.0	1.622	100.0	0.037

3. Rotation	Swiss chard		Turnip leaves		Turnip roots	
TRR [mg eq/kg] =	0.211		0.095		0.012	
Compound	% TRR	mg eq/ kg	% TRR	mg eq/ kg	% TRR	mg eq/ kg
AE C656948, a.s.	38.6	0.081	64.2	0.061	91.7	0.010
fluopyram methyl-sulfoxide (M45)	0.5	0.001	-	-	-	-
fluopyram pyridyl-carboxylic acid (M43)	0.2	0.000	1.1	0.001	-	-
fluopyram 7-hydroxy-glc-MA (M12), isomer 1	-	-	1.7	0.002	-	-
fluopyram 7-hydroxy-glc-MA (M12), isomer 2	-	-	1.9	0.002	-	-
fluopyram 8-hydroxy-glc-MA (M19)	-	-	-	-	-	-
fluopyram 7-OH-SA (M10)	13.5	0.029	0.4	0.000	-	-
fluopyram 7-hydroxy-glc (M11)	1.3	0.003	1.6	0.001	-	-
fluopyram phenol-glc (M06)	-	-	14.7	0.014	-	-
fluopyram 7-hydroxy (M08)	38.6	0.081	8.3	0.008	6.1	0.001
fluopyram 8-hydroxy (M18)	1.3	0.003	0.4	0.000	-	-
Total identified	94.0	0.198	94.2	0.090	97.8	0.012
Characterized by HPLC retention*	4.2	0.009	4.5	0.004	-	-
Characterized by diastase treatment	-	-	-	-	-	-
Total extractable	98.2	0.207	98.7	0.094	97.8	0.011
Total bound residues (PES)	-	-	-	-	-	-
Not analysed	1.8	0.004	1.3	0.001	2.2	< 0.001
Accountability	100.0	0.211	100.0	0.095	100.0	0.012

* up to 13 minor metabolites characterised by extraction and chromatographic behaviour, all of them ≤ 1.3% of the TRR and < 0.022 mg eq/kg

III. Conclusions

After application of [pyridyl-2,6-^{14}C]fluopyram on bare soil, most of the recovered radioactivity was detected in the dry leaf RACs wheat straw (1.622–6.663 mg eq/kg) and hay (0.709–1.802 mg eq/kg). Medium amounts of radioactivity were found in the fresh leaf RACs wheat forage (0.157–0.568 mg eq/kg), Swiss chard (0.211–0.570 mg eq/kg) and turnip leaves (0.095–0.565 mg eq/kg), but also wheat grain of the1st rotation (0.412 mg eq/kg).

Only minor residues were detected in grain of the 2nd and 3rd rotation (0.037–0.072 mg eq/kg) and turnip roots (0.010–0.036 mg eq/kg). A significant decline (factor of 2 to 6) of the radioactive residues was observed for all RACs except wheat forage and hay, where the residues remained at a similar level.

In total, 27 components were detected in the various samples of this study, and 11 of them, including parent and major metabolites, were identified.

Apart from wheat grain, the parent compound was the main compound in all RACs of all rotations and covered 57–86% of the TRR in the RACs of the 1st rotation, 37–95% of the TRR in the RACs of the 2nd rotation and 39–92% of the TRR in the RACs in the 3rd rotation. In wheat grain it accounted for 20.4–33.4% of the TRR in the three rotations.

In wheat grain, two label specific metabolites represented by fluopyram-methyl-sulfoxide and fluopyram-pyridyl carboxylic acid were main metabolites. The residues of these metabolites were in the range of 50–65% of the TRR. fluopyram-methyl-sulfoxide amounted to 1.2%–49.0%–20.3% of the TRR (1st–2nd–3rd rotation) while fluopyram-pyridyl carboxylic acid represented 55.9%–16.4%–28.6% of the TRR (1st–2nd–3rd rotation). The relative high amount of fluopyram-pyridyl-carboxylic acid in wheat grains is considered to be the result of the phloem transport into the grains (as a phloem sink) rather than the result of metabolism of the parent compound in this compartment, in analogy to the behaviour of other organic acids in higher plants.

In the other RACs these label specific metabolites were of less importance accounting for ≤ 6% (sum of both) of the TRRs. However, fluopyram-pyridyl carboxylic acid was a prominent metabolite in wheat forage of the 1st rotation accounting for 17% of the TRR but decreased to < 1% of the TRR in the following rotations.

Fluopyram-7-hydroxy and its various conjugates with glucose, malonic acid (2 isomers) and sulphuric acid were important metabolites mainly in Swiss chard, where fluopyram-7-hydroxy increased from 28% to about 39% of the TRR. In the other RACs, the amount of fluopyram-7-hydroxy was distinctively lower than in Swiss chard, only 2–7% of the TRR in the 1st rotation but also increasing from rotation to rotation up to 2–10%.

The sulphuric acid conjugate of fluopyram-7-hydroxy, fluopyram-7-OH-SA, was also a prominent metabolite in Swiss chard increasing from 8% of TRR in the 1st rotation to 17% and 14% of the TRR in the 2nd and 3rd rotation. The sum of the four 7-hydroxy conjugates reached a maximum of fraction of the TRR of 18% in Swiss chard of the 2nd rotation. The maximum absolute amount of 0.652 mg eq/kg was reached in wheat straw in the 1st rotation.

fluopyram-8-hydroxy and its conjugate were only of minor importance. Both or at least one of them were detected in all RACs but at levels < 2.9% of the TRR in sum.

fluopyram-phenol-glc was detected in turnip leaves only, where it amounted to 12%, 18% and 15% of the TRR of the 1st, 2nd and 3rd rotation, respectively.

The main metabolic transformations detected were:

- hydroxylation of the ethyl linking group of the parent compound forming fluopyram-7-hydroxy and -8-hydroxy metabolites
- hydroxylation of the phenyl ring and subsequent conjugation with glucose leading to fluopyram-phenol-glc (turnip leaves only)
- conjugation of the hydroxylated metabolites with glucose, malonic acid and sulphuric acid
- hydrolytic cleavage and subsequent oxidation to fluopyram-pyridyl carboxylic acid and fluopyram-methyl sulfoxide
- formation of polar, probably natural compounds which were incorporated into the starch matrix of grains

The proposed metabolic pathway for [pyridyl-2,6-^{14}C]fluopyram in rotational crops is given in Figure 6.6.1-2.

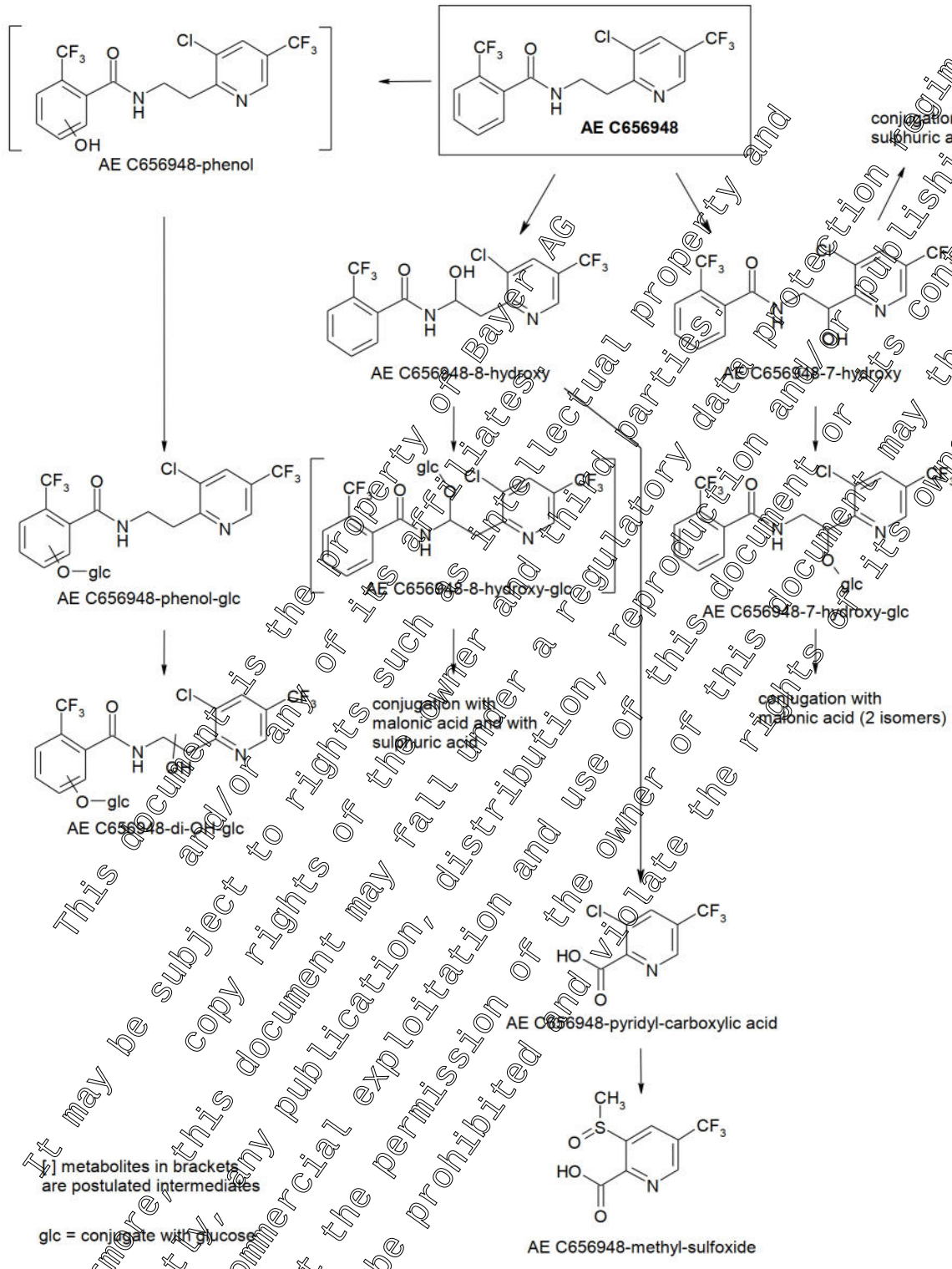


Figure 6.6.1-2: Proposed metabolic pathway of [pyridyl-2,6-^{14}C]AE C656948 (fluopyram) in confined rotational crops (CRC)

Assessment and conclusion by applicant:

The study is valid and acceptable.

CA 6.6.2 Magnitude of residues in rotational crops

Risk assessment residue definition (EFSA Journal 2013;11(4):3052)

In food of plant origin : Sum of fluopyram and fluopyram-benzamide (M25), expressed as fluopyram

Additional analytical targets in rotational field residues trials:

- Rotational crops: FLU-PCA, FLU-7-OH and FLU-methyl-sulfoxide.
- Rotational crops since 2019**:** FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide and TFA

During the first submission of fluopyram in EU in 2009 (DAR, 2014), rotational crop studies were submitted and are summarized again in the present dossier (KCA 6.6.2/01 to KCA 6.6.2/07).

Some trials were conducted in the USA as the dossier was a joined review (KCA 6.6.2/05 to KCA 6.6.2/07). These US studies are not summarized here as EU data are required for this renewal dossier. They are overruled by studies KCA 6.6.2-01 to KCA 6.6.2-04 and by KCA 6.6.2-08 to KCA 6.6.2-25.

According to the GAPs supported at that time, the trials were conducted at 500g ai/ha following either a soil application or a primary crop application. An overview of these 4 EU studies (12 trials) is presented in Table 6.6.2- 1.

Additional trials were conducted in EU at the same dose for different crops after a plant back interval around 30 days (KCA 6.6.2/08 to KCA 6.6.2/25). An overview of these 18 studies (35 trials) is presented in Table 6.6.2- 2.

Considering the whole package of residue results from the trials conducted at 500 g ai/ha, median residues values were calculated and are presented in Table 6.6.2- 3. Rotational crops field trials were conducted at a dose rate of application covering the max PECsoil for parent (~ 1.2N) (Germany, 2011; EFSA 2014; EFSA 2020).

As the limited crop rotational studies show residues in some of the trials and according to the OECD guidance document on residues in rotational crops ENV/JM/MONO(2018)9 from 22 May 2018, a batch of extended rotational trials started in 2019. Studies are conducted on carrot (4 trials), leek (4 trials), wheat (4 trials), corn (4 trials), lettuce (4 trials), cabbage (4 trials), broccoli (4 trials), rape (4 trials), pea (4 trials), strawberry (4 trials), cucumber (4 trials) and potato (4 trials).

At the start of the trials, some uncertainty remained around the new PECsoil value to calculate the long-term residue accumulation plateau. Thus, an exaggerated application dose rate of 1200 g ai/ha was selected.

In addition to the fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (AE C657188), fluopyram-pyridyl-acetic acid, fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122) the analysis of the trifluoroacetic acid (TFA) metabolite are conducted in the 2019 study package because this compound may be generated in soil.

As agreed with the RMS, an updated MCA section 06 document will be submitted in January 2022 when the final reports are available for 12 studies. The 3 remaining studies (wheat (2 trials), rape (2 trials), strawberry (2 trials)) will be made available in Q2 2022.

Table 6.6.2- 1: Overview of EU trials from 1st submission (3 rotations)

reference	author (report date)	formu-lation	Trial region plots	application on...	dose ai.ha	rot. nb	crop	PBI (days)	FLU (mg/kg)	FLU-benzamide (mg/kg)	FLU-PCA (mg/kg)	FLU-7-OH (mg/kg)	FLU-methyl-sulfoxide (mg/kg)
RA-2649/06 M-296608-01-1	[redacted] (21/01/2008)	FLU 500SC	1 NEU 3 plots	soil (≤ 8cm)	500g	3	turnip	30	0.01-0.04 leaf	<0.01	<0.01	<0.01	<0.01
							lettuce	30	0.02-0.11	<0.01	<0.01	<0.01	<0.01
							wheat	30	0.28 straw	<0.01	<0.01	0.11 straw	0.09 grain 0.07 straw
				Lettuce	500g		turnip	90	0.03 leaf	<0.01	<0.01	<0.01	<0.01
							lettuce	90	0.01	<0.01	<0.01	<0.01	<0.01
							wheat	146	0.17 straw	<0.01	<0.01	0.10-0.17 straw	0.05 grain
				Lettuce	500g		turnip	320	0.01 leaf	<0.01	<0.01	<0.01	<0.01
							lettuce	320	0.01	<0.01	<0.01	<0.01	<0.01
							wheat	286	0.06 straw	<0.01	<0.01	<0.01	0.02 grain
RA-2648/06 M-296625-02-1	[redacted] (26/09/2008)	FLU 500SC	1 NEU 3 plots	soil (≤ 8cm)	500g	3	turnip	30	0.02 leaf 0.02 root	<0.01	<0.01	<0.01	<0.01
							lettuce	30	0.01-0.03	<0.01	<0.01	<0.01	<0.01
							wheat	28	0.07 straw	<0.01	<0.01	<0.01	0.01 grain
				Lettuce	500g		turnip	216	0.04 leaf 0.02 root	<0.01	<0.01	<0.01	<0.01
							lettuce	230	0.01	<0.01	<0.01	<0.01	<0.01
							wheat	100	0.09 straw	<0.01	<0.01	0.05 straw	0.04 grain
				Lettuce	500g		turnip	301	0.01 leaf	<0.01	<0.01	<0.01	<0.01
							lettuce	301	0.01	<0.01	<0.01	<0.01	<0.01
							wheat	363	0.01	<0.01	<0.01	<0.01	<0.01

reference	author (report date)	formu-lation	Trial region plots	application on...	dose ai.ha	rot. nb	crop	PBI	FLU (mg/kg)	FLU-benzamide (mg/kg)	FLU-PCA (mg/kg)	FLU-7-OH (mg/kg)	FLU-methyl-sulfoxide (mg/kg)
RA-2650/06 M-296652-02-1	[redacted] (29/09/2008)	FLU 500SC	1 SEU 3 plots	soil (≤ 8cm)	500g	3	carrot	30	0.02-0.04 leaf 0.05 root	<0.01	<0.01	<0.01	<0.01
							lettuce	30	0.03-0.09	<0.01	<0.01	<0.01	<0.01
							wheat	30	0.01 grain 0.15 straw	<0.01	<0.01	0.08 straw	0.03 grain
				Lettuce	500g		carrot	240	0.01 leaf 0.02 root	<0.01	<0.01	<0.01	<0.01
							lettuce	240	0.02	<0.01	<0.01	<0.01	<0.01
							wheat	120	<0.01	<0.01	<0.01	<0.01	0.02 grain
							carrot	289	0.01 leaf 0.02 root	<0.01	<0.01	<0.01	<0.01
							lettuce	290	0.01-0.03	<0.01	<0.01	<0.01	<0.01
							wheat	363	0.08 straw	<0.01	<0.01	0.06 straw	<0.01
RA-2651/06 M-296671-02-1	[redacted] (30/09/2008)	FLU 500SC	1 SEU 3 plots	soil (≤ 8cm)	500g	3	carrot	36	0.01 leaf 0.02 root	<0.01	<0.01	<0.01	<0.01
							lettuce	30	<0.01	<0.01	<0.01	<0.01	<0.01
							wheat	49	0.05 straw	0.14 straw	<0.01	<0.01	0.01 grain 0.06 straw
				Lettuce	500g		carrot	154	0.02 leaf 0.03 root	<0.01	<0.01	<0.01	<0.01
							lettuce	155	<0.01	<0.01	<0.01	<0.01	<0.01
							wheat	154	0.19 straw	<0.01	<0.01	0.05 straw	0.04 grain
							carrot	344	0.01 leaf/root	<0.01	<0.01	<0.01	<0.01
							lettuce	347	<0.01	<0.01	<0.01	<0.01	<0.01
							wheat	357	<0.01	0.05 straw	<0.01	<0.01	0.03 grain

Table 6.6.2- 2: Overview of not peer reviewed EU trials

reference	author (report date)	formu-lation	Trial region plots	application	dose ai.ha	rot. nb	crop	PBI	FLU (mg/kg)	FLU-benzamide (mg/kg)	FLU-PCA (mg/kg)	FLU-7-OH (mg/kg)	FLU-methyl-sulfoxide (mg/kg)
08-2160 M-350816-01-1	[redacted] (03/07/2009)	FLU 500SC	2 NEU	soil (≤ 8cm)	500g	1	potato	28-30	0.01-0.02	<0.01	<0.01	<0.01	<0.01
08-2171 M-350747-01-1	[redacted] (03/07/2009)	FLU 500SC	2 SEU	soil (≤ 8cm)	500g	1	potato	30-31	<0.01	<0.01	0.01	<0.01	<0.01
08-2161 M-352225-01-1	[redacted] (21/01/2009)	FLU 500SC	2 NEU	soil (≤ 8cm)	500g	1	onion	28-30	<0.01	<0.01	<0.01	<0.01	<0.01
08-2172 M-355319-01-1	[redacted] (07/09/2009)	FLU 500SC	2 SEU	soil (≤ 8cm)	500g	1	onion	29-31	<0.01	<0.01	<0.01	<0.01	<0.01
08-2165 M-352213-01-1	[redacted] (23/07/2009)	FLU 500SC	2 NEU	soil (≤ 8cm)	500g	1	tomato	28-30	<0.01	<0.01	<0.01	<0.01	<0.01
08-2176 M-355320-02-1	[redacted] (07/09/2009)	FLU 500SC	2 SEU	soil (≤ 8cm)	500g	1	tomato	30	<0.01	<0.01	<0.01	<0.01	<0.01
08-2167 M-354235-01-1	[redacted] (25/08/2009)	FLU 500SC	2 NEU	soil (≤ 8cm)	500g	1	pea	28-30	<0.01	<0.01	0.02 dry	<0.01	<0.01
08-2178 M-354237-01-1	[redacted] (25/08/2009)	FLU 500SC	2 SEU	soil (≤ 8cm)	500g	1	pea	28	<0.01	<0.01	0.02 green 0.03 dry	<0.01	<0.01
08-2168 M-355324-01-1	[redacted] (07/09/2009)	FLU 500SC	2 NEU	soil (≤ 8cm)	500g	1	corn	31-32	0.03 BBCH34	<0.01	<0.01	<0.01	<0.01
08-2179 M-355327-01-1	[redacted] (07/09/2009)	FLU 500SC	2 SEU	soil (≤ 8cm)	500g	1	corn	28-34	0.05-0.07 BBCH34	0.01 BBCH34	<0.01	0.03 BBCH34	<0.01

reference	author (report date)	formu-lation	Trial region plots	application	dose ai.ha	rot. nb	crop	PBI	FLU (mg/kg)	FLU-benzamide (mg/kg)	FLU-PCA (mg/kg)	FLU-7-OH (mg/kg)	FLU-methyl-sulfoxide (mg/kg)
08-2164 M-357777-01-1	[redacted] (22/10/2009)	FLU 500SC	2 NEU	soil (≤ 8cm)	500g	1	leek	30-31	0.03-0.04	<0.01	<0.01	<0.01	<0.01
08-2175 M-357762-01-1	[redacted] (22/10/2009)	FLU 500SC	2 SEU	soil (≤ 8cm)	500g	1	leek	29	0.02	<0.01	0.02	<0.01	<0.01
08-2166 M-357953-01-1	[redacted] (27/10/2009)	FLU 500SC	2 NEU	soil (≤ 8cm)	500g	1	strawberry	31-32	<0.01	<0.01	<0.01	<0.01	<0.01
08-2177 M-357930-01-1	[redacted] (27/10/2009)	FLU 500SC	2 SEU	soil (≤ 8cm)	500g	1	strawberry	28	<0.01	<0.01	<0.01	<0.01	<0.01
08-2071 M-357943-01-1	[redacted] (27/10/2009)	FLU 500SC	2 NEU	soil (≤ 8cm)	500g	1	spinach	28-29	0.03	0.02-0.08	<0.01	0.05-0.26	<0.01
08-2170 M-357959-01-1	[redacted] (27/10/2009)	FLU 500SC	2 SEU	soil (≤ 8cm)	500g	1	spinach	28-29	0.09	0.01-0.05	<0.01	0.04-0.22	<0.01
08-2169 M-350532-02-1	[redacted] (10/09/2009)	FLU 500SC	1 NEU	soil (≤ 8cm)	500g	1	Summer rape	33	0.01	<0.01	<0.01	<0.01	<0.01
08-2180 M-359808-01-1	[redacted] (02/12/2009)	FLU 500SC	2 SEU	soil (≤ 8cm)	500g	1	Winter rape	29-30	0.01	<0.01	<0.01	<0.01	<0.01

Table 6.6.2- 3: Result overview of all EU rotational trials conducted at 500 g ai/ha

Gap at 500 g ai/ha		FLU		FLU-benzamide		FLU-PCA		FLU-7-OH		FLU-methyl-sulfoxide		Total residue calc	
	Rotation	STMR	HR	STMR	HR	STMR	HR	STMR	HR	STMR	HR	STMR	HR
Corn GM	R1	<0.01	<0.01	0.06	0.07	0.0.1	0.01	<0.01	<0.01	<0.01	<0.01	0.05	0.08
Corn kernel	R1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	<0.02
Corn milky	R1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	<0.02
Tomato	R1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	<0.02
Spinach*	R1	0.01	0.07	0.02	0.08	<0.01	<0.01	0.07	0.10	<0.01	<0.01	0.075	0.13
Potato*	R1	0.02	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.03	0.03
Onion*	R1	0.01	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	0.03
Leek*	R1	0.025	0.04	<0.01	<0.01	0.01	0.02	<0.01	<0.01	<0.01	<0.01	0.03	0.05
Pea green seed	R1	<0.01	<0.01	<0.01	<0.01	0.02	0.02	<0.01	<0.01	<0.01	<0.01	<0.02	<0.02
Pea dry seed	R1	<0.01	<0.01	<0.01	<0.01	0.025	0.03	<0.01	<0.01	<0.01	<0.01	<0.02	<0.02
Strawberry	R1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	<0.02
Rape pod	R1	0.02	0.06	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.03	0.07
Rape seed	R1	0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	0.02
Wheat grain	R1	0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	0.09	0.02	0.02
	R2	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.04	0.05	<0.02	<0.02
	R3	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.025	0.03	<0.02	<0.02
Wheat GM	R1	0.11	0.12	0.05	0.07	<0.01	<0.01	<0.01	<0.01	0.035	0.05	0.12	0.13
	R2	0.06	0.08	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	0.06	0.08	0.09
	R3	0.04	0.1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.06	0.07	0.11
Wheat straw	R1	0.11	0.28	0.05	0.14	<0.05	<0.05	0.065	0.11	0.055	0.07	0.20	0.33
	R2	0.13	0.19	<0.05	<0.05	<0.05	<0.05	0.05	0.1	<0.05	<0.05	0.14	0.22
	R3	0.055	0.08	0.05	0.05	<0.05	<0.05	0.05	0.06	<0.05	<0.05	0.11	0.13
Carrot leaves	R1	0.015	0.04	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.025	0.05
	R2	0.01	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	0.03
	R3	0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	0.02
Carrot root	R1	0.035	0.05	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.045	0.06
	R2	0.02	0.03	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.025	0.04
	R3	0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	0.03
Turnip body	R1	0.01	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	0.03
	R2	0.015	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.025	0.03
	R3	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	<0.02
Turnip leaves	R1	0.03	0.04	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.04	0.11
	R2	0.035	0.04	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.045	0.05
	R3	0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	0.02
Lettuce	R1	0.025	0.11	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.035	0.10
	R2	0.01	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	0.03
	R3	0.01	0.03	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	0.04

* : at harvest
GM : green material
R1 : 1st rotation (Plant Back Interval ±30 days), R2 : 2nd rotation (PBI range 90-240d), R3 : 3rd rotation (PBI range 286-363d)
Total residue calculated : Sum of Fluopyram + Fluopyram-benzamide

Data Point:	KCA 6.6.2/01
Report Author:	[redacted]
Report Year:	2008
Report Title:	Determination of the residues of AE C656948 in/on the field rotational crops turnip, lettuce and winter wheat after spraying of AE C656948 (500 SC) in the field in Northern France
Report No:	RA-2649/06
Document No:	M-296608-01-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC guidance working document 7524/VI/95 rev. 7 (1997-07-22) OECD Guideline for testing of Chemicals, Residues in rotational crops (limited field studies), No. 504 Equivalent to US EPA OPPTS Guideline No. 860.1900 (SEP)
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted rev. 1 to Vol 3 of DAR B7, August 2012 (reference relied on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Methods

The purpose of the study was to determine the magnitude of the relevant residues of fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188), fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122), in turnip, lettuce and wheat, grown as succeeding crops in northern Europe (Northern France) and following one spray application of fluopyram SC500 on soil or on target crop lettuce. Residues of the active substance fluopyram and its metabolites were determined in the succeeding crops only. The target crop lettuce was not analysed.

The test item was applied to the treated plots in a different manner, but the application rate of 1.0 L of test item/ha (0.5 kg a.s./ha) was the same.

For the plot A (trials R 2006 0864/0, R 2006 0865/9 and R 2006 0866/7) – the test item was applied to the bare soil followed by incorporation (maximum 8 cm depth) to avoid photodegradation. The investigated plant back interval was 30 days for all crops (turnip, lettuce and winter wheat) and corresponds to the standard plant back interval in case of crop failure.

For the plot B (trial R 2006 0867/5) – the test item was applied on lettuce as the target crop two weeks after planting. Plot B represents a plant back interval of 90-240 days.

For the plot C (trial R 2006 0868/3) – the test item was applied on lettuce as the target crop two weeks after planting. Plot C represents a plant back interval of 290-365 days.

Each Plot was subdivided into 3 subplots for the 3 crops (turnip, lettuce, cereals) to be planted as rotational crops. Explanation of the study plot designs is illustrated in the Table 6.6.2- 4.

Table 6.6.2- 4: Plot design and plant back intervals

Trial No.	Requested PBI (days) / Application on	actual Plant Back Interval (PBI)			Remarks
		Root crop DAT (days)	Leafy crop DAT (days)	Cereals DAT (days)	
R 2006 0864/0 R 2006 0865/9 R 2006 0866/7	Plot A: 30 / soil	turnip 30 (R 2006 0864/0)	lettuce 30 (R 2006 0865/9)	winter wheat 30 (R 2006 0866/7)	Application on bare soil; planting/sowing of rotational crop after 30 days
R 2006 0867/5	Plot B: 90-240 / lettuce	turnip 90*	lettuce 90*	winter wheat 146	Application on lettuce two weeks after planting; harvest/ploughing of lettuce; planting/sowing of rotational crops
R 2006 0868/3	Plot C: 290-365 / lettuce	turnip 320	lettuce 320	spring wheat 286	Application on lettuce two weeks after planting; harvest/ploughing of lettuce; cultivation break; planting/sowing of rotational crops

DAT= days after treatment
* = Due to long growing time (lettuce 8-10 weeks; turnip 12-14 weeks) and to avoid a risk of frost sowing, PBI was 90 days instead of originally requested 120 days.

In trials R 2006 0867/5 and R 2006 0868/3: on plot B, the target crop lettuce was harvested at normal harvest, 60 days after planting, on plot C lettuce was harvested after 43 days. No lettuce samples were taken for analysis.

The harvest leftovers were destroyed by grinding and ploughed in (in order to capture all possible residues and not remove them from the plot prior to planting the rotational crops).

Untreated plots were prepared before treated plots.

At the 30-day (Plot A), 90-240-day (Plot B) and 290-365-day plant back intervals (Plot C) the plots were prepared for crop planting following normal agronomic practices for each crop type in the regions. For the longer-term Plot B (90-240 days) and Plot C (290-365 days) a bare soil status had to be maintained after harvest of lettuce, i.e. no cover crop is sown.

Samples were taken, prepared in the field where necessary, transported and stored.

The first sampling of rotational crops such as turnips and lettuce was taken at early harvest (14 days prior to normal harvest) followed by sampling at normal harvest maturity for each crop type. For wheat green material was sampled at growth stage BBCH 29-30 and at normal harvest maturity (BBCH 89) (grain and straw). All sampling equipment was cleaned prior to entering any plot that was to be harvested.

The field samples from all trials were stored deep-frozen within 24 hours after sampling. All field samples were shipped by deep-freeze lorry and arrived in good condition. The field sub samples were stored in a freezer at -18 °C or below until preparation of the examination samples. For the preparation of examination samples, the deep-frozen field sub samples were shredded with dry ice in a cutter. Representative parts of the shredded samples were transferred into polystyrene boxes separately for analysis (UP samples) and archiving (UR samples) and stored at -18°C or below until analysis.

Residues of fluopyram and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide) were determined by LC-MS/MS according to method 00984 ([redacted], 05/02/2007, M-283301-01-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues of these compounds were extracted from 10 g of sample material (5 g for wheat straw) by two successive extractions using a high-speed blender with a mixture of acetonitrile:water (80:20; v:v). After centrifugation the extract volume was adjusted. Then, the extracts were diluted ten times by adding the internal standards:

- One dilution was performed under acidic conditions: this extract allowed the determination of FLU-PCA and FLU-methyl-sulfoxide.
- In parallel, another dilution was performed under basic conditions: this extract allowed the determination of FLU, FLU-benzamide and FLU-7-OH.

An aliquot of the extracts was injected into LC-MS/MS operated in Electrospray Ionization mode:

- One injection in positive electrospray ionization for the determination of FLU, FLU-benzamide and FLU-7-OH.
- Another injection in negative electrospray ionization for the determination of FLU-PCA and FLU-methyl-sulfoxide under different conditions.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels. For each calibration curve, the correlation coefficient R was above 0.99.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.05 mg/kg for wheat straw and 0.01 mg/kg for all other matrices.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev. 4.

Findings

In order to check the performance of the analytical method, recovery determinations were concurrently performed to the analyses of control and treated samples from the study in each set of analyses. The data demonstrate acceptable method performance during sample analysis. All the recovery results and details are given in the Table 6.6.2- 5.

Table 6.6.2- 5: Recovery data for fluopyram (AE C656948) and its metabolites in rotational crop matrices

Crop/Sample material	FL (mg/kg)	Single values (%)	Mean value (%)	RSD* (%)
Fluopyram (AE C656948)				
turnip leaf, normal	0.01	99	99	--
	0.10	96; 102	99	--
		Overall recovery (n=3)	**99**	**3.0**
turnip body	0.01	95; 81	88	--
	0.10	96; 104	100	--
		Overall recovery (n=4)	**94**	**10.2**
lettuce head	0.01	103	103	--
	0.10	96; 106	101	--
		Overall recovery (n=3)	**102**	**5.0**
wheat green material	0.01	85	85	--
	0.10	97	97	--
	0.2	100	100	--
		Overall recovery (n=3)	**94**	**8.4**
Wheat straw	0.05	85	85	--
	0.5	95	95	--
	2.5	95	95	--
		Overall recovery (n=3)	**92**	**6.3**
Wheat grain	0.01	75; 96	86	--
	0.10	103	103	--
		Overall recovery (n=3)	**91**	**16.0**
Fluopyram-benzamide (AEF148815)				
turnip leaf, normal	0.01	101	101	--

Crop/Sample material	FL (mg/kg)	Single values (%)	Mean value (%)	RSD* (%)
	0.10	93; 105	99	--
		Overall recovery (n=3)	**100**	**6.1**
turnip body	0.01	101; 89	95	--
	0.10	95; 99	97	--
		Overall recovery (n=4)	**96**	**5.5**
lettuce head	0.01	102	102	--
	0.10	91; 103	97	--
		Overall recovery (n=3)	**99**	**6.7**
wheat green material	0.01	84	84	--
	0.10	94	94	--
	0.5	**90**	**90**	--
		Overall recovery (n=3)	**89**	**5.6**
Wheat straw	0.05	89	89	--
	0.5	92	92	--
	2.5	89	89	--
		Overall recovery (n=3)	**90**	**1.9**
Wheat grain	0.01	89; 106	98	--
	0.10	95	95	--
		Overall recovery (n=3)	**97**	**8.9**
Fluopyram-methylsulfoxide (AE1344122)				
turnip leaf, normal	0.01	111**	111	--
	0.10	104; 109	107	--
		Overall recovery (n=3)	**108**	**3.3**
turnip body	0.10	80	80	--
	0.10	73; 82	78	--
		Overall recovery (n=3)	**78**	**6.0**
lettuce head	0.01	94	94	--
	0.10	102; 107	105	--
		Overall recovery (n=3)	**101**	**6.5**
wheat green material	0.01	133	133	--
	0.10	101	101	--
	0.5	101	101	--
		Overall recovery (n=3)	**112**	**16.5**
Wheat straw	0.05	109	109	--
	0.5	100	100	--
	2.5	99	99	--
		Overall recovery (n=3)	**103**	**5.4**
Wheat grain	0.01	98; 86	92	--
	0.1	97	97	--
		Overall recovery (n=3)	**94**	**7.1**
Fluopyram-pyridyl-carboxylic-acid (AEC 657188)				
turnip leaf, normal	0.01	105	105	--
	0.10	98; 103	101	--
		Overall recovery (n=3)	**102**	**3.5**
turnip body	0.01	86; 99	93	--
	0.10	92; 96	94	--
		Overall recovery (n=4)	**93**	**6.0**
lettuce head	0.01	78	78	--
	0.10	94; 99	97	--
		Overall recovery (n=3)	**90**	**12.1**
wheat green material	0.01	99	99	--
	0.10	88	88	--
	0.5	92	92	--
		Overall recovery (n=3)	**93**	**6.0**

Crop/Sample material	FL (mg/kg)	Single values (%)	Mean value (%)	RSD* (%)
Wheat straw	0.05	92	92	--
	0.5	92	92	--
	2.5	90	90	--
		Overall recovery (n=3)	**91**	**1.3**
Wheat grain	0.01	100; 91	96	--
	0.1	94	94	--
		Overall recovery (n=3)	**95**	**4.8**
Fluopyram-7-hydroxy (BCS-AA10065)				
Turnip leaf, normal	0.01	90	90	--
	0.10	92; 101	97	--
		Overall recovery (n=3)	**94**	**6.2**
Turnip body	0.01	94; 90	92	--
	0.10	96; 106	101	--
		Overall recovery (n=4)	**97**	**7.1**
Lettuce head	0.01	97	97	--
	0.10	96; 106	101	--
		Overall recovery (n=3)	**100**	**5.5**
winter wheat green material	0.01	79	79	--
	0.10	96	96	--
	0.5	93	93	--
		Overall recovery (n=3)	**89**	**10.2**
winter wheat straw	0.05	83	83	--
	0.5	95	95	--
	2.5	91	91	--
		Overall recovery (n=3)	**90**	**6.8**
winter wheat grain	0.01	82; 92	87	--
	0.1	100	100	--
		Overall recovery (n=3)	**91**	**9.9**

FL: fortification level, RSD - Relative Standard Deviation
* some RSDs were not calculated as there were only two individual recoveries given
** Recovery corrected with the control interference (0.0021 mg/kg); recovery not corrected = 132%
Final determination as: fluopyram Residues calculated as: fluopyram
Final determination as: FLU-benzamide Residues calculated as: fluopyram
Final determination as: FLU-methylsulfoxide Residues calculated as: fluopyram
Final determination as: FLU-PCA Residues calculated as: fluopyram
Final determination as: FLU-7OH Residues calculated as: fluopyram

No residue of fluopyram or related metabolites were found above the LOQs in any of the control samples of rotational crop matrices analysed.

The residue levels of fluopyram and its relevant metabolites in the rotational crop matrices of turnip, lettuce and wheat are summarized in the tables below.

The storage period of deep-frozen samples was up to 342 days for lettuce, 310 days for turnip leaves, 308 days for turnip body, 169 days for wheat green material, 57 days for wheat grain and 66 days for wheat straw.

Residue measured in the relevant succeeding crop matrices are summarized below and overall trials and residue summaries are presented in the Table 6.6.2- 6.

Residues in Turnip

In trial R 2006 0864/0 (plot A, PBI 30 days), residues of fluopyram (AE C656948) were 0.10 and 0.04 mg/kg in turnip leaf 91 and 105 days after treatment (DAT), respectively. In turnip body the residues of fluopyram were <0.01 mg/kg at DAT 91 and 105.

