Update

Document Title

Summary of the residues in or on treated products, food and feed for Flufenacet (FOE 5043)

Data Requirements

EU Regulation 1107/2009 & EU Regulation 283/2013
Document MCA
Section 6: Residues in or on treated products, food and feed

According to the guidance document, SANCO 10181/2013, for preparing dossiers for the approval of a chemical active substance

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Author(s)

[redacted]

Bayer AG,

Crop Science Division

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Version history

Date	Data points containing amendments or additions[1] and brief description	Document identifier and version number
2014-03-12	Originally submitted document	M-482131-01-1
2016-07-07	Update during generation dRAR by RMS Poland; inclusion of information on storage stability in animal matrices	M-482131-02-1

[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

Flufenacet was included in Annex I of Directive 91/414/EEC on 01/01/2004, as notified in Directive 2003/84/EC dated 25 September 2003 wherein there is no specific provision under Part B which needs to be considered related to the metabolism and residue data.

The Monograph prepared by the Rapporteur Member State France in the context of the inclusion of flufenacet in Annex 1 of the Council Directive 91/414/EEC, the Review Report for flufenacet (7469/VI/98-Final – 3rd July 2003) and the EFSA's Reasoned Opinion on the review of existing maximum residue levels (MRLs) for flufenacet according to Article 12 of Regulation (EC) No 396/2005 (EFSA Journal 2012;10(4):2689) are considered to provide the relevant scientific information for the review of the active substance. Information on the residue definition can be taken from the Complete List of Endpoints, Report of ECCO 73, Annex 2, 5 Residue Section.

CA 6.1 Storage stability of residues

Storage stability data was reported in chapter 6 of the Annex II dossier ([redacted], L., L.; 1997; M-002426-01)). The freezer storage stability of flufenacet (FOE 5043) and 5 of its metabolites (FOE-oxalate, FOE sulfonic acid, FOE thioglycolate sulfoxide, FOE methylsulfoxide, and FOE methylsulfone) was examined in commodities of three different crops, representing oil-, starch- and water containing materials. Field grown corn grain, forages, and fodder, soybean seeds, forage, and hay; and turnip roots and tops were fortified at a nominal rate of 1 mg/kg with the radiolabeled compounds. The first study covers a storage period of 11 months for all commodities. In the addendum, freezer storage stability data for turnips up to 20 months and for corn and soybean commodities up to 28 months were reported. The results show that residues of flufenacet and its metabolites are stable in all tested matrices under frozen conditions for at least as long as the storage stability studies lasted. Storage stability data were considered appropriate in the Monograph (Annex B 6) and in the EFSA Reasoned Opinion on existing MRLs (EFSA Journal 2012;10(4): 2689). Thus, in principle, no further data is required. The data already evaluated is briefly summarised in Table 6.1-1.

Table 6.1-1: Maximum demonstrated storage stability for flufenacet and metabolites in plant matrices

Compound	Commodity	Maximum storage period (months) AII 6.3.3/01	Maximum storage period (months) AII 6.3.3/02	Storage conditions	Reference
Flufenacet, FOE-oxalate, FOE-sulfonic acid, FOE-thioglycolate sulfoxide, FOE-methylsulfoxide, FOE-methylsulfone	Corn grain	11	28	≤ -21°C	KCA 6.1/01 Monograph Annex B 6 EFSA Reasoned Opinion 2012
	Corn forage	11	28		
	Corn fodder	11	28		
	Soybean seed	11	28		
	Soybean forage	11	28		
	Soybean hay	11	28		
	Turnip roots	11	20		
	Turnip tops	11	20		

Additional storage stability information is reported in a study from the US on wheat commodities (wheat forage, grain and straw) for flufenacet and the 5 metabolites mentioned above for storage periods up to 21 months. The study was not evaluated during the EU peer review and in principle no additional data were considered necessary relative to the uses evaluated for Annx I listing. For sake of completeness the study on wheat commodities is summarised below since it may provide supplementary information relative to the representative use on cereals.

In addition data were generated to demonstrate storage stability for additional commodity groups of high protein content (dry bean seed) and high acid content (orange fruit) as outlined in OECD guideline 506 (stability of pesticide residues in stored commodities). The study is also summarized below.

Report:	**KCA 6.1/04, [redacted].; 1997; M-002424-01**
Title:	The storage stability of FOE 5043 and metabolites in wheat forage, grain, and straw
Report No. & Document No.:	107137 dated April 22, 1997 M-002424-01-1
Guidelines:	Fulfils data requirement of US EPA 171-4(e) Storage Stability - Crops
GLP:	Yes; Deviations: none

Material and Methods

Freezer storage stability of flufenacet (FOE 5043) and 5 of its metabolites (FOE oxalate, FOE sulfonic acid, FOE thioglycolate sulfoxide, FOE methylsulfoxide, and FOE methylsulfone) was examined in commodities of wheat (grain, straw and forage). The study was performed using [fluorophenyl-UL-^{14}C]flufenacet and metabolites with the ^{14}C label in the fluorophenyl ring. Sample materials were fortified with different treating solutions:

i) Solution A containing 1.01 ppm flufenacet + 1.02 ppm FOE thioglycolate sulfoxide (FAMSOC) in flufenacet equivalents
ii) Solution B containing 0.99 ppm FOE oxalate + 1.10 ppm FOE methylsulfoxide (FAMSO) in flufenacet equivalents
iii) Solution C containing 1.02 ppm FOE sulfonic acid (FASO3H) + 1.02 ppm FOE methylsulfone (FAMSO2) in flufenacet equivalents.

Fortification: 10 g samples of pulverized and frozen wheat forage, grain, and straw were weighed into glass jars. The jars were closed, labeled, and placed in frozen storage (-24±5°C) until fortification. Three unfortified samples of each matrix were designated as blank controls. The samples for spiking were removed from the freezer, allowed to warm to room temperature, and were fortified with 1 mL of solution A, B or C, nominally at 1 mg/kg for each analyte. The samples were manually shaken and rotated to distribute the fortification solution on the matrix. Three of the samples of each matrix/fortification solution combination were selected for immediate (zero-time) extraction and analysis; the remaining samples were returned to frozen storage.

Sample extraction: At each sampling interval, replicate fortified samples (triplicate at zero time and duplicate at 6 and 21 months) of each matrix were extracted by repeated blending with methanol for 2 to 3 minutes and filtered. The filtered extracts were combined, radioassayed by LSC, and analyzed by HPLC.

Analytical methodology: Liquid samples were radioassayed by LSC. Aliquots of solid samples were combusted using a sample oxidizer and the resulting $^{14}CO_2$ was trapped in alkaline solution and measured by LSC. HPLC analyses were conducted on a C8 column preceded by a reverse-phase precolumn and radioactivity was quantified using a radioactivity monitor. The peaks observed during HPLC analysis of the extracts corresponded to the peaks for the compounds fortified in the samples. To verify the identity of each peak, extracts representing each dosing solution and each matrix were spiked with standards for each analyte and subjected to co-chromatography by HPLC. The corresponding standard for each analyte was shown to co-elute with the identified peak.

Findings

The recoveries of flufenacet and the 5 metabolites in the extract for each time point are given in Tables 6.1-2 to 6.1-7. The analytical method was suitable for determining residues in the storage stability study. Samples were fortified with [^{14}C]-labeled analytes, and analyses were performed by HPLC. Peak identification was verified by co-chromatography with known standards of each analyte. Recoveries of radioactivity from the HPLC column at each storage interval were as follows: 92-103% at time-zero, 89-107% at 6 months, and 91-122% at 21-months. After 21 months of storage, recoveries of flufenacet-related residues ranged from 84 to 120% (calculated as percent of measured time-zero residue) for wheat forage, grain, and straw fortified with each analyte at ~1 mg/kg.

Conclusion

Under freezer conditions -24±5°C, flufenacet and 5 of its metabolites (FOE-oxalate, FOE sulfonic acid, FOE thioglycolate sulfoxide, FOE methylsulfoxide, and FOE methylsulfone) were found to be stable for at least 21 months in wheat forage, grain and straw. No significant degradation was observed for any of the analytes after 21 months. After 21 months of storage, recoveries of flufenacet-related residues (calculated as percent of measured time-zero residue) ranged from 84 to 120% for wheat forage, grain, and straw fortified with each analyte at ~1 mg/kg.

Table 6.1-2: Storage stability of [^{14}C]flufenacet in wheat commodities

Sample material	Spike level (mg/kg)	Storage interval (months)	Recoveries in extract (mg/kg)	Mean (mg/kg)	% apparent stored recovery[1]
Forage	1.02	0	0.93; 0.96; 0.94	0.94	--
	1.02	6	0.92; 0.96;	0.94	100
	1.02	21	0.99; 0.89	0.94	100
Grain	1.02	0	0.89; 0.88; 0.86	0.88	--
	1.02	6	0.83; 0.86	0.84	95
	1.02	21	0.74; 0.74	0.74	84
Straw	1.02	0	0.98; 0.94; 0.93	0.95	--
	1.02	6	0.79; 0.75	0.77	81
	1.02	21	0.86; 0.83	0.84	88

[1] % Apparent stored recovery = (Recovered residue after storage/Recovered residue at time-zero) x 100. Values calculated using the average recovered residue at each storage interval. No concurrent recoveries were determined for the stored samples. Therefore, no corrections were made based on concurrent recovery values.

Table 6.1-3: Storage stability of [^{14}C] FOE thioglycolate sulfoxide (FAMSOC) in wheat commodities

Sample material	Spike level	Storage interval	Recoveries in	Mean	% apparent

	(mg/kg)	(months)	extract (mg/kg)	(mg/kg)	stored recovery[1]
Forage	1.01	0	1.16; 1.19; 1.29	1.21	--
	1.01	6	1.24; 1.20	1.22	101
	1.01	21	1.05; 1.25	1.15	95
Grain	1.01	0	1.02; 0.90; 0.94	0.95	--
	1.01	6	0.84; 0.83	0.84	88
	1.01	21	1.17; 1.10	1.14	120
Straw	1.01	0	1.00; 1.04; 0.98	1.01	--
	1.01	6	0.77; 0.72	0.74	73
	1.01	21	1.17; 1.12	1.14	113

[1] % Apparent stored recovery = (Recovered residue after storage/Recovered residue at time-zero) x 100. Values calculated using the average recovered residue at each storage interval. No concurrent recoveries were determined for the stored samples. Therefore, no corrections were made based on concurrent recovery values.

Table 6.1-4: Storage stability of [^{14}C] FOE oxalate in wheat commodities

Sample material	Spike level (mg/kg)	Storage interval (months)	Recoveries in extract (mg/kg)	Mean (mg/kg)	% apparent stored recovery[1]
Forage	0.99	0	1.01; 0.96; 0.91	0.96	--
	0.99	6	0.99; 1.00	1.00	104
	0.99	21	0.96; 0.95	0.96	100
Grain	0.99	0	0.88; 0.86; 0.80	0.85	--
	0.99	6	0.68; 0.77	0.72	85
	0.99	21	0.72; 0.74	0.73	86
Straw	0.99	0	0.93; 0.87; 0.95	0.92	--
	0.99	6	0.71; 0.69	0.70	76
	0.99	21	0.88; 0.88	0.88	96

[1] % Apparent stored recovery = (Recovered residue after storage/Recovered residue at time-zero) x 100. Values calculated using the average recovered residue at each storage interval. No concurrent recoveries were determined for the stored samples. Therefore, no corrections were made based on concurrent recovery values.

Table 6.1-5: Storage stability of [^{14}C] FOE methylsulfoxide (FAMSO) in wheat commodities

Sample material	Spike level (mg/kg)	Storage interval (months)	Recoveries in extract (mg/kg)	Mean (mg/kg)	% apparent stored recovery[1]
Forage	1.10	0	1.12; 1.11; 1.10	1.11	--
	1.10	6	1.06; 0.99	1.02	92
	1.10	21	1.16; 1.06	1.11	100
Grain	1.10	0	1.06; 0.99;1.01	1.02	--
	1.10	6	0.85; 0.93	0.89	87
	1.10	21	0.95; 0.93	0.94	92
Straw	1.10	0	1.02; 0.97; 1.06	1.02	--
	1.10	6	0.82; 0.77	0.80	78
	1.10	21	0.99; 1.02	1.0	98

[1] % Apparent stored recovery = (Recovered residue after storage/Recovered residue at time-zero) x 100. Values calculated using the average recovered residue at each storage interval. No concurrent recoveries were determined for the stored samples. Therefore, no corrections were made based on concurrent recovery values.

Table 6.1-6: Storage stability of [^{14}C] FOE sulfonic acid (FASO3H) in wheat commodities

Sample material	Spike level (mg/kg)	Storage interval (months)	Recoveries in extract (mg/kg)	Mean (mg/kg)	% apparent stored recovery[1]
Forage	1.02	0	1.06; 1.07; 1.08	1.07	
	1.02	6	1.00; 1.06	1.03	96
	1.02	21	1.16; 1.14	1.15	107
Grain	1.02	0	0.90; 0.89; 0.89	0.89	
	1.02	6	0.85; 0.89	0.87	98
	1.02	21	0.94; 0.89	0.92	103
Straw	1.02	0	0.92; 0.89; 0.94	0.92	--
	1.02	6	0.79; 0.83	0.81	88
	1.02	21	1.04; 1.03	1.04	113

[1] % Apparent stored recovery = (Recovered residue after storage/Recovered residue at time-zero) x 100. Values calculated using the average recovered residue at each storage interval. No concurrent recoveries were determined for the stored samples. Therefore, no corrections were made based on concurrent recovery values.

Table 6.1-7: Storage stability of [^{14}C] FOE methylsulfone (FAMSO2) in wheat commodities

Sample material	Spike level (mg/kg)	Storage interval (months)	Recoveries in extract (mg/kg)	Mean (mg/kg)	% apparent stored recovery[1]
Forage	1.02	0	0.98; 1.03; 1.04	1.02	--
	1.02	6	0.90; 0.98	0.94	92
	1.02	21	0.88; 0.93	0.90	88
Grain	1.02	0	0.93; 0.96; 0.97	0.95	--
	1.02	6	0.75; 0.82	0.78	82
	1.02	21	0.81; 0.82	0.82	86
Straw	1.02	0	0.96; 0.97; 1.02	0.98	--
	1.02	6	0.85; 0.90	0.88	90
	1.02	21	0.88; 0.86	0.88	90

[1] % Apparent stored recovery = (Recovered residue after storage/Recovered residue at time-zero) x 100. Values calculated using the average recovered residue at each storage interval. No concurrent recoveries were determined for the stored samples. Therefore, no corrections were made based on concurrent recovery values.

Report:	**KCA 6.1/02, [redacted],; [redacted]; 2013; M-439517-02**
Title:	Amendment no. 1 to report no: P642100741 - Storage stability of flufenacet and metabolites in/on orange fruit and dry bean seeds for 24 months
Report No. & Document No.:	MR-10/006, dated October 08, 2012 ; amended 2013-11-05 M-439517-02-1
Guidelines:	– 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 (reference to document no. 7032/VI/95 rev.5 Appendix H) – US EPA Residue Chemistry Test Guideline OCSPP 860.1380 Storage Stability Data – OECD Test Guideline 506, adopted 16 October 2007 – PMRA Ref.: DACO 7.3, Storage Stability
GLP:	Yes; Deviations: none

Material and Methods

To determine the freezer storage stability of flufenacet (FOE 5043) and its metabolites in plant materials, individual 5-g control samples of orange fruit (high acid content) and bean seed (high protein content) were spiked with parent flufenacet or a 1/1/1 mixture of its metabolites FOE oxalate hydrate, FOE thioglycolate sulfoxide, FOE sulfonic acid separately, resulting in a fortification level of either 0.10 mg/kg of flufenacet or the metabolite mixture. All fortification levels are expressed as parent equivalents. Except for the day-0 analysis, samples were stored in amber glass bottles in a deep-freezer at -18°C or below for later use. For day-0 analysis, five spiked samples of each sample material and two blank control samples were analysed. In addition, two concurrent recoveries spiked at the respective LOQ level were performed. Further samples were also analysed after nominal storage intervals of 1 (only dry bean seed), 2 (only orange fruit), 6, 12 and 24 months (both commodities). At each of these intervals, three treated samples and three control samples of each material were removed from storage and analysed. Two control samples were fortified for the determination of concurrent recoveries. Samples used for concurrent recoveries were fortified freshly on the day of analysis at the same magnitude as the spiked storage samples.

The total residue of flufenacet (flufenacet and its metabolites containing the N-fluorophenyl-N-isopropyl amine moiety) in/on matrices of plant origin was analytically determined as 4-fluoro-N-isopropylaniline using analytical method 01100 by LC-MS/MS ([redacted], P.; 2010; M-362575-02). The LOQ is 0.01 mg/kg expressed as flufenacet equivalents.

Findings

Data on procedural recoveries are summarized in Tables 6.1-8 and 6.1-9. Storage stability data for flufenacet and the metabolite mix are summarized in Table 6.1-10 to 6.1-13.
Mean procedural recoveries analysed alongside with the stored samples were within the range of 81-119% for both matrices for the parent compound (overall at 0.1 mg/kg 87-97 % per matrix). For the metabolite mix, procedural recoveries ranged from 61- 99 % for both sample materials and all storage intervals (overall mean 77-85 %). RSDs were always below 20%. Residues in the control samples were below 30% of the LOQ for each storage interval and both matrices.

After a deep-freezer storage period of about 24 months, the mean recovery rate for flufenacet from the stored samples of orange fruit was 98 % (111 % normalized to day 0). In samples of dry bean seed the mean recovery was 87 % (99 % normalized to day 0). After the longest storage period of 24 months, recoveries fortified with the metabolite mix (FOE oxalate hydrate, FOE thioglycolate sulfoxide, FOE sulfonic acid) were 68 % (94% normalized to day 0) and 71% (108% normalized to day 0) in orange fruit and dry bean seed, respectively. Recoveries of the metabolite mix were generally lower for both, the stored samples and the freshly fortified samples, compared to the parent compound. However, normalized to the recoveries at day 0 it is evident that the lower values do not indicate any degradation.

Conclusion

The study results demonstrate stability of flufenacet and the representative metabolites FOE oxalate, FOE thioglycolate sulfoxide and FOE sulfonic acid containing the N-fluorophenyl-N-isopropyl amine moiety for at least 24 months in frozen storage at ≤ -18 °C in the tested plant commodities (dry bean seed, orange fruit) representing the commodity groups of high protein content and high acid content.

Table 6.1-8: Concurrent Recoveries for Flufenacet (FOE 5043)

Sample Material	Nominal Storage Interval [d]	Concurrent Recoveries [%]			
		0.01 mg/kg fort. level		0.10 mg/kg fort. level	
		Single Values	Mean	Single Values	Mean
Orange fruit	0	118, 71	95	-	-
	60	-	-	91, 88	90
	180	-	-	85, 94	90
	360	-	-	85, 90	88
	720	-	-	81, 80	81
Overall mean, RSD		Overall mean = 95		Overall mean = 87, RSD = 5.6	
Dry bean seed	0	106, 101	104	-	-
	30	-	-	105, 132	119
	180	-	-	106, 101	104
	360	-	-	87, 80	84
	720	-	-	90, 76	83
Overall mean, RSD		Overall mean = 104		Overall mean = 97, RSD = 18.5	

determined as 4-fluoro-N-isopropylaniline, calculated and expressed as flufenacet

Table 6.1-9: Concurrent Recoveries for Analyte Mixture of FOE oxalate hydrate, FOE thioglycolate sulfoxide, FOE sulfonic acid

Sample Material	Nominal Storage Interval [d]	Concurrent Recoveries [%]			
		0.01 mg/kg fort. level		0.10 mg/kg fort. level	
		Single Values	Mean	Single Values	Mean
Orange fruit	0	72, 70	71	-	-
	60	-	-	76, 74	75
	180	-	-	94, 98	96
	360	-	-	72, 78	75
	720	-	-	59, 63	61
Overall mean, RSD		Overall mean = 71		Overall mean = 77, RSD = 17.7	
Dry bean seed	0	69, 73	71	-	-
	30	-	-	86, 85	86
	180	-	-	95, 103	99
	360	-	-	73, 93	83
	720	-	-	66, 78	72
Overall mean, RSD		Overall mean = 71		Overall mean = 85, RSD = 14.4	

determined as 4-fluoro-N-isopropylaniline, calculated and expressed as flufenacet

Table 6.1-10: Storage stability data for Flufenacet (FOE 5043) in orange fruit

Commodity	Nominal Storage Period (days)	Residue Level in Stored Samples			Day-0 Normalized Recovery[a]	Average % of Fresh Concurrent Recoveries	Average Corrected % Recovery[b]
		mg/kg (ppm)	% of nominal spiking level	Average % recovery			
Orange Fruit	**Flufenacet (FOE 5043)**						
	0	0.087 0.090 0.094 0.090 0.079	87 90 94 90 79	88	100	95	93
	60	0.096 0.091 0.070	96 91 70	86	98	90	96
	180	0.104 0.102 0.098	104 102 98	101	115	90	112
	360	0.081 0.094 0.099	81 94 99	91	103	88	103
	720	0.089 0.101 0.103	89 101 103	98	111	81	121

determined as 4-fluoro-N-isopropylaniline, calculated and expressed as flufenacet

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

[b] Corrected percent recovery = (Average % recovery (stored) / Average of fresh concurrent recoveries) X 100%

Table6.1-11: Storage stability data for analyte mixture of FOE oxalate hydrate, FOE thioglycolate sulfoxide, FOE sulfonic acid in orange fruit

Commodity	Nominal Storage Period (days)	Residue Level in Stored Samples			Day-0 Normalized Recovery[a]	Average % of Fresh Concurrent Recoveries	Average Corrected % Recovery[b]
		mg/kg (ppm)	% of nominal spiking level	Average % recovery			
Orange Fruit	**Metabolite mix: FOE oxalate hydrate, FOE thioglycolate sulfoxide, FOE sulfonic acid**						
	0	0.074 0.067 0.074 0.070 0.073	74 67 74 70 73	72	100	71	101
	60	0.077 0.075 0.075	77 75 75	76	106	75	101
	180	0.082 0.089 0.086	82 89 86	86	120	96	90
	360	0.070 0.068 0.068	70 68 68	69	96	75	92
	720	0.066 0.073 0.065	66 73 65	68	94	61	111

determined as 4-fluoro-N-isopropylaniline, calculated and expressed as flufenacet

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

[b] Corrected percent recovery = (Average % recovery (stored) / Average of fresh concurrent recoveries) X 100%

Table 6.1-12: Storage stability data for Flufenacet (FOE 5043) in dry bean seed

Commodity	Nominal Storage Period (days)	Residue Level in Stored Samples			Day-0 Normalized Recovery[a]	Average % of Fresh Concurrent Recoveries	Average Corrected % Recovery[b]
		mg/kg (ppm)	% of nominal spiking level	Average % recovery			
Dry bean seed	**Flufenacet (FOE 5043)**						
	0	0.071 0.097 0.082 0.091 0.098	71 97 82 91 98	88	100	104	85
	30	0.109 0.094 0.109	109 94 109	104	119	119	87
	180	0.094 0.101 0.107	94 101 107	101	115	104	97
	360	0.073 0.084 0.080	73 84 80	79	90	84	94
	720	0.087 0.100 0.074	87 100 74	87	99	83	105

determined as 4-fluoro-N-isopropylaniline, calculated and expressed as flufenacet

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

[b] Corrected percent recovery = (Average % recovery (stored) / Average of fresh concurrent recoveries) X 100%

Table6.1-13 Storage stability data for analyte mixture of FOE oxalate hydrate, FOE thioglycolate sulfoxide, FOE sulfonic acid in dry bean seed

Commodity	Nominal Storage Period (days)	Residue Level in Stored Samples			Day-0 Normalized Recovery[a]	Average % of Fresh Concurrent Recoveries	Average Corrected % Recovery[b]
		mg/kg (ppm)	% of nominal spiking level	Average % recovery			
Dry bean seed	**Metabolite mix: FOE oxalate hydrate, FOE thioglycolate sulfoxide, FOE sulfonic acid**						
	0	0.075 0.067 0.073 0.056 0.059	75 67 73 56 59	66	100	71	93
	30	0.078 0.065 0.078	78 65 78	74	112	86	86
	180	0.094 0.095 0.098	94 95 98	96	145	99	97
	360	0.069 0.073 0.070	69 73 70	71	108	83	86
	720	0.070 0.064 0.079	70 64 79	71	108	72	99

determined as 4-fluoro-N-isopropylaniline, calculated and expressed as flufenacet

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

[b] Corrected percent recovery = (Average % recovery (stored) / Average of fresh concurrent recoveries) X 100%

In 3 trials from residue studies 12-2001 and 12-2002 from 2012 for some field samples the requested storage temperature of -18°C was exceeded due to problems during shipment. In order to address this deviation a short term storage stability study was conducted. The storage conditions tested were such that the most unfavorable conditions which were determined for all shipments are covered.

Table 6.1-14: Deviations in conditions of storage temperature for field samples

Study number	Trial number	Maximum temperature reached	Total duration above -18°C	Average temperature above -18°C
12-2001	12-2001-01	-10°C	08 h, 10 m	-14.8°C
12-2002	12-2002-03	1°C	4 d 04 h 15 m	-13.9°C
12-2002	12-2002-03	-0.5°C	3 d 17 h 15 m	-11.6°C
12-2002	12-2002-04	-5.6°C	6 d, 15 h, 00 m	-15.8°C

d = day, h = hour, m = minutes

Report:	**KCA 6.1/03, [redacted],; [redacted], 2013; M-467724-02-1**
Title:	7 days freezer storage stability study of flufenacet (FOE5043) and its metabolites in tomato and wheat grain
Report No. & Document No.:	S13-02753, dated 2013-10-08, amended 2013-11-19 M-467724-02-1
Guidelines:	– 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 – PMRA Ref.: DACO 7.3, Storage Stability
GLP:	Yes, deviations: none

Material and Methods

The objective of the study was to evaluate the stability of flufenacet (FOE 5043) and its metabolites after storage for a period of 4 hours at +1°C following 7 days at -10°C in tomato and wheat grain as representatives for two different commodity groups.

Individual aliquots of plant material from tomato and wheat grain were fortified with 1.0 mg/kg of a mixture of flufenacet (FOE 5043) and its metabolites FOE oxalate hydrate, FOE sulfonic acid (as Na salt) and FOE thioglycolate sulfoxide (3/1/1/1). The samples were stored in plastic containers at an average temperature of +1°C for 4 hours and at -10°C for the following 7 days and were analysed at the nominal storage intervals of 0 and 7 days.

On day 0, for each matrix, six samples were prepared with 5 g of specimen material. Then, five containers were fortified with a mixture of Flufenacet (FOE 5043) and its metabolites FOE oxalate hydrate, FOE sulfonic acid (as Na salt) and FOE thioglycolate sulfoxide (3/1/1/1) at 1.0 mg/kg and one was used without fortification as a control specimen. The samples were analysed directly.

For analysis at day 7, for each matrix, eight samples were prepared with 5 g of specimen material. Five containers were fortified with a mixture of Flufenacet (FOE 5043) and its metabolites FOE oxalate hydrate, FOE sulfonic acid (as Na salt) and FOE thioglycolate sulfoxide (3/1/1/1) at 1.0 mg/kg. Three containers were stored without fortification to be used as control material and procedural recoveries. The storage containers were placed in a freezer at +1(±0.5)°C immediately after

the fortification. After 4 hours the storage containers were placed in a freezer at -10°C for seven days. The temperature of the freezers was continually recorded with a data recorder.

The five freshly fortified tomato and wheat grain specimen fortified at 1.0 mg/kg on day 0 also served as method validation recoveries. Two concurrent recoveries were conducted at 1.0 mg/kg in tomato and wheat grain, at 7 days of storage.

The total residue of flufenacet (flufenacet and its metabolites containing the N-fluorophenyl-N-isopropyl amine moiety) in/on matrices of plant origin was analytically determined as 4-fluoro-N-isopropylaniline using analytical Method 01100/M002 ([redacted], S.; [redacted], L.; 2013; M-448503-01-1).

Samples were extracted under acidic and oxidative conditions. After steam distillation of the common moiety 4-fluoro-N-isopropylaniline, samples were analysed with high performance liquid chromatography and tandem mass spectrometry (HPLC-MS/MS) using an internal standard for quantification.

Findings

The recoveries in the freshly fortified samples proved the method performance. Mean recoveries for the amount of total residues of flufenacet ranged between 71% and 84%. Two mass transitions were monitored and provided comparable results.
In addition, 2 concurrent recoveries per commodity were conducted at the nominal storage intervals of 7 days. Recoveries were at 70% and 85% for tomato and wheat grain, respectively. Validation and procedural recoveries are summarised in Table 6.1-15.
In the control samples of tomato and wheat grain, total residues of flufenacet were below the LOQ (0.01 mg/kg).

The recoveries of the stored samples showed that the total residue of flufenacet, determined as 4-fluoro-N-isopropylaniline, is stable in plant matrices (tomato and wheat grain) for at least 4 hours at +1°C followed by 7 days at -10°C. After 7 days of storage, recoveries were 71% for tomato and 82% for wheat grain (normalised to day 0: 99% and 98% for tomato and wheat grain, respectively). Table 6.1-16 summarises the total residues of flufenacet in tomato and wheat grain stored spiked samples, as well as the corresponding mean concurrent recovery data.

Conclusion

The findings from short-term storage stability study demonstrate that the temperature deviations during shipment did not result in a negative impact on the quality of the residue studies concerned.
The storage conditions tested were such that the most unfavorable conditions which were determined for all shipments are covered. Residues of flufenacet proved to be stable under the experimental conditions tested.

Table 6.1-15: Procedural recovery data for the total residue of flufenacet

Plant Material	Fortification Level [mg/kg]	Date of Extraction	Storage Interval (days)	Flufenacet (FOE5043) and metabolites Single Recoveries [%]					Mean [%]	RSD [%]	Standard Deviation [%]
Tomato	1.0	2013-07-04	0	74	68	72	72	71	71	3.1	2.2
	1.0	2013-07-11	7	79	61	-	-		70	-	-
	Overall Mean, RSD and standard deviation [%]								71	7.8	5.5
Wheat Grain	1.0	2013-07-04	0	80	75	100	85	79	84	12	9.7
	1.0	2013-07-11	7	82	87	-	-		85	-	-
	Overall Mean, RSD and standard deviation [%]								84	9.6	8.1

RSD: relative standard deviation

Table 6.1-16: Storage stability data for flufenacet in tomato and wheat grain

Commodity	Storage Period (days)	Residue Level in Stored Spiked Samples			Day-0 Normalized Recovery[a]	Average % of Fresh Concurrent Recoveries	Average Corrected % Recovery[b]
		mg/kg (ppm)	% of nominal spiking level	Average % recovery			
Tomato	0	0.740	74	71	100	NA	NA
		0.680	68				
		0.720	72				
		0.720	72				
		0.710	71				
	7	0.700	70	71	99	70	101
		0.710	71				
		0.700	70				
		0.730	73				
		0.700	70				
Wheat Grain	0	0.800	80	84	100	NA	NA
		0.750	75				
		1.000	100				
		0.850	85				
		0.790	79				
	7	0.890	89	82	98	85	97
		0.730	75				
		0.770	77				
		0.880	88				
		0.830	83				

determined as 4-fluoro-N-isopropylaniline, calculated and expressed as flufenacet

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

[b]Corrected percent recovery = (Average % recovery (stored spiked sample) / Average of fresh concurrent recoveries) X 100%

NA = Not applicable

Animal Matrices

Since feeding studies using non-radio labelled active substance were not triggered according to the EU data requirements (DAR/Monograph and EFSA 2012) the need to evaluate storage stability data for animal commodities did not arise.

Nevertheless information on storage stability for FOE-oxalate which forms a predominant plant metabolite and other flufenacet metabolites containing the fluorophenyl-isopropyl moiety can be obtained from the goat metabolism studies.

In the livestock metabolism studies with the goat using both [Fluorophenyl-UL-14C]flufenacet and [Fluorophenyl-UL-14C]flufenacet oxalate, storage stability data were generated at ≤ -24°C for periods of 6-21.5 months for flufenacet derived residues relevant to the residue definition (metabolites containing the fluorophenyl-isopropyl moiety) (Table 6.1-17). Samples from the cow feeding study ([redacted], F. K.; 1995) were stored concurrently with the samples from the [14C]FOE oxalate goat metabolism study in the same freezer and under the same storage conditions.

Reanalysis of the goat matrices showed that FOE oxalate was stable in goat tissues and milk for 18 to 21 months; hence, FOE oxalate was assumed to be stable in cow tissues and milk from the feeding study which were analyzed within 6 months after collection as well.

Table 6.1-17: Summary of stability data achieved at ≤ -18°C (unless stated otherwise)

Matrix	Characteristics of the matrix	Acceptable maximum storage duration, months	Reference
Data relied on in EU			
Animal products (Goat) (investigated in livestock metabolism studies)*			
flufenacet and other metabolites containing the fluorophenyl-isopropyl moiety			
Fat		8	Report of ECCO 73; Annex 2; LoEP, 1999 [redacted], R. G.; [redacted], P. L.; 1995; M-002250-01
Liver		6	
Muscle		8	
Milk		8.5	
Kidney		8.5	
FOE oxalate			
Fat		20	Report of ECCO 73; Annex 2; LoEP, 1999 [redacted], F. K.; [redacted], P. L.; [redacted], C. M.; [redacted], R. G.; 1995; M-004478-01-1
Liver		21.5	
Muscle		21.5	
Milk		21.6	
Kidney		18	

Additionally, an EFSA Reasoned Opinion on existing MRLs of Flufenacet confirmed the conclusions drawn in the EU review process: "

On the basis of the animal metabolism studies it is concluded that, after exposure to the maximum dietary burden (about 200 times lower than the dose level in the metabolism studies [ie. 5 mg/kg bw/d];...), residue levels in livestock commodities are expected to remain below the enforcement LOQ of 0.01 mg/kg in milk, 0.02 mg/kg in liver and 0.05 mg/kg in fat, eggs, kidney and muscle. Hence, no

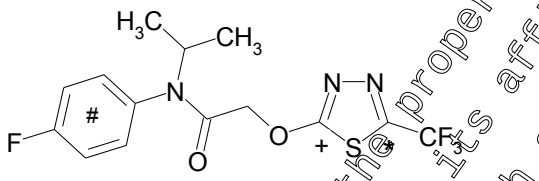
livestock feeding study is needed; MRLs and risk assessment values for the relevant commodities in ruminants, pigs and poultry can be established at the LOQ level."(EFSA Journal 2012:10(4):2689, p.29/30).

It is therefore concluded that there is still no need to investigate the storage stability of flufenacet residues in animal commodities.

CA 6.2 Metabolism, distribution and expression of residues

CA 6.2.1 Plants

Flufenacet was ^{14}C-labelled at three different positions of the molecule for investigation of metabolism studies in plants and animals:



fluorophenyl-UL-^{14}C
\+ thiadiazole-2-^{14}C
* thiadiazole-5-^{14}C

Most of the plant metabolism studies were conducted with [fluorophenyl-UL-^{14}C]flufenacet. These studies included maize/corn, soybeans and cotton (all pre-planting treatment) as well as the rotated crops kale, turnip and wheat with different plant back intervals. For soybeans (pre-planting treatment) and the rotated crops the [thiadiazole-2-^{14}C] label was used additionally. These studies were submitted with the dossier for Annex I listing of flufenacet according to EU directive 91/414/EEC and reported in the Tier 2 summary for the active substance, under Annex IIA, Point 6.1 (1996). As a consequence, they were already evaluated during the Annex I listing process and considered appropriate to describe the metabolism in plants.

A summary of the results of these studies is given in the original dossier for Annex I application (Section 6.10). The initial metabolic reaction is cleavage of the molecule into the thiadone and acetamide moiety. While the resulting thiadone (M09) itself was not observed, various conjugates were formed, the most important being the corresponding N-glucoside (M25). In soybeans, the malonylalanine conjugate (M34) predominated.

The fluorophenyl-acetamide portion is directly conjugated with glutathione (GSH) or homoglutathione (hGSH) and further metabolized yielding the transient FOE cysteine conjugate (M23). All subsequent metabolites can be considered as hydrolysis, oxidation and conjugation products of the glutathione pathway. However, the FOE oxalate (M01) most likely arose through direct oxidation of the transient hydrolysis product of flufenacet, the primary alcohol (FOE alc, M03).

Residue definition for food of plant origin

From these studies a conclusion on the residue definition in food of plant origin was made: "The metabolism of the flufenacet results in a number of metabolites, which all have the common moiety N-

isopropyl-4-fluorophenyl. Although no parent compound was found in any study and only three metabolites were of quantitative significance (M01: FOE oxalate; M02: FOE sulfonic acid, M04: FOE thioglycolate sulfoxide) a **"total residue" approach is proposed, based on the total amount of N-fluorophenyl-N-isopropyl derived residues**." (Monograph on FOE 5043 (flufenacet), Annex B.6, Section B.6.3.

Additional plant metabolism studies were conducted later which were not included in the original Annex II dossier and thus not evaluated by a peer review on EU level. These are studies of [fluorophenyl-UL-^{14}C]flufenacet on potato (pre-planting and post-emerging treatment) and on wheat and maize (both post-emerging treatment). The studies were submitted and evaluated in different EU Member States in support of uses in potatoes and maize. They will now also be reported in this summary.

To complete the knowledge on the metabolic pathway in plants additional metabolism studies were conducted on wheat (post-emerging treatment), potato (pre-emerging treatment) and on the rotated crops turnip, Swiss chard and wheat. All of these later studies were conducted with flufenacet radiolabeled in the [thiadiazole-5-^{14}C] position. Finally, [thiadiazole-5-^{14}C]flufenacet was also used in a supporting metabolism study in the rat.

An overview of all plant metabolism studies of radiolabeled flufenacet and the different positions of the ^{14}C-label is compiled in Table 6.2.1-1.

Table 6.2.1- 1: Overview of all plant metabolism studies with ^{14}C-labeled flufenacet in primary crops

Study type	Crop	Application scenario	Label	Report	Submission	
					EU baseline dossier Annex II , Section 4, Point 6	Reported in supplementary dossier Section 6.2
Plant metabolism	Corn (maize)	Pre-emergence application	[Fluorophenyl-UL-^{14}C]	[redacted]; 1994; M-002270-01-1	KCA 6.2.1/01	-
	Soybean	Pre-emergence application	[Fluorophenyl-UL-^{14}C] [Thiadiazole-2-^{14}C]	[redacted] Z; [redacted]; 1995; M-002278-01-1	KCA 6.2.1/02	-
	Cotton	Pre-emergence application	[Fluorophenyl-UL-^{14}C]	[redacted] T; [redacted]; 1995; M-002277-01-1	KCA 6.2.1/03	-
	Plant cell suspension cultures	--	[Fluorophenyl-UL-^{14}C] [Thiadiazole-2-^{14}C]	[redacted]; 1995; M-002366-01-1	KCA 6.2.1/04	--
	Potato	Pre- and post-emergence application	[Fluorophenyl-UL-^{14}C	[redacted] E. C.; [redacted] S. I.; 2000; M-020428-01-1		KCA 6.2.1/07
	Wheat	Post emergence application	[Fluorophenyl-UL-^{14}C	[redacted] S; [redacted]; 1997; M-002275-01		KCA 6.2.1/05
	Corn (maize)	Post emergence application	[Fluorophenyl-UL-^{14}C	[redacted] Z; [redacted]; 1998; M-005755-01		KCA 6.2.1/06
	Wheat	Post emergence application	[Thiadiazole-5-^{14}C]	[redacted]; 2013; M-444475-01-1		KCA 6.2.1/09
	Potato	Pre-emergence application	[Thiadiazole-5-^{14}C]	[redacted]; 2012; M-441506-02-1		KCA 6.2.1/08

Additional plant metabolism studies with [fluorophenyl-UL-^{14}C]flufenacet

The following additional plant metabolism studies were conducted on potato, wheat and corn for registration in USA applying a higher application rate than used in Europe. These studies were not included in the original dossier submitted for Annex I inclusion. However, in the meantime they supported also registrations in these crops in European Member States at lower application rates and were evaluated on national level and for the review of existing MRLs according to Article 12 of Regulation (EC) 396/2005. They are now added to complete the picture on the metabolism of flufenacet in plants and to confirm common basic metabolic transformations.

Potato

Report:	**KCA 6.2.1/07, [redacted], 2000, M-020428-01** **also filed KCA 4.1.2**
Title:	The Metabolism of [fluorophenyl-UL-^{14}C]Flufenacet in Potatoes
Document No:	M-020428-01-1
Report No:	109226, dated 2000-04-28
Guidelines:	US-EPA OPPTS 860.1300, Nature of Residues - Plants
GLP	Yes; deviations: none

Executive Summary

The metabolism of [fluorophenyl-UL-^{14}C]flufenacet was investigated in potatoes following two techniques of application: pre-emergent soil treatment at a use rate of 2.30 lb. ai/acre (2.58 kg as/ha) and post-emergent foliar treatment at a rate of 2.69 lb. ai/acre (3.01 kg as/ha). At harvest, mature tubers contained total radioactive residues (TRR) at a level of 0.35 mg equ/kg (soil treatment) or 0.32 mg equ/kg (foliar treatment). The tubers were homogenized under liquid nitrogen and extracted with methanol at room temperature and refluxed with methanol. The release of residues was completed by hydrolysis of the matrix with hydrochloric acid at room temperature. The extracted residues were separated by reversed phase HPLC and identified by LC-MS/MS and co-elution with authentic reference standards.

A total of 16 metabolites were detected in the tubers grown after soil treatment and a total of 13 metabolites in the tubers grown after foliar treatment. 63% of TRR was identified in the tubers after soil treatment and even 80% of TRR in the tubers after foliar treatment. Two major metabolites were identified in both trials. Most prominent was FOE cysteine (FACS, M23) amounting to 44% of TRR after soil treatment and to 52% of TRR after foliar treatment. The second major metabolite was identified as FOE sulfanyl lactic acid glucoside (FAMSL-Glu, M41) amounting to 19% of TRR after soil treatment and to 17% of TRR after foliar treatment. Two minor metabolites were tentatively identified in the tubers after foliar treatment, i.e. FOE thioglycolate sulfoxide (FAMSOC, M4) amounting to 7% of TRR and FOE sulfonic acid (FASO3H, M2) amounting to 4% of TRR. A lot of minor unknown metabolites were also detected in both trials, all of them at a level of < 10% of TRR.