The residues of FLU-benzamide, FLU-methylsulfoxide, FLU-PCA, FLU-7OH in all turnip matrices were <0.01 mg/kg.

In trial R 2006 0867/5 (plot B, PBI 90 days) residues of fluopyram (AE C656948) were 0.03 mg/kg in turnip leaf, normal at DAT 91 and 105 and in turnip body residues of fluopyram were <0.01 mg/kg.

The residues of FLU-benzamide, FLU-methylsulfoxide, FLU-PCA, FLU-7OH in all turnip matrices were <0.01 mg/kg.

In trial R 2006 0868/3 (plot C, PBI 320 days) residues of fluopyram (AE C656948) were 0.01 mg/kg in turnip leaf at DAT 381 and in turnip body residues of fluopyram were < 0.01 mg/kg. The residues of FLU-benzamide, FLU-methylsulfoxide, FLU-PCA, FLU-7OH in all turnip matrices were <0.01 mg/kg.

Residues in Lettuce

In trial R 2006 0865/9 (plot A, PBI 30 days), residues of fluopyram (AE C656948) were 0.11 mg/kg and 0.02 mg/kg in lettuce head 59 and 73 days after treatment (DAT), respectively.

The residues of FLU-benzamide, FLU-methylsulfoxide, FLU-PCA, FLU-7OH in all lettuce matrices were <0.01 mg/kg.

In trial R 2006 0867/5 (plot B, PBI 90 days) residues of fluopyram (AE C656948) were 0.01 mg/kg in lettuce head samples at DAT 129 and 143. The residues of FLU-benzamide, FLU-methylsulfoxide, FLU-PCA, FLU-7OH in all lettuce matrices were <0.01 mg/kg.

In trial R 2006 0868/5 (plot C, PBI 320 days) residues of fluopyram (AE C656948) were <0.01 mg/kg in lettuce head samples at DAT 354 and 368. The residues of FLU-benzamide, FLU-methylsulfoxide, FLU-PCA, FLU-7OH in all lettuce matrices were <0.01 mg/kg.

Residues in Wheat

In trial R 2006 0866/7 (plot A, PBI 30 days), residues of fluopyram (AE C656948) were 0.12 mg/kg (in green material), 0.28 mg/kg (in wheat straw), and <0.01 mg/kg in wheat grain at DAT (days after treatment), 191, 308, and 308, respectively.

The residues of FLU-benzamide and FLU-PCA in all three wheat matrices were <LOQ.

The residues of FLU-methylsulfoxide were at 0.05 mg/kg in green material (DAT 191), 0.07 mg/kg in straw (DAT 308), 0.09 mg/kg in grain (DAT 308).

The residues of FLU-7OH were <0.01 mg/kg in wheat green material and grain and 0.11 mg/kg in wheat straw.

In trial R 2006 0867/5 (plot B, PBI 146 days), residues of fluopyram (AE C656948) were 0.05 mg/kg (in green material), <0.01 mg/kg (in wheat grain), and 0.17 mg/kg in wheat straw at DAT (days after treatment), 307, 425, and 425, respectively.

The residues of FLU-benzamide and FLU-PCA in all three wheat matrices were <LOQ.

The residues of FLU-methylsulfoxide were at 0.06 mg/kg in green material (DAT 307), 0.05 mg/kg in grain (DAT 425), <0.05 mg/kg in straw (DAT 425).

The residues of FLU-7OH were <0.01 mg/kg in wheat green material and grain and 0.10 mg/kg in wheat straw.

In trial R 2006 0868/5 (plot C, PBI 286 days)

residues of fluopyram (AE C656948) were 0.10 mg/kg (in green material), <0.01 mg/kg (in wheat grain), and 0.06 mg/kg in wheat straw at DAT (days after treatment), 336, 425, and 425, respectively.

The residues of FLU-benzamide, FLU-PCA and FLU-7OH in all three wheat matrices were <LOQ.

The residues of FLU-methylsulfoxide were at 0.01 mg/kg in green material (DAT 336), 0.02 mg/kg in grain (DAT 425), <0.05 mg/kg in straw (DAT 425).

Conclusion

Following one spray application conducted with fluopyram SC500 to bare soil or lettuce as a target crop at a rate of 0.5 kg a.s./ha, residues in succeeding crops were analysed for fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA; AE C657188) and fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122) using a validated selective HPLC-MS/MS analytical method.

These data show that residues in turnip, lettuce and wheat as succeeding crop are adequately covered by the current EU MRL.

Assessment and conclusion by applicant:
The study is acceptable

Table 6.6.2- 6: Results of rotational crop trials conducted with fluopyram

Trial No./ Location/ Year	Plot (commodity)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment to bare soil or target crop			Dates of treatment or no. of treatments and last date	Portion analysed	Growth stage at sam-pling	Residues (mg/kg)						PHI (days)
			kg a.s./ha	Water (L/ha)	g a.s./hl				AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residue calc.	
	(a)	(b)				(c)		(d)							(e)
R 2006 0864/0 0864-06 / 01 France, north F-95000 Cergy Europe, North F 2006	**Plot A** Application to bare soil **PBI: 30 d** Rotational crop: Turnip, edible (Aramis)	1) 05.07.2006 3) 15.09.2006 - 30.09.2006	0.500	300	0.1667	05.06.2006	leaves	47	0.10	<0.01	<0.01	<0.01	<0.01	0.11	91
								49	0.04	<0.01	<0.01	<0.01	<0.01	0.05	105
							body	47	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	91
								49	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	105
R 2006 0865/9 0865-06 / 01 France, north F-95000 Cergy Europe, North F 2006	**Plot A** Application to bare soil **PBI : 30 d** Rotational crop: Lettuce (Estelle)	1) 05.07.2006 3) 14.08.2006 - 25.08.2006	0.500	300	0.1667	05.06.2006	head	47	0.11	<0.01	<0.01	<0.01	<0.01	0.12	59
								49	0.02	<0.01	<0.01	<0.01	<0.01	0.03	73
R 2006 0866/7 0866-06 / 01 France, north	**Plot A** Application to bare soil **PBI: 30 d**	1) 26.10.2006	0.500	300	0.1667	26.09.2006	green material	30	0.12	<0.01	<0.01	<0.01	0.05	0.13	191
							grain	89	<0.01	<0.01	<0.01	<0.01	0.09	<0.02	308

Trial No./ Location/ Year	Plot (commodity)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment to bare soil or target crop			Dates of treatment or no. of treatments and last date	Portion analysed	Growth stage at sampling	Residues (mg/kg)						PHI (days)
			kg a.s./ha	Water (L/ha)	g a.s./hL				AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residue calc.	
	(a)	(b)				(c)		(d)							(e)
F-95000 Cergy Europe, North F 2006	Rotational crop: Wheat, winter						straw	89	0.28	<0.05	<0.05	0.11	0.07	0.33	308
R 2006 0867/5 0867-06 / 02 France, north F-95000 Cergy Europe, North F 2006	**Plot B:** Target crop: lettuce, Admiration **PBI: 90*** days Rotational crop: Turnip edible Stanis	1) 19.05.2006 (lettuce) 1) 31.08.2006 (turnip)	0.500	300	0.1667	02.06.2006	Leaves	47	0.03	<0.01	<0.01	<0.01	<0.01	0.04	151
								49	0.03	<0.01	<0.01	<0.01	<0.01	0.04	165
							Body	47	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	151
								49	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	165
	Plot B : Target crop: lettuce, Admiration **PBI: 90 d** Rotational crop: Lettuce, Estelle	1) 19.05.2006 (lettuce) 1) 31.08.2006 (lettuce)	0.500	300	0.1667	02.06.2006	head	48	0.01	<0.01	<0.01	<0.01	<0.01	<0.02	129
								49	0.01	<0.01	<0.01	<0.01	<0.01	<0.02	143
	Plot B:	1) 19.05.2006 (lettuce)	0.500	300	0.1667	02.06.2006	green material	30	0.05	<0.01	<0.01	<0.01	0.06	0.06	307

Trial No./ Location/ Year	Plot (commodity)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment to bare soil or target crop			Dates of treatment or no. of treatments and last date	Portion analysed	Growth stage at sampling	Residues (mg/kg)						PHI (days)
			kg a.s./ha	Water (L/ha)	g a.s./hL				AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residue calc.	
	(a)	(b)				(c)		(d)							(e)
	Target crop: lettuce, Admiration **PBI: 146** days Rotational crop: Wheat, winter PR 22 R2	1) 26.10.2006 (wheat)					grain	89	<0.01	<0.01	<0.01	<0.01	0.05	<0.02	425
							straw	89	0.17	<0.05	<0.05	0.10	<0.05	0.22	425
R 2006 0868/3 0868-06 / 03 France, north F-95000 Cergy Europe, North F 2006	**Plot C** Target crop : lettuce, Admiration **PBI: 320 d** Rotational crop: Turnip, edible Atlantis	1)19.05.2006 (lettuce) 1)18.04.2007 (turnip) 3) 14.08.2006 – 25.08.2006 (lettuce)	0.500	300	0.1667	02.06.2006	leaves	47	0.01	<0.01	<0.01	<0.01	<0.01	<0.02	381
							body	47	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	381
								49	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	397
	Plot C Target crop : lettuce, Admiration **PBI: 320 d** Rotational crop: Lettuce, Estelle	1) 18.04.2007	0.500	300	0.1667	02.06.2006	head	41	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	354
								49	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	368
	Plot C	1) 13.03.2007	0.500	300	0.1667	02.06.2006	green material	30	0.10	<0.01	<0.01	<0.01	0.01	0.11	336

Trial No./ Location/ Year	Plot (commodity)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment to bare soil or target crop			Dates of treatment or no. of treatments and last date	Portion analysed	Growth stage at sampling	Residues (mg/kg)						PHI (days)
			kg a.s./ha	Water (L/ha)	g a.s./hL				AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residue calc.	
	(a)	(b)				(c)		(d)							(e)
	Target crop : lettuce, Admiration **PBI: 286 d** Rotational crop: Wheat, winter Panifor R1						grain	89	<0.01	<0.01	<0.01	<0.01	0.02	<0.02	425
							straw	89	0.06	<0.05	<0.05	<0.05	<0.05	0.11	425

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions, Reference to analytical method and information which metabolites are included
(g) Study reference
* prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

*Due to long growing time (lettuce 8-10 weeks; turnip 12-14 weeks), and therefore the risk of frost sowing took place earlier, at 90 days after treatment instead of originally requested 120 days.

Total residue calculated : sum of fluopyram+FLU-benzamide

Data Point:	KCA 6.6.2/02
Report Author:	[redacted]
Report Year:	2008
Report Title:	Determination of the residues of AE C656948 in/on the field rotational crops turnip, lettuce and winter wheat after spraying of AE C656948 (500 SC) in the field in Germany
Report No:	RA-2648/06
Document No:	M-296625-02-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC guidance working document 7524/VI/95 rev. 2 (1997-07-22) OECD Guideline for testing of chemicals; Residues in rotational crops (limited field studies), No. 504
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted rev. 1 to Vol. 3 of DAR B7 August 2012 (reference cited in)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and Methods

The purpose of the study was to determine the magnitude of the relevant residues of fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148159), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188) and fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122) in turnip, lettuce and wheat, grown as rotational crops in northern Europe (Germany) following one spray application of fluopyram SC500 on soil or on target crop lettuce. Residues of the active substance fluopyram and its metabolites were determined in the succeeding crops only. The target crop lettuce was not analysed.

The test item was applied to the treated plots in a different manner, but the application rate of 1.0 L of test item/ha (0.5 kg a.s./ha) was the same.

For the plot A (trials R 2006 0858/6, R 2006 0859/4 and R 2006 0860/8) – the test item was applied to the bare soil followed by incorporation (maximum 8 cm depth) to avoid photodegradation. The investigated plant back interval was 30 days for all crops (turnip, lettuce and wheat) and corresponds to the standard plant back interval in case of crop failure.

For the plot B (trial R 2006 0861/6) – the test item was applied on lettuce as the target crop two weeks after planting. Plot B represents a plant back interval of 100-240 days.

For the plot C (trial R 2006 0862/4) – the test item was applied on lettuce as the target crop two weeks after planting. Plot C represents a plant back interval of 290-365 days.

Plots B and C were subdivided into 3 subplots for the 3 crops (turnip, lettuce, cereals) to be planted as rotational crops. Explanation of the study plot designs is illustrated in the

Table 6.6.2- 7.

Table 6.6.2- 7: Plot design and plant back intervals

Trial No.	Requested PBI (days) / Application on	actual Plant Back Interval (PBI)			Remarks
		Root crop DAT (days)	Leafy crop DAT (days)	Cereals DAT (days)	
R 2006 0858/6 R 2006 0859/4 R 2006 0860/8	Plot A: 30 / soil	turnip 30 (R 2006 0864/0)	lettuce 30 (R 2006 0865/9)	winter wheat 28 (R 2006 0866/7)	Application on bare soil; planting/sowing of rotational crop after 30 days
R 2006 0861/6	Plot B: 90-240 / lettuce	turnip 216	lettuce 230	winter wheat 190	Application on lettuce two weeks after planting; harvest/ploughing of lettuce; planting/sowing of rotational crops
R 2006 0862/4	Plot C: 290-365 / lettuce	turnip 301	lettuce 301	spring wheat 363	Application on lettuce two weeks after planting; harvest/ploughing of lettuce; cultivation break; planting/sowing of rotational crops

DAT= days after treatment

Harvest of target crop lettuce (Plot B, trial R 2006 0861/6 and Plot C trial R 2006 0862/4):
On plots B and C the target crop lettuce was harvested at normal harvest, 43 days after planting. No lettuce samples were taken for analysis.
The harvest leftovers were destroyed by grinding and ploughed in (in order to capture all possible residues and not remove them from the plot prior to planting the rotational crops.)
Planting rotational crops:
Untreated plots were prepared before treated plots.
At the 30-day (Plot A), the 100 – 240-day (Plot B) and the 290 – 365-day (Plot C) plant back intervals the plots were prepared for crop planting following normal agronomic practices for each crop type in the regions. For the longer term Plots B (100 – 240 days) and C (290 – 365 days) a bare soil status had to be maintained after harvest of lettuce, i.e. no cover crop is sown.

Samples were taken, prepared in the field where necessary, transported and stored.

The first sampling of turnips and lettuce was taken at early harvest (14 days prior to normal harvest) followed by sampling at normal harvest maturity for each crop type. For wheat green material was sampled at growth stage BBCH 29-30 and at normal harvest maturity (BBCH 89) (grain and straw). All sampling equipment was cleaned prior to entering any plot that was to be harvested.

The field samples from all trials were stored deep-frozen within 24 hours after sampling. All field samples were shipped by deep-freeze lorry and arrived in good condition. The field sub samples were stored in a freezer at ≤-18 °C until preparation of the examination samples. For the preparation of examination samples, the deep-frozen field sub samples were shredded with dry ice in a cutter. Representative parts of the shredded samples were transferred into polystyrene boxes separately for analysis (UP samples) and archiving (UR samples) and stored at ≤-18 °C until analysis.

Residues of fluopyram and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide) were determined by LC-MS/MS according to method 00984 ([redacted], 05/02/2007, M-283301-01-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues of these compounds were extracted from 10 g of sample material (5 g for wheat straw) by two successive extractions using a high-speed blender with a mixture of acetonitrile:water (80:20; *v:v*). After

centrifugation the extract volume was adjusted. Then, the extracts were diluted ten times by adding the internal standards:

- One dilution was performed under acidic conditions: this extract allowed the determination of FLU-PCA and FLU-methyl-sulfoxide.
- In parallel, another dilution was performed under basic conditions: this extract allowed the determination of FLU, FLU-benzamide and FLU-7-OH.

An aliquot of the extracts was injected into LC-MS/MS operated in Electrospray Ionization mode:

- One injection in positive electrospray ionization for the determination of FLU, FLU-benzamide and FLU-7-OH.
- Another injection in negative electrospray ionization for the determination of FLU-PCA and FLU-methyl-sulfoxide under different conditions.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels. For each calibration curve, the correlation coefficient R was above 0.99.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.05 mg/kg for wheat straw and 0.01 mg/kg for all other matrices.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Findings

In order to check the performance of the analytical method, recovery determinations were concurrently performed to the analyses of control and treated samples from the study in each set of analyses. The data demonstrate acceptable method performance during sample analysis. All the recovery results and details are given in the Table 6.6.2- 8.

Table 6.6.2- 8: Recovery data for fluopyram (AE C656948) and its metabolites in rotational crop matrices

Crop/Sample material	FL (mg/kg)	Single values (%)	Mean value (%)	RSD* (%)
Fluopyram (AE C656948)				
turnip leaf, normal	0.01	90; 93; 92; 98	93	3.6
	0.10	103; 108; 102; 96; 85	99	8.9
		Overall recovery (n=9)	96	**7.5**
turnip body	0.01	94; 100; 96; 97	97	2.6
	0.10	114; 105; 106; 99	106	5.8
		Overall recovery (n=8)	**101**	**6.5**
lettuce head	0.01	86; 97	92	--
	0.10	105	105	--
		Overall recovery (n=3)	**96**	**9.9**
wheat green material	0.01	85; 85; 83; 80	83	2.8
	0.10	92; 88; 91; 90	90	1.9
		Overall recovery (n=8)	**87**	**4.8**
wheat straw	0.05	86; 86	86	--
	0.5	89	89	--
		Overall recovery (n=3)	**87**	**2.0**
wheat grain	0.01	77; 78	78	--

Crop/Sample material	FL (mg/kg)	Single values (%)	Mean value (%)	RSD* (%)
	0.10	83	83	--
		Overall recovery (n=3)	**79**	**4.1**
Fluopyram-benzamide (AEF148815)				
turnip leaf, normal	0.01	98; 100; 107; 97	101	4.5
	0.10	105; 97; 100; 98; 100	100	3.1
		Overall recovery (n=9)	**100**	**3.5**
turnip body	0.01	101; 97; 89; 110	99	8.8
	0.10	98; 94; 100; 101	98	3.2
		Overall recovery (n=8)	**99**	**6.2**
lettuce head	0.01	95; 101	98	--
	0.10	104	104	--
		Overall recovery (n=3)	**100**	**4.6**
wheat green material	0.01	88; 81; 87; 78	84	5.7
	0.10	94; 86; 87; 92	90	4.3
		Overall recovery (n=8)	**87**	**6.0**
wheat straw	0.05	89; 107	**98**	--
	0.5	109	**109**	--
		Overall recovery (n=3)	**102**	**10.8**
wheat grain	0.01	91; 88	90	--
	0.10	124	124	--
		Overall recovery (n=3)	**101**	**19.8**
Fluopyram-methylsulfoxide (AE1344122)				
turnip leaf, normal	0.01	75; 83; 93; 95	87	10.7
	0.10	104; 104; 100; 92; 107	101	5.7
		Overall recovery (n=9)	**95**	**11.1**
turnip body	0.10	87; 84; 106	92	12.9
	0.10	101; 74; 80; 106; 74	87	17.7
		Overall recovery (n=8)	**89**	**15.2**
lettuce head	0.01	78; 90	84	--
	0.10	107	107	--
		Overall recovery (n=3)	**92**	**15.9**
wheat green material	0.01	96; 106; 95; 102	100	5.2
	0.10	79; 81; 89; 102	88	11.9
		Overall recovery (n=8)	**94**	**10.6**
wheat straw	0.05	108; 112	110	--
	0.5	107	107	--
		Overall recovery (n=3)	**109**	**2.4**
wheat grain	0.01	79; 103	91	--
	0.1	102	102	--
		Overall recovery (n=3)	**95**	**14.3**
Fluopyram-pyridyl-carboxylic-acid (AEC 657188)				
turnip leaf, normal	0.01	105; 87; 100; 101	98	7.9
	0.10	91; 91; 89; 89; 97	91	3.6
		Overall recovery (n=9)	**94**	**6.8**
turnip body	0.01	94; 80; 73; 106	88	16.7
	0.10	88; 89; 93; 110	95	10.8
		Overall recovery (n=8)	**92**	**13.4**
lettuce head	0.01	78; 96	87	--
	0.10	92	92	--
		Overall recovery (n=3)	**89**	**10.7**
wheat green material	0.01	96; 101; 95; 90	96	4.7
	0.10	79; 78; 78; 87	81	5.4
		Overall recovery (n=8)	**88**	**10.2**
wheat straw	0.05	94; 103	99	--

Crop/Sample material	FL (mg/kg)	Single values (%)	Mean value (%)	RSD* (%)
	0.5	80	80	--
		Overall recovery (n=3)	**92**	**12.6**
wheat grain	0.01	90; 81	86	--
	0.1	95	95	--
		Overall recovery (n=1)	**89**	**8.0**
Fluopyram-7-hydroxy (BCS-AA10065)				
Turnip leaf, normal	0.01	98; 93; 93; 96	95	2.6
	0.10	110; 103; 106; 95; 78	98	12.9
		Overall recovery (n=9)	**97**	**9.5**
Turnip body	0.01	87; 86; 83; 96	88	6.4
	0.10	106; 101; 98; 88	98	7.7
		Overall recovery (n=8)	**93**	**8.9**
Lettuce head	0.01	90; 97	94	--
	0.10	105	105	--
		Overall recovery (n=3)	**97**	**7.7**
wheat green material	0.01	82; 85; 82; 85	84	2.1
	0.10	97; 91; 94; 95	94	2.7
		Overall recovery (n=8)	**89**	**6.8**
wheat straw	0.05	87; 88	88	--
	0.5	96	96	--
		Overall recovery (n=3)	**90**	**5.5**
wheat grain	0.01	82; 92	87	--
	0.1	87	87	--
		Overall recovery (n=3)	**87**	**5.7**

FL: fortification level, RSD - Relative Standard Deviation
* some RSDs were not calculated as there were only two individual recoveries given
** Recovery corrected with the control interference (0.002 mg/kg), recovery not corrected = 132%
Final determination as: fluopyram Residues calculated as: fluopyram
Final determination as: FLU-benzamide Residues calculated as: fluopyram
Final determination as: FLU-methylsulfoxide Residues calculated as: fluopyram
Final determination as: FLU-PCA Residues calculated as: fluopyram
Final determination as: FLU-7OH Residues calculated as: fluopyram

No residue of fluopyram or related metabolites were found above the LOQs in any of the control samples of rotational crop matrices analysed.

The residue levels of fluopyram and its relevant metabolites in the rotational crop matrices of turnip, lettuce and wheat are summarized in the Table 6.6.2- 9.

The storage period of deep-frozen samples was up to 290 days for lettuce, 372 days for turnip leaves, 372 days for turnip body, 334 days for wheat green material, 71 days for wheat grain and 80 days for wheat straw.

Residues in Turnip

In trial R 2006 0858/6 (plot A, PBI 30 days), residues of fluopyram (AE C656948) were 0.02 mg/kg in turnip leaf, normal at DAT (days after treatment), 86 and 100. In turnip body samples the residues of fluopyram were 0.02 and 0.01 mg/kg at DAT 86 and 100, respectively.

The residues of FLU-benzamide, FLU-methylsulfoxide, FLU-PCA, FLU-7OH in all turnip matrices were <0.01 mg/kg.

In trial R 2006 0861/6 (plot B, PBI 216 days) residues of fluopyram (AE C656948) were 0.04 mg/kg in turnip leaf, normal at DAT 323 and 337, and in turnip body residues of fluopyram were 0.02 mg/kg at DAT of 323 and 337. The residues of FLU-benzamide, FLU-methylsulfoxide, FLU-PCA, FLU-7OH in all turnip matrices were <0.01 mg/kg.

In trial R 2006 0862/4 (plot C, PBI 301 days) residues of fluopyram (AE C656948) were <0.01 mg/kg and 0.01 mg/kg in turnip leaf, normal at DAT of 372 and 386, respectively. In turnip body residues of

fluopyram were <0.01 mg/kg at DAT of 372 and 386. The residues of FLU-benzamide, FLU-methylsulfoxide, FLU-PCA, FLU-7OH in all turnip matrices were <0.01 mg/kg.

Residues in Lettuce

In trial R 2006 0859/4 (plot A, PBI 30 days), residues of fluopyram (AE C656948) were 0.03 mg/kg and 0.01 mg/kg in lettuce head at DAT (days after treatment), 66 and 80, respectively.

The residues of FLU-benzamide, FLU-methylsulfoxide, FLU-PCA, FLU-7OH in all lettuce matrices were <0.01 mg/kg.

In trial R 2006 0861/6 (plot B, PBI 216 days) residues of fluopyram (AE C656948) were 0.01 mg/kg in lettuce head samples at DAT 261 and 275. The residues of FLU-benzamide, FLU-methylsulfoxide, FLU-PCA, FLU-7OH in all lettuce matrices were <0.01 mg/kg.

In trial R 2006 0862/4 (plot C, PBI 301 days) residues of fluopyram (AE C656948) were 0.01 mg/kg in lettuce head samples at DAT 338 and <0.01 mg/kg at DAT 352. The residues of FLU-benzamide, FLU-methylsulfoxide, FLU-PCA, FLU-7OH in all lettuce matrices were <0.01 mg/kg.

Residues in Wheat

In trial R 2006 0860/8 (plot A, PBI 28 days), residues of fluopyram (AE C656948) were 0.07 mg/kg (in green material), 0.07 mg/kg (in wheat straw), and <0.01 mg/kg in wheat grain at DAT (days after treatment), 190, 314, and 314, respectively.

The residues of FLU-benzamide, FLU-PCA and FLU-7OH in all three wheat matrices were below relevant LOQ. The residues of FLU-methylsulfoxide were at <0.01 mg/kg in green material (DAT 190), <0.05 mg/kg in straw (DAT 314), 0.01 mg/kg in grain (DAT 314).

In trial R 2006 0861/6 (plot B, PBI 100 days), residues of fluopyram (AE C656948) were 0.08 mg/kg (in green material), <0.01 mg/kg (in wheat grain), and 0.09 mg/kg in wheat straw at DAT (days after treatment), 240, 328, and 328, respectively.

The residues of FLU-benzamide, FLU-PCA and FLU-7OH in all three wheat matrices were below relevant LOQ.

The residues of FLU-methylsulfoxide were at 0.02 mg/kg in green material (DAT 240), 0.04 mg/kg in grain (DAT 328), <0.05 mg/kg in straw (DAT 328).

In trial R 2006 0862/4 (plot C, PBI 363 days), residues of fluopyram (AE C656948) were 0.02 mg/kg (in green material), <0.01 mg/kg (in wheat grain), and <0.05 mg/kg in wheat straw at DAT (days after treatment), 414, 691, and 691, respectively.

The residues of FLU-benzamide, FLU-methylsulfoxide, FLU-PCA, FLU-7OH in all three wheat matrices were below relevant LOQ.

Conclusion

Following one spray application conducted with fluopyram SC500 to bare soil or lettuce as a target crop at a rate of 0.5 kg a.s./ha, residues in succeeding crops were analysed for fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188) and fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122) using a validated selective HPLC-MS/MS analytical method.

These data show that residues in turnip, lettuce and wheat as succeeding crop are adequately covered by the current EU MRL.

Assessment and conclusion by applicant:
The study is acceptable

Table 6.6.2- 9: Results of rotational crop trials conducted with fluopyram

Trial No./ Location/ Year	Plot (commodity) (a)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting (b)	Application rate per treatment to bare soil or target crop: kg a.s./ha	Water (L/ha)	g a.s./hL	Dates of treatment or no. of treatments and last date (c)	Portion analysed	Growth stage at sampling (d)	Residues (mg/kg): AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total resude calc.	PHI (days) (e)
R 2006 0858/6 0858-06 / 01 Germany D-51399 Burscheid, Trial Station Höfchen Europe, North F, 2006	**Plot A** Application to bare soil **PBI: 30 d** Rotational crop : Turnip, edible Rondo	1) 18.08.2006 3) 27.10.2006	0.500	300	0.1667	19.07.2006	leaves	47 49	0.02 0.02	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	0.03 0.03	86 100
							body	47 49	0.02 0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	0.03 0.02	86 100
R 2006 0859/4 0859-06 / 01 Germany D-51399 Burscheid (Versuchsgut Höfchen) Europe, North F, 2006	**Plot A** Application to bare soil **PBI: 30 d** Rotational crop Lettuce Gisela: Butterhead variety	1) 03.08.2006 3) 22.09.2006	0.500	300	0.1667	04.07.2006	head	42 49	0.03 0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	0.04 <0.02	66 80

Trial No./ Location/ Year	Plot (commodity)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment to bare soil or target crop			Dates of treatment or no. of treatments and last date	Portion analysed	Growth stage at sampling	Residues (mg/kg)						PHI (days)
			kg a.s./ha	Water (L/ha)	g a.s./hl				AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total resude calc.	
	(a)	(b)				(c)		(d)							(e)
R 2006 0860/8 0860-06 / 01 Germany D-51399 Burscheid Europe, North F, 2006	**Plot A** Application to bare soil **PBI: 28 d** Rotational crop: Wheat, winter Thommy	1) 04.10.2006 2) 22.05.2007 - 29.05.2007 3) 17.07.2007	0.500	300	0.1667	06.09.2006	green material	30	0.07	<0.01	<0.01	<0.01	<0.01	0.08	190
							grain	89	<0.01	<0.01	<0.01	<0.01	0.01	<0.02	314
							straw	89	0.07	<0.05	<0.05	<0.05	<0.05	0.12	314
R 2006 0861/6 0861-06 / 02 Germany D-51399 Burscheid (Versuchsgut Höfchen) Europe, North F 2006	**Plot B** Target crop: Lettuce Gisela **PBI:216 d** Rotational crop: Turnip, edible Mairübe	1) 27.03.2007	0.500	300	0.1667	23.08.2006	leaves	48	0.04	<0.01	<0.01	<0.01	<0.01	0.05	323
								49	0.04	<0.01	<0.01	<0.01	<0.01	0.05	337
							body	48	0.02	<0.01	<0.01	<0.01	<0.01	0.03	323
								49	0.02	<0.01	<0.01	<0.01	<0.01	0.03	337
	Plot B Target crop: Lettuce Gisela **PBI: 230 d** Rotational crops: Lettuce Gisela	1) 10.04.2007	0.500	300	0.1667	23.08.2006	head	46	0.01	<0.01	<0.01	<0.01	<0.01	<0.02	261
								49	0.01	<0.01	<0.01	<0.01	<0.01	<0.02	275

Trial No./ Location/ Year	Plot (commodity)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment to bare soil or target crop			Dates of treatment or no. of treatments and last date	Portion analysed	Growth stage at sampling	Residues (mg/kg)						PHI (days)
			kg a.s./ha	Water (L/ha)	g a.s./hl				AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total resude calc.	
	(a)	(b)				(c)		(d)							(e)
	Plot B Target crop: Lettuce Gisela **PBI: 100 d** Rotational crop: Wheat, winter	1) 01.12.2006	0.500	300	0.1667	23.08.2006	green material	30	0.08	<0.01	<0.01	<0.01	0.02	0.09	240
							grain	89	<0.01	<0.01	<0.01	<0.01	0.04	<0.02	328
							straw	89	0.09	<0.05	<0.05	0.05	<0.05	0.14	328
R 2006 0862/4 0862-06 / 03 Germany D-51399 Burscheid (Versuchsgut Höfchen) Europe, North F 2006	**Plot C** Target crop: Lettuce Gisela **PBI: 301 d** Rotational crop: Turnip, edible Mairübe Lettuce,Gisela	1) 20.06.2007	0.500	300	0.1667	23.08.2006	leaves	47	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	372
								49	0.01	0.01	<0.01	<0.01	<0.01	0.02	386
							body	47	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	372
								49	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	386
	Plot C Target crop: Lettuce Gisela **PBI: 301 d** Rotational crop: Wheat winter,Ackerkohl	1) 20.06.2007	0.500	300	0.1667	23.08.2006	head	45	0.01	<0.01	<0.01	<0.01	<0.01	0.02	338
								49	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	352

Trial No./ Location/ Year	Plot (commodity)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment to bare soil or target crop			Dates of treatment or no. of treatments and last date	Portion analysed	Growth stage at sampling	Residues (mg/kg)						PHI (days)
			kg a.s./ha	Water (L/ha)	g a.s./hl				AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total resude calc.	
	(a)	(b)				(e)		(d)							(e)
	Plot C Target crop: Lettuce Gisela **PBI: 363 d** Rotational crops: Turnip, edible Mairübe	1) 21.08.2007	0.500	300	0.1667	23.08.2006	green material	29	0.02	<0.01	<0.01	<0.01	<0.01	0.03	414
							grain	89	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	691
							straw	89	<0.05	<0.05	<0.05	<0.05	<0.05	0.10	691

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI, underlined)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) Study reference
* prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Total residue calculated : sum of fluopyram+FLU-benzamide

Data Point:	KCA 6.6.2/03
Report Author:	[redacted]
Report Year:	2008
Report Title:	Determination of the residues of AE C656948 in/on the field rotational crops carrot, lettuce and winter wheat after spraying of AE C656948 (500 SC) in the field in Italy
Report No:	RA-2650/06
Document No:	M-296652-02-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC guidance working document 7524/VI/95 rev. 2 (1997-07-22) OECD Guideline for testing of chemicals; Residues in rotational crops (limited field studies), No. 504
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted rev. 1 to Vol. 3 of DAR B7 August 2012 (reference cited in)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and Methods

The purpose of the study was to determine the magnitude of residues of fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188) and fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122) in carrot, lettuce, winter wheat grown as rotational crops in southern Europe (Italy) following one spray application of fluopyram SC 500. Residues of the active substance fluopyram and its metabolites were determined in the succeeding crops only. The target crop lettuce was not analysed.

The test item was applied to the treated plots in a different manner, but the application rate of 1.0 L of test item/ha (0.5 kg a.s./ha) was the same.

For the plot A (trials R 2006 0869/1, R 2006 0870/5 and R 2006 0871/3) – the test item was applied to the bare soil followed by incorporation (maximum 8 cm depth) to avoid photodegradation. The investigated plant back interval was 30 days for all crops (carrot, lettuce and winter wheat) and corresponds to the standard plant back interval in case of crop failure.

For the plot B (trial R 2006 0872/1) – the test item was applied on lettuce as the target crop two weeks after planting. Plot B represents a plant back interval of 100-240 days.

For the plot C (trial R 2006 0874/8) – the test item was applied on lettuce as the target crop two weeks after planting. Plot C represents a plant back interval of 290-365days.

Plots B and C were subdivided into 3 subplots for the 3 crops (carrot, lettuce, cereals) to be planted as rotational crops. Explanation of the study plot designs is illustrated in the Table 6.6.2- 10.

Table 6.6.2- 10: Plot design and plant back intervals

Trial No.	Requested PBI (days) / Application on	actual Plant Back Interval (PBI)			Remarks
		Root crop DAT (days)	Leafy crop DAT (days)	Cereals DAT (days)	
R 2006 0869/1 R 2006 0870/5 R 2006 0871/3	Plot A: 30 / soil	carrot 30 (R 2006 0869/1)	lettuce 30 (R 2006 0870/5)	winter wheat 30 (R 2006 0871/3)	Application on bare soil; planting/sowing of rotational crop after 30 days
R 2006 0872/1	Plot B: 90-240 / lettuce	carrot 240	lettuce 240	winter wheat 120	Application on lettuce two weeks after planting; harvest/ploughing of lettuce; planting/sowing of rotational crops
R 2006 0874/8	Plot C: 290-365 / lettuce	carrot 289	lettuce 290	spring wheat 363	Application on lettuce two weeks after planting; harvest/ploughing of lettuce; cultivation break; planting/sowing of rotational crops

DAT= days after treatment

Harvest of target crop lettuce (Plot B, R 2006 0872/1 and Plot C, R 2006 0874/8):
On plot B the target crop lettuce was harvested at normal harvest, 32 days after planting. On plot C the target crop lettuce was harvested at normal harvest, 56 days after planting.
No lettuce samples were taken for analysis.
The harvest leftovers were destroyed by grinding and ploughed in (in order to capture all possible residues and not remove them from the plot prior to planting the rotational crops.)

Planting rotational crops:
Untreated plots were prepared before treated plots.

At the 30-day (Plot A), the 100-240-day (Plot B) and the 290-365-day (Plot C) plant back intervals the plots were prepared for crop planting following normal agronomic practices for each crop type in the regions.

For the longer-term Plots B (100-240 days) and C (290-365 days) a bare soil status had to be maintained after harvest of lettuce, i.e. no cover crop is sown.

Samples were taken, prepared in the field where necessary, transported and stored.

The first sampling of succeeding crops carrots and lettuce was taken at early harvest (14 days prior to normal harvest) followed by sampling at normal harvest maturity for each crop type.

For wheat, green material was sampled at growth stage 29-30 BBCH and at normal harvest maturity (grain and straw). All sampling equipment was cleaned prior to entering any plot that was to be harvested.

The field samples from all trials were stored deep-frozen within 24 hours after sampling. All field samples were shipped by deep-freeze lorry and arrived in good condition. The field sub samples were stored in a freezer at ≤-18°C until preparation of the examination samples. For the preparation of examination samples, the deep-frozen field sub samples were shredded with dry ice in a cutter. Representative parts of the shredded samples were transferred into polystyrene boxes separately for analysis (UR samples) and archiving (UR samples) and stored at ≤-18°C until analysis.

Residues of fluopyram and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide), were determined by LC-MS/MS according to method 00984 ([redacted], 05/02/2007, M-283301-01-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method

are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues of these compounds were extracted from 10 g of sample material (5 g for wheat straw) by two successive extractions using a high-speed blender with a mixture of acetonitrile:water (80:20; v:v). After centrifugation the extract volume was adjusted. Then, the extracts were diluted ten times by adding the internal standards:

- One dilution was performed under acidic conditions: this extract allowed the determination of FLU-PCA and FLU-methyl-sulfoxide.
- In parallel, another dilution was performed under basic conditions: this extract allowed the determination of FLU, FLU-benzamide and FLU-7-OH.

An aliquot of the extracts was injected into LC-MS/MS operated in Electrospray Ionization mode:

- One injection in positive electrospray ionization for the determination of FLU, FLU-benzamide and FLU-7-OH.
- Another injection in negative electrospray ionization for the determination of FLU-PCA and FLU-methyl-sulfoxide under different conditions.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels. For each calibration curve, the correlation coefficient R was above 0.99.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.05 mg/kg for wheat straw and 0.01 mg/kg for all other matrices.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Findings

In order to check the performance of the analytical method, recovery determinations were concurrently performed to the analyses of control and treated samples from the study in each set of analyses. The data demonstrate acceptable method performance during sample analysis. All the recovery results and details are given in the Table 6.6.2- 11.

Table 6.6.2- 11: Recovery data for fluopyram (AE C656948) and its metabolites in rotational crop matrices (carrot leaf and root, lettuce head, winter wheat green material, winter wheat straw, and winter wheat grain)

Crop/Sample material	FL (mg/kg)	Single values (%)	Mean value (%)	RSD* (%)
Fluopyram (AE C656948)				
carrot leaf, normal	0.01	98; 104; 96; 100	100	3.4
	0.10	103; 108; 101; 97	102	4.5
		Overall recovery (n=8)	101	**4.0**
carrot root	0.01	103; 93; 105; 106	102	5.9
	0.10	94; 101; 104; 99	100	4.2
		Overall recovery (n=8)	**101**	**4.9**
lettuce head	0.01	104; 105	105	--
	0.10	105; 99	102	--
		Overall recovery (n=4)	**103**	**2.8**
winter wheat green material	0.01	100	100	--
	0.10	91	91	--
	0.5	86	86	--

Crop/Sample material	FL (mg/kg)	Single values (%)	Mean value (%)	RSD* (%)
		Overall recovery (n=3)	**92**	**7.7**
winter wheat straw	0.05	87; 83	85	--
	5.0	108	108	--
		Overall recovery (n=3)	**93**	**14.5**
winter wheat grain	0.01	85; 92; 84	87	5.0
	0.10	96	96	--
		Overall recovery (n=4)	**89**	**6.4**
Fluopyram-benzamide (AEF148815)				
carrot leaf, normal	0.01	98; 85; 100; 105	97	8.8
	0.10	102; 99; 102; 100	101	1.5
		Overall recovery (n=8)	**99**	**6.1**
carrot root	0.01	99; 86; 93; 101	93	10.1
	0.10	95; 95; 100; 107	99	5.7
		Overall recovery (n=8)	**96**	**8.2**
lettuce head	0.01	105; 90	98	--
	0.10	100; 109	105	--
		Overall recovery (n=4)	**101**	**8.1**
winter wheat green material	0.01	96	96	--
	0.10	87	87	--
	0.5	107	107	--
		Overall recovery (n=3)	**97**	**10.4**
winter wheat straw	0.05	107; 101	104	--
	5.0	96	96	--
		Overall recovery (n=3)	**101**	**5.4**
winter wheat grain	0.00	89; 92	91	--
	0.10	97	97	--
		Overall recovery (n=3)	**93**	**4.4**
Fluopyram-methylsulfoxide (AE1344122)				
carrot leaf, normal	0.01	94; 92; 81; 92	90	6.6
	0.10	91; 88; 93; 88	90	2.7
		Overall recovery (n=8)	**90**	**4.7**
carrot root	0.10	83; 78; 86; 100	87	10.9
	0.10	85; 94; 96; 93	91	5.1
		Overall recovery (n=8)	**89**	**8.2**
lettuce head	0.01	82; 88	85	--
	0.10	109; 95	102	--
		Overall recovery (n=4)	**94**	**12.4**
winter wheat green material	0.01	80	80	--
	0.10	100	100	--
	0.50	100	100	--
		Overall recovery (n=3)	**93**	**12.4**
winter wheat straw	0.05	125; 108	117	--
	5.0	92	92	--
		Overall recovery (n=3)	**108**	**15.2**
winter wheat grain	0.01	110; 96	103	--
	0.1	91	91	--
		Overall recovery (n=3)	**99**	**9.9**
Fluopyram-pyridyl-carboxylic-acid (AEC657188)				
carrot leaf, normal	0.01	61; 60; 64; 96	70	24.6
	0.10	66; 68; 69; 90	73	15.3
		Overall recovery (n=8)	**72**	**18.9**
carrot root	0.01	101; 94; 91; 107	98	7.3
	0.10	86; 85; 95; 88	89	5.1

Crop/Sample material	FL (mg/kg)	Single values (%)	Mean value (%)	RSD* (%)
		Overall recovery (n=8)	**93**	**8.2**
lettuce head	0.01	102; 98	100	--
	0.10	101; 96	99	--
		Overall recovery (n=4)	**99**	**2.8**
winter wheat green material	0.01	109	109	--
	0.10	93	93	--
	0.5	80	80	--
		Overall recovery (n=3)	**94**	**15.5**
winter wheat straw	0.05	95; 101	98	--
	5.0	93	93	--
		Overall recovery (n=3)	**96**	**4.3**
winter wheat grain	0.01	79; 76	78	--
	0.1	78	78	--
		Overall recovery (n=3)	**78**	**2.0**
Fluopyram-7-hydroxy (BCS-AA10065)				
carrot leaf, normal	0.01	93; 89; 94; 99	93	3.5
	0.10	102; 102; 100; 96	100	2.8
		Overall recovery (n=8)	**97**	**4.8**
carrot root	0.01	99; 91; 86; 101	94	7.4
	0.10	96; 89; 92; 89	92	3.6
		Overall recovery (n=8)	**93**	**5.7**
lettuce head	0.01	97; 95	96	--
	0.10	104; 90	97	--
		Overall recovery (n=4)	**97**	**6.0**
winter wheat green material	0.01	97	97	--
	0.10	91	91	--
	0.5	96	96	--
		Overall recovery (n=3)	**95**	**4.0**
winter wheat straw	0.05	90; 79	85	--
	5.0	99	99	--
		Overall recovery (n=3)	**89**	**11.2**
winter wheat grain	0.01	87; 86	87	--
	0.1	87	87	--
		Overall recovery (n=3)	**87**	**0.7**

FL: fortification level, RSD - Relative Standard Deviation
* some RSDs were not calculated as there were only two individual recoveries given
** Recovery corrected with the control interference (0.002 mg/kg), recovery not corrected = 132%
Final determination as: fluopyram Residues calculated as: fluopyram
Final determination as: FLU-benzamide Residues calculated as: fluopyram
Final determination as: FLU-methylsulfonide Residues calculated as: fluopyram
Final determination as: FLU-PCA Residues calculated as: fluopyram
Final determination as: FLU-7OH Residues calculated as: fluopyram

No residue of fluopyram or related metabolites were found above the relevant LOQs in any of the control samples of rotational crop matrices analysed.

The residue levels of fluopyram and its relevant metabolites in the rotational crop matrices of carrot, lettuce and wheat are summarized in the tables below.

The storage period of deep-frozen samples was up to 363 days for lettuce, 315 days for carrot leaves, 315 days for carrot root, 221 days for wheat green material, 109 days for wheat grain and 107 days for wheat straw.

Residue measured in the relevant succeeding crop matrices are summarized below and overall trials and residue summaries are presented in Table 6.6.2- 12.

Residues in Carrot

In trial R 2006 0869/1 (plot A, PBI 30 days), residues of fluopyram (AE C656948) were 0.02 mg/kg and 0.04 mg/kg in carrot leaf 108 and 122 days after treatment (DAT), respectively. In carrot root samples, the residues of fluopyram were 0.05 mg/kg at DAT 108 and 122.

The residues of FLU-benzamide, FLU-methylsulfoxide, FLU-PCA and FLU-7OH in all carrot matrices were <0.01 mg/kg.

In trial R 2006 0872/1 (plot B, PBI 100-240 days) residues of fluopyram (AE C656948) were 0.01 mg/kg in carrot leaf, normal at DAT 338 and 352, and in carrot root residues of fluopyram were 0.02 mg/kg at DAT of 338 and 352. The residues of FLU-benzamide, FLU-methylsulfoxide, FLU-PCA and FLU-7OH in all carrot matrices were <0.01 mg/kg.

In trial R 2006 0874/8 (plot C, PBI 290-365 days) residues of fluopyram (AE C656948) were 0.01 mg/kg in carrot at DAT of 363 and 377, respectively. In carrot root residues of fluopyram were 0.02 mg/kg and 0.01mg/kg at DAT of 363 and 377, respectively. The residues of FLU-benzamide, FLU-methylsulfoxide, FLU-PCA and FLU-7OH in all carrot root matrices were <0.01 mg/kg.

Residues in Lettuce

In trial R 2006 0870/5 (plot A, PBI 30 days), residues of fluopyram (AE C656948) were 0.09 mg/kg and 0.03 mg/kg in lettuce head 58 and 72 days after treatment (DAT), respectively.
The residues of FLU-benzamide, FLU-methylsulfoxide, FLU-PCA and FLU-7OH in all lettuce matrices were <0.01 mg/kg.

In trial R 2006 0872/1 (plot B, PBI 100 – 240 days) residues of fluopyram (AE C656948) were 0.02 mg/kg and <0.01 mg/kg in lettuce head samples at DAT 296 and 310, respectively. The residues of FLU-benzamide, FLU-methylsulfoxide, FLU-PCA and FLU-7OH in all lettuce matrices were <0.01 mg/kg.

In trial R 2006 0874/8 (plot C, PBI 290 – 365 days) residues of fluopyram (AE C656948) were 0.03 mg/kg and 0.01 mg/kg in lettuce head samples at DAT 318 and 332, respectively. The residues of FLU-benzamide, FLU-methylsulfoxide, FLU-PCA and FLU-7OH in all lettuce matrices were <0.01 mg/kg.

Residues in Wheat

In trial R 2006 0871/3 (plot A, PBI 30 days), residues of fluopyram (AE C656948) were 0.11 mg/kg (in green material), 0.13 mg/kg (in wheat straw), and 0.01 mg/kg in wheat grain 139, 265, and 265 days after treatment (DAT), respectively.
The residues of FLU-methylsulfoxide in wheat green material were 0.02 mg/kg (DAT 139), in straw <0.05 mg/kg (DAT 265), and 0.03 mg/kg in wheat grain (at DALT 265).
The residues of FLU-7OH in wheat green material and grain were <0.01 mg/kg (DAT 139 and 265) and at 0.08 mg/kg in wheat straw.
The residues of FLU-benzamide and FLU-PCA in all wheat matrices were below their respective LOQs.

In trial R 2006 0872/1 (plot B, PBI 100-240 days), residues of fluopyram (AE C656948) were 0.07 mg/kg (in green material), <0.01 mg/kg (in wheat grain), and <0.05 mg/kg in wheat straw 242, 352, and 352 days after treatment (DAT), respectively.
The residues of FLU-methylsulfoxide were at 0.01 mg/kg in green material (DAT 242), 0.02 mg/kg in grain (DAT 352), <0.05 mg/kg in straw (DAT 352).
The residues of FLU-benzamide, FLU-PCA and FLU-7OH in all three wheat matrices were below relevant LOQ.

In trial R 2006 0874/8 (plot C, PBI 290-365 days), residues of fluopyram (AE C656948) were 0.06 mg/kg (in green material), <0.01 mg/kg (in wheat grain), and 0.08 mg/kg in wheat straw 478, 611, and 611 days after treatment (DAT), respectively.

The residues of FLU-methylsulfoxide in wheat green material were at the level of 0.01 mg/kg at DAT 478, <0.01 mg/kg in wheat grain, and <0.05 mg/kg in wheat straw at DAT 611.
The residues of FLU-7OH in wheat green material and grain were <0.01 mg/kg at DAT 478 and 611, and at the level of 0.06 mg/kg in wheat straw (DAT 611).
The residues of FLU-benzamide and FLU-PCA in all wheat matrices were below their respective LOQs.

Conclusions

Following one spray application conducted with fluopyram SC500 to bare soil or lettuce as a target crop at a rate of 0.5 kg a.s./ha, residues in succeeding crops were analysed for fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188) and fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122) using a validated selective HPLC-MS/MS analytical method.

These data show that residues in carrot, lettuce and wheat as succeeding crop are adequately covered by the current EU MRL.

Assessment and conclusion by applicant:
The study is acceptable

Table 6.6.2- 12: Results of rotational crop trials conducted with fluopyram

Trial No./ Location/ Year	Plot (commodity)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment to bare soil or target crop			Dates of treatment or no. of treatments and last date	Portion analysed	Growth stage at sampling	Residues (mg/kg)						PHI (days)
			kg a.s./ha	Water (L/ha)	g a.s./hL				AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residue calc.	
	(a)	(b)				(c)		(d)							(e)
R 2006 0869/1 0869-06 / 01 Italy I-37050 Albaro Europe, South F 2006	**Plot A** Application to bare soil **PBI:30 d** Rotational crop : Carrot, Nantese di Chioggia	1) 10.08.2006 3) 05.11.2006 - 15.11.2006	0.50	300	0.1667	11.07.2006	leaves	39 49	0.02 0.04	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	0.03 0.05	108 122
							root	39 49	0.05 0.04	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	0.06 0.06	108 122
R 2006 0870/5 0870-06 / 01 Italy I-37050 Albaro Europe, South F 2006	**Plot A** Application to bare soil **PBI: 30 d** Rotational crop Lettuce,Potomac (Numens); Butterhead variety	1) 10.08.2006 3) 17.09.2006 - 24.09.2006	0.50	300	0.1667	11.07.2006	head	44 49	0.09 0.03	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	0.10 0.04	58 72
R 2006 0871/3 0871-06 / 01 Italy	**Plot A** Application to bare soil **PBI: 30 d**	1) 26.10.2006 2) 25.05.2007 - 15.06.2007 3) 25.06.2007 - 05.07.2007	0.50	300	0.1667	26.09.2006	green material	30	0.11	<0.01	<0.01	<0.01	0.02	0.12	139
							grain	89	0.01	<0.01	<0.01	<0.01	0.03	0.02	265

Trial No./ Location/ Year	Plot (commodity)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment to bare soil or target crop			Dates of treatment or no. of treatments and last date	Portion analysed	Growth stage at sampling	Residues (mg/kg)						PHI (days)
			kg a.s./ha	Water (L/ha)	g a.s./hL				AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residue calc.	
	(a)	(b)				(c)		(d)							(e)
I-37050 Albaro Europe, South F 2006	Rotational crop: Wheat, winter Guadalupe						straw	89	0.15	0.05	0.05	0.08	<0.05	0.20	265
R 2006 0872/1 0872-06 / 02 Italy I-37050 Albaro Europe, South F 2006	**Plot B** Target crop: lettuce (target crop) **PBI: 240 d** Rotational crop: Carrot N/A	1) 28.02.2007	0.5	500	0.1000	03.07.2006	leaves	47	0.01	<0.01	<0.01	<0.01	<0.01	<0.02	338
								49	0.01	<0.01	<0.01	<0.01	<0.01	<0.02	352
							root	47	0.02	<0.01	<0.01	<0.01	<0.01	<0.02	338
								49	0.02	<0.01	<0.01	<0.01	<0.01	<0.02	352
	Plot B Target crop: lettuce (target crop) **PBI: 240 d** Rotational crops: Lettuce Funly	1) 28.02.2007	0.5	500	0.1000	03.07.2006	head	47	0.02	<0.01	<0.01	<0.01	<0.01	0.03	296
								49	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	310
	Plot B Target crop:	1) 31.10.2006	0.5	500	0.1000	03.07.2006	green material	30	0.07	<0.01	<0.01	<0.01	0.01	0.08	242

Trial No./ Location/ Year	Plot (commodity)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment to bare soil or target crop			Dates of treatment or no. of treatments and last date	Portion analysed	Growth stage at sampling	Residues (mg/kg)						PHI (days)
			kg a.s./ha	Water (L/ha)	g a.s./hL				AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residue calc.	
	(a)	(b)				(c)		(d)							(e)
	lettuce (target crop) **PBI: 120 d** Wheat, winter Serio						grain	89	<0.01	<0.01	<0.01	<0.01	0.02	<0.02	352
							straw	89	<0.05	<0.05	<0.05	<0.05	<0.05	0.10	352
R 2006 0874/8 0874-06 / 03 Italy I-37050 Albaro Europe, South F 2006	**Plot C** Target crop: lettuce **PBI: 289 d** Rotational crop: Carrot N/A	1) 09.08.2007	0.50	500	0.1000	24.10.2006	leaves	47	0.01	<0.01	<0.01	<0.01	<0.01	0.02	363
								49	0.01	<0.01	<0.01/0.01**	<0.01	<0.01	0.02	377
							root	47	0.02	<0.01	<0.01	<0.01	<0.01	0.03	363
								49	0.01	<0.01	<0.01	<0.01	<0.01	0.02	377
	Plot C Target crop: lettuce **PBI: 290 d** Rotational crop: Lettuce Estony	1) 10.08.2007	0.50	500	0.1000	24.10.2006	head	45	0.03	<0.01	<0.01	<0.01	<0.01	0.04	318
								49	0.01	<0.01	<0.01	<0.01	<0.01	0.02	332
	Plot C Target crop: lettuce **PBI: 303 d** Rotational	1) 22.10.2007	0.50	500	0.1000	24.10.2006	green material	29	0.06	<0.01	<0.01	<0.01	0.01	0.07	478
							grain	89	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	611

Trial No./ Location/ Year	Plot (commodity)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment to bare soil or target crop			Dates of treatment or no. of treatments and last date	Portion analysed	Growth stage at sampling	Residues (mg/kg)						PHI (days)
	(a)	(b)	kg a.s./ha	Water (L/ha)	g a.s./hL	(c)		(d)	AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residue calc.	(e)
	crops: Wheat, winter Enesco						straw	89	0.08	<0.05	0.05	0.06	<0.05	0.13	611

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
(e) Days after last application (Label pre-harvest interval, PHI underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) Study reference
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
(l) Method validation
(m) Storage (max)
- based on date of analysis
P based on production date

G greenhouse F field * prior to last treatment ** residue in control # no data available

Total residue calculated : sum of fluopyram+FLU-benzamide

Data Point:	KCA 6.6.2/04
Report Author:	[redacted]
Report Year:	2008
Report Title:	Determination of the residues of AE C656948 in/on the field rotational crops carrot, lettuce and winter wheat after spraying of AE C656948 (500 SC) in the field in Spain
Report No:	RA-2651/06
Document No:	M-296671-02-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC guidance working document 7524/VI/95 rev. 2 (1997-07-22) OECD Guideline for testing of Chemicals; Residues in rotational crops (limited field studies), No. 504
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted rev. 1 to Vol. 3 of DAR B7 August 2012 (references relied on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and Methods

The purpose of the study was to determine the magnitude of the relevant residues of fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188) and fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122) in carrot, lettuce and winter wheat as rotational crops in southern Europe (Spain) following one spray application of fluopyram SC500 on soil or on target crop lettuce. Residues of the active substance fluopyram and its metabolites were determined in the succeeding crops only. The target crop lettuce was not analysed.

The test item was applied to the treated plots in a different manner, but the application rate of 1.0 L of test item/ha (0.5 kg a.s./ha) was the same.

For the plot A (trials R 2006 0875/6, R 2006 0876/4 and R 2006 0877/2) – the test item was applied to the bare soil followed by incorporation (maximum 8 cm depth) to avoid photodegradation. The investigated plant back interval was 30 days for all crops (carrot, lettuce and winter wheat) and corresponds to the standard plant back interval in case of crop failure.

For the plot B (trial R 2006 0878/0) – the test item was applied on lettuce as the target crop two weeks after planting. Plot B represents a plant back interval of 120-240 days.

For the plot C (trial R 2006 0879/9): – the test item was applied on lettuce as the target crop two weeks after planting. Plot C represents a plant back interval of 290-365 days.

Plots B and C were subdivided into 3 subplots for the 3 crops (carrot, lettuce, cereals) to be planted as rotational crops. Explanation of the study plot designs is illustrated in the

Table 6.6.2- 13.

Table 6.6.2- 13: Plot design and plant back intervals

Trial No.	Requested PBI (days) / Application on	actual Plant Back Interval (PBI)			Remarks
		Root crop DAT (days)	Leafy crop DAT (days)	Cereals DAT (days)	
R 2006 0875/6 R 2006 0876/4 R 2006 0877/2	Plot A: 30 / soil	carrot 36 (R 2006 0864/0)	lettuce 30 (R 2006 0865/9)	winter wheat 49 (R 2006 0866/7)	Application on bare soil; planting/sowing of rotational crop after 30 days
R 2006 0878/0	Plot B: 90-240 / lettuce	carrot 154	lettuce 155	winter wheat 154	Application on lettuce two weeks after planting; harvest/ploughing of lettuce; planting/sowing of rotational crops
R 2006 0879/9	Plot C: 290-365 / lettuce	carrot 344	lettuce 337	spring wheat 357	Application on lettuce two weeks after planting; harvest/ploughing of lettuce; cultivation break; planting/sowing of rotational crops

DAT= days after treatment

Harvest of target crop lettuce (Plot B, R 2006 0878/0 and Plot C, R 2006 0879/9):

On plots B, the target crop lettuce was harvested at normal harvest, 86 days after planting. On Plot C the target crop lettuce was harvested at normal harvest, 91 days after planting.

No lettuce samples were taken for analysis.

The harvest leftovers were destroyed by grinding and ploughed in (in order to capture all possible residues and not remove them from the plot prior to planting the rotational crops.)

Planting rotational crops:

Untreated plots were prepared before treated plots.

At the 30-day (Plot A), the 100-240-day (Plot B) and the 290-365-day (Plot C) plant back intervals the plots were prepared for crop planting following normal agronomic practices for each crop type in the regions.

For the longer-term Plots B (100-240 days) and C (290-365 days) a bare soil status had to be maintained after harvest of lettuce, i.e. no cover crop is sown.

Samples were taken, prepared in the field where necessary, transported and stored.

The first sampling of carrots and lettuce was taken at early harvest (14 days prior to normal harvest) followed by sampling at normal harvest maturity for each crop type.

For wheat green material was sampled at growth stage 30 BBCH and at normal harvest maturity (BBCH 89) (grain and straw). All sampling equipment was cleaned prior to entering any plot that was to be harvested.

The field samples from all trials were stored deep-frozen within 24 hours after sampling. All field samples were shipped by deep-freeze lorry and arrived in good condition. The field sub samples were stored in a freezer at ≤-18 °C until preparation of the examination samples. For the preparation of examination samples, the deep-frozen field sub samples were shredded with dry ice in a cutter. Representative parts of the shredded samples were transferred into polystyrene boxes separately for analysis (UP samples) and archiving (UR samples) and stored at ≤-18 °C until analysis.

Residues of fluopyram and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide), were determined by LC-MS/MS according to method 00984 ([redacted], 05/02/2007, M-283301-01-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method

are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues of these compounds were extracted from 10 g of sample material (5 g for wheat straw) by two successive extractions using a high-speed blender with a mixture of acetonitrile:water (80:20; *v*:*v*). After centrifugation the extract volume was adjusted. Then, the extracts were diluted ten times by adding the internal standards:

- One dilution was performed under acidic conditions: this extract allowed the determination of FLU-PCA and FLU-methyl-sulfoxide.
- In parallel, another dilution was performed under basic conditions: this extract allowed the determination of FLU, FLU-benzamide and FLU-7-OH.

An aliquot of the extracts was injected into LC-MS/MS operated in Electrospray Ionization mode:

- One injection in positive electrospray ionization for the determination of FLU, FLU-benzamide and FLU-7-OH.
- Another injection in negative electrospray ionization for the determination of FLU-PCA and FLU-methyl-sulfoxide under different conditions.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels. For each calibration curve, the correlation coefficient R was above 0.99.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.05 mg/kg for wheat straw and 0.01 mg/kg for all other matrices.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Findings

In order to check the performance of the analytical method, recovery determinations were concurrently performed to the analyses of control and treated samples from the study in each set of analyses. The data demonstrate acceptable method performance during sample analysis. All the recovery results and details are given in the Table 6.6.2- 14.

Table 6.6.2- 14: Recovery data for fluopyram (AE C656948) and its metabolites in rotational crop matrices (carrot leaf, normal, carrot root, lettuce head, winter wheat green material, winter wheat straw, winter wheat grain)

Crop/Sample material	FL (mg/kg)	Single values (%)	Mean value (%)	RSD*(%)
Fluopyram (AE C656948)				
carrot leaf, normal	0.01	109	109	--
	0.10	100; 91	96	--
	0.15	100	100	--
		Overall recovery (n=4)	**100**	**7.3**
carrot root	0.01	83	83	--
	0.10	96; 97	97	--
		Overall recovery (n=3)	**92**	**8.5**
lettuce head	0.01	86; 98	92	--
	0.10	102; 98	100	--
		Overall recovery (n=4)	**96**	**7.2**
winter wheat green	0.01	92	92	--

Crop/Sample material	FL (mg/kg)	Single values (%)	Mean value (%)	RSD*(%)
material	0.15	95	95	--
	0.5	85	85	--
		Overall recovery (n=3)	**91**	**5.7**
winter wheat straw	0.05	91; 81	86	--
	0.5	108	108	--
		Overall recovery (n=3)	**93**	**14.6**
winter wheat grain	0.01	93; 78	86	--
	0.10	84	84	--
		Overall recovery (n=3)	**85**	**8.9**
Fluopyram-benzamide (AEF148815)				
carrot leaf, normal	0.01	109	109	--
	0.10	95; 104	100	--
	0.15	98	98	--
		Overall recovery (n=4)	**102**	**6.2**
carrot root	0.01	89	89	--
	0.10	86; 91	89	--
		Overall recovery (n=3)	**89**	**2.8**
lettuce head	0.01	92; 98	95	--
	0.10	99; 109	104	--
		Overall recovery (n=4)	**100**	**7.1**
winter wheat green material	0.01	108; 90	99	--
	0.10	93	93	--
		Overall recovery (n=3)	**97**	**9.9**
winter wheat straw	0.05	96; 103	100	--
	0.5	87	87	--
		Overall recovery (n=3)	**95**	**8.4**
winter wheat grain	0.01	85; 103	94	--
	0.10	100	100	--
		Overall recovery (n=3)	**96**	**10.0**
Fluopyram-methylsulfoxide (AE1344122)				
carrot leaf, normal	0.01	104	104	--
	0.10	86; 89	88	--
	0.15	104	104	--
		Overall recovery (n=4)	**96**	**10.0**
carrot root	0.01	96	96	--
	0.10	87; 82	85	--
		Overall recovery (n=3)	**88**	**8.0**
lettuce head	0.01	78; 82	78	--
	0.10	110; 102	106	--
		Overall recovery (n=4)	**92**	**18.7**
winter wheat green material	0.01	109	109	--
	0.15	107	107	--
	0.5	99	99	--
		Overall recovery (n=3)	**105**	**5.0**
winter wheat straw	0.05	113; 119	116	--
	0.5	101	101	--
		Overall recovery (n=3)	**111**	**8.3**
winter wheat grain	0.01	99; 106	103	--
	0.1	97	97	--
		Overall recovery (n=3)	**101**	**4.7**
Fluopyram-pyridyl-carboxylic-acid (AEC657188)				
carrot leaf, normal	0.01	89	89	--
	0.10	88; 77	83	--
	0.15	98	98	--

Crop/Sample material	FL (mg/kg)	Single values (%)	Mean value (%)	RSD*(%)
		Overall recovery (n=4)	**88**	**9.8**
carrot root	0.01	89	89	--
	0.10	96; 83	90	--
		Overall recovery (n=3)	**89**	**7.3**
lettuce head	0.01	99; 93	96	--
	0.10	96; 91	94	--
		Overall recovery (n=4)	**95**	**3.7**
winter wheat green material	0.01	74	74	--
	0.15	92	92	--
	0.5	84	84	--
		Overall recovery (n=3)	**83**	**10.8**
winter wheat straw	0.05	101; 77	89	--
	0.5	90	90	--
		Overall recovery (n=3)	**89**	**13.4**
winter wheat grain	0.01	96; 112	104	--
	0.1	84	84	--
		Overall recovery (n=3)	**97**	**13.9**
Fluopyram-7-hydroxy (BCS-AA10065)				
carrot leaf, normal	0.01	93	93	--
	0.10	101; 91	96	--
	0.15	100	100	--
		Overall recovery (n=4)	**96**	**5.2**
carrot root	0.01	81	81	--
	0.10	95; 83	89	--
		Overall recovery (n=3)	**86**	**8.8**
lettuce head	0.01	78; 95	87	--
	0.10	103; 91	97	--
		Overall recovery (n=4)	**92**	**11.4**
winter wheat green material	0.01	93	93	--
	0.15	96	96	--
	0.50	93	93	--
		Overall recovery (n=3)	**94**	**1.8**
winter wheat straw	0.05	87; 85	86	--
	5	83	83	--
		Overall recovery (n=3)	**85**	**2.4**
winter wheat grain	0.01	89; 83	86	--
	0.1	81	81	--
		Overall recovery (n=3)	**84**	**4.9**

FL: fortification level, RSD - Relative Standard Deviation
* some RSDs were not calculated as there were only two individual recoveries given
** Recovery corrected with the control interference (0.0021 mg/kg); recovery not corrected = 132%
Final determination as: fluopyram Residues calculated as: fluopyram
Final determination as: FLU-benzamide Residues calculated as: fluopyram
Final determination as: FLU-methylsulfoxide Residues calculated as: fluopyram
Final determination as: FLU-PCA Residues calculated as: fluopyram
Final determination as: FLU-7OH Residues calculated as: fluopyram

No residue of fluopyram or related metabolites were found above the LOQs in any of the control samples of rotational crop matrices analysed, except for the trial R 2006 0879/9 in which the residue of AEC657188 in carrot root was at 0.02 mg/kg.

The residue levels of Fluopyram and its relevant metabolites in the rotational crop matrices of carrot, lettuce and wheat are summarized in the Table 6.6.2- 15.

The storage period of deep-frozen samples was up to 290 days for lettuce, 139 days for carrot leaves, 145 days for carrot root, 334 days for wheat green material, 71 days for wheat grain and 80 days for wheat straw.

Residues in Carrot

In trial R 2006 0875/6 (plot A, PBI 30 days), residues of fluopyram (AE C656948) were < 0.01 mg/kg and 0.01 mg/kg in carrot leaf 206 and 220 days after treatment (DAT), respectively. In carrot root samples the residues of fluopyram were 0.02 mg/kg at DAT 206 and 220.

The residues of FLU-benzamide, FLU-methylsulfoxide, FLU-PCA, FLU-7OH in all carrot matrices were <0.01 mg/kg.

In trial R 2006 0878/4 (plot B, PBI 120-240 days) residues of fluopyram (AE C656948) were 0.01 mg/kg and 0.02 mg/kg in carrot leaf (DAT 271 and 285), and in carrot root residues of fluopyram were 0.02 mg/kg and 0.03 mg/kg (DAT of 271 and 285), respectively. The residues of FLU-benzamide, FLU-methylsulfoxide, FLU-PCA, FLU-7OH in all carrot matrices were <0.01 mg/kg.

In trial R 2006 0879/9 (plot C, PBI 290-365 days) residues of fluopyram (AE C656948) were 0.1 mg/kg in all carrot matrices DAT of 532 and 546. The residues of FLU-benzamide, FLU-methylsulfoxide, FLU-PCA, FLU-7OH in all carrot matrices were <0.01 mg/kg.

Residues in Lettuce

In trial R 2006 0876/4 (plot A, PBI 30 days), residues of fluopyram (AE C656948) were <0.01 mg/kg in lettuce head 136 and 150 days after treatment (DAT).
The residues of FLU-benzamide, FLU-methylsulfoxide, FLU-PCA, FLU-7OH in all lettuce matrices were <0.01 mg/kg.

In trial R 2006 0878/0 (plot B, PBI 120 – 240 days) residues of fluopyram (AE C656948) were <0.01 mg/kg in lettuce head samples at DAT 225 and 239. The residues of FLU-benzamide, FLU-methylsulfoxide, FLU-PCA, FLU-7OH in all lettuce matrices were <0.01 mg/kg.

In trial R 2006 0879/9 (plot C, PBI 290 – 365 days) residues of fluopyram (AE C656948) were <0.01 mg/kg in lettuce head samples at DAT 449 and 463. The residues of FLU-benzamide, FLU-methylsulfoxide, FLU-PCA, FLU-7OH in all lettuce matrices were <0.01 mg/kg.

Residues in Wheat

In trial R 2006 0877/2 (plot A, PBI 30 days), residues of fluopyram (AE C656948) were 0.11 mg/kg (in green material), 0.05 mg/kg (in wheat straw), and <0.01 mg/kg in wheat grain 126, 219, and 219 days after treatment (DAT), respectively.
The residues of FLU-benzamide were 0.01 mg/kg (in green material), 0.14 mg/kg (in wheat straw), and <0.01 mg/kg in wheat grain at DAT 126, 219, and 219, respectively.
The residues of FLU-methylsulfoxide were 0.02 mg/kg (in green material), 0.06 mg/kg (in wheat straw), and 0.01 mg/kg in wheat grain at DAT 126, 219, and 219, respectively
The residues of FLU-PCA and FLU-7OH in all three wheat matrices were below relevant LOQ.

In trial R 2006 0878/0 (plot B, PBI 120-240 days), residues of fluopyram (AE C656948) were 0.05 mg/kg (in green material), 0.19 mg/kg (in wheat straw), and <0.01 mg/kg in wheat grain at DAT (days after treatment), 211, 266, and 266, respectively.
The residues of FLU-benzamide and FLU-PCA were below relevant LOQ in all wheat matrices.
The residues of FLU-methylsulfoxide were 0.02 mg/kg in green material at DAT 211, <0.05 mg/kg in wheat straw at DAT 266, and 0.04 mg/kg wheat grain at DAT 266.
The residues of FLU-7OH in wheat green material (at DAT 211) and wheat grain (at DAT 266) were <0.01 mg/kg and 0.05 mg/kg in wheat straw (at DAT 266).

In trial R 2006 0879/9 (plot C, PBI 290-365 days), residues of fluopyram (AE C656948) were 0.02 mg/kg (in green material), <0.05 mg/kg in wheat straw and <0.01 mg/kg (in wheat grain) at DAT (days after treatment), 437, 582, and 582, respectively.
The residues of FLU-methylsulfoxide were 0.06 mg/kg (in green material), <0.05 mg/kg in wheat straw and 0.03 mg/kg (in wheat grain) at DAT (days after treatment), 437, 582, and 582, respectively.
The residues of FLU-benzamide were <0.01 mg/kg (in green material and grain) and 0.05 mg/kg in wheat straw.
The residues of FLU-PCA and FLU-7OH in all three wheat matrices were below relevant LOQ.

Conclusion

Following one spray application conducted with fluopyram SC500 to bare soil or lettuce as a target crop at a rate of 0.5 kg a.s./ha, residues in succeeding crops were analysed for fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188) and fluopyram-7-hydroxy (BCS-AA10063) and fluopyram-methyl sulfoxide (AE 1344122) using a validated selective HPLC-MS/MS analytical method.

These data show that residues in carrot, lettuce and wheat as succeeding crop are adequately covered by the current EU MRL.