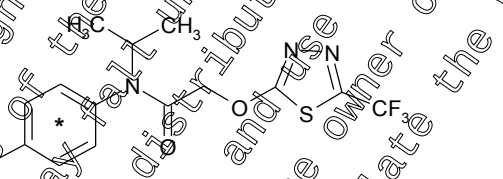
The non-extractable residues accounted for 4% of TRR in both trials. The parent substance flufenacet was not observed in the tubers of any treatment.

From the pattern of metabolites observed the initial step of flufenacet metabolism in potato tubers is assumed to be a glutathionate conjugation of the acetamide moiety of the molecule. The transient glutathionate degraded to FOE cysteine being the main residue component in potatoes. Subsequent metabolic steps are hydrolysis and oxidation of FOE cysteine followed by conjugation with glucose forming minor metabolites. The same metabolic pathway was also observed in soybean, corn and wheat, also conducted with [fluorophenyl-UL-^{14}C]flufenacet. A metabolism study with [thiadiazole-5-^{14}C] labeled flufenacet completed the metabolic pathway in potato (see below). The proposed metabolic pathway of flufenacet in potato tubers is shown in Figure 6.2.1-1.

The extraction of flufenacet residues according to the residue analytical method via oxidative hydrolysis and determination of the common moiety "4-fluorophenyl-N-isopropy-amine" was complete when compared to the total amount of identified residue components in this metabolism study.

Material and Methods

Test Material

Structural formula	 * denotes the ^{14}C label
Chemical name	N-(4-Fluorophenyl)-N-isopropyl-2-(5-trifluoromethyl-[1,3,4]thiadiazol-2-yloxy)-acetamide (IUPAC); Acetamide, N-(4-fluorophenyl)-N-(1-methylethyl)-2-[[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]oxy]- (9Cl; CAS)
Common name	flufenacet
CAS RN	142459-58-3
Empirical formula	$C_{13}H_{13}F_4N_3O_2S$
Company code	FOE 5043
Molar mass (non-labelled)	363.34 g/mol
Label	[fluorophenyl-UL-^{14}C]flufenacet
Specific radioactivity	47.9 mCi/mmol (0.132 mCi/mg, 4.878 MBq/mg)
Radiochemical purity	>99% (radio-HPLC)

Test Plants

Test plant	Potato (*Solanum tuberosum*)
Variety	Kennebec
Growth stage at application	Two parallel trials: (1) Pre-emergent soil treatment at the same day as planting of the potatoes (2) Post-emergent foliar treatment approx. 4 weeks after emergence
Harvested commodities	Tubers, immature (only soil treatment) and mature

(1) Pre-emergent treatment:

Planting of seed potatoes, preparation and application of the test mixture

The radiolabelled test substance (dissolved in acetonitrile) was mixed the same amount of stable labelled [isopropyl-1,3-^{13}C]flufenacet and with a blank formulation resulting in a 60WP formulation. The solvent was removed by rotary evaporation and water was added to yield the application mixture. An aliquot was taken for radio-HPLC analysis. The application rate corresponded to 2.30 lb. ai/acre (2.58 kg ai/ha).

Sandy loam (68.8% sand, 18.4% silt, 12.8% clay, 1.12% organic matter, pH 6.4) was filled in five 5-gal plastic buckets (approx. 19 L). Four seed potatoes were placed at a depth of 3 inches (7.5 cm) in each of the buckets. The upper 1 inch (2.5 cm) soil layer was removed and mixed with the application mixture in a tumbling mixer. The treated soil was returned to the buckets with the seed potatoes as top soil layer.

The potatoes were grown initially in a greenhouse. Following emergence (approx. 2 weeks after planting) they were thinned to one or two shoots per bucket. Further cultivation happened outdoors in a fenced patio in Stilwell, Kansas, USA, during spring and summer 1999 until harvest approx. 3.5 months after planting and soil treatment.

(2) Post-emergent treatment:

Planting of seed potatoes, preparation and application of the test mixture

Four seed potatoes were planted in 5-gal plastic buckets with sandy soil as done for the pre-emergent treatment. The potatoes were first grown in a greenhouse, thinned after emergence and further cultivated under outdoor conditions in Stilwell, during spring and summer 1999 until harvest approx. 3.5 months after planting.

The radiolabelled test substance was mixed the ^{13}C-labelled test substance, with WP60 formulation blank and with water as done for the pre-emergent treatment. The spray mixture was evenly sprayed to the leaf surface and the surrounding soil approx. 4 weeks after emergence using a hand-held plastic pump sprayer. An aliquot of the spray mixture was analysed by radio-HPLC. The application rate corresponded to 2.69 lb. ai/acre (3.01 kg ai/ha).

Harvest and processing

Mature potatoes (109 day after pre-emergent and 67 days after post-emergent treatment) were dug out from the soil. The vines were cut away. The tubers were gently rinsed with water to remove soil, combined from all buckets of the same treatment type, cut into pieces and homogenized in liquid nitrogen using a high speed mixer. Following evaporation of the liquid nitrogen in a freezer the pulverized tubers were radioassayed. Aliquots of the tuber samples were used for initial extraction (6 days after harvest). The remaining samples were stored in a freezer at approx. -20°C.

The homogenized tubers were extracted three times with methanol at room temperature, followed by 4-hours refluxing with methanol and hydrolyzed with 1 N hydrochloric acid at ambient temperature for 8 hours. The acid hydrolysate was adjusted to pH 6 and extracted with chloroform. All liquid phases were radioassayed. The final solids were first air-dried and radioassayed via combustion.

To examine for potential glucoside conjugates, a major radioactive residue component was isolated by preparative HPLC, evaporated to dryness and re-dissolved in a sodium phosphate buffer solution. This solution was incubated with ß-glucosidase (37°C, 24 hours), then concentrated to dryness, re-dissolved in acidic methanol (0.1% acetic acid)/water (4/1) and analyzed by radio-HPLC.

Radioassaying and analysis

Radioassaying (measurement of the radioactivity) was conducted by liquid scintillation counting (LSC). Quenching was automatically compensated using an external standard. Solid samples were firstly combusted and the formed $^{14}CO_2$ absorbed in an alkaline scintillation liquid. The limit of quantification (LOQ) was set to twice the background radioactivity for radioassaying of solid samples. Given the aliquot amount of combustion and the specific radioactivity used in this study the LOQ for radioassaying was 0.0005 mg parent equivalents/kg (0.0005 mg equ/kg).

Radio-HPLC was conducted on a RP8 column (250 x 10 mm, 5 µm particle size) operated with a gradient mixture of water and methanol (both containing 0.1% acetic acid). The HPLC system was equipped with a radiomonitor with a glass scintillator. The linearity of the radiomonitor response was examined by injection of various amounts of radioactivity. The limit of detection was derived from detector-response curve and the specific radioactivity of the test substance amounting to 0.002 – 0.007 µg of the test substance.

LC-MS/MS analyses were performed with a combination of a TSQ mass spectrometer connected to a HPLC system with a RP8 column (250 x 4.6mm, 5 µm particle size) and a radiomonitor. A gradient mixture of aqueous ammonium acetate or formic acid and methanol served as mobile phase. The MS system was operated in the negative ion electrospray ionization mode.

Findings

Total radioactive residues and their extractability in potato tubers

The total radioactive residues (TRR) amounted to 1.77 mg equ/kg in immature tubers 40 days after soil (pre-emergence) treatment. In mature tubers, TRR amounted to 0.35 mg equ/kg 109 days after soil treatment at a rate of 2.58 kg as/ha and to 0.32 mg equ/kg 67 days after foliar treatment at a rate of 3.01 kg as/ha.

The extractable portions of TRR using the different techniques are shown in Table 6.2.1- 2. Most the residues could already be released by conventional extraction with methanol at ambient temperature accounting for 76 – 79% of TRR. Refluxing with methanol and hydrolysis with hydrochloric acid at room temperature almost completed the release of residues leaving only a small portion of non-extractable residues (4% of TRR, 0.01 mg equ/kg).

Residues in potato tubers following pre- and post-emergence treatment with flufenacet

The composition of the radioactive residues in mature potato tubers following pre- and post-emergence soil and foliar treatment is presented in Table 6.2.1- 3. A total of 16 components were extracted from the tubers after soil treatment and a total of 13 components after foliar treatment. The parent substance was not observed in the tuber either after soil of after foliar treatment.

Two metabolites revealed to be the main residue components, i.e. P2, FOE cysteine (FACS, M23) amounting to 44% of TRR (0.16 mg equ/kg) after soil and to 52% of TRR (0.17 mg equ/kg) after foliar treatment and P1, FOE sulfanyl lactic acid glucoside (FAMSL-Glu, M23) amounting to 19% of TRR (0.07 mg equ/kg) after soil and to 17% of TRR (0.05 mg equ/kg) after foliar treatment. These metabolites were isolated by radio-HPLC and identified by LC-MS making use of additional ^{13}C-labelling. The glucoside conjugation of FOE sulfanyl lactic acid was confirmed by enzymatic splitting off of glucose with glucosidase.

After foliar treatment, two additional metabolites could be identified in the tubers, i.e. FOE thioglycolate-sulfoxide (FAMSOC, M4) amounting to 7% of TRR (0.02 mg equ/kg) and FOE-sulfonic acid (FASO3H, M2) amounting to 4% of TRR (0.01 mg equ/kg). These metabolites were tentatively identified by co-chromatography with authentic references standards.

From these residue components the following metabolic transformation reactions were concluded: The primary transformation was a glutathionate conjugation of the fluorophenyl-isopropyl-acetamide moiety of flufenacet followed by hydrolytic release of alanine and glutamic acid to form FOE cysteine. Subsequent metabolic reactions were hydrolysis forming transient FOE sulfanyl lactic acid and FOE thioglycolate sulfoxide and oxidation of the sulfur to FOE sulfonic acid. FOE sulfanyl lactic acid was finally conjugated as glucoside. The proposed metabolic pathways are presented in Figure 6.2.1- 1.

Extraction efficiency of the residue analytical method[1]

The extraction efficiency of the analytical method (accountability of residue method) was examined using potato tubers with incurred residues from the pre-emergent and post-emergent application of radiolabelled flufenacet. TRR levels of tubers used for this test amounted to 0.37 or 0.34 mg equ/kg

[redacted] (1995). An analytical method for the determination of FOE 5043 residues in plant matrices, report 106406 of Bayer Corp., Stilwell, KS, USA, Comp. No. M-041601-01-1; now replaced by the current version (2013) without derivatization and direct HPLC-MS/MS determination of the common moiety, Comp. No. M-448503-01-1.

after pre- or post-emergent application. These levels were slightly higher (approx. 6%) than the initial levels, probably due to desiccation during freezer storage.

Following oxidation, hydrolysis and steam distillation of the residues in tubers from post-emergent application the distillate contained a radioactivity level of 0.28 mg equ/kg. 0.26 mg equ/kg partitioned into dichloromethane and 0.24 mg equ/kg was quantified as the derivatized analytical target N-4-fluorophenyl-N-isopropyl-trifluoroacetamide. Compared to the total extractability with methanol determined in the metabolism experiment (0.25 mg equ/kg, Table 6.2.1- 3) this figure represented an extraction efficiency of 96%.

The distillate from tubers grown in pre-emergent treated soil contained 0.31 mg equ/kg, and 0.28 mg equ/kg partitioned into dichloromethane. 0.26 mg equ/kg was quantified as the derivatized analytical target N-4-fluorophenyl-N-isopropyl-trifluoroacetamide. Compared to the total extractability with methanol determined in the metabolism experiment (0.23 mg equ/kg, Table 6.2.1- 3) this figure represented an extraction efficiency of 113%.

Therefore, it is concluded that the extraction efficiency of the analytical method from potato tubers is excellent when compared with the amount of all identified residue components detected in this metabolism study.

Storage stability of flufenacet residues in potato tubers

The initial extraction of the tubers following both soil and foliar treatment was performed within 6 days after sample collection. Re-extraction was performed on frozen samples 169 days after harvest. The major metabolites in both trials, FOE cysteine (FACS) and FOE sulfanyl lactic acid glucoside (FAMSL-Glu), were found to be stable upon storage. Also, the minor metabolites in tubers following foliar treatment were stable upon storage. Therefore, the stability of flufenacet residues in potato tubers was shown for a storage period of approx. 6 months at approx. -20°C.

Conclusion

The metabolism of [fluorophenyl-UL-^{14}C]flufenacet was investigated in potatoes following pre-emergent soil treatment at a use rate of 2.30 lb. ai/acre (2.58 kg as/ha) and following post-emergent foliar treatment at a rate of 2.69 lb. ai/acre (3.01 kg as/ha). At harvest, mature tubers contained total radioactive residues (TRR) at a level of 0.35 mg equ/kg (soil treatment) or 0.32 mg equ/kg (foliar treatment).

A total of 16 metabolites were detected in the tubers grown after soil treatment and a total of 13 metabolites in the tubers grown after foliar treatment. Two major metabolites were identified in both trials. Most prominent was FOE cysteine (FACS, M23) amounting to 44% of TRR after soil treatment and to 52% of TRR after foliar treatment. The second major metabolite was identified as FOE sulfanyl lactic acid glucoside (FAMSL-Glu, M41) amounting to 19% of TRR after soil treatment and to 17% of TRR after foliar treatment. Two minor metabolites were detected in the tubers after foliar treatment, i.e. FOE thioglycolate sulfoxide (FAMSOC, M4) amounting to 7% of TRR and FOE sulfonic acid (FASO3H, M2) amounting to 4% of TRR. The parent substance flufenacet was not observed in the tubers of any treatment.

From the pattern of metabolites observed the initial step of flufenacet metabolism in potato tubers is assumed to be a glutathionate conjugation of the acetamide moiety of the molecule. The transient glutathionate degraded to FOE cysteine being the main residue component in potatoes. Subsequent metabolic steps are hydrolysis and oxidation of FOE cysteine followed by conjugation with glucose forming minor metabolites. The same metabolic pathway was also observed in soybean[2], corn[3,4] and wheat[5]. All of these metabolism studies were conducted with [fluorophenyl-UL-^{14}C]flufenacet. A metabolism study with [thiadiazole-5-^{14}C] labeled flufenacet completed the metabolic pathway in potato[6]. The proposed metabolic pathway of flufenacet in potato tubers is shown in Figure 6.2.1-1.

The extraction of flufenacet residues according to the residue analytical method via oxidative hydrolysis and determination of the common moiety "4-fluorophenyl-N-isopropy-amine" was complete when compared to the total amount of identified residue components in this metabolism study.

Table 6.2.1- 2: Extractability of radioactive residues from mature potato tubers treated with [fluorophenyl-UL-^{14}C]flufenacet

Treatment type	**Soil treatment, pre-emergent**		**Foliar treatment, post-emergent**	
Application rate [kg as/ha]	2.58		3.01	
Days after treatment	109		67	
TRR [mg equ/kg]	0.35		0.32	
Extraction with	[% of TRR]	[mg equ/kg]	[% of TRR]	[mg equ/kg]
Methanol, room temperature	79	0.28	76	0.24
Methanol, refluxing	7	0.02	8	0.03
1N HCl, room temperature	10	0.04	12	0.04
- Partition into chloroform	1	< 0.01	-	-
- Partition into water	10	0.04	-	-
Non extractable (solids)	4	0.01	4	0.01
Total	100	0.035	100	0.32

[2] [redacted], M. E. and [redacted], L. L. (1995): The metabolism of FOE 5043 in soybeans, Bayer AG, Div. Report No. MR105187, Comp. No. M-002278-01-1

[3] [redacted], J. H. (1994): The metabolism of [fluorophenyl-UL-^{14}C]FOE 5043 in corn, Bayer AG, Div. Report No. MR105027, Comp. No. M-002270-01-1

[4] [redacted], M. E. and [redacted], L. L. (1998): The metabolism of [fluorophenyl-UL-^{14}C]FOE 5043 in corn after postemergent foliar application, Bayer AG, Div. Report No. 108497, Comp. No. M-005755-01

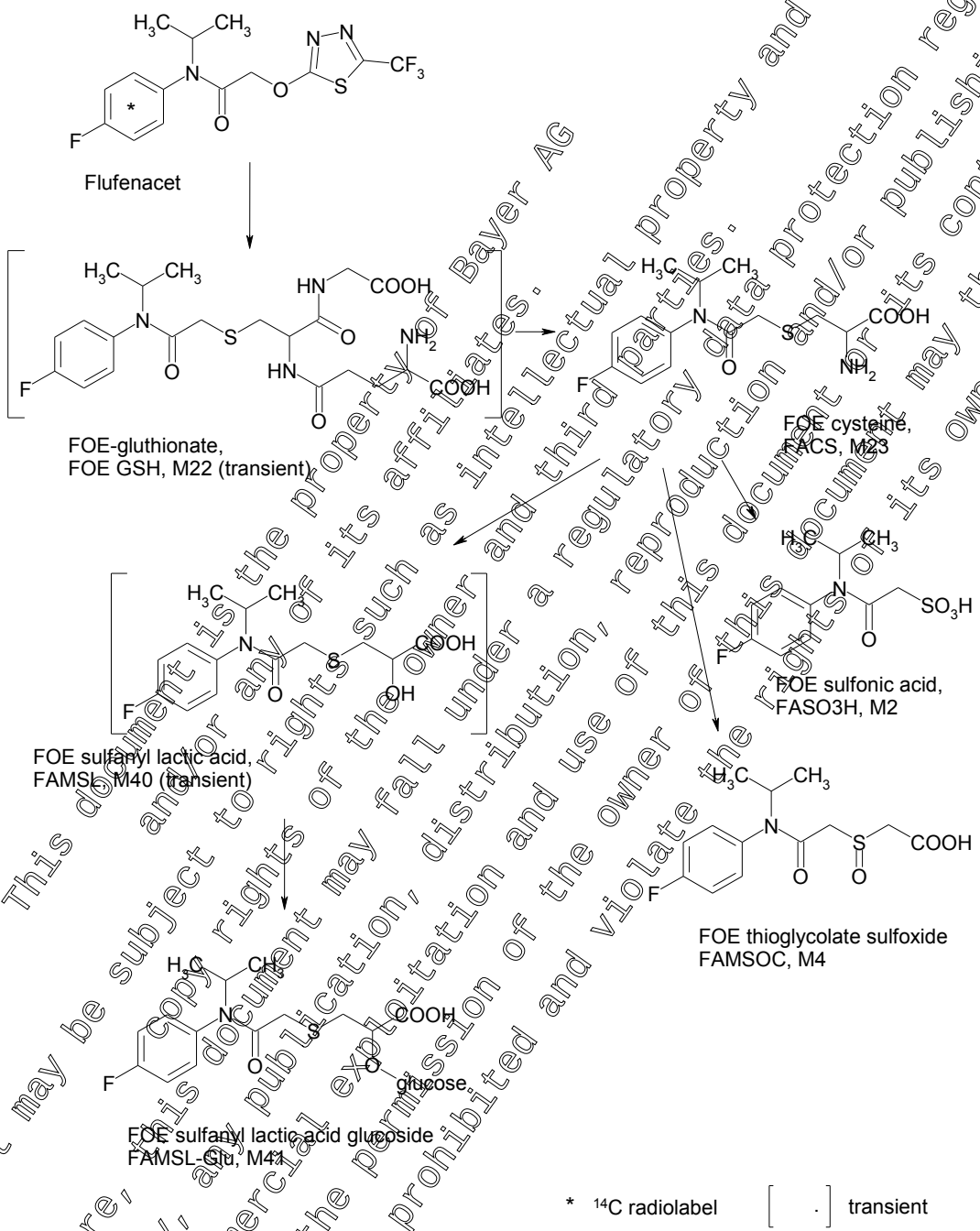
[5] [redacted], M. E. and [redacted], L. L. (1997): The metabolism of [fluorophenyl-UL-^{14}C]FOE 5043 in wheat after postemergent foliar spray application, Bayer AG Div Report 107399, Comp. No. M-002275-01-1

[6] [redacted], R. (2012): Metabolism of [thiadiazole-5-^{14}C]flufenacet in potatoes, report EnSa-12-0537 of Bayer CropScience, Comp. No. M-441506-02-1

Table 6.2.1- 3: Composition of residues in mature potato tubers treated with [fluorophenyl-UL-^{14}C]flufenacet (sum of the respective components in all extraction fractions)

Treatment type	**Soil treatment, pre-emergent**		**Foliar treatment, post-emergent**	
Application rate [kg as/ha]	2.58		3.01	
Days after treatment	109		67	
TRR [mg equ/kg]	0.35		0.32	
Metabolites	[% of TRR]	[mg equ/kg]	[% of TRR]	[mg equ/kg]
P1, FOE sulfanyl lactic acid glucoside (FAMSL-Glu, M41)	19	0.07	17	0.05
P2, FOE cysteine (FACS, M23)	44	0.16	52	0.17
P3 – P16, unknown	< 1 - 6	< 0.01 – 0.02	-	-
P17, FOE sulfonic acid (FASO3H, M2)	-	-	4	0.01
P19, FOE thioglycolate sulfoxide (FAMSOC, M4)	-	-	2	0.02
P18, P20 – P27, unknown	-	-	< 1 - 3	< 0.01 – 0.01
Total identified	63	0.23	80	0.25

Figure 6.2.1- 1: Proposed metabolic pathway of flufenacet in potato tubers after soil and foliar application



Wheat

Report:	**KCA 6.2.1/05, [redacted]; [redacted]; 1997; M-002275-01** **also filed KCA 4.1.2**
Title:	The Metabolism of [Fluorophenyl-UL-^{14}C]FOE 5043 in Wheat After Postemergent Foliar Application
Document No:	M-002275-01-1
Report No:	107399, dated 1997-11-04
Guidelines:	US-EPA OPPTS 860.1300, Nature of Residues - Plants
GLP	yes

Executive Summary

The metabolism of [fluorophenyl-UL-^{14}C]flufenacet was investigated in spring wheat following post-emergent foliar application to young shoots (4-tiller growth stage) at a use rate of 0.46 lb. ai/acre (0.52 kg as/ha). Agricultural commodities of wheat were collected as immature forage, immature hay, mature straw and grain. All commodity samples were homogenized under liquid nitrogen and aliquots were radioassayed by combustion and liquid scintillation counting (LSC). The total radioactive residues (TRR) amounted to 1.93; 3.50; 2.04 and 0.62 mg equ/kg in forage, hay, straw and grain. Extraction with methanol at ambient temperature and under reflux revealed a high extractability of the radioactive residues accounting for 92, 94, 86 and 80% of TRR for forage, hay, straw and grain. Following further acid and alkaline hydrolysis of the residues non-extractable from plant matrix were negligible (≤ 3 – 4% of TRR). The extracted residues were separated by reversed phase HPLC and identified by LC-MS/MS and co-elution with authentic reference standards.

The metabolism of flufenacet in wheat was extensive. While no parent substance was observed in any of the plant commodities 12 metabolites were detected in forage and straw, and 9 metabolites in hay and grain, respectively. FOE oxalate (FOEOX, M1) revealed to be a major metabolite in all commodities. It proved to be predominant in wheat grain amounting to 65% of TRR (corresponding to 0.40 mg equ/kg). Other metabolites in grain appeared at a very low level (≤ 2% of TRR). In forage, hay and straw two other major metabolites were identified as FOE sulfinyl lactic acid I (FAMSOL I, M33) and FOE sulfanyl lactic acid glucoside (FAMSL-Glu, M41). In straw, a further metabolite FOE sulfonic acid (FOESO3H, M2) amounted to 15% of TRR.

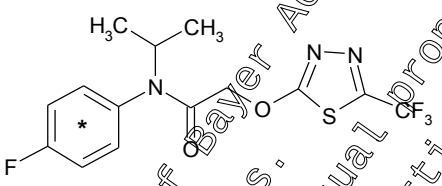
The main metabolite present in all commodities, i.e. FOE oxalate, most likely arose from oxidation of transient primary alcohol hydrolysis product. All other metabolites were formed by hydrolysis, oxidation and conjugation of a primary transient metabolite formed by initial conjugation with glutathione. From the pattern of metabolites observed in this study with [fluorophenyl-UL-^{14}C] labeled flufenacet a metabolic pathway of flufenacet in wheat is proposed in Figure 6.2.1- 2. The parent substance was not observed in any commodity of forage, hay, straw and grain. All major metabolites in these commodities contained the common moiety fluorophenyl-N-isopropyl amine.

Comparative extraction of the residues using methanol (this metabolism study) and determination of the residues using the residue analytical method (oxidative acid hydrolysis and quantification of the

hereby formed N-fluorophenyl-N-isopropyl amine) showed a good agreement of amount of residue compounds containing the common moiety.

Material and Methods

Test Material

Structural formula	 * denotes the ^{14}C label
Chemical name	N-(4-Fluorophenyl)-N-isopropyl-2-(5-trifluoromethyl-[1,3,4]thiadiazol-2-yloxy)-acetamide (IUPAC); Acetamide, N-(4-Fluorophenyl)-N-(1-methylethyl)-2-[[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]oxy]- (9CI, CAS)
Common name	Flufenacet
CAS RN	142459-58-3
Empirical formula	$C_{14}H_{13}F_4N_3O_2S$
Company code	FOE 5043
Molar mass (non-labelled)	363.34 g/mol
Label	[fluorophenyl-UL-^{14}C]Flufenacet
Specific radioactivity	47.9 mCi/mmol (0.132 mCi/mg, 4.878 MBq/mg)
Radiochemical purity	96% (radio-HPLC), 92% after formulation with slight degradation to FOE alcohol (FOEALC, M3, identified by HPLC-MS)

Test Plants

Test plant	Spring wheat (*Triticum vulgare*)
Origin	Farmers Union Cooperative, Spring Hill, Kansas, USA
Growth stage at application	4-tiller growth stage, 46 days after seed planting
Harvested commodities	Forage, hay, straw, grain

Planting of wheat grain, preparation and application of the test mixture

Loam soil (49.2% sand, 32.8% silt, 18.0% clay, 2.51% organic matter, pH 6.4) was filled in a trough with a surface area of 18.4 ft² (1.70 m²) and a depth of 14 inches (35 cm). Wheat seeds were placed in furrows on the soil surface, approx. 6 inches (15 cm) apart, at approx. 1-cm intervals. The furrows were finally covered with a 0.5 cm soil layer. The wheat was grown outdoors in spring and summer 1995 at the Bayer Research Park in Stilwell, Kansas, USA.

The radiolabelled test substance was mixed 60WP blank formulation and water resulting in the spraying mixture. This spraying mixture was evenly sprayed across the surface of the trough with the wheat plants in the 4-tillering stage (46 days after sowing) using a plastic pump sprayer. The actual application rate corresponded to 0.461 lb. ai/acre (0.52 kg as/ha).

Harvest, processing and extraction

The wheat plants were harvested at the following growth stages:

Forage:	at BBCH 26, 6-tillering growth stage, 64 days after sowing
Hay:	at BBCH 85, soft dough growth stage
Straw and grain:	at full maturity, 105 and 112 days after sowing

Plants were cut off at the soil surface level. They were cut into 1-inch pieces and homogenized under liquid nitrogen using a high-speed tissue mixer. The liquid nitrogen was allowed to evaporate in a freezer a < -10°C. Aliquots of the resulting tissue powder were radioassayed and the remainder stored in the freezer for later analysis.

In case of grain and straw sampling, ripe heads were first cut from the stalks using scissors. Then, the remaining plant (straw) was cut above the soil. The wheat heads were rubbed across a No. 10 soil sieve to remove the seeds. The sifted and winnowed (using a gentle nitrogen stream) grain was pulverized in a Warring blender. The straw was cut into pieces and homogenized under liquid nitrogen as done with forage and hay.

Homogenized forage was extracted with methanol (3x) at ambient temperature followed by refluxing with methanol. Aliquots of the methanol extracts were evaporated to dryness, re-dissolved in 0.1% acetic acid and analyzed by radio-HPLC. Each fraction was radioassayed.

Homogenized hay was extracted with methanol/water (3/1, 1x) and pure methanol (3x) at room temperature followed by refluxing with methanol. The methanol extracts were concentrated, and analyzed by radio-HPLC. The remaining solids were suspended successively in 1 N hydrochloric acid and in 2 N aqueous sodium hydroxide, both at ambient temperature. The aqueous phases were neutralized and partitioned against chloroform. The remaining solids were refluxed successively with 6 N aqueous hydrochloric acid and 6 N aqueous sodium hydroxide. All fractions/phases were radioassayed.

Homogenized straw and grain were extracted separately with methanol/water (4/1, 1x) following steeping at room temperature for half an hour. Extraction was continued with pure methanol (2x) at ambient temperature and under reflux, with hydrochloric acid and sodium hydroxide at room temperature and under reflux as done with hay. The aqueous phases were neutralized and partitioned against chloroform. Between acid/basic hydrolysis at room temperature and under reflux an additional extraction step with methanol/water (3/1) under ultrasonication was inserted. All fractions/phases were radioassayed.

Extraction efficiency of the residue analytical method[7]

[7] [redacted]. (1995). An analytical method for the determination of FOE 5043 residues in plant matrices, report 106406 of Bayer Corp., Stilwell, KS, USA, Comp. No. M-041601-01-1; now replaced by the current version (2013) without derivatization and direct HPLC-MS/MS determination of the common moiety, Comp. No. M-448503-01-1.

Samples of grain and straw were processed and analyzed according to the analytical residue method for flufenacet in plants; this is a common moiety method with analysis for split-off "N-fluorophenyl-N-isopropyl amine".

The sample was hydrolyzed and oxidized with sulfuric acid and potassium permanganate. Surplus permanganate was reduced by added sodium bisulfite. The hydrolysis was completed by addition of concentrated sulfuric acid and refluxing for 24 hours. The resulting mixture was cooled down, made strongly basic with sodium hydroxide and the formed N-fluorophenyl-N-isopropyl amine distilled off together with water (steam distillation). This amine was purified by partitioning with methylene chloride, derivatized with trifluoroacetic anhydride in pyridine. The final reaction mixture was radioassyed and analyzed by HPLC.

Radioassaying and analysis

Radioassaying (measurement of the radioactivity) was conducted by liquid scintillation counting (LSC). Quenching was automatically compensated using an external standard. Solid samples were firstly combusted and the formed $^{14}CO_2$ absorbed in an alkaline scintillation liquid. The limit of quantification (LOQ) was set to twice the background radioactivity for radioassaying of solid samples. Given the aliquot amount of combustion and the specific radioactivity used in this study the LOQ for radioassaying was 0.00077 mg parent equivalents/kg (0.00077 mg equ/kg) for liquid samples and 0.0011 mg equ/kg for solid samples.

Radio-HPLC was conducted on a RP8 or RP18 column (250 x 4.0 mm, 5 µm particle size) operated with a gradient mixture of water and methanol (both containing 0.1% acetic acid). The HPLC system was equipped with a radiomonitor with a glass scintillator. The linearity of the radiomonitor response was examined by injection of various amounts of radioactivity. The limit of detection was derived from detector response curve and the specific radioactivity of the test substance. It amounted to 0.0093 µg of the test substance.

Radio-TLC of the straw hydrolysis fraction was conducted on TLC plates (5 x 20 cm) coated with Silicagel 60 F_{254}. The plates were developed with tetrahydrofuran/methanol (9/1). Radioactive zones were detected using a radio-TLC-scanner.

LC-MS/MS analyses were performed with a combination of a mass spectrometer connected to a HPLC system. The MS system was operated in both the positive and negative ion electrospray ionization (ESI) mode.

Findings

Total radioactive residues and their extractability in wheat commodities

The total radioactive residues (TRR) amounted to 1.93 mg equ/kg in wheat forage 18 days post treatment, to 3.50 mg equ/kg in wheat hay 33 days post treatment, to 2.04 mg equ/kg in wheat straw 66 days post treatment and to 0.62 mg equ/kg in grain 59 - 66 days post treatment.

The extractable portions of TRR using the different techniques are shown in Table 6.2.1- 4 for wheat forage and hay and in Table 6.2.1- 5 for wheat straw and grain. Most the residues could already be released by conventional extraction with methanol at ambient temperature accounting for 64 (grain) – 92% (forage) of TRR. Refluxing with methanol released additional 4 - 16% of TRR resulting in a total of 80 (grain) – 96% (forage) of TRR. Sonication with methanol/water released an additional portion of 8% of TRR from wheat grain. Since most of the residues had already been released by the previous extraction steps succeeding acid and basic hydrolysis were not efficient. The portion of non-extractable residues finally was negligible amounting to 4% of TRR in forage (no acid or basic hydrolysis of the matrix performed), to <1% of TRR in hay, to 3% of TRR in straw and to 2% of TRR in grain samples.

Residues in wheat commodities originating from foliar application of ^{14}C-flufenacet

The composition of the radioactive residues in wheat forage and hay following foliar treatment of [fluoro-phenyl-UL-14C]flufenacet are summarized in Table 6.2.1- 6. The respective composition of residues in wheat straw and grain is shown in Table 6.2.1- 7. A total of 12 metabolites were detected in forage and straw and 9 metabolites in hay and grain. The metabolites were identified by comparison of their HPLC retention to those of authentic reference standards and by individual collection following HPLC separation and identification by HPLC-MS.

The chromatographic profiles of the methanol extracts of forage, hay and straw were very similar. Common major metabolites were identified as FOE oxalate, M1 (14 – 36% of TRR) and FOE sulfinyl lactic acid I, M33 (20 – 26% of TRR). At the earlier growth stages forage and hay two additional metabolites were observed at relevant amounts, i.e. FOE sulfanyl lactic acid glucoside, M41 (8 – 21% of TRR) and FOE sulfinyl lactic acid glucoside, M37 (6 – 10% of TRR), whereas at maturity FOE sulfonic acid, M2 (15% of TRR) was found in straw. Other metabolites appeared at a minor extent (<10% of TRR).

The grain extract comprised mainly of a single component (65% of TRR corresponding to 0.40 mg equ/kg) which was identified as FOE oxalate, M1. Other metabolites were quantified as very minor (≤ 2% of TRR).

The parent substance was not observed in any commodity of forage, hay, straw and grain. All major metabolites in these commodities contained the common moiety “fluorophenyl-N-isopropyl amine”. The proposed metabolic pathway of flufenacet in wheat is shown in Figure 6.2.1- 2.

Extraction efficiency of the residue analytical method

The extraction efficiency of the analytical method (accountability of residue method) was examined using grain and straw with incurred residues from the current wheat metabolism study. TRR levels of

grain and straw samples used for this test amounted to 0.55 and 1.96 mg equ/kg. These levels were slightly lower than the initial levels, probably due to hydration of the dried grain and straw during freezer storage.

Following oxidation, hydrolysis and steam distillation of formed common moiety N-fluorophenyl-N-isopropyl amine from wheat grain the distillate contained 97% of TRR in the original grain sample. 84% of TRR partitioned into the organic phase after addition of sodium hydroxide. Subsequent derivatisation revealed the analytical target N-4-fluorophenyl-N-isopropyl-trifluoroacetamide representing 81% of TRR in the original grain sample. Compared to the total extractability with methanol determined in the metabolism experiment (80% of TRR extractable at room temperature and under reflux conditions, with 66% of TRR identified as metabolites containing the common moiety, Table 6.2.1- 5) this figure represented a complete extraction of those residue components that contain the respective N-fluorophenyl-N-isopropyl amine moiety.

Applying the same method to a straw sample resulted in 86% of TRR in the distillate with 76% of TRR in the organic phase prior to derivatisation. The derivatized sample contained 70% of TRR in the original straw sample, which was identified as N-4-fluorophenyl-N-isopropyl-trifluoroacetamide. Compared to the total extractability with methanol determined in the metabolism experiment (86% of TRR extractable at room temperature and under reflux conditions, with 74% of TRR identified as metabolites containing the common moiety, Table 6.2.1- 5) this figure represented also a complete extraction of those residue components that contain the respective N-fluorophenyl-N-isopropyl amine moiety.

Storage stability of residues in the freezer

Initial extraction of all commodities was made one month after sample collection. All extractions and quantitative measurements were completed within 6 months of sample collection. Therefore, no additional storage stability data are required according to OECD Guideline 501 (2007) on "Metabolism in Crops" to support this study.

Conclusion

The metabolism of [fluorophenyl-UL-^{14}C]flufenacet was investigated in spring wheat following post-emergent foliar application to young shoots (4-tiller growth stage) at an use rate of 0.46 lb. ai/acre (0.52 kg as/ha). The following crop commodities were collected and analysed: immature forage, immature hay, mature straw and grain. The total radioactive residues (TRR) amounted to 1.93; 3.50; 2.04 and 0.62 mg equ/kg in forage, hay, straw and grain. Extraction with methanol at ambient temperature and under reflux revealed a high extractability of the radioactive residues accounting for 92, 94, 86 and 80% of TRR for forage, hay, straw and grain. Following additional acid and alkaline hydrolysis of the plant matrix the non-extractable residues were negligible (≤ 3 – 4% of TRR).

The metabolism of flufenacet was extensive in wheat. While no parent substance was observed in any of the plant commodities 12 metabolites were detected in forage and straw, and 9 metabolites in hay and grain, respectively. FOE oxalate (FOEOX, M1) revealed to be a major metabolite in all commodities. It proved to be predominant in wheat grain amounting to 65% of TRR (corresponding to 0.40 mg equ/kg). Other metabolites in grain appeared at a very low level (≤ 2% of TRR). In forage,

hay and straw two other major metabolites were identified as FOE sulfinyl lactic acid I (FAMSOL I, M33) and FOE sulfanyl lactic acid glucoside (FAMSL-Glu, M41). In straw, a further metabolite FOE sulfonic acid (FOESO3H, M2) amounted to 15% of TRR.

The main metabolite present in all commodities, i.e. FOE oxalate, most likely arose from oxidation of transient primary alcohol hydrolysis product. All other metabolites were formed by hydrolysis, oxidation and conjugation of a primary transient metabolite formed by initial conjugation with glutathione. A similar metabolic pathway of flufenacet was also found in soybeans, corn and cotton[8]. All of these metabolism studies were conducted with [fluorophenyl-UL-^{14}C]flufenacet. From the pattern of detected metabolites a metabolic pathway of flufenacet in wheat is proposed in Figure 6.2.1-2. A metabolism study with [thiadiazole-5-^{14}C] labelled flufenacet completed the metabolic pathway in wheat[9] (see below).

Comparative extraction of the residues using methanol (this metabolism study) and determination of the residues using the residue analytical method (oxidative acid hydrolysis and quantification of the hereby formed N-fluorophenyl-N-isopropyl amine) showed a good agreement of amount of residue compounds containing the common moiety.