Assessment and conclusion by applicant:
The study is acceptable

Table 6.6.2- 15: Results of rotational crop trials conducted with fluopyram

Trial No./ Location/ Year	Plot (commodity) (a)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting (b)	Application rate per treatment to bare soil or target crop: kg a.s./ha	Water (L/ha)	g a.s./hL	Dates of treatment or no. of treatments and last date (c)	Portion analysed	Growth stage at sampling (d)	Residues (mg/kg): AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residue calc.	PHI (days) (e)
R 2006 0875/6 0875-06 / 01 Spain E-41310 Brenes Sevilla Europe, South F 2006	**Plot A** Application to bare soil **PBI: 36* d** Rotational crop : carrot, Navarino F1	1) 14.11.206 3) 15.04.2007 – 15.06.2007	0.50	300	0.1667	09.10.2006	leaves	45	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	206
								49	0.01	<0.01	<0.01	<0.01	<0.01	<0.02	220
							root	45	0.02	<0.01	<0.01	<0.01	<0.01	0.03	206
								49	0.02	<0.01	<0.01	<0.01	<0.01	0.03	220
R 2006 0876/4 0876-06 / 01 Spain E-41310 Brenes, Sevilla Europe, South F, 2006	**Plot A** Application to bare soil **PBI: 30 d** Rotational crop lettuce, Carolus	1) 15.11.2006 3) 30.11.2006 – 15.03.2007	0.50	300	0.1667	16.10.2006	head	45	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	136
								49	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	150
R 2006 0877/2 0877-06 / 01 Spain	**Plot A** Application to bare soil **PBI: 49* d**	1) 18.12.2006 2) 12.04.2007 – 20.04.2007 3) 15.06.2007 – 15.07.2007	0.46	276	0.1667	30.10.2006	green material	30	0.11	0.01	<0.01	<0.01	0.02	0.12	126
							grain	89	<0.01	<0.01	<0.01	<0.01	0.01	<0.02	220

Trial No./ Location/ Year	Plot (commodity)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment to bare soil or target crop			Dates of treatment or no. of treatments and last date	Portion analysed	Growth stage at sampling	Residues (mg/kg)						PHI (days)
			kg a.s./ha	Water (L/ha)	g a.s./hL				AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-70H as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residue calc.	
	(a)	(b)				(c)		(d)							(e)
E-41310 Brenes Sevilla Europe, South F 2006	Rotational crop: Winter wheat, Don Pedro						straw	89	0.05	0.14	<0.03	<0.03	0.06	0.19	220
R 2006 0878/0 0878-06 / 02 Spain E-41310 Brenes, Sevilla Europe, South F 2006	**Plot B** Target crop: lettuce, Stilo **PBI: 154 d** Rotational crop:carrot, Navarino F1	1) 07.03.2007	0.47	376	0.125	04.10.2006	leaves	45	0.01	<0.01	<0.01	<0.01	<0.01	0.02	271
								49	0.02	0.01	<0.01	<0.01	<0.01	0.03	285
							root	45	0.02	<0.01	<0.01	<0.01	<0.01	0.03	271
								49	0.03	<0.01	<0.01	<0.01	<0.01	0.04	285
	Plot B Target crop: lettuce, Silo **PBI: 155 d** Rotational crops: lettuce, Carolus	1) 08.03.2007	0.47	376	0.125	04.10.2006	head	45	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	225
								49	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	239
	Plot B Target crop: lettuce, Silo	1) 07.03.2007	0.47	376	0.125	04.10.2006	green material	30	0.05	<0.01	<0.01	<0.01	0.02	0.06	211
							grain	89	<0.01	<0.01	<0.01	<0.01	0.04	<0.02	266

Trial No./ Location/ Year	Plot (commodity)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment to bare soil or target crop			Dates of treatment or no. of treatments and last date	Portion analysed	Growth stage at sampling	Residues (mg/kg)						PHI (days)
			kg a.s./ha	Water (L/ha)	g a.s./hL				AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residue calc.	
	(a)	(b)				(c)	(d)								(e)
	PBI: 154 d Rotational crop: winter wheat, Cajeme						straw	89	0.49	<0.05	<0.05	0.05	<0.05	0.24	266
R 2006 0879/9 0879-06 / 03 Spain E_41310 Brenes (Sevilla) Europe, South F 2006	**Plot C** Target crop: lettuce, Filipo **PBI: 344 d** Rotational crop: carrot, Navarino F1	1) 09.10.2007	0.50	400	0.1667	30.10.2006	leaves	47	0.01	<0.01	<0.01	<0.01	<0.01	0.02	532
								49	0.01	<0.01	<0.01	<0.01	<0.01	0.02	546
							root	47	0.01	<0.01	<0.01	<0.01	<0.01	0.02	532
								49	0.01	<0.01	<0.01/0.02**	<0.01	<0.01	0.02	546
	Plot C Target crop: lettuce, Filipo **PBI:337 d** Rotational crop: lettuce Filipus	1) 02.10.2007	0.50	400	0.1667	30.10.2006	head	49	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	449
								49	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	463
	Plot C Target crop: lettuce, Flipo **PBI: 357 d** Rotational crops: winter wheat, Don Pedro	1) 22.10.2007	0.50	400	0.1667	30.10.2006	green material	30	0.02	<0.01	<0.01	<0.01	0.06	0.03	437
							grain	89	<0.01	<0.01	<0.01	<0.01	0.03	<0.02	582
							straw	89	<0.05	0.05	<0.05	<0.05	<0.05	0.10	582

* = due to heavy rainfall sowing of carrot and winter wheat was not possible at day 30 after application.

(a)	According to CODEX Classification / Guide	(e)	Days after last application (Label pre-harvest interval, PHI, underline)	(h)	Formulation type	(l)	Method validation		
(b)	Only if relevant	(f)	Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included	(i)	Application method	(m)	Storage (max)		
(c)	Year must be indicated			(j)	Method information		! based on date of analysis		
(d)	Either growth stage description or BBCH Code	(g)	Study reference	(k)	LOQ		P based on production date		
G	greenhouse	F	field	*	prior to last treatment	**	residue in control	#	no data available

Total residue calculated : sum of fluopyram+FLU-benzamide

Data Point:	KCA 6.6.2/05
Report Author:	[redacted]
Report Year:	2008
Report Title:	AE C656948 500 SC - Magnitude of the residue in alfalfa (rotational crop tolerance)
Report No:	RAGMP105
Document No:	M-306501-01-1
Guideline(s) followed in study:	EPA Ref.: OPPTS 860.1500, Crop Field Trials PMRA Ref.: DACO 7.4.4, Field Crop Rotational Trial Study
Deviations from current test guideline:	--
Previous evaluation:	
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	

This US study was part of the baseline dossier but is not summarized here as EU data are required for this renewal dossier

Data Point:	KCA 6.6.2/06
Report Author:	[redacted]
Report Year:	2008
Report Title:	AE C656948 500 SC - Magnitude of the residue in cotton (rotational crop tolerance)
Report No:	RAGMY004
Document No:	M-307306-01-1
Guideline(s) followed in study:	OPPTS 860.1500, Crop Field Trials DACO 7.4.4, Field Crop Rotational Trial Study
Deviations from current test guideline:	--
Previous evaluation:	
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	

This US study was part of the baseline dossier but is not summarized here as EU data are required for this renewal dossier

Data Point:	KCA 6.6.2/07
Report Author:	[redacted]
Report Year:	2008
Report Title:	AE C656948 500 SC - Magnitude of the residue in field rotational crops (2.00 day plant back interval)
Report No:	RAGMP061
Document No:	M-306586-01-1
Guideline(s) followed in study:	EPA Ref.: OPPTS 860.1900, Field Accumulation in Rotational Crops PMRA Ref.: DACO 7.4.4, Field Crop Rotational Trial Study
Deviations from current test guideline:	--
Previous evaluation:	
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	

This US study was part of the baseline dossier but is not summarized here as EU data are required for this renewal dossier

Data Point:	KCA 6.6.2/08
Report Author:	[redacted]
Report Year:	2009
Report Title:	Determination of the residues of AE C656948 in/on potato after spraying of fluopyram SC 500 in the field in France (North) and Germany
Report No:	08-2160
Document No:	M-350816-01-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC guidance working document 7029/VI/95 rev. 5 (1997-07-22)
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted Study was not found in DAR/RAR and the Addenda
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and Methods

The purpose of the study was to determine the magnitude of residues of fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188),fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122), in/on potato (tuber) harvested after one spraying application with Fluopyram SC 500 on bare soil 30 days before planting of potatoes in northern Europe (Northern France and Germany). The investigated plant back interval was 30 days.

For application the formulation Fluopyram SC 500 was used, a suspension concentrate formulation containing 500 g/L of Fluopyram. The application was done on bare soil followed by incorporation into the soil (< 8 cm) 30 days (trial 08-2160-01) and 28 days (trial 08-2160-02) before the planting of potatoes.

In both trials the application was done with 1.0 L of test item/ha and a water rate of 300 L/ha, corresponding to a spray concentration of the test item of 0.33% (0.17% of active substance in the spray liquid). The application rate was 0.50 kg fluopyram /ha.

For residue analysis samples of potato (tuber) were taken from the treated and the control plots. In order to obtain representative samples of the raw commodity, samples were taken at random along the treated and the control plot.

Samples of potato (tuber) were taken from the treated and untreated plot in both trials 14 days before harvest and at harvest.

The field samples were stored deep-frozen within 24 hours after sampling and until dispatch. The field samples of trial 08-2160-01 were shipped by deep-freeze lorry, the field samples of trial 08-2160-02 were shipped by car with dry ice and arrived in good condition. The field samples were stored in a freezer at ≤ -18°C until preparation of the examination samples. For the preparation of examination samples, the deep-frozen field samples were shredded with dry ice in a cutter. Representative parts of the shredded field samples were transferred into polystyrene boxes separately for analysis or archiving and stored at ≤-18°C.

Residues of fluopyram and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide), were determined by LC-MS/MS according to method 00984 ([redacted], 05/02/2007, M-283301-01-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues of these compounds were extracted from 10 g of sample material (5 g for wheat straw) by two successive extractions using a high-speed blender with a mixture of acetonitrile:water (80:20; v:v). After centrifugation the extract volume was adjusted. Then, the extracts were diluted ten times by adding the internal standards:

- One dilution was performed under acidic conditions: this extract allowed the determination of FLU-PCA and FLU-methyl-sulfoxide.
- In parallel, another dilution was performed under basic conditions: this extract allowed the determination of FLU, FLU-benzamide and FLU-7-OH.

An aliquot of the extracts was injected into LC-MS/MS operated in Electrospray Ionization mode:

- One injection in positive electrospray ionization for the determination of FLU, FLU-benzamide and FLU-7-OH.
- Another injection in negative electrospray ionization for the determination of FLU-PCA and FLU-methyl-sulfoxide under different conditions.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels. For each calibration curve, the correlation coefficient R was above 0.99.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.05 mg/kg for wheat straw and 0.01 mg/kg for all other matrices.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Findings

In order to check the performance of the analytical method, recovery determinations were concurrently performed to the analyses of control and treated samples from the study in each set of analyses. The means of the concurrent recoveries were for all fortification levels within the acceptable range of 70 – 110 % except for FLU-methyl-sulfoxide at 114 % and 115 % respectively at 0.1 mg/kg and 1 mg/kg. All overall mean recoveries are in the acceptable range of 70 – 110 %; therefore, the results are considered valid. Recovery results are presented in Table 6.6.2- 16.

No residue of fluopyram or related metabolites were found above the LOQ in any of the control samples of rotational crop matrices analysed.

The residue levels of fluopyram and its relevant metabolites in the rotational crop matrices of potato tubers are summarized in the Table 6.6.2- 17.

The storage period of deep-frozen samples ranged between 271 and 316 days.

Residues in potato tubers

In trial 08-2160-01 (PBI 30 days), residues of fluopyram (AE C656948) were 0.02 mg/kg in potato tuber 14 days before harvest and at harvest.

The residues of the metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide) were < 0.01 mg/kg 14 days before harvest and at harvest.

In trial 08-2160-02 (PBI 28 days) residues of fluopyram (AE C656948) were 0.02 mg/kg and 0.01 mg/kg in potato tubers 14 days before harvest and at harvest, respectively.

The residues of the metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide) were < 0.01 mg/kg 14 days before harvest and at harvest.

Conclusions

Following one spray application conducted with fluopyram SC500 to bare soil at a rate of 0.5 kg a.s./ha, residues in succeeding crops were analysed for fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657288) and fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122) using a validated selective HPLC-MS/MS analytical method.

These data show that residues in potato (tuber) as succeeding crop are covered by the current EU-MRL.

Assessment and conclusion by applicant:
The study is acceptable

Table 6.6.2- 16: Recovery data for fluopyram (AE C656948) and its metabolites in potato, tuber

Crop/Sample material	FL (mg/kg)	Single values (%)	Mean value (%)	RSD* (%)
Fluopyram (AE C656948)				
Potato, tuber	0.01	82; 95; 84	87	8.0
	0.10	97; 96; 96	96	0.6
	1.0	83	83	--
		Overall recovery (n=7)	**90**	**7.7**
Fluopyram-benzamide (AEF148815)				
Potato, tuber	0.01	89; 78; 84	84	6.6
	0.10	90; 91; 90	90	0.6
	1.0	89	89	--
		Overall recovery (n=7)	**87**	**5.4**
Fluopyram-pyridyl-carboxilic-acid (AE C657188)				
Potato, tuber	0.01	72; 70; 69	70	2.2
	0.10	87; 97; 97	94	6.2
	1.0	90	90	--
		Overall recovery (n= 7)	**83**	**15.1**
Fluopyram-7-hydroxy (BCS-AA10065)				
Potato, tuber	0.01	89; 93; 87	90	3.4
	0.10	96; 99; 96	97	1.8
	1.0	91	91	--
		Overall recovery (n= 7)	**93**	**4.6**
Fluopyram-methylsulfoxide (AEC344122)				
Potato, tuber	0.01	78; 93; 89	87	9.0
	0.10	114; 115; 114	114	0.5
	1.0	115	115	--
		Overall recovery (n= 7)	**103**	**15.2**

FL: fortification level, RSD - Relative Standard Deviation
* some RSDs were not calculated as there were only two individual recoveries given
Final determination as: fluopyram Residues calculated as: fluopyram
Final determination as: FLU-benzamide Residues calculated as: fluopyram
Final determination as: FLU -methylsulfoxide Residues calculated as: fluopyram
Final determination as: FLU-PCA Residues calculated as: fluopyram
Final determination as: FLU-7OH Residues calculated as: fluopyram

Table 6.6.2- 17: Results of rotational crop trials conducted with fluopyram

Trial No./ Location/ Year	Plot (commodity) (a)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting (b)	Application rate per treatment to bare soil or target crop: kg a.s./ha	Water (L/ha)	kg a.s./hL	Dates of treatment or no. of treatments and last date (c)	Portion analysed	Growth stage at sampling (d)	Residues (mg/kg): AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residue calc.	PHI (days) (e)
08-2160-01 France, north 37230 Fondettes Centre Europe, North F 2008	Application to bare soil **PBI: 30 d** Rotational crop: Potato Juliette	1) 16.05.2008 2) 07.07.2008 - 23.07.2008 3) 10.09.2008 - 25.09.2008	0.5	300	0.17	16.04.2008	tuber	49 49	0.02 0.02	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	0.03 0.03	141 155
08-2160-02 Germany 51399 Burscheid Nordrhein-Westfalen Europe, North F 2008	Application to bare soil **PBI: 28 d** Rotational crop: Potato Cilena	1) 02.05.2008 2) 15.06.2008 - 30.06.2008 3) 20.08.2008	0.5	300	0.17	04.04.2008	tuber	47 49	0.02 0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	0.03 0.02	124 138

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH-Code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) Study reference
prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Total residue calculated : sum of fluopyram+FLU-benzamide

Data Point:	KCA 6.6.2/09
Report Author:	[redacted]
Report Year:	2009
Report Title:	Determination of the residues of AE C656948 in/on potato after spraying of fluopyram SC 500 in the field in Italy and Spain
Report No:	08-2171
Document No:	M-350747-01-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8; Residues in or on Treated Products, Food and Feed; Equivalent to US EPA OPPTS 860.1500 (Supplemental)
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted Study was not found in DAR/RAR and the Addenda
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and Methods

The purpose of the study was to determine the magnitude of residues of fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188),fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122), in/on potato (tuber) harvested after one spraying application with Fluopyram SC 500 on bare soil 30 days before planting of potatoes in southern Europe (Spain and Italy). The investigated plant back interval was 30 days.

For application the formulation Fluopyram SC 500 was used, a suspension concentrate formulation containing 500 g/L of fluopyram. The application was done on bare soil 31 days (trial 08-2171-01) and 30 days (trial 08-2171-02) before planting of potatoes. After the application the test item was incorporated into the soil (08-2171-01: 8-10 cm depth and 08-2171-02: < 8 cm depth).

In both trials the application was done with 1.0 L of test item/ha and a water rate of 300 L/ha, corresponding to a spray concentration of the test item of 0.33% (0.17% of active substance in the spray liquid). The application rate was 0.50 kg fluopyram /ha.

For residue analysis samples of potato (tuber) were taken from the treated and the control plots. In order to obtain representative samples of the raw commodity, samples were taken at random along the treated and the control plot.

Samples of potato (tuber) were taken from the treated and untreated plot in both trials 14 days before harvest and at harvest.

The field samples were stored deep-frozen within 24 hours after sampling and until dispatch. The field samples were shipped by deep-freeze lorry and arrived in good condition. The field samples were stored in a freezer at ≤-18°C until preparation of the examination samples. For the preparation of examination samples, the deep-frozen field samples were shredded with dry ice in a cutter. Representative parts of the shredded field samples were transferred into polystyrene boxes separately for analysis or archiving and stored at ≤-18°C

Residues of fluopyram and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide), were determined by LC-MS/MS according to method 00984 ([redacted], 05/02/2007, M-283301-01-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method

are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues of these compounds were extracted from 10 g of sample material (5 g for wheat straw) by two successive extractions using a high-speed blender with a mixture of acetonitrile:water (80:20; v:v). After centrifugation the extract volume was adjusted. Then, the extracts were diluted ten times by adding the internal standards:

- One dilution was performed under acidic conditions: this extract allowed the determination of FLU-PCA and FLU-methyl-sulfoxide.
- In parallel, another dilution was performed under basic conditions: this extract allowed the determination of FLU, FLU-benzamide and FLU-7-OH.

An aliquot of the extracts was injected into LC-MS/MS operated in Electrospray Ionization mode:

- One injection in positive electrospray ionization for the determination of FLU, FLU-benzamide and FLU-7-OH.
- Another injection in negative electrospray ionization for the determination of FLU-PCA and FLU-methyl-sulfoxide under different conditions.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels. For each calibration curve, the correlation coefficient R was above 0.99.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.05 mg/kg for wheat straw and 0.01 mg/kg for all other matrices.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Findings

In order to check the performance of the analytical method, recovery determinations were concurrently performed to the analyses of control and treated samples from the study in each set of analyses. The means of the concurrent recoveries were for all fortification levels within the acceptable range of 70 – 110 % except for FLU-methyl-sulfoxide at 111 % and 120 % respectively at 0.1 mg/kg and 1 mg/kg. All overall average recoveries are in the acceptable range of 70 – 110 %; therefore, the results are considered valid. Recovery results are presented in Table 6.6.2- 18.

No residue of fluopyram or related metabolites were found above the LOQ in any of the control samples of rotational crop matrices analysed.

The residue levels of fluopyram and its relevant metabolites in the rotational crop matrices of potato tubers are summarized in the Table 6.6.2-19.

The storage period of deep-frozen samples ranged between 316 and 345 days.

Residues in potato tubers

In trial 08-2171-01 (PBI 31 days), residues of fluopyram (AE C656948) were 0.02 mg/kg in potato tuber 14 days before harvest and at harvest.

The residues of the metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide) were < 0.01 mg/kg 14 days before harvest and at harvest.

In trial 08-2171-02 (PBI 30 days) residues of fluopyram (AE C656948) were 0.02 mg/kg in potato tubers 14 days before harvest and at harvest.

The residues of the metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide) were < 0.01 mg/kg 14 days before harvest and at harvest.

Conclusions

Following one spray application conducted with fluopyram SC500 to bare soil at a rate of 0.5 kg a.s./ha, residues in succeeding crops were analysed for fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188) and fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122) using a validated selective HPLC-MS/MS analytical method.

These data show that residues in potato (tuber) as succeeding crop are covered by the current EU MRL.

Assessment and conclusion by applicant:
The study is acceptable

Table 6.6.2- 18: Recovery data for fluopyram (AE C656948) and its metabolites in potato, tuber

Crop/Sample material	FL (mg/kg)	Single values (%)	Mean value (%)	RSD* (%)
Fluopyram (AE C656948)				
Potato, tuber	0.01	88; 93	91	--
	0.10	98	98	--
	1.0	106	106	--
		Overall recovery (n=4)	**96**	**8.0**
Fluopyram-benzamide (AEF148815)				
Potato, tuber	0.01	80; 76	78	--
	0.10	94	94	--
	1.0	98	98	--
		Overall recovery (n=4)	**86**	**14.6**
Fluopyram-pyridyl-carboxilic-acid (AE C657188)				
Potato, tuber	0.01	74; 85	80	--
	0.10	88	88	--
	1.0	96	96	--
		Overall recovery (n= 7)	**86**	**10.6**
Fluopyram-7-hydroxy (BCS-AA10065)				
Potato, tuber	0.01	90; 93	92	--
	0.10	101	101	--
	1.0	104	104	--
		Overall recovery (n= 7)	**97**	**6.8**
Fluopyram-methylsulfoxide (AE1344122)				
Potato, tuber	0.01	83; 79	81	--
	0.10	111	111	--
	1.0	120	120	--
		Overall recovery (n= 7)	**98**	**20.7**

FL: fortification level, RSD = Relative Standard Deviation
* some RSDs were not calculated as there were only two individual recoveries given
Final determination as: fluopyram Residues calculated as: fluopyram
Final determination as: FLU-benzamide Residues calculated as: fluopyram
Final determination as: FLU-methylsulfoxide Residues calculated as: fluopyram
Final determination as: FLU-PCA Residues calculated as: fluopyram
Final determination as: FLU-7OH Residues calculated as: fluopyram

Table 6.6.2- 19: Results of rotational crop trials conducted with fluopyram

Trial No./ Location/ Year	Plot (commodity)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment to bare soil or target crop			Dates of treatment or no. of treatments and last date	Portion analysed	Growth stage at sampling	Residues (mg/kg)						PHI (days)
			kg a.s./ha	Water (L/ha)	kg a.s./hL				FLU AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residue calc.	
	(a)	(b)				(c)		(d)							(e)
08-2171-01 Spain 46230 Alginet Comunidad Valenciana Europe, South F 2008	Application to bare soil **PBI: 31 d** Rotational crop: Potato, Nicola	1) 14.04.2008 2) 01.06.2008 - 20.06.2008 3) 01.07.2008 - 30.07.2008	0.5	300	0.17	14.03.2008	tuber	48 49	0.02 0.02	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	0.03 0.03	116 130
08-2171-02 Italy 00055 Ladispoli (RM) Lazio Europe, South F 2008	Application to bare soil **PBI: 30 d** Rotational crop: Potato, Veronie	1) 01.05.2008 2) 13.06.2008 - 07.07.2008 3) 15.07.2008 - 15.08.2008	0.5	300	0.17	01.04.2008	tuber	95 99	0.02 0.02	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	0.03 0.03	113 127

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) Study reference
* prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Total residue calculated : sum of fluopyram+FLU-benzamide

Data Point:	KCA 6.6.2/10
Report Author:	[redacted]
Report Year:	2009
Report Title:	Determination of the residues of AE C656948 in/on onion after spraying of fluopyram SC 500 in the field in France (North) and Germany
Report No:	08-2161
Document No:	M-352225-01-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted Study was not found in DAR/RAR and the Addenda
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and Methods

The purpose of the study was to determine the magnitude of residues of fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188),fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122), in/on onion (bulb) harvested after one spray application with Fluopyram SC 500 on bare soil followed by incorporation, 30 days before sowing of onions in northern Europe (northern France and Germany).

For application the formulation Fluopyram SC 500 was used, a suspension concentrate formulation containing 500 g/L of Fluopyram. The application was done with 1.0 L test item per ha and 300 L water per ha on bare soil followed by incorporation into the soil (< 8 cm) 30 days before the sowing of onions (28 days in trial 08-2161-02). The investigated plant back interval was 30 days.

In both trials the application was done with 1.0 L test item per ha and 300 L water per ha, corresponding to a spray concentration of the test item of 0.33 % (0.17% of active substance in the spray liquid). The application rate was 0.50 kg fluopyram/ha.

For residue analysis samples of onion (bulb) were taken 14 days before harvest and at harvest in both trials corresponding to 160 and 174 days (08-2161-01) and 124 and 138 days (08-2161-02) after last treatment. Samples of onion (bulb) were taken from the treated and the control plots at both sampling events. In order to obtain representative samples of the raw commodity, samples were taken according to different sampling procedures from various parts of each treated and control plot.

The field samples were stored deep-frozen within 24 hours after sampling and until dispatch. The field samples of trial 08-2161-01 were shipped by deep-freeze lorry, the field samples of trial 08-2161-02 were shipped by car with dry ice and arrived in good condition. The field samples were stored in a freezer at ≤-18°C until preparation of the examination samples. For the preparation of examination samples, the deep-frozen field samples were shredded with dry ice in a cutter. Representative parts of the shredded field samples were transferred into polystyrene boxes separately for analysis or archiving and stored at ≤-18°C.

Residues of fluopyram and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide) were determined by LC-MS/MS according to method 00984 ([redacted], 05/02/2007, M-283301-01-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues of these compounds were extracted from 10 g of sample material (5 g for wheat straw) by two successive extractions using a high-speed blender with a mixture of acetonitrile:water (80:20; v:v). After centrifugation the extract volume was adjusted. Then, the extracts were diluted ten times by adding the internal standards:

- One dilution was performed under acidic conditions: this extract allowed the determination of FLU-PCA and FLU-methyl-sulfoxide.
- In parallel, another dilution was performed under basic conditions: this extract allowed the determination of FLU, FLU-benzamide and FLU-7-OH.

An aliquot of the extracts was injected into LC-MS/MS operated in Electrospray Ionization mode:

- One injection in positive electrospray ionization for the determination of FLU, FLU-benzamide and FLU-7-OH.
- Another injection in negative electrospray ionization for the determination of FLU-PCA and FLU-methyl-sulfoxide under different conditions.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels. For each calibration curve, the correlation coefficient R was above 0.99.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.05 mg/kg for wheat straw and 0.01 mg/kg for all other matrices.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Findings

In order to check the performance of the analytical method recovery determinations were concurrently performed to the analyses of control and treated samples from the study in each set of analyses. The overall means of the concurrent recoveries were for all fortification levels within the acceptable range of 70 – 110 %, except for FLU-methyl-sulfoxide at 114 % at 0.1 mg/kg. All overall mean recoveries are in the acceptable range of 70 – 110 %; therefore the results are considered valid. Recovery results are presented in Table 6.6.2- 20.

No residue of fluopyram or related metabolites were found above the LOQ in any of the control samples of rotational crop matrices analysed.

The residue levels of fluopyram and its relevant metabolites in the rotational crop matrices of onion bulbs are summarized in the Table 6.6.2- 21.

The storage period of deep-frozen samples ranged between 262 and 301 days.

Residues in onion bulbs

In trial 08-2161-01 (PBI 30 days), residues of fluopyram (AE C656948) were at the level of 0.01 mg/kg in onion tuber 14 days before harvest and at harvest.

The residues of metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide) were < 0.01 mg/kg, 14 days before harvest and at harvest.

In trial 08-2161-02 (PBI 28 days) residues of fluopyram (AE C656948) and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide) were < 0.01 mg/kg, 14 days before harvest and at harvest.

Conclusions

Following one spray application conducted with fluopyram SC500 to bare soil at a rate of 0.5 kg a.s./ha, residues in succeeding crops were analysed for fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188) and fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122) using a validated selective HPLC-MS/MS analytical method.

These data show that residues in onion (bulb) as succeeding crop are covered by the current EU MRL.

Assessment and conclusion by applicant:
The study is acceptable

Table 6.6.2- 20: Recovery data for fluopyram (AE C656948) and its metabolites in onion, bulb

Crop/Sample material	FL (mg/kg)	Single values (%)	Mean value (%)	RSD*(%)
Fluopyram (AE C656948)				
Onion, bulb	0.01	81; 74; 83; 79	79	4.9
	0.10	97; 97; 98; 91	95	3.2
		Overall recovery (n=8)	**88**	**10.8**
Fluopyram-benzamide (AEF148815)				
Onion, bulb	0.01	83; 73; 73; 87	79	9.0
	0.10	92; 96; 92; 95	94	2.2
		Overall recovery (n=8)	**86**	**10.7**
Fluopyram-pyridyl-carboxilic-acid (AE C657188)				
Onion, bulb	0.01	65; 66; 77; 70	70	7.8
	0.10	89; 89; 94; 94	92	3.2
		Overall recovery (n=8)	**81**	**15.4**
Fluopyram-7-hydroxy (BCS-AA10065)				
Onion, bulb	0.01	82; 77; 83; 103	86	13.3
	0.10	90; 90; 93; 91	91	1.6
		Overall recovery (n=8)	**89**	**9.0**
Fluopyram-methylsulfoxide (AE1344122)				
Onion, bulb	0.01	90; 104; 96; 84	94	9.1
	0.10	114; 119; 108; 114	114	4.0
		Overall recovery (n=8)	**104**	**12.1**

FL: fortification level, RSD - Relative Standard Deviation
* some RSDs were not calculated as there were only two individual recoveries given
Final determination as: fluopyram Residues calculated as: fluopyram
Final determination as: FLU-benzamide Residues calculated as: fluopyram
Final determination as: FLU-methylsulfoxide Residues calculated as: fluopyram
Final determination as: FLU-PCA Residues calculated as: fluopyram
Final determination as: FLU-7OH Residues calculated as: fluopyram

Table 6.6.2- 21: Results of rotational crop trials conducted with fluopyram

Trial No./ Location/ Year	Plot (commodity)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment to bare soil or target crop			Dates of treatment or no. of treatments and last date	Portion analysed	Growth stage at sampling	Residues (mg/kg)						PHI (days)
			kg a.s./ha	Water (L/ha)	kg a.s./hL				AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residue calc.	
	(a)	(b)				(c)		(d)							(e)
08-2161-01 France, north 8410 Bouafle Ile-de-France Europe, North F 2008	Application to bare soil **PBI: 30 d** Rotational crop: Onion Paille des vertus	1) 18.04.2008 3) 01.09.2008 - 15.09.2008	0.5	300	0.17	19.03.2008	bulb	48 49	0.01 0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	0.02 0.02	160 174
08-2161-02 Germany 51399 Burscheid Nordrhein-Westfalen Europe, North F 2008	Application to bare soil **PBI: 28 d** Rotational crop: Onion Sherpa	1) 02.05.2008 3) 20.08.2008	0.5	300	0.17	04.04.2008	bulb	95 99	0.02 0.02	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	0.03 0.03	124 138

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse
F field
(e) Days after last application (Label pre-harvest-interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) Study reference
* prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Total residue calculated : sum of fluopyram+FLU-benzamide

Data Point:	KCA 6.6.2/11
Report Author:	[redacted]
Report Year:	2009
Report Title:	Determination of the residues of AE C656948 in/on onion after spraying of fluopyram SC 500 in the field in Italy and Spain
Report No:	08-2172
Document No:	M-355319-01-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC guidance working document 7029/VI/95 rev. 5 (1997-07-22)
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted Study was not found in DAR/RAR and the Addenda
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and Methods

The purpose of the study was to determine the magnitude of residues of fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188),fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122), in/on onion (bulb) harvested after one spraying application with Fluopyram SC 500 on bare soil followed by incorporation 30 days before sowing/planting of onion in southern Europe (Spain and Italy).

For application the formulation Fluopyram SC 500 was used a suspension concentrate formulation containing 500 g/L of Fluopyram. The application was done with 1.0 L test item per ha and 300 L water per ha on bare soil followed by incorporation into the soil (~ 8 cm) 30 days before the sowing of onions (31-29 days in trials 08-2161-01 & 02 respectively). The investigated plant back interval was 30 days.

In both trials the application was done with 1.0 L test item per ha and 300 L water per ha, corresponding to a spray concentration of the test item of 0.33 % (0.17% of active substance in the spray liquid). The application rate was 0.50 kg fluopyram /ha.

For residue analysis samples of onion (bulb) were taken 14 days before harvest and at harvest in both trials corresponding to 139 and 153 days (08-2172-01) and 133 and 146 days (08-2172-02) after last treatment. Samples of onion (bulb) were taken from the treated and the control plots at both sampling events. In order to obtain representative samples of the raw commodity, samples were taken according to different sampling procedures from various parts of each treated and control plot.

The field samples were stored deep-frozen within 24 hours after sampling and until dispatch. The field samples were shipped by deep-freeze lorry and arrived in good condition. The field samples were stored in a freezer at ≤-18 °C until preparation of the examination samples. For the preparation of examination samples, the deep-frozen field samples were shredded with dry ice in a cutter. Representative parts of the shredded field samples were transferred into polystyrene boxes separately for analysis or archiving and stored at ≤-18°C.

Residues of fluopyram and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide), were determined by LC-MS/MS according to method 00984 ([redacted], 05/02/2007, M-283301-01-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues of these compounds were extracted from 10 g of sample material (5 g for wheat straw) by two successive extractions using a high-speed blender with a mixture of acetonitrile:water (80:20; *v:v*). After centrifugation the extract volume was adjusted. Then, the extracts were diluted ten times by adding the internal standards:

- One dilution was performed under acidic conditions: this extract allowed the determination of FLU-PCA and FLU-methyl-sulfoxide.
- In parallel, another dilution was performed under basic conditions: this extract allowed the determination of FLU, FLU-benzamide and FLU-7-OH.

An aliquot of the extracts was injected into LC-MS/MS operated in Electrospray Ionization mode:

- One injection in positive electrospray ionization for the determination of FLU, FLU-benzamide and FLU-7-OH.
- Another injection in negative electrospray ionization for the determination of FLU-PCA and FLU-methyl-sulfoxide under different conditions.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels. For each calibration curve, the correlation coefficient R was above 0.99.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.05 mg/kg for wheat straw and 0.01 mg/kg for all other matrices.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Findings

In order to check the performance of the analytical method recovery determinations were concurrently performed to the analyses of control and treated samples from the study in each set of analyses. The means of the concurrent recoveries were for all fortification levels within the acceptable range of 70 – 110 % except for FLU-methyl-sulfoxide at 116 % for the fortification 0.1 mg/kg. All overall mean recoveries are in the acceptable range of 70 – 110 %; therefore, the results are considered valid. Recovery results are presented in Table 6.2- 22.

No residue of fluopyram or related metabolites were found above the LOQ in any of the control samples of rotational crop matrices analysed.

The residue levels of fluopyram and its relevant metabolites in the rotational crop matrices of onion bulbs are summarized in the Table 6.6.2- 23.

The storage period of deep-frozen samples ranged from 272 to 326 days.

Residues in onion bulbs

In trial 08-2172-01 (PBI 91 days), residues of residues of fluopyram (AE C656948) and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide) were < 0.01 mg/kg, 14 days before harvest and at harvest.

In trial 08-2172-02 (PBI 29 days) residues of residues of fluopyram (AE C656948) and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide) were < 0.01 mg/kg, 14 days before harvest and at harvest.

Conclusions

Following one spray application conducted with fluopyram SC500 to bare soil at a rate of 0.5 kg a.s./ha, residues in succeeding crops were analysed for fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188) and fluopyram-

7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122) using a validated selective HPLC-MS/MS analytical method.

These data show that residues in onion (bulb) as succeeding crop are covered by the current EU MRL.

Assessment and conclusion by applicant:
The study is acceptable

Table 6.6.2- 22: Recovery data for fluopyram (AE C656948) and its metabolites in onion, bulb

Crop/Sample material	FL (mg/kg)	Single values (%)	Mean value (%)	RSD*(%)
Fluopyram (AE C656948)				
Onion, bulb	0.01	87	87	--
	0.10	83; 85	84	1.7
		Overall recovery (n=3)	**85**	**2.4**
Fluopyram-benzamide (AEF148815)				
Onion, bulb	0.01	91	91	--
	0.10	88; 89	89	0.8
		Overall recovery (n=3)	**89**	**1.7**
Fluopyram-pyridyl-carboxilic-acid (AE C657188)				
Onion, bulb	0.01	77	77	--
	0.10	100; 96	98	2.9
		Overall recovery (n=3)	**91**	**13.5**
Fluopyram-7-hydroxy (BCS-AA10065)				
Onion, bulb	0.01	78	78	--
	0.10	95; 93	94	1.5
		Overall recovery (n=3)	**89**	**10.5**
Fluopyram-methylsulfoxide (AE1344122)				
Onion, bulb	0.01	97	97	--
	0.10	116; 116	116	0.0
		Overall recovery (n=3)	**110**	**10.0**

FL: fortification level, RSD - Relative Standard Deviation
* some RSDs were not calculated as there were only two individual recoveries given
Final determination as: fluopyram Residues calculated as: fluopyram
Final determination as: FLU-benzamide Residues calculated as: fluopyram
Final determination as: FLU-methylsulfoxide Residues calculated as: fluopyram
Final determination as: FLU-PCA Residues calculated as: fluopyram
Final determination as: FLU-7OH Residues calculated as: fluopyram

Table 6.6.2- 23: Results of rotational crop trials conducted with fluopyram

Trial No./ Location/ Year	Plot (commodity)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment to bare soil or target crop			Dates of treatment or no. of treatments and last date	Portion analysed	Growth stage at sampling	Residues (mg/kg)						PHI (days)
			kg a.s./ha	Water (L/ha)	kg a.s./hL				AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-PAM as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residue calc.	
	(a)	(b)				(c)		(d)							(e)
08-2172-01 Spain 46230 Alginet Comunidad Valenciana Europe, South F 2008	Application to bare soil **PBI: 31 d** Rotational crop: Onion Liria	1) 10.05.2008 3) 01.09.2008 - 30.09.2008	0.5	300	0.17	09.04.2008	bulb	48 49	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.02 <0.02	139 153
08-2172-02 Italy 40128 Bologna Emilia - Romagna Europe, South F 2008	Application to bare soil **PBI: 29 d** Rotational crop: Onion Density	1) 04.04.2008 3) 20.07.2008 - 30.07.2008	0.5	300	0.17	06.03.2008	bulb	47 48	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.02 <0.02	133 146

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH-Code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) Study reference
prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Total residue calculated : sum of fluopyram+FLU-benzamide

Data Point:	KCA 6.6.2/12
Report Author:	[redacted]
Report Year:	2009
Report Title:	Determination of the residues of AE C656948 in/on tomato after spraying of fluopyram SC 500 in the field in France (North) and Germany
Report No:	08-2165
Document No:	M-352213-01-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted Study was not found in DAR/RAR and the Addenda
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and Methods

The purpose of the study was to determine the magnitude of the relevant residues of fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188),fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122), in/on tomato (fruits) harvested after one spraying application with Fluopyram SC 500 on bare soil 30 days before sowing/planting of tomato in northern Europe (northern France and Germany). The investigated plant back interval was 30 days.

For application the formulation Fluopyram SC 500 was used, a suspension concentrate formulation containing 500 g/L of Fluopyram. The application was done on bare soil followed by incorporation into the soil (< 8 cm) 30 days before the sowing of tomato.

In both trials the application was done with 1.0 L of test item per ha and 300 L of water per ha, corresponding to a spray concentration of 0.33% (0.17% of active substance in the spray liquid). The application rate was 0.50 kg fluopyram /ha.

In both trials the application was done on bare soil 30 days (08-2165-01) and 28 days (08-2165-02) before the planting of tomato. The test item was incorporated into the soil (08-2165-01: 5-6 cm depth and 08-2165-02: < 8cm depth).

For residue analysis samples of tomato (fruit) were taken from the treated and the control plots. In order to obtain representative samples of the raw commodity, samples were taken at random along the treated and the control plot. Samples of tomato (fruit) were taken from the treated and untreated plot in both trials at harvest.

The field samples were stored deep-frozen within 24 hours after sampling and until dispatch. All field samples were shipped by deep-freeze lorry (08-2165-01) and by car with dry ice (08-2165-02) and arrived in good condition. The field samples were stored in a freezer at ≤ -18°C until preparation of the examination samples. For the preparation of examination samples, the deep-frozen field samples were shredded with dry ice in a cutter. Representative parts of the shredded field samples were transferred into polystyrene boxes separately for analysis or archiving and stored at ≤ -18°C.

Residues of fluopyram and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide), were determined by LC-MS/MS according to method 00984 ([redacted], 05/02/2007, M-283301-01-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method

are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues of these compounds were extracted from 10 g of sample material (5 g for wheat straw) by two successive extractions using a high-speed blender with a mixture of acetonitrile:water (80:20; v:v). After centrifugation the extract volume was adjusted. Then, the extracts were diluted ten times by adding the internal standards:

- One dilution was performed under acidic conditions: this extract allowed the determination of FLU-PCA and FLU-methyl-sulfoxide.
- In parallel, another dilution was performed under basic conditions: this extract allowed the determination of FLU, FLU-benzamide and FLU-7-OH.

An aliquot of the extracts was injected into LC-MS/MS operated in Electrospray Ionization mode:

- One injection in positive electrospray ionization for the determination of FLU, FLU-benzamide and FLU-7-OH.
- Another injection in negative electrospray ionization for the determination of FLU-PCA and FLU-methyl-sulfoxide under different conditions.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels. For each calibration curve, the correlation coefficient R was above 0.99.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.05 mg/kg for wheat straw and 0.01 mg/kg for all other matrices.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Findings

In order to check the performance of the analytical method, recovery determinations were concurrently performed to the analyses of control and treated samples from the study in each set of analyses. The overall means of the concurrent recoveries were within the acceptable range of 70 – 110 % and all the results are considered valid. Recovery results are presented in Table 6.6.2- 24.

No residue of fluopyram or related metabolites were found above the LOQ in any of the control samples of rotational crop matrices analysed.

The residue levels of fluopyram and its relevant metabolites in the rotational crop matrices of tomato fruits are summarized in the Table 6.6.2- 25.

The storage period of deep-frozen samples ranged from 290 to 321 days.

Residues in tomato fruits

In trial 08-2172-01 (PBI 30 days), residues of fluopyram (AE C656948) and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide) were all < 0.01 mg/kg in tomato fruit at harvest.

In trial 08-2172-02 (PBI 28 days) residues of fluopyram (AE C656948) and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide) were all < 0.01 mg/kg in tomato fruit at harvest.

Conclusions

Following one spray application conducted with fluopyram SC500 to bare soil at a rate of 0.5 kg a.s./ha, residues in succeeding crops were analysed for fluopyram (AE C656948) and its metabolites fluopyram-

benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188) and fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122) using a validated selective HPLC-MS/MS analytical method.

These data show that residues in tomato (fruit) as succeeding crop are covered by the current EU MRL.

Assessment and conclusion by applicant:
The study is acceptable

Table 6.6.2- 24: Recovery data for fluopyram (AE C656948) and its metabolites in tomato, fruit

Crop/Sample material	FL (mg/kg)	Single values (%)	Mean value (%)	RSD* (%)
Fluopyram (AE C656948)				
Tomato, fruit	0.01	83	83	--
	0.10	91	91	--
	1.0	97	97	--
		Overall recovery (n=3)	**90**	**7.8**
Fluopyram-benzamide (AEF148815)				
Tomato, fruit	0.01	81	81	--
	0.10	90	90	--
	1.0	93	93	--
		Overall recovery (n=3)	**88**	**7.1**
Fluopyram-pyridyl-carboxilic-acid (AE C657188)				
Tomato, fruit	0.01	75	75	--
	0.10	89	89	--
	1.0	92	92	--
		Overall recovery (n=3)	**85**	**10.6**
Fluopyram-7-hydroxy (BCS-AA10065)				
Tomato, fruit	0.01	86	86	--
	0.10	81	81	--
	1.0	81	81	--
		Overall recovery (n=3)	**83**	**3.5**
Fluopyram-methylsulfoxide (AE1344122)				
Tomato, fruit	0.01	71	71	--
	0.10	80	80	--
	1.0	88	88	--
		Overall recovery (n=3)	**80**	**10.7**

FL: fortification level, RSD = Relative Standard Deviation
* some RSDs were not calculated as there were only two individual recoveries given
Final determination as: fluopyram Residues calculated as: fluopyram
Final determination as: FLU-benzamide Residues calculated as: fluopyram
Final determination as: FLU-methylsulfoxide Residues calculated as: fluopyram
Final determination as: FLU-PCA Residues calculated as: fluopyram
Final determination as: FLU-7OH Residues calculated as: fluopyram

Table 6.6.2- 25: Results of rotational crop trials conducted with fluopyram

Trial No./ Location/ Year	Plot (commodity)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment to bare soil or target crop			Dates of treatment or no. of treatments and last date	Portion analysed	Growth stage at sampling	Residues (mg/kg)						PHI (days)
			kg a.s./ha	Water (L/ha)	kg a.s./hL				AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residue calc.	
	(a)	(b)				(c)		(d)							(e)
08-2165-01 France, north 37230 Fondettes Centre Europe, North F 2008	Application to bare soil **PBI: 30 d** Rotational crop: Tomato Super Marmande	1) 02.04.2008 1) 16.05.2008 2) 09.06.2008 - 04.07.2008 3) 04.08.2008 - 15.08.2008	0.5	300	0.17	16.04.2008	fruit	89	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	114
08-2165-02 Germany 51399 Burscheid Nordrhein-Westfalen Europe, North F 2008	Application to bare soil **PBI: 28 d** Rotational crop: Tomato Hoffmanns Rentita	1) 23.05.2008 2) 15.06.2008 - 01.09.2008 3) 08.09.2008	0.5	300	0.17	25.04.2008	fruit	89	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	136

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) Study reference
* prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Total residue calculated : sum of fluopyram+FLU-benzamide

Data Point:	KCA 6.6.2/13
Report Author:	[redacted]
Report Year:	2010
Report Title:	Determination of the residues of AE C656948 in/on tomato after spraying of fluopyram SC 500 in the field in Italy and Spain
Report No:	08-2176
Document No:	M-355320-02-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC guidance working document 7029/VI/95 rev. 5 (1997-07-22)
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted rev. 1 to Vol.3 of DAR B7 August 2012 (references relied on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and Methods

The purpose of the study was to determine the magnitude of residues of fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188), fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122), in/on tomato (fruits) harvested after one spray application with Fluopyram SC 500 on bare soil 30 days before sowing/planting of tomato in southern Europe (in Italy and Spain). The investigated plant back interval was 30 days.

Samples were taken at growth stage BBCH 87 (corresponding to DALT 125, trial 08-2176-01) and BBCH 89 (corresponding to DALT 114, trial 08-2176-02).

For application the formulation Fluopyram SC 500 was used, a suspension concentrate formulation containing 500 g/L of Fluopyram. The application was done on bare soil followed by incorporation into the soil (< 8 cm) 30 days before the sowing of tomato.

In both trials the application was done with 1.0 L of test item per ha and 300 L of water per ha, corresponding to a spray concentration of 0.33% (0.17% of active substance in the spray liquid). The application rate was 0.50 kg fluopyram /ha.

In both trials the application was done on bare soil 30 days (08-2165-01) and 28 days (08-2165-02) before the planting of tomato. The test item was incorporated into the soil (08-2176-01: 8-10 cm depth; 08-2176-02: <8 cm depth).

For residue analysis samples of tomatoes were taken from the treated and the control plots. In order to obtain representative samples of the raw commodity, samples were taken at random all along the treated and the control plot. Samples were taken at harvest : growth stage BBCH 87 (trial 08-2176-01) and BBCH 89 (trial 08-2176-02).

The field samples were stored deep-frozen within 24 hours after sampling and until dispatch. All field samples were shipped by deep-freeze lorry and arrived in good condition. The field samples were stored in a freezer at ≤-18°C until preparation of the examination samples. For the preparation of examination samples, the deep-frozen field samples were shredded with dry ice in a cutter. Representative parts of the shredded field samples and laboratory samples were transferred into polystyrene boxes separately for analysis or archiving and stored at ≤-18°C.

Residues of fluopyram and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide), were determined by LC-MS/MS according to method 00984 ([redacted], 05/02/2007, M-283301-01-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues of these compounds were extracted from 10 g of sample material (5 g for wheat straw) by two successive extractions using a high-speed blender with a mixture of acetonitrile:water (80:20, v:v). After centrifugation the extract volume was adjusted. Then, the extracts were diluted ten times by adding the internal standards:

- One dilution was performed under acidic conditions: this extract allowed the determination of FLU-PCA and FLU-methyl-sulfoxide.
- In parallel, another dilution was performed under basic conditions: this extract allowed the determination of FLU, FLU-benzamide and FLU-7-OH.

An aliquot of the extracts was injected into LC-MS/MS operated in Electrospray Ionization mode:

- One injection in positive electrospray ionization for the determination of FLU, FLU-benzamide and FLU-7-OH.
- Another injection in negative electrospray ionization for the determination of FLU-PCA and FLU-methyl-sulfoxide under different conditions.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels. For each calibration curve, the correlation coefficient R was above 0.99.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.05 mg/kg for wheat straw and 0.01 mg/kg for all other matrices.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Findings

In order to check the performance of the analytical method, recovery determinations were concurrently performed to the analyses of control and treated samples from the study in each set of analyses. The overall means of the concurrent recoveries were within the acceptable range of 70 – 110 % except for FLU-PCA (65% at 0.01 mg/kg spiking level). All overall mean recoveries are in the acceptable range of 70 – 110 %; therefore, the results are considered valid. Recovery results are presented in Table 6.6.2-26.

No residue of fluopyram or related metabolites were found above the LOQ in any of the control samples of rotational crop matrices analysed.

The residue levels of fluopyram and its relevant metabolites in the rotational crop matrices of tomato fruits are summarized in the Table 6.6.2- 27.

The storage period of deep-frozen samples ranged between 259 and 348 days.

Residues in tomato fruits

In trial 08-2176-01 (PBI 30 days), residues of fluopyram (AE C656948) and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide) were all < 0.01 mg/kg in tomato fruit at harvest.

In trial 08-2176-02 (PBI 30 days) residues of fluopyram (AE C656948) and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide) were all < 0.01 mg/kg in tomato fruit at harvest.

Conclusions

Following one spray application conducted with fluopyram SC500 to bare soil at a rate of 0.5 kg a.s./ha, residues in succeeding crops were analysed for fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188) and fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122) using a validated selective HPLC-MS/MS analytical method.

These data show that residues in tomato (fruit) as succeeding crop are covered by the current EU MRL.

Assessment and conclusion by applicant
The study is acceptable

Table 6.6.2- 26: Recovery data for fluopyram (AE C656948) and its metabolites in tomato, fruit

Crop/Sample material	FL (mg/kg)	Single values (%)	Mean value (%)	RSD* (%)
Fluopyram (AE C656948)				
Tomato, fruit	0.01	84; 94; 90	89	5.6
	0.10	97; 106; 100	101	4.5
		Overall recovery (n=6)	**95**	**8.1**
Fluopyram-benzamide (AEF148815)				
Tomato, fruit	0.01	77; 80; 85	81	5.0
	0.10	90; 85; 92	89	4.1
		Overall recovery (n=6)	**85**	**6.7**
Fluopyram-pyridyl-carboxilic-acid (AE C657188)				
Tomato, fruit	0.01	68; 66; 61	65	5.5
	0.10	94; 90; 90	91	2.5
		Overall recovery (n=6)	**78**	**18.8**
Fluopyram-7-hydroxy (BCS-AA10065)				
Tomato, fruit	0.01	98; 94; 92	95	3.2
	0.10	101; 108; 99	103	4.6
		Overall recovery (n=6)	**99**	**5.7**
Fluopyram-methylsulfoxide (AE1344122)				
Tomato, fruit	0.01	73; 97; 95	88	15.1
	0.10	118; 109; 124	120	2.7
		Overall recovery (n=6)	**104**	**18.7**

FL: fortification level, RSD - Relative Standard Deviation
* some RSDs were not calculated as there were only two individual recoveries given
Final determination as: fluopyram Residues calculated as: fluopyram
Final determination as: FLU-benzamide Residues calculated as: fluopyram
Final determination as: FLU-methylsulfoxide Residues calculated as: fluopyram
Final determination as: FLU-PCA Residues calculated as: fluopyram
Final determination as: FLU-7OH Residues calculated as: fluopyram

Table 6.6.2- 27: Results of rotational crop trials conducted with fluopyram

Trial No./ Location/ Year	Plot (commodity)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment to bare soil or target crop			Dates of treatment or no. of treatments and last date	Portion analysed	Growth stage at sampling	Residues (mg/kg)						PHI (days)
			kg a.s./ha	Water (L/ha)	kg a.s./hL				AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residue calc.	
	(a)	(b)				(c)		(d)							(e)
08-2176-01 Spain 46230 Alginet Comunidad Valenciana Europe, South F 2008	Application to bare soil **PBI: 30 d** Rotational crop: Tomato Vilma	1) 18.07.2008 2) 01.09.2008 - 01.10.2008 3) 10.09.2008 - 10.10.2008	0.5	300	0.17	18.06.2008	fruit	87	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	125
08-2176-02 Italy 00055 Ladispoli (RM) Lazio Europe, South F 2008	Application to bare soil **PBI: 30 d** Rotational crop: Tomato Perfect Peel PS 1296	1) 01.05.2008 2) 25.05.2008 - 24.07.2008 3) 15.07.2008 - 15.08.2008	0.5	300	0.17	01.04.2008	fruit	89	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	114

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) Study reference
* prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Total residue calculated : sum of fluopyram+FLU-benzamide

Data Point:	KCA 6.6.2/14
Report Author:	[redacted]
Report Year:	2009
Report Title:	Determination of the residues of AE C656948 in/on pea, after spraying of fluopyram SC 500 in the field in Germany and Netherlands
Report No:	08-2167
Document No:	M-354235-01-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8; Residues in or on Treated Products, Food and Feed
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted rev. 1 to Vol.3 of DAR B7 August 2012 (references relied on)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and Methods

The purpose of the study was to determine the magnitude of residues of fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188),fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122), in/on pea, field (dry seed and green seed) harvested after one spray application with Fluopyram SC 500 on bare soil 30 days before sowing/planting of pea in northern Europe (the Netherlands and Germany). The investigated plant back interval was 30 days.

The formulation Fluopyram SC 500 is a suspension concentrate formulation containing 500 g/L of Fluopyram. In trial 08-2167-01 and 08-2167-02 the application was done on bare soil followed by incorporation into the soil (~ 8 cm) 30 days (trial 08-2167-01) and 28 days (trial 08-2167-02) before sowing of pea, field.

In both trials the application was done with 1.0 L test item per ha and 300 L water per ha, corresponding to a spray concentration of the test item of 0.33 % (0.17% of active substance in the spray liquid). The application rate was 0.50 kg fluopyram /ha.

For residue analysis samples of pea, field, green seed and dry seed, were taken from the treated and the control plots. In order to obtain representative samples of the raw commodity, samples were taken at random along the treated and the control plot.

Samples of pods were taken in both trials at growth stage BBCH 79. Samples of pods were separated in pods and green seed. Samples taken at normal harvest (growth stage BBCH 89) were also separated in pods and dry seed.

The field samples and lab sample were stored deep-frozen within 24 hours after sampling and until dispatch. The field samples and lab samples of trial 08-2167-01and trial 08-2167-02 were shipped by car with dry ice and arrived in good condition. The field samples and lab samples were stored in a freezer at ≤-18°C until preparation of the examination samples. For the preparation of examination samples, the deep-frozen field samples and lab samples were shredded with dry ice in a cutter. Representative parts of the shredded field samples were transferred into polystyrene boxes separately for analysis or archiving and stored at ≤-18°C.

Residues of fluopyram and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide), were determined by LC-MS/MS according to method 00984 ([redacted], 05/02/2007, M-283301-01-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues of these compounds were extracted from 10 g of sample material (5 g for wheat straw) by two successive extractions using a high-speed blender with a mixture of acetonitrile:water (80:20; v:v). After centrifugation the extract volume was adjusted. Then, the extracts were diluted ten times by adding the internal standards:

- One dilution was performed under acidic conditions: this extract allowed the determination of FLU-PCA and FLU-methyl-sulfoxide.
- In parallel, another dilution was performed under basic conditions: this extract allowed the determination of FLU, FLU-benzamide and FLU-7-OH.

An aliquot of the extracts was injected into LC-MS/MS operated in Electrospray ionization mode:

- One injection in positive electrospray ionization for the determination of FLU, FLU-benzamide and FLU-7-OH.
- Another injection in negative electrospray ionization for the determination of FLU-PCA and FLU-methyl-sulfoxide under different conditions.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels. For each calibration curve, the correlation coefficient R was above 0.99.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.05 mg/kg for wheat straw and 0.01 mg/kg for all other matrices.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Findings

In order to check the performance of the analytical method, recovery determinations were concurrently performed to the analyses of control and treated samples from the study in each set of analyses. The overall means of the concurrent recoveries were within the acceptable range of 70 – 110 % and all the results are considered valid. Recovery results are presented in Table 6.6.2- 28.

No residue of fluopyram or related metabolites were found above the LOQ in any of the control samples of rotational crop matrices analysed.

The residue levels of fluopyram and its relevant metabolites in the rotational crop matrices of pea, field are summarized in the Table 6.6.2- 29.

The storage period of deep-frozen samples is up to 362 days for green seeds and 335 days for dry seeds..

Residues in pea, field

In trial 08-2167-01 (PBI 30 days), residues of fluopyram (AE C656948) and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide) were all < 0.01 mg/kg in pea, field matrices (both green seed and dry seed) at harvest and at BBCH 79.

In trial 08-2167-02 (PBI 28 days) residues of fluopyram (AE C656948) and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide) were all < 0.01 mg/kg in pea, field matrices (both green seed and dry seed) at harvest and at BBCH 79 except for FLU-PCA in dry pea seed in which the residues were at the level of 0.02 mg/kg at harvest.

Conclusions

Following one spray application conducted with fluopyram SC500 to bare soil at a rate of 0.5 kg a.s./ha, residues in succeeding crops were analysed for fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188) and fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122) using a validated selective HPLC-MS/MS analytical method.

These data show that residues in pea (seed) as succeeding crop are covered by the current EU MRL.

Assessment and conclusion by applicant:
The study is acceptable

Table 6.6.2- 28: Recovery data for fluopyram (AE C656948) and its metabolites in pea, field

Crop/Sample material	FL (mg/kg)	Single values (%)	Mean value (%)	RSD*(%)
Fluopyram (AE C656948)				
Pea, field Dry seed	0.01	104	104	--
	0.10	100	100	--
	1.0	93	93	--
		Overall recovery (n=3)	**99**	**5.6**
Pea, field Green seed	0.01	116	116	--
	0.10	109	109	--
	1.0	99	99	--
		Overall recovery (n=3)	**108**	**7.9**
Fluopyram-benzamide (AEF148815)				
Pea, field Dry seed	0.01	80	80	--
	0.10	94	94	--
	1.0	84	84	--
		Overall recovery (n=3)	**86**	**8.4**
Pea, field Green seed	0.01	82	82	--
	0.10	96	96	--
	1.0	91	91	--
		Overall recovery (n=3)	**90**	**7.9**
Fluopyram-pyridyl-carboxilic-acid (AE C657188)				
Pea, field Dry seed	0.01	120	120	--
	0.10	99	99	--
	1.0	88	88	--
		Overall recovery (n=3)	**102**	**15.9**
Pea, field Green seed	0.01	123	123	--
	0.10	94	94	--
	1.0	92	92	--
		Overall recovery (n=3)	**103**	**16.8**
Fluopyram-7-hydroxy (BCS-AA10065)				
Pea, field Dry seed	0.01	90	90	--
	0.10	99	99	--
	1.0	93	93	--
		Overall recovery (n=3)	**94**	**4.9**
Pea, field Green seed	0.01	94	94	--
	0.10	105	105	--
	1.0	95	95	--
		Overall recovery (n=3)	**98**	**6.2**
Fluopyram-methylsulfoxide (AE1344122)				
Pea, field Dry seed	0.01	85	85	--
	0.10	106	106	--
	1.0	105	105	--
		Overall recovery (n=3)	**99**	**12.0**
Pea, field Green seed	0.01	95	95	--
	0.10	115	115	--
	1.0	111	111	--
		Overall recovery (n=3)	**107**	**9.9**

FL: fortification level, RSD: Relative Standard Deviation
* some RSDs were not calculated as there were only two individual recoveries given
Final determination as: fluopyram Residues calculated as: fluopyram
Final determination as: FLU-benzamide Residues calculated as: fluopyram
Final determination as: FLU-methylsulfoxide Residues calculated as: fluopyram
Final determination as: FLU-PCA Residues calculated as: fluopyram
Final determination as: FLU-7OH Residues calculated as: fluopyram

Table 6.6.2- 29: Results of rotational crop trials conducted with fluopyram

Trial No./ Location/ Year	Plot (commodity)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment to bare soil or target crop			Dates of treatment or no. of treatments and last date	Portion analysed	Growth stage at sampling	Residues (mg/kg)						PHI (days)
			kg a.s./ha	Water (L/ha)	kg a.s./hL				AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residue calc.	
	(a)	(b)				(c)		(d)							(e)
08-2167-01 Netherlands 1681 ND Zwaagdijk-Oost Noord-Holland Europe, North F 2008	Application to bare soil **PBI: 30 d** Rotational crop: Pea, field Kelvedon	1) 14.05.2008 2) 01.07.2008 - 01.08.2008 3) 29.08.2008 - 05.09.2008	0.5	300	0.17	14.04.2008	seed, green	79	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	121
							seed, dry	89	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	140
08-2167-02 Germany 51399 Burscheid Nordrhein-Westfalen Europe, North F 2008	Application to bare soil **PBI: 28 d** Rotational crop: Pea, field Konto	1) 02.05.2008 2) 06.06.2008 - 15.06.2008 3) 30.07.2008 - 02.08.2008	0.5	300	0.17	04.04.2008	seed, green	79	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	90
							seed, dry	89	<0.01	<0.01	0.02	<0.01	<0.01	<0.02	117

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) Study reference
prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Total residue calculated : sum of fluopyram+FLU-benzamide

Data Point:	KCA 6.6.2/15
Report Author:	[redacted]
Report Year:	2009
Report Title:	Determination of the residues of AE C656948 in/on pea, after spraying of fluopyram SC 500 in the field in Italy and Spain
Report No:	08-2178
Document No:	M-354237-01-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8; Residues in or on Treated Products, Food and Feed
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted Study was not found in DAR/RAR and the Addenda
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and Methods

The purpose of the study was to determine the magnitude of residues of fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188),fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122), in/on pea, field (dry seed and green seed) harvested after one spraying application with Fluopyram SC 500 on the bare soil 30 days before sowing/planting of pea in southern Europe (Spain and Italy). The investigated plant back interval was 30 days.

The formulation Fluopyram SC 500 is a suspension concentrate formulation containing 500 g/L of Fluopyram. The application was done with 1.0 L of test item per ha and 300 L water per ha on bare soil followed by incorporation into the soil (~ 8 cm) 30 days before the sowing of pea, fields.

In trial 08-2178-01 330 L water per ha were used, corresponding to a spray concentration of 0.33 %. The application rate was 0.55 kg/ha of the active substance (a.s.) Fluopyram.

In trial 08-2178-02, 300 L water per ha were used, corresponding to a spray concentration of 0.33 %. The application rate was 0.50 kg/ha of active substance (a.s.) Fluopyram.

In both trials the application was done on bare soil followed by incorporation (08-2178-01: no information on incorporation depth; 08-2178-02: 5-10 cm depth) 28 days before the sowing of pea, field.

For residue analysis samples of pea, field, green seed and dry seed, were taken from the treated and the control plots. In order to obtain representative samples of the raw commodity, samples were taken all along the treated and the control plot (trial 08-2178-01) and according to S-sampling (trial 08-2178-02).

Samples of pods were taken in both trials at growth stage BBCH 79. Samples of pods were separated in pods and green seed. Samples taken at normal harvest (growth stage BBCH 89) were also separated in pods and dry seed.

In trial 08-2178-02 dry seeds were sampled in growth stage BBCH 87 because the pods were open very early and seeds dropped off, therefore, the plots had to be harvested earlier.

The field samples and laboratory samples were stored deep-frozen within 24 hours after sampling and until dispatch. All field samples and laboratory samples were shipped by deep-freeze lorry and arrived in good condition. The field samples and laboratory samples were stored in a freezer at ≤-18°C until preparation of the examination samples. For the preparation of examination samples, the deep-frozen

field samples and laboratory samples were shredded with dry ice in a cutter. Representative parts of the shredded field samples and laboratory samples were transferred into polystyrene boxes separately for analysis or archiving and stored at ≤-18°C.

Residues of fluopyram and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide), were determined by LC-MS/MS according to method 00984 ([redacted], 05/02/2007, M-283301-01-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues of these compounds were extracted from 10 g of sample material (5 g for wheat straw) by two successive extractions using a high-speed blender with a mixture of acetonitrile:water (80:20; *v:v*). After centrifugation the extract volume was adjusted. Then, the extracts were diluted ten times by adding the internal standards:

- One dilution was performed under acidic conditions; this extract allowed the determination of FLU-PCA and FLU-methyl-sulfoxide.
- In parallel, another dilution was performed under basic conditions; this extract allowed the determination of FLU, FLU-benzamide and FLU-7-OH.

An aliquot of the extracts was injected into LC-MS/MS operated in Electrospray Ionization mode:

- One injection in positive electrospray ionization for the determination of FLU, FLU-benzamide and FLU-7-OH.
- Another injection in negative electrospray ionization for the determination of FLU-PCA and FLU-methyl-sulfoxide under different conditions.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels. For each calibration curve, the correlation coefficient R was above 0.99.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.05 mg/kg for wheat straw and 0.01 mg/kg for all other matrices.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Findings

In order to check the performance of the analytical method, recovery determinations were concurrently performed to the analyses of control and treated samples from the study in each set of analyses. The means of the concurrent recoveries were for all fortification levels within the acceptable range of 70 – 110 % except for FLU-methyl-sulfoxide at 112 % at 0.1 mg/kg. All overall mean recoveries are in the acceptable range of 70 – 110 %; therefore, the results are considered valid. Recovery results are presented in Table 6.6.2- 30.

No residue of fluopyram or related metabolites were found above the LOQ in any of the control samples of rotational crop matrices analysed.

The residue levels of fluopyram and its relevant metabolites in the rotational crop matrices of pea, field are summarized in the Table 6.6.2- 31.

The storage period of deep-frozen samples is up to 365 days for green seeds and 352 days for dry seeds.

Residues in pea, field

In trial 08-2178-01 (PBI 28 days), residues of fluopyram (AE C656948) and its metabolites (FLU-benzamide, FLU-7-OH, FLU-methyl-sulfoxide) were all < 0.01 mg/kg in pea, field matrices (both green

seed and dry seed) at harvest and at BBCH 79. Residues of FLU-PCA were 0.02 mg/kg in green seeds and 0.03mg/kg at harvest in dry seeds.

In trial 08-2178-02 (PBI 28 days) residues of fluopyram (AE C656948) and its metabolites (FLU-benzamide, FLU-7-OH, FLU-methyl-sulfoxide) were all < 0.01 mg/kg in pea, field matrices (both green seed and dry seed) at harvest and at BBCH 79. Residues of FLU-PCA were 0.01 mg/kg in green seeds and 0.03mg/kg at harvest in dry seeds.

Conclusions

Following one spray application conducted with fluopyram SC500 to bare soil at a rate of 0.5 kg a.s./ha, residues in succeeding crops were analysed for fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188) and fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122) using a validated selective HPLC-MS/MS analytical method.

These data show that residues in pea (seed) as succeeding crop are covered by the current EU MRL.

Assessment and conclusion by applicant:
The study is acceptable

Table 6.6.2- 30: Recovery data for fluopyram (AE C656948) and its metabolites in pea, field

Crop/Sample material	FL (mg/kg)	Single values (%)	Mean value (%)	RSD*(%)
Fluopyram (AE C656948)				
Pea, field Dry seed	0.01	86; 92; 83	87	5.3
	0.10	96; 99; 94	96	2.6
	1.0	94	94	--
		Overall recovery (n=7)	**92**	**6.1**
Pea, field Green seed	0.01	89; 95; 93	92	3.3
	0.10	109; 106; 105	107	2.0
	1.0	97	97	--
		Overall recovery (n=7)	**99**	**7.6**
Fluopyram-benzamide (AEF148815)				
Pea, field Dry seed	0.01	67; 87; 84	79	17.7
	0.10	91; 89; 87	89	2
	1.0	82	82	--
		Overall recovery (n=7)	**81**	**13.5**
Pea, field Green seed	0.01	89; 87; 83	86	3.5
	0.10	98; 95; 96	96	1.6
	1.0	90	90	--
		Overall recovery (n=7)	**91**	**5.9**
Fluopyram-pyridyl-carboxilic acid (AE C657188)				
Pea, field Dry seed	0.01	84; 98; 89	90	7.9
	0.10	89; 90; 92	90	1.7
	1.0	80	80	--
		Overall recovery (n=7)	**89**	**6.4**
Pea, field Green seed	0.01	98; 83; 80	87	11.1
	0.10	93; 95; 103	97	5.5
	1.0	91	91	--
		Overall recovery (n=7)	**92**	**8.8**
Fluopyram-7-hydroxy (BCS-AA10065)				
Pea, field Dry seed	0.01	78; 81; 75	78	3.8
	0.10	96; 103; 100	100	3.5
	1.0	90	90	--
		Overall recovery (n=7)	**89**	**12.5**
Pea, field Green seed	0.01	89; 90; 88	89	1.1
	0.10	103; 101; 101	102	1.1
	1.0	95	95	--
		Overall recovery (n=7)	**95**	**6.7**
Fluopyram-methylsulfoxide (AE1344122)				
Pea, field Dry seed	0.01	83; 104; 74	87	17.7
	0.10	105; 101; 107	104	2.9
	1.0	102	102	--
		Overall recovery (n=7)	**97**	**13.2**
Pea, field Green seed	0.01	73; 90; 77	80	11.1
	0.10	113; 113; 109	112	2.1
	1.0	104	104	--
		Overall recovery (n=7)	**97**	**17.5**

FL: fortification level, RSD - Relative Standard Deviation
* some RSDs were not calculated as there were only two individual recoveries given
Final determination as: fluopyram Residues calculated as: fluopyram
Final determination as: FLU-benzamide Residues calculated as: fluopyram
Final determination as: FLU-methylsulfoxide Residues calculated as: fluopyram
Final determination as: FLU-PCA Residues calculated as: fluopyram
Final determination as: FLU-7OH Residues calculated as: fluopyram

Table 6.6.2- 31: Results of rotational crop trials conducted with fluopyram

Trial No./ Location/ Year	Plot (commodity)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment to bare soil or target crop			Dates of treatment or no. of treatments and last date	Portion analysed	Growth stage at sampling	Residues (mg/kg)						PHI (days)
			kg a.s./ha	Water (L/ha)	kg a.s./hL				AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	PLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residue calc.	
	(a)	(b)				(c)		(d)							(e)
08-2178-01 Spain 08850 Gava - Barcelona Cataluña Europe, South F 2008	Application to bare soil **PBI: 28 d** Rotational crop: Pea, field Utrillo	1) 11.04.2008 2) 20.05.2008 - 30.06.2008 3) 20.06.2008 - 30.06.2008	0.55	330	0.17	14.03.2008	seed, green	79	<0.01	<0.01	0.02	<0.01	<0.01	<0.02	105
							seed, dry	89	<0.01	<0.01	0.03	<0.01	<0.01	<0.02	118
08-2178-02 Italy 40128 Bologna Emilia - Romagna Europe, South F 2008	Application to bare soil **PBI: 28 d** Rotational crop: Pea, field Pepone	1) 09.04.2008 2) 01.05.2008 - 15.05.2008 3) 01.07.2008 - 15.07.2008	0.50	300	0.17	12.03.2008	seed, green	79	<0.01	<0.01	0.01	<0.01	<0.01	<0.02	106
							seed, dry	87	<0.01	<0.01	0.03	<0.01	<0.01	<0.02	119

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) Study reference
* prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Total residue calculated : sum of fluopyram+FLU-benzamide

Data Point:	KCA 6.6.2/16
Report Author:	[redacted]
Report Year:	2009
Report Title:	Determination of the residues of AE C656948 in/on maize/corn after spraying of fluopyram SC 500 in the field in France (North) and Germany
Report No:	08-2168
Document No:	M-355324-01-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC guidance working document 7029/VI/95 rev. 5 (1997-07-22)
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted Study was not found in DAR/RAR and the Addenda
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and Methods

The purpose of the study was to determine the magnitude of the relevant residues of fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188),fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122), in/on maize/corn (green material, kernel and immature kernel (milk ripe)) harvested after one spray application with Fluopyram SC 500 on bare soil 30 days before sowing of maize/corn in northern Europe (northern France and Germany). The investigated plant back interval was 30 days.

The formulation Fluopyram SC 500 is a suspension concentrate formulation containing 500 g/L of Fluopyram. The application was done with 1.0 L test item per ha and 300 L water per ha on bare soil followed by incorporation into the soil < 5 cm, 32 days and < 8 cm, 31 days before the planting of maize in trial 08-2168-01 and 08-2168-02 respectively.

In both trials the application was done with 1.0 L of test item/ha and a water rate of 300 L/ha, corresponding to a spray concentration of the test item of 0.33% (0.17% of active substance in the spray liquid). The application rate was 0.50 kg fluopyram /ha.

For residue analysis samples of maize/corn (green material, kernel and immature kernel (milk ripe)) were taken from the treated and the control plots. In order to obtain representative samples of the raw commodity, samples were taken according to S-sampling (trial 08-2168-01) and at random (trial 08-2168-02) along the treated and the control plot.

Samples of green material were taken in both trials at growth stage BBCH 34. Samples of ear without husk were taken at BBCH 75 and then were used to generate laboratory samples of immature kernel (milk ripe). Samples of kernel were taken at harvest (BBCH 89).

The field samples from all trials were stored deep-frozen within 24 hours after sampling and until dispatch. All field and laboratory samples of trial 08-2168-01 were shipped by deep-freeze lorry, those of trial 08-2168-02 by car with dry ice and arrived in good condition. The field samples and laboratory samples were stored in a freezer at ≤-18°C until preparation of the examination samples. For the preparation of examination samples, the deep-frozen field and laboratory samples were shredded with dry ice in a cutter. Representative parts of the shredded field and laboratory samples were transferred into polystyrene boxes separately for analysis or archiving and stored at ≤-18°C.

Residues of fluopyram and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide), were determined by LC-MS/MS according to method 00984 ([redacted], 05/02/2007, M-283301-01-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues of these compounds were extracted from 10 g of sample material (5 g for wheat straw) by two successive extractions using a high-speed blender with a mixture of acetonitrile:water (80:20; v:v). After centrifugation the extract volume was adjusted. Then, the extracts were diluted ten times by adding the internal standards:

- One dilution was performed under acidic conditions: this extract allowed the determination of FLU-PCA and FLU-methyl-sulfoxide.
- In parallel, another dilution was performed under basic conditions: this extract allowed the determination of FLU, FLU-benzamide and FLU-7-OH.

An aliquot of the extracts was injected into LC-MS/MS operated in Electrospray ionization mode:

- One injection in positive electrospray ionization for the determination of FLU, FLU-benzamide and FLU-7-OH.
- Another injection in negative electrospray ionization for the determination of FLU-PCA and FLU-methyl-sulfoxide under different conditions.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels. For each calibration curve, the correlation coefficient R was above 0.99.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.05 mg/kg for wheat straw and 0.01 mg/kg for all other matrices.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Findings

In order to check the performance of the analytical method, recovery determinations were concurrently performed to the analyses of control and treated samples from the study in each set of analyses. The means of the concurrent recoveries were for all fortification levels within the acceptable range of 70 – 110 % and all the results are considered valid. Recovery results are presented in Table 6.6.2- 32.

No residue of fluopyram or related metabolites were found above the LOQ in any of the control samples of rotational crop matrices analysed.

The residue levels of fluopyram and its relevant metabolites in the rotational crop matrices of maize/corn are summarized in the Table 6.6.2- 33.

The storage period of deep-frozen samples is up to 279 days in immature kernels, 281 days in kernel and 352 days in green material.

Residues in maize/corn

In trial 08-2168-01 (PBI 32 days), residues in corn green material (BBCH 34) were at the level of 0.07 mg/kg (fluopyram), 0.01 mg/kg (FLU-benzamide), 0.03 (FLU-7-OH) and <0.01 mg/kg (FLU-PCA and FLU-methylsulfoxide). The residues of fluopyram (AE C656948) and its metabolites in other corn matrices (kernel & immature kernel) were all <0.01 mg/kg.

In trial 08-2168-02 (PBI 31 days), residues of fluopyram (AE C656948) and its metabolites were all < 0.01 mg/kg in corn matrices except for fluopyram (AE C656948) in corn green material (BBCH 34) in which the residues were at the level of 0.05 mg/kg.

Conclusions

Following one spray application conducted with fluopyram SC500 to bare soil at a rate of 0.5 kg a.s./ha, residues in succeeding crops were analysed for fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188) and fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1349122) using a validated selective HPLC-MS/MS analytical method.

These data show that residues in corn (kernel) as succeeding crop are covered by the current EU MRL.