[8] [redacted], M. E. and [redacted], L. L. (1995): The metabolism of FOE 5043 in cotton, Bayer AG Div. Report No. 106666, Comp. No. M-002277-01-1

[9] [redacted], R. and [redacted], D. (2013): Metabolism of [thiadiazole-5-14C]flufenacet in wheat, unpublished report EnSa-12-0536 of Bayer CropScience AG, Comp. No. M-444475-01-1

Table 6.2.1- 4: Extractability of radioactive residues from wheat forage and hay following foliar treatment with [fluorophenyl-UL-^{14}C]flufenacet at a use rate of 0.52 kg as/ha

Agricultural commodity	**Wheat forage**		**Wheat hay**	
Days after treatment	18		33	
TRR [mg equ/kg]	1.93		3.50	
Extraction with	[% of TRR]	[mg equ/kg]	[% of TRR]	[mg equ/kg]
Methanol, room temperature	92	1.78	88	3.01
Methanol, refluxing	4	0.08	6	0.21
1N HCl, room temperature				
- Partition into chloroform	-	-	<1	<0.01
- Partition into water	-	-	2	0.02
2 N NaOH, room temperature				
- Partition into chloroform	-	-	<1	<0.01
- Partition into water	-	-	<1	<0.01
Methanol/water sonication	-	-	1	0.01
6 N HCl, reflux	-	-	<1	<0.01
6 N NaOH, reflux	-	-	<1	<0.01
Non-extractable (solids)	4	0.08	1	<0.01
Total*	100	1.94	100	3.37

* slight differences from TRR determination measured by combustion due to rounding of subfractions

Table 6.2.1- 5: Extractability of radioactive residues from wheat straw and grain following foliar treatment with [fluorophenyl-UL-^{14}C]flufenacet at a use rate of 0.52 kg as/ha

Agricultural commodity	**Wheat straw**		**Wheat grain**	
Days after treatment	66		59 - 66	
TRR [mg equ/kg]	2.04		0.62	
Extraction with	[% of TRR]	[mg equ/kg]	[% of TRR]	[mg equ/kg]
Methanol, room temperature	76	1.51	64	0.36
Methanol, refluxing	10	0.20	16	0.09
1N HCl, room temperature				
- Partition into chloroform	<1	<0.01	<1	<0.01
- Partition into water	3	0.06	3	0.02
2 N NaOH, room temperature				
- Partition into chloroform	1	0.02	1	<0.01
- Partition into water	4	0.08	3	0.02
Methanol/water sonication	2	0.04	8	0.04
6 N HCl, reflux				
- Partition into chloroform	<1	<0.01	2	<0.01
- Partition into water	<1	<0.01	1	<0.01
6 N NaOH, reflux	<1	<0.01	<1	<0.01
Non-extractable (solids)	3	0.06	2	0.01
Total*	100	1.97	100	0.54

* slight differences from TRR determination measured by combustion due to rounding of subfractions

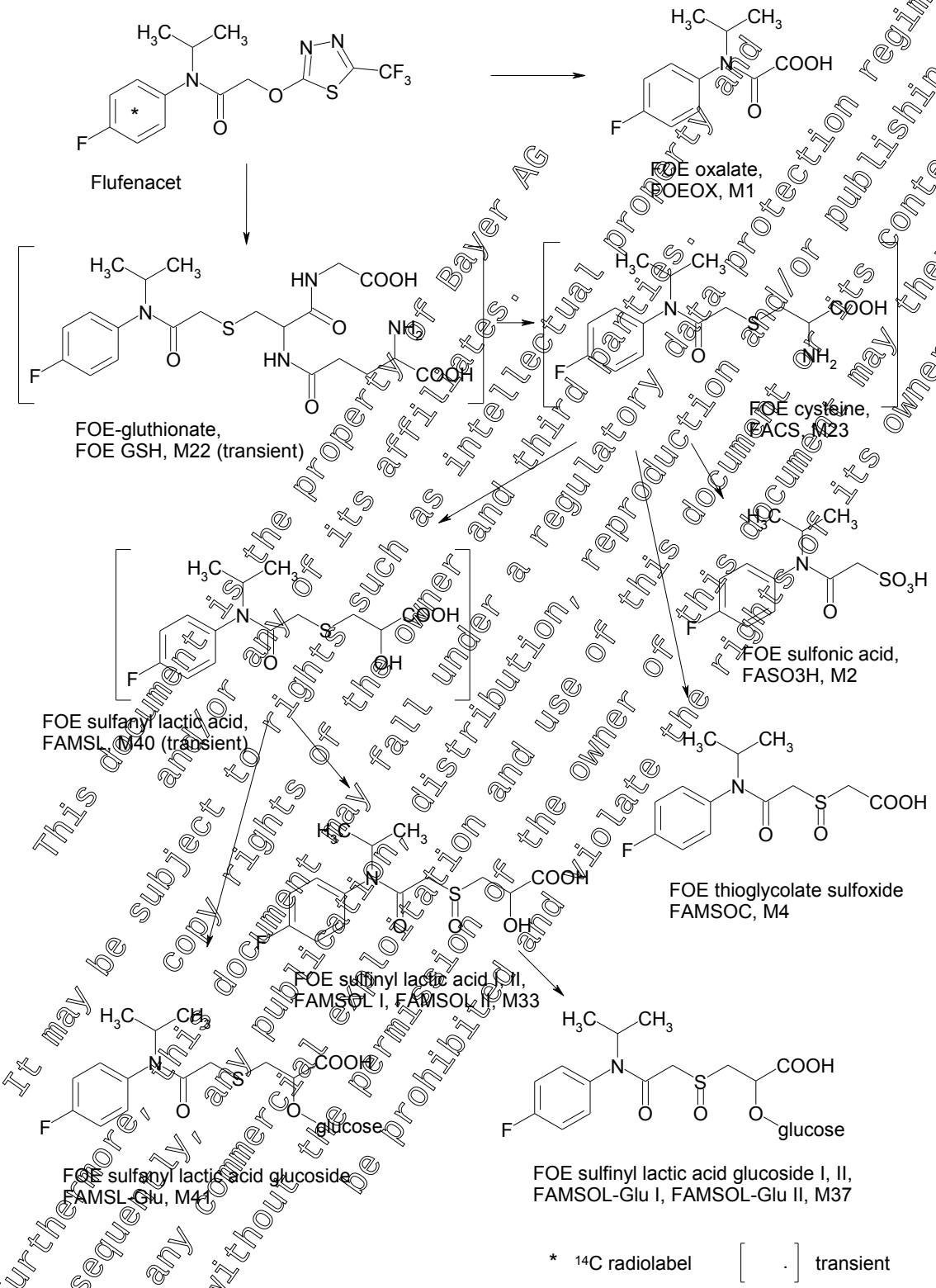
Table 6.2.1- 6: Composition of residues in wheat forage and hay treated with [fluorophenyl-UL-^{14}C]flufenacet at a use rate of 0.52 kg as/ha

Agricultural commodity	**Wheat forage**		**Wheat hay**	
Days after treatment	18		33	
TRR [mg equ/kg]	1.93		3.50	
Metabolites extracted with MeOH at RT and MeOH refluxing	[% of TRR]	[mg equ/kg]	[% of TRR]	[mg equ/kg]
Unknown 1	<1	<0.02	-	-
FOE oxalate (FOEOX, M1)	19	0.37	36	1.26
Unknown 2	-	-	<1	<0.04
FOE sulfinyl lactic acid glucoside I (FAMSOL-Glu I, M37)	6	0.12	10	35
FOE sulfinyl lactic acid glucoside II (FAMSOL-Glu II, M37)	6	0.12	5	0.18
FOE thioglycolate sulfoxide (FAMSOC, M4)	2	0.04	4	0.14
FOE sulfinyl lactic acid I (FAMSOL I, M33)	23	0.44	20	0.70
FOE sulfinyl lactic acid I I (FAMSOL II, M33)	7	0.14	4	0.14
Unknown 3	3	0.06	2	0.07
Unknown 4	2	0.04	-	-
FOE sulfanyl lactic acid glucoside (FAMSL-Glu, M41)	21	0.21	8	0.28
Unknown 5	<1	<0.02	-	-
Unknown 6	<1	<0.02	-	-
Total	89	1.74	89	3.12
Total identified	84	1.64	87	1.68

Table 6.2.1- 7: Composition of residues in wheat straw and grain treated with [fluorophenyl-UL-^{14}C]flufenacet at a use rate of 0.52 kg as/ha

Agricultural commodity	**Wheat straw**		**Wheat grain**	
Days after treatment	66		59 - 66	
TRR [mg equ/kg]	2.04		0.62	
Metabolites extracted with MeOH at RT and MeOH refluxing	[% of TRR]	[mg equ/kg]	[% of TRR]	[mg equ/kg]
Unknown 1	-	-	1	<0.01
FOE sulfonic acid (FASO3H, M2)	15	0.31	-	-
FOE oxalate (FOEOX, M1)	14	0.29	65	0.40
Unknown 2			2	<0.01
FOE sulfinyl lactic acid glucoside I (FAMSOL-Glu I, M37)	2	0.04	<1	<0.01
FOE sulfinyl lactic acid glucoside II (FAMSOL-Glu II, M37)	1	0.02	1	<0.01
FOE thioglycolate sulfoxide (FAMSOC, M4)	7	0.14	-	-
Unknown 3	1	0.02	<1	<0.01
FOE sulfinyl lactic acid I (FAMSOL I, M33)	26	0.53	-	-
FOE sulfinyl lactic acid I I (FAMSOL II, M33)	9	0.18	-	-
Unknown 4	1	0.02	-	<0.01
Unknown 5	-	-	<1	<0.01
FOE sulfanyl lactic acid glucoside (FAMSL-Glu, M41)	<1	0.02	-	-
Unknown 6	<1	<0.02	<1	<0.01
Unknown 7	2	0.04	<1	<0.01
Total	78	1.61	69	0.41
Total identified	74	1.53	66	0.40

Figure 6.2.1- 2: Proposed metabolic pathway of flufenacet in wheat following post-emergent foliar application



Corn

Report:	**KCA 6.2.1/06, [redacted]; 1998; M-005755-01**
Title:	~~The Metabolism of [Fluorophenyl-UL-^{14}C]FOE 5043 in Corn After Postemergent~~

	Foliar Application
Document No:	M-005755-01-1
Report No:	108497, dated 1998-09-23
Guidelines:	US-EPA OPPTS 860.1300, Nature of Residues - Plants
GLP	Yes; deviation: none

Executive Summary

The metabolism of [fluorophenyl-UL-^{14}C]flufenacet was investigated in corn/maize following post-emergent foliar application to young shoots (4 - 5 leaf growth stage) at an use rate of 1.30 lb. ai/acre (1.46 kg as/ha). Agricultural commodities of corn were collected as immature forage (82 day post treatment) and mature fodder and grain (129 days post treatment). All commodity samples were homogenized with dry ice and aliquots were radioassayed by combustion and liquid scintillation counting (LSC). The total radioactive residues (TRR) amounted to 0.62; 1.91 and 0.11 mg equ/kg in forage, fodder and grain. Extraction with methanol at ambient temperature released a very high portion of radioactive residues from animal feed commodities, i.e. forage accounting for 92% of TRR and fodder accounting for 82% of TRR. The extractability with methanol was lower from grain accounting for 47% of TRR at room temperature and additional 6% by refluxing. Relevant portions the residues in grain could be released by acidic hydrolysis of the matrix (11% by agitation with 1N HCl at room temperature and additional 14% with 6 N HCl under reflux). These residues proved to be mainly polar. The extracted residues were separated by reversed phase HPLC and identified by LC-MS/MS and co-elution with authentic reference standards.

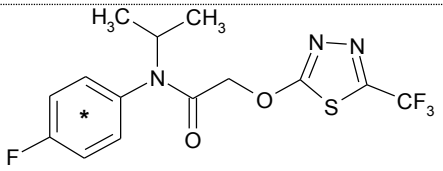
Flucenacet was extensively metabolized in corn. While no parent substance was observed in any of the plant commodities 7 metabolites were detected in forage, 10 metabolites in fodder and 6 metabolites in grain, respectively. FOE oxalate (FOEOX, M1) revealed to be a major metabolite in animal feed commodities forage and fodder, but was absent in grain. The main metabolite in grain was identified as FOE sulfinyl lactic acid glucoside (FAMSOL-Glu, M37) amounting to 23% of TRR (0.02 mg equ/kg). This metabolite was also major in fodder (18% of TRR), but minor in forage (<10% of TRR). Exclusively in forage, the conjugate FOE malonylcysteine (FAMS-MalCys, M42) was observed at a significant extent (25% of TRR). A lot of other metabolites were detected in grain, fodder and forage, all of them containing the common moiety N-fluorophenyl-N-isopropyl amine.

The forage and fodder metabolite, i.e. FOE oxalate, most likely arose from oxidation of transient primary alcohol hydrolysis product. All other metabolites were formed by hydrolysis, oxidation and conjugation of a primary transient metabolite formed by initial conjugation with glutathione. From the pattern of metabolites observed in this study with [fluorophenyl-UL-^{14}C] labelled flufenacet a metabolic pathway of flufenacet in corn is proposed in Figure 6.2.1- 3.

Material and Methods

Test Material

Structural formula	

	 * denotes the ^{14}C-label
Chemical name	N-(4-Fluorophenyl)-N-isopropyl-2-(5-trifluoromethyl-[1,3,4]thiadiazol-2-yloxy)-acetamide (IUPAC); Acetamide, N-(4-Fluorophenyl)-N-(1-methylethyl)-2-[[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]oxy]- (9CI, CAS)
Common name	Flufenacet
CAS RN	142459-58-3
Empirical formula	$C_{14}H_{13}F_4N_3O_2S$
Company code	FOE 5043
Molar mass (non-labelled)	363.34 g/mol
Label	[fluorophenyl-UL-^{14}C]Flufenacet
Specific radioactivity	Originally: 47.9 mCi/mmol (0.132 mCi/mg, 4.878 MBq/mg), Used in the study: 146000 dpm/µg (2.433 MBq/mg, 0.066 mCi/mg)
Radiochemical purity	100% (radio-HPLC)

Test Plants

Test plant	Corn (*Zea mays*)
Variety	Great Lakes 533
Origin	Bayer Research Farm at Howe, Illinois, USA
Growth stage at application	4 – 5 leaf stage, 14 days after planting
Harvested commodities	Immature forage, fodder and grain at maturity

Planting of corn, preparation and application of the test mixture

Loam soil (49.2% sand, 32.8% silt, 18.0% clay, 2.51% organic matter, pH 6.4) was filled into twelve 5-gal (approx. 19 L) plastic buckets with drainage holes to a depth of 12 inches (approx. 30 cm). Several corn seeds were sown into each bucket. Following emergence the corn shoots were twice thinned to finally one plant per bucket. The corn plants were first grown outdoors in summer 1994 on a patio at the Bayer Research Park in Stilwell, Kansas, USA, and then matured in a greenhouse.

The radiolabelled test substance dissolved in ethyl acetate was blended with the same amount of non-labelled test substance and mixed with 60WP formulation blank. Following thorough mixing the solvent was evaporated and water was added resulting in the spray mixture. This mixture was sonicated and then transferred to a hand-held plastic pump sprayer. The young corn plants at the 4- to 5-leaf stage were evenly sprayed with the spray mixture 14 days after planting. The actual application rate was 1.30 lb ai/acre (1.46 kg as/ha).

Harvest and processing

Agricultural commodities of corn plants were harvested at the two following growth stages:

Forage: at BBCH 85-86, 82 days post treatment at the late dough/early dent stage

Fodder and grain: at BBCH 97, 129 days post treatment at maturity

Immature plants were cut off few inches above the soil surface level. They were cut into 5-6-inch pieces and pulverized in a food processor in presence of dry ice. The dry ice was allowed to sublime off in a freezer (< -10°C).

In case of mature plants, the ears were first removed from the stalks and husks. Dry grains were manually removed from each cob, processed in a food processor with dry ice and subsequently pulverized more finally in a blender also with dry ice. The remaining husks and cobs were added to the stalks (representing the fodder) and homogenized in a food processor with dry ice. The dry ice was allowed to sublime at < -10°C. Aliquots of all homogenized samples were radioassayed. The remainder was stored under frozen condition until extraction and analysis.

Extraction of forage

Homogenized forage was extracted with methanol (3x) at ambient temperature followed by refluxing with methanol. Aliquots of the methanol extracts were evaporated to dryness, re-dissolved in aqueous 0.1% acetic acid and analyzed by radio-HPLC. The solids remaining after extraction were suspended successively in 1 N hydrochloric acid and in 2 N aqueous sodium hydroxide, both at ambient temperature. The aqueous phases were neutralized and partitioned against chloroform. The filtered solids were suspended in methanol/water (3/1) and sonicated for 2 hours at room temperature. Each fraction was radioassayed.

Extraction of fodder

Homogenized fodder was first steeped in methanol/water (4/1) and the extracted with the same solvent mixture followed by two extractions with pure methanol. The combined extract was concentrated and analyzed by radio-HPLC. The remaining solid was refluxed with methanol and the organic extract concentrated and analyzed by radio-HPLC.

The remaining solids were suspended successively hydrolyzed with 1 N hydrochloric acid and 2 N sodium hydroxide followed by sonication in methanol/water (3/1) as mentioned above. The remaining solids were refluxed successively with 6 N aqueous hydrochloric acid and 6 N aqueous sodium hydroxide. All fractions/phases were radioassayed.

Extraction of grain

Homogenized grain powder was first steeped in methanol/water (4/1) and the extracted with the same solvent mixture followed by two extractions with pure methanol as conducted with fodder. The combined extract was concentrated and analyzed by radio-HPLC. The remaining solid was refluxed with methanol and the organic extract concentrated and analyzed by radio-HPLC.

The remaining solids were extracted successively with hexane (1x) and acetonitrile (2x). Then, the solids were refluxed with methanol and hydrolyzed with 1 N hydrochloric acid and 2 N aqueous sodium hydroxide at ambient temperature. The aqueous phase of the acid hydrolysis was partitioned against chloroform. The remaining solids were then sonicated in methanol/water (3/1) and finally hydrolyzed with 6 N hydrochloric acid and 6 N sodium hydroxide under reflux. The aqueous hydrolyzates were partitioned against chloroform. All fractions/phases were radioassayed.

Radioassaying and analysis

Radioassaying (measurement of the radioactivity) was conducted by liquid scintillation counting (LSC). Quenching was automatically compensated using an external standard. Solid samples were firstly combusted and the formed $^{14}CO_2$ absorbed in an alkaline scintillation liquid. The limit of quantification (LOQ) was set to twice the background radioactivity for radioassaying of solid samples. Given the aliquot amount of combustion and the specific radioactivity used in this study the LOQ for radioassaying was 0.00031 mg parent equivalents/kg (0.00031 mg equ/kg) for liquid samples and 0.00042 mg equ/kg for solid samples.

Radio-HPLC was conducted on a RP8 or RP18 column (250 x 10 mm, 5 or 10 µm particle size) operated with a gradient mixture of water and methanol (both containing 0.1% acetic acid). The HPLC system was equipped with UV detector and a radiomonitor with a glass scintillator. The linearity of the radiomonitor response was examined by injection of various amounts of radioactivity. The limit of detection was derived from detector-response curve and the specific radioactivity of the test substance amounting to 0.0188 µg of the test substance. In addition, a straight phase HPLC system was used for purification of isolated metabolites operating with a normal phase column (250 x 10 mm, 5µm particle size) and a gradient of the solvents hexane and 0.2% acetic acid in IPA (isopropyl alcohol).

Radio-TLC of the fodder isolated metabolites was conducted on TLC plates (5 x 20 cm) coated with Silicagel 60 F_{254}. The plates were developed with tetrahydrofuran/methanol (9/1). Radioactive zones were detected using a radio-TLC-scanner.

LC-MS/MS analyses were performed with a combination of a mass spectrometer connected to a HPLC system. The MS system was operated in both the positive and negative ion electrospray ionization (ESI) mode.

Findings

Total radioactive residues and their extractability in corn commodities

The total radioactive residues (TRR) amounted to 0.62 mg equ/kg in corn forage 82 days post treatment, to 1.91 mg equ/kg in fodder and to 0.11 mg equ/kg in grain both harvested 129 days post treatment.

Residues extractable from corn forage and fodder are shown in Table 6.2.1- 8. Most the residues in forage and fodder could already be released by conventional extraction with methanol at ambient temperature accounting for 92% of TRR in forage and 82% of TRR in fodder. Refluxing with methanol released additional 2 - 6% of TRR resulting in a total of 94% (forage) and 88% (fodder) of TRR. [redacted] portions of residues were additionally released by acid and alkaline hydrolysis of the matrix and sonication with methanol and water. The non-extractable residues at the end of the extraction steps amounted to 1% of TRR

The extractable portion of TRR from corn grain is shown in Table 6.2.1- 9. Extraction with methanol released only 47% of TRR at room temperature and additional 6% by refluxing. Relevant portions of the radioactive residues could also be released by mild acidic hydrolysis of the matrix at room temperature (2% of TRR being unpolar and 9% being polar) and drastic acidic hydrolysis with 6 N

HCl under reflux (3% of TRR unpolar and 14% polar). [redacted] portions were released by alkaline hydrolysis. The non-extractable residues amounted to 5% of TRR.

Residues in corn commodities originating from foliar application of ^{14}C-flufenacet

The composition of the radioactive residues in corn forage and fodder following foliar treatment of [fluorophenyl-UL-14C]flufenacet is summarized in Table 6.2.1- 10. The respective composition of residues in corn grain is shown in Table 6.2.1- 11. A total of 7 metabolites were detected in forage, a total of 10 metabolites in corn fodder, and 6 metabolites in grain. The metabolites were identified by comparison of their HPLC characteristics to authentic reference standards and already identified metabolites from other plant commodities, and by individual collection following HPLC separation and identification by HPLC-MS.

The chromatographic profiles of the methanol extracts of forage and fodder were similar. Common major metabolites were identified as FOE-oxalate and M4 (22 - 27% of TRR) and FOE sulfinyl lactic acid I, M33 (16 - 19% of TRR). In corn forage, FOE sulfanyl lactic acid glucoside, M41 (25% of TRR) was observed as additional major metabolite, whereas FOE malonylcysteine, M42 (16% of TRR) was the analogue major metabolite in fodder. Corn fodder contained also FOE sulfinyl lactic acid glucoside, M37 (18% of TRR) as a major metabolite.

The grain extract comprised mainly of a single component (23% of TRR corresponding to 0.02 mg equ/kg) which was identified as FOE-sulfinyl lactic acid glucoside, M37 (two diasteromers, non-separated). Apart from FOE thioglycolate sulfoxide, M4 (9% of TRR) and FOE-methyl sulfoxide, M6 (7% of TRR), other metabolites were quantified as minor (≤ 4% of TRR).

The parent substance was not observed in any commodity forage, fodder and grain. All of the major metabolites in these commodities contained the common moiety "fluorophenyl-N-isopropyl amine". The proposed metabolic pathway of flufenacet in corn is shown in Figure 6.2.1- 3.

Storage stability of residues in the freezer

Initial extraction and analyses of plant samples were performed within 10 days after sample collection. Some samples were stored frozen for up to 13 months to repeat analysis. In addition, the storage stability of flufenacet residues at -26±5°C was shown in a separate report using corn, soybean and turnip with incurred residues for at least 20 or 28 months[10].

[10] [redacted], L. L. (1995): The storage stability of FOE 5043 and metabolites in corn, soybean, and turnip raw agricultural commodities, unpublished report 106971 of Bayer Corp., Stilwell, Kansas, USA, Comp. No. M-002426-01-1.

Conclusion

The metabolism of [fluorophenyl-UL-^{14}C]flufenacet was investigated in corn following post-emergent foliar application to young shoots (4-5 leaf growth stage) at an use rate of 1.30 lb ai/acre (1.46 kg as/ha). The following crop commodities were collected and analysed: immature forage, mature fodder (stalks, husk and cobs) and grain. The total radioactive residues (TRR) amounted to 0.62; 1.91 and 0.11 mg equ/kg in forage, fodder and grain. Extraction with methanol at ambient temperature revealed a high extractability of the radioactive residues from forage and fodder accounting for 92% of TRR (forage) and 82% of TRR (fodder). The extractability with methanol from grain was lower accounting for 47% of TRR at room temperature and additional 6% by refluxing. Relevant portions the residues in grain could be released by acidic hydrolysis of the matrix (11% by agitation with 1N HCl at room temperature and additional 14% with 6 N HCl under reflux). These residues proved to be mainly polar.

Flufenacet was extensively metabolized in corn. While no parent substance was observed in any of the plant commodities 7 metabolites were detected in forage, 10 metabolites in fodder and 6 metabolites in grain, respectively. FOE oxalate (FOEOX, M1) revealed to be a major metabolite in animal feed commodities forage and fodder, but was absent in grain. The main metabolite in grain was identified as FOE sulfinyl lactic acid glucoside (FAMSOL-Glu, M37) amounting to 23% of TRR (0.02 mg equ/kg). This metabolite was also major in fodder (18% of TRR), but minor in forage (<10% of TRR). Exclusively in forage, the conjugate FOE malonylcysteine (FAMS-MalCys, M42) was observed at a significant extent (25% of TRR). A lot of other metabolites were detected in grain, fodder and forage, all of them containing the common moiety "N-fluorophenyl-N-isopropyl amine".

The forage and fodder metabolite, i.e. FOE oxalate, most likely arose from oxidation of transient primary alcohol hydrolysis product. All other metabolites were formed by hydrolysis, oxidation and conjugation of a primary transient metabolite formed by initial conjugation with glutathione. A similar metabolic pathway of flufenacet was also found in soybeans, wheat[11] and cotton.

From the pattern of metabolites observed in this study with [fluorophenyl-UL-^{14}C] labelled flufenacet a metabolic pathway of flufenacet in wheat is proposed in Figure 6.2.1- 3.

[11] [redacted], M. E, and [redacted], L. L. (1997): The metabolism of [fluorophenyl-UL-^{14}C]flufenacet in wheat after postemergent foliar application, Bayer AG, Div. Agriculture Report 107399, Comp. No. M-002275-01-1

Table 6.2.1- 8: Extractability of radioactive residues from corn forage and fodder following foliar treatment with [fluorophenyl-UL-^{14}C]flufenacet at a use rate of 1.46 kg as/ha

Agricultural commodity	**Corn forage**		**Corn fodder**	
Days after treatment	82		129	
TRR [mg equ/kg]	0.62		1.91	
Extraction with	[% of TRR]	[mg equ/kg]	[% of TRR]	[mg equ/kg]
Methanol, room temperature	92	0.54	82	1.53
Methanol, refluxing	2	0.01	6	0.11
1N HCl, room temperature				
- Partition into chloroform	<1	<0.01	<1	0.01
- Partition into water	1	<0.01	6	0.10
2 N NaOH, room temperature				
- Partition into chloroform	1	<0.01	1	<0.01
- Partition into water	1	<0.01	2	0.04
Methanol/water sonication	<1	<0.01	2	0.04
6 N HCl, reflux	-	-	1	<0.01
6 N NaOH, reflux	-	-	<1	<0.01
Non-extractable (solids)	1	<0.01	1	0.02
Total*	98	0.56	99	1.85

* slight differences from TRR determination measured by combustion due to rounding of subfractions

Table 6.2.1- 9: Extractability of radioactive residues from grain of corn following foliar treatment with [fluorophenyl-UL-^{14}C]flufenacet at a use rate of 1.46 kg as/ha

Agricultural commodity	**Corn grain**	
Days after treatment	129	
TRR [mg equ/kg]	0.11	
Extraction with	[% of TRR]	[mg equ/kg]
Methanol, room temperature	47	0.05
Hexane, room temperature	3	<0.01
Acetonitrile, room temperature	<1	<0.01
Methanol, refluxing	6	0.01
1N HCl, room temperature		
- Partition into chloroform	2	<0.01
- Partition into water	9	0.01
2 N NaOH, room temperature		
- Partition into chloroform	3	<0.01
- Partition into water	5	<0.01
Methanol/water sonication	2	
6 N HCl, reflux		
- Partition into chloroform	3	<0.01
- Partition into water	14	0.01
6 N NaOH, reflux		
- Partition into chloroform	<1	<0.01
- Partition into water	<1	<0.01
Non-extractable (solids)	5	<0.01
Total*	99	0.08

* slight differences from TRR determination measured by combustion due to rounding of subfractions

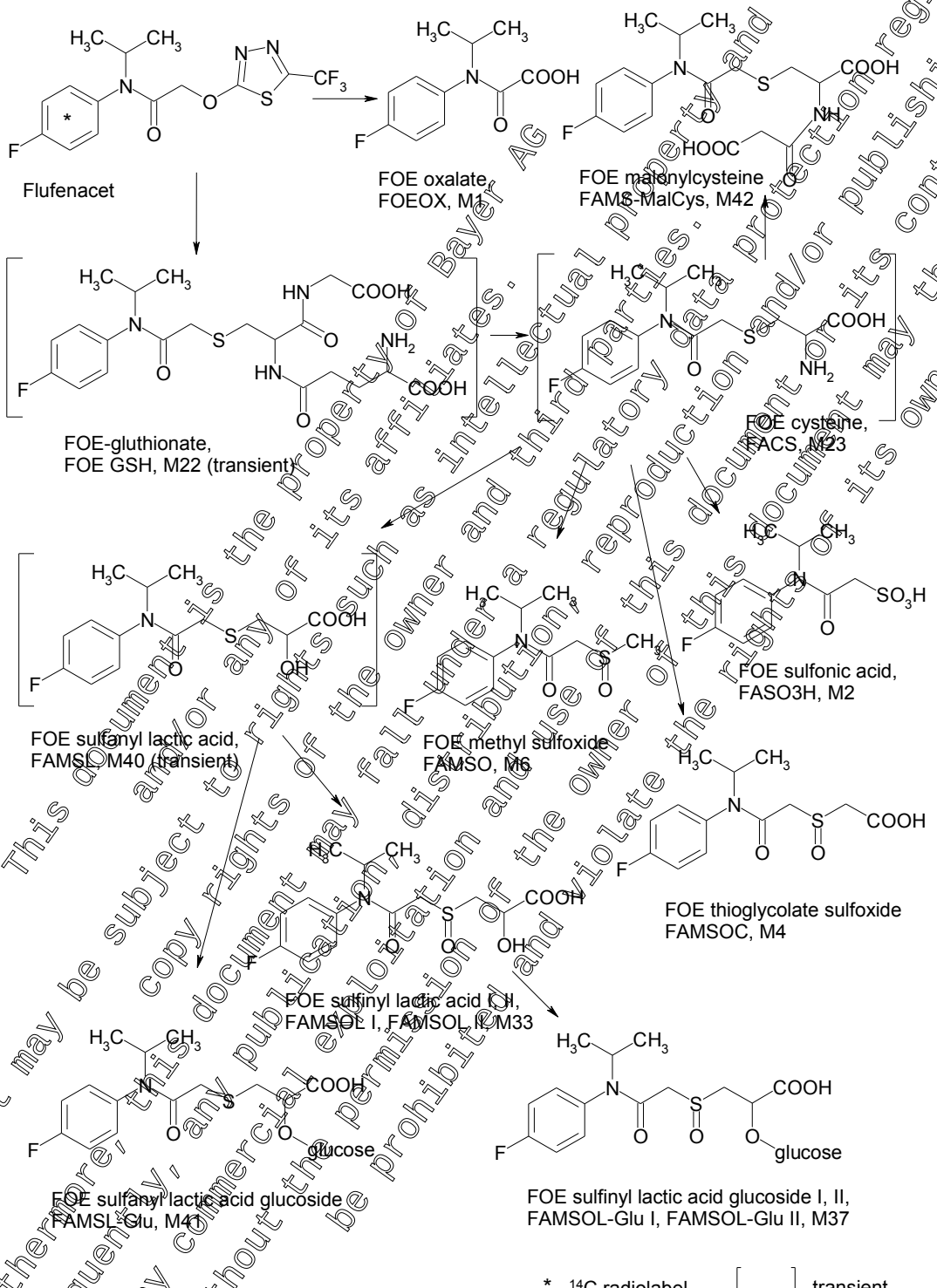
Table 6.2.1- 10: Composition of residues in corn forage and fodder treated with [fluorophenyl-UL-^{14}C]flufenacet at a use rate of 1.46 kg as/ha

Agricultural commodity	**Corn forage**		**Corn fodder**	
Days after treatment	82		129	
TRR [mg equ/kg]	0.62		1.91	
Metabolites released by MeOH at RT, MeOH refluxing and 1 N HCl at RT	[% of TRR]	[mg equ/kg]	[% of TRR]	[mg equ/kg]
Unknown 1	-	-	1	0.02
FOE oxalate (FOEOX, M1)	27	0.17	22	0.42
Unknown 2	-	-	4	0.08
Unknown 3	-	-	1	0.02
FOE sulfinyl lactic acid glucoside I , II (FAMSOL-Glu, M37)	6	0.03	18	0.34
FOE thioglycolate sulfoxide (FAMSOC, M4)	7	0.04	5	0.10
FOE sulfinyl lactic acid I (FAMSOL I, M33)	5	0.03	5	0.10
FOE sulfinyl lactic acid II (FAMSOL II, M33)	19	0.12	16	0.30
Unknown 4	3	0.02	3	0.06
FOE sulfonyl lactic acid glucoside (FAMSL-Glu, M41)	25	0.15	-	-
FOE malonylcysteine (FAMS-MalCys, M42)	-	-	16	0.30
Total	92	0.56	91	1.74
Total identified	89	0.54	82	1.56

Table 6.2.1- 11: Composition of residues in of the methanol extract of corn grain treated with [fluorophenyl-UL-^{14}C]flufenacet at a use rate of 1.46 kg as/ha

Agricultural commodity	**Corn grain**	
Days after treatment	129	
TRR [mg equ/kg]	0.11	
Metabolites extracted with MeOH at RT	[% of TRR]	[mg equ/kg]
FOE sulfonic acid (FASO3H, M2)	4	<0.01
FOE oxalate (FOEOX, M1)	-	-
FOE sulfinyl lactic acid glucoside I , II (FAMSOL-Glu I, M37)	23	0.02
FOE thioglycolate sulfoxide (FAMSOC, M4)	9	0.01
FOE sulfinyl lactic acid I (FAMSOL I, M33)	2	<0.01
FOE sulfinyl lactic acid I I (FAMSOL II, M33)	2	<0.01
FOE methyl sulfoxide (FAMSO, M6)	7	0.01
Total	47	0.04
Total identified	47	0.04

Figure 6.2.1- 3: Proposed metabolic pathway of flufenacet in corn following post-emergent foliar application



Additional plant metabolism studies with [thiadiazole-5-^{14}C]flufenacet

As mentioned before the older metabolism studies of flufenacet on plants were conducted with [fluorophenyl-UL-^{14}C]- and the [thiadiazole-2-^{14}C]flufenacet. To complete the pattern of all potential metabolites and metabolic pathways additional metabolism studies were recently conducted with [thiadiazole-5-^{14}C]flufenacet on potatoes, wheat and rotated crops. These studies have still not been evaluated by registration authorities. They are summarized and presented in the following.

Remark about formation of trifluoroacetate (TFA) under environmental and physiological conditions

Metabolism studies of [thiadiazole-5-^{14}C]flufenacet in primary and confined rotational crops often indicate trifluoroacetate (TFA) as a major metabolite. This metabolite is denoted misleadingly as trifluoroacetic acid, although the matrix of its formation (soil) or crops following uptake via the roots do not get acidic.

Under physiological and environmental conditions metabolic formation of TFA does not result in trifluoroacetic acid (TFA-H), rather than in formation of a trifluoroacetate salt (consists of TFA anion and counter cation). This is because of the very high acidity of TFA-H as characterized by its low pKa of 1.3[12] (for comparison, pKa of acetic acid: 4.76) indicating complete dissociation at higher pH.

During metabolic formation of TFA the acidity of the forming matrix (e.g. soil with microoganisms) does not change indicating that TFA cannot be present as carboxylic acid TFA-H. The dissociating proton of the carboxylic acid is immediately captured and neutralized by soil constituents due to the high buffer capacity of the soil. In its acid form it would damage the roots of plants rather than be taken up.

TFA is formed as trifluoroacetate anion with an undefined counter cation depending on the environment. Since the counter cation is undefined the TFA is usually denoted by the name of its parent acid, trifluoroacetic acid, keeping in mind that their salts are meant.

While the acid TFA-H is known to be highly irritant due to its high acidity, the TFA anion combined with an environmentally appearing cation behaves like an inert salt. Therefore, toxicological evaluation must not be conducted with TFA-H, but with a TFA salt.

[12] [redacted]., 2011: Trifluoro acetic acid (AE C502988): Determination of the dissociation constant in water, unpublished report 20100672.02 of Siemens Prozess-Sicherheit, Frankfurt, Germany, for Bayer CropScience, Comp. No. M-418628-01-1

Potato

Report:	**KCA 6.2.1/06,** [redacted]; 2012; M-441506-02-1
Title:	Metabolism of [thiadiazole-5-^{14}C]Flufenacet in Potatoes
Document No:	M-441506-02-1
Report No:	EnSa-12-0537, dated 2012-12-10
Guidelines:	OECD guideline 501: Metabolism in Crops, adopted 8-January-2007, US EPA OCSPP Residue Chemistry Guideline OPPTS 860.1300
GLP	Yes; deviations: none

Executive Summary

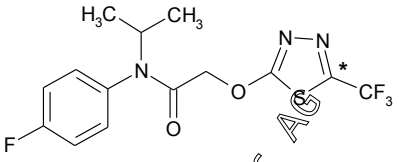
The metabolism of [thiadiazole-5-^{14}C]flufenacet was investigated in potatoes after pre-emergent application at a rate of approximately 630 g as/ha to the soil where seed potatoes have been planted one day before application. This use rate exceeded the intended field rate of 600 g as/ha by 5%. At maturity, 112 days after application, the potato plants were harvested, separated into tubers and foliage (leaves and stems), radioassayed for the level of total radioactive residues (TRR) and analyzed for the nature of these residues. In potato tubers, TRR amounted to 0.867 mg parent equivalents/kg (mg equ/kg) and in foliage to 40.52 mg equ/kg. Extraction of these residues with acetonitrile/water (8/2, v/v) was nearly complete amounting to 99% or 98% of TRR in tubers or foliage, respectively.

The predominant portion of the residues consisted of ^{14}C-labelled trifluoroacetate (TFA) contributing to 92% of TRR (corresponding to 0.801 mg equ/kg) in tubers and to 90% of TRR (corresponding to 36.45 mg equ/kg) in foliage. FOE-thiadone-glycoside was additionally detected as minor metabolite amounting to 1.8% of TRR (corresponding to 0.015 mg equ/kg) in tubers and to 4.4% of TRR (corresponding to 1.80 mg equ/kg) in foliage. Five additional unknown metabolites were also detected at a very low level, the sum of them accounted for 3.9% of TRR (corresponding to 0.034 mg equ/kg) in tubers and for 3.5% of TRR (corresponding to 1.41 mg equ/kg) in foliage. The portion of non-extractable residues was negligible amounting to 1% of TRR in tubers and 2% of TRR in foliage. The parent substance flufenacet was not observed in tubers or foliage.

From these results it was concluded that the thiadiazole ring is cleaved from the parent molecule and absorbed by the potato plants at a very low extent followed by formation of a glycoside conjugate. However, the predominant metabolic pathway proceeds via extensive degradation of the thiadiazole ring to form TFA that is widely taken up by the potato plants and translocated mainly into the green parts of the plants. A metabolic pathway is proposed in Figure 6.2.1- 4.

Material and Methods

Test Material

Structural formula	 * denotes the ^{14}C label
Chemical name	N-(4-Fluoro-phenyl)-N-isopropyl-2-(5-trifluoromethyl-[1,3,4]thiadiazol-2-yloxy)-acetamide (IUPAC); Acetamide, N-(4-fluorophenyl)-N-(1-methylethyl)-2-[[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]oxy]- (9Cl; CAS)
Common name	Flufenacet
CAS RN	142459-58-3
Empirical formula	$C_{14}H_{13}F_4N_3O_2S$
Company code	FOE 5043
Molar mass (non-labelled)	363.34 g/mol
Label	[thiadiazole-5-^{14}C]Flufenacet
Specific radioactivity	1.9 MBq/mg (used in the study; the original test substance had a specific radioactivity of 3.81 MBq/mg or 103.04 µCi/mg)
Radiochemical purity	>99% by TLC and HPLC (radio-detection)
Chemical purity	>99% by HPLC (UV detection at 210 nm)

Test Plants

Test plant	Potato
Variety	Cilena
Growth stage at application	Soil treatment one day after seeding of the tubers and before emergence of the plants
Harvested commodities	Mature tubers (BBCH 97 – 99) together with potato vines

Planting of seed potatoes, preparation and application of the spray mixture

A plant container (surface area 1 m^2) was filled with a sandy loam soil (67% sand, 18% silt, 15% clay, 1.2% organic carbon, pH 6.9 ($CaCl_2$)). Six seed potato tubers were planted in the soil one day before application of the spray mixture to the soil.

The original radiolabelled test substance was diluted with non-labelled flufenacet resulting in a specific radioactivity of 1.9 MBq/mg. Addition of a blank formulation yielded a SC 500 formulation with a concentration of the active substance of 42.4% (w/w). Addition of water finally resulted in the spray mixture of a volume of 104.5 mL.

The spray solution was applied to the bare soil surface of the prepared plant container using a computer controlled track sprayer fitted with a flat fan nozzle. The actual application rate amounted to 631 g as/ha being 5% higher than the intended field rate of 600 g as/ha. The stability the test substance in the spray mixture was demonstrated by radio-HPLC before and after application.

Cultivation of the test plants

The treated plant container was placed in an open vegetation hall with a glass roof and the plants were grown under outdoor conditions between April and August 2011. The mean temperatures ranged from 16 to 22°C and the mean sunshine periods between 83 to 231 hours/month.

Harvest and processing of the potatoes

Mature potato plants (BBCH 97 – 99) were dogged out of the soil 112 days after application of the test substance. The plants were separated into tubers and foliage (leaves and stems). Soil adhering to the tubers was removed after air-drying. Afterwards the tubers were washed with water, cut into slices and homogenized under liquid nitrogen using a high-speed stirrer (Polytron). Potato foliage was also homogenized as done with the tubers. Aliquots of the homogenates were extracted and the remaining homogenates stored at ≤ -18°C. The tuber wash, the extracts and the extracted solids were radioassayed.

Radioassaying, extraction and analysis of the plant samples

Radioassaying (measurement of the radioactivity) was conducted by liquid scintillation counting (LSC). The counting was repeated three times. Quenching was automatically compensated using an external standard. Solid samples were firstly combusted and the formed $^{14}CO_2$ absorbed in an alkaline scintillation liquid. The limit of quantification (LOQ) was set to twice the background radioactivity for radioassaying of solid samples. Given the aliquot amount of combustion and the specific radioactivity used in this study the LOQ for radioassaying was 0.002 mg equ/kg.

Homogenized plant samples were extracted three times with acetonitrile/water (8/2, v/v) using a high speed stirrer (Polytron) followed by one extraction with pure acetonitrile. The radioactivity contents of the extracts and the remaining solids (and in case to tuber the tuber wash) were numerically summarized to yield the total radioactive residues (TRR) of the original sample. The extracts were combined, concentrated and analysed for metabolite profiling by radio-HPLC and radio-TLC (TLC only done for polar HPLC fractions).

Radio-HPLC was conducted on a RP18 column (250 x 4.6 mm, 5 µm particle size) operated with a gradient mixture of water/formic acid (99/1, v/v) and acetonitrile/formic acid (99/1, v/v) at 40°C. The HPLC system was equipped with a UV-detector (254 nm) and a radiomonitor with a glass scintillator (cell size 370 µL). Column recovery (97.9% for tuber analysis) was proven by comparison of the eluted and injected radioactivity. The LOQ for HPLC determination was derived from the background noise and the smallest radio-peak of the respective sample. HPLC-LOQs for tuber and foliage samples were set to 0.004 and 0.07 mg equ/kg. Radiolabelled parent substance, trifluoroacetate (isolated and identified in a metabolism study on rotated crops[13]) and FOE thiadone glycoside (isolated and identified in a metabolism study on wheat) as well as non-labelled FOE-thiadone were used as reference standards for co-chromatography.

[13] [redacted], R. (2012): Metabolism of [thiadiazole-5-^{14}C]Flufenacet in Confined Rotational Crops, unpublished report EnSa-12-0535 of Bayer CropScience AG, Comp. No. M-443538-01-1.

One-dimensional radio-TLC was conducted on a silica gel TLC plates (20 x 20 cm, layer thickness 0.25 mm). Development of the spotted plates was performed with a solvent mixture consisted of ethyl acetate/ 2-propanol/water/acetic acid (65/24/11/1, v/v/v/v) after chamber saturation. The radioactive spots on the developed plates were visualized and quantified using a Bio-Imaging Analyzer.

LC-MS was conducted on a combination of RP18-HPLC (operated with a gradient mixture of 0.1% formic acid in water and in acetonitrile) and an Orbitrap mass spectrometer using electro-spray for ionization.

Potato tubers or foliage samples were extracted 13 or 22 days after harvest and storage at ≤ -18 °C. The extracts were chromatographically analyzed for the composition of residues within one day after extraction.