Assessment and conclusion by applicant:
The study is acceptable

Table 6.6.2- 32: Recovery data for fluopyram (AE C656948) and its metabolites in corn

Crop/Sample material	FL (mg/kg)	Single values (%)	Mean value (%)	RSD*(%)
Fluopyram (AE C656948)				
Corn, green material	0.01	80, 88, 67, 86	80	11
	0.10	92, 91, 96, 84	91	5
	1.0	97	97	--
		Overall recovery (n=9)	**87**	**11**
Corn, immature kernel	0.01	93, 91, 79, 100	91	10
	0.10	87, 108, 99, 86	95	11
	1.0	96	96	--
		Overall recovery (n=9)	**93**	**9**
Corn, kernel	0.01	83, 88, 93, 87	88	4
	0.10	101, 92, 93, 92	95	5
	1.0	94	94	--
		Overall recovery (n=9)	**92**	**6**
Fluopyram-benzamide (AEF148815)				
Corn, green material	0.01	91, 95, 110, 80	94	13
	0.10	94, 86, 89, 84	88	5
	1.0	84	84	--
		Overall recovery (n=9)	**90**	**10**
Corn, immature kernel	0.01	96, 98, 97, 110	100	6
	0.10	84, 86, 93, 88	88	4
	1.0	98	98	--
		Overall recovery (n=9)	**94**	**8**
Corn, kernel	0.01	91, 108, 94, 105	99	8
	0.10	65, 81, 86, 86	79	12
	1.0	87	87	--
		Overall recovery (n=9)	**89**	**14**
Fluopyram-pyridyl-carboxilic-acid (AE C657188)				
Corn, green material	0.01	78, 108, 78, 79	86	18
	0.10	78, 81, 77, 79	79	2
	1.0	84	84	--
		Overall recovery (n=9)	**82**	**12**
Corn, immature kernel	0.01	97, 93, 88, 64	86	18
	0.10	93, 80, 79, 80	83	8
	1.0	87	87	--
		Overall recovery (n=9)	**85**	**12**
Corn, kernel	0.01	77, 84, 84, 96	85	9
	0.10	81, 82, 73, 79	79	5
	1.0	88	88	--
		Overall recovery (n=9)	**83**	**8**
Fluopyram-7-hydroxy (BCS-AA10065)				
Corn, green material	0.01	69, 93, 95, 105	90	17
	0.10	83, 85, 97, 84	87	8
	1.0	88	88	--
		Overall recovery (n=9)	**89**	**12**
Corn, immature kernel	0.01	73, 95, 92, 101	90	13
	0.10	83, 84, 96, 89	88	7
	1.0	94	94	--
		Overall recovery (n=9)	**90**	**9**
Corn, kernel	0.01	82, 94, 92, 100	92	8
	0.10	87, 96, 94, 91	92	4
	1.0	88	88	--

Crop/Sample material	FL (mg/kg)	Single values (%)	Mean value (%)	RSD*(%)
		Overall recovery (n=9)	**92**	**6**
Fluopyram-methylsulfoxide (AE1344122)				
Corn, green material	0.01	95, 80, 90, 95	90	8
	0.10	82, 72, 78, 75	77	6
	1.0	80	80	--
		Overall recovery (n=9)	**83**	**10**
Corn, immature kernel	0.01	103, 89, 88, 85	91	9
	0.10	87, 80, 76, 69	78	10
	1.0	84	84	--
		Overall recovery (n=9)	**84**	**11**
Corn, kernel	0.01	89, 88, 92, 82	89	5
	0.10	89, 82, 78, 78	82	6
	1.0	77	77	--
		Overall recovery (n=9)	**84**	**8**

FL: fortification level, RSD - Relative Standard Deviation
* some RSDs were not calculated as there were only two individual recoveries given
Final determination as: fluopyram Residues calculated as: fluopyram
Final determination as: FLU-benzamide Residues calculated as: fluopyram
Final determination as: FLU-methylsulfoxide Residues calculated as: fluopyram
Final determination as: FLU-PCA Residues calculated as: fluopyram
Final determination as: FLU-7OH Residues calculated as: fluopyram

Table 6.6.2- 33: Results of rotational crop trials conducted with fluopyram

Trial No./ Location/ Year	Plot (commodity)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment to bare soil or target crop			Dates of treatment or no. of treatments and last date	Portion analysed	Growth stage at sampling	Residues (mg/kg)						PHI (days)
			kg a.s./ha	Water (L/ha)	kg a.s./hL				AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residue calc.	
	(a)	(b)				(c)		(d)							(e)
08-2168-01 France, north 71570 Saint Symphorien d'Ancelles Bourgogne Europe, North F 2008	Application to bare soil **PBI: 32 d** Rotational crop: Maize / Corn, field PR37Y12	1) 10.05.2008 2) 30.07.2008 - 10.08.2008 3) 15.10.2008 - 21.10.2008	0.50	300	0.17	08.04.2008	green material	34	0.07	0.01	<0.01	0.05	<0.01	0.08	93
							kernel, immature	75	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	141
							kernel	89	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	192
08-2168-02 Germany 51399 Burscheid Nordrhein-Westfalen Europe, North F 2008	Application to bare soil **PBI: 31 d** Rotational crop: Maize / Corn, field Oldham	1) 05.05.2008 2) 15.07.2008 - 30.07.2008 3) 16.09.2008	0.50	300	0.17	04.04.2008	green material	34	0.05	<0.01	<0.01	<0.01	<0.01	0.06	67
							kernel, immature	75	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	140
							kernel	80	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	165

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH-Code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) Study reference
prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Total residue calculated : sum of fluopyram+FLU-benzamide

Data Point:	KCA 6.6.2/17
Report Author:	[redacted]
Report Year:	2009
Report Title:	Determination of the residues of AE C656948 in/on maize/corn after spraying of fluopyram SC 500 in the field in Italy and Spain
Report No:	08-2179
Document No:	M-355327-01-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC guidance working document 7029/VI/95 rev. 5 (1997-07-22)
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted Study was not found in DAR/RAR and the Addenda
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and Methods

The purpose of the study was to determine the magnitude of the relevant residues of fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188), fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122), in/on maize/corn (green material, kernel and immature kernel (milk ripe)) harvested after one spray application with Fluopyram SC 500 on bare soil 30 days before sowing of maize/corn in southern Europe (Spain and Italy). The investigated plant back interval was 30 days.

The formulation Fluopyram SC 500 is a suspension concentrate formulation containing 500 g/L of Fluopyram. The application was done with 1.0 L test item per ha and 300 L water per ha on bare soil followed by incorporation into the soil < 8 cm, 34 days and 5-10 cm, 28 days before the planting of maize in trial 08-2179-01 and 08-2179-02 respectively.

In both trials the application was done with 1.0 L of test item/ha and a water rate of 300 L/ha, corresponding to a spray concentration of the test item of 0.33% (0.17% of active substance in the spray liquid). The application rate was 0.50 kg fluopyram /ha.

For residue analysis samples of maize/corn, green material, kernel and kernel, immature (milk ripe) were taken from the treated and the control plots. In order to obtain representative samples of the raw commodity, samples were taken according to different sampling procedures from various parts of each treated and control plot.

Samples of green material were taken in both trials at growth stage BBCH 34. Samples of ear without husk were taken at BBCH 75 and then were used to generate laboratory samples of immature kernel (milk ripe). Samples of kernel were taken at harvest (BBCH 89).

The field samples from all trials were stored deep-frozen within 24 hours after sampling and until dispatch. All field and laboratory samples of trial 08-2168-01 were shipped by deep-freeze lorry, those of trial 08-2168-02 by car with dry ice and arrived in good condition. The field samples and laboratory samples were stored in a freezer at ≤-18°C until preparation of the examination samples. For the preparation of examination samples, the deep-frozen field and laboratory samples were shredded with dry ice in a cutter. Representative parts of the shredded field and laboratory samples were transferred into polystyrene boxes separately for analysis or archiving and stored at ≤-18°C.

Residues of fluopyram and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide), were determined by LC-MS/MS according to method 00984 ([redacted], 05/02/2007, M-283301-01-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues of these compounds were extracted from 10 g of sample material (5 g for wheat straw) by two successive extractions using a high-speed blender with a mixture of acetonitrile:water (80:20; v:v). After centrifugation the extract volume was adjusted. Then, the extracts were diluted ten times by adding the internal standards:

- One dilution was performed under acidic conditions: this extract allowed the determination of FLU-PCA and FLU-methyl-sulfoxide.
- In parallel, another dilution was performed under basic conditions: this extract allowed the determination of FLU, FLU-benzamide and FLU-7-OH.

An aliquot of the extracts was injected into LC-MS/MS operated in Electrospray ionization mode:

- One injection in positive electrospray ionization for the determination of FLU, FLU-benzamide and FLU-7-OH.
- Another injection in negative electrospray ionization for the determination of FLU-PCA and FLU-methyl-sulfoxide under different conditions.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels. For each calibration curve, the correlation coefficient R was above 0.99.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.05 mg/kg for wheat straw and 0.01 mg/kg for all other matrices.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Findings

In order to check the performance of the analytical method, recovery determinations were concurrently performed to the analyses of control and treated samples from the study in each set of analyses. The means of the concurrent recoveries were for all fortification levels within the acceptable range of 70 – 110 % and all the results are considered valid. Recovery results are presented in Table 6.6.2- 34.

No residue of fluopyram or related metabolites were found above the LOQ in any of the control samples of rotational crop matrices analysed.

The residue levels of fluopyram and its relevant metabolites in the rotational crop matrices of maize/corn are summarized in the Table 6.6.2- 35.

The storage period of deep-frozen samples is up to 318 days in immature kernels, 264 days in kernel and 353 days in green material.

Residues in maize/corn

In trial 08-2179-01 (PBI 34 days), residues of fluopyram (AE C656948) and its metabolites were all < 0.01 mg/kg in corn matrices except for fluopyram (AE C656948) in corn green material (BBCH 34) in which the residues were at the level of 0.03 mg/kg.

In trial 08-2179-02 (PBI 28 days), residues of fluopyram (AE C656948) and its metabolites were all < 0.01 mg/kg in all corn matrices (green material BBCH 34, immature kernel and kernel).

Conclusions

Following one spray application conducted with fluopyram SC500 to bare soil at a rate of 0.5 kg a.s./ha, residues in succeeding crops were analysed for fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188) and fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122) using a validated selective HPLC-MS/MS analytical method.

These data show that residues in corn (kernel) as succeeding crop are covered by the current EU MRL.

Assessment and conclusion by applicant:
The study is acceptable

Table 6.6.2- 34: Recovery data for fluopyram (AE C656948) and its metabolites in corn

Crop/Sample material	FL (mg/kg)	Single values (%)	Mean value (%)	RSD*(%)
Fluopyram (AE C656948)				
Corn, green material	0.01	84	84	--
	0.10	85; 87	86	--
		Overall recovery (n=3)	**85**	**2**
Corn, immature kernel	0.01	89	89	--
	0.10	92	92	--
	1.0	79	79	--
		Overall recovery (n=3)	**87**	**8**
Corn, kernel	0.01	85	85	--
	0.10	86	86	--
	1.0	97	97	--
		Overall recovery (n=3)	**89**	**7**
Fluopyram-benzamide (AEF148815)				
Corn, green material	0.01	110	110	--
	0.10	92; 87	90	--
		Overall recovery (n=3)	**96**	**13**
Corn, immature kernel	0.01	87	87	--
	0.10	93	93	--
	1.0	85	85	--
		Overall recovery (n=3)	**88**	**5**
Corn, kernel	0.01	91	91	--
	0.10	96	96	--
	1.0	95	95	--
		Overall recovery (n=3)	**94**	**3**
Fluopyram-pyridyl-carboxilic-acid (AE C657188)				
Corn, green material	0.01	83	83	--
	0.10	84	84	--
	1.0	90	90	--
		Overall recovery (n=3)	**86**	**4**
Corn, immature kernel	0.01	87	87	--
	0.10	81	81	--
	1.0	77	77	--
		Overall recovery (n=3)	**82**	**6**
Corn, kernel	0.01	81	81	--
	0.10	74	74	--
	1.0	89	89	--
		Overall recovery (n=3)	**81**	**9**
Fluopyram-7-hydroxy (BCS-AA10065)				
Corn, green material	0.01	78	78	--
	0.10	91; 79	85	--
		Overall recovery (n=3)	**83**	**9**
Corn, immature kernel	0.01	80	80	--
	0.10	96	96	--
	1.0	75	75	--
		Overall recovery (n=3)	**84**	**13**
Corn, kernel	0.01	78	78	--
	0.10	87	87	--
	1.0	88	88	--
		Overall recovery (n=3)	**84**	**7**
Fluopyram-methylsulfoxide (AE1344122)				
	0.01	80	80	--

Crop/Sample material	FL (mg/kg)	Single values (%)	Mean value (%)	RSD*(%)
Corn, green material	0.10	79	79	--
	1.0	80	80	--
		Overall recovery (n=3)	**80**	**1**
Corn, immature kernel	0.01	87	87	--
	0.10	77	77	--
	1.0	76	76	--
		Overall recovery (n=3)	**80**	**8**
Corn, kernel	0.01	83	83	--
	0.10	73	73	--
	1.0	76	76	--
		Overall recovery (n=3)	**77**	**7**

FL: fortification level, RSD - Relative Standard Deviation
* some RSDs were not calculated as there were only two individual recoveries given
Final determination as: fluopyram Residues calculated as: fluopyram
Final determination as: FLU-benzamide Residues calculated as: fluopyram
Final determination as: FLU-methylsulfoxide Residues calculated as: fluopyram
Final determination as: FLU-PCA Residues calculated as: fluopyram
Final determination as: FLU-7OH Residues calculated as: fluopyram

Table 6.6.2- 35: Results of rotational crop trials conducted with fluopyram

Trial No./ Location/ Year	Plot (commodity)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment to bare soil or target crop			Dates of treatment or no. of treatments and last date	Portion analysed	Growth stage at sampling	Residues (mg/kg)						PHI (days)
			kg a.s./ha	Water (L/ha)	kg a.s./hL				AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residue calc.	
	(a)	(b)				(c)		(d)							(e)
08-2179-01 Spain 46230 Alginet Comunidad Valenciana Europe, South F 2008	Application to bare soil **PBI:34 d** Rotational crop: Maize / Corn, field DKC 6418	1) 13.05.2008 2) 10.07.2008 - 25.07.2008 3) 15.09.2008 - 15.10.2008	0.50	300	0.17	09.04.2008	green material	34	0.03	<0.01	<0.01	<0.01	<0.01	0.04	78
							kernel, immature	75	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	113
							kernel	89	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	156
08-2179-02 Italy 40128 Bologna Emilia - Romagna Europe, South F 2008	Application to bare soil **PBI: 28 d** Rotational crop: Maize / Corn, field pr33a46	1) 09.04.2008 2) 20.06.2008 - 30.06.2008 3) 20.08.2008 - 10.09.2008	0.50	300	0.17	12.03.2008	green material	34	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	86
							kernel, immature	75	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	121
							kernel	89	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	175

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse
F field
(e) Days after last application (Label pre-harvest-interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) Study reference
* prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Total residue calculated : sum of fluopyram+FLU-benzamide

Data Point:	KCA 6.6.2/18
Report Author:	[redacted]
Report Year:	2009
Report Title:	Determination of the residues of AE C656948 in/on leek after spraying of fluopyram SC 500 in the field in France (North) and Germany
Report No:	08-2164
Document No:	M-357777-01-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8; Residues in or on Treated Products, Food and Feed EC guidance working document 7029/VI/95 rev. 5 (1997-07-22)
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

I. Materials and Methods

The purpose of the study 08-2164 was to determine the magnitude of residues of fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188), fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122), in/on leek (whole plant without root) harvested after one spray application with Fluopyram SC 500 on bare soil 30 days before planting of leek in northern Europe (Northern France and Germany). The investigated plant back interval was 30 days.

For application the formulation Fluopyram SC 500 was used, a suspension concentrate formulation containing 500 g/L of Fluopyram. The application was done on bare soil followed by incorporation into the soil (< 8 cm) 30 days (trial 08-2164-01) and 31 days (trial 08-2164-02) before the planting of leeks.

In both trials the application was done with 1.0 L of test item/ha and a water rate of 300 L/ha, corresponding to a spray concentration of the test item of 0.33% (0.17% of active substance in the spray liquid). The application rate was 0.50 kg fluopyram /ha.

For residue analysis samples of leeks were taken from the treated and the control plots. In order to obtain representative samples of the raw commodity samples were taken at random along the treated and the control plot.

In both trials, samples were taken from the treated and untreated plot in both trials 14 days before harvest and at harvest.

The field samples were stored deep-frozen within 24 hours after sampling and until dispatch. The field samples were shipped by deep-freeze lorry (08-2164-01) and by car with dry ice (08-2164-02) and arrived in good condition. The field samples and lab samples were stored in a freezer at ≤-18°C until preparation of the examination samples. For the preparation of examination samples, the deep-frozen field samples and lab samples were shredded with dry ice in a cutter. Representative parts of the shredded field samples were transferred into polystyrene boxes separately for analysis or archiving and stored at ≤-18°C.

Residues of fluopyram and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide), were determined by LC-MS/MS according to method 00984 ([redacted], 05/02/2007, M-283301-01-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method

are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues of these compounds were extracted from 10 g of sample material (5 g for wheat straw) by two successive extractions using a high-speed blender with a mixture of acetonitrile:water (80:20; v:v). After centrifugation the extract volume was adjusted. Then, the extracts were diluted ten times by adding the internal standards:

- One dilution was performed under acidic conditions: this extract allowed the determination of FLU-PCA and FLU-methyl-sulfoxide.
- In parallel, another dilution was performed under basic conditions: this extract allowed the determination of fluopyram, FLU-benzamide and FLU-7-OH.

An aliquot of the extracts was injected into LC-MS/MS operated in Electrospray Ionization mode:

- One injection in positive electrospray ionization for the determination of fluopyram, FLU-benzamide and FLU-7-OH.
- Another injection in negative electrospray ionization for the determination of FLU-PCA and FLU-methyl-sulfoxide under different conditions.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels. For each calibration curve, the correlation coefficient R was above 0.99.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.05 mg/kg for wheat straw and 0.01 mg/kg for all other matrices.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Findings

In order to check the performance of the analytical method recovery determinations were concurrently performed to the analyses of control and treated samples from the study in each set of analyses. The means of the concurrent recoveries were for all fortification levels within the acceptable range of 70 – 110 % except for FLU-methyl-sulfoxide at 118 % 8 at 0.1 mg/kg. All overall mean recoveries are in the acceptable range of 70 – 111 %, therefore, the results are considered valid. Recovery results are presented in Table 6.6.2-36.

No residue of fluopyram or related metabolites were found above the LOQ in any of the control samples of rotational crop matrices analysed.

The residue levels of fluopyram and its relevant metabolites in the rotational crop matrices of leeks are summarized in the in Table 6.6.2-37.

The maximum storage period of deep-frozen samples was 363 days.

Residues in leek (whole plant without roots)

In trial 08-2164-01 (PBI 30 days), residues of fluopyram (AE C656948) were at 0.04 mg/kg in leeks 14 days before harvest and at harvest. The residues of the metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide) were < 0.01 mg/kg 14 days before harvest and at harvest.

In trial 08-2164-02 (PBI 31 days) residues of fluopyram (AE C656948) were 0.02 mg/kg and 0.03 mg/kg in potato tubers 14 days before harvest and at harvest, respectively. The residues of the metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide) were < 0.01 mg/kg 14 days before harvest and at harvest.

Conclusions

Following one spray application conducted with fluopyram SC500 to bare soil at a rate of 0.5 kg a.s./ha, residues in succeeding crops were analysed for fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188) and fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122) using a validated selective HPLC-MS/MS analytical method.

These data show that residues in leek (whole plant without root) as succeeding crop are covered by the current EU MRL.

Assessment and conclusion by applicant:
The study is acceptable

Table 6.6.2- 36: Recovery data for fluopyram and its metabolites in leek

Crop/Sample material	Fortification level (mg/kg)	Single values (%)	Mean value (%)	RSD* (%)
Fluopyram (AE C656948)				
Leek (whole plant without root)	0.01	69	-	-
	0.10	96, 100	98	-
	Overall recovery (n=3)		**88**	**19.1**
Fluopyram-benzamide (AEF148815)				
Leek (whole plant without root)	0.01	73	-	-
	0.10	85, 86	86	-
	Overall recovery (n=3)		**81**	**8.9**
Fluopyram-pyridyl-carboxilic-acid (AE C657188)				
Leek (whole plant without root)	0.01	100	-	-
	0.10	92, 95	94	-
	Overall recovery (n=3)		**96**	**4.2**
Fluopyram-7-hydroxy (BCS-AA10065)				
Leek (whole plant without root)	0.01	73	-	-
	0.10	90, 90	90	-
	Overall recovery (n=3)		**84**	**11.6**
Fluopyram-methylsulfoxide (AE1344122)				
Leek (whole plant without root)	0.01	96	-	-
	0.10	116, 120	118	-
	Overall recovery (n=3)		**111**	**11.6**

FL: fortification level, RSD - Relative Standard Deviation
* some RSDs were not calculated as there were only two individual recoveries given
Final determination as: fluopyram Residues calculated as: fluopyram
Final determination as: FLU-benzamide Residues calculated as: fluopyram
Final determination as: FLU-methylsulfoxide Residues calculated as: fluopyram
Final determination as: FLU-PCA Residues calculated as: fluopyram
Final determination as: FLU-7OH Residues calculated as: fluopyram

Table 6.6.2- 37: Results of rotational crop trials conducted with fluopyram

Trial No./ Location/ Year	Plot (commodity)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment to bare soil or target crop			Dates of treatment or no. of treatments and last date	Portion analysed	Growth stage at sampling	Residues (mg/kg)						PHI (days)
			kg a.s./ha	Water (L/ha)	kg a.s./hL				AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residue calc.	
	(a)	(b)				(c)		(d)							(e)
08-2164-01 France, north 78410 Bouafle Ile-de-France Europe, North F 2008	Application to bare soil **PBI: 30 d** Rotational crop: Leek Saint victor	1) 18.07.2008 3) 28.10.2008 - 30.11.2008	500	300	170	18.06.2008	whole plant without root	48 49	0.04 0.04	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	0.05 0.05	124 138
08-2164-02 Germany 51399 Burscheid Nordrhein- Westfalen Europe, North F 2008	Application to bare soil **PBI: 31 d** Rotational crop: Leek Axima	1) 05.05.2008 3) 24.07.2008	500	300	170	04.04.2008	whole plant without root	47 49	0.02 0.03	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	0.03 0.04	97 111

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) Study reference
* prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Total residue calculated: sum of fluopyram+FLU-benzamide

Data Point:	KCA 6.6.2/19
Report Author:	[redacted]
Report Year:	2009
Report Title:	Determination of the residues of AE C656948 in/on leek after spraying of fluopyram SC 500 in the field in Italy and Spain
Report No:	08-2175
Document No:	M-357762-01-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8; Residues in or on Treated Products, Food and Feed EC guidance working document 7029/VI/95 rev. 5 (1997-07-22)
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted Study was not found in DAR/RAR and the Addenda
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and Methods

The purpose of the study was to determine the magnitude of residues of fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188),fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122), in/on leek (whole plant without root) harvested after one spraying application with Fluopyram SC 500 on bare soil 30 days before planting of leek in Southern Europe (Italy and Spain). The investigated plant back interval was 30 days.

For application the formulation Fluopyram SC 500 was used, a suspension concentrate formulation containing 500 g/L of Fluopyram. The application was done on bare soil followed by incorporation into the soil 29 days before the planting of leeks in the two trials.

In both trials the application was done with 1.0 L of test item/ha and a water rate of 300 L/ha, corresponding to a spray concentration of the test item of 0.33% (0.17% of active substance in the spray liquid). The application rate was 0.50 kg fluopyram /ha.

For residue analysis samples of leek were taken from the treated and the control plots. In order to obtain representative samples of the raw commodity, samples were taken at random along the treated and the control plot.

In both trials, samples were taken from the treated and untreated plot in both trials 14 days before harvest and at harvest.

The field samples were stored deep-frozen within 24 hours after sampling and until dispatch. All field samples were shipped by deep-freeze lorry and arrived in good condition. The field samples and lab samples were stored in a freezer at ≤-18°C until preparation of the examination samples. For the preparation of examination samples, the deep-frozen field samples and lab samples were shredded with dry ice in a cutter. Representative parts of the shredded field samples were transferred into polystyrene boxes separately for analysis or archiving and stored at ≤-18°C.

Residues of fluopyram and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide), were determined by LC-MS/MS according to method 00984 ([redacted], 05/02/2007, M-283301-01-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method

are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues of these compounds were extracted from 10 g of sample material (5 g for wheat straw) by two successive extractions using a high-speed blender with a mixture of acetonitrile:water (80:20; v:v). After centrifugation the extract volume was adjusted. Then, the extracts were diluted ten times by adding the internal standards:

- One dilution was performed under acidic conditions: this extract allowed the determination of FLU-PCA and FLU-methyl-sulfoxide.
- In parallel, another dilution was performed under basic conditions: this extract allowed the determination of FLU, FLU-benzamide and FLU-7-OH.

An aliquot of the extracts was injected into LC-MS/MS operated in Electrospray Ionization mode:

- One injection in positive electrospray ionization for the determination of FLU, FLU-benzamide and FLU-7-OH.
- Another injection in negative electrospray ionization for the determination of FLU-PCA and FLU-methyl-sulfoxide under different conditions.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels. For each calibration curve, the correlation coefficient R was above 0.99.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.05 mg/kg for wheat straw and 0.01 mg/kg for all other matrices.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Findings

In order to check the performance of the analytical method, recovery determinations were concurrently performed to the analyses of control and treated samples from the study in each set of analyses. The means of the concurrent recoveries were for all fortification levels within the acceptable range of 70 – 110 % except for FLU-methyl-sulfoxide at 111 % at 0.1 mg/kg. All overall mean recoveries are in the acceptable range of 70 – 110 %; therefore, the results are considered valid. Recovery results are presented in Table 6.6.2- 38.

No residue of fluopyram or related metabolites were found above the LOQ in any of the control samples of rotational crop matrices analysed, except for trial 08-2175-02 in which FLU-PCA was at the level of 0.02 mg/kg in leek whole plant without root 14 days before harvest.

The residue levels of fluopyram and its relevant metabolites in the rotational crop matrices of leek are summarized in the Table 6.6.2- 39.

The storage period of deep-frozen samples ranged between 273 and 369 days.

Residues in leek

In trial 08-2175-01 (PBI 29 days), residues of fluopyram (AE C656948) and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide) in leek whole plant without root were all <0.01 mg/kg 14 days before harvest and at harvest.

In trial 08-2175-02 (PBI 29 days), residues of fluopyram (AE C656948) were at the level of 0.02 mg/kg 14 days before harvest and at harvest. Residues of fluopyram metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide) were < 0.01 mg/kg in leek whole plant without root 14 days before harvest and at harvest except for FLU-PCA for which the level was 0.02 mg/kg 14 days before harvest.

Conclusions

Following one spray application conducted with fluopyram SC500 to bare soil at a rate of 0.5 kg a.s./ha, residues in succeeding crops were analysed for fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188) and fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122) using a validated selective HPLC-MS/MS analytical method.

These data show that residues in leek (whole plant without root) as succeeding crop are covered by the current EU MRL.

Assessment and conclusion by applicant:
The study is acceptable

Table 6.6.2- 38: Recovery data for fluopyram (AE C656948) and its metabolites in leek

Crop/Sample material	FL (mg/kg)	Single values (%)	Mean value (%)	RSD*(%)
Fluopyram (AE C656948)				
Leek, whole plant without root	0.01	78; 87; 84; 77; 68	79	9.3
	0.10	103; 94; 92; 90; 92	94	5.4
		Overall recovery (n=10)	**87**	**11.6**
Fluopyram-benzamide (AEF148815)				
Leek, whole plant without root	0.01	84; 83; 79; 76; 88	82	5.7
	0.10	82; 89; 89; 89; 82	86	4.4
		Overall recovery (n=10)	**84**	**5.4**
Fluopyram-pyridyl-carboxilic-acid (AE C657188)				
Leek, whole plant without root	0.01	85; 121; 92; 76; 107	96	18.6
	0.10	110; 97; 88; 98; 88	96	9.4
		Overall recovery (n=10)	**96**	**13.9**
Fluopyram-7-hydroxy (BCS-AA10065)				
Leek, whole plant without root	0.01	81; 78; 80; 80; 70	78	5.8
	0.10	92; 98; 88; 89; 91	92	4.3
		Overall recovery (n=10)	**85**	**9.8**
Fluopyram-methylsulfoxide (AE1344122)				
Leek, whole plant without root	0.01	108; 96; 88; 102; 72	93	15.0
	0.10	115; 120; 100; 111; 108	111	6.8
		Overall recovery (n=10)	**102**	**13.8**

FL: fortification level, RSD - Relative Standard Deviation
* some RSDs were not calculated as there were only two individual recoveries given
Final determination as: fluopyram Residues calculated as: fluopyram
Final determination as: FLU-benzamide Residues calculated as: fluopyram
Final determination as: FLU-methylsulfoxide Residues calculated as: fluopyram
Final determination as: FLU-PCA Residues calculated as: fluopyram
Final determination as: FLU-7OH Residues calculated as: fluopyram

Table 6.6.2- 39: Results of rotational crop trials conducted with fluopyram

Trial No./ Location/ Year	Plot (commodity)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment to bare soil or target crop			Dates of treatment or no. of treatments and last date	Portion analysed	Growth stage at sampling	Residues (mg/kg)						PHI (days)
			kg a.s./ha	Water (L/ha)	kg a.s./hL				AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residue calc.	
	(a)	(b)				(c)		(d)							(e)
08-2175-01 Spain 41310 Brenes Sevilla Andalucia Europe, South F 2008	Application to bare soil **PBI:29 d** Rotational crop: Leek Shelton	1) 15.05.2008 3) 01.09.2008 - 31.10.2008	0.47	381	0.17	16.04.2008	whole plant without root	48 49	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.02 <0.02	145 159
08-2175-02 Italy 40128 Bologna Emilia - Romagna Europe, South F 2008	Application to bare soil **PBI: 29 d** Rotational crop: Leek Linx	1) 10.04.2008 3) 10.07.2008 - 25.07.2008	0.50	300	0.17	12.03.2008	whole plant without root	45 49	0.02 0.02	<0.01 <0.01	0.02/0.02** <0.01	<0.01 <0.01	<0.01 <0.01	<0.03 <0.03	107 121

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse
F field
(e) Days after (last)application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) Study reference
* prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Total residue calculated: sum of fluopyram+FLU-benzamide

Data Point:	KCA 6.6.2/20
Report Author:	[redacted]
Report Year:	2009
Report Title:	Determination of the residues of AE C656948 in/on strawberry after spraying of fluopyram SC 500 in the field in France (North) and Germany
Report No:	08-2166
Document No:	M-357953-01-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC guidance working document 7029/VI/95 rev. 5 (1997-07-22)
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted Study was not found in DAR/RAR and the Addenda
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and Methods

The purpose of the study was to determine the magnitude of residues of fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188),fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122), in/on strawberry (fruits) harvested after one spraying application with Fluopyram SC 500 on bare soil 30 days before sowing/planting of strawberry in northern Europe (France and Germany). The investigated plant back interval was 30 days.

For application the formulation Fluopyram SC 500 was used, a suspension concentrate formulation containing 500 g/L of Fluopyram. The application was done with 1.0 L test item per ha and 300 L water per ha on bare soil followed by incorporation into the soil (< 8 cm) 31 days (trial 08-2166-01) and 32 days (trial 08-2166-02) before the planting of strawberries. Documentation of the incorporation in trial 08-2166-02 is missing.

In both trials the application was done with 1.0 L of test item/ha and a water rate of 300 L/ha, corresponding to a spray concentration of the test item of 0.33% (0.17% of active substance in the spray liquid). The application rate was 0.50 kg fluopyram /ha.

For residue analysis samples of strawberries (fruit) were taken from the treated and the control plots. In order to obtain representative samples of the raw commodity, samples were taken according to S-sampling (trial 08-2166-01) and at random (trial 08-2166-02) along the treated and the control plot. Samples of strawberries (fruit) were taken from the treated and untreated plot in both trials at harvest (BBCH 87).

The field samples were stored deep-frozen within 24 hours after sampling and until dispatch. The field samples were shipped by deep-freeze lorry (trial 08-2166-01) and by car with dry ice (trial 08-2166-02) and arrived in good condition. The field samples were stored in a freezer at ≤-18 °C or below until preparation of the examination samples. For the preparation of examination samples, the deep-frozen field samples were shredded with dry ice in a cutter. Representative parts of the shredded field samples were transferred into polystyrene boxes separately for analysis or archiving and stored at ≤-18°C.

Residues of fluopyram and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide), were determined by LC-MS/MS according to method 00984 ([redacted], 05/02/2007, M-

283301-01-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues of these compounds were extracted from 10 g of sample material (5 g for wheat straw) by two successive extractions using a high-speed blender with a mixture of acetonitrile:water (80:20; v:v). After centrifugation the extract volume was adjusted. Then, the extracts were diluted ten times by adding the internal standards:

- One dilution was performed under acidic conditions: this extract allowed the determination of FLU-PCA and FLU-methyl-sulfoxide.
- In parallel, another dilution was performed under basic conditions: this extract allowed the determination of FLU, FLU-benzamide and FLU-7-OH.

An aliquot of the extracts was injected into LC-MS/MS operated in Electrospray Ionization mode:

- One injection in positive electrospray ionization for the determination of FLU, FLU-benzamide and FLU-7-OH.
- Another injection in negative electrospray ionization for the determination of FLU-PCA and FLU-methyl-sulfoxide under different conditions.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels. For each calibration curve, the correlation coefficient R was above 0.99.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.05 mg/kg for wheat straw and 0.01 mg/kg for all other matrices.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Findings

In order to check the performance of the analytical method, recovery determinations were concurrently performed to the analyses of control and treated samples from the study in each set of analyses. The means of the concurrent recoveries were for all fortification levels within the acceptable range of 70 – 110 % except for FLU-methyl-sulfoxide at 61 % at 0.01 mg/kg. All overall mean recoveries are in the acceptable range of 70 – 110 %; therefore, the results are considered valid. Recovery results are presented in Table 6.6.2- 40.

No residue of fluopyram or related metabolites were found above the LOQ in any of the control samples of rotational crop matrices analysed.

The residue levels of fluopyram and its relevant metabolites in the rotational crop matrices of strawberry fruits are summarized in the Table 6.6.2- 41.

The storage period of deep-frozen samples ranged between 328 and 349 days.

A new storage stability study of fluopyram-pyridyl-acetic-acid in acidic matrix (strawberry) is ongoing and will be submitted by January 2022 to support a storage longer than 6 months.

Residues in strawberry fruits

In trial 08-2166-01 (PBI 31 days), residues of fluopyram (AE C656948) and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide) were all < 0.01 mg/kg in strawberry fruit at harvest.

In trial 08-2166-02 (PBI 32 days) residues of fluopyram (AE C656948) and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide) were all < 0.01 mg/kg in strawberry fruit at harvest.

Conclusions

Following one spray application conducted with fluopyram SC500 to bare soil at a rate of 0.5 kg a.s./ha, residues in succeeding crops were analysed for fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188) and fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122) using a validated selective HPLC-MS/MS analytical method.

These data show that residues in strawberry (fruit) as succeeding crop are covered by the current EU MRL.

Assessment and conclusion by applicant:
The study is acceptable

Table 6.6.2- 40: Recovery data for fluopyram (AE C656948) and its metabolites in strawberry

Crop/Sample material	FL (mg/kg)	Single values (%)	Mean value (%)	RSD*(%)
Fluopyram (AE C656948)				
Strawberry, fruit	0.01	87	87	--
	0.10	107	107	--
	1.0	99	99	--
		Overall recovery (n=3)	**98**	**10.3**
Fluopyram-benzamide (AEF148815)				
Strawberry, fruit	0.01	80	80	--
	0.10	104	104	--
	1.0	95	95	--
		Overall recovery (n=3)	**93**	**13.0**
Fluopyram-pyridyl-carboxilic-acid (AE C657188)				
Strawberry, fruit	0.01	76	76	--
	0.10	91	91	--
	1.0	103	103	--
		Overall recovery (n=3)	**90**	**15.0**
Fluopyram-7-hydroxy (BCS-AA10065)				
Strawberry, fruit	0.01	85	85	--
	0.10	100	100	--
	1.0	95	95	--
		Overall recovery (n=3)	**93**	**8.2**
Fluopyram-methylsulfoxide (AE1344122)				
Strawberry, fruit	0.01	61	61	--
	0.10	101	101	--
	1.0	132	132	--
		Overall recovery (n=3)	**98**	**36.3**

FL: fortification level, RSD: Relative Standard Deviation
* some RSDs were not calculated as there were only two individual recoveries given
Final determination as: fluopyram Residues calculated as: fluopyram
Final determination as: FLU-benzamide Residues calculated as: fluopyram
Final determination as: FLU-methylsulfoxide Residues calculated as: fluopyram
Final determination as: FLU-PCA Residues calculated as: fluopyram
Final determination as: FLU-7OH Residues calculated as: fluopyram

Table 6.6.2- 41: Results of rotational crop trials conducted with fluopyram

Trial No./ Location/ Year	Plot (commodity)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment to bare soil or target crop			Dates of treatment or no. of treatments and last date	Portion analysed	Growth stage at sampling	Residues (mg/kg)						PHI (days)
			kg a.s./ha	Water (L/ha)	kg a.s./hL				AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residue calc.	
	(a)	(b)				(c)		(d)							(e)
08-2166-01 France, north 80700 Cremery Picardie Europe, North F 2008	Application to bare soil **PBI: 31 d** Rotational crop: Strawberry Isadora	1) 26.05.2008 2) 05.07.2008 - 24.07.2008 3) 10.08.2008 - 20.08.2008	0.5	300	0.17	25.04.2008	fruit	87	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	91
08-2166-02 Germany 51399 Burscheid Nordrhein-Westfalen Europe, North F 2008	Application to bare soil **PBI: 32 d** Rotational crop: Strawberry Elsanta	1) 19.05.2008 2) 01.06.2008 - 20.06.2008 3) 01.07.2008 - 15.07.2008	0.5	300	0.17	17.04.2008	fruit	87	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	78

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) Study reference
* prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Total residue calculated : sum of fluopyram+FLU-benzamide

Data Point:	KCA 6.6.2/21
Report Author:	[redacted]
Report Year:	2009
Report Title:	Determination of the residues of AE C656948 in/on strawberry after spraying of fluopyram SC 500 in the field in Italy and Spain
Report No:	08-2177
Document No:	M-357930-01-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC guidance working document 7029/VI/95 rev. 5 (1997-07-22)
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted Study was not found in DAR/RAR and the Addenda
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and Methods

The purpose of the study was to determine the magnitude of the relevant residues of fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188),fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122), in/on strawberry (fruits) harvested after one spraying application with Fluopyram SC 500 on bare soil 30 days before sowing/planting of strawberry in southern Europe (Spain and Italy). The investigated plant back interval was 30 days.