Findings

Total radioactive residues

Seed potatoes were planted one day before application and matured potatoes were harvested 112 days after application of [thiadiazole-3-^{14}C]flufenacet to soil at a use rate of 631 g as/ha. The total radioactive residues (TRR) in the harvested tubers amounted to 0.867 mg parent equivalents/kg (mg equ/kg) and in foliage to 40.52 mg equ/kg. Very low radioactivity could be washed from the surface of the tubers with water amounting to 0.1% of TRR and corresponding to 0.001 mg equ/kg.

Extraction of residues from potato tubers and foliage

The radioactive residues could be extracted almost completely using acetonitrile/water (8/2, v/v). A portion of 98.0% of TRR (corresponding to 0.850 mg equ/kg) was extracted from the tubers and 97.8% of TRR (corresponding to 39.66 mg equ/kg) from the foliage. In turn, the non-extractable portion accounted for 1.0% of TRR in tubers and 2.1% of TRR in foliage. The procedural losses were ≤ 1% of the respective TRR. Summing up these portions, the resulting mass balance was complete for tubers and foliage.

Nature of residues in potato tubers and foliage (Table 6.2.1- 12)

The residues extracted from the tubers and foliage was analyzed by radio-HPLC on a reversed phase and radio-TLC on a straight phase and thus using two different chromatographic separation mechanisms. The main portion of the radiolabelled residues comprised of ^{14}C-trifluoro acetate (TFA, M45) accounting for 90% of TRR (corresponding to 36.45 mg equ/kg) in the foliage and 92% of TRR (corresponding to 0.801 mg equ/kg) in the tubers. FOE-thiadone-glycoside (M25) appeared as minor metabolite amounting to 1.8% of TRR (corresponding to 0.015 mg equ/kg) in tubers and to 4.4% of TRR (corresponding to 1.80 mg equ/kg) in foliage. The parent substance flufenacet was not present in tubers and foliage. Five minor unknown metabolites accounted in sum to 3.9% of TRR in tubers and to 3.5% of TRR in foliage. The non-extractable portion of residues was negligible accounting for 1% of TRR in tubers and for 2% of TRR in the foliage.

Conclusion

Seed potatoes were planted into soil. One day after planting [thiadiazole-5-^{14}C]flufenacet was applied to the soil surface at a use rate of approximately 630 g as/ha in the pre-emergence stage. Following cultivation till maturity the plants were harvested and analyzed for the composition of radiolabelled residue in tubers and foliage. The predominant portion of these residues consisted of ^{14}C-labelled trifluoroacetate (TFA, M45). TFA amounted to ≥ 90% of TRR in both tubers (corresponding to 0.801 mg equ/kg) and foliage (corresponding to 40.52 mg equ/kg). A minor metabolite FOE-thiadone-glycoside (M25) appeared also in tubers and foliage at a portion of less than 5% of TRR. The unchanged parent substance was not detected in potatoes.

Obviously, the thiadiazole ring was split off of the parent substance, taken up by the potato plants at a very low extent and conjugated to a glycoside. However the main metabolic pathway proceeded by an extensive degradation of the thiadiazole ring to form TFA (M45) that is widely absorbed by potato plants and translocated particularly into the foliage. The metabolic pathway is proposed in Figure 6.2.1- 4.

Table 6.2.1- 12: Composition of the radioactive residues in potatoes after pre-emergence application of [thiadiazole-5-^{14}C]flufenacet at a use rate of 630 g as/ha to soil

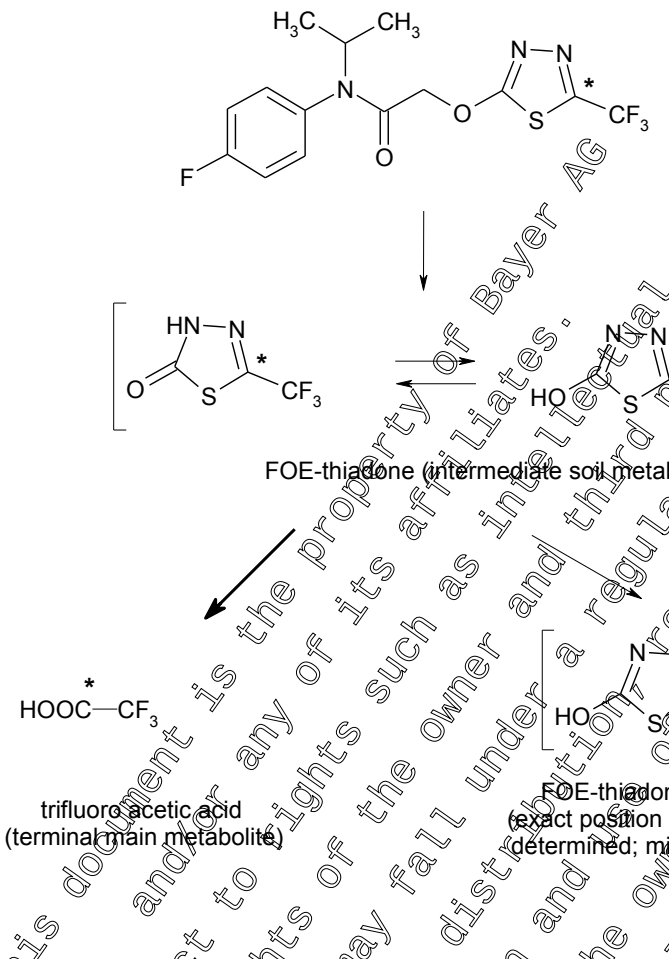
Potato	Tubers		Foliage	
	TRR = 0.867 mg equ/kg		TRR = 40.52 mg equ/kg	
	% of TRR	mg/kg#	% of TRR	mg/kg#
TFA (trifluoroacetic acid)	92.3	0.801	90.0	36.45
FOE-thiadone-glycoside	1.8	0.015	4.4	1.80
Total identified	94.1	0.816	94.4	38.25
Unknown 1	0.6	0.005	---	---
Unknown 2	---	---	0.7	0.28
Unknown 3	1.6	0.014	2.5	0.99
Unknown 4	0.8	0.007	0.3	0.13
Unknown 5	0.9	0.008	---	
Total characterised*	3.9	0.034	3.5	1.41
Tuber wash	0.1	0.001	---	---
Procedural loss	0.8	0.007	<0.1	0.01
Total extractable	99.0	0.858	97.9	39.67
Non-extractable (PES) **	1.0	0.009	2.1	0.85
Accountability	100.0	0.867	100.0	40.52

* The non-identified compounds were characterised by their extraction and chromatographic behaviour.

** PES = post extraction solids

\# mg/kg means mg parent equivalents/kg

Figure 6.2.1- 4: Metabolic pathway of [thiadiazole-5-^{14}C]flufenacet in potatoes after pre-emergent application to soil at a use rate of 630 g as/ha



Wheat

Report:	**KCA 6.2.1/07,** [redacted], R.; [redacted], D.; 2013; M-444475-01-1
Title:	Metabolism of [thiadiazole-5-^{14}C]Flufenacet in Wheat
Document No:	M-444475-01-1
Report No:	EnSa-12-0536, dated 2013-01-07
Guidelines:	OECD Guideline 501: Metabolism in Crops, adopted 8-January-2007 US EPA OCSPP Residue Chemistry Guideline OPPTS 860.1300
GLP	Yes; deviations: none

Executive Summary

The metabolism of [thiadiazole-5-^{14}C]flufenacet was investigated in wheat following a foliar treatment at a use rate of 270 g as/ha in the mid-tillering growth stage. This use rate exceeded the intended field rate of 240 g as/ha by 12.5%. The total amount and the nature of residues was disclosed in wheat forage sampled four days after treatment (DAT), in wheat hay sampled 56 DAT and in wheat straw and grain harvested 84 DAT, respectively.

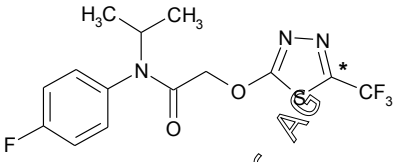
The total radioactive residues (TRR) amounting to 5.145 mg equ/kg in forage, 2.689 mg equ/kg in hay, 2.974 mg equ/kg in straw and to 0.704 mg equ/kg in wheat grain could almost completely be extracted with acetonitrile/water (8+2, v/v, 4x) at room temperature followed by extraction with acetonitrile/water (1+1, v/v) plus formic acid at elevated temperatures.

Whereas the parent substance flufenacet revealed to be the prominent residue component in wheat forage it was almost completely metabolized in wheat hay and straw and was no more detectable in wheat grain. The metabolite trifluoroacetate (TFA) was still not detected in wheat forage, but proved to be the main residue component in wheat and straw. In grain practically the complete radioactive residues consisted of radiolabelled TFA. An intermediate metabolite, FOE-thiadone-glycoside, appeared already in the forage commodity at a relevant portion and increased slightly to approximately one third of the total residues in hay and straw, but was almost completely degraded to TFA in grain. The portion of non-extractable residues was negligible in all wheat commodities not exceeding 1% of TRR.

Obviously, the thiadiazole ring was rapidly split off of the parent substance and conjugated to a glycoside to a moderate extent. The further metabolic pathway proceeded in an extensive degradation of the thiadiazole ring to form TFA as the main residue component in hay and straw and as the terminal and nearly exclusive residue component in wheat grain. The metabolic pathway is proposed in Figure 6.2.1-5.

Material and Methods

Test Material

Structural formula	 * denotes the ^{14}C label
Chemical name	N-(4-Fluoro-phenyl)-N-isopropyl-2-(5-trifluoromethyl-[1,3,4]thiadiazol-2-yloxy)-acetamide (IUPAC); Acetamide, N-(4-fluorophenyl)-N-(1-methylethyl)-2-[[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]oxy]- (9Cl; CAS)
Common name	Flufenacet
CAS RN	142459-58-3
Empirical formula	$C_{14}H_{13}F_4N_3O_2S$
Company code	FOE 5043
Molar mass (non-labelled)	363.34 g/mol
Label	[thiadiazole-5-^{14}C]Flufenacet
Specific radioactivity	1.9 MBq/mg (used in the study; the original test substance had a specific radioactivity of 3.81 MBq/mg or 103.04 µCi/mg)
Radiochemical purity	>99% by TLC and HPLC (radio-detection)
Chemical purity	>99% by HPLC (UV detection at 210 nm)

Test Plants

Test plant	Spring wheat
Variety	Thasos
Growth stage at application	Post-emergent foliar application at growth stage BBCH 21 – 25 (beginning of first tillering – five tillers detectable)
Harvested commodities	Wheat forage (BBCH 29, end of tillering), PHI: 4 days Wheat hay (BBCH 75-83, medium milk – early dough stage), PHI: 56 days Wheat grain and straw (BBCH 89, full ripe grain), PHI: 84 days

Sowing of wheat, preparation and application of the spray mixture

A plant container (surface area 1 m^2) was filled with a sandy loam soil (67% sand, 18% silt, 15% clay) having an organic carbon content of 1.2% and a pH ($CaCl_2$) of 6.9. Wheat was sown in 10 rows at a sowing density of approximately 500 seeds/m^2.

The original radiolabelled test substance was diluted with non-labelled flufenacet resulting in a specific radioactivity of 1.9 MBq/mg. Addition of a blank formulation yielded a SC 500 formulation with a concentration of the active substance of 42.4% (w/w). Addition of water finally resulted in the spray mixture of a volume of 105 mL.

The spray mixture was sprayed to the wheat plants grown in the plant container using a computer controlled track sprayer fitted with a flat jet nozzle at the mid tillering growth stage BBCH 21 - 25.

The actual application rate amounted to 270 g as/ha, being 12.5% higher than the intended field rate of 240 g as/ha. The stability the test substance in the spray mixture was demonstrated by radio-HPLC before and after application.

Cultivation of the test plants

The treated plant container was placed in an open vegetation hall with a glass roof and the plants were grown under outdoor conditions between April and August 2011. During sunshine periods the glass roof was opened. The mean temperatures ranged from 16 to 22°C and the mean sunshine periods between 83 to 231 hours/month. Commercial cereals fungicides and insecticides were applied when required according to agricultural practice.

Harvest and processing of the wheat commodities

Wheat forage (BBCH 29): The plants of two of the ten rows were cut above the soil, cut into small pieces and homogenized under liquid nitrogen with use of a high-speed stirrer (Polytron). An aliquot of the homogenate was extracted and the remaining material stored at ≤ -18°C.

Wheat hay (BBCH 75 – 83): The plants of another two rows were cut above the soil, dried for four days at room temperature, cut into small pieces and homogenized and stored as mentioned for wheat forage.

Wheat straw and grain (BBCH 89): The remaining plants were cultivated until full maturity and then cut above the soil. The seeds were pulled out the ears by hand yielding the grain sample. The remaining ears and chaffs were combined with the straw and cut into small pieces. Grain and straw were separately homogenized under liquid nitrogen and stored as described for wheat hay.

Radioassaying, extraction and analysis of the plant samples

Radioassaying (measurement of the radioactivity) was conducted by liquid scintillation counting (LSC). The counting was repeated three times. Quenching was automatically compensated using an external standard. Solid samples were first combusted and the formed $^{14}CO_2$ absorbed in an alkaline scintillation liquid. The limit of quantification (LOQ) was set to twice the background radioactivity for radioassaying of solid samples. Given the aliquot amount of combustion and the specific radioactivity used in this study the LOQ for radioassaying was 0.002 mg parent equivalents/kg (0.002 mg equ/kg).

Immature homogenized plant samples were extracted three times with acetonitrile/water (8/2, v/v) using a high speed stirrer (Polytron) followed by one extraction with pure acetonitrile (conventional extraction). Wheat hay, straw and grain were successively extracted with acetonitrile/water (1/1, v/v) and acetonitrile/water (1/1, v/v) plus formic acid at elevated temperatures with microwave assistance (exhaustive extraction) to complete the extraction. The radioactivity contents of the extracts and the remaining solids were numerically summarized to yield the total radioactive residues (TRR) of the original sample. The conventional and exhaustive extracts were separately combined, concentrated and analysed for metabolite profiling by radio-HPLC and radio-TLC (TLC only done for polar HPLC fractions).

Radio-HPLC was conducted on a RP18 column (250 x 4.6 mm, 5 µm particle size) operated with a gradient mixture of water/formic acid (99/1, v/v) and acetonitrile/formic acid (99/1, v/v) at 40°C. The

HPLC system was equipped with a UV detector (254 nm) and a radiomonitor with a glass scintillator (cell size 370 µL). Column recovery was proven by comparison of the eluted and injected radioactivity. It was excellent amounting to 97.6 – 99.6% for analysis of forage, hay, straw and grain extract. The LOQ for HPLC determination was derived from the background noise and the smallest radio-peak of the respective sample. HPLC-LOQs for the different extracts were set to 0.002 (grain) to 0.018 (forage) mg equ/kg. Radiolabelled parent substance, trifluoroacetate (isolated and identified in a metabolism study on rotated crops and non-labelled FOE-thiadone were used as reference standards for co-chromatography.

One-dimensional radio-TLC was conducted on a silica gel TLC plates (20 x 20 cm, layer thickness 0.25 mm). Development of the spotted plates was performed with a solvent mixture consisted of ethyl acetate/ 2-propanol/water/acetic acid (65/24/11/1, v/v/v/v) after chamber saturation. The radioactive spots on the developed plates were visualized and quantified out using a Bio-Imaging Analyzer.

LC-MS of parent flufenacet and FOE-thiadone-glycoside was conducted on a combination of RP18-HPLC (operated with a gradient mixture of 0.1% formic acid in water and in acetonitrile) and an Orbitrap mass spectrometer using electro-spray for ionization.

Wheat samples (forage, hay, straw and grain) were extracted one to twelve days after harvest and storage at ≤ -18°C. The extracts were chromatographically analyzed for the composition of residues within one to two days after extraction.

Findings

Total radioactive residues

Spring wheat was sprayed with [thiadiazole-5-^{14}C]flufenacet at a use rate of 270 g as/ha in the mid tillering growth stage. Plant commodities were samples after different intervals after treatment: forage 4 days, hay 56 days, straw and grain 84 days. The total radioactive radioactivity (TRR) in these commodities amounted to 5.145 mg equ/kg in forage, 2.689 mg equ/kg in hay, 2.974 mg equ/kg in straw and 0.704 mg equ/kg in grain.

Extraction of residues from wheat commodities (Table 6.2.1- 13)

The radioactive residues could be extracted almost completely from all wheat commodities using acetonitrile/water (8/2, v/v; "conventional extraction") at room temperature and acetonitrile/water (1/1, v/v, partly with formic acid; "exhaustive extraction") at elevated temperature. The totally extractable residues amounted to 99.0 – 99.7% of TRR. In turn, the non-extractable portion accounted for 0.3 – 1.0% of TRR. The prominent portion of residues could already be extracted at room temperature ranging from 75.2% of TRR (grain) to 98.3% of TRR (forage).

Nature of residues in wheat commodities (Table 6.2.1- 14)

The residues extracted from the wheat forage, hay, straw and grain were analyzed by radio-HPLC on a reversed phase and radio-TLC on a straight phase and thus using two different chromatographic separation mechanisms.

The parent substance flufenacet was the main residue component in wheat forage sampled four days after application. It amounted to 76.7% of TRR (3.944 mg/kg). However, flufenacet was almost completely metabolised in hay and straw (≤ 1.8% of TRR) and did no longer appear in wheat grain.

Trifluoroacetate (TFA, M45) did still not appear in wheat forage, but proved to get the main residue component in hay (63.1% of TRR; 1.697 mg equ/kg) and straw (61.7% of TRR; 1.836 mg equ/kg). In wheat grain, almost the total residues consisted of TFA (M45) amounting to 99.2% of TRR corresponding to 0.698 mg equ/kg.

FOE-thiadone-glycoside (M25) was already formed in wheat forage, four days after application of flufenacet, amounting to 21.6% of TRR (1.113 mg equ/kg). In hay and straw, it contributed to approximately one third of the total residues (30.5 – 33.5% of TRR, corresponding to 0.822 mg equ/kg in hay and to 0.997 mg equ/kg in straw). It decreased to a very minor metabolite in wheat grain accounting for 0.4% of TRR (0.003 mg equ/kg).

Conclusion

Following foliar treatment of spring wheat with [thiadiazole-5-^{14}C]flufenacet at a use rate of 270 g as/ha the radioactive residues were investigated in wheat forage sampled four days after treatment (DAT), in wheat hay sampled 56 DAT and in wheat straw and grain harvested 84 DAT.

Whereas the parent substance flufenacet revealed to be the prominent residue component in wheat forage it was almost completely metabolized in wheat hay and straw and was no more detectable in wheat grain. The metabolite trifluoroacetate (TFA, M45) could still not be detected in wheat forage, but proved to be the main residue component in wheat and straw. In grain, practically the complete radioactive residues consisted of radiolabelled TFA. An intermediate metabolite, FOE-thiadone-glycoside (M25), appeared already in the forage commodity at a relevant portion and increased slightly to approximately one third of the total residues in hay and straw, but was almost completely degraded to TFA in grain.

Obviously, the thiadiazole ring was rapidly split off of the parent substance and conjugated to a glycoside at a moderate extent. The further metabolic pathway proceeded in an extensive degradation of the thiadiazole ring to form TFA as the main residue component in hay and straw and as the terminal and nearly exclusive residue component in wheat grain. The metabolic pathway is proposed in Figure 6.2.1- 5.

Table 6.2.1- 13: Extractability of radioactive residues from wheat commodities after foliar application of [thiadiazole-5-^{14}C]flufenacet at a use rate of 270 g as/ha

Wheat	Forage, 4 DAT##		Hay, 56 DAT		Straw, 84 DAT		Grain, 84 DAT	
	%TRR	mg/kg#	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
TRR	100	5.145	100	2.689	100	2.974	100	0.704
Conventional extraction *	**98.3**	**5.057**	**93.5**	**2.514**	**94.0**	**2.796**	**75.2**	**0.529**
Exhaustive extraction **	--	--	5.9	0.157	5.2	0.155	24.4	0.172
Procedural loss	0.7	0.034	0.3	0.009	0.2	0.006	--	--
Total extractable	99.0	5.091	99.7	2.680	99.4	2.957	99.7	0.701
Non-extractable (PES) ***	1.0	0.053	0.3	0.009	0.6	0.017	0.3	0.002
Accountability	**100.0**	**5.145**	**100.0**	**2.689**	**100.0**	**2.974**	**100.0**	**0.704**

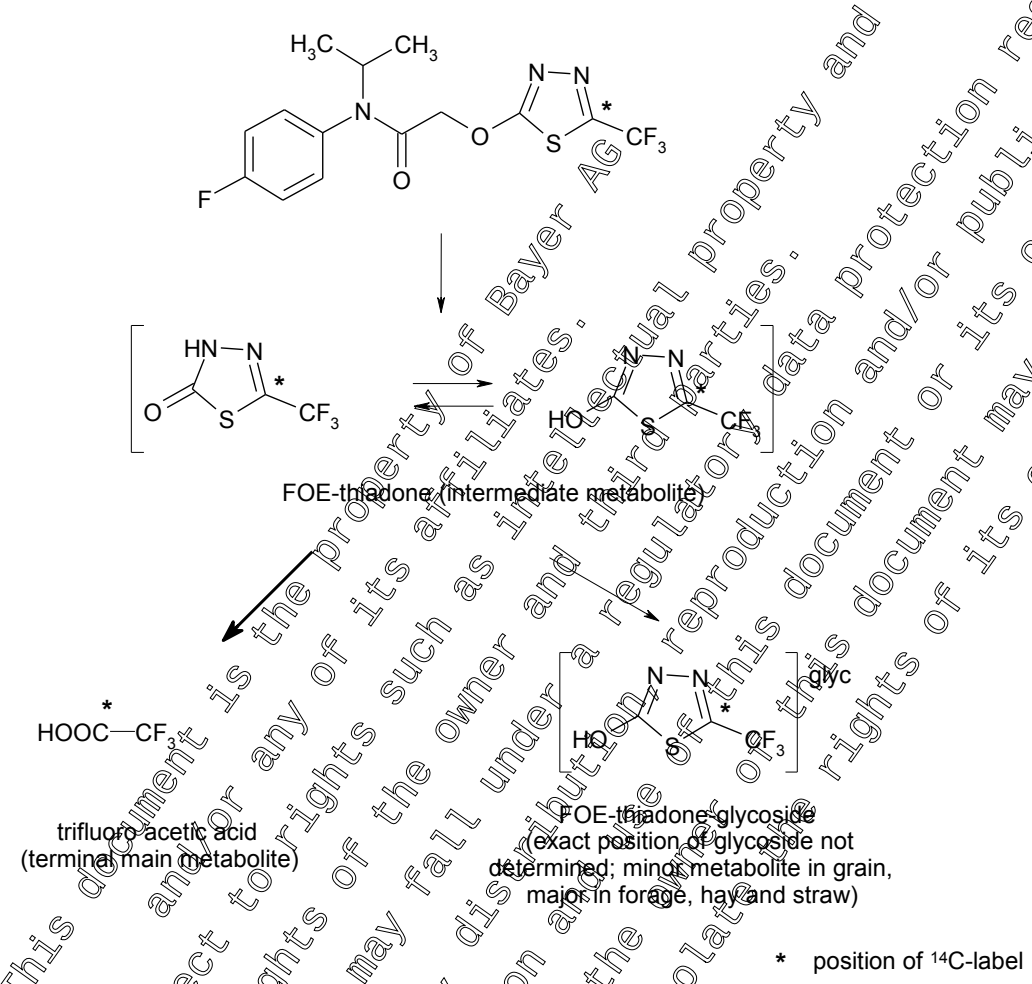
* Extraction with acetonitrile/water (8/2, v/v) at room temperature
** Succeeding extraction with acetonitrile/water (1/1, v/v) plus formic acid at elevated temperature
*** PES: post extraction solids
# mg/kg: mg parent equivalents/kg (mg equ/kg)
4 DAT: sampling 4 days after treatment

Table 6.2.1- 14: Composition of the radioactive residues in wheat commodities after foliar application of [thiadiazole-5-^{14}C]flufenacet at a use rate of 270 g as/ha

Wheat	Forage		Hay		Straw		Grain	
	%TRR	mg/kg#	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
Conventional extraction (at room temperature)								
Flufenacet	76.7	3.944	1.8	0.048	0.4	0.013	--	--
TFA (trifluoroacetate)	--	--	58.9	1.583	58.0	1.726	74.8	0.526
FOE-thiadone-glycoside	21.6	1.113	28.9	0.778	32.0	0.952	0.4	0.003
Unknown 1	--	--	--	--	0.6	0.019	--	--
Unknown 2	--	--	0.6	0.015	0.7	0.020	--	--
Unknown 3	--	--	0.4	0.011	--	--	--	--
Unknown 3	--	--	1.8	0.048	1.0	0.029	--	--
Unknown 5	--	--	0.6	0.017	1.3	0.038	--	--
Unknown 6	--	--	0.6	0.015	--	--	--	--
Procedural loss	0.7	0.034	0.3	0.009	0.2	0.006	--	--
Exhaustive extraction (at elevated temperature, performed after conventional extraction)								
TFA (trifluoroacetate)	--	--	4.2	0.114	3.7	0.110	24.4	0.172
FOE-thiadone-glycoside	--	--	1.6	0.044	1.5	0.045	--	--
Summary of extraction								
Flufenacet	76.7	3.944	1.8	0.048	0.4	0.013	--	--
TFA (trifluoroacetate)	--	--	63.1	1.697	61.7	1.836	99.2	0.698
FOE-thiadone-glycoside	21.6	1.113	30.5	0.822	33.5	0.997	0.4	0.003
Total identified	**98.3**	**5.057**	**95.4**	**2.566**	**95.7**	**2.845**	**99.7**	**0.701**
Total characterized *	--	--	3.9	0.106	3.6	0.106	--	--
Non-extractable (PES) **	1.0	0.053	0.3	0.009	0.6	0.017	0.3	0.002
Accountability	**100.0**	**5.145**	**100.0**	**2.689**	**100.0**	**2.974**	**100.0**	**0.704**

* Characterized by the extraction and chromatographic behaviour
** PES: post extraction solids
# mg/kg: mg parent equivalents/kg (mg eq/kg)

Figure 6.2.1- 5: Metabolic pathway of [thiadiazole-5-^{14}C]flufenacet in wheat after foliar application at a use rate of 270 g as/ha



Summary of the metabolism of flufenacet in plants including the new metabolism studies

From the metabolism studies submitted for approval in the EU and USA a conclusion of a common metabolic pathway of flufenacet in plants was made. The initial metabolic reaction is a cleavage of the molecule into the thiadone and acetamide moiety by glutathione (GSH) conjugation of the acetamide part resulting in the transient gluathionate conjugated FOE GSH (M22).

This transient glutathione conjugate is further metabolized by splitting off glycine and glutamine acid yielding the FOE cysteine conjugate (M23). All further metabolites can be considered as hydrolysis, oxidation and conjugation products of the FOE cysteine conjugate. However, the FOE oxalate (M01) most likely arose through direct oxidation of a transient primary alcohol hydrolysis product of Flufenacet (FOE alcohol, M03).

Due to the initial cleavage of the parent molecule caused by glutathionate conjugation, trifluoromethyl thiadone (M09) was released. While this transient moiety was not observed, various conjugates were formed, the quantitatively most important being the corresponding N-glucoside (M 25). In soybeans, the malonylalanine conjugate (M34) predominated.

The additional studies with [fluorophenyl-UL-^{14}C]flufenacet on potato (pre- and post-emergence application), wheat and corn (both post-emergence application) confirmed this metabolic pathway. Additional plant metabolism studies with [thiadiazole-5-^{14}C]flufenacet in potato (pre-emergence application), wheat (post-emergence application) and in the rotational crops wheat, turnip and Swiss chard disclosed an already known metabolite, a glycoside conjugate of FOE thiadone, probably THNG (M25), and a new metabolite, i.e. trifluoroacetate, TFA (denoted as the parent substance trifluoroacetic acid, since the counter cation depends from the surrounding medium, and therefore varies and is not defined). Trifluoroacetate proved to be the main residue component in all plant metabolism and confined rotational crop studies with the [thiadiazole-5-^{14}C]-label. The combined metabolic pathway of flufenacet in plants is shown in Figure 6.2.1- 6. In order to find common major metabolites as potential marker substances for a residue analytical method all major metabolites of flufenacet in all investigated plants are compiled in a summary is presented in Table 6.2.1- 15 (given in % of TRR) and Table 6.2.1- 16 (given in mg equ/kg).

The parent substance flufenacet did not occur in any crop. The main flufenacet metabolites in corn, cotton, soybean, potato and wheat are marked in **bold** in the summary Table 6.2.1- 15 and Table 6.2.1- 16.

However, no metabolite can be found that proved to be major in all crops and can be selected as marker substance. Therefore, a common moiety method was developed as alternative method. Using the [fluorophenyl-UL-^{14}C]-labelled flufenacet all the metabolites containing a common moiety, i.e. "N-(4-fluorophenyl)-N-isopropyl amine" are compiled in the bluish array of Table 6.2.1- 15 and Table 6.2.1- 16. Based on these metabolites the residue definition of flufenacet residues in plants was proposed as parent substance and all metabolites containing the common moiety. When summing up the metabolites with the common moiety the resulting sum represents the major portion of TRR in most of the examined raw agricultural commodities, except in corn kernels with no identified residues ([redacted], 1994). This corn/maize study can be replaced by the study of [redacted] and [redacted] (1997)

conducted with the same plant species. Metabolites containing this common moiety are all located inside the blue frame in Figure 6.2.1- 6.

Using flufenacet radiolabeled as [thiadiazole-2-^{14}C] or [thiadiazole-5-^{14}C] flufenacet this results in other label-specific metabolites derived from the thiadone ring of flufenacet (highlighted in red).

EFSA, in principle, accepted the current residue definition in their "Reasoned opinion of the review of existing maximum residue levels (MRLs) of flufenacet" as published in the EFSA Journal 2012; 10(4): 2689. However, EFSA also mentioned that the 'common moiety residue definition' might not be "not the most adequate for enforcement proposes" and therefore proposed to investigate the option to include six individual metabolites in a multiresidue method. New residue trials would not be needed as the current common moiety method includes all of these metabolites.

In presentations held at the 9th European Pesticide Residue Workshop in Vienna (Austria) on 27-June-2012 and at the 7th International Fresenius Conference (Düsseldorf, 16 May 2013) a representative of the EFSA Pesticide Unit outlined EFSA's role and view relative to setting enforcement residue definitions.
Since flufenacet is included in the presentation as a case study this reference is considered to provide valuable information. In the contribution on "Potential and possible solutions for simplifying complex residue definitions" it is concluded that based on the metabolite pattern in plants the complex residue definition based on the N-fluorophenyl-N-isopropyl moiety is needed. The marker concept would not be an appropriate solution for deriving a residue method for enforcement of flufenacet residues; instead the common moiety approach would be more appropriate in this case. It is concluded that as a consequence a common moiety method has to be maintained.

This topic is further addressed in section CA 4.2 (analytical methods).

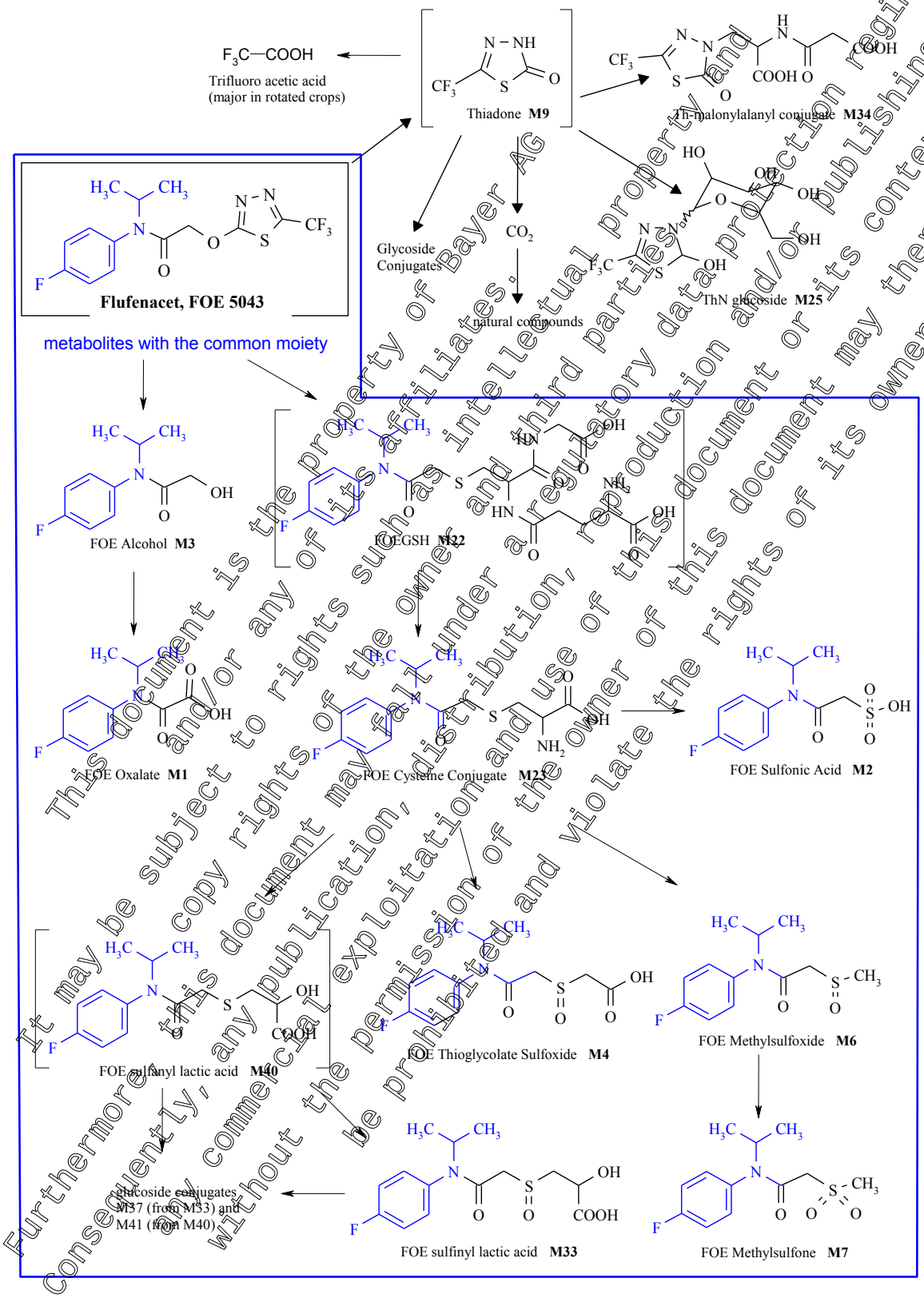
Table 6.2.1- 15: Metabolites of flufenacet in plant metabolism studies following pre- and post-emerg. application using three label positions (% TRR)

Crop (radiolabel)	Corn (F-phenyl)		Cotton (F-phenyl)		Soybean (F-phenyl)		Soybean (thia-2)		Potato (F-phenyl)		Wheat (F-phenyl)		Corn (F-phenyl)		Potato (thia-5)	Wheat (thia-5)	
Appl Rate [kg as/ha]	1.370 (pre)		1.778 (pre)		1.485 (pre)		1.380 (pre)		2.58 (pre)	3.01 (post)	0.52 (post)		1.46 (post)		0.630 (pre)	0.270 (post)	
Agricultural Commodity	Ker-nels	Fod-der	Seeds	Total plant	Beans	Fo-rage	Beans	Hay	Tuber	Tuber	Grain	Straw	Grain	Fod-der	Tuber	Grain	Straw
TRR [mg equ/kg]	0.012	0.498	0.067	1.54	1.02	8.49	0.68	5.78	0.35	0.32	0.62	2.04	0.11	1.91	0.867	0.704	2.974
A.S.	-	-	-	-	-	-	-	-	-	-	-	-	-	-			0.4
M1, -oxalate	-	**41**	-	11	6	18					**65**	14	-	**22**			
M2, -sulfonic acid	-	5	-	**66**	5	**42**			-	4		15	4				
M4, -thioglycolate sulfoxide	-	11	-	6	**26**	17			7		7		9	5			
M6, -methyl sulfoxide	-	1	-	-	6	6				-	-	-	-	-			
M7, -methyl sulfone	-	3	-	2	4	9			-	-			-	-			
M33, -sulfinyl lactic acid I, II	-	9	-	-	-	-			-	-		**35**	4	21			
M37, -sulfinyl lactic acid glucoside I, II	-	-	-	-	-	-				-	-	3	**23**	18			
M41, sulfanyl lactic acid glucoside	-	-	-	-	-	-			9	17	-	-	-	-			
M23, -cysteine	-	-				-			**44**	**52**	-	-	-				
M42, malonyl cysteine	-	-	-	-	-	-			-		-	-	-	16			
M25, THNG							-	**66**							1.8	0.4	33.5
M34, Th-malonyl-alanine							**66**	-							-	-	-
Other Th-conjugates							-	13							-	-	-
TFA							-	-							**92.3**	**99.2**	**61.7**
Reference	[redacted], 1994 M-002270-01-1		[redacted], 1995 M-002277-01-1		[redacted], [redacted], 1995 M-002279-01-1				[redacted], [redacted], 2000 M-020428-01-1		[redacted], [redacted], 1997 M-002275-01-1		[redacted], [redacted], 1998 M-005755-01-1		[redacted], 2012 M-441506-02-1	[redacted], [redacted], 2013 M-444475-01-1	

Table 6.2.1- 16: Metabolites of flufenacet in plant metabolism studies following pre- and post-emerg. application using three label positions (mg equ/kg)

Crop (radiolabel)	**Corn (F-phenyl)**		**Cotton (F-phenyl)**		**Soybean (F-phenyl)**		**Soybean (thia-2)**		**Potato (F-phenyl)**		**Wheat (F-phenyl)**		**Corn (F-phenyl)**		**Potato (thia-5)**	**Wheat (thia-5)**	
Appl Rate [kg as/ha]	1.370 (pre)		1.778 (pre)		1.485 (pre)		1.380 (pre)		2.58 (pre)	3.01 (post)	0.52 (post)		1.46 (post)		0.630 (pre)	0.270 (post)	
Agricultural Commodity	Ker-nels	Fod-der	Seeds	Total plant	Beans	Fo-rage	Beans	Hay	Tuber	Tuber	Grain	Straw	Grain	Fod-der	Tuber	Grain	Straw
TRR [mg equ/kg]	0.012	0.498	0.067	1.54	1.02	8.49	0.68	5.78	0.35	0.32	0.62	2.04	0.11	1.91	0.867	0.704	2.974
A.S.	-	-	-	-	-	-	-	-			-	-	-	-	-	-	0.4
M1, -oxalate	-	**0.205**	-	0.17	0.06	1.53					**0.30**	0.29	-	**0.42**			
M2, -sulfonic acid	-	0.026	-	**1.02**	0.05	**3.57**			-	0.01	-	0.31					
M4, -thioglycolate sulfoxide	-	0.056	-	0.09	**0.27**	1.44			-	0.02		0.14	0.01	0.10			
M6, -methyl sulfoxide	-	0.003	-	-	0.06	0.51			-	-	-	-	0.01				
M7, -methyl sulfone	-	0.016	-	0.03	0.04	0.76			-	-	-			-			
M33, -sulfinyl lactic acid I, II	-	0.045	-	-	-	-			-			**0.71**	-	0.40			
M37, -sulfinyl lactic acid glucoside I, II	-	-	-	-	-	-			-	-		0.06	**0.02**	0.34			
M41, sulfanyl lactic acid glucoside	-	-	-	-	-	-			0.07	0.05	-	-	-	-			
M23, -cysteine	-	-	-						**0.15**	**0.17**	-	-	-				
M42, malonyl cysteine	-		-	-	-	-			-		-	-	-	0.30			
M25, THNG							-	**3.81**							0.015	0.003	0.997
M34, Th-malonyl-alanine							**0.41**	-							-	-	-
Other Th-conjugates							-	0.75							-	-	-
TFA							-	-							**0.801**	**0.698**	**1.836**
Reference	[redacted], 1994 M-002270-01-1		[redacted], 1995 M-002277-01-1		[redacted], [redacted], 1995 M-002279-01-1				[redacted], [redacted], 2000 M-020428-01-1		[redacted], 1997 M-002275-01-1		[redacted], 1998 M-005755-01-1		[redacted], 2012 M-441506-02-1	[redacted], [redacted], 2013 M-444475-01-1	

Figure 6.2.1- 6: Proposed metabolic pathway of flufenacet in plants, combination of all plant metabolism studies with three different radiolabels



CA 6.2.2 Poultry

The nature of flufenacet resides in laying hen was investigated in the framework of Directive 91/414/EEC. The studies used [fluorophenyl-UL-^{14}C]flufenacet , [thiadiazole-2-^{14}C]flufenacet and [fluorophenyl-UL-^{14}C]flufenacet oxalate, the latter one being the main plant metabolite in poultry and ruminant feed. The studies were reviewed in the Monograph.

In the EFSA reasoned opinion a detailed assessment is provided on the review of the existing maximum residue levels according to Art 12 of Regulation (EC) no. 396/2005 (2012). The general metabolic pathways in rodents and livestock were found to be comparable.

Since the parent compound degrades rapidly in plants and is not detectable in animal feeding items the metabolism study using [fluorophenyl-UL-^{14}C] FOE oxalate provides the most relevant information. Oral administration of [fluorophenyl-U-^{14}C]flufenacet oxalate to ruminant and poultry showed its metabolic stability. Flufenacet oxalate is essentially not metabolised by the animal. The low residue levels in tissue, milk and eggs suggest that flufenacet oxalate is minimally absorbed and rapidly excreted. This metabolic stability was confirmed by a bio-availability study of flufenacet oxalate in rats[14]. Following oral administration of radiolabeled flufenacet oxalate to three rats at a dose rate of approx. 1 mg/kg bw 19 – 37% of the dose was excreted with urine and 61 – 80% was excreted with faeces as unchanged flufenacet oxalate.

The metabolism studies performed with flufenacet indicate a wide range of metabolites are formed containing the N-fluorophenyl-N-isopropyl moiety. Therefore, EFSA concluded that for commodities of animal origin, it is desirable to include all metabolites containing the N-fluorophenyl-N-isopropyl moiety in the residue definition, both for enforcement and risk assessment.

New plant metabolism studies with thiadiazole-5-^{14}C]flufenacet in primary and succeeding plants revealed trifluoroacetate (M45) as a major metabolite in edible plant parts and in plant parts intended as feeding stuff for livestock animals. For a complete dietary risk assessment including residues in food of animal origin, a potential residue transfer of trifluoroacetate from feeding stuff to food of animal origin has been investigated. Therefore, metabolism studies on ^{14}C-labelled trifluoroacetate in goat and hen were conducted.

Table 6.2.2- 1 provides an overview on the metabolism studies on laying hen.

[14] Part of the study of [redacted], M. E. and [redacted], L. L. (1995): Metabolism of FOE 5043 in Soybeans, unpublished report 105187 of Miles Inc. Kansas, USA, now Bayer CropScience, Comp. No. M-002278-01-1.

Table 6.2.2- 1: Overview of hen metabolism studies with ^{14}C-label flufenacet

Animal	Label	Report	Submission	
			EU baseline dossier, Annex II , Section 4, Point 6	Reported in supplementary dossier Section 6
laying hen	[Fluorophenyl-UL-^{14}C] FOE 5043	[redacted], R. G.; [redacted], P. L.; 1995; M-002251-01-1	KCA 6.2.2/01	-
	[Thiadiazole-2-^{14}C] FOE 5043	[redacted], F. K.; et al.; 1995; M-002253-01-1	KCA 6.2.2/02	-
	[Fluorophenyl-UL-^{14}C] FOE oxalate	[redacted], F. K.; et al.; 1995; M-004474-01-1	KCA 6.2.2/03	-
	[1-^{14}C] Trifluoroacetic acid	[redacted]; [redacted]; 2013; M-463376-01-1	-	KCA 6.2.2/04

Metabolism of trifluoroacetic acid in laying hen

Under natural, physiological and environmental conditions TFA is dissociated and appears as TFA salt. The counter cation depends on the chemical surrounding and is thus not defined. Therefore, TFA is expressed as the parent compound of the salts, i.e. as TFA-acid, keeping in mind that a TFA salt was administered to the animals.

Report:	**KCA 6.2.2/04,** [redacted]; [redacted]; 2013; M-463376-01-1
Title:	[1-^{14}C]Trifluoroacetic acid - Metabolism in the Laying Hen
Document No:	M-463376-01-1
Report No:	EnSa-12-0648, dated 2013-09-02
Guidelines and data requirements:	OECD guideline 503, Metabolism in Livestock, adopted 8-January-2007, U.S.OPPTS guideline 860.1300, Nature of Residues – Plants, Livestock, 1996 Compliant with EU Regulation (EC) No. 1107/2009 amended by Commission Regulation (EU) No 283/2013
GLP	yes

Executive Summary

A metabolism study with ^{14}C-labelled Na-TFA was conducted with six laying hens as TFA revealed to be a major metabolite in plants that were treated with flufenacet and are intended as poultry feed. This study is needed for a dietary risk assessment including food of animal origin to address the transfer of TFA residue from feedstuffs to eggs and edible animal tissue.

TFA was orally administered per gavage as ^{14}C-TFA-Na to the hens for 14 consecutive days with one dose per day. The dose level expressed as trifluoroacetic acid, TFA, was 0.50 mg/kg bw/day corresponding to 7.84 mg TFA/kg dry feed/day.

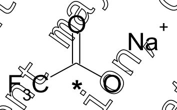
The radioactive residues in eggs reached a mean residue plateau of 0.391 mg TFA parent equivalents/kg (mg equ/kg) after 8 daily administrations. The birds were slaughtered six hours after the last dose and the radioactive residues were determined in edible organs and tissues. Average residues from six birds accounted for 0.090 mg equ/kg in fat, 0.615 mg equ/kg in muscle, 0.760 mg equ/kg in liver, 1.343 mg equ/kg in kidneys, and 1.101 mg equ/kg in skin.

The samples were extracted with acetonitrile/water and the extracts analysed by radio-HPLC (reversed phase) and radio-TLC (straight phase). Identification of the radioactivity in all samples using co-chromatography with authentic ^{14}C-TFA reference and two chromatographic methods with different modes of separation generally showed only one chromatographic peak that was unambiguously identified as TFA. No other radioactive peak appeared in any sample. Therefore, the conclusion has to be drawn that the total radioactivity in eggs, organs and tissues consisted of the unchanged TFA.

By comparison of the residue levels in feed, eggs, organs and tissues the following transfer factors for the residue transfer of TFA from animal fodder to food of animal origin could be derived: eggs (at plateau): 0.0499; muscle: 0.0784; fat: 0.0115; liver: 0.0969.

Material and methods

Test Material

Structural formula	 * denotes the ^{14}C label
Chemical name	Sodium trifluoroacetate
CAS RN	2932-18-4
Empirical formula	$C_2F_3NaO_2$
Company code	BCS-AZ56567
Molar mass (non-labelled)	136.01 g/mol
Label	1-^{14}C
Specific radioactivity	4.08 MBq/mg = 110.14 µCi/mg
Radiochemical purity	>98% by TLC and HPLC (radio-detection)
Remark	Trifluoroacetate appeared as anion under physiological and environmental conditions. The corresponding cation depends on the chemical surrounding and, thus, is not defined. Therefore, the residue levels of trifluoroacetate are expressed as the parent substance trifluoro-acetic acid (TFA). A conversion is conducted via the ratio of the molar masses: MM (trifluoroacetic acid) / MM (sodium trifluoro acetate) = 114.02/136.01 = 0.8383 The specific radioactivity of the respective trifluoroacetic acid (TFA) is

	therefore: 4.08 MB/mg / 0.8383 = 4.87 MBq/mg

Test Animal

Species	Hen (*Gallus gallus domesticus*)
Breed	White Leghorn
Sex, number	Six female laying hen
Mean body weight	1.57 kg at test start (1.45 – 1.65 kg)
Age	Approx. 6 months
Acclimatization	14 days before administration
Housing	Each 1 bird per stainless steel metabolism cage, approx. 24°C, approx. 31% rel. humidity, 16/8 hours light/dark cycle, 10-15 air changes per hour
Identification	Individual animal number using cage cards and wing tags
Feed and water	Commercial hen feed supplemented by eggshells and crushed marine shells, *ad libitum*. Tap water from local supplier, *ad libitum*
Health status	Acceptable according to veterinary investigation

Preparation of the dosing mixtures and administration

The radiolabelled solid sodium trifluoroacetate was dissolved in water resulting in a concentration of 0.59 mg/mL (corresponding to 0.49 mg TFA/mL). The exact concentration, radiochemical purity and the identity were determined by radioassaying, radio-TLC and LC-MS/MS using small aliquots of the dosing solution. Dosing aliquots of 1.0 mL/kg bw were orally administered by gavage using a syringe attached to an animal-feeding knob cannula. Directly after dosage, the swallowing reflex was supported by a gentle massage of the throat in direction of the crop. Each bird received one dose per day for 14 consecutive days. The average daily dose was 0.79 mg TFA per bird corresponding to 0.50 mg TFA/kg bw/day. With reference to the daily feed consumption this dose corresponded to 7.84 mg TFA/kg dry feed/day. This dose was tolerated without any observable toxicological effects.

Collection and processing of eggs and excreta

During the test, the grates of the cages were inspected for egg production once daily and the number of eggs was recorded for all hens. The eggs were collected during the 24 hour period after each administration and labelled with animal number and date. After removal of the shells, the contents of each egg were weighed and thoroughly mixed afterwards. An aliquot of each homogenate radioassayed and the remaining samples were stored in a freezer until metabolite analysis.

The excreta of each hen were collected from the collecting tins as far as possible quantitatively in daily intervals until sacrifice. The individual samples were homogenized after adding of water, before the total weights were recorded. An aliquot of each fraction was radioassayed and the remaining samples were stored in a freezer until metabolite analysis.

Sacrifice and collection of organs and tissues

The animals were sacrificed approx. 6 hours after the last dose. Each hen was transferred into a special cage, weighed and anaesthetized using carbon dioxide gas. Under general anaesthesia the animals were sacrificed by decapitation followed by exsanguination. The following organs and tissues were dissected: muscle (leg and thorax), fat (subcutaneous), liver (without gall bladder), skin (without subcutaneous fat) kidney and eggs from the ovary as well as oviduct.

The tissue samples were weighed and passed several times through a mincing machine in half-frozen state. The resulting homogeneous pulp was radioassayed and stored frozen (≤ - 18°C) until metabolite analysis.

Radioassaying and processing of samples

Radioactivity measurements (radioassaying) were conducted by liquid scintillation counting (LSC); aliquots of liquid samples were directly measured, aliquots of solid samples were first combusted using a sample oxidizer, the formed $^{14}CO_2$ was absorbed in an alkaline scintillation cocktail and the resulting solution radioassayed by LSC. The limit of quantification (LOQ) of radioassaying depended on the specific radioactivity of the test substance, the amount of aliquot measured and the background radioactivity. It was exemplarily given as 0.0005 mg equ/kg.

For metabolism investigations, aliquot samples from eggs, muscle and liver were conventionally extracted with acetonitrile/water (8/2; v/v, 3x) and pure acetonitrile using a high-speed stirrer. Fat was extracted with n-heptane/acetonitrile (9/1; v/v) and acetonitrile/water/n-heptane (7/2/1; v/v/v). The liquid phases were filtrated from the solids. In case of fat, the extracts were separated in an unpolar (n-heptane) and a polar (acetonitrile/water) fraction. The unpolar fraction was again extracted with acetonitrile/water and the polar fraction with n-heptane. The total radioactivity extracted from fat finally partitioned into the combined polar acetonitrile/water phase. The acetonitrile/water extracts were concentrated and analyzed by radio-HPLC and radio-TLC. The remaining solids were radioassayed via combustion.

Radio-chromatography and mass spectrometry of samples

Radio-HPLC was conducted using a reversed-phase column (RP18, 250 x 4.6 mm, 5 µm particles) that was operated with a gradient mixture of water/formic acid (99/1, v/v) and acetonitrile/formic acid (99/1, v/v) at 40°C. The system was equipped with an UV detector (254 nm) and a radiomonitor with a solid glass scintillator (cell volume 370 µL). The LOQ was derived from background level of the baseline and the highest peak in the chromatogram. It ranged from 0.001 mg equ/kg (muscle and fat extract) to 0.004 mg equ/kg (egg extract). Column recovery was determined by comparison of the eluted radioactivity with column and detector and without column and detector. It generally accounted for 99.7%. ^{14}C-labelled TFA was co-injected to identify the residues in the samples.

Radio-TLC was conducted on a silica gel TLC plate (20 x 20 cm) that was developed with a solvent mixture of ethyl acetate/2-propanol/water/acetic acid (65/24/22/1, v/v/v/v). Following development the radioactive spots were detected by radioluminography via exposure of an imaging plate for 14 hours. The detection limit was approximately 5-10 dpm/spot after an exposure period of at least 14 hours. ^{14}C-TFA was also used as reference standard.

The test substance TFA was identified by LC-MS/MS consisting of anion exchange chromatography and a high resolution mass spectrometer. For chromatography an anion exchanging Dionex column was eluted with an aqueous solution of 20 mmol KOH as isocratic liquid phase. A Q-Exactive mass spectrometer was operated in the mode of electro-spray ionization.

Findings

Recovery of radioactivity in eggs, excreta and analyzed organs and tissues

Six hours after the last of 14 oral doses of ^{14}C-labelled TFA at a dose rate of 0.50 mg/kg bw/day 94.97% of the total radioactivity was recovered in eggs, excreta, muscle, fat liver and kidney. The remaining 5% of the total dose were assumed to be associated with the gastro-intestinal tract and the remaining body.

88.01% of the total dose was detected in the excreta. 1.91% of the total dose were found in the eggs and 5.06% were detected in the dissected edible organs and tissues with approx. 70% of this radioactivity (3.53% of dose) being associated with the skeletal muscle (assuming 40% of the body weight for skeletal muscle).

Radioactive residues in the eggs

The total radioactive residues (TRR) in the eggs ranged from 0.123 mg equ/kg at day two to 0.408 mg equ/kg at day 13. The time course of the TRR showed a more or less linear increase until seven administrations at a dose rate of 7.84 mg TFA/kg dry feed/day. By the eighth administration, TRR reached a pronounced residue plateau. The weighted mean amounted to 0.391 mg equ/kg between the 7th and 13th day (8th – 14th administration). The residue level of the last egg sample (0.607 mg equ/kg) was excluded from plateau calculation since the interval between dosing and egg collection (0.25 day) was significantly shorter than at the other days. Daily TRR levels in the eggs are compiled in Table 6.2.2- 2.

Radioactive residues in dissected organs and tissues

The TRR in edible organs and tissues ranged from fat amounting to 0.090 mg equ/kg to kidney amounting to 1.343 mg equ/kg. Skeletal muscle accounted for 0.615 mg equ/kg and skin for 1.101 mg equ/kg. The residue levels in all edible tissues of hen are compiled in Table 6.2.2- 3.

Extraction efficiency and identification of extracted residues

The majority of the radioactive residues (99.9% - 100% of TRR) in eggs, muscle, liver and excreta (Day 13) was extractable with acetonitrile/water (8/2; v/v) and pure acetonitrile. From fat, 95% of TRR could be extracted with heptane and acetonitrile/water that completely partitioned into the polar phase. Negligible amounts of ≤ 0.1% of the TRR (≤ 0.001 mg/kg) remained unextractable. Following concentration, 99.5% to 100% of the TRR in the extracts were analysed and quantified by radio-HPLC and radio-TLC.

The radio-chromatographic profiles of all extracts (eggs, muscle, liver, kidney, fat, and excreta) showed only one polar radioactive peak. Co-chromatography with the reference standard ^{14}C-TFA resulted in the same single peak that was unambiguously identified as radiolabelled TFA since two different chromatographic systems (reversed phase HPLC and straight phase TLC) were used. No other peak could be observed. Therefore, the total radioactivity in all samples represented unchanged TFA. Thus, the rate of identification in the samples was excellent amounting to 99.5 – 100% of TRR in all extracts.

Transfer factors of residue transfer of TFA from animal fodder to food of animal origin

The TFA transfer factors (TF) were calculated as mean ratio between the radioactive residues in animal fodder (based on dry mass) and the total radioactive residues in eggs, and edible organs and tissues of the six hens. Any correction for metabolic conversion products of TFA is not needed as total radioactive residue was represented by the administered test substance (see before). These transfer factors ranging from 0.0115 (fat) to 0.1713 (kidney) are listed in detail in Table 6.2.2- 4.

Conclusion

Following repeated oral administration of ^{14}C-labelled sodium trifluoroacetate (TFA-Na) to six laying hens for 14 consecutive days at a dose level of 0.50 mg TFA/kg bw/day (corresponding to 7.84 mg TFA/ kg dry feed/day) the radioactive residues in eggs reached a plateau level of 0.391 mg equ/kg after 7 daily administrations. 14 days after the first administration the hens were slaughtered and radioactive residues were determined in edible organs and tissues. These residues accounted for 1.101 mg equ/kg in fat, 0.615 mg equ/kg in muscle, 0.760 mg equ/kg in liver and 1.343 mg equ/kg in kidney. The samples were extracted with acetonitrile/water and the extracts analysed by radio-HPLC (reversed phase) and radio-TLC (straight phase). Identification of the radioactivity in all samples using co-chromatography with authentic ^{14}C-TFA and two chromatographic methods with different modes of separation generally showed only one chromatographic peak that was unambiguously identified as TFA. No other radioactive peak appeared in any sample. As a conclusion it can be stated that TFA is metabolically stable in poultry. It was rapidly excreted as not more than 5% of the total dose was detected in organs and tissues 6 hours after administration of the last dose.

By comparison of the residue levels in feed, eggs, organs and tissues the following transfer factors for the residue transfer of TFA from animal fodder to food of animal origin could be derived: eggs (at plateau): 0.0499; muscle: 0.0784; fat: 0.0115; liver: 0.0969; kidney: 0.1713.

Table 6.2.2- 2: Total radioactive residues (TRR) in eggs of hens orally administered with ^{14}C-TFA at a dose of 7.84 mg TFA/kg dry feed/day for 14 consecutive days (mean of 6 hens)

Time after the 1st administration	No. of administration	TRR in freshly laid eggs	Remark
[days]		[mg equ/kg]	
0	1	no egg sampled	
1	2	no egg sampled	
2	3	0.123	Continuous increase of residue level
3	4	0.218	
4	5	0.262	
5	6	0.310	
6	7	0.362	
7	8	0.396	**Plateau level of residues**
8	9	0.406	
9	10	0.410	
10	11	0.402	
11	12	0.395	
12	13	0.405	
13	14	0.408	
13.25	---	0.607	Short collection period
Weighted mean plateau level of 8th – 14th administration (days 7-13)		**0.391**	

Table 6.2.2- 3: Radioactive residues in organs and tissues of hens 6 hours after the last of 14 doses of ^{14}C-TFA at a dose level of 7.84 mg TFA/kg dry feed/day (mean of 6 hens)

Organ/Tissue	Mean Residue Level [mg equ/kg]
Liver	0.760
Kidney	1.343
Skeletal muscle, total	0.615
Leg muscle	0.712
Thorax muscle	0.507
Skin without fat	1.101
Subcutaneous fat	0.090
Eggs from ovary/oviduct	0.754

Table 6.2.2- 4: Transfer factors for residue transfer of ^{14}C-TFA from animal feed to eggs, muscle, fat, liver and kidney of hens following repeated administration at a dose level of 7.84 mg TFA/kg dry feed/day

Milk/Organ/Tissue	Residue level [mg equ/kg]	Transfer factor (TF)
Eggs (at residue plateau)	0.391	0.0499
Muscle	0.615	0.0784
Fat	0.090	0.0115
Liver	0.760	0.0969
Kidney	1.343	0.1713
Skin	1.101	0.1404

CA 6.2.3 Lactating ruminants

The nature of flufenacet resides in goat was investigated in the framework of Directive 91/414/EEC. The studies used [fluorophenyl-UL-^{14}C]flufenacet, [thiadiazole-2-^{14}C] flufenacet, [fluorophenyl-UL-^{14}C]flufenacet oxalate and [thiadiazole-2-^{14}C]thiadone-N-glucoside, the later two substances being the main plant metabolites in ruminant feed.

All studies except the later one were reported in the Monograph. In the EFSA reasoned opinion on the review of the existing maximum residue levels according to Art. 12 of Regulation (EC) no. 396/2005 (2012) a detailed assessment is provided. The general metabolic pathways in rodents and ruminants were found to be comparable.

The metabolism of [thiadiazole-2-^{14}C]thiadone-N-glucoside in the lactating goat was performed on request of the USEPA. It was not submitted with the former EU application. Therefore it is summarized in this submission.

Table 6.2.3- 1 provides an overview on the metabolism studies of flufenacet and major plant metabolites on lactating goat.

Table 6.2.3- 1: Overview of goat metabolism studies with ^{14}C-label flufenacet

Animal	Label	Report	Submission	
			EU baseline dossier, Annex II , Section 4, Point 6	Presented in supplementary dossier Section 6
Lactating goat	[Fluorophenyl-UL-^{14}C] FOE 5043	[redacted], R. G.; [redacted], P. L.; 1995; M-002250-01-1	KCA 6.2.3/01	-
	[Thiadiazole-2-^{14}C] FOE 5043	[redacted], F. K.; et al.; 1995; M-002248-01-1	KCA 6.2.3/02	
	[Fluorophenyl-UL-^{14}C] FOE oxalate	[redacted], F. K.; et al.; 1995; M-004478-01-1	KCA 6.2.3/03	
	[Thiadiazole-2-^{14}C] thiadone-N-glucoside	[redacted], M. E et al.; 2002; M-079251-01-1		KCA 6.2.3/04
	[1-^{14}C] Trifluoroacetic acid	[redacted], J. C. et al.; 2013; M-444659-01-1	-	KCA 6.2.3/05

Metabolism of thiadone-N-glucoside (THNG) in the lactating goat

In metabolism studies of [thiadiazole-2-^{14}C]flufenacet in soybeans and rotational crops (e.g. wheat) a major residue component in ruminant feed (forage, hay, straw) was detected as thiadone-N-glucoside (M25, THNG), whereas the parent substance was not present. Therefore, a metabolism study with a lactating goat was conducted using this metabolite to discover the residues in food of animal origin. This study was performed on request of the USEPA to investigate the metabolic fate and bioavailability of THNG in a lactating ruminant.

Report:	**KCA 6.2.3/04,** [redacted]; 2002; M-079251-01-1
Title:	The metabolism of FOE 5043 Thiadone N-Glycoside in a Lactating Goat.
Document No:	M-079251-01-1
Report No:	E3041002
Guidelines and data requirements	EPA Ref.: 860.1300 – Nature of residue – livestock; 870.7485 – Metabolism and pharmacokinetics
GLP	yes

Executive Summary

To determine the metabolic fate of thiadone-N-glucoside (THNG, M25) in ruminants [thiadiazole-2-^{14}C]THNG was administered orally as a single dose to a lactating pygmy goat at a dose rate of 0.432 mg/kg bw. corresponding to 16.3 mg/kg feed.

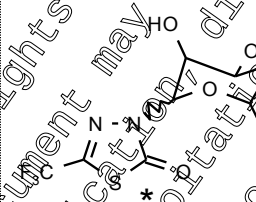
[Thiadiazole-2-^{14}C]THNG was well absorbed and metabolized. Recovered radioactivity accounted to 91% of the dose. The majority of radioactivity was (72% of the dose) was excreted with the urine, and a smaller amount was excreted with the faeces (7% of the dose). Very little of the dose (< 1%) was observed in the milk. The maximum residue level in milk (0.040 mg THNG equ/kg) was detected in the milk secreted at the day of administration.

Residue levels found in tissues were 0.215 mg equ/kg in whole blood, 0.175 mg equ/kg in kidneys, 0.125 mg equ/kg in the liver, 0.059 mg equ/kg in the GIT, 0.025 mg equ/kg in muscle tissue, and 0.008 – 0.040 mg equ/kg in milk sampled until day 7 after administration.

The metabolism of THNG was through oxidative and hydrolytic processes and conjugation as concluded from the metabolites excreted with the urine. The main residue in liver, kidney, muscle and fat was free thiadone. However, thiadone is expected to be negligible in food of animal origin as the goat in this study was significantly overdosed with THNG. In milk, no thiadone was detectable. From the metabolites found in the urine a proposed metabolic pathway was concluded. It is shown in Figure 6.2.3- 1.

Material and methods

Test Material

Structural formula	 * denotes the ^{14}C label
Chemical name	Thiadone-N-glucoside
Empirical formula	$C_9H_{11}F_3N_2O_6S$
Company code	THNG
Molar mass (non-labelled)	332.26 g/mol
IUPAC name	3-hexopyranosyl-5-(trifluoromethyl)-2,3-dihydro-1,3,4-thiadiazol-2-one; 3-glucosyl-5-trifluoromethyl-1,3,4-thiadiazol-2(3*H*)-one
Label	[thiadiazole-2-^{14}C]
Specific radioactivity	9.41 mCi/mmol = 63000 dpm/µg (1.048 MBq/mg)
Radiochemical purity	>99% by HPLC (radio-detection)

Test Animal

Species	Pygmy goat
In-vivo phase	Southwest Biolabs, Inc.; Las Cruces, NM, USA
Analytical phase	Bayer Research Park, Stilwell, KS, USA
Sex, number	One female lactating goat
Body weight	18.6 kg at receiving
Age	Approx. 2.5 years
Acclimatization	Two days before administration
Housing	Stainless steel metabolism cage, 19-31°C, 20-79% rel. humidity, 14/10 hours light/dark cycle
Feed and water	Ruminant feed, alfalfa pellets, hay, *ad libitum* Fresh potable water, *ad libitum*
Health status	Normal and acceptable according to veterinary investigation

Preparation of the dosing mixtures and administration

The solid radiolabelled test substance was dissolved in a small amount of methanol and filled into a gelatin capsule that contained α-lactose. The methanol was allowed to evaporate, and the capsule was sealed at ambient temperature. The sealed capsule was orally administered using a balling gun. The actual dose rate was 0.432 mg/kg bw/day corresponding to 16.3 mg/kg in feed based on an average feed consumption of 0.493 kg feed/day.

Collection of milk, urine and faeces

The goat was milked twice daily in the morning and evening until 168 hours post dose. The milk samples were weighed, subsampled and stored frozen.

Urine and faeces were separately collected on a daily basis until 168 hours post dose (additional urine collection: 6 and 12 hours after dosing). Faeces samples were blended with distilled water until homogenous. Aliquots of the milk and excreta samples were radioassayed.

Sacrifice and collection of organs and tissues

A major portion of blood was collected prior to termination of the animal. On day 7, the goat was humanly terminated with a captive bolt pistol. At necropsy, bile, liver, kidneys, fat, muscle, the GI tract and the residual carcass were collected, weighed and stored frozen. Liver, kidney and fat samples were homogenized with dry ice.

All samples were shipped to the Bayer Research Park in frozen stage for analysis.

Sample extraction and processing

Urine profiles were determined by radio-HPLC for each collection point. The identification and characterization of the radioactive compounds were made for a composite urine sample from all time points.

Faeces samples were extracted four times with acetonitrile/water (9/1, v/v). The combined extracts were radioassayed and purified by passing through a C18 solid phase extraction cartridge, concentrated. The solids were further extracted with methanol, 1N aqueous hydrochloric acid and 2N

sodium hydroxide in succeeding steps, each time for 18 hours under reflux. Each subsample was radioassayed.

Day-1 milk samples were lyophilized and the resulting solid extracted three times with methanol followed by extraction with water/acetonitrile (9/1, v/v). The methanol extract was radioassayed and analyzed by radio-HPLC.

Blood samples were mixed with acetonitrile and the resulting suspension separated by centrifugation. The supernatant was evaporated to dryness and dissolved in water/acetonitrile (9/1, v/v) for radioassaying and analysis by radio-HPLC.

Homogenized liver, kidney and muscle samples were separately extracted three times with acetonitrile/water (9/1, v/v). The combined extracts were concentrated to dryness and redissolved in acetonitrile. In case of liver, the resulting acetonitrile solution was partitioned against n-hexane (3x). In each case, the acetonitrile solution was radioassayed and analyzed by radio-HPLC.

Fat samples were extracted three times with hexane. The combined hexane solution was partitioned against acetonitrile. The residual solids from the hexane extraction were extracted three times with acetonitrile. The acetonitrile partition from the hexane extracts and the acetonitrile extracts were combined, radioassayed and analyzed by radio-HPLC.

Homogenized GIT sample was extracted three times with acetonitrile. The combined extract was radioassayed and analyzed by radio-HPLC.

Radioassaying

Radioactivity measurements (radioassaying) were conducted by liquid scintillation counting (LSC); aliquots of liquid samples were directly measured, aliquots of solid samples were first combusted using a sample oxidizer. The formed $^{14}CO_2$ was absorbed in an alkaline scintillation cocktail and the resulting solution radioassayed by LSC. The minimum sensitivity of LSC was 0.00055 mg equ/kg for liquid and 0.0006 mg equ/kg for solid samples.

Radio-chromatography and mass spectrometry of the extracts

Radio-HPLC was conducted using a reversed-phase column (RP18, 250 x 4.6 mm, 10 µm particles) that was operated with a gradient mixture of 0.1% aqueous acetic acid and methanol. The system was equipped with an UV detector and a radiomonitor.

A combination of liquid chromatography/electrospray mass spectrometry (LC/ESI-MS) was employed for structure evaluation. Mass spectrometry was performed in both the positive and negative ionization modes.

^{19}F-NMR spectroscopy

^{19}F-NMR spectra of isolated urine metabolites and reference standards were recorded in methanol solutions. The magnetic field strength was 14.0 Tesla. The observation frequency was 564.717 MHz for ^{19}F. Chemical shifts were reported as parts per million (ppm) downfield from external trifluoro acetic acid.

Findings

Recovery of radioactivity in milk, excreta and analyzed organs and tissues (Table 6.2.3- 2)
At study termination, 7 days after the oral dose of ^{14}C-THNG, the total recovery of the ^{14}C-label amounted to 91% of the administered dose. The predominant portion of the dose was excreted with the urine (72% of the dose), while only a tenth of the urinary radioactivity was found in the faeces (7% of the dose). Less than 1% of the dose was detected in the (total) milk and 1% of the dose in liver. Kidney, muscle and fat contained less than 1% of the dose.

Residue levels in milk, blood and organs and tissues (Table 6.2.3- 2)
The highest residue level was detected in the blood amounting to 0.215 mg THNG equ/kg followed by the excretory and metabolizing organs kidney (0.175 mg equ/kg) and liver (0.125 mg equ/kg). Muscle and fat amounted to 0.025 and 0.059 mg equ/kg. The highest residue level in milk was found at the first day after dosing amounting to 0.040 mg equ/kg.

Composition of residues in milk and dissected organs and tissues (Table 6.2.3- 3)
Milk samples of day one after dosing (morning and evening milk) showed 4 radioactive peaks in the radio-HPLC analysis. None of these peaks could be identified, but thiadone (TH) could definitely be excluded by comparison the HPLC elution times.

In the extracts of liver, kidney, muscle, fat and blood only one radio-peak was detected that could be attributed to thiadone by comparison of the HPLC elution time and by LC/MS.

Radioactive residues in urine and faeces (Table 6.2.3- 3)
The major portion of renally excreted residues originating from THNG (thiadone-N-glucoside) was the oxidation product THNGA (thiadone-N-glucuronic acid, 37% of the dose) and the original test compound THNG conjugated with an additional glucuronic acid (THNG-GA, 4% of the dose). Instead of this extra glucuronic acid also sulfuric acid can be linked to form THNGSA (7% of the dose). In these conjugates, the C-N bond between thiadone and endocons remained intact as indicated by ^{19}F-NMR analysis. A small portion of free thiadone (TH) was detected in the urine amounting to of 7% of the dose.

In faeces, only 1% of the dose was detected as the oxidation product THNGA and another 1% of the dose as free thiadone (TH).

Conclusion

Following administration of the radiolabelled thiadone-N-glucoside (THNG) to a lactating goat the radioactive residue was well absorbed and almost completely excreted. The main route was through the urine, with a renal-to-fecal excretion ratio of 10:1. The main metabolic conversion of THNG (in urine) was the oxidation of the glucoside endocon to glucuronic acid and an additional conjugation with glucuronic or sulfuric acid. None of the conjugated metabolites were formed from free thiadone (TH).

While free thiadone was detected in edible tissues of the goat, the residue levels were low at 1x feeding level as the goat in this study was administered with an exaggerated dose. No free thiadone was detected in the milk.

The proposed metabolic pathway of THNG in the goat was derived from the metabolites in urine. The major detoxification proceeded initially through oxidation and conjugation reactions of THNG prior to excretion. The pathway is shown in Figure 6.2.3- 1.

Table 6.2.3- 2: Distribution of radioactive residues in excreta, milk and organs and tissue of a goat 7 days after a single oral dose of [thiadiazole-2-^{14}C]thiadone-N-glucoside (THNG) at a feeding level of 16.3 mg/kg feed/day

(given in % of dose and mg equ. of THNG/kg)

Excreta/Milk/Organ/Tissue	Residue level	
	% of dose	[mg equ/kg]
Urine (total)	72	-
Faeces (total)	7	-
Milk (total)	< 1	max. 0.040 at the first day
Liver	1	0.125
Kidney	< 1	0.175
Muscle	< 1	0.025
Fat	< 1	0.059
Gastrointestinal tract	3	0.057
Bile	< 1	0.014
Blood	2	0.215
Residual carcass*)	6	0.001
Cage wash	< 1	-
Total recovery	91	-

*) Radioactivity in the residual carcass was estimated based on the carcass weight and the average residues in muscle and fat

Table 6.2.3- 3: Radioactive residues in excreta, milk and organs and tissue of a goat 7 days after a single oral dose of [thiadiazole-2-^{14}C]thiadone-N-glucoside (THNG) at a level 16.3 mg/kg feed/day

(given in % of dose or % of TRR in milk/organ/tissues)

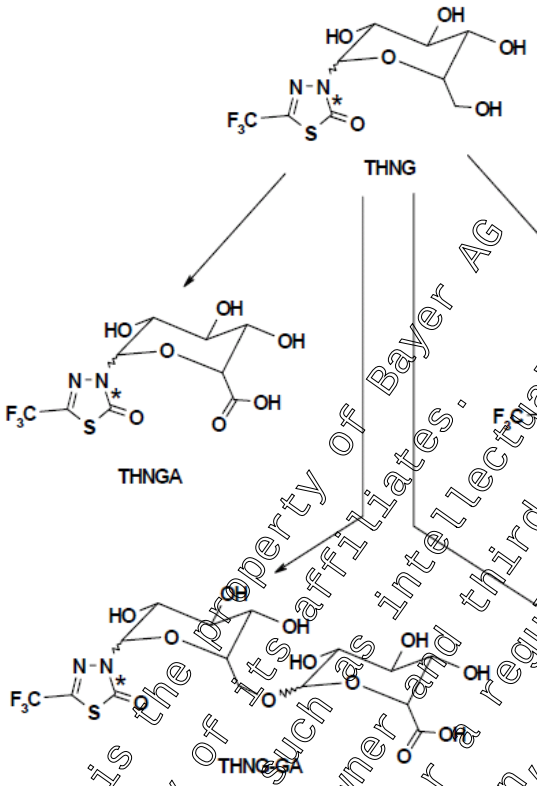
	Residue component				
	THNGA + THNG-GA[#]) (M24)	THNG-SO3H	THNG (M25)	TH (M9)	unknowns
Excreta/carcass	[% of dose]				
Urine (total)	41	7	12	7	6
Faeces (total)	1	-	-	1	-
Residual carcass	-	-	-	6	-
Milk/organ/tissue	[% of TRR in milk/organ/tissue]				
Milk (total)	-	-	-	-	100 (4 peaks)*)
Liver	-	-	-	92	-
Kidney	-	-	-	90	-
Muscle	-	-	-	95	-
Fat	-	-	-	48	-
Blood	-	-	-	90	-

[#]) mixture of the two components THNGA (37%) and THNG-GA (4%)

*) The radiopeaks in milk did not co-eluate with TH

Figure 6.2.3- 1: Proposed metabolic pathway of thiadone-N-glucoside in the lactating goat

(concluded from the metabolites observed in urine)



* position of ^{14}C-label

Metabolism of trifluoroacetic acid in lacating goat

Under natural, physiological and environmental conditions TFA is dissociated and appears as TFA salt. The counter cation depends on the chemical surrounding and is, thus, not defined. Therefore, TFA is expressed as the parent compound of the salts, i.e. as TFA-acid, keeping in mind that a TFA salt was administered to the animals.

Report:	**KCA 6.2.3/05,** [redacted]; 2013; M-444459-01-1
Title:	[1-^{14}C]Trifluoroacetic acid - Metabolism in the Lactating Goat.
Document No:	M-444459-01-1
Report No:	EnSa-12-0628
Guidelines and data requirements:	OECD guideline 503, Metabolism in Livestock, adopted 8-January-2007, US-OPPTS guideline 860.1300, Nature of Residues – Plants, Livestock, 1996 Compliant with EU Regulation (EC) No. 1107/2009
GLP:	yes

Executive Summary

A metabolism study with ^{14}C-labelled Na-TFA was conducted with a lactating goat as TFA revealed to be a major metabolite in plants that were treated with flufenacet and are intended as ruminant feed. This study is needed for a dietary risk assessment including food of animal origin to address the transfer of TFA residue from feedstuffs to milk and edible animal tissue.

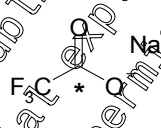
TFA was orally administered per gavage as ^{14}C-Na-TFA to the goat for five consecutive days with one dose per day. The dose level expressed as trifluoroacetic acid, TFA, was 0.90 mg/kg bw/day corresponding to 11.9 mg TFA/kg dry feed/day.

The radioactive residues in milk reached a steady state at approximately 30 hours after the first dose amounting to a plateau level of 0.102 mg parent equivalents/kg (mg equ/kg). Five days after the first administration the goat was slaughtered and radioactive residues were determined in edible organs and tissues. These residues accounted for 0.091 mg equ/kg in fat, 0.347 mg equ/kg in muscle, 0.551 mg equ/kg in liver and 0.967 mg equ/kg in kidney. The samples were extracted with acetonitrile/water and the extracts analyzed by radio-HPLC (reversed phase) and radio-TLC (straight phase). All radio-chromatograms showed only one chromatographic ^{14}C-peak, Co-chromatography with authentic ^{14}C-TFA using two chromatographic methods with different modes of separation unambiguously identified the radioactive peak as TFA. No other radioactive peak appeared in any sample. Therefore, the conclusion is drawn that the total radioactivity in milk, organs and tissues consisted of the unchanged TFA.

By comparison of the residue levels in feed, milk, organs and tissues the following transfer factors for the residue transfer of TFA from animal fodder to food of animal origin could be derived: milk (at plateau): 0.0086; muscle: 0.0292; fat: 0.0076; liver: 0.0463; kidney: 0.0813.

Material and methods

Test Material

Structural formula	 * denotes the ^{14}C label
Chemical name	Sodium trifluoroacetate
CAS RN	2932-18-4
Empirical formula	$C_2F_3NaO_2$
Company code	BCS-AZ56567
Molar mass (non-labelled)	136.01 g/mol
Label	1-^{14}C
Specific radioactivity	4.08 MBq/mg = 110.14 µCi/mg
Radiochemical purity	>98% by TLC and HPLC (radio-detection)
Remark	Trifluoro acetate appeared as anion under physiological and environmental conditions. The corresponding cation depends on the chemical surrounding and, thus, is not defined. Therefore, the residue

	levels of trifluoro acetate are expressed as the parent substance trifluoro acetic acid (TFA). A conversion is conducted via the ratio of the molar masses: MM (trifluoroacetic acid) / MM (sodium trifluoro acetate) = 114.02/136.01 = 0.8383 The specific radioactivity of the respective trifluoroacetic acid (TFA) is therefore: 4.08 MB/mg / 0.8383 = 4.87 MBq/mg

Test Animal

Species	Goat (*Capra hircus*)
Breed	"Weiße deutsche Edelziege"
Sex, number	One female lactating goat
Body weight	52 kg at first administration, 51 kg at sacrifice
Age	Approx.15 months
Acclimatization	Two week before administration
Housing	Stainless steel metabolism cage, 18°C, approx. 60% rel. humidity, 12/12 hours light/dark cycle, 10-15 air changes per hour
Feed and water	Ruminant feed, hay, hay pellets, carrot, *ad libitum* Tap water from local supplier, *ad libitum*
Health status	Acceptable according to veterinary investigation

Preparation of the dosing mixtures and administration

Aliquots of the solid radiolabelled test substance were filled into five gelatin capsules. The sealed capsules were stored at ≤ -18°C until administration. Remaining test substance was used for identification via LC-MS/MS and to demonstrate the storage stability during the dosing period via radio-TLC. One capsule per day was orally administered in the morning for five succeeding days using a capsule applicator. The average daily dose amounted to 30.9 mg sodium trifluoroacetate (corresponding to 25.9 mg trifluoroacetic acid, TFA). Referred to the daily feed consumption and the body weight, this dose corresponded to a dose level of 11.9 mg TFA/kg dry feed or 0.50 mg TFA/kg bw/day. This dose was tolerated without any observable toxicological effects.

Collection of milk, urine and faeces

The goat was milked in the morning immediately prior to each administration, and eight hours after administration and directly before sacrifice. The collection intervals for milk sampling were: 0-8, 8-24, 24-32, 32-48, 48-56, 56-72, 72-80, 80-96, and 96-120 hours after the first administration. The milk samples were weighed, radioassayed via liquid scintillation counting (LSC) and stored at ≤ -18°C for 97 days.

Urine and faeces were collected on a daily basis. Urine was collected in plastic vessels under dry ice cooling. The faeces samples were homogenized after addition of water to yield a wet paste. Aliquots of the excreta were radioassayed.

Sacrifice and collection of organs and tissues

Six hours after the last dose, the goat was sedated and anaesthetized by injection of Xylazin/Rompun, Ketamin and Pentobarbital-Na. Under deep anaesthesia, the animal was exsanguinated by cannulating the jugular vein and finally terminated by intracardiac injection of the veterinary drug "T 61®". Then, the goat was slaughtered and the following organs and tissues were dissected and stored at ≤ -18°C

until analysis (103 - 124 days): round and loin muscle, omental and perennial fat, liver (without gall bladder), and kidneys.

Radioassaying and processing of samples

Radioactivity measurements (radioassaying) were conducted by liquid scintillation counting (LSC); aliquots of liquid samples were directly measured, aliquots of solid samples were first combusted using a sample oxidizer, the formed $^{14}CO_2$ was absorbed in an alkaline scintillation coektail and the resulting solution radioassayed by LSC.

For metabolism investigations, a composite sample of milk collected from 0 h to 102 h (time of sacrifice) after the first administration and composite samples of muscle (loin and round muscle) and fat (perirenal and omental) were prepared. The composite milk, muscle and fat samples and the complete liver, both kidneys and one faeces sample (72 - 96 h) were thoroughly homogenized and kept frozen until extraction. Each sample (except fat) was extracted with acetonitrile/water (8/2, v/v) and pure acetonitrile using a high-speed stirrer. The fat was extracted with mixtures of n-heptane and acetonitrile/water (8/2, v/v) also using a high-speed stirrer followed by separation of the heptane and the aqueous layer. All acetonitrile/water extracts were concentrated and analyzed by radio-HPLC and radio-TLC.

Radio-chromatography and mass spectrometry of the extracts

Radio-HPLC was conducted using a reversed-phase column (RP18, 250 x 4.6 mm, 5 µm particles) that was operated with a gradient mixture of water/formic acid (99/1, v/v) and acetonitrile/formic acid (99/1, v/v) at 40°C. The system was equipped with an UV detector (254 nm) and a radiomonitor with a solid glass scintillator (cell volume 370 µL). The limit of quantification (LOQ) was derived from background noise level of the baseline and the highest peak in the chromatogram. It ranged from 0.001 mg equ/kg (milk, fat, kidney) to 0.005 mg equ/kg (liver). Column recovery was determined by comparison of injected and eluted radioactivity. In each case, it accounted for 96.2 – 99.6%. ^{14}C-labelled TFA was co-injected to identify the residues in the samples.