For application the formulation Fluopyram SC 500 was used a suspension concentrate formulation containing 500 g/L of Fluopyram. The application was done with 1.0 L test item per ha and 300 L water per ha on bare soil followed by incorporation into the soil 28 before the planting of strawberries. In trial 08-2177-01 the incorporation was done by rotovator (the depth of incorporation is unknown) and in trial 08-2177-02 the incorporation was done by rotary tiller at 5 – 10 cm depth.

In both trials the application was done with 1.0 L of test item/ha and a water rate of 300 L/ha, corresponding to a spray concentration of the test item of 0.33% (0.17% of active substance in the spray liquid). The application rate was 0.50 kg fluopyram /ha.

For residue analysis, samples were taken from the treated and the control plots. In order to obtain representative samples of the raw commodity, samples were taken according to different sampling procedures from various parts of each treated and control plot. Samples of strawberries (fruit) were taken from the treated and untreated plot in both trials at harvest (BBCH 87) and 14 days before harvest trial 08-2177-01 only.

In trial 08-2177-01 the sample weight at harvest was only 794g instead of 1 kg; mostly all fruits were lightly affected by diseases.

The field samples from all trials were stored deep-frozen within 24 hours after sampling and until dispatch. All field samples were shipped by deep-freeze lorry and arrived in good condition. The field samples were stored in a freezer at ≤-18°C until preparation of the examination samples. For the preparation of examination samples, the deep-frozen field samples were shredded with dry ice in a cutter. Representative parts of the shredded field samples were transferred into polystyrene boxes separately for analysis or archiving and stored at ≤-18°C.

Residues of fluopyram and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide), were determined by LC-MS/MS according to method 00984 ([redacted], 05/02/2007, M-283301-01-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues of these compounds were extracted from 10 g of sample material (5 g for wheat straw) by two successive extractions using a high-speed blender with a mixture of acetonitrile:water (80:20; v:v). After centrifugation the extract volume was adjusted. Then, the extracts were diluted ten times by adding the internal standards:

- One dilution was performed under acidic conditions: this extract allowed the determination of FLU-PCA and FLU-methyl-sulfoxide.
- In parallel, another dilution was performed under basic conditions: this extract allowed the determination of FLU, FLU-benzamide and FLU-7-OH.

An aliquot of the extracts was injected into LC-MS/MS operated in Electrospray ionization mode:

- One injection in positive electrospray ionization for the determination of FLU, FLU-benzamide and FLU-7-OH.
- Another injection in negative electrospray ionization for the determination of FLU-PCA and FLU-methyl-sulfoxide under different conditions.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels. For each calibration curve, the correlation coefficient R was above 0.99.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.05 mg/kg for wheat straw and 0.01 mg/kg for all other matrices.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Findings

In order to check the performance of the analytical method, recovery determinations were concurrently performed to the analyses of control and treated samples from the study in each set of analyses. The means of the concurrent recoveries were for all fortification levels within the acceptable range of 70 – 110 % except for FLU-PCA at 63 % at 0.01 mg/kg. All overall mean recoveries are in the acceptable range of 70 – 110 %; therefore, the results are considered valid. Recovery results are presented in Table 6.6.2- 42.

No residue of fluopyram or related metabolites were found above the LOQ in any of the control samples of rotational crop matrices analysed.

The residue levels of fluopyram and its relevant metabolites in the rotational crop matrices of strawberry fruits are summarized in the Table 6.6.2- 43.

The storage period of deep-frozen samples ranged between 319 and 393 days.

A new storage stability study of fluopyram-pyridyl-acetic-acid in acidic matrix (strawberry) is ongoing and will be submitted by January 2022 to support a storage longer than 6 months.

Residues in strawberry fruits

In trial 08-2177-01 (PBI, 28 days), residues of fluopyram (AE C656948) and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide) were all < 0.01 mg/kg in strawberry fruit 14 days before harvest and at harvest.

In trial 08-2177-02 (PBI 28 days) residues of fluopyram (AE C656948) and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide) were all < 0.01 mg/kg in strawberry fruit at harvest.

Conclusions

Following one spray application conducted with fluopyram SC500 to bare soil at a rate of 0.5 kg a.s./ha, residues in succeeding crops were analysed for fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188) and fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1349122) using a validated selective HPLC-MS/MS analytical method.

These data show that residues in strawberry (fruit) as succeeding crop are covered by the current EU MRL.

Assessment and conclusion by applicant:
The study is acceptable

Table 6.6.2- 42: Recovery data for fluopyram (AE C656948) and its metabolites in strawberry

Crop/Sample material	FL (mg/kg)	Single values (%)	Mean value (%)	RSD*(%)
Fluopyram (AE C656948)				
Strawberry, fruit	0.01	92	92	--
	0.10	100	100	--
	1.0	89	89	--
		Overall recovery (n=3)	**94**	**6.1**
Fluopyram-benzamide (AEF148815)				
Strawberry, fruit	0.01	90	90	--
	0.10	94	94	--
	1.0	82	82	--
		Overall recovery (n=3)	**89**	**6.9**
Fluopyram-pyridyl-carboxilic-acid (AE C657188)				
Strawberry, fruit	0.01	63	63	--
	0.10	95	95	--
	1.0	80	80	--
		Overall recovery (n=3)	**79**	**20.2**
Fluopyram-7-hydroxy (BCS-AA10065)				
Strawberry, fruit	0.01	88	88	--
	0.10	109	109	--
	1.0	92	92	--
		Overall recovery (n=3)	**96**	**11.6**
Fluopyram-methylsulfoxide (AE1344122)				
Strawberry, fruit	0.01	84	84	--
	0.10	101	101	--
	1.0	101	101	--
		Overall recovery (n=3)	**95**	**10.3**

FL: fortification level, RSD - Relative Standard Deviation
* some RSDs were not calculated as there were only two individual recoveries given
Final determination as: Fluopyram Residues calculated as: fluopyram
Final determination as: FLU-benzamide Residues calculated as: fluopyram
Final determination as: FLU-methylsulfoxide Residues calculated as: fluopyram
Final determination as: FLU-PCA Residues calculated as: fluopyram
Final determination as: FLU-7OH Residues calculated as: fluopyram

Table 6.6.2- 43: Results of rotational crop trials conducted with fluopyram

Trial No./ Location/ Year	Plot (commodity)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment to bare soil or target crop			Dates of treatment or no. of treatments and last date	Portion analysed	Growth stage at sampling	Residues (mg/kg)						PHI (days)
			kg a.s./ha	Water (L/ha)	kg a.s./hL				AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	PLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residue calc.	
	(a)	(b)				(c)		(d)							(e)
08-2177-01 Spain 08850 Gava - Barcelona Cataluña Europe, South F 2008	Application to bare soil **PBI: 28 d** Rotational crop: Strawberry Albion	1) 11.04.2008 2) 10.05.2008 - 01.08.2008 3) 15.07.2008 - 15.08.2008	0.5	300	0.17	14.03.2008	fruit	87 89	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.02 <0.02	126 140
08-2177-02 Italy 40128 Bologna Emilia - Romagna Europe, South F 2008	Application to bare soil **PBI: 28 d** Rotational crop: Strawberry Clery	1) 28.03.2008 1) 28.03.2008 2) 25.04.2008 3) 15.05.2008	0.5	300	0.17	29.02.2008	fruit	87	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	80

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) Study reference
* prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Total residue calculated : sum of fluopyram+FLU-benzamide

Data Point:	KCA 6.6.2/22
Report Author:	[redacted]
Report Year:	2009
Report Title:	Determination of the residues of AE C656948 in/on spinach after spraying of fluopyram SC 500 in the field in France (North) and Germany
Report No:	08-2071
Document No:	M-357943-01-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC guidance working document 7029/VI/95 rev. 5 (1997-07-22)
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted Study was not found in DAR/RAR and the Addenda
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and Methods

The purpose of the study was to determine the magnitude of residues of fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188),fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122), in/on spinach (leaf) harvested after one spray application with Fluopyram SC 500 on bare soil 30 days before sowing of spinach in northern Europe (northern France and Germany). The investigated plant back interval was 30 days.

In both trials the application was done with 1.0 L of test item/ha and a water rate of 300 L/ha, corresponding to a spray concentration of the test item of 0.33% (0.17% of active substance in the spray liquid). The application rate was 0.50 kg fluopyram/ha.

In both trials the application was done on bare soil (followed by incorporation to 5-6 cm depth in trial 08-2071-01; incorporation depth was not documented in trial 08-2071-02).

The applications were done 29 days (trial 08- 2071- 01) and 28 days (trial 08-2071-02) before sowing of spinach

For residue analysis samples of spinach were taken from the treated and the control plots. In order to obtain representative samples of the raw commodity, samples were taken according to S-sampling (trial 08-2071-01) and at random all along the treated and the control plot (trial 08-2071-02).

In both trials, samples were taken from the treated and untreated plot in both trials 14 days before harvest and at harvest (BBCH 49).

The field samples were stored deep-frozen within 24 hours after sampling and until dispatch. All field samples were shipped by deep-freeze lorry (08-2071-01) or by car with dry ice (08-2071-02) and arrived in good condition. The field samples were stored in a freezer at ≤ -18°C or below until preparation of the examination samples. For the preparation of examination samples, the deep-frozen field samples were shredded with dry ice in a cutter.

Representative parts of the shredded field samples and laboratory samples were transferred into polystyrene boxes separately for analysis or archiving and stored at ≤ -18°C.

Residues of fluopyram and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide), were determined by LC-MS/MS according to method 00984 ([redacted], 05/02/2007, M-283301-01-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method

are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues of these compounds were extracted from 10 g of sample material (5 g for wheat straw) by two successive extractions using a high-speed blender with a mixture of acetonitrile:water (80:20; v:v). After centrifugation the extract volume was adjusted. Then, the extracts were diluted ten times by adding the internal standards:

- One dilution was performed under acidic conditions: this extract allowed the determination of FLU-PCA and FLU-methyl-sulfoxide.
- In parallel, another dilution was performed under basic conditions: this extract allowed the determination of FLU, FLU-benzamide and FLU-7-OH.

An aliquot of the extracts was injected into LC-MS/MS operated in Electrospray Ionization mode:

- One injection in positive electrospray ionization for the determination of FLU, FLU-benzamide and FLU-7-OH.
- Another injection in negative electrospray ionization for the determination of FLU-PCA and FLU-Methyl-sulfoxide under different conditions.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels. For each calibration curve, the correlation coefficient R was above 0.99.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.05 mg/kg for wheat straw and 0.01 mg/kg for all other matrices.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Findings

In order to check the performance of the analytical method, recovery determinations were concurrently performed to the analyses of control and treated samples from the study in each set of analyses. The means of the concurrent recoveries were for all fortification levels within the acceptable range of 70 – 110 %. All the recovery results are considered valid. Recovery results are presented in Table 6.6.2- 44.

No residue of fluopyram or related metabolites were found above the LOQ in any of the control samples of rotational crop matrices analysed.

The residue levels of fluopyram and its relevant metabolites in the rotational crop matrices of spinach leaves are summarized in the Table 6.6.2- 45.

The storage period of deep-frozen samples ranged between 280 and 417 days.

Residues in spinach leaves

In trial 08-2071-01 and 08-2071-02 (PBI 29 days),

14 days before harvest:

- residues of fluopyram (AE C656948) were at 0.03 mg/kg
- residues of FLU-benzamide range between 0.02 mg/kg and 0.07 mg/kg.
- residues of FLU-7-OH range between 0.08 mg/kg and 0.26 mg/kg.
- residues of FLU-PCA and FLU-methyl-sulfoxide were < 0.01 mg/kg.

At harvest:

- residues of fluopyram (AE C656948) range between <0.01 mg/kg and 0.01 mg/kg
- residues of FLU-benzamide range between 0.02 mg/kg and 0.08 mg/kg.
- residues of FLU-7-OH range between 0.05 mg/kg and 0.10 mg/kg.

- residues of FLU-PCA and FLU-methyl-sulfoxide were < 0.01 mg/kg.

Conclusions

Following one spray application conducted with fluopyram SC500 to bare soil at a rate of 0.5 kg a.s./ha, residues in succeeding crops were analysed for fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188) and fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122) using a validated selective HPLC-MS/MS analytical method.

These data show that residues in spinach (leaves) as succeeding crop are covered by the current EU MRL.

Assessment and conclusion by applicant:
The study is acceptable

These data support a 30-day, PBIs requested for tuber vegetable crops after the use of fluopyram SC500

Table 6.6.2- 44: Recovery data for fluopyram (AE C656948) and its metabolites in spinach, leaf

Crop/Sample material	FL (mg/kg)	Single values (%)	Mean value (%)	RSD*(%)
Fluopyram (AE C656948)				
Spinach, leaf	0.01	80; 82; 87; 83	83	3.5
	0.10	93; 99; 95	96	3.2
	1.0	81	81	--
		Overall recovery (n=8)	**88**	**8.3**
Fluopyram-benzamide (AEF148815)				
Spinach, leaf	0.01	86; 76; 81; 69	78	9.3
	0.10	82; 81; 80; 103	87	12.8
	1.0	79	79	--
		Overall recovery (n=9)	**82**	**11.2**
Fluopyram-pyridyl-carboxilic-acid (AE C657188)				
Spinach, leaf	0.01	67; 77; 63; 79	72	10.8
	0.10	81; 87; 81	83	4.2
	1.0	88	88	--
		Overall recovery (n=8)	**78**	**11.4**
Fluopyram-7-hydroxy (BCS-CA10065)				
Spinach, leaf	0.01	81; 80; 80; 84	81	2.3
	0.10	93; 92; 94; 111	98	9.3
	1.0	86	86	--
		Overall recovery (n=9)	**88**	**11.2**
Fluopyram-methylsulfoxide (AE1344122)				
Spinach, leaf	0.01	68; 75; 72; 79	74	6.3
	0.10	88; 94; 94	92	3.8
	1.0	96	96	--
		Overall recovery (n=8)	**83**	**13.3**

FL: fortification level, RSD: Relative Standard Deviation
* some RSDs were not calculated as there were only two individual recoveries given
Final determination as: fluopyram Residues calculated as: fluopyram
Final determination as: FLU-benzamide Residues calculated as: fluopyram
Final determination as: FLU-methylsulfoxide Residues calculated as: fluopyram
Final determination as: FLU-PCA Residues calculated as: fluopyram
Final determination as: FLU-7OH Residues calculated as: fluopyram

Table 6.6.2- 45: Results of rotational crop trials conducted with fluopyram

Trial No./ Location/ Year	Plot (commodity)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment to bare soil or target crop			Dates of treatment or no. of treatments and last date	Portion analysed	Growth stage at sampling	Residues (mg/kg)						PHI (days)
			kg a.s./ha	Water (L/ha)	kg a.s./hL				FLU AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residue calc.	
	(a)	(b)				(c)		(d)							(e)
08-2071-01 08-2071-01-T France, north 78410 Bouafle Ile-de-France Europe, North F 2008	Application to bare soil **PBI: 29 d** Rotational crop : Spinach, Samos F1	1) 19.06.2008 3) 28.07.2008 - 12.08.2008	0.5	300	0.17	21.05.2008	leaves	37 49	0.03 <0.01	0.07 0.08	<0.01 <0.01	0.26 0.10	<0.01 <0.01	0.1 0.09	64 78
08-2071-02 08-2071-02-T Germany 51399 Burscheid Nordrhein-Westfalen Europe, North F 2008	Application to bare soil **PBI: 28 d** Rotational crop: Spinach, Cezanne	1) 02.05.2008 3) 10.06.2008 - 25.06.2008	0.5	300	0.17	04.04.2008	leaves	48 49	0.05 0.01	0.02 0.02	<0.01 <0.01	0.08 0.05	<0.01 <0.01	0.05 0.03	60 74

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) Study reference
prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Total residue calculated : sum of fluopyram+FLU-benzamide

Data Point:	KCA 6.6.2/23
Report Author:	[redacted]
Report Year:	2009
Report Title:	Determination of the residues of AE C656948 in/on spinach after spraying of fluopyram SC 500 in the field in Italy and Spain
Report No:	08-2170
Document No:	M-357959-01-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC guidance working document 7029/VI/95 rev. 5 (1997-07-22)
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and Methods

The purpose of the study was to determine the magnitude of residues of fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188),fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122), in/on spinach (leaf) harvested after one spray application with Fluopyram SC 500 on bare soil 30 days before sowing of spinach in southern Europe (Spain and Italy). The investigated plant back interval was 30 days.

For application the formulation Fluopyram SC 500 was used, a suspension concentrate formulation containing 500 g/L of Fluopyram.

In both trials the application was done with 1.0 L of test item/ha and a water rate of 300 L/ha, corresponding to a spray concentration of the test item of 0.33% (0.17% of active substance in the spray liquid). The application rate was 0.50 kg fluopyram /ha.

The applications were done on bare soil 28 days (trial 08-2170-01) and 29 days (trial 08-2170-02) before sowing of spinach.

For residue analysis, samples were taken from the treated and the control plots. In order to obtain representative samples of the raw commodity, samples were taken according to different sampling procedures from various parts of each treated and control plot.

In both trials, samples were taken from the treated and untreated plot in both trials 14 (or 12) days before harvest and at harvest.

The field samples from all trials were stored deep-frozen within 24 hours after sampling and until dispatch. All field samples were shipped by deep-freeze lorry and arrived in good condition. The field samples were stored in a freezer at ≤ -18°C until preparation of the examination samples. For the preparation of examination samples, the deep-frozen field samples were shredded with dry ice in a cutter. Representative parts of the shredded field samples were transferred into polystyrene boxes separately for analysis or archiving and stored at ≤- 18°C.

Residues of fluopyram and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide), were determined by LC-MS/MS according to method 00984 ([redacted], 05/02/2007, M-283301-01-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method

are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues of these compounds were extracted from 10 g of sample material (5 g for wheat straw) by two successive extractions using a high-speed blender with a mixture of acetonitrile:water (80:20; v:v). After centrifugation the extract volume was adjusted. Then, the extracts were diluted ten times by adding the internal standards:

- One dilution was performed under acidic conditions: this extract allowed the determination of FLU-PCA and FLU-methyl-sulfoxide.
- In parallel, another dilution was performed under basic conditions: this extract allowed the determination of FLU, FLU-benzamide and FLU-7-OH.

An aliquot of the extracts was injected into LC-MS/MS operated in Electrospray Ionization mode:

- One injection in positive electrospray ionization for the determination of FLU, FLU-benzamide and FLU-7-OH.
- Another injection in negative electrospray ionization for the determination of FLU-PCA and FLU-Methyl-sulfoxide under different conditions.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels. For each calibration curve, the correlation coefficient R was above 0.99.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.05 mg/kg for wheat straw and 0.01 mg/kg for all other matrices.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Findings

In order to check the performance of the analytical method, recovery determinations were concurrently performed to the analyses of control and treated samples from the study in each set of analyses. All overall average recoveries were within the acceptable range of 70 - 110 %. All the recovery results are considered valid. Recovery results are presented in Table 6.6.2-46.

No residue of fluopyram or related metabolites were found above the LOQ in any of the control samples of rotational crop matrices analysed.

The residue levels of fluopyram and its relevant metabolites in the rotational crop matrices of spinach leaves are summarized in the Table 6.6.2-47.

The storage period of deep-frozen samples ranged between 356 and 380 days.

Residues in spinach leaves

In trial 08-2070-01 and 08-2070-02 (PBI 28-29 days),

14 days before harvest:

- residues of fluopyram (AE C656948) range between 0.02 mg/kg and 0.09 mg/kg.
- residues of FLU-benzamide range between 0.04 mg/kg and 0.05 mg/kg.
- residues of FLU-7-OH range between 0.08 mg/kg and 0.22 mg/kg.
- residues of FLU-PCA and FLU-methyl-sulfoxide were < 0.01 mg/kg.

At harvest:

- residues of fluopyram (AE C656948) range between <0.01 mg/kg and 0.07 mg/kg
- residues of FLU-benzamide range between 0.01 mg/kg and 0.02 mg/kg.
- residues of FLU-7-OH range between 0.045 mg/kg and 0.08 mg/kg.

- residues of FLU-PCA and FLU-methyl-sulfoxide were < 0.01 mg/kg.

Conclusions

Following one spray application conducted with fluopyram SC500 to bare soil at a rate of 0.5 kg a.s./ha, residues in succeeding crops were analysed for fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188) and fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122) using a validated selective HPLC-MS/MS analytical method.

These data show that residues in spinach (leaves) as succeeding crop are covered by the current EU MRL.

Assessment and conclusion by applicant:
The study is acceptable

Table 6.6.2- 46: Recovery data for fluopyram (AE C656948) and its metabolites in spinach, leaf

Crop/Sample material	FL (mg/kg)	Single values (%)	Mean value (%)	RSD*(%)
Fluopyram (AE C656948)				
Spinach, leaf	0.01	98	98	--
	0.10	67	67	--
	1.0	91	91	--
		Overall recovery (n=3)	**85**	**19.1**
Fluopyram-benzamide (AEF148815)				
Spinach, leaf	0.01	91	91	--
	0.10	61	61	--
	1.0	86	86	--
		Overall recovery (n=3)	**79**	**20.3**
Fluopyram-pyridyl-carboxilic-acid (AE C657188)				
Spinach, leaf	0.01	83	83	--
	0.10	90	90	--
	1.0	86	86	--
		Overall recovery (n=3)	**86**	**4.1**
Fluopyram-7-hydroxy (BCS-AA10065)				
Spinach, leaf	0.01	89	89	--
	0.10	61	61	--
	1.0	86	86	--
		Overall recovery (n=3)	**79**	**19.5**
Fluopyram-methylsulfoxide (AE1344122)				
Spinach, leaf	0.01	61	61	--
	0.10	109	109	--
	1.0	102	102	--
		Overall recovery (n=3)	**91**	**28.6**

FL: fortification level, RSD - Relative Standard Deviation
* some RSDs were not calculated as there were only two individual recoveries given
Final determination as: fluopyram Residues calculated as: fluopyram
Final determination as: FLU-benzamide Residues calculated as: fluopyram
Final determination as: FLU-methylsulfoxide Residues calculated as: fluopyram
Final determination as: FLU-PCA Residues calculated as: fluopyram
Final determination as: FLU-7OH Residues calculated as: fluopyram

Table 6.6.2- 47: Results of rotational crop trials conducted with fluopyram

Trial No./ Location/ Year	Plot (commodity)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment to bare soil or target crop			Dates of treatment or no. of treatments and last date	Portion analysed	Growth stage at sampling	Residues (mg/kg)						PHI (days)
			kg a.s./ha	Water (L/ha)	kg a.s./hL				AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residue calc.	
	(a)	(b)				(c)		(d)							(e)
08-2170-01 Spain 08850 Gava - Barcelona Cataluña Europe, South F 2008	Application to bare soil **PBI: 28 d** Rotational crop : Spinach Apollo	1) 11.04.2008 3) 25.05.2008 - 15.06.2008	0.5	300	0.17	14.03.2008	leaves	47 49	0.02 <0.01	0.05 <0.01	<0.01 <0.01	0.08 0.04	<0.01 <0.01	0.07 0.03	67 81
08-2170-02 Italy 37050 Albaro Veneto Europe, South F 2008	Application to bare soil **PBI: 29 d** Rotational crop: Spinach Seven R	1) 04.04.2008	0.5	300	0.17	06.03.2008	leaves	16 49	0.09 0.07	0.04 0.01	<0.01 <0.01	0.22 0.08	<0.01 <0.01	0.13 0.08	65 77

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) Study reference
* prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Total residue calculated : sum of fluopyram+FLU-benzamide

Data Point:	KCA 6.6.2/24
Report Author:	[redacted]
Report Year:	2009
Report Title:	Determination of the residues of AE C656948 in/on summer rape after spraying of Fluopyram SC 500 in the field in northern France
Report No:	08-2169
Document No:	M-350532-02-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC guidance working document 7029/VI/95 rev. 5 (1997-07-22) OECD Guideline for testing of Chemicals; Residues in rotational crops (limited field studies), No. 504, 8 Jan. 2007
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Methods

The purpose of the study 08-2169 was to determine the magnitude of residues of fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188), fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122), in/on rape (pod and seed). One spray application with Fluopyram SC 500, a suspension concentrate (SC) formulation containing 500 g/L fluopyram was done to bare soil followed by sowing of summer rape with plant back interval of 30 days. The crop was sowed 33 days instead of 30 days after application on bare soil due to bad weather conditions.

One supervised residue trial was conducted in northern Europe (northern France) during the 2008 growing season.

One spray application of Fluopyram SC 500 was done to bare soil at an application rate of 1 L/ha (equivalent to 0.50 kg/ha fluopyram) with a water rate of 300 L/ha. The application procedure was followed by incorporation of the test item into the soil to a depth of 6 cm 33 days after application. The treatment was made at the scheduled rate.

For residue analysis, samples of rape (pod and seed) were taken from the treated and the control plots. Samples of pod were taken 29 days prior harvest at a growth stage BBCH 79 and at harvest maturity growth stage BBCH 89.

The field samples were stored deep-frozen within 24 hours after sampling and until dispatch. All field samples were shipped by deep-freeze lorry and arrived in good condition. The field samples were stored in a freezer at ≤-18°C until preparation of the examination samples. For the preparation of examination samples, the deep frozen field samples were shredded with dry ice in a cutter. Representative parts of the shredded field samples were transferred into polystyrene boxes separately for analysis (examination samples) and archiving (retain samples) and stored for analysis or archiving at ≤-18°C until analysis.

Residues of fluopyram and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide), were determined by LC-MS/MS according to method 00984 ([redacted], 05/02/2007, M-

283301-01-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues were extracted from 10 g of sample material by two successive extractions using a high-speed blender with a mixture of acetonitrile:water (80:20; v:v). After centrifugation the extract volume was adjusted. Then, the extracts were diluted ten times by adding the internal standards:

- One dilution was performed under acidic conditions: this extract allowed the determination of FLU-PCA and FLU-methyl-sulfoxide.
- In parallel, another dilution was performed under basic conditions: this extract allowed the determination of fluopyram, FLU-benzamide and FLU-7-OH.

An aliquot of the extracts was injected into LC-MS/MS operated in Electrospray Ionization mode:

- One injection in positive electrospray ionization for the determination of fluopyram, FLU-benzamide and FLU-7-OH.
- Another injection in negative electrospray ionization for the determination of FLU-PCA and FLU-methyl-sulfoxide under different conditions.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels. For each calibration curve, the correlation coefficient R was above 0.99.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.05 mg/kg for wheat straw and 0.01 mg/kg for all other matrices.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Findings

In order to check the performance of the analytical method, recovery determinations were concurrently performed to the analyses of control and treated samples from the study in each set of analyses. The means of the concurrent recoveries were for all fortification levels within the acceptable range of 70 – 110 % except for FLU-methyl-sulfoxide at 111 % at 0.1 mg/kg in rape pod. All overall mean recoveries are in the acceptable range of 70 - 110 %, therefore, the results are considered valid. Recovery results are presented in Table 6.6.2- 48.

No residues above the LOQs were found in the control samples. The detailed results obtained in the rotational crop of rape are summarized in Table 6.6.2- 49. The results were not corrected for concurrent recoveries.

The maximum storage period of deep-frozen samples was 313 days for pods and 284 for seeds.

Residues in rape

In trial 08-2169-01 (PBI 33 days), residues of fluopyram (AE C656948) in pods were 0.02 mg/kg at BBCH 79 and 0.01 mg/kg in seeds at harvest.

Residues of the metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide) in pods were <0.01 mg/kg at BBCH 79 and <0.01 mg/kg in seeds at harvest.

Conclusions

Following one spray application conducted with fluopyram SC500 to bare soil at a rate of 0.5 kg a.s./ha, residues in succeeding crops were analysed for fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188) and fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122) using a validated selective HPLC-MS/MS analytical method.

These data show that residues in rape (seed) as succeeding crop are covered by the current EU MRL.

Assessment and conclusion by applicant:
The study is acceptable

Table 6.6.2- 48: Recovery data for fluopyram and its metabolites in rape

Crop/Sample material	Fortification level (mg/kg)	Single values (%)	Mean value (%)	RSD* (%)
Fluopyram (AE C656948)				
Rape (pod)	0.01	94, 101	98	-
	0.10	100, 102	101	-
	Overall recovery (n=4)		**99**	**3.6**
Rape (seed)	0.01	72	-	-
	0.10	70	-	-
	Overall recovery (n=2)		**71**	
Fluopyram-benzamide (AEF148815)				
Rape (pod)	0.005	86, 92	89	-
	0.05	100, 96	98	-
	Overall recovery (n=4)		**94**	**6.4**
Rape (seed)	0.005	75	-	-
	0.05	70	-	-
	Overall recovery (n=2)		**73**	**-**
Fluopyram-pyridyl-carboxilic-acid (AE C657188)				
Rape (pod)	0.006	104, 104	104	-
	0.06	104, 104	104	-
	Overall recovery (n=4)		**104**	**0**
Rape (seed)	0.006	96	-	-
	0.06	103	-	-
	Overall recovery (n=2)		**100**	**-**
Fluopyram-7-hydroxy (BCS-AA10065)				
Rape (pod)	0.01	92, 100	96	-
	0.10	98, 100	99	-
	Overall recovery (n=4)		**98**	**3.9**
Rape (seed)	0.01	62	-	-
	0.10	68	-	-
	Overall recovery (n=2)		**65**	**-**
Fluopyram-methylsulfoxide (AE1344122)				
Rape (pod)	0.006	86, 90	88	-
	0.06	111, 111	111	-
	Overall recovery (n=4)		**100**	**13.4**
Rape (seed)	0.006	115	-	-
	0.06	98	-	-
	Overall recovery (n=2)		**107**	**-**

FL: fortification level; RSD - Relative Standard Deviation
* some RSDs were not calculated as there were only two individual recoveries given
Final determination as: fluopyram Residues calculated as: fluopyram
Final determination as: FLU-benzamide Residues calculated as: fluopyram
Final determination as: FLU-methylsulfoxide Residues calculated as: fluopyram
Final determination as: FLU-PCA Residues calculated as: fluopyram
Final determination as: FLU-7OH Residues calculated as: fluopyram

Table 6.6.2- 49: Results of rotational crop trial conducted with fluopyram

Trial No./ Location/ Year	Plot (commodity)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment to bare soil or target crop			Dates of treatment or no. of treatments and last date	Portion analysed	Growth stage at sampling	Residues (mg/kg)						PHI (days)
			kg a.s./ha	Water (L/ha)	kg a.s./hL				AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-7OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residue calc.	
	(a)	(b)				(c)		(d)							(e)
08-2169-01 France, north 37310 Chambourg sur Indre Centre Europe, North F 2008	Application to bare soil **PBI:33 d** Rotational crop: Rape, summer Olindigo	1) 16.04.2008 2) 04.07.2008 - 15.07.2008 3) 29.08.2008	0.5	300	0.167	14.03.2008	rod	76	0.02	0.01	0.01	0.01	<0.01	0.03	139
							seed	89	0.01	<0.01	<0.01	<0.01	<0.01	0.02	168

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information when metabolites are included
(g) Study reference
prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Total residue calculated : sum of fluopyram+FLU-benzamide

Data Point:	KCA 6.6.2/25
Report Author:	[redacted]
Report Year:	2009
Report Title:	Determination of the residues of AE C656948 in/on winter rape after spraying of Fluopyram SC 500 in the field in Italy and Spain
Report No:	08-2180
Document No:	M-359808-01-1
Guideline(s) followed in study:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC guidance working document 7029/VI/95 rev. 5 (1997-07-22)
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Materials and Methods

The purpose of the study 08-2180 was to determine the magnitude of residues of fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188), fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122), in/on rape (pod and seed). One spray application with Fluopyram SC 500, a suspension concentrate (SC) formulation containing 500 g/L fluopyram was done to bare soil followed by sowing of summer rape with plant back interval of 29-30 days.

Two supervised residue trial was conducted in southern Europe (Italy and Spain) during the 2008 growing season.

In trial 08-2180-01, the application of Fluopyram SC 500 was done to bare soil at an application rate of 0.88 L/ha (equivalent to 0.44 kg/ha fluopyram) with a water rate of 265 L/ha. The application procedure was followed by incorporation of the test item into the soil (using a rotovator three hours after the application) 29 days before sowing of winter rape. The treatment was 12% underdosed due to clogging of one nozzle.

In trial 08-2180-02, the application of Fluopyram SC 500 was done to bare soil at an application rate of 1 L/ha (equivalent to 0.50 kg/ha fluopyram) with a water rate of 300 L/ha. The application procedure was followed by incorporation of the test item into the soil to a depth < 8 cm and 30 days before sowing of winter rape. The treatment was made at the scheduled rate.

For residue analysis, samples of rape (pod and seed) were taken from the treated and the control plots. Samples of pod were taken 35-37 days prior harvest at a growth stage BBCH 79 and at harvest (BBCH 89).

The field samples from both trials were stored deep-frozen within 24 hours after sampling and until dispatch. All field samples were shipped by deep-freeze lorry and arrived in good condition. The field samples were stored in a freezer at ≤-18°C until preparation of the examination samples. For the preparation of examination samples, the deep frozen field samples were shredded with dry ice in a cutter. Representative parts of the shredded field samples were transferred into polystyrene boxes separately for analysis (examination samples) and archiving (retain samples) and stored for analysis or archiving at ≤-18°C until analysis.

Residues of fluopyram and its metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide), were determined by LC-MS/MS according to method 00984 ([redacted], 05/02/2007, M-283301-01-1, see MCA section 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues were extracted from 10 g of sample material by two successive extractions using a high-speed blender with a mixture of acetonitrile:water (80:20; *v:v*). After centrifugation the extract volume was adjusted. Then, the extracts were diluted ten times by adding the internal standards:

- One dilution was performed under acidic conditions: this extract allowed the determination of FLU-PCA and FLU-methyl-sulfoxide.
- In parallel, another dilution was performed under basic conditions: this extract allowed the determination of fluopyram, FLU-benzamide and FLU-7-OH.

An aliquot of the extracts was injected into LC-MS/MS operated in Electrospray Ionization mode:

- One injection in positive electrospray ionization for the determination of fluopyram, FLU-benzamide and FLU-7-OH.
- Another injection in negative electrospray ionization for the determination of FLU-PCA and FLU-methyl-sulfoxide under different conditions.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels. For each calibration curve, the correlation coefficient R was above 0.99.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

The Limit of Quantification (LOQ), expressed as fluopyram, defined as the lowest validated fortification level, was 0.05 mg/kg for wheat straw and 0.01 mg/kg for all other matrices.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Findings

The method performance could have been checked by concurrent recoveries. Unfortunately, the recovery samples which were extracted the same day as the examination samples were invalid, because of a dilution error. Therefore, the concurrent recoveries were extracted and analysed three days later. This deviation was accepted because the method 00984 was fully validated on rape seed. Details on concurrent recovery data are shown in Table 6.6.2- 50. No mean of recoveries and no RSD can be calculated because only one individual recovery was performed by level. The overall average recoveries (n=2) ranged between 92 - 128%.

No residues above the LOQs were found in the control samples. The detailed results obtained in the rotational crop of rape are summarized in Table 6.6.2- 51.The results were not corrected for concurrent recoveries.

The maximum storage period of deep-frozen samples was 158 days for pods and 123 for seeds.

Residues in rape

In pods, in trial the two trials (PBI 29-30 days), residues of fluopyram (AE C656948) were between 0.01 and 0.06 mg/kg at BBCH 79.

Residues of the metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide) in pods were <0.01 mg/kg at BBCH 79.

In seeds, in trial the two trials (PBI 29-30 days), residues of fluopyram (AE C656948) were between <0.01 and 0.01 mg/kg at BBCH 89.

Residues of the metabolites (FLU-benzamide, FLU-PCA, FLU-7-OH, FLU-methyl-sulfoxide) in seeds were <0.01 mg/kg at BBCH 89.

Conclusions

Following one spray application conducted with fluopyram SC500 to bare soil at a rate of 0.5 kg a.s./ha, residues in succeeding crops were analysed for fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815), fluopyram-pyridyl carboxylic acid (FLU-PCA, AE C657188) and fluopyram-7-hydroxy (BCS-AA10065) and fluopyram-methyl sulfoxide (AE 1344122) using a validated selective HPLC-MS/MS analytical method.

These data show that residues in rape (seed) as succeeding crop are covered by the current EU MRL.