Radio-TLC was conducted on a silica gel TLC plate (20 x 20 cm) that was developed with a solvent mixture of ethyl acetate/2-propanol/water/acetic acid (65/24/22/1, v/v/v/v). Following development the radioactive spots were detected by radioluminography via exposure of an imaging plate to the radioactive spots. The detection limit was approximately 5-10 dpm/spot after an exposure period of at least 14 hours. ^{14}C-TFA was also used as reference standard.

The test substance TFA was identified by LC-MS/MS consisting of anion exchange chromatography and a high resolution mass spectrometer. For ion exchange chromatography a Dionex column was eluted with an aqueous solution of 20 mmol KOH as liquid phase. The mass spectrometer was operated in the mode of electro-spray ionization. This test substance was also used as reference standard in radio-HPLC and radio-TLC of the extracts.

Findings

Recovery of radioactivity in milk, excreta and analyzed organs and tissues

Six hours after the last of five oral doses of 0.50 mg/kg bw/day of ^{14}C-labelled TFA approximately 69% of the total radioactivity was recovered in milk, excreta, muscle, fat, liver and kidney. The remaining 31% of the total dose were assumed to be associated with the gastro-intestinal tract and the remaining body. 47.3% of the total dose was excreted with the urine and 15.1% with the faeces. 1.14% of the total dose was secreted into the milk and 5.1% were detected in the dissected edible organs and tissues with 4.1% of the dose being associated with the muscular tissue (assuming 30% of the body weight to the muscular mass).

Radioactive residues in the milk

The total radioactive residues (TRR) in the milk ranged from 0.079 mg equ/kg to 0.145 mg equ/kg in the collection period 48 to 80 hours after the first administration. At the time of sacrifice a further increase to 0.171 mg equ/kg was observed due to the shorter time interval between the last dosing and sampling (ca. 6 hours). The time course of radioactivity in milk showed a typical diurnal pattern with temporal peaks eight hours after each administration and sinks shortly before the next dosing (Table 6.2.3- 4). A plateau level was reached approximately 30 hours after the first administration. This level was calculated as mean value of the mass weighted daily averages of the milk samples between the second and the fourth administration. The resulting steady state level in milk amounted to 0.10 mg equ/kg. (The residue levels of the first day were excluded from the plateau calculation since the residues were still increasing at the beginning of milk collection. The residue level of the last milk sample was also excluded since the interval between dosing and milking was shorter than at the other days and a second milk sample was not available due to slaughtering.)

Radioactive residues in dissected organs and tissues

The TRR in edible organs and tissues ranged from fat amounting to 0.091 mg equ/kg (mean of perirenal and omental fat) to kidney amounting to 0.967 mg equ/kg (Table 6.2.3- 5). The radioactivity concentrations of the total muscle and fat referred to 4.08% and 0.43% of the total dose assuming a value of 30% and 12% of the body weight for these tissues, respectively. Altogether, the test radioactive residues in all dissected organs and tissue samples accounted for about 5.14% of the total dose.

Identification of the radioactive residues

Radio-HPLC and radio-TLC profiles of the extracts of all samples (milk, liver, kidney, muscle, fat, urine and faeces) showed only one polar radioactive peak. Co-chromatography with the reference standard ^{14}C-TFA resulted in the same single peak. No other peak could be observed. This peak was unambiguously identified as radiolabelled TFA since two chromatographic systems with different separation modes (reversed phase HPLC and straight phase TLC) were used for co-chromatography. Therefore, the total radioactivity in all samples represented unchanged TFA. The rate of identification in the samples was excellent amounting to 98.6 – 100% of TRR in all extracts.

Transfer factors of residue transfer of TFA from animal fodder to food of animal origin

The TFA transfer factors (TF) were calculated as ratio between the radioactive residues in animal fodder and the total radioactive residues in milk, and edible organs and tissues of the goat. Any correction for formation of transformation products of TFA is not needed as total radioactive residue was represented by the administered test substance (see before). These transfer factors ranging from 0.01 (milk, fat) to 0.08 (kidney) are listed in detail in Table 6.2.3- 6.

Conclusion

Following repeated oral administration of ^{14}C-labelled sodium trifluoroacetate (TFA-Na) to a lactating goat for five consecutive days at a dose level of 0.50 mg TFA-acid/kg bw/day (corresponding to 11.9 mg TFA-acid/ kg dry feed/day) the radioactive residues in milk reached a steady state at approximately 30 hours after the first dose amounting to a plateau level of 0.102 mg equ/kg. Five days after the first administration the goat was slaughtered and radioactive residues were determined in edible organs and tissues. These residues accounted for 0.091 mg equ/kg in fat, 0.347 mg equ/kg in muscle, 0.551 mg equ/kg in liver and 0.967 mg equ/kg in kidney. The samples were extracted with acetonitrile/water and the extracts analyzed by radio-HPLC (reversed phase) and radio-TLC (straight phase). Identification of the radioactivity in all samples using co-chromatography with authentic ^{14}C-TFA and two chromatographic methods with different modes of separation generally showed only one chromatographic peak that was unambiguously identified as TFA. No other radioactive peak appeared in any sample. As a conclusion it can be stated that TFA is metabolically stable. It was rapidly excreted as not more than 5% of the total dose was detected in the dissected organs and tissues 6 hours after administration of the last dose.

By comparison of the residue levels in feed, milk, organs and tissues the following transfer factors for the residue transfer of TFA from animal fodder to food of animal origin could be derived: milk (at plateau): 0.0086; muscle: 0.0292; fat: 0.0076; liver: 0.0463; kidney: 0.0813.

Table 6.2.3- 4: Radioactive residues in milk of a goat administered with ^{14}C-TFA at a dose level of 11.9 mg TFA/kg dry feed/day

Time schedule after the first administration [hours]	Number of administration	Weight of milk sample [kg]	Residue level in milk sample [mg equ/kg]	Residue level in milk, daily average [mg equ/kg]
0	1	-----	-----	
8		1.27836	0.101**)	
24 *)		2.20244	0.057**)	0.073**)
24	2	-----	-----	
32		1.19898	0.132	
48 *)		2.30380	0.079	0.097
48	3	-----	-----	
56		1.10321	0.138	
72 *)		2.36203	0.079	0.098
72	4	-----	-----	
80		1.16649	0.145	
96 *)		2.34357	0.095	0.112
96	5	-----	-----	
102		0.85857	0.171***)	-
Residue plateau in milk (30–96 hours after first administration)				**0.102**

*) Milking immediately before the next administration

**) Not used for calculation of the residue plateau in milk since residues are still increasing at the beginning of the collection period

***) Not used for calculation of the residue plateau in milk since period between dosing and milking was shorter (only 6 hours) and no data of the second milking was available.

Table 6.2.3- 5: Radioactive residues in organs and tissues of a goat 6 hours after the last of 5 doses of ^{14}C-TFA at a dose level of 11.9 mg TFA/kg dry feed/day

Organ/Tissue	Residue level [mg equ/kg]
Liver	0.551
Kidney	0.967
Round muscle (sample)	0.346
Loin muscle (sample)	0.352
Total body muscle *)	0.347
Perirenal fat (sample)	0.064
Omental fat (sample)	0.107
Total body fat *)	0.091

*) Weighed mean residue levels in total body muscle and fat were calculated from the sample masses of the two types of muscle and fat and the total radioactive residues in that samples, respectively.

Table 6.2.3- 6: Transfer factors for residue transfer of ^{14}C-TFA from animal feed to milk, muscle, fat, liver and kidney of a goat following repeated administration at a dose level of 11.9 mg TFA/kg dry feed/day

Milk/Organ/Tissue	Residue level [mg equ/kg]	Transfer factor (TF)
Milk (at residue plateau)	0.102	0.0086
Muscle	0.347	0.0292
Fat	0.091	0.0076
Liver	0.551	0.0463
Kidney	0.967	0.0813

Rat metabolism study with [thiadiazole-5-^{14}C]flufenacet

Please refer to Section CA 5.1.1 of the Flufenacet dossier, Report KCA 5.1.1/01.

[redacted], R. 2012: [Thiadiazole-5-^{14}C]Flufenacet: Supportive Experiment for Identification of Metabolites in the Urine of the Rat, unpublished report of Bayer CropScience Comp. No. M-441499-01-1.

The result of this study is summarized in the following.

Following oral administration of [thiadiazole-5-^{14}C]flufenacet to rats (1 mg/kg bw) most of the radioactivity was already excreted within 24 hours with renal excretion being the predominant route of elimination. The excretion pattern was similar to that of a former study on the metabolism of radiolabelled flufenacet in the rat[15]. A polar metabolite detected in urine and blood plasma revealed to be trifluoroacetate (M45) reaching a level of approximately 10% of the administered dose. Therefore, it is concluded that this metabolite is covered in toxicological studies of the parent substance.

Summary of transfer factors for a potential residue transfer of TFA from fodder plants to food of animal origin resulting from livestock animals

For a dietary exposure assessment the potential residues of TFA in food of animal origin have to be included. The transfer of TFA into eggs, milk, meat, liver and kidneys were determined in the

[15] [redacted], M.E., [redacted], C.M., [redacted], L.L., Sahali, Y. (1995): The metabolism of FOE 5043 in rats. Unpublished report 106665 of Miles Inc., Stilwell, KS, USA, now Bayer CropScience, Comp. No. M-002247-01-1.

metabolism studies on ^{14}C-TFA in goat[16] and hen[17] described above. TFA transfer factors derived in these studies are presented in Table 6.2.3- 7:

Table 6.2.3- 7: Summary of TFA transfer factors from animal feed to edible commodities of livestock animals

Edible Commodity	Goat 5 x 0.50 mg TFA/kg bw/day 11.9 mg TFA/kg dry feed	Hen 14 x 0.50 mg TFA/kg bw/day 7.84 mg TFA/kg dry feed
Milk (plateau)	0.0086	-
Egg (plateau)	-	0.0499
Muscle	0.0292	0.0784
Fat	0.0076	0.0115
Liver	0.0463	0.0969
Kidney	0.0813	0.1713

CA 6.2.4 Pigs

The parent substance flufenacet is metabolized in rat, goat and hen via the same principle metabolic reactions. These reactions comprise a first cleavage of the molecule between the N-fluorophenyl-N-isopropyl acetamide group and the trifluoromethyl thiadiazole group by reaction of glutathione transferase with the acetamide moiety. The resulting glutathionate conjugate (M22) is further metabolized by hydrolytic, oxidative and cleavage steps to FOE cysteine (M23), FOE methylsulfone (M7) and FOE des-isopropyl-methylsulfone (M15) and via acetylation to FOE acetyl cysteine (M10). Further cleavage reactions resulted in formation of FOE fluorophenyl acetamide (M23). The other part of the parent molecule remaining after glutathione conjugation is the major metabolite trifluoromethyl thiadone (M9) that was partly conjugated to thiadone glucuronide (M24).

All of these described livestock metabolism studies were already submitted with the original dossier for EU registration of flufenacet and evaluated according to EU directive 91/414 EEC.

New livestock metabolism studies in goat and hen were conducted with the newly detected main metabolite trifluoroacetate (TFA). These studies showed the metabolic stability of TFA with absolutely no metabolic conversion or conjugation in both goat and hen.

Summing up, the same metabolic reactions (or metabolic stability) were observed in rat, goat and hen when feeding the parent substance flufenacet or the main residue components of flufenacet in animal

[16] [redacted], J., [redacted], R., [redacted], K., (2013): [1-^{14}C]Trifluoroacetic acid – Metabolism in the Lactating Goat, unpublished report EnSa-12-0628 of Bayer CropScience AG, Comp. No. M-444459-01-1

[17] [redacted], J., [redacted] K., [redacted], R. (2013): [1-^{14}C]Trifluoroacetic acid – Metabolism in the Laying Hen, unpublished report EnSa-12-0648 of Bayer CropScience AG, Comp. No. M-463376-01-1

feed, i.e. FOE oxalate or trifluoroacetate. Therefore, an extra metabolism study in pigs is unlikely to provide new information on the nature of residues in food of animal origin and is consequently not required.

CA 6.2.5 Fish

Since no guideline on a metabolism study in fish and its composition of feedstuff is currently available a bioconcentration study with bluegill sunfish also reporting metabolism data in fish is summarized instead. The main objective of this kind of study was the determination of a potential bioaccumulation of a test substance in fish during long-term exposure in the fishwater. However, the nature of residues of radiolabelled flufenacet in fillet and viscera of the fish was also disclosed in this study following a 28-day uptake of continuously added [fluorophenyl-U-^{14}C]flufenacet with the inflowing water in flow-through study. As this study yields the same information as a metabolism study in fish, it can be used as surrogate study according to Section 6.2.5 of the official data requirements (EU) No. 283/2013 of 1-March-2013 in accordance with Regulation (EC) No. 1107/2009. This study has already been submitted in the Ecotoxicology Section of the original dossier under Section Number 8.2.3 for authorization according to EU Directive 91/414/EEC and has been evaluated in the Monograph including addenda.

The study is divided in two sections and reported in two reports. The first report of [redacted] describes the in-life phase and the determination of the steady-state BCF on basis of radioactivity measurements. The second report of [redacted] and [redacted] describes the nature of residues in the fish following uptake from of radiolabelled flufenacet from the fish water.

Report	**KCA 6.2.5/01,** [redacted] Ö; 1994; M-003803-01
Title:	Uptake, Depuration and Bioaccumulation of Phenyl-[^{14}C]FOE 5043 Technical by Bluegill Sunfish (*Lepomis macrochirus*) Under Flow-Through Conditions
Document No:	M-003803-01-1
Report No:	106760 of Miles Inc. Stilwell, Kansas, USA, now Bayer CropScience AG, dated 1994-07-08
Guidelines:	US EPA Guidelines for Pesticide Registration: Subdivision N, Section 165-4 Accumulation in Fish
GLP	yes

Report:	**KCA 6.2.5/02, [redacted]; [redacted]; 1994; M-003804-01**
Title:	Identification of Radioactive Residues of Phenyl-[^{14}C]FOE 5043 in Bluegill Sunfish (*Lepomis macrochirus*)
Document No:	M-003804-01-1
Report No:	106577 of Miles Inc. Stilwell, Kansas, USA, now Bayer CropScience AG, dated 1994-07-13
Guidelines:	US EPA Guidelines for Pesticide Registration: Subdivision N, Section 165-4 Accumulation in Fish
GLP	yes

Executive Summary

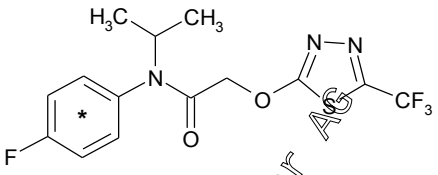
[Fluorophenyl-UL-^{14}C]flufenacet was introduced into several aquaria with the inflowing water holding bluegill sunfish in a flow-through experiment for a total exposure period of 28 days. The concentration of the test substance in the aquarium for investigation of fish metabolism was kept constant at a level of approx. 100 µg/L. Several fish were collected after 21- and 28-day of exposure. The total radioactive residues (TRR) in fillet and viscera were essentially the same for both exposure periods amounting to approx. 1.7 (fillet) and 14 (viscera) mg equ/kg. The pattern of metabolites was also nearly identical at both periods. This indicates that residues and the metabolism had reached a steady state.

A total of nine metabolites were identified, but four of these were greater than 5% of TRR in the respective tissue. The data indicate that the primary metabolic pathway starts with a glutathionate conjugation of the isopropyl acetanilide moiety (M22) of the parent molecule followed by subsequent formation of FOE cysteine (M23) and its acetylated derivative, the mercapturic acid or FOE acetyl cysteine (M10). A minor metabolic pathway in fish is the hydroxylation of the isopropyl group followed by conjugation with glucuronic acid. A proposal of the metabolic pathway of flufenacet in fish is presented in Figure 6.2.5- 1. The same metabolic reactions were, in principle, also found in the laboratory animal rat and in the livestock animals goat and hen.

In separated trials, some fish from other aquaria were collected after different exposure periods and radioassayed for determination of the bioconcentration factor (BCF) This BCF value (applying for a steady state between uptake and elimination) was reached after approx. 7 days of exposure and amounted to 68 – 71 for the whole body.

Material and methods

Test Material

Structural formula	 * denotes the ^{14}C label
Chemical name	N-(4-Fluorophenyl)-N-isopropyl-2-(5-trifluoromethyl-[1,3,4]thiadiazol-2-yloxy)-acetamide (IUPAC); Acetamide, N-(4-Fluorophenyl)-N-(1-methylethyl)-2-[[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]oxy]- (9Cl; CAS)
Common name	Flufenacet
CAS RNo.	142459-58-3
Empirical formula	$C_{14}H_{13}F_4N_3O_2S$
Company code	FOE 5043
Molar mass (non-labelled)	363.34 g/mol
Water solubility	51 mg/L at pH 6.9 and 20°C[18]
Label	[fluorophenyl-UL-^{14}C]Flufenacet
Specific radioactivity	Actually used: 12755 dpm/µg (0.765 MBq/µg; 0.02 mCi/µg) following blending of radiolabelled (66.5 mCi/mmol; 0.183 mCi/mg, 6.77 MBq/mg) and non-labelled Flufenacet
Radiochemical purity	Original: 98.9%, re-analysis by radio-TLC: 95.3%
Chemical purity of the non-labelled test substance	96.8%
Solvent for stock solution	Acetone

Test Animals

Species	Bluegill sunfish (*Lepomis macrochirus*)
Breed	Osaga Catfisheries, Osaga Beach, Missouri, USA
Number	Approx. 95 smaller fish per aquarium at the beginning for BCF determination; also used for investigation of the metabolism in fish; 6 lager fish per aquarium after removal the small fish to support in the disclosure of the metabolites
Body length	Smaller fish: approx. 19 mm; lager fish: 4 – 6 inch (10 – 15 cm)
Acclimatization	Smaller fish: 1 month Larger fish: 4 days
Husbandry	Two 100 L glass aquaria with a standpipe for drainage, filled with 78 L water, temperature 22 ± 2°C, pH 7.1 – 7.5, 16-hour daylight period
Feed	Newly hatched brine shrimp and commercial fish food, daily feeding
Water turn overs in the flow-through system	Approx. 10.5 – 11.2 volumes per 24 hours, the inflowing water passed an ultraviolet sterilizer

[18] [redacted] 2012: Flufenacet (FOE 5043, AE F133402), pure substance: Solubility in distilled water (flask method), unpublished report PA12/059 of Bayer CropScience AG, Comp. No. M-438187-01-1.

Duration (only uptake)	Smaller fish: 28 days at maximum, Lager fish: additional 7 days (1st aquarium), 14 days (2nd aquarium)

Exposure of fish to radiolabelled test substance

Three 100-L glass aquaria holding initially 150 smaller fish each (body length approx. 19 mm, body weight approx. 0.17 g) were kept in flow-through condition for a total uptake period of 28 days and a subsequent depuration period of 14 days (two aquaria with the test substance, one control aquarium without test substance). During the uptake period radiolabelled flufenacet was added to the inflowing water to reach a concentration in the fish water of approx. 100 µg/L. During the depuration period pure water with no test substance was introduced.

Following complete removal of the smaller fish six larger fish (body length approx. 10 – 15 cm) were inserted in each of the two aquaria and exposed to radiolabelled flufenacet in the same way as done with the smaller fish.

Collection of fish and extraction of fish

The smaller fish of the BCF trial were sampled after different exposure periods, i.e. 0, 1, 3, 7, 14, 21, and 28 days. They were directly radioassayed (following cutting in suitable pieces) or first dissected into fillet (edible) and viscera (non-edible tissue). Respective fractions were ground to powder under liquid nitrogen using mortar and pestle. The liquid nitrogen was allowed to sublime in a freezer at -20°C. The fillet and viscera samples were also radioassayed to determine the total radioactive residues (TRR) in the whole body, fillet and viscera.

Fillet and viscera samples of collection days 21 and 28 were extracted with methanol and a mixture of methanol and 0.1N hydrochloric acid at room temperature. The methanol extract was partitioned against hexane. The hexane solution was discarded. The methanol fraction was concentrated, centrifuged and analyzed by radio-HPLC.

The larger fish were collected after a 7-day (first aquarium) and 14-day exposure (second aquarium). These fish were dissected and their bladders were carefully removed, punctured and drained. The removed urine was centrifuged and analyzed by radio-HPLC.

Extraction of fish water

Water samples were taken at the same time as fish were collected. Radioactive residues in these water samples were extracted with dichloromethane, the extracts concentrated to dryness and re-constituted in methanol. Alternatively, radioactive residues in water samples were also extracted by solid-phase extraction using a C18 cartridge. Adsorbed residues were eluted by flushing with methanol. The methanol extracts were concentrated and analyzed by radio-HPLC.

Radioassaying of samples

Radioassaying (radioactivity measurements) were conducted by liquid scintillation counting (LSC); aliquots of liquid samples were directly measured, aliquots of solid samples were first combusted and the formed $^{14}CO_2$ was absorbed in an alkaline scintillation cocktail. The minimum counting efficiency (LOD) was derived from the lowest net count rate of the LSC-counter, the specific activity of the test

substance and the sample size used for LSC counting. For fish water a LOD of 5.77 x 10^{-3} µg equ/L, for fish tissue a LOD of 0.096 µg equ/kg was reported.

Radio-HPLC and LC-MS of sample extracts

Radio-HPLC was conducted using RP8 columns (250 x 9.4 cm, particle size 10 µm and 250 x 4.6 cm, particle size 5 µm) operated with gradient mixtures of aqueous 0.1% acetic acid or trifluoroacetic acid and methanol or acetonitrile. The systems were equipped with a radiomonitor with a 400 or 500 µL cell with a solid scintillator.

LC-MS was conducted by a combination of a HPLC system, a radiomonitor and a mass spectrometer. The HPLC system used a RP18 separation column (150 x 4.9 mm, particle size 5 µm) and was operated with gradient mixture of aqueous 0.1% acetic acid and methanol. Determination of the separated compounds was performed by a double focusing mass spectrometer with a thermospray interface.

Findings

TRR in fish and derived BCF values ([redacted], 1994)

Bluegill sunfish were exposed to dissolved radiolabelled flufenacet in fishwater at a concentration of approx. 100 µg/L for different exposure periods. The total radioactive residue (TRR) in fish tissue amounted to 833 - 2213 µg equ/kg in edible fillet, to 5899 - 10846 µg equ/kg in non-edible viscera and to 3315 - 9900 µg equ/kg in whole fish. Comparing the residue levels in fish tissue and fish water resulted in daily bioconcentration factors (BCF values) of 8.4 – 22.1 for fillet, 59.2 – 111 for viscera and 33.3 – 98.0 for the whole body. The plateau levels (steady-state levels) were already reached after approximately 7 days of exposure.

The mean steady-state BCF for the whole body was determined to 68 (mean BCF of the last four sampling dates 7, 14, 21 and 28 days of uptake) or 71.4 when calculated using the BIOFAC model operating on the basis of an uptake and depuration rate constant.

TRR in fish water and hydrolytic stability of the test substance ([redacted], [redacted], 1994)

Radioassaying of fish water at the different collection days resulted in a radioactivity concentration in the range of 95.9 – 100.0 µg equ/L. Determination of the intact test substance amounting to 86.7 – 95.0 µg/L indicated no significant degradation of the test substance in the aquaria.

Composition of radioactive residues in fish tissue

The composition of the radioactive residues in viscera and fillet of bluegill sunfish following 21 and 28-day exposure of radiolabelled flufenacet are presented in Table 6.2.5- 1 and Table 6.2.5- 2. The structures of the metabolites were derived from their mass spectra and by comparison of the retention behavior in reversed phase HPLC. The composition of residues in viscera and fillet was almost identical during the 21 and 28-day exposure indicating a steady state metabolism.

The major metabolites in non-edible viscera were identified as FOE cysteine conjugate (FACS, M23) amounting to approximately 50% of TRR and its acetylated derivative FOE acetyl cysteine (FANACS, "mercapturic acid", M10) amounting to approximately 24% of TRR. Other four minor metabolites (<10% of TRR) were also identified. The parent substance flufenacet was observed at a low level of approximately 5% of TRR.

The major metabolites in edible fillet proved to be also FOE cysteine conjugate (FACS, M23) amounting to approximately 37% of TRR and FOE acetyl cysteine (FANACS, M10) amounting to approximately 16% of TRR. Eight unknown minor metabolites could be characterized according to their polarity (retention behavior in reversed phase liquid chromatography). The parent compound flufenacet contributed significantly to pattern of residues accounting for 18% of TRR.

Conclusion

The bioconcentration factor (BCF) of [fluorophenyl-UL-^{14}C]flufenacet in bluegill sunfish amounted to 68 – 71 for the whole body based on radioactivity measurements. The respective steady state of uptake and elimination was reached after approx. 7 days of exposure at a concentration of 100 µg/L.

The metabolism of [fluorophenyl-UL-^{14}C]flufenacet was investigated in bluegill sunfish after 21 and 28-day exposure in the fish water a concentration of approx. 100 µg/L. The TRR levels in the fillet and viscera were essentially the same for both exposure periods amounting to approx. 1.7 (fillet) and 11 (viscera) mg equ/kg. The pattern of metabolites was also nearly identical at both periods. This indicates that residues and the metabolism had reached a steady state.

A total of nine metabolites were identified, four of these were greater than 5% of TRR in the respective tissue. The data indicate that the primary metabolic pathway starts with a glutathionate conjugation of the isopropyl acetanilide moiety (M22) of the parent molecule followed by subsequent formation of FOE cysteine (M23) and its acetylated derivative, the mercapturic acid or FOE acetyl cysteine (M10). A minor metabolic pathway in fish is the hydroxylation of the isopropyl group followed by conjugation with glucuronic acid. A proposal of the metabolic pathway of flufenacet in fish is presented in Figure 6.2.5-1.

The same principle metabolic reactions were also found in the laboratory animal rat and in the livestock animals goat and hen.

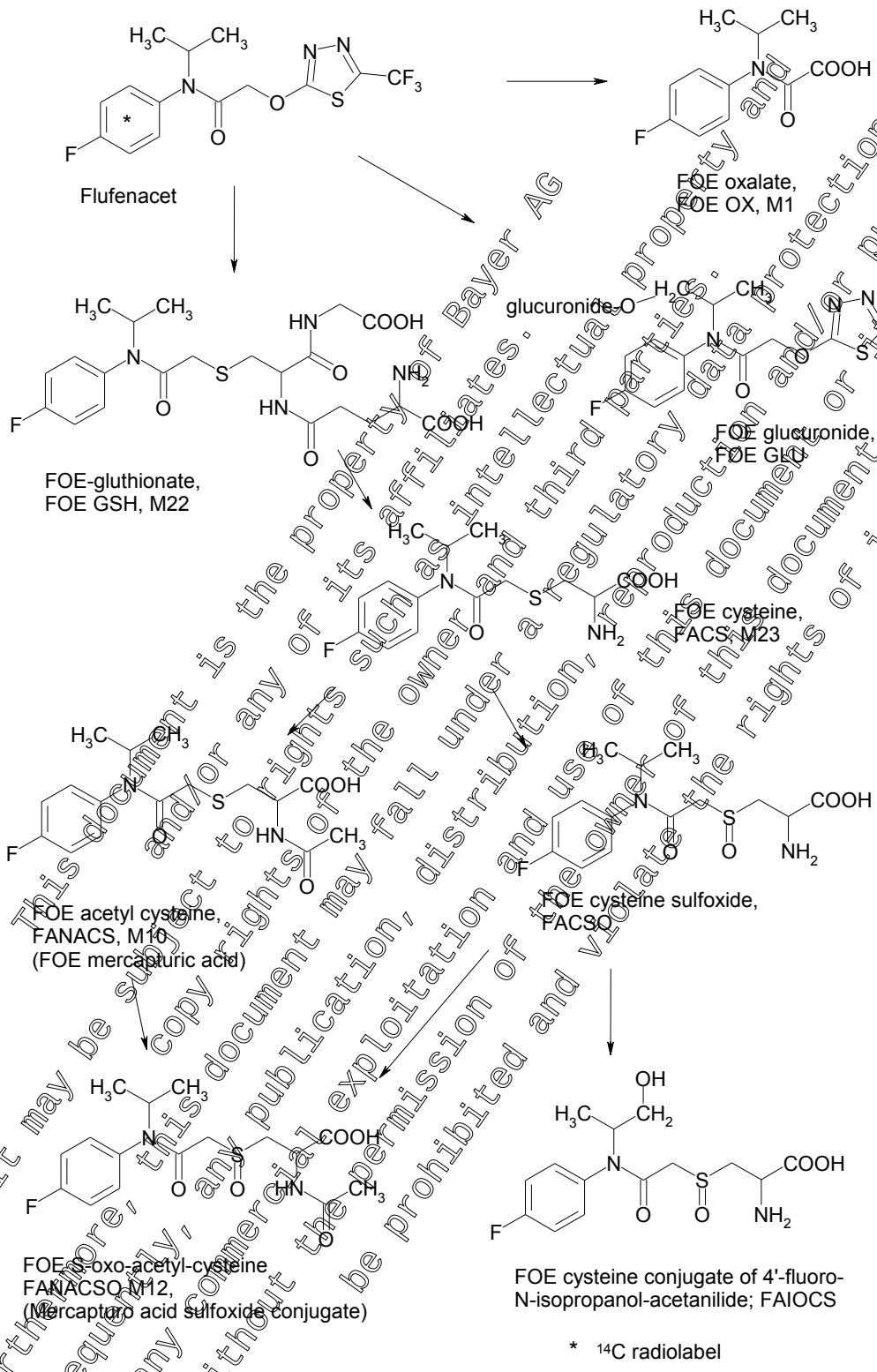
Table 6.2.5- 1: Radioactive residues in viscera of bluegill sunfish following 21 and 28-day exposure of [fluorophenyl-UL-^{14}C]flufenacet at a concentration of 100 µg/L fish water in a flow-through study

Exposure period	**21 Days**		**28 Days**	
TRR [mg equ/kg] (after combustion)	10.99		10.22	
Metabolite detected by radio-HPLC	[% of TRR]	[mg equ/kg]	[% of TRR]	[mg equ/kg]
FOE isopropyl hydroxy cysteine (FAIOCS) and FOE oxalate (FOE OX, M1)	2.6	0.258	1.8	0.191
FOE cysteine sulfoxide conjugate (FACSO, M39)	3.3	0.327	1.1	0.114
FOE S-oxo-acetylcysteine (FANACSO, M12)	4.4	0.444	0.9	0.097
FOE glutathionate (FOE GSH, M22)	3.5	0.335	2.3	0.239
FOE cysteine conjugate (FACS, M23)	46.9	4.694	54.8	5.719
FOE acetyl cysteine (FANACS, M10)	24.0	2.408	23.3	2.431
FOE isopropanol glucuronide (FOE GLU)	5.2	0.520	4.5	0.467
Flufenacet (FOE 5043, parent substance)	3.8	0.381	5.7	0.599
Unextracted	6.3	0.631	5.6	0.586
Total	100	10.018	100	10.443

Table 6.2.5- 2: Radioactive residues in the fillet of bluegill sunfish following 21 and 28-day exposure of [fluorophenyl-UL-^{14}C]flufenacet at a concentration of 100 µg/L fish water in a flow-through study

Exposure period	**21 Days**		**28 Days**	
TRR [mg equ/kg] (after combustion)	1.79		1.76	
Metabolite detected by radio-HPLC	[% of TRR]	[mg equ/kg]	[% of TRR]	[mg equ/kg]
Unknown 1	1.3	0.026	1.5	0.026
Unknown 2	1.4	0.027	2.1	0.036
Unknown 3	1.2	0.022	2.4	0.041
Unknown 4	2	0.039	3.0	0.052
FOE cysteine conjugate (FACS, M23)	36.2	0.692	37.3	0.631
FOE acetyl cysteine (FANACS, M10)	17.1	0.326	15.3	0.260
Unknown 5	2.0	0.038	1.9	0.032
Unknown 6	1.4	0.027	1.6	0.027
Unknown 7	1.6	0.030	0.7	0.001
Unknown 8	1.6	0.030	0.8	0.014
Flufenacet (FOE 5043, parent substance)	18.1	0.345	17.6	0.297
Unextracted	16.0	0.308	15.8	0.268
Total identified	69.3	1.326	68.4	1.159
Total	100	1.910	100	1.696

Figure 6.2.5- 1: Proposed metabolic pathway of [fluorophenyl-UL-^{14}C]flufenacet of bluegill sunfish



CA 6.3 Magnitude of residue trials in plants

The herbicide flufenacet is mainly used to control annual grasses and broad-leaved weeds in cereals (wheat, rye, triticale, barley and oat). It may be applied either pre- or post-emergence of the cereals. Flufenacet is usually co-formulated with other herbicides such as diflufenican. The representative formulation for the renewal of the approval of flufenacet is 'Flufenacet + Diflufenican SC 600', a soluble concentrate formulation containing 400 g/L of flufenacet and 200 g/L of diflufenican.
The product 'Flufenacet + Diflufenican SC 600'was also the representative formulation for evaluation of diflufenican in the EU peer review process (2008).[19].

CA 6.3.1 Cereals (wheat, rye, triticale, barley and oats)

According to the 'guideline on comparability, extrapolation, group tolerances and data requirements for setting MRLs', SANCO 7525/VI/95 rev. 9 (March 2011), extrapolation of residue data obtained from any of the crops (wheat, rye, triticale, barley, oats) for an active substance is possible if the use pattern involves treatments early in the growing season (last application before consumable parts of the crop have started to form).
Therefore combined data sets obtained from residue studies on wheat and barley are reported in this chapter in order to support flufenacet uses.

[19] In the initial version of the Annex II dossier that was issued in November 2003 a different representative use was supported, namely autumn application to winter cereals at the rate of 187.5 g as/ha up to the growth stage BBCH 25 (5 tillers detectable). This use corresponded to autumn application of the formulation JAVELIN® (500 g/L isoproturon + 62.5 g/L diflufenican). Autumn application of HEROLD®SC600 (Flufenacet + Diflufenican SC 600) in winter cereals was proposed as a second representative use and dealt with separately in an Annex III dossier. In January 2004 the Rapporteur Member State and Bayer CropScience agreed to consider only the use of HEROLD®SC600 as the representative use for the EU review.

Representative uses for renewal of approval of flufenacet

The representative uses supported for the renewal of approval for flufenacet are summarised in Table 6.3.1-1.

Table 6.3.1-1: Summary of the representative uses supported for renewal of approval for flufenacet in the product 'Flufenacet + Diflufenican SC 500'

Crop	Region*	F, G or I**	Maximum Number of Applications	Growth stage at application	Maximum Rate flufenacet (g a.s./ha)	Minimum PHI (days)
Cereals (winter wheat, winter barley, winter rye)	EU-N	F	1	Early post-emergence BBCH 10-13 (autumn use)	240	n.a.
Cereals (winter wheat, winter barley, winter rye)	EU-N	F	1	Pre-emergence; early post-emergence BBCH 00-22	120	n.a.
Cereals (wheat, barley)	EU-S	F	1	Early post-emergence BBCH 11-13	240	n.a.
Cereals (wheat, barley)	EU-S	F	1	Early post-emergence BBCH 11-13	160	n.a.

* EU-N northern Europe, EU-S southern Europe ** F Field; G Greenhouse; I Indoor
n.a. not applicable, the PHI is covered by the vegetation period of the crop from treatment to harvest.

Representative use included in the Annex II dossier and evaluated for Annex I inclusion

The representative use considered during the EU review of flufenacet (and taken into account for Annex I inclusion of the active substance) **is pre-emergence/early post-emergence application to winter cereals** (wheat, rye, triticale, barley) **in autumn at the rate of 240 g as/ha.** Since the use pattern referred to autumn application no specific growth stage for the crop was defined for the latest possible application. The application is typically made pre-emergence or during leaf development or tillering.

The representative product in the Annex II dossier to support the critical GAP for flufenacet in wheat, rye, triticale and barley at the European level was a straight formulation WG 60, containing 60% flufenacet. The use was supported in the north European climatic zone. The use evaluated with the Annex II dossier corresponds to the critical GAP for flufenacet in northern Europe and forms the basis for the MRL as established in Regulation (EC) 396/2005.

The GAP of the representative use in cereals supported with the Annex II dossier and taken into account for Annex I inclusion is summarised in Table 6.3.1-2.

Table 6.3.1- 1: Summary of the representative use of Flufenacet WG 60 considered for Annex I inclusion of the active substance flufenacet

Crop	Region *	F, G or I**	Growth stage	Maximum Number of Applications	Maximum Rate (g as/ha)	Minimum PHI (days)
Winter wheat Winter barley Winter rye	EU-N	F	pre-emergence to early post emergence (autumn) 2nd leaf stage of weeds	1	240	n.a.

*EU-N: northern Europe **F Field; G Greenhouse; I Indoor.
n.a. : not applicable. The pre-harvest interval covers the vegetation period of the crop until harvest.

Summary of the residue trials supporting the representative use dealt with in the Annex II dossier:

A total of 18 residue trials on winter barley (7 trials, 1 trial yielding green plant material only), winter rye (2 trials) and winter wheat (9 trials), which were performed at different sites in northern Europe during the 1993/94 and 1994/95 growing seasons, are reviewed in the Annex II Section 4 for flufenacet. The plants were treated post-emergence between mid-October and mid-March at growth stages ranging from BBCH 11 (first leaf unfolded) to BBCH 25 (5 tillers detectable). A straight WG formulation containing 60% w/w of flufenacet (WG 60) was applied at a nominal rate of 0.4 kg/ha, which corresponds to 240 g as/ha. In two trials the actually achieved rate slightly differed from the nominal rate, at either 188 g as/ha (ca. 22% less) or 260 g as/ha (ca. 8% more). Harvest was between 120 and 271 days after application. Residues were determined at various development stages of the treated plants.

- Depending on the growth stage and season when the treatment was performed, the residues in the green plants at the growth stage BBCH 29 (end of tillering) ranged between < 0.05 and 0.25 mg/kg and were < 0.05 mg/kg at the growth stage BBCH 51 (beginning of heading).
- The residues of flufenacet in grain and straw at harvest were always below the respective limit of quantification, i.e. 0.05 mg/kg in grain and 0.10 mg/kg in straw.

The residue trials considered to grant Annex I inclusion of flufenacet are summarized in Table 6.3.1-3 below. They are not reported again in detail, however, the tier 1 summary forms are included in document ([redacted], M.; 2014; M-478066-01-1) for sake of easy reference.

Table 6.3.1-3 Number of residue trials conducted per geographical region and vegetation period considered for Annex I inclusion of the active substance flufenacet

Crop	Formula-tion	Year	Applica-tion rate (g as/ha)	Growth stage at application	Country (No. of trials)	Report No.	Annex II Baseline dossier reference Report
Northern Europe							
Winter barley	WG 60 (60 %)	1993/94	240 240	BBCH 12-21 BBCH 22-24	Germany (3**) France N (2)*	RA-2054/93	KCA 6.3.1/02 [redacted]; 1995; M-002284-01-2
Winter wheat	WG 60 (60 %)	1993/94	240 240 240	BBCH 11-12 BBCH 13-22 BBCH 12	Germany (3) France N (2)* the Netherlands (2)*	RA-2054/93	KCA 6.3.1/02 [redacted]; M.; 1995; M-002284-01-2
Winter wheat	WG 60 (60 %)	1994/95	240 186	BBCH 21 BBCH 13	Germany (1) France N (1)	RA-2008/94	KCA 6.3.1/01 [redacted]M; 1996; M-002280-01-2
Winter barley	WG 60 (60 %)	1994/95	240 200	BBCH 13 BBCH 21	Germany (1) France N (1)	RA-2008/94	KCA 6.3.1/01 [redacted]; 1996; M-002280-01-2
Winter rye	WG 60 (60 %)	1994/95	240 240	BBCH 21 BBCH 25	Germany (2)	RA-2008/94	KCA 6.3.1/01 [redacted]; 1996; M-002280-01-2

*application carried out in March **only green material, but no grain and straw were sampled in one trial

The samples from the trials supporting the representative use of Flufenacet WG 60 dealt with in the Annex II dossier were analysed for residues of flufenacet according to the method 00346 ([redacted], M.; 1995;M-018864-02) , which yields the combined level of the parent compound and all its metabolites containing the N-fluorophenyl-N-isopropyl functional group with a limit of quantification of 0.03 mg/kg in grain and green plant material and 0.1 mg/kg in straw.

Before analysis the grain and straw samples were stored frozen for up to 250 days (8.5 months), while samples of green material were stored for a maximum storage period of 350 days (12 months). The maximum storage periods are covered by the storage period investigated in the storage stability study (refer to chapter CA 6.1).

Concurrent recoveries were performed during the analysis of the study samples. Recovery means were within the range of 70-110% in grain, straw and green material. The relative standard deviation was < 20% for all sample materials and at all fortification levels.

Residues in the control samples were below the respective LOQs (0.05 mg/kg for grain and green material and 0.1 mg/kg for straw). The residue levels in the samples of green plant material, grain and straw from the trials supporting the representative use are summarised in Table 6.3.1-4

Table 6.3.1-4: Summary of flufenacet residue data supporting the representative use considered for Annex I inclusion of the active substance flufenacet

Application	Sample material	n	Residue level (mg/kg)		
			Min.	Max.	Median
Northern Europe					
240 (186-260) g as/ha at latest BBCH 25 (application November to March)	Grain	17	< 0.05	< 0.05	< 0.05
	Straw	17	< 0.1	< 0.1	< 0.1
	Green material (BBCH 51)	18	< 0.05	< 0.05	< 0.05

The residue trials considered to grant Annex I inclusion of flufenacet support application of flufenacet to cereals at the rate of 240 g as/ha at pre- or early post emergence growth stages up to mid of tillering. The applications were performed between November and March (BBCH 10 to 25) and were considered suitable to support the autumn/winter use of the product Flufenacet WG 60.

Annex I renewal process:

The representative uses supported for the renewal of approval for flufenacet are summarised in Table 6.3.1-1 above.

For the northern climatic zone, the critical use pattern of the representative product 'Flufenacet + Diflufenican SC 600' involves the same application parameters relative to flufenacet as 'Flufenacet WG 60' considered to grant Annex I inclusion. For both products the maximum supported application rate of flufenacet amounts to 240 g as/ha.

The trials reviewed in the Annex II dossier of flufenacet were performed using a WG formulation which is known to produce comparable residue levels to SC formulations. Therefore, both formulation types can be used interchangeably to support either of the products (cf. 'guideline on comparability, extrapolation, group tolerances and data requirements for setting MRLs', SANCO 7525/VI/95 rev 9 (March 2011) and 'OECD guideline for the testing of chemicals'-crop field trial, 509).

Thus, the residue trials reviewed in the Annex II dossier of flufenacet are considered to adequately support the representative use of 'Flufenacet + Diflufenican SC 600' in northern Europe. In principle, no further trials are required.

Supplementary trials are available to support the representative use patterns relevant for renewal of approval in northern and southern European climatic zones. The studies were conducted using mixture products, either a 2 way mixture with diflufenican or a product containing 3 active substances (i.e. flufenacet, diflufenican and flurtamone). The supplementary studies cover application rates from 110 to 254 g as/ha addressing the representative uses at the corresponding rates. An overview on the studies is compiled in Table **6.3.1-** . In principle, the studies involving application rates at 240 g as/ha

(actual 220-254 g as/ha) are considered adequate to also support use patterns involving lower rates of flufenacet since they reflect the critical GAPs for the active substance in both climatic regions.

In order to support the active substance flufenacet only residue data pertaining to flufenacet are summarized below. Data on the mixing partner diflufenican may be found in the Tier 1 summary forms.

The detailed tables (Tier 1) of the trials evaluated for Annex I inclusion (only northern Europe) and the supplementary trials for northern and southern Europe are submitted in a separate document as additional information for the evaluator.

Report:	**KCA 6.3.1/13, [redacted], 2014, M-478066-01-1**
Title:	Tier 1 Summary of the residue data and processing studies for flufenacet and residue data supporting the representative product 'Flufenacet + Diflufenican SC 600'
Document No:	M-478066-01-1
GLP	Not applicable

Table 6.3.1- 5: Supplementary residue trials conducted per geographical region and formulation

Crop	Formula-tion	Year	Applica-tion rate Flufenacet (g as/ha)	Growth stage at application	No. of trials	Report No.	KCA reference Report .	Documentation reference number
Northern Europe								
Wheat Barley Rye	FFA+DFF WG 60	1993/94	240	BBCH 13-25	4	RA-2010/94	KCA 6.3.1/03	M-004451-01-2
Wheat Barley	FFA+DFF SC 600	2000/01	240	BBCH 13	2	RA-2144/00	KCA 6.3.1/06	M-058156-01-1
Wheat	FFA+DFF+ FLT SC360	2011	110-120	BBCH 25	2	11-2095	KCA 6.3.1/09	M-459755-01-1
Barley	FFA+DFF+ FLT SC360	2011	120	BBCH 25	2	11-2094	KCA 6.3.1/12	M-460003-01-1
Wheat barley	FFA+DFF+ FLT SC360	2011/12	120	BBCH 22-25	4	12-2001	KCA 6.3.1/10	M-459795-01-1
Southern Europe								
Wheat Barley	FFA+DFF SC 600	2000/01	240-254	BBCH 13	2	RA-2144/00	KCA 6.3.1/06	M-058156-01-1
Barley	FFA+DFF SC 600	2008/09	240	BBCH 13	3	09-2048	KCA 6.3.1/07	M-361495-01-1
Wheat	FFA+DFF SC 600	2008/09	220-240	BBCH 13-21	4	09-2052	KCA 6.3.1/08	M-363200-02-1
Wheat	FFA+DFF+ FLT SC360	2011	120*	BBCH 29-30	2	11-2095	KCA 6.3.1/09	M-459755-01-1
Barley	FFA+DFF+ FLT SC360	2011	120*	BBCH 25-29	2	11-2094	KCA 6.3.1/12	M-460003-01-1
Wheat Barley	FFA+DFF+ FLT SC360	2011/12	120*	BBCH 22-25	3 (4**)	12-2002	KCA 6.3.1/11	M-459799-01-1
Wheat Barley	FFA+DFF WG70	1997	126*	BBCH 13	3	RA-2153/97	KCA 6.3.1/04	M-012486-02-1
Wheat Barley	FFA+DFF WG70	1998	126*	BBCH 13	2	RA-2185/98	KCA 6.3.1/05	M-033163-01-1

FFA+DFF WG 60: wettable granule formulation containing 40% flufenacet + 20% diflufenican
FFA+DFF SC600: suspension concentrate containing 400 g/L flufenacet +200 g/L diflufenican
FFA+DFF+FLT SC 360: suspension concentrate containing 120 g/L flufenacet +120 g/L diflufenican + 120 g/L flurtamone
FFA+DFF WG 70 wettable granule formulation containing 35% flufenace t + 35% diflufenican

* residue trials at a rate of 120 g as/ha are considered appropriate to also support the GAP involving 160 g as/ha since the rate is within the acceptable 25% range of comparabiltity (Guideline on comparability, extrapolation, group tolerances and data requirements for setting MRLs, SANCO 7525/VI/95 rev 9)

**One trial was underdosed by 7% and thus out of the acceptable range for comparability of 25% relative to the supported use pattern.

Table 6.3.1- 6: Overall summary of supplementary residue data on cereals supporting the representative GAPs for renewal of approval

Application Rate flufenacet (g as/ha)	Region	Formulation	Crop	Sample material	n	Residue level (mg/kg) flufenacet		
						Min.	Max.	STMR
240	EU-N	FFA+DFF WG 60 FFA+DFF SC 600	wheat, barley	grain	6	<0.05	<0.05	<0.05
				straw	6	<0.10	<0.10	<0.10
110-120	EU-N	FFA+FLT+DFF SC 360	Wheat, barley	grain	8	<0.01	0.022	<0.01
				straw	8	<0.05	<0.05	<0.05
220-254	EU-S	FFA+DFF SC 600	Wheat, barley	grain	9	<0.01	0.05	<0.01
				straw	9	<0.05	0.11	0.06
120-126	EU-S	FFA+FLT+DFF SC 360 FFA+DFF WG 70	Wheat, barley	grain	12	<0.01 <0.05	0.035/ <0.05	0.022
				straw	12	<0.05	0.069	<0.05

EU-N northern Europe EU-S southern Europe n: number of trials

FFA+DFF WG 60 containing 40% flufenacet and 20% diflufenican
FFA+ DFF SC600 containing 400 g/L flufenacet and 200 g/L diflufenican
FFA+FLT+DFF SC 360 containing 120 g/L flufenacet, 120 g/L flurtamone and 120 g/L diflufenican
FFA+DFF WG 70 containing 35% flufenacet and 35% diflufenican

Table 6.3.1- 7: Compilation of individual residue levels for flufenacet in supplementary trials

Report No.	Application rate Flufenacet (g as/ha)	Residue levels grain (mg/kg)	Residue levels straw (mg/kg)
Northern Europe			
RA-2010/94	240	<0.05/ <0.05/ <0.05 /<0.05	<0.1/ <0.1/ <0.1/ <0.1
RA-2144/00	240	<0.05/ <0.05	<0.1/ <0.1
		STMR < 0.05	**STMR <0.1**
11-2095	110-120	<0.01/ 0.022	<0.05/ <0.05
11-2094	120	<0.01/ 0.017	<0.05/ <0.05
12-2001	120	<0.01/ <0.01/ <0.01/ <0.01	<0.05/ <0.05/ <0.05/ <0.05
		STMR <0.01	**STMR <0.05**
Southern Europe			
RA-2144/00	240-254	<0.05/< 0.05	< 0.1/ 0.11
09-2048	240	<0.01/ <0.01/ <0.01	< 0.05/ 0.06/ 0.06
09-2052	220-240	<0.01/ <0.01/ 0.01/ 0.05	<0.05/ <0.05/ <0.05/ 0.09
		STMR <0.01	**STMR 0.06**
11-2095	120	0.02/ 0.035	<0.05/ <0.05
11-2094	120	<0.01/ <0.01	<0.05/ 0.059
12-2002	120	<0.01/ <0.01/ <0.01	<0.05/ <0.05/ 0.069
RA-2153/97	126	<0.05/ <0.05/ < 0.05/	<0.05/ <0.05/ < 0.05/
RA-2185/98	126	<0.05/ <0.05	<0.05/ <0.05
		STMR 0.028	**STMR <0.05**

Supplementary field trials – <u>northern Europe</u> (application rate 240 g as/ha)

Report:	**KCA 6.3.1/03,** [redacted]; 1996; M-004451-01-2
Title:	Determination of residues of FOE 5043 & Diflufenican 60 WG in/on winter barley, winter rye and winter wheat following early post-emergence spray application in Germany
Document No & Report No:	M-004451-01-2 RA-2010/94 dated 1996-03-25
Guidelines:	Not indicated, fulfils EU 7029/VI/95 rev.5 dated 22 July 1997
GLP	Yes; Deviations: none

Material and methods

Four trials on winter cereals (1 trial on barley, 1 trial on winter rye, and 2 trials on winter wheat) were conducted during the 1994-1995 growing season in Germany using a WG formulation containing 20% diflufenican + 40% flufenacet. The plants were treated in autumn (November), at growth stages ranging from BBCH 13 (3 leaves unfolded) to BBCH 25 (5 tillers detectable). The application rate was 240 g flufenacet/ha.

Green plant samples were taken for analysis at the growth stages BBCH 29 (end of tillering) and BBCH 51 (beginning of heading). Grain and straw samples were taken at normal harvest, which was between 246 and 253 days after application.

All samples were analysed for residues of flufenacet according to the method 00346 ([redacted], M.; 1995; M-018864-02) which yields the combined level of the parent compound and all its metabolites containing the N-fluorophenyl-N-isopropyl functional group. The method was reported in the original Annex II dossier (point 4). The procedure involves oxidation of the residues with potassium permanganate, hydrolysis with sulfuric acid, steam distillation, liquid/liquid partitioning, derivatisation with trifluoroacetic anhydride and GC/MS determination of the this obtained 2,2,2-trifluoro-N-(4-fluorophenyl)-N-isopropylacetamide (trifluoroacetamide). Residues are expressed as parent flufenacet.

Before analysis the samples were stored frozen for less than 8 months (237 days) for green material and less than 4 months (112 days) for grain and straw. These storage periods are adequately covered by the storage stability data for flufenacet.

Findings

Recovery rates were determined prior to analysis in order to validate the analytical method and concurrently with the sample analysis in order to check the accuracy of the residue analysis. Fortification was performed by spiking control samples with one of the following compounds or a mixture thereof: parent flufenacet, flufenacet oxalate, flufenacet sulfonic acid, flufenacet thioglycolate sulfoxide. The recovery-rates and corresponding relative standard deviations (RSD) were satisfactory as shown in Table 6.3.1-8. The limit of quantification was 0.05 mg/kg in green plant and grain, and 0.10 mg/kg in straw. The residues in the barley, wheat, and rye samples from the individual trials are summarised in Tables 6.3.1-9.

No apparent residues were found in any of the untreated samples, i.e. residues were < LOQ for flufenacet.

Flufenacet residues ranged between < 0.05 mg/kg to 0.1 mg/kg in green material collected at growth stage BBCH 29, while at the later growth stage (BBCH 51) residues have declined below the LOQ (0.05 mg/kg). In all trials, residues in grain and straw were below the LOQ of 0.05 mg/kg and 0.1 mg/kg, respectively.

Table 6.3.1-8: Procedural recovery data for Flufenacet

The LOQ is marked in bold.

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./meta-bolite	n	Fortification level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
RA-2010/94 40044/0 0044-94 GLP: yes 1994	Barley, winter	green material	total residue flufenacet	3	**0.05**	84; 85; 91	84	91	87	4.4
				3	overall mg/kg		84	91	87	4.4
		grain	total residue flufenacet	3	**0.05**	81; 85; 91	81	91	86	5.9
				3	overall mg/kg		81	91	86	5.9
		straw	total residue flufenacet	3	**0.1**	80; 90; 92	80	92	87	7.4
				3	overall mg/kg		80	92	87	7.4
RA-2010/94 40045/9 0045-94 GLP: yes 1994	Rye, winter	green material	total residue flufenacet	12	**0.05**	79; 84; 87; 87; 90; 94; 94; 94; 94; 95; 96; 97	79	97	91	6.1
				8	0.5	81; 82; 86; 88; 92; 93; 93; 93	81	93	89	5.7
				20	overall mg/kg		79	97	90	6.0
		grain	total residue flufenacet	12	**0.05**	76; 79; 80; 86; 88; 89; 89; 90; 95; 96; 101; 105	76	105	90	9.2
				8	0.5	71; 79; 82; 84; 87; 90; 93; 94	71	94	85	9.1
				20	overall mg/kg		71	105	88	9.4
		straw	total residue flufenacet	12	**0.1**	79; 81; 82; 85; 85; 87; 88; 90; 90; 91; 93; 95	79	95	87	5.7
				8	1.0	69; 79; 79; 80; 81; 85; 87; 88	69	88	81	7.4
				20	overall mg/kg		69	95	85	7.2
RA-2010/94 40046/7 0046-94 and 40047/5 0047-94 GLP: yes 1994	Wheat, winter	green material	total residue flufenacet	6	**0.05**	71; 81; 84; 88; 95; 97	71	97	86	11.2
				6	overall mg/kg		71	97	86	11.2
		grain	total residue flufenacet	4	**0.05**	81; 91; 99; 109	81	109	95	12.5
				4	overall mg/kg		81	109	95	12.5
		straw	total residue flufenacet	3	**0.10**	79; 81; 94	79	94	85	9.6
				3	overall mg/kg		79	94	85	9.6

Table 6.3.1-9: Residues of flufenacet in barley, wheat and rye after post-emergence application of flufenacet + diflufenican WG 60 (containing 40% flufenacet + 20% diflufenican) in northern Europe

Study Trial No. Trial SubID GLP Year	Crop Variety	Country	Application FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Residues Portion analysed	DALT (days)	total residue flufenacet* (mg/kg)
RA-2010/94 40044/0 0044-94 GLP yes 1994	Barley, winter Loreley	Germany [redacted] Europe, North	60 WG	1	0.24	0.08	13	green material grain straw	124 201 253 253	<0.05 <0.05 <0.05 <0.1
RA-2010/94 40045/9 0045-94 GLP yes 1994	Rye, winter Gambit	Germany [redacted] Europe, North	60 WG	1	0.24	0.08	21	green material grain straw	94 172 246 246	0.06 <0.05 <0.05 <0.1
RA-2010/94 40046/7 0046-94 GLP yes 1994	Wheat, winter Contra	Germany [redacted] Europe, North	60 WG	1	0.24	0.08	25	green material grain straw	119 191 247 247	<0.05 <0.05 <0.05 <0.10
RA-2010/94 40047/5 0047-94 GLP yes 1994	Wheat winter Contra	Germany [redacted] Europe, North	60 WG	1	0.24	0.08	21	green material grain straw	133 190 246 246	0.10 <0.05 <0.05 <0.1

*Residues for total residue flufenacet determined as FOE 5043 Trifluoro acetamide and calculated as flufenacet
DALT : Days after last treatment

Conclusion

Four trials on winter cereals (1 trial on barley, 1 trial on winter rye, and 2 trials on winter wheat) were conducted during the 1994-1995 growing season in Germany to investigate the residues of flufenacet in cereals after application of 240 g flufenacet/ha and 120 g diflufenican/ha using a mixed WG formulation of the two substances. The plants were treated in autumn (November), at growth stages ranging from BBCH 13 (3 leaves unfolded) to BBCH 25 (5 tillers detectable). At mature harvest, the residues of flufenacet were < 0.05 mg/kg in grain and < 0.10 mg/kg in straw.

Supplementary field trials – Northern and southern Europe (application rate 240- 254 g as/ha)

Report:	**KCA 6.3.1/06, [redacted]; 2002; M-058156-01**
Title:	Determination of residues of FOE 5043 in/on wheat and barley following spray application of FOE 5043 & Diflufenican (600 SC) to winter wheat and winter barley in the field in Northern and Southern France, Germany and Spain
Document No & Report No:	M-058156-01-1 RA-2144/00, dated 2002-04-12
Guidelines:	Directive 94/414/EEC Residues in or on treated products, food and feed, EU 7029/VI/95 rev.5 dated 22 July 1997
GLP	Yes; Deviations: none

Material and methods

Two trials on winter wheat and two trials on winter barley were conducted during the 2000-2001 growing season in northern and southern France, Germany and Spain using 'Flufenacet + Diflufenican SC 600'. The plants were treated at the growth stage BBCH 13-13 leaves unfolded), which was usually in autumn (October - December), except in the Spanish trial, in which treatment was in February. The application rate was 240 g flufenacet /ha, except in the Spanish trial, in which the applied rate was slightly higher (254 g flufenacet/ha).

Grain and straw samples were taken at normal harvest, which was between 148 and 254 days after application.

All the samples were analysed for residues of flufenacet according to the method 00346 ([redacted], M.; 1995; M-018864-02) which yields the combined level of the parent compound and all its metabolites containing the N-fluorophenyl-N-isopropyl functional group. Residues are expressed as parent flufenacet.

Before analysis the grain and straw samples were stored frozen for less than 8 months (226 days). This storage period is adequately covered by the available storage stability data for flufenacet.

Findings

Recovery rates were determined prior to analysis in order to validate the analytical method and concurrently with the sample analysis in order to check the accuracy of the residue analysis. Fortification was performed by spiking control samples with one of the following compounds or a mixture thereof: parent flufenacet, flufenacet oxalate, flufenacet sulfonic acid, flufenacet thioglycolate sulfoxide. The recovery-rates and corresponding relative standard deviations (RSD) were satisfactory as shown in Table 6.3.1-10. The limit of quantification was 0.05 mg/kg in grain and 0.10 mg/kg in straw.
No residues were found in any of the untreated samples, i.e. residues were < LOQ for flufenacet.

The residues found in wheat and barley samples from the individual trials were below the LOQ for grain. Residues in straw were less than the LOQ in 3 trials and 0.11 mg/kg in the Spanish trial. The findings are summarised in Tables 6.3.1-11 (northern Europe) and 6.3.1-12 (southern Europe).

Table 6.3.1-10: Procedural recovery data for Flufenacet
The LOQ is marked in bold.

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
RA-2144/00 R 2000 0566/0 0566-00 and R 2000 0567/9 0567-00 GLP: yes 2000	Wheat, winter	grain	total residue flufenacet	11	**0.05**	94; 84; 81; 98; 98; 91; 81; 87; 80; 80; 79	77	98	86	9.3
				1	0.5	73	73	73	73	
				12	overall mg/kg		73	98	85	10.0
		straw	total residue flufenacet	11	**0.10**	101; 93; 79; 88; 90; 92; 84; 81; 77; 83; 81	77	101	86	8.4
				1	1.0	75	75	75	75	
				12	overall mg/kg		75	101	85	8.9
RA-2144/00 R 2000 0568/7 0568-00 and R 2000 0570/9 0570-00 GLP: yes 2000	Barley, winter	grain	total residue flufenacet	11	**0.05**	111; 104; 89; 84; 89; 73; 74; 73; 72; 77; 81	72	111	84	15.6
				1	0.5	80	80	80	80	
				12	overall mg/kg		72	111	84	15.0
		straw	total residue flufenacet	11	**0.10**	86; 83; 81; 79; 82; 74; 85; 81; 78; 95; 97	74	97	84	8.3
				1	1.0	81	81	81	81	
				12	overall mg/kg		74	97	84	8.0

Fortified with flufenacet, flufenacet oxalate, flufenacet sulfonic acid, flufenacet thioglycolate sulfoxide or a mixture thereof; determined as FOE 5043 trifluoroacetamide and calculated as flufenacet equivalent

Table 6.3.1-11: Residues of flufenacet in barley and wheat after post-emergence application of Flufenacet + Diflufenican SC 600 (containing 400 g/L flufenacet + 200 g/L diflufenican) in northern Europe

Study Trial No. Trial SubID GLP Year	Crop Variety	Country	Application FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Residues Portion analysed	DALT (days)	total residue flufenacet* (mg/kg)
RA-2144/00 R 2000 0566 0 0566-00 GLP yes 2000	Wheat, winter Isen-grain	France [redacted] Europe, North	600 SC	1	0.24	0.08	13	grain straw	243 243	<0.05 <0.10
RA-2144/00 R 2000 0568 7 0568-00 GLP yes 2000	Barley, winter Theresa	Germany [redacted] Europe, North	600 SC	1	0.24	0.08	13	grain straw	254 254	0.05 <0.10

*Residues for total residue flufenacet (determined as FOE 5043 Trifluoro acetamide and calculated as flufenacet)
DALT : Days after last treatment

Table 8.3.1-12: Residues of flufenacet in barley and wheat after post-emergence application of Flufenacet + Diflufenican SC 600 (containing 400 g/L flufenacet + 200 g/L diflufenican) in southern Europe

Study Trial No. Trial SubID GLP Year	Crop Variety	Country	Application FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Residues Portion analysed	DALT (days)	total residue flufenacet* (mg/kg)
RA-2144/00 R 2000 0567 9 0567-00 GLP yes 2000	Wheat, winter Soissons	France [redacted] Europe, South	600 SC	1	0.24	0.08	13	grain straw	196 196	<0.05 <0.10
RA-2144/00 R 2000 0570 9 0570-00 GLP yes 2001	Barley, winter Graphic	Spain [redacted] Europe, South	600 SC	1	0.254	0.08	13	grain straw	148 148	<0.05 0.11

*Residues for total residue flufenacet (determined as FOE 5043 Trifluoro acetamide and calculated as flufenacet)
DALT : Days after last treatment

Conclusion

Three trials on cereals (2 trials on wheat and 1 trial on barley) were conducted during the 2000-2001 growing season in northern and southern France, and in Germany using the 'Flufenacet + Diflufenican SC 600' formulation. The plants were treated in autumn at the growth stage BBCH 13 (3 leaves unfolded). Following application of 240 g flufenacet/ha, the residues of flufenacet at harvest were < 0.05 mg/kg in grain and < 0.10 mg/kg in straw.
A fourth trial with the 'Flufenacet + Diflufenican SC 600' formulation was performed on barley in Spain during the 2001 growing season. The plants were treated in February at the growth stage BBCH 13 (3 leaves unfolded). The application rate slightly exceeded the target rate at 254 g flufenacet/ha. At harvest, the residues of flufenacet were < 0.05 mg/kg in grain and 0.11 mg/kg in straw.

Supplementary field trials – <u>southern Europe</u> (application rate 220 - 240 g as/ha)

Report:	**KCA 6.3.1/07, [redacted]; 2010; M-361495-01**
Title:	Determination of the residues of diflufenican and flufenacet in/on winter barley after spraying of Flufenacet & Diflufenican SC 600 in the field in France (South)
Document No & Report No:	M-361495-01-1 09-2048 dated 2010-01-12
Guidelines:	Directive 94/414/EC Residues in or on treated products, food and feed EU 7029/VI/95 rev.5 dated 22 July 1997
GLP	Yes; Deviations: none

Material and methods

Three trials on barley were conducted during the 2008-2009 growing season in southern France using the formulation 'Flufenacet + Diflufenican SC 600'. The plants were treated at the growth stage BBCH 13 (3 leaves unfolded) in late autumn (December). The application rate was 240 g flufenacet/ha.

Green plant samples were taken for analysis at the growth stage BBCH 13 immediately after application. Grain and straw samples were taken at normal harvest, which was between 188 and 203 days after application.

All the samples were analysed for residues of flufenacet according to the method 01179 ([redacted]; 2010; M-362716-01) , which yields the combined level of the parent compound and all its metabolites containing the N-fluorophenyl-N-isopropyl functional group. The procedure involves oxidation of the residues with potassium permanganate, hydrolysis with sulfuric acid, steam distillation, liquid/liquid partitioning, and LC-MS/MS determination of the thus obtained 4-fluoro-N-isopropylaniline. Residues are expressed as parent flufenacet.

Before analysis, samples were stored frozen for less than 11 months for green material and 4 months for grain and straw (329 days for green material, 113 days for grain and 115 days for straw). These storage periods are adequately covered by the storage stability data for flufenacet.

Findings

Recovery rates were determined concurrently with the sample analysis in order to check the accuracy of the residue analysis. For flufenacet, fortification was performed by spiking control samples with one of the following compounds or a mixture thereof: parent flufenacet, flufenacet oxalate hydrate, flufenacet sulfonic acid sodium salt, flufenacet thioglycolate sulfoxide. The overall mean recoveries for total residue of flufenacet were within the acceptable range of 70 – 110% (RSD < 20%) with the exception of green material when fortified with flufenacet and for straw when fortified with the mixture of the metabolites where values were just below guideline requirements (67 and 69% respectively).
The limit of quantification was 0.01 mg/kg in grain and green material, and 0.05 mg/kg in straw.
No apparent residues were found in any of the untreated samples i.e. residues were < LOQ for flufenacet.

Flufenacet derived residues in grain were < 0.01 mg/kg and ranged from < 0.05 – 0.06 mg/kg in straw.
The residues found in the barley samples from the individual trials are summarised in Table 6.3.1-14.

Table 6.3.1-13: Procedural recoveries for flufenacet in winter barley

The LOQ is marked in bold.

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
Fortified with flufenacet										
09-2048 09-2048-01 09-2048-02 09-2048-03 GLP: yes 2008	Barley, winter	green material	total residue flufenacet	4	**0.01**	66;68;74;67	66	74	69	5.2
				1	5.0	60	60	60	60	
				1	12	66	66	66	66	
				6	overall		60	74	67	6.7
		grain	total residue flufenacet	3	**0.01**	92;79;62	62	92	78	19.4
				4	0.10	71;65;69;80	65	80	71	8.9
				7	overall		62	92	74	14.0
		straw	total residue flufenacet	2	**0.05**	87;87	87	87	87	
				2	0.50	62;62	62	62	62	
				4	overall		62	87	75	19.4
Fortified with mixture of flufenacet oxalate hydrate/flufenacet sulfonic acid sodium salt/flufenacet thioglycolate sulfoxide (1/1/1)										
09-2048 09-2048-01 09-2048-02 09-2048-03 GLP: yes 2008	Barley, winter	green material	total residue flufenacet	1	**0.01**	116	-	-	-	-
				1	2.4	90	-	-	-	
				2	overall		90	116	103	-
		grain	total residue flufenacet	1	**0.01**	83	-	-	-	-
				1	0.10	67	-	-	-	-
				2	overall		67	83	75	-
		straw	total residue flufenacet	2	**0.05**	71, 67	67	71	69	
				2	overall		67	71	69	-

Table 6.3.1-14: Residues of flufenacet in barley after post-emergence application of flufenacet + diflufenican SC 600 (containing 400 g/L flufenacet + 200 g/L diflufenican) in southern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues		
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	total residue flufenacet* (mg/kg)
09-2048 09-2048-01 GLP: yes 2008	Barley, winter Platine	France [redacted] Europe, South	600 SC	1	0.24	0.080	13	green material	0	9.2
								grain	197	<0.01
								straw	197	<0.05
09-2048 09-2048-02 GLP: yes 2008	Barley, winter Baraka	France [redacted] Europe, South	600 SC	1	0.24	0.080	13	green material	0	11
								grain	188	<0.01
								straw	188	0.06
09-2048 09-2048-03 GLP: yes 2008	Barley, winter Esterel	France [redacted] South	600 SC	1	0.24	0.080	13	green material	0	9.5
								grain	203	<0.01
								straw	203	0.06

*Residues for total residue flufenacet (determined as 4-fluoro-N-isopropylaniline and calculated as flufenacet)
DALT : Days after last treatment

Conclusion

Three trials on barley were conducted in the southern part of France during the 2008/2009 growing season. The product 'Flufenacet + Diflufenican SC 600' was applied at a rate of 0.6 L/ha corresponding to 240 g flufenacet/ha and 120 kg diflufenican/ha. Treatment was performed in autumn at the growth stage BBCH 13. At mature harvest flufenacet residues were < 0.01 mg/kg in grain and < 0.05-0.06 mg/kg in straw.

Report:	**KCA 6.3.1/08, [redacted],,[redacted]; 2010; M-363200-02**
Title:	Determination of the residues of diflufenican and flufenacet in/on winter wheat after spraying of Flufenacet & Diflufenican SC 600 in the field in France (south)
Document No & Report No:	M-363200-02-1 09-2052 dated 2010-08-05
Guidelines:	Directive 94/414/EEC Residues in or on treated products, food and feed EU 7029/VI/95 rev.5 dated 22 July 1997
GLP	yes

Material and methods

Four trials on winter wheat were conducted during the 2008/2009 growing season in southern France using a SC formulation containing 200 g/L diflufenican and 400 g/L flufenacet. The plants were treated in late autumn and winter (December-January), at growth stages ranging from BBCH 13 (3 leaves unfolded) to BBCH 21 (first tiller detectable). The application rate was 240 g flufenacet /ha in 3 trials. In one trial the application rate was underdosed by 7% (220 g flufenacet /ha).

Green plant samples were taken for analysis at the growth stages BBCH 13 immediately after application. Grain and straw samples were taken at normal harvest, which was between 153 and 220 days after application.

All samples were analysed for residues of flufenacet according to the method 01179 ([redacted]; 2010; M-362716-01) , which yields the combined level of the parent compound and all its metabolites containing the N-fluorophenyl-N-isopropyl functional group. Residues are expressed as parent flufenacet.

Before analysis for flufenacet the samples were stored frozen for less than 12 months (352 days) for green material and about 5 months (155 and 143 days) for grain and straw, respectively.
All storage periods are adequately covered by the storage stability data for flufenacet.

Findings

Recovery rates were determined concurrently with the sample analysis in order to check the accuracy of the residue analysis. For flufenacet fortification was performed by spiking control samples with one of the following compounds or a mixture thereof: parent flufenacet, flufenacet oxalate hydrate, flufenacet sulfonic acid sodium salt, flufenacet thioglycolate sulfoxide. The recovery-rates and corresponding RSD were satisfactory (cf. Table 6.3.1-15). The limit of quantification was 0.01 mg/kg in green plant and grain, and 0.05 mg/kg in straw.
No residues were found in any of the untreated samples. Flufenacet derived residues in grain ranged from < 0.01-0.05 mg/kg. In straw residues were < 0.05 mg/kg in 3 trials and 0.09 mg/kg in one trial.
The residues found in the wheat samples from the individual trials are summarised in Tables 6.3.1-16.

Table 6.3.1-15: Procedural recoveries for flufenacet in winter wheat

The LOQ is marked in bold.

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
Fortified with flufenacet										
09-2052 09-2052-01 09-2052-02 09-2052-03 09-2052-04 GLP: yes 2008	Wheat, winter	green material	total residue flufenacet	1	**0.10**	74	74	74	74	
				1	20	75	75	75	75	
				3	30	82; 87; 95	82	95	88	7.5
				5	overall		74	95	83	10.6
		grain	total residue flufenacet	1	**0.01**	97	97	97	97	
				2	0.10	103; 91	91	103	97	
				3	overall		91	103	97	6.2
		straw	total residue flufenacet	1	**0.05**	70	70	70	70	
				2	0.50	87; 84	84	87	86	
				3	overall		70	87	80	11.3
Fortified with mixture of flufenacet oxalate hydrate/flufenacet sulfonic acid sodium salt/flufenacet thioglycolate sulfoxide (1/1/1)										
09-2052 09-2052-01 09-2052-02 09-2052-03 09-2052-04 GLP: yes 2008	Wheat, winter	green material	total residue flufenacet	1	**0.01**	69		-	-	-
				2	18	72; 73	72	73	73	-
				1	27	66	-	-	-	-
				2	30	67; 65	65	67	66	-
				6	overall		65	73	69	4.8
		grain	total residue flufenacet	3	**0.01**	83; 73; 72	72	83	76	8.0
				3	0.10	79; 89; 77	77	89	82	7.9
				6	overall		72	89	79	8.1
		straw	total residue flufenacet	1	**0.05**	71	-	-	-	-
				4	0.6	69; 74; 85; 92	69	92	80	13
				5	overall		69	92	78	13

Table 6.3.1-16: Residues of flufenacet in wheat after post-emergence application of flufenacet + diflufenican SC 600 (containing 400 g/L flufenacet + 200 g/L diflufenican) in southern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues		
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	total residue flufenacet* (mg/kg)
09-2052 09-2052-01 GLP: yes 2008	Wheat, winter Arlequin	France [redacted] Europe, South	600 SC	1	0.24	0.080	13	green material	0	17
								grain	209	0.01
								straw	209	<0.05
09-2052 09-2052-02 GLP: yes 2009	Wheat, winter Aubusson	France [redacted] Europe, South	600 SC	1	0.24	0.080	21	green material	0	22
								grain	153	<0.01
								straw	153	<0.05
09-2052 09-2052-03 GLP: yes 2008	Wheat, winter Aubusson	France [redacted] Europe, South	600 SC	1	0.22	0.085	13	green material	0	17
								grain	196	0.05
								straw	196	0.09
09-2052 09-2052-04 GLP: yes 2008	Wheat, winter Mendel	France [redacted] Europe, South	600 SC	1	0.24	0.080	13	green material	0	24
								grain	220	<0.01
								straw	220	<0.05

*Residues for total residue flufenacet (determined as 4-fluoro-N-isopropylaniline and calculated as flufenacet)
DALT : Days after last treatment

Conclusion

Four trials on winter wheat were performed during the 2008/2009 growing season in southern France to investigate the residues of flufenacet (and diflufenican) in cereals after application of 240 g flufenacet/ha (and 120 g diflufenican /ha) using a mixed SC formulation of the two substances. The plants were treated in autumn or winter in case of wet weather conditions (December-January), at growth stages BBCH 13 (3 leaves unfolded) to BBCH 21 (first tiller detectable). At mature harvest, the residues of flufenacet amounted to < 0.01-0.05 mg/kg in grain and <0.05-0.09 mg/kg in straw.

Conclusion for the use of flufenacet in northern and southern Europe with use patterns involving 240 g flufenacet/ha

The set of residue data on wheat, barley and rye conducted with the straight formulation WG 60 and evaluated for Annex I inclusion is considered appropriate to also support the representative use for the mixed product 'flufenacet + diflufenican SC 600' at a rate of 240 g flufenacet/ha in northern Europe. The use pattern for both products involve the same application parameters and residue data obtained from trials using a WG formulation are considered appropriate to also support SC formulations. Both formulations types are known to produce comparable residues, particularly if the application is conducted early during the crop development. In all trials, residues have shown to be less than the LOQ for grain (< 0.05 mg/kg) and straw (< 0.1 mg/kg).
Nevertheless, 6 trials on wheat, barley and rye are reported for the northern region with WG and SC formulations at an application rate of 240 g flufenacet/ha which demonstrate that the residue behaviour of flufenacet does not alter when applied in a mixture with diflufenican. Applications were performed early post-emergence during leaf development until mid of tillering (BBCH 13-25). Residues in grain and straw were always below the LOQ of 0.05 or 0.1 mg/kg, respectively.

No residue data for flufenacet from the southern region were evaluated for Annex I inclusion. With the present dossier 9 trials are submitted to support the use pattern at 240 g as/ha with early post-emergence application. The trials were already evaluated at a national level (evaluating member state France, product name FOSBURI). Flufenacet was applied at rates ranging from 220 – 254 g as/ha during leaf development until beginning of tillering (BBCH 13-21). The trials on wheat and barley were conducted over two growing seasons. Residues in grain ranged from < 0.01 to 0.05 mg/kg (median (< 0.01 mg/kg), and in straw from 0.05 to 0.11 mg/kg (median 0.06 mg/kg).

The data sets from the northern and southern region are considered to represent the critical GAPs for flufenacet.
The data sets were recently reviewed by the RMS France and EFSA and the data set from southern Europe forms the basis for the new MRL proposal of 0.1 mg/kg as published with the EFSA Reasoned Opinion (EFSA Journal 2012;10(4):2689).

Supplementary field trials in northern Europe (application rate 120 g as/ha)

Report:	**KCA 6.3.1/10, [redacted],; [redacted]; 2013; M-459795-01**
Title:	Determination of the residues of flufenacet and flurtamone in/on winter barley and winter wheat after Spraying of DFF & FFA & FLT SC 360 in the field in Germany, Belgium and the Netherlands
Document No Report No	M-459795-01-1 Study no. 12-2001 dated 2013-07-09
Guidelines:	• Regulation (EC) no 1107/2009 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 (July 22, 1997) • OECD 509 Adopted 2009-09-07; Crop Field Trial • US EPA OCSPP Guideline No. 860.1500
GLP	Yes ; Deviations : none

Material and methods

Four trials on winter wheat (2) and winter barley (2) were conducted during the 2011/2012 growing season in Germany (2), Belgium (1), and the Netherlands (1) using an SC formulation containing 120 g/L flufenacet, 120 g/L diflufenican and 120 g/L flurtamone (DFF+FFA+FLT SC 360). The plants were treated in late autumn (November) in 3 trials, at growth stage BBCH 22/23. In one trial the requested growth stage was not reached in autumn and thus the application was conducted in spring (April) at BBCH 25. The application was at the required rate in all trials (120 g flufenacet /ha).

Green plant samples were taken for analysis at the growth stages BBCH 49 (forage stage) and at BBCH 83 (silage stage, whole plant without root). Grain and straw samples were taken at normal harvest, which was between 112 and 263 days after application.

All samples were analysed for residues of flufenacet according to the method 01100/M002 ([redacted], S.; [redacted], L.; 2013; M-448503-01), which yields the combined level of the parent compound and all its metabolites containing the N-fluorophenyl-N-isopropyl functional group. Residues are expressed as parent flufenacet.

Before analysis for flufenacet the samples were stored frozen up to 12 months (371 days) for green material/whole plants without root and up to 10 months (300 days) for grain and straw.
All storage periods are adequately covered by the storage stability data for flufenacet.

Findings

Recovery rates were determined concurrently with the sample analysis in order to check the accuracy of the residue analysis. The recovery-rates and relative standard deviations (RSD) were satisfactory (cf. Table 6.3.1-17). The limit of quantification was 0.01 mg/kg in green plant material and grain, and 0.05 mg/kg in straw.
The residues of flufenacet in the untreated samples were < LOQ. The residues found in the wheat and barley samples from the individual trials are summarised in Table 6.3.1-18. Flufenacet derived residues in green material at forage stage and silage stage, in grain and straw were less than the LOQ

(< 0.01 mg/kg for green plant material and grain and < 0.05 mg/kg for straw) in all trials.

Table 6.3.1-17: Procedural recovery data for Flufenacet
The LOQ is marked in bold

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
12-2001 12-2001-01 12-2001-02 12-2001-03 12-2001-04 GLP: yes 2011	Barley, winter	green material[#]	total residue flufenacet	6	**0.01**	77;82;96;98; 103; 105	77	105	94	12.2
				4	0.10	84;88;90;104	84	104	92	9.5
				3	1.0	91;92;102	91	102	95	6.4
				1	20	77	77	77	77	
				14	overall		77	105	92	10.5
		grain	total residue flufenacet	4	**0.01**	69;79;94;95	69	95	84	14.9
				2	0.10	89;91	89	91	90	
				6	overall		69	95	86	11.8
		straw	total residue flufenacet	2	**0.05**	88;97	88	97	93	
				2	0.50	94;97	94	97	96	
				4	overall		88	97	94	4.5

Sample materials green material and whole plant without root are grouped to the sample group cereals green material.
Fortified with flufenacet, determined as 4-fluoro-N-isopropylaniline and calculated as flufenacet
The recoveries were performed during the conduct of the study 12-2001, 12-2002 (and 12-2003, not reported).

Table 6.3.1- 18: Residues of flufenacet in wheat and barley after post-emergence application of flufenacet + diflufenican + flurtamone SC 360 (containing 120 g/L flufenacet + 120 g/L diflufenican + 120 g/L flurtamone) in northern Europe

Study Trial No. GLP Year	Crop Variety	Country	Application					Residues			
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Growth stage (BBCH)	total residue flufenacet * (mg/kg)
12-2001 12-2001-01 GLP: yes 2011	Barley, winter Meridian	Germany [redacted] Europe, North	360 SC	1	0.12	0.040	23	green material	170	49	<0.01
								whole plant without roots	216	83	<0.01
								grain	244	92	<0.01
								straw	244	92	<0.05
12-2001 12-2001-02 GLP: yes 2011	Barley, winter Saskia (early 6-rows variety, mid height)	Belgium [redacted] Europe, North	360 SC	1	0.12	0.040	22	green material	181	49	<0.01
								whole plant without roots	209	83	<0.01
								grain	252	89	<0.01
								straw	252	89	<0.05
12-2001 12-2001-03 GLP: yes 2011	Wheat, winter Inspiration	Germany [redacted] Europe, North	360 SC	1	0.12	0.040	22	green material	192	49	<0.01
								whole plant without roots	239	83	<0.01
								grain	263	89	<0.01
								straw	263	89	<0.05
12-2001 12-2001-04 GLP: yes 2012	Wheat, winter Taureq winter	Netherlands [redacted] Europe, North	360 SC	1	0.12	0.040	25	green material	41	49	<0.01
								whole plant without roots	83	83	<0.01
								grain	112	89	<0.01
								straw	112	89	<0.05

*Residues for total residue flufenacet (determined as 4-fluoro-N-isopropylaniline and calculated as flufenacet)
DALT : Days after last treatment

Conclusion

Four trials on winter wheat and winter barley were performed during the 2011/2012 growing season in northern Europe to investigate the residues of flufenacet (and flurtamone) in cereals after application of 120 g flufenacet/ha using a triple mixture also containing flurtamone and diflufenican. The plants were treated in autumn at growth stages BBCH 22/23 or in spring in one trial in case of delayed development (BBCH 25). Residues of flufenacet were < 0.01 mg/kg in green material at forage and silage stage. At mature harvest, flufenacet derived residues were < 0.01 mg/kg in grain and <0.05 mg/kg in straw.