Assessment and conclusion by applicant:
The study is acceptable

Table 6.6.2- 50: Recovery data for fluopyram and its metabolites in rape

Crop/Sample material	Fortification level (mg/kg)	Single values (%)	Mean value (%)	RSD* (%)
Fluopyram (AE C656948)				
Rape (seed)	0.01	156	-	-
	0.10	100		-
	Overall recovery (n=2)		**128**	-
Fluopyram-benzamide (AEF148815)				
Rape (seed)	0.005	91		-
	0.05	98		-
	Overall recovery (n=2)		**95**	-
Fluopyram-pyridyl-carboxilic-acid (AE C657188)				
Rape (seed)	0.006	97		-
	0.06	97	-	-
	Overall recovery (n=2)		**97**	-
Fluopyram-7-hydroxy (BCS-AA10065)				
Rape (seed)	0.01	85	-	-
	0.10	99		-
	Overall recovery (n=2)		**92**	
Fluopyram-methylsulfoxide (AE1344122)				
Rape (seed)	0.006	101		-
	0.06	100		-
	Overall recovery (n=2)		**101**	-

FL: fortification level, RSD - Relative Standard Deviation
* some RSDs were not calculated as there were only two individual recoveries given
Final determination as: fluopyram Residues calculated as: fluopyram
Final determination as: FLU-benzamide Residues calculated as: fluopyram
Final determination as: FLU-methylsulfoxide Residues calculated as: fluopyram
Final determination as: FLU-PCA Residues calculated as: fluopyram
Final determination as: FLU-7OH Residues calculated as: fluopyram

Table 6.6.2- 51: Results of rotational crop trial conducted with fluopyram

Trial No./ Location/ Year	Plot (commodity)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment to bare soil or target crop			Dates of treatment or no. of treatments and last date	Portion analysed	Growth stage at sampling	Residues (mg/kg)						PHI (days)
			g a.s./ha	Water (L/ha)	g a.s./hL				AE C656948 as AE C656948	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	Total residue calc.	
	(a)	(b)				(c)		(d)							(e)
08-2180-01 Spain 41310 Brenes Sevilla Andalucia Europe, South F 2008	Application to bare soil **PBI:29 d** Rotational crop: Rape, winter Jura	1) 07.11.2008 2) 20.03.2009 - 20.04.2009 3) 01.05.2009 - 15.06.2009	439	265	166	09.10.2008	pod	79	<0.06	<0.01	<0.01	<0.01	<0.01	0.07	200
							seed	89	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	237
08-2180-01 Spain 41310 Brenes Sevilla Andalucia Europe, South F 2008	Application to bare soil **PBI:30 d** Rotational crop: Rape, winter Livius	1) 25.09.2008 2) 16.03.2009 - 23.04.2009 3) 15.05.2009 - 15.06.2009	500	300	167	09.10.2008	pod	79	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	240
							seed	89	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	275

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (Label: pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) Study reference
* prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available

Total residue calculated : sum of fluopyram+FLU-benzamide

CA 6.7 Proposed residue definitions and maximum residue levels

CA 6.7.1 Proposed residue definitions

Plants

During the development and very first inclusion submission of fluopyram (data generation), for the selection of the metabolites possibly relevant for risk assessment, consideration was given to the relative levels found in the available metabolism studies (see MCA 6.2). Metabolites were envisaged for inclusion into the residue definition for risk assessment, if they were major metabolites.

The metabolism studies were conducted with two different radiolabeled test items (phenyl label and pyridyl label) to cover both sides of the molecule after cleavage. Considering that all metabolites which were not previously excluded from the assessment are covered by the toxicological profile of parent, the TRR is considered to give a good overview of the toxicological exposure.

Metabolism in primary crops

- Phenyl label : the two main compounds (fluopyram and fluopyram-benzamide) represent more than 75% of the TRR in grapes, potato, bean, rice (all foliar applications) in pepper (drip application) and 54-68% in wheat (seed treatment).
- Pyridyl label : parent fluopyram is the most abundant compound in most of the studies but fluopyram-pyridyl-acetic acid (M40) and fluopyram-pyridyl-carboxylic acid (M43) are also major metabolites after cleavage of the parent in some matrices (potato tuber, succulent, dry bean, wheat straw and pepper fruit).

Metabolism in rotational crops

The metabolic pathway in the rotational crop studies was shown to be similar than in primary crops. However, fluopyram-7-OH (M08) and its conjugates occurred in much higher proportions (swiss chard, wheat straw and hay), and metabolite fluopyram-methyl-sulfoxide (M45) occurred in significant amounts in wheat matrices.

Based on the available metabolism data, **for the first inclusion dossier** (please refer to DAR Vol. 3, B-7); **fluopyram and fluopyram-benzamide (M25),** as well as **fluopyram-pyridyl-acetic acid (M40) and fluopyram-pyridyl-carboxylic acid (M43)** were measured in residue trials (primary crops). For rotational crops, **fluopyram and fluopyram-benzamide (M25), Fluopyram-7-hydroxy (M08), Fluopyram-pyridyl-carboxylic acid (M43)** and **fluopyram-methyl-sulfoxide (M45)** were determined.

Subsequent to the generation and evaluation of this data, low residue levels were observed for fluopyram-pyridyl-acetic acid (M40), fluopyram-pyridyl-carboxylic acid (M43) and (for rotational crops), fluopyram-7-hydroxy (M08) and fluopyram-methyl-sulfoxide (M45). The respective contribution to the livestock dietary burden and to the consumer risk assessment turned out to be relatively low to very low (please refer to revised DAR Vol. 3 B-7, August 2012, from p130).

As a result, EFSA did not included these metabolites in the residue definition **for risk assessment** (EFSA Journal 2013;11(4):3052) both for primary and rotational crops and defined it as **parent fluopyram and fluopyram-benzamide**.

Moreover, because fluopyram-benzamide is **expressed as parent** and included in the definition of residue for risk assessment, **it covers also the pyridine side** when the dietary risk assessment is made and exposure compared to the toxicological endpoints of parent. For these reasons, there is no need to amend the DoR for risk assessment.

For monitoring purposes, **parent fluopyram** is a good marker and the following residue definition is proposed : Fluopyram.

Processed products

The radiolabeled high temperature hydrolysis studies for fluopyram and its metabolites fluopyram-benzamide, fluopyram-7-hydroxy (M08), fluopyram-pyridyl carboxylic acid (M43) and fluopyram-pyridyl-acetic-acid (M40), which simulate the conditions associated with baking, brewing and boiling, showed that all compounds remained stable following the processing procedures except fluopyram-pyridyl-acetic-acid. As residues of fluopyram-pyridyl-acetic-acid in raw agricultural commodities are expected to be very low (revised DAR, Vol. 3, B-7, p133-134), it is considered appropriate to maintain the current residue definitions (risk assessment and monitoring) for processed commodities, which are aligned with the residue definitions defined for the primary crops.

Animal commodities

Fluopyram, fluopyram-benzamide and fluopyram-olefins are the major metabolites identified from the poultry and ruminant metabolism studies with labeled fluopyram on the phenyl group. Fragments identified from the pyridyl labeled fluopyram show also the major presence of parent fluopyram and fluopyram-olefins. Additionally, hydroxylated metabolites and conjugates are measured in ruminant liver and kidney but those compounds are considered to be covered by the toxicological profile of the parent and are not proposed to be included in the residue definition.

For these reasons, the proposed residue definition for risk assessment in animal commodities is :

- The sum of fluopyram, fluopyram-benzamide and fluopyram-olefins expressed as fluopyram.

For monitoring purposes, fluopyram benzamide appears to be a good marker and the following residue definition is proposed :

- The sum of fluopyram and fluopyram benzamide expressed as fluopyram

This conclusion is in line with the 2020 review of the EU existing MRL according to Art. 12 of the Regulation (EC) No 396/2005. Please see below the extract of the document “Review of the existing maximum residue levels for fluopyram according to Article 12 of Regulation (EC) No 396/2005” (EFSA Journal 2020;18(4):6059):

A wide range of growing conditions and crop groups was investigated (spraying in fruits, pulses, and tuber crops; drip irrigation in fruits; as well as cereals, root crops and leafy crops grown in rotation). Fluopyram is also authorised as primary seed treatment on oil seeds and as a local treatment (pre-forcing) on chicory roots (witloofs). As the metabolite pattern is essentially the same in all crop categories even under different application systems, the above studies are considered to cover also the latter uses. Overall, the studies experimental designs were representative of the authorized uses and no further study is required.

As the parent compound was found to be a sufficient marker in all crops investigated, the residue definition for enforcement is proposed as ‘fluopyram’ only.

An analytical method for the enforcement of the proposed residue definition at the LOQ of 0.01 mg/kg in all four main plant matrices is available (EFSA, 2013a). According to the EURLs, the LOQ of 0.002 mg/kg in high water content and high acid content commodities and the LOQ of 0.01 mg/kg in high oil content and dry commodities is achievable by using the QuEChERS method in routine analyses (EURL, 2018).

The metabolic pathway of fluopyram in plants can be regarded as essentially the same in all crops investigated, with the parent compound being one of the major constituents of the residues. The metabolic pathway primarily consists of the hydroxylation of parent compound (M08), followed by cleavage of the hydroxylated parent compound leading to metabolite M25 (fluopyram-benzamide) from the phenyl moiety and metabolites M40 (primary crops only, including its hexose-conjugate M42), M45 (rotational crop only) and M43 from the pyridyl moiety of the active substance.

In the supervised field trials assessed in the current review M25 was detected only in a few commodities (up to a level of 0.16 mg/kg in rape seed) (see Section 1.2.1). In rotational crop field trials, solely M25 and M08 were found in significant amounts, and only in straw (see Section 1.2.2). However, as the relative contribution of M08 is little and would have very limited impact on the animal burden, if at all, its inclusion in the residue definition for risk assessment that would be specific to rotational cereals (straw) is not proposed. The peer review concluded that metabolite M40 does not need to be included in the residue definition as is of no toxicological concern at the levels detected in supervised field trials, and it may be covered by the concurrently detected phenyl specific M25, included in the residue definition (Germany, 2011).

M08, M25, M40 and its conjugate M42 were considered covered by the toxicological profile of the parent compound (EFSA, 2013a). M43 and M45, are common metabolites with active substance fluopicolide. In the light of their levels in food and feed items and the conclusion for fluopicolide, the peer review considered these metabolites as toxicologically not relevant (Germany, 2011).

Altogether, the residue definition for risk assessment is proposed to remain 'sum of fluopyram and fluopyram-benzamide (M25), expressed as fluopyram' as set by the peer review (EFSA, 2013a).

Note :

- M-02 & M03 : fluopyram-olefins, FLU-olefins
- M08 : fluopyram-7-hydroxy, FLU-7OH
- M25 : fluopyram-benzamide, FLU-benzamide
- M40 : fluopyram-pyridyl-acetic-acid, FLU-PAA
- M43 : fluopyram-pyridyl carboxylic acid, FLU-PCA
- M45 : fluopyram-methyl-sulfoxide, FLU-methylsulfoxide

Consideration of all the above, leads to the following proposed residue definitions for fluopyram:

Table 6.7.1- 1 Residue definitions for fluopyram

Category	Residue definition
Enforcement (post-registration) residue definition in plant commodities	Fluopyram
Enforcement (post-registration) residue definition for products of livestock origin:	Sum of flyopyram and fluopyram-benzamide expressed as fluopyram
Risk assessment residue definition in plant commodities	Sum of flyopyram and fluopyram-benzamide expressed as fluopyram
Risk assessment residue definition for products of livestock origin	Sum of flyopyram, fluopyram-benzamide and fluopyram-E/Z-olefins expressed as fluopyram
Risk assessment residue definition for processed plant commodities	Sum of flyopyram and fluopyram-benzamide expressed as fluopyram

Data Point:	KCA 6.7.1/01
Report Author:	[redacted]
Report Year:	2008
Report Title:	U.S. plant residue definition for AE C656948: Communication with U. S. EPA ChemSAC
Report No:	201868
Document No:	M-299913-01-1
Guideline(s) followed in study:	OPPTS 860.1300 (Supplemental)
Deviations from current test guideline:	--
Previous evaluation:	
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	

CA 6.7.2 Proposed maximum residue levels (MRLs) and justification of the acceptability of the levels proposed

No MRL are proposed in the frame of this active substance renewal application.

CA 6.7.3 Proposed maximum residue levels (MRLs) and justification of the acceptability of the levels proposed for imported products (import tolerance)

No MRL for imported products are proposed in the frame of this active substance renewal application.

CA 6.8 Proposed safety intervals

There is no need to propose safety intervals.

CA 6.9 Estimation of the potential and actual exposure through diet and other sources

In order to evaluate the potential chronic and acute exposures to fluopyram residues through the diet, calculations were done using the EFSA PRIMo model (revision 3.1) and the following toxicological endpoints.

Acceptable Daily Intake (ADI) and Dietary Exposure Calculation

According to the first EU inclusion peer review (EFSA, 2013), the ADI is at the level of 0.012 mg/kg bw/day.

Acute Reference Dose (ARfD) and Dietary Exposure Calculation

According to the first EU inclusion peer review (EFSA, 2013), the ARfD is at the level of 0.5 mg/kg bw.

A risk based on TMDI is not presented as it overpasses the 100% of ADI contribution. A more pertinent IEDI calculation is proposed. In the frame of this renewal the consumer risk is evaluated regarding the contribution of the submitted data presented in Table 6.9- 1 :

- Residues from representative uses according to the residue definition for risk assessment (RA)
- Residues in succeeding crops taken at the plant back interval showing the most critical values

- Residues in animal commodities from the submitted feeding studies at the worst-case feeding dose (tissues) or at a more realistic dose (milk)
- Residues in honey as presented in MCA 6.10.1

Table 6.9- 1: Input values for the consumer risk assessment

Commodity	Chronic exposure assessment		Acute exposure assessment	
	Input (mg/kg)	Comment	Input (mg/kg)	Comment
Apple [1)]	0.07	$STMR_{RA}$ rep. use	0.22	HR_{RA} rep. use
Table grape	0.025	$STMR_{RA}$ rep. use	0.05	HR_{RA} rep. use
Wine grape	0.025	$STMR_{RA}$ rep. use	0.05	HR_{RA} rep. use
Strawberry	<LOQ	$STMR_{RA}$ rotational	<LOQ	HR_{RA} rotational
Potato	0.03	$STMR_{RA}$ rotational	0.03	HR_{RA} rotational
Carrot	0.05	$STMR_{RA}$ rotational	0.06	HR_{RA} rotational
Bulbs (onion. garlic...)	0.02	$STMR_{RA}$ rotational	0.03	HR_{RA} rotational
Tomato	<LOQ	$STMR_{RA}$ rotational	<LOQ	HR_{RA} rotational
Sweet corn	<LOQ	$STMR_{RA}$ rotational	LOQ	HR_{RA} rotational
Lettuces	1.61	$STMR_{RA}$ rep. use	10	HR_{RA} rep. use
Spinaches	0.08	$STMR_{RA}$ rotational	0.43	HR_{RA} rotational
Pea(without pods)	<LOQ	$STMR_{RA}$ rotational	<LOQ	HR_{RA} rotational
Leek	0.03	$STMR_{RA}$ rotational	0.05	HR_{RA} rotational
Pulses (dry)	LOQ	$STMR_{RA}$ rotational	<LOQ	HR_{RA} rotational
Rape seed	0.02	$STMR_{RA}$ rotational	0.02	HR_{RA} rotational
Barley	0.02	$STMR_{RA}$ rep. use	0.09	HR_{RA} rep. use
Wheat	0.02	$STMR_{RA}$ rotational	0.02	HR_{RA} rotational
Maize	<LOQ	$STMR_{RA}$ rotational	LOQ	HR_{RA} rotational
Mammalian meat	0.32	Cow feeding$_{RA}$*	0.32	Cow feeding$_{RA}$*
Mammalian fat	0.31	Cow feeding$_{RA}$*	0.31	Cow feeding$_{RA}$*
Mammalian liver	1.95	Cow feeding$_{RA}$*	1.95	Cow feeding$_{RA}$*
Mammalian kidney	0.31	Cow feeding$_{RA}$*	0.31	Cow feeding$_{RA}$*
Poultry meat	0.04	Poultry feeding$_{RA}$*	0.04	Poultry feeding$_{RA}$*
Poultry fat	0.06	Poultry feeding$_{RA}$*	0.06	Poultry feeding$_{RA}$*
Poultry liver	0.18	Poultry feeding$_{RA}$*	0.18	Poultry feeding$_{RA}$*
Poultry kidney	0.18	Poultry feeding$_{RA}$*	0.18	Poultry feeding$_{RA}$*
Milk	0.04	Cow feeding$_{RA}$*	0.04	Cow feeding$_{RA}$*
Eggs	0.09	Poultry feeding$_{RA}$*	0.09	Poultry feeding$_{RA}$*
Honey	LOQ	$STMR_{RA}$	<LOQ	HR_{RA}

RA Crops : sum of fluopyram and fluopyram-benzamide expressed as fluopyram

RA Animal commodities : sum of fluopyram, fluopyram-benzamide and fluopyram-olefins expressed as fluopyram

*In tissues, as a worse case the residue results from the 1X group is used as input data to calculate the risk assessment.

*In milk the dietary burden of 0.076 mg/kg bw/day in dairy cattle is close to the 0.1 X dose value of 0.04 mg/kg bw/day. Values in milk from the 0.1 X dose group are considered to calculate the dietary risk assessment.

As shown in Table 6.9- 2, the highest chronic risk is calculated for the NL toddler diet and represents 40% of the ADI. The highest contributors are cattle milk (20%) and apples (6%).

The results indicate that there is no unacceptable chronic risk to human health from the consumption of commodities treated with fluopyram according to the representative intended uses/GAPs.

The short term exposure show the following acute risk according to the EFSA Primo 3.1 calculation:

- for children, risk is below 100% of ARfD, lettuce being the highest contributor (76% of ARfD).
- for adults, risk is below 100% of ARfD, lettuce being the highest contributor (24% of ARfD).
- Acute risk from processed commodities remain below 1% of ARfD, in all population categories

A short-term intake of residues of fluopyram according to the presented representative uses is therefore unlikely to present a public health concern.

Table 6.9- 2: IEDI calculation for fluopyram (EFSA Primo 3.1)

European Food Safety Authority

EFSA PRIMo revision 3.1; 2019/03/19

Fluopyram

LOQs (mg/kg) range from: 0.02 to: 0.05

Toxicological reference values

ADI (mg/kg bw/day):	0.012	ARfD (mg/kg bw):	0.5
Source of ADI:	EFSA	Source of ARfD:	EFSA
Year of evaluation:	2013	Year of evaluation:	2013

Input values

Details - chronic risk assessment | Supplementary results - chronic risk assessment

Details - acute risk assessment/children | Details - acute risk assessment/adults

Comments:

Normal mode

Chronic risk assessment: JMPR methodology (IEDI/TMDI)

No of diets exceeding the ADI :

TMDI/NEDI/IEDI calculation (based on average food consumption)

Calculated exposure (% of ADI)	MS Diet	Exposure (µg/kg bw per day)	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	Exposure resulting from MRLs set at the LOQ (in % of	Exposure resulting from commodities not under assessment (in % of ADI)
40%	NL toddler	4.86	20%	Milk: Cattle	6%	Apples	3%	Swine: Liver	0.0%	
25%	SE general	2.95	12%	Bovine: Muscle/meat	5%	Lettuces	4%	Milk: Cattle	0.0%	
24%	UK infant	2.92	13%	Milk: Cattle	3%	Bovine: Muscle/meat	3%	Bovine: Edible offals (other than liv	0.0%	
24%	NL child	2.83	8%	Milk: Cattle	3%	Apples	3%	Swine: Muscle/meat	0.0%	
23%	ES child	2.78	6%	Lettuces	4%	Milk: Cattle	4%	Bovine: Muscle/meat	0.0%	
22%	FR child 3 15 yr	2.66	8%	Milk: Cattle	4%	Bovine: Muscle/meat	4%	Swine: Muscle/meat	0.0%	
22%	DK child	2.64	6%	Swine: Muscle/meat	4%	Milk: Cattle	3%	Bovine: Muscle/meat		
22%	FR toddler 2 3 yr	2.61	10%	Milk: Cattle	3%	Bovine: Muscle/meat	3%	Swine: Muscle/meat	0.0%	
21%	DE child	2.50	7%	Apples	2%	Milk: Cattle	2%	Swine: Muscle/meat	0.0%	
20%	GEMS/Food G07	2.34	3%	Lettuces	3%	Swine: Muscle/meat	2%	Bovine: Muscle/meat		
17%	ES adult	2.02	7%	Lettuces	2%	Bovine: Muscle/meat	2%	Swine: Muscle/meat	0.0%	
17%	IE adult	2.01	4%	Sheep: Liver	3%	Other farmed animals: Muscle/meat	2%	Sheep: Edible offals (other than liv		
17%	GEMS/Food G08	2.00	5%	Swine: Muscle/meat	3%	Lettuces	2%	Milk: Cattle		
16%	GEMS/Food G10	1.94	4%	Lettuces	2%	Bovine: Muscle/meat	2%	Swine: Muscle/meat		
16%	GEMS/Food G15	1.94	4%	Swine: Muscle/meat	2%	Milk: Cattle	2%	Swine: Liver		
16%	UK toddler	1.87	7%	Milk: Cattle	3%	Bovine: Muscle/meat	1.0%	Apples	0.0%	
14%	GEMS/Food G11	1.73	3%	Swine: Muscle/meat	3%	Milk: Cattle	2%	Bovine: Muscle/meat		
13%	DE general	1.57	4%	Milk: Cattle	3%	Swine: Muscle/meat	1%	Apples	0.0%	
12%	DE women 14-50 yr	1.49	4%	Milk: Cattle	2%	Swine: Muscle/meat	2%	Lettuces	0.0%	
12%	NL general	1.47	3%	Milk: Cattle	3%	Swine: Muscle/meat	2%	Bovine: Muscle/meat	0.0%	
12%	RO general	1.43	4%	Milk: Cattle	3%	Swine: Muscle/meat	0.9%	Potatoes	0.0%	
11%	FR infant	1.31	6%	Milk: Cattle	1%	Apples	1.0%	Swine: Muscle/meat	0.0%	
10%	DK adult	1.24	2%	Swine: Muscle/meat	2%	Milk: Cattle	1%	Bovine: Muscle/meat		
9%	FR adult	1.12	2%	Swine: Muscle/meat	2%	Bovine: Muscle/meat	1%	Milk: Cattle	0.0%	
9%	LT adult	1.06	3%	Swine: Muscle/meat	1%	Milk: Cattle	1%	Apples		
8%	GEMS/Food G06	0.95	1%	Lettuces	1%	Wheat	0.8%	Milk: Cattle		
7%	IT adult	0.78	5%	Lettuces	0.7%	Wheat	0.5%	Apples		
6%	UK adult	0.75	2%	Bovine: Muscle/meat	2%	Lettuces	1.0%	Milk: Cattle	0.0%	
6%	IT toddler	0.71	4%	Lettuces	1%	Wheat	0.5%	Apples		
5%	PT general	0.59	1%	Lettuces	1%	Potatoes	0.7%	Wheat		
5%	UK vegetarian	0.57	2%	Lettuces	1%	Milk: Cattle	0.3%	Apples		
3%	IE child	0.37	1%	Milk: Cattle	0.5%	Swine: Muscle/meat	0.3%	Swine: Fat tissue	0.0%	
3%	FI 6 yr	0.36	1%	Lettuces	1.0%	Potatoes	0.3%	Apples	0.0%	
3%	FI 3 yr	0.35	1%	Potatoes	0.5%	Apples	0.5%	Lettuces	0.0%	
3%	FI adult	0.34	2%	Lettuces	0.3%	Apples	0.3%	Potatoes		
2%	PL general	0.30	1%	Apples	0.9%	Potatoes	0.2%	Lettuces		

Conclusion:
The estimated long-term dietary intake (TMDI/NEDI/IEDI) was below the ADI.
The long-term intake of residues of Fluopyram is unlikely to present a public health concern.

Table 6.9- 3: IESTI calculation for fluopyram (EFSA Primo 3.1)

Acute risk assessment /children	Acute risk assessment / adults / general population
Details - acute risk assessment /children	Details - acute risk assessment/adults

The acute risk assessment is based on the ARfD.
The calculation is based on the large portion of the most critical consumer group.

Show results for all crops

Unprocessed commodities

Results for children
No. of commodities for which ARfD/ADI is exceeded (IESTI): ---

IESTI

Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
76%	Lettuces	0 / 10	381
5%	Apples	0 / 0.22	24
3%	Bovine: Liver	1.95 / 1.95	16
3%	Bovine: Edible offals	1.95 / 1.95	14
2%	Other farmed animals:	1.5 / 1.5	10
1%	Swine: Edible offals	1.95 / 1.95	5.9
1.0%	Milk: Cattle	0.04 / 0.04	5.0
0.9%	Potatoes	0 / 0.03	4.6
0.8%	Swine: Muscle/meat	0.32 / 0.32	3.9
0.8%	Carrots	0 / 0.06	3.8
0.7%	Table grapes	0 / 0.05	3.6
0.6%	Leeks	0 / 0.05	2.9
0.6%	Spinaches	0 / 0.13	2.9
0.5%	Swine: Liver	1.95 / 1.95	2.4
0.5%	Bovine: Muscle/meat	0.32 / 0.32	2.3

Expand/collapse list

Results for adults
No. of commodities for which ARfD/ADI is exceeded (IESTI): ---

IESTI

Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
24%	Lettuces	0 / 10	121
2%	Other farmed animals:	1.5 / 1.5	8.4
2%	Bovine: Liver	1.95 / 1.95	7.8
1%	Bovine: Edible offals (other	1.95 / 1.95	6.5
1%	Apples	0 / 0.22	6.2
1%	Sheep: Liver	1.95 / 1.95	5.5
1%	Swine: Edible offals (other	1.95 / 1.95	5.1
0.6%	Swine: Liver	1.95 / 1.95	2.8
0.4%	Bovine: Muscle	0.32 / 0.32	1.8
0.3%	Table grapes	0 / 0.05	1.7
0.3%	Swine: Muscle/meat	0.32 / 0.32	1.5
0.3%	Milk: Cattle	0.04 / 0.04	1.5
0.3%	Equine: Muscle/meat	0.32 / 0.32	1.5
0.3%	Sheep: Muscle/meat	0.32 / 0.32	1.5
0.3%	Sheep: Edible offals (other	1.95 / 1.95	1.3

Total number of commodities exceeding the ARfD/ADI in children and adult diets (IESTI calculation)

Processed commodities

Results for children
No of processed commodities for which ARfD/ADI is exceeded (IESTI): ---

IESTI

Highest % of ARfD/ADI	Processed commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
0.8%	Apples / juice	0 / 0.07	3.8
0.6%	Leeks / boiled	0 / 0.05	2.9
0.6%	Potatoes / fried	0 / 0.03	2.8
0.4%	Spinaches / frozen; boiled	0 / 0.13	1.8
0.4%	Carrots / juice	0 / 0.05	1.8
0.4%	Potatoes / dried (flakes)	0 / 0.14	1.8
0.2%	Wine grapes / juice	0 / 0.03	1.1
0.0%	Wheat / milling (flour)	0 / 0.02	0.24
0.0%	Wheat / milling (wholemea	0 / 0.02	0.11
0.0%	Barley / cooked	0 / 0.02	0.07
0.0%	Barley / milling (flour)	0 / 0.02	0.04
0.0%	Rapeseeds / oils	0 / 0.04	0.01
#NOMBRE!	#NOMBRE!	#NOMBRE!	#NOMBRE!
#NOMBRE!	#NOMBRE!	#NOMBRE!	#NOMBRE!
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Expand/collapse list

Results for adults
No of processed commodities for which ARfD/ADI is exceeded (IESTI): ---

IESTI

Highest % of ARfD/ADI	Processed commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
0.5%	Apples / juice	0 / 0.07	2.3
0.2%	Spinaches / frozen; boiled	0 / 0.13	1.1
0.2%	Leeks / boiled	0 / 0.05	0.87
0.1%	Wine grapes / juice	0 / 0.03	0.52
0.09%	Wine grapes / wine	0 / 0.05	0.47
0.08%	Carrots / canned	0 / 0.05	0.41
0.06%	Table grapes / raisins	0 / 0.24	0.29
0.06%	Onions / boiled	0 / 0.03	0.28
0.05%	Potatoes / chips	0 / 0.03	0.25
0.03%	Potatoes / dried (flakes)	0 / 0.14	0.17
0.03%	Barley / beer	0 / 0	0.14
0.02%	Wheat / bread/pizza	0 / 0.02	0.09
0.02%	Wheat / pasta	0 / 0.02	0.08
0.01%	Wheat / bread	0 / 0.02	0.07
#NOMBRE!	#NOMBRE!	#NOMBRE!	#NOMBRE!

Conclusion
No exceedance of the toxicological reference value was identified for any unprocessed commodity.
A short term intake of residues of Fluopyram is unlikely to present a public health risk.

For processed commodities, no exceedance of the ARfD/ADI was identified.

CA 6.10 Other studies

CA 6.10.1 Effect on the residue level in pollen and bee products

Following the publication of the guideline for setting MRLs in honey SANTE 11956 2016 rev 9, four tunnel residue trials on the surrogate crop Phacelia tanacetifolia followed by honey analysis.

Data Point:	KCA 6.10.1/01
Report Author:	[redacted]
Report Year:	2020
Report Title:	Determination of residues of fluopyram and its metabolites in honey after two applications of FLU SC 250 in Phacelia tanacetifolia at 4 sites in Northern and Southern Europe in 2019
Report No:	S19-01064
Document No:	M-681608-01-1
Guideline(s) followed in study:	OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) EC (2018) Technical guidelines for determining the magnitude of pesticide residues in honey and setting Maximum Residue Levels in honey (SANTE/11956/2016 rev. 9) Commission Regulation (EU) 283/2013 and 284/2013 (implementing Regulation (EC) 1107/2009 (Oct. 2009)
Deviations from current test guideline:	--
Previous evaluation:	
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	

Test system

In order to support the intended and most critical uses of fluopyram on melliferous crops and in order to determine the resulting residues of fluopyram in honey, four GLP honey trials were conducted on *Phacelia tanacetifolia* in northern and southern European zones under semi-field conditions during the 2019 season.

The trials were performed in Germany (2), southern France and Spain.

Two spray applications of the formulation FLU SC 250, a suspension concentrate containing 250 g fluopyram/L were applied at 1 L/ha during flowering (BBCH 62-65) with an interval of 6-7 days, representing the most critical GAP of the crops intended for authorisation in EU MSs and covering the whole flowering period.

Table 6.10.1- 1: use pattern applied for the honey residue trials after spray application of fluopyram under semi-field conditions

Description	Application Type	Formulation	No. of appl.	Growth stage at last application	Application rate per treatment (g a.s./ha)	Interval (days)
Phacelia tanacetifolia (Honey tunnel trials)	Semi-field	SC 250*	2	62-65 (flowering)	250	6-7

* Suspension Concentrate containing 250 g fluopyram/L

On each trial site one tunnel confining the bees was established on the control and the treated plot. One bee hive was set up per tunnel for the control and treated plot, each. Colony assessments were performed before set-up of the hives in the tunnels and after sampling of the honey.

Honey was collected from initially empty combs which were introduced in the hives shortly before the last application. Honey was collected once mature at the end of flowering or if the water content was < 20% or after comb closure – whatever occurred first - for subsequent residue analysis. In case the water content was >20% at the end of flowering a subsample was dried in a compartment dryer at the laboratory at conditions simulating the bee hive conditions.

In one trial (S19-01064-04) honey was sampled before end of flowering and with a water content > 20% as rainy weather was forecasted, and new honey was only available at low amounts in the colonies. In order not to risk losing the produced honey, the honey was sampled already before the end of flowering. An additional honey sample was collected in this trial at the end of flowering to obtain honey with water content of < 20%.

Honey samples were taken 2 to 10 days after the last application.

All honey samples were transported on dry ice from the field to the test facility, with exception of samples destinated for further drying which were transported at ambient temperature. Samples were stored deep frozen within 24 hours after sampling, or after end of drying, respectively. The field samples were stored in a freezer at -18 °C or below until preparation of the examination samples.

The samples were analysed for fluopyram (AE C656948) and its metabolites fluopyram-benzamide (AE F148815, FLU-benzamide), fluopyram-pyridyl-carboxylic-acid (AE C657188, FLU-PCA), fluopyram-pyridyl-acetic-acid (BCS-AA 10139, FLU-PAA), fluopyram-7-hydroxy (BCS-AA10065, FLU-7-OH)with the analytical method 01594 (Roth A., 20/02/2020, M-681002-02-2, see section MCA 4.1.2). Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

The samples were fully diluted with an acetonitrile/water mixture (1+1, v/v). An aliquot was analysed by high performance liquid chromatography, chromatographed under reversed phase gradient conditions and detected by Tandem Mass Spectrometry with electrospray ionisation.

The Limit of Quantification (LOQ) in/on honey was 0.01 mg/kg for fluopyram and its metabolites, expressed as fluopyram.

The linearity was demonstrated in each analytical batch with a 1/x weighted calibration curve established with at least 5 concentration levels. For each calibration curve, the correlation coefficient R was above 0.99.

The quantification was done by internal standardization using isotopically stable labelled internal standards.

Findings

In order to check the performance of the analytical method, recovery determinations were concurrently performed to the analyses of control and treated samples from the study in each set of analyses. For control material honey from local market was used for concurrent recoveries. The means of the concurrent recoveries were for all fortification levels within the acceptable range of 70 – 110 % and RSD values were <20%. All overall mean recoveries are in the acceptable range of 70 – 110 %; therefore, the results are considered valid. Recovery results are presented in Table 6.10.1- 2. The storage periods of deep-frozen samples intended for the analysis of fluopyram and its metabolites was 28 to 104 days.

- Residue results: After two spray applications of Fluopyram SC 250 to Phacelia tanacetifolia, the residues of fluopyram and its metabolites were determined in honey sampled 2-10 days after the last application to the flowering Phacelia tanacetifolia.

No residues of fluopyram and its five metabolites (FLU-benzamide, FLU-PCA, FLU-PAA, FLU-7OH and FLU-methylsulfoxide) were found above the LOQ in any of the control and treated samples of honey. The detailed results obtained for honey in EU tunnel trials are summarized in the

Table 6.10.1- 3.

Conclusion

Following two fluopyram applications at a high dose rate under protected conditions on a meliferous crop at flowering, no residues of fluopyram and its metabolites were measured in honey.

Table 6.10.1- 2: recovery data for fluopyram and its metabolites in honey

Sample material	FL (mg/kg)	Single values (%)	Mean value (%)	RSD*(%)
Fluopyram (AE C656948)				
honey	0.01	105,98,102	102	3.5
	0.10	111, 112, 108	110	1.9
		Overall recovery (n=6)	106	5.1
Fluopyram-benzamide (AEF148815)				
honey	0.01	103, 98, 76	92	15.6
	0.10	97, 99, 98	98	1.0
		Overall recovery (n=6)	95	10.1
Fluopyram-pyridyl-carboxilic-acid (AE C657188)				
honey	0.01	97, 94, 95	95	1.6
	0.10	95, 94, 99	96	2.8
		Overall recovery (n=6)	96	2.1
Fluopyram-pyridyl-acetic-acid (BCS AA10139)				
honey	0.01	94, 91, 77	87	10.4
	0.10	97, 98, 95	97	1.6
		Overall recovery (n=6)	92	8.4
Fluopyram-7-hydroxy (BCS-AA10065)				
honey	0.01	103, 100, 102	102	1.5
	0.10	110, 111, 107	109	1.9
		Overall recovery (n=6)	106	4.3
Fluopyram-methylsulfoxide (AE1344122)				
honey	0.01	97, 90, 92	93	3.9
	0.10	95, 94, 100	96	3.3
		Overall recovery (n=6)	95	3.8

FL: fortification level, RSD - Relative Standard Deviation
Final determination as: fluopyram Residues calculated as: fluopyram
Final determination as: FLU-benzamide Residues calculated as: fluopyram
Final determination as: FLU-PCA Residues calculated as: fluopyram
Final determination as: FLU-PAA Residues calculated as: fluopyram
Final determination as: FLU-7OH Residues calculated as: fluopyram
Final determination as: FLU-methylsulfoxide Residues calculated as: fluopyram

Table 6.10.1- 3: results of fluopyram honey tunnel trials

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)						PHI (days)
			g a.s./ha	Water (L/ha)	g a.s./hL					FLU AE C656948 as sum	FLU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-PAA as AE C656948	FLU-70H as AE C656948	FLU-methyl-sulfoxide as AE C656948	
	(a)	(b)				(c)	(d)		(d)							(e)
S19-01064-01 Germany 75177 Pforzheim, Baden-Württemberg Europe, North F, 2019	Phacelia Tanacetifolia Balo	1) 29-03-2019	248 250	397 406	62.5 62.6	14-06-2019/0 21-06-2019/7	65	Honey fresh	68	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	7
								Honey dried	68	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	7
S19-01064-02 Germany 76927 Stutensee, Baden-Württemberg Europe, North F, 2019	Phacelia Tanacetifolia Balo	1) 15-04-2019	251 251	402 402	62.4 62.4	18-06-2019/0 25-06-2019/7	65	Honey fresh	69	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	7

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment	Portion analyzed	Growth stage at sampling	Residues (mg/kg)						PHI (days)
			g a.s./ha	Water (L/ha)	g a.s./hL					AE C656948 as AE C656948	PEU-benzamide as AE C656948	FLU-PCA as AE C656948	FLU-PAA as AE C656948	FLU-3OH as AE C656948	FLU-methyl-sulfoxide as AE C656948	
	(a)	(b)				(c)	(d)		(d)							(e)
S19-01064-03 France, south 47460 Monheurt, Lot-et-Garonne Europe, South F, 2019	Phacelia Tanacetifolia Stala	1) 22-02-2019 2	251 253	401 403	62.6 62.8	25-05-2019/0 01-06-2019/7	65	Honey fresh	65	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	10
S19-01064-04 Spain 46820, Anna, Comunidad Valenciana Europe, South F, 2019	Phacelia Tanacetifolia Stala	1) Natural emergence from 2018 seeding	270 243	432 389	62.5 62.5	09-04-2019/0 15-04-2019/6	64	Honey fresh	64-65 66-67	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	2 10
								Honey dried	64-65 66-67	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	2 10

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Either growth stage description or BBCH Code
G greenhouse F field
(e) Days after last application (Label pre-harvest interval, PHI, underline)
(f) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included
(g) study reference
* prior to last treatment
(h) Formulation type
(i) Application method
(j) Method information
(k) LOQ
** residue in control
(l) Method validation
(m) Storage (max)
! based on date of analysis
P based on production date
no data available