Supplementary field trials in northern and southern Europe (application rate 110-120 g as/ha)

Report:	**KCA 6.3.1/12, [redacted]; 2013; M-460003-01**
Title:	Determination of the residues of diflufenican, flufenacet and flurtamone in/on winter barley after spray application of DFF & FFA & FLT SC 360 in Germany, the United Kingdom, southern France and Italy
Document No Report No	M-460003-01-1 Study No. 11-2094 dated 2013-07-11
Guidelines:	• Regulation (EC) No 1107/2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC • OECD 509 Adopted 2009-09-07, Crop Field Trial • EC Guidance working document 7029/VI/95 rev.5 (1997-07-22) • US EPA OCSPP Guideline No. 860.1500
GLP	Yes ; Deviations : none

Report:	**KCA 6.3.1/09, [redacted]; 2013; M-459755-01**
Title:	Determination of the residues of diflufenican, flufenacet and flurtamone in/on winter wheat after spray application of DFF & FFA & FLT SC 360 in Germany, the Netherlands, southern France and Spain
Document No Report No	M-459755-01-1 Study No. 11-2095 dated 2013-07-10
Guidelines:	• Regulation (EC) No 1107/2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC • OECD 509 Adopted 2009-09-07, Crop Field Trial • EC Guidance working document 7029/VI/95 rev.5 (1997-07-22) • US EPA OCSPP Guideline No. 860.1500
GLP	Yes ; Deviations : none

Material and methods

In total 8 trials on winter wheat and winter barley were conducted during the 2010/2011 growing season. Four trials were conducted in the northern European climatic zone (2 trials on barley, 2 trials on wheat) in Germany (2), the Netherlands (1) and the United Kingdom (1). Four trials (2 trials on barley, 2 trials on wheat) were conducted in the southern European climatic zone: France (2), Spain (1) and Italy (1). For all trials an SC formulation containing 120 g/L flufenacet, 120 g/L diflufenican and 120 g/L flurtamone (DFF+FFA+FLT SC 360) has been used.

The application schedule called for application of 1 L product/ha (corresponding to 120 g flufenacet/ha) at growth stage BBCH 25. In the northern zone, the plants were treated between January and April at growth stage BBCH 25. Due to extreme dry weather conditions, in one trial delayed germination resulted in a range of growth stages at application (actual BBCH 23 to 27), however, the average was estimated to be at BBCH 25.

In the southern zone plants were treated in March at growth stage BBCH 25 to 30. Due to unfavourable weather conditions the treatment was slightly delayed in 3 trials.

The application was at the required rate (120 g flufenacet /ha) in all trials except one from the northern zone where the application rate was slightly underdosed (110 g flufenacet /ha).

Samples of green material at early growth stages were taken to generate residue data needed to refine the ecotoxicological evalutaion (day 0, 1, 3, 5, 14).
Green plant samples were taken for analysis at the growth stages BBCH 51 (forage stage) and at BBCH 83 (silage stage, whole plant without root).
Grain and straw samples were taken at normal harvest, which was between 117 and 262 days after application for the northern European trials and 80 and 119 days for the southern European trials.

All samples were analysed for residues of flufenacet according to the method 01100/M002 ([redacted], S.; [redacted], L.; 2013; M-448503-01) which yields the combined level of the parent compound and all its metabolites containing the N-fluorophenyl-N-isopropyl functional group. Residues are expressed as parent flufenacet.

Before analysis for flufenacet the samples were stored frozen up to 25 months (738 and 748 days) in study 11-2094 and 11-2095. All storage periods are adequately covered by the storage stability data for flufenacet.

Findings

Recovery rates were determined concurrently with the sample analysis in order to check the accuracy of the residue analysis. The recovery-rates and relative standard deviations (RSD) were satisfactory (cf. Table 6.3.1-20). The limit of quantification was 0.01 mg/kg in green plant material and grain, and 0.05 mg/kg in straw.

No residues were found in the untreated samples, i.e. residues were < LOQ for flufenacet except for barley green material samples (for three trials) collected on the day of treatment where the residues ranged from 0.012 to 0.022 mg/kg and one exception at 0.016 mg/kg in barley green material sample at BBCH 51 (DALT 18). Residues found in control samples were identified as contaminations in the water steam distilleries originating from the high residues of flufenacet found in the treated samples of green material at day 0 - 6. Since analysis of green material at early growth stages (day 0 to 14) intended for ecotoxicological evaluations was not needed for flufenacet, it was decided not to re-analyze these samples. All apparatus was thoroughly cleaned and tested for any further analyses.

The relative dry matter of control and treated samples of cereals green material harvested at forage stage (BBCH 51) and whole plant without root at silage stage (BBCH 83) was determined for studies 11-2094 and 11-2095. The determination of relative dry matter content was not conducted according to GLP. The results of the determination of relative dry matter for these samples are shown in Table 6.3.1-19.

The residues found in the wheat and barley samples from the individual trials are summarised in Tables 6.3.1-21 (northern Europe) and 6.3.1-22 (southern Europe).
Northern Europe: Flufenacet derived residues in green material at forage stage (BBCH 51) ranged between < 0.01 and 0.077 mg/kg, and residues in whole plant without root (BBCH 83) were between <0.01 and 0.019 mg/kg. Residues in grain amounted to <0.01 – 0.022 mg/kg and were less than the LOQ (0.05 mg/kg) in straw.
Southern Europe: Residues at forage stage of green plant material were between 0.027 and

0.081 mg/kg and at silage stage between 0.017 and 0.061 mg/kg. In grain at harvest, residues ranged from <0.01 to 0.035 mg/kg and from <0.05 to 0.059 mg/kg in straw.

Table 6.3.1- 19: Relative dry matter content of control and treated samples at forage and silage stage

Trial no. Country	Control (C) / Treated (T)	Growth stage [BBCH]	DALT	Crop	Sample material	Relative dry matter [%]
North European climatic zone						
11-2095-01 Germany	C	51	51	wheat	green material	21.9
	T	51	51		green material	24.9
	C	83	93		whole plant without root	41.9
	T	83	93		whole plant without root	39.1
11-2095-02 Netherlands	C	47-57*	43	wheat	green material	23.4
	T	47-57*	43		green material	22.3
	C	83	95		whole plant without root	43.6
	T	83	95		whole plant without root	43.1
11-2094-01 Germany	C	51	181	barley	green material	17.3
	T	51	181		green material	15.4
	C	83	209		whole plant without root	29.8
	T	83	209		whole plant without root	30.3
11-2094-02 United Kingdom	C	51	119	barley	green material	19.4
	T	51	119		green material	22.3
	C	83	164		whole plant without root	34.3
	T	83	164		whole plant without root	38.3
South European climatic zone						
11-2095-03 France	C	51	57	wheat	green material	21.9
	T	51	57		green material	21.3
	C	83	90		whole plant without root	33.3
	T	83	90		whole plant without root	33.6
11-2095-04 Spain	C	51	42	wheat	green material	30.3
	T	51	42		green material	25.8
	C	83	68		whole plant without root	43.6
	T	83	68		whole plant without root	44.8
11-2094-03 France	C	51	55	barley	green material	27.9
	T	51	55		green material	25.8
	C	83	83		whole plant without root	40.7
	T	85	83		whole plant without root	43.8
11-2094-04 Italy	C	51	28	barley	green material	23.1
	T	51	28		green material	22.7
	C	83	50		whole plant without root	30.6

Trial no. Country	Control (C) / Treated (T)	Growth stage [BBCH]	DALT	Crop	Sample material	Relative dry matter [%]
	T	83	50		whole plant without root	30.6

* Due to extreme dry weather conditions, germination was partly delayed resulting in a range of different growth stages at sampling.

Table 6.3.1- 20: Procedural recoveries for flufenacet in/on wheat and barley

The LOQ is marked in bold

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
11-2094 11-2094-01 to 11-2094-04 11-2095 11-2095-01 to 11-2095-04	Barley, Wheat	green material*	total residue flufenacet	3	**0.01**	106;109; 115	106	115	110	4.2
				4	0.10	90;96; 104;118	90	118	102	11.9
				1	1.0	79	79	79	79	
				1	10	85	85	85	85	
				1	20	77	77	77	77	
				10	overall		77	118	98	15.0
GLP: yes 2011		grain	total residue flufenacet	3	**0.01**	83;91; 114	83	114	96	16.8
				9	0.10	88;91; 97;98; 101;108; 111;116; 113	88	116	103	9.7
				1	1.0	116	116	116	116	
				1	10	76	76	76	76	
				2	20	75;96	75	96	86	
				16	overall		75	116	98	14.0
		straw	total residue flufenacet	1	**0.05**	78**	78	78	78	
				1	0.50	93**	93	93	93	
				2	overall		78	93	86	

FL = Fortification level; RSD = Relative standard deviation, n = number of tests, LOQ = Practical limit of quantification

Fortified with flufenacet, determined as 4-fluoro-N-isopropylaniline and calculated as flufenacet

*Samples of green material and whole plant without root were combined to “green material” for calculation of the mean value and RSD.

** These recoveries exclusively were conducted during the study 11-2094 in barley straw which is also representative for wheat straw.

Table 6.3.1- 21: Residues of flufenacet in wheat and barley after post-emergence application of flufenacet + diflufenican + flurtamone SC 360 (containing 120 g/L flufenacet + 120 g/L diflufenican + 120 g/L flurtamone) in northern Europe

Study Trial No. GLP Year	Crop Variety	Country	Application FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Residues Portion analysed	DALT (days)	Growth stage (BBCH)	total residue flufenacet (mg/kg)
11-2094 11-2094-01 GLP: yes 2011	Barley, winter Ketos winter barley	Germany [redacted] Europe, North	360 SC	1	0.12	0.040	25	green material	0 1 3 5 14 181	25 25 25 25 29 51	3.3 1.7 1.6 1.6 1.0 <0.01/0.016*
								whole plant without roots	209	83	0.019
								grain	262	89	0.017
								straw	262	89	<0.05
11-2094 11-2094-02 GLP: yes 2012	Barley, winter Carat Winter Barley	United Kingdom [redacted] Europe, North	360 SC	1	0.12	0.060	25	green material	0 1 3 4 14 119	25 25 25 25 26 51	14/0.022* 14 2.5 3.9 1.8 0.037
								whole plant without roots	164	83	<0.01
								grain	203	89	<0.01
								straw	203	89	<0.05
11-2095 11-2095-01 GLP: yes 2011	Wheat, winter Akteur	Germany 59457 [redacted] Europe, North	360 SC	1	0.12	0.040	25	green material	0 1 3 5 14 51	25 25 26 27 31 51	4.7/0.012* 3.6 2.5 1.6 0.42 0.020
								whole plant without roots	93	83	0.015
								grain	117	89	0.022
								straw	117	89	<0.05
11-2095 11-2095-02 GLP: yes 2011	Wheat, winter Tabasco	Netherlands [redacted] Europe, North	360 SC	1	0.1104	0.0399	25-27	green material	0 1 3 5 14 43	25 25 27 26 28 57	16/0.022* 12 5.0 3.8 0.66 0.077
								whole plant without roots	95	83	<0.01
								grain	121	89	<0.01
								straw	121	89	<0.05

Total residue flufenacet: Final determination as 4-fluoro-N-isopropylaniline, residues calculated as flufenacet.
* Residues found in control samples were identified as contaminations in the water steam distilleries originating from the high residues of flufenacet found in the treated samples of green material at day 0-5. All apparatus was thoroughly cleaned and tested for any further analyses.

Table 6.3.1- 22: Residues of flufenacet in wheat and barley after post-emergence application of flufenacet + diflufenican + flurtamone SC 360 (containing 120 g/L flufenacet + 120 g/L diflufenican + 120 g/L flurtamone) in southern Europe

Study Trial No. GLP Year	Crop Variety	Country	Application FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Residues Portion analysed	DALT (days)	Growth stage (BBCH)	total residue flufenacet (mg/kg)
11-2094 11-2094-03 GLP: yes 2011	Barley, winter Kétos Winter Barley	France [redacted] Europe, South	360 SC	1	0.12	0.040	29	green material	0 1 3 6 14 55	29 29 29 29 30 51	5.2 5.1 3.3 1.4 0.48 0.027
								whole plant without roots	83	85	0.031
								grain	108	89	<0.01
								straw	108	89	<0.05
11-2094 11-2094-04 GLP: yes 2011	Barley, winter Aldebaran winter variety	Italy [redacted] Europe, South	360 SC	1	0.12	0.030	25	green material	0 1 3 5 14 28	25 25 26 26 31 51	3.0/0.024* 2.9 1.8 1.2 0.44 0.081
								whole plant without roots	50	83	0.044
								grain	80	89	<0.01
								straw	80	89	0.059
11-2095 11-2095-03 GLP: yes 2011	Wheat, winter Cezanne	France [redacted] Europe, South	360 SC	1	0.12	0.040	29	green material	0 1 3 6 14 57	29 29 29 29 30 51	12/0.013* 8.6 4.3 1.9 0.70 0.033
								whole plant without roots	90	83	0.017
								grain	119	89	0.020
								straw	119	89	<0.05
11-2095 11-2095-04 GLP: yes 2011	Wheat, winter Moncada; sowing seed production	Spain [redacted] Europe, South	360 SC	1	0.12	0.040	30	green material	0 1 2 5 14 42	30 30 30 31 32 51	8.3/0.015* 8.3 5.1 0.84 0.41 0.071
								whole plant without roots	68	83	0.061
								grain	103	89	0.035
								straw	103	89	<0.05

Total residue flufenacet: Final determination as 4-fluoro-N-isopropylaniline, residues calculated as flufenacet.
* Residues found in control samples were identified as contaminations in the water steam distilleries originating from the high residues of flufenacet found in the treated samples of green material at day 0-6. All apparatus was thoroughly cleaned and tested for any further analyses.

Conclusion:
Four trials per geographical region on wheat and barley were performed during the 2010/2011 growing season (8 trials in total) to investigate the residues of flufenacet (as well as flurtamone and diflufenican) in cereals after application of 120 g flufenacet/ha (and 120 g flurtamone/ha, 120 g diflufenican/ha) using a triple mixture. The use pattern called for application at mid tillering, howevwe, due to unfavourable weather conditions the application was slightly delayed up to BBCH 29/30 in 3 southern European trials but still within tillering stage.
Northern Europe: Flufenacet derived residues in green material at forage stage (BBCH 31) ranged between < 0.01 and 0.077 mg/kg, and residues in whole plant without root (BBCH 83) were between <0.01 and 0.019 mg/kg. Residues in grain amounted to <0.01 – 0.022 mg/kg and were less than the LOQ (0.05 mg/kg) in straw.
Southern Europe: Residues at forage stage of green material were between 0.027 and 0.081 mg/kg and at silage stage between 0.017 and 0.061 mg/kg. In grain at harvest, residues ranged between <0.01 and 0.035 mg/kg and were <0.05-0.059 mg/kg in straw.
The deviation to the rate of the supported GAP (160 g as/ha) is within the EU's tolerance criteria for comparability (-25%).

Supplementary field trials in southern Europe (application rate flufenacet: 120 g as/ha)

Report:	**KCA 6.3.1/11, [redacted] [redacted], 2013, M-459799-01**
Title:	Determination of the residues of flufenacet and flurtamone in/on winter barley and winter wheat after spray application of DFF & FFA & FLT SC 360 in Southern France, Italy, Spain and Portugal
Document No Report No	M-459799-01-1 Study No. 12-2002 dated 2013-07-09
Guidelines:	• Regulation (EC) No 1107/2009 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 (July 22, 1997) • OECD 509 Adopted 2009-09-07, OECD Guideline for the testing of chemicals; • Crop Field Trial; US EPA OCSPP Guideline No. 860.1500
GLP	Yes ; Deviations: none

Material and methods
Four trials on winter wheat (2) and winter barley (2) were conducted during the 2011/2012 growing season in southern France, Italy, Spain and Portugal using an SC formulation containing 120 g/L flufenacet, 120 g/L diflufenican and 120 g/L flurtamone (DFF+FFA+FLT SC 360). The application was at the required rate in all trials (120 g flufenacet /ha) except in one trial (03) where the dose rate was slightly less (-7% of the target rate). Since the latter trial is out of the 25% range for comparability relative to the application rate of the supported GAP, this trial is disregarded in the following tables.
The plants in the remaining 3 trials were treated in late autumn (November, December) or beginning of March when the requested growth stage was not reached in autumn. Treatments were conducted at growth stage BBCH 22 to 25.

Green plant samples were taken for analysis at the growth stages BBCH 49 (forage stage) and at BBCH 83 (silage stage, whole plant without root). Grain and straw samples were taken at normal harvest, which was between 119 and 213 days after application.

All samples were analysed for residues of flufenacet according to the method 01100/M002 ([redacted] S.; [redacted], L.; 2013; M-448503-01) which yields the combined level of the parent compound and all its metabolites containing the N-fluorophenyl-N-isopropyl functional group. Residues are expressed as parent flufenacet.

Before analysis for flufenacet the samples were stored frozen up to 14 months (414 days) for green material/whole plants without root and up to 12 months (346 days) for grain and straw.
All storage periods are adequately covered by the storage stability data for flufenacet.

Findings

Recovery rates were determined concurrently with the sample analysis in order to check the accuracy of the residue analysis. The recovery-rates and relative standard deviations (RSD) were satisfactory (cf. Table 6.3.1-23). The limit of quantification was 0.01 mg/kg in green plant material and grain, and 0.05 mg/kg in straw.
The residues of flufenacet in the untreated samples were < LOQ. The residues found in the barley and wheat samples from the individual trials are summarised in Tables 6.1.3-24. Residues of flufenacet ranged between < 0.01 and 0.035 mg/kg in green plant material at forage stage and between < 0.01 and 0.045 mg/kg at silage stage (whole plant without root). At harvest, flufenacet derived residues in grain were < 0.01 mg/kg. In straw, residues amounted to < 0.05 - 0.069 mg/kg.

Table 6.3.1-23: Procedural recovery data for Flufenacet

The LOQ is marked in bold

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
12-2002 12-2002-01 12-2002-02 12-2002-04 yes 2011	Barley, winter	green material[#]	total residue flufenacet	6	**0.01**	77;82;96;98; 103; 105	77	105	94	12.2
				4	0.10	84;88;90;104	84	104	92	9.5
				3	1.0	91;92;102	91	102	95	6.4
				1	20	77	77	77	77	
				14	overall		77	105	92	10.5
		grain	total residue flufenacet	4	**0.01**	69;79;94;95	69	95	84	14.9
				2	0.10	89;91	89	91	90	
				6	overall		69	95	86	11.8
		straw	total residue flufenacet	2	**0.05**	88;97	88	97	93	
				2	0.50	94;97	94	97	96	
				4	overall		88	97	94	4.5

Sample materials green material and whole plant without root are grouped to the sample group cereals green material.
Fortified with flufenacet, determined as 4-fluoro-N-isopropylaniline and calculated as flufenacet
Recoveries were performed during the conduct of the study 12-2001, 12-2002 (and 12-2003, not reported).

Table 6.3.1- 24: Residues of flufenacet in wheat and barley after post-emergence application of flufenacet + diflufenican + flurtamone SC 360 (containing 120 g/L flufenacet + 120 g/L diflufenican + 120 g/L flurtamone) in southern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues		
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	total residue flufenacet* (mg/kg)
12-2002 12-2002-01 GLP: yes 2011	Barley, winter Platine	France [redacted] Europe, South	360 SC	1	0.12	0.040	25	green material	121	0.035
								whole plant without roots	155	0.045
								grain	192	0.01
								straw	192	0.069
12-2002 12-2002-02 GLP: yes 2012	Barley, winter Amillis	Italy [redacted] Europe, South	360 SC	1	0.12	0.040	23	green material	46	0.027
								whole plant without roots	76	0.025
								grain	105	<0.01
								straw	105	<0.05
12-2002 12-2002-04 GLP: yes 2011	Wheat, winter Hystar	Portugal [redacted] Europe, South	360 SC	1	0.12	0.040	22	green material	129	<0.01
								whole plant without roots	185	<0.01
								grain	213	<0.01
								straw	213	<0.05

*Residues for total residue flufenacet (determined as 4-fluoro-N-isopropylaniline and calculated as flufenacet)
DALT : Days after last treatment

Conclusion:

Three trials on winter wheat (1) and winter barley (2) were performed during the 2011/2012 growing season in southern Europe to investigate the residues of flufenacet (and flurtamone) in cereals after application of 120 g flufenacet/ha using a triple mixture also containing flurtamone and diflufenican. The plants were treated at growth stages BBCH 22-25 in autumn/winter depending on the crop development. Residues of flufenacet ranged between < 0.01 and 0.035 mg/kg in green plant material at forage stage and between < 0.01 and 0.045 mg/kg at silage stage (whole plant without root). At harvest, flufenacet derived residues in grain were < 0.01 mg/kg and ranged from < 0.05- 0.069 mg/kg in straw.
The trials are considered appropriate to support the representative GAP in southern Europe with an application rate of 160 g as/ha since the deviation to the rate of the supported GAP is within the EU's tolerance criteria for comparability (-25%).

Supplementary field trials in <u>southern Europe</u> (application rate flufenacet: 126 g as/ha)

Report:	**KCA 6.3.1/04, [redacted] M; [redacted]; 1999; M-012486-02**
Title:	Determination of the residues of FOE 5043 & Diflufenican 70 WG in/on winter barley and winter wheat in the field in France
Document No Report No	**M-012486-02-1** Study No. RA-2153/97 dated 1999-07-29
Guidelines:	Directive 94/414/EEC Residues in or on treated products, food and feed
GLP	Yes ; Deviations : none

Material and methods

Three trials on winter wheat (1) or winter barley (2) were conducted during the 1997/1998 growing season in southern France using a WG formulation containing 35% flufenacet and 35% diflufenican (WG 70). The plants were treated in late autumn (November, December) at growth stage BBCH 13 (3 leaves unfolded). The application rate for flufenacet was 126 g as/ha.

Grain and straw samples were taken at normal harvest, which was between 209 and 229 days after application.

All samples were analysed for residues of flufenacet according to the method 00346 ([redacted], M.; 1995; M-018864-02), which yields the combined level of the parent compound and all its metabolites containing the N-fluorophenyl-N-isopropyl functional group. The procedure involves oxidation of the residues with potassium permanganate, hydrolysis with sulfuric acid, steam distillation, liquid/liquid partitioning, derivatization with trifluoroacetic anhydride and GC/MS determination of the thus obtained 2,2,2-trifluoro-N-(4-fluorophenyl)-N-isopropylacetamide (trifluoroacetamide). Residues are expressed as parent flufenacet. The method was evaluated with the original Annex II dossier.
The limit of quantification (LOQ) of flufenacet was 0.05 mg/kg in grain and in straw. Other than stated in the report on the employed residue analysis method, the required method validation conducted prior to and concurrently with the analysis of treated samples allowed for an LOQ of 0.05 mg/kg not only for grain but also for straw.

Before analysis for flufenacet the samples were stored frozen up to 3.5 months (106 days) for grain and straw. The storage period is adequately covered by the storage stability data for flufenacet.

Findings

The accuracy of the residue determination was established by determining recoveries prior to analysis in order to validate the method and by procedural recoveries from control samples of straw and grain fortified with flufenacet. For flufenacet, fortification was performed by spiking control samples with one of the following compounds or a mixture thereof: parent flufenacet, flufenacet oxalate hydrate, flufenacet sulfonic acid sodium salt, flufenacet thioglycolate sulfoxide. The average recoveries and relative standard deviations (RSD) were satisfactory as shown in Table 6.3.1-25.
Residues for flufenacet were < LOQ in untreated control samples. The residues found in the wheat and barley samples from the individual trials are summarised in Tables 6.1.3-26. Flufenacet derived residues in grain and straw were less than the LOQ in all trials.

Table 6.3.1-25: Procedural recovery data for Flufenacet

The LOQ is marked in bold

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
RA-2153/97 70258/7 0258-97 70731/7 0731-97 GLP: yes 1997	Barley, winter	grain	total residue flufenacet	9	**0.05**	74; 77; 82; 82; 82; 82; 83; 83; 93	74	93	82	6.3
				9	overall mg/kg		74	93	82	6.3
		straw	total residue flufenacet	11	**0.05**	77; 86; 89; 90; 92; 94; 94; 94; 94; 103; 109	77	109	93	8.9
				11	overall mg/kg		77	109	93	8.9
RA-2153/97 70732/5 0732-97 GLP: yes 1997	Wheat, winter	grain	total residue flufenacet	11	**0.05**	75; 76; 76; 76; 78; 78; 80; 80; 81; 87; 88	75	88	80	5.5
				11	overall mg/kg		75	88	80	5.5
		straw	total residue flufenacet	9	**0.05**	80; 80; 82; 90; 92; 94; 94; 95; 95	80	95	89	7.4
				9	overall mg/kg		80	95	89	7.4

Fortified with flufenacet, flufenacet oxalate hydrate, flufenacet sulfonic acid sodium salt, flufenacet thioglycolate sulfoxide or a mixture thereof; determined as FOE 5043 trifluoroacetamide and calculated as flufenacet equivalent

Table 6.3.1- 26: Residues of flufenacet in wheat and barley after post-emergence application of flufenacet + diflufenican WG 70 (containing 35% flufenacet + 35% diflufenican) in southern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues		
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	total residue flufenacet* (mg/kg)
RA-2153/97 70258/7 0258-97 GLP: yes 1997	Barley, winter Vertige	France [redacted] Europe, South	70 WG	1	0.126	0.045	13	grain	215	<0.05
								straw	215	<0.05
RA-2153/97 70731/7 0731-97 GLP: yes 1997	Barley, winter Pastoral	France [redacted] Europe, South	70 WG	1	0.126	0.045	13	grain	229	<0.05
								straw	229	<0.05
RA-2153/97 70732/5 0732-97 GLP: yes 1997	Wheat, winter Soissons	France [redacted] Europe, South	70 WG	1	0.126	0.045	13	grain	209	<0.05
								straw	209	<0.05

*Residues for total residue flufenacet (determined as FOE 5043 Trifluoroacetamide and calculated as flufenacet)
DALT : Days after last treatment

Conclusion

Three trials on winter cereals (2 trials on barley, 1 trial on winter wheat) were conducted during the 1997-1998 growing season in southern France to investigate the residues of flufenacet in cereals after application of 126 g flufenacet/ha (and 126 g diflufenican/ha) using a mixed WG formulation of the two substances. The plants were treated in autumn (November, December), at growth stage BBCH 13 (3 leaves unfolded). At mature harvest, the residues of flufenacet were < 0.05 mg/kg in grain and in straw.

The trials are considered appropriate to support the representative GAP in southern Europe with an application rate of 160 g as/ha since the deviation to the rate of the supported GAP is within the EU's tolerance criteria for comparability (-21%).

Report:	**KCA 6.3.1/05, [redacted] 2000; M-033163-01**
Title:	Determination of residues of FOE 5043 on winter wheat after spray application of FOE 5043 & Diflufenican 70 WG in the field in France
Document No Report No	**M-033163-01-1** Study No. RA-2185/98 dated 2000-05-12
Guidelines:	Directive 94/414/EEC Residues in or on treated products, food and feed
GLP	Yes ; Deviations : none

Material and methods

Two trials on winter wheat were conducted during the 1998/1999 growing season in southern France using a WG formulation containing 35% flufenacet and 35% diflufenican (WG 70). The plants were treated in late autumn (October, December) at growth stage BBCH 13 (3 leaves unfolded). The application rate of flufenacet was 126 g as/ha.

Grain and straw samples were taken at normal harvest, which was between 206 and 266 days after application.

All samples were analysed for residues of flufenacet according to the method 00346 ([redacted], M.; 1995; M-018864-02), which yields the combined level of the parent compound and all its metabolites containing the N-fluorophenyl-N-isopropyl functional group (see above).
The limit of quantification (LOQ) of flufenacet was 0.05 mg/kg in grain and in straw. Other than stated in the report on the employed residue analysis method, the required method validation conducted prior to and concurrently with the analysis of treated samples allowed for an LOQ of 0.05 mg/kg not only for grain but also for straw.

Before analysis for flufenacet the samples were stored frozen up to 4.5 months (138 days) for grain and straw. The storage period is covered by the storage stability data for flufenacet.

Findings

The accuracy of the residue determination was established by determining recoveries prior to analysis in order to validate the method and by procedural recoveries from control samples of straw and grain fortified with flufenacet. For flufenacet, fortification was performed by spiking control samples with one of the following compounds or a mixture thereof: parent flufenacet, flufenacet oxalate hydrate, flufenacet sulfonic acid sodium salt, flufenacet thioglycolate sulfoxide. The average recoveries and relative standard deviations (RSD) were satisfactory as shown in Table 6.3.1-27.
Residues for flufenacet were < LOQ in untreated control samples. The residues found in the wheat samples from the individual trials are summarised in Tables 6.1.3-28. Flufenacet derived residues in grain and straw were less than the LOQ in both trials.

Table 6.3.1-27: Procedural recovery data for Flufenacet
The LOQ is marked in bold

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
RA-2185/98 R 1998 1726/0 1726-98 and R 1998 1727/9 1727-98 GLP: yes 1998	Wheat, winter	grain	total residue flufenacet	11	**0.05**	76; 80; 81; 81; 82; 82; 83; 85; 85; 86; 90	76	90	83	4.4
				11	overall mg/kg		76	90	83	4.4
		straw	total residue flufenacet	9	**0.05**	77; 78; 80; 82; 85; 86; 88; 89; 92	77	92	84	6.2
				9	overall mg/kg		77	92	84	6.2

Fortified with flufenacet, flufenacet oxalate hydrate, flufenacet sulfonic acid sodium salt, flufenacet thioglycolate sulfoxide or a mixture thereof; determined as FOE 5043 trifluoroacetamide and calculated as flufenacet equivalent

Table 6.3.1- 28: Residues of flufenacet in wheat and barley after post-emergence application of flufenacet + diflufenican WG 70 (containing 35% flufenacet + 35% diflufenican) in southern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application FI	No	kg/ha (a.s.)	kg/hl (a.s.)	GS	Residues Portion analysed	DALT (days)	total residue flufenacet* (mg/kg)
RA-2185/98 R 1998 1726/0 1726-98 GLP: yes 1998	Wheat, winter Sideral	France [redacted] Europe, South	70 WG	1	0.1260	0.04515	13	grain straw	266 266	<0.05 <0.05
RA-2185/98 R 1998 1727/9 1727-98 GLP: yes 1998	Wheat, winter Isengrain	France [redacted] Europe, South	70 WG	1	0.1260	0.04515	13	grain straw	206 206	<0.05 <0.05

*Residues for total residue flufenacet (determined as FOE 5043 Trifluoroacetamide and calculated as flufenacet)
DALT : Days after last treatment

Conclusion

Two trials on winter wheat were conducted during the 1998-1999 growing season in southern France to investigate the residues of flufenacet in cereals after application of 126 g flufenacet/ha using a mixed WG formulation with diflufenican. The plants were treated in autumn (October, December), at growth stage BBCH 13 (3 leaves unfolded). At mature harvest, the residues of flufenacet were < 0.05 mg/kg in grain and in straw.
The trials are considered appropriate to support the representative GAP in southern Europe with an application rate of 160 g as/ha since the deviation to the rate of the supported GAP is within the EU's tolerance criteria for comparability (-21%).

Conclusion for the use of flufenacet with use patterns involving 120 g flufenacet/ha in northern Europe and 160 g as/ha in southern Europe

The data set evaluated for Annex I inclusion was considered suitable to support a use pattern at 240 g as/ha in northern Europe with application in autumn. In the evaluated trials winter cereals were treated between November and March at growth stages ranging from BBCH 11 to 25. In all trials, residues have shown to be less than the LOQ for grain (< 0.05 mg/kg) and straw (< 0.1 mg/kg).
With this dossier 6 supplementary residue trials are reported where flufenacet has been applied in mixture with diflufenican with WG and SC formulations at growth stages ranging between BBCH 13 and 25. Thus, a large data set of 23 trials is available to support the representative use at 240 g as/ha.
This data set is considered appropriate to support the critical GAP involving 240 g as/ha for the northern climatic zone. Since the second representative use involving an application rate of 120 g as/ha can be considered to be less critical the data set supporting the high rate can be considered suitable to also support the lower application rate.

However, 8 supplementary trials are reported with the present dossier with mixture products which support a use pattern for the representative use at 120 g as/ha at growth stages up to BBCH 22 (actual BBCH 22-25). Since a slightly later growth stage is targeted the growth stage was given priority in the design of the residue trials, because the application may not always be possible in autumn. Thus the residue trials cover scenarios with treatments between November and April. The findings demonstrate that residue levels range between the lowered LOQ (< 0.01 mg/kg) and 0.022 mg/kg (median < 0.01 mg/kg) in grain and remain below the lower LOQ (< 0.05 mg/kg) in straw. It may be concluded that a possibly later application does not result in higher residues at a rate of maximum 120 g as/ha.

For the southern European climatic zone a data set of 9 trials is reported involving an application rate of 240 g as/ha and application at growth stage BBCH 13 and BBCH 21 in one trial. The trials were all performed using the representative formulation 'Flufenacet + Diflufenican SC 600'. In the southern zone cereals are typically sown continuously during autum and winter. Thus, in order to reflect this agricultural practice applications were made early post-emergence between December and February. Residue levels in grain ranged between < 0.01 and 0.05 mg/kg (median < 0.01 mg/kg) and < 0.05 and 0.11 mg/kg (median 0.06 mg/kg) in straw.
This GAP can be considered as the critical GAP for the southern region. This data set is considered appropriate to cover the less critical GAP of the second representative use for the southern region at a rate of 160 g as/ha. However, 12 supplementary trials are submitted using mixture products. The application rates for these trials were 21-25% less relative to the target rate (160 g as/ha), however

within the 25% range of the tolerance criteria for comparability. Treatments were made between October and March at growth stages ranging from BBCH 13 to 30. Residue levels ranged between less than the LOQ (< 0.01 or < 0.05 mg/kg) to 0.035 mg/kg in grain and < 0.05 to 0.069 mg/kg in straw. The supplementary trials broaden the database and confirm the representative use at 240 g as/ha as the critical GAP.

CA 6.4 Feeding studies

Evaluation during the EU peer review process

During the EU evaluation process the dietary burden for livestock was assessed based on uses in cereals, corn, sunflower and soybean as relevant feeding items. Since i) no residues above the LOQ (0.05 mg/kg in green material of plants (at forage stage), cereal grain, sunflower and soybean seed, maize kernel and 0.1 mg/kg in straw) were determined and ii) the data from metabolism studies do not indicate a possible transfer from residues in feeding items to food of animal origin, it was concluded in the Monograph that livestock feeding studies are not required. However, a cow feeding study conducted for the US was submitted and has been evaluated. In this study, cows were administered highly exaggerated doses of FOE 5043-oxalate which constitutes the main plant metabolite. The results show that even at an exaggerated dose of 7.8 ppm (10x dose in the study: 0.555 mg/kg bw/d) no flufenacet derived residues can be expected in tissues or products of animals which have been fed flufenacet treated crops.

In the Report of ECCO 73, Annex 2, Complete List of Endpoints it is concluded that no residues can be expected in animal tissues or products and, thus, it was proposed to delete all MRLs for products of animal origin.

Evaluation in the EFSA Reasoned Opinion on the review of the existing maximum residue levels (MRLs) for flufenacet according to Art 12 of Regulation (EC) No 396/2005 (EFSA Journal 2012;10(4):2689

Based on the uses reported by the RMS, significant intakes were calculated for ruminants, poultry and pigs. EFSA calculated the dietary burden based on all authorized uses for crops that might be fed to livestock (potatoes, sunflower seed, soya bean, barley, maize, rye, wheat) and the corresponding by-products which may be used as feeding items (cereal bran, oilseed meals). In the EFSA Reasoned Opinion, the median and maximum dietary burdens were therefore calculated for different groups of livestock using the agreed European methodology (EC, 1996). The input values for all relevant commodities have been selected according to the recommendations of JMPR (FAO, 2009) and are summarized in Table 6.4-1 (corresponds to Table 3-4 of the Reasoned Opinion). For cereal bran and sunflower seed meal default processing factors of 8 and 2, respectively, have been included in the calculation in order to consider potential concentration of residues in these commodities. The default processing factor for soya bean has not been applied as processing studies submitted with the Annex II dossier show that residues of flufenacet are below the LOQ in both the RACs and the processed products and no concentration of flufenacet is observed.

Table 6.4-1: Input values for the dietary burden calculation

Commodity	Median dietary burden		Maximum dietary burden	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
Cereal grain (small)	0.05	Median residue	0.05	Median residue
Maize grain	0.05	Median residue	0.05	Median residue
Cereal bran	0.4	Median residue × 8	0.4	Median residue × 8
Cereal straw	0.1	Median residue	0.11	Highest residue
Potatoes	0.05	Median residue	0.11	Highest residue
Sunflower seed	0.05	Median residue	0.05	Median residue
Sunflower seed meal	0.1	Median residue × 2	0.1	Median residue × 2
Soya bean	0.05	Median residue	0.05	Median residue
Soya bean meal	0.05	Median residue	0.05	Median residue

The results of the calculations are reported in Table 6.4-2 (corresponds to Table 3-5 of the Reasoned Opinion). The calculated dietary burdens for all groups of livestock were found to exceed the trigger value of 0.1 mg/kg DM.

Table 8.4-2: Results of the dietary burden calculation

	Median dietary burden (mg/kg bw/d)	Maximum dietacy burden (mg/kg bw/d)	Highest contributing commodity	Max dietary burden (mg/kg DM)	Trigger exceeded(Y/N)
Dairy ruminants	0.0090	0.0135	Potatoes	0.3704	Y
Meat ruminants	0.0134	0.0238	Potatoes	0.5555	Y
Poultry	0.0092	0.0143	Wheat bran	0.2257	Y
Pigs	0.0125	0.0221	Potatoes	0.5531	Y

Evaluation of the magnitude of residues in livestock (EFSA Reasoned Opinion, 2012:
"On the basis of the animal metabolism studies it is concluded that, after exposure to the maximum dietary burden (about 200 times lower than the dose level in the metabolism studies, [5 mg/kg bw/d]*), residue levels in livestock commodities are expected to remain below the enforcement LOQ of 0.01 mg/kg in milk, 0.02 mg/kg in liver and 0.05 mg/kg in fat, eggs, kidney and muscle. Hence, no livestock feeding study is needed; MRLs and risk assessment values for the relevant commodities in ruminants, pigs and poultry can be established at the LOQ level."* (p.29/30).

The EFSA Reasoned Opinion confirms the conclusion drawn in the EU review process relative to the evaluation of the dairy cattle feeding study conducted in accordance with the US EPA guidance: "*The results of the study show that no detectable residues of flufenacet oxalate are to be expected in products of animal origin which have been fed crops treated with flufenacet according to the GAPs (…)*".

The lowest dose rate in the US feeding study amounted to 7.8 mg/kg in feed corresponding to 14times the maximum diatary burden for meat ruminants (0.555 mg/kg DM) and 21times the diatary burden for dairy ruminants (0.370 mg/kg DM) as shown in the calculation above.

In animal tissues, FOE oxalate residues were only detected at the highly exaggerated treatment rates.

At the lowest dosing rate rate (14N), only the kidney just barely showed a measurable residue (up to 0.057 mg/kg). Therefore, no detectable residues of flufenacet are to be expected in meat from cattle, which are fed at the 1× rate.

In milk no residues above the LOQ (= 0.01mg/kg) was found even in samples obtained from cows fed at the highest dose rate (148× rate corresponding to 82 mg/kg feed).

It has to be noted that Bayer CropScience intends to limit the flufenacet uses to cereals, potatoes and maize, thus resulting in a slightly lower dietary burden for dairy ruminants, poultry and pigs but without any impact on the dietary burden for meat ruminants.

Table 6.4-3: Results of the dietary burden calculation (based on supported crops cereals, maize, potato)

	Median dietary burden (mg/kg bw/d)	Maximum dietary burden (mg/kg bw/d)	Highest contributing commodity	Max dietary burden (mg/kg DM)	Trigger exceeded(Y/N)
Dairy ruminants	0.0078	0.0122	Potatoes	0.3355	Y
Meat ruminants	0.0134	0.0238	Potatoes	0.5555	Y
Poultry	0.0084	0.0135	Potatoes	0.2141	Y
Pigs	0.0116	0.0212	Potatoes	0.5299	Y

Calculation of the dietary burden according to the OECD guidance document on residues in livestock (4 September 2013)

The new EU data requirement as published with Regulation (EC) 283/2013 for active substances state the need for feeding studies where intake is above 0.004 mg/kg bw/d. However, the circumstances in which feeding studies are required also have to take into consideration where metabolism studies indicate that residues at levels of above 0.01 mg/kg may not occur in edible animal tissue, milk, eggs or fish, taking into account the residue levels in potential feeding items, obtained at the 1 × dose rate, calculated on the dry weight basis.

Table 6.4-4 compiles the input data for the dietary burden calculation. The crops which will be supported in the future are taken into account (i.e. cereals, potato, corn (maize)). Although from the available trials with application rate of 240 g as/ha residues in forage were always less than the LOQ of 0.05 mg/kg, the higher residue levels for green plant material at forage stage - and in addition at silage stage - from the trials with a lower rate (120 g as/ha) were used for the calculation (where relevant). The higher residue levels at early growth stages resulted from the relatively later application dates at BBCH ≥25 and thus a shorter interval until forage stage is reached. These residue levels are considered as a worse case concerning the dietary burden. (Adding hay from cereals as feeding item does not alter the calculation when residue levels from straw are used as surrogate).

Table 6.4-5 provides the results of the dietary burden calculation for Europe according to the OECD guidance document on residues in livestock (ENV/JM/MONO(2013)8, 04-Sep-2013) and the feeding tables provided therewith and by using the RWCF approach (Reasonable Worst Case Feed).

Table 6.4-4: Input data for dietary burden calculation according to OECD guidance document

Category	Crop	Commodity	IFN Code	Classification	Residue input	DM (%)	Residue level on fresh / total weight basis (mg/kg) EU
Forages / Fodders	Barley	forage	2-00-511	R	HR	30	0.081
Forages / Fodders	Barley	straw	1-00-498	R	HR	89	0.110
Forages / Fodders	Barley	silage	3-00-512	R	HR	40	0.061
Forages / Fodders	Oat	forage	2-03-292	R	HR	30	0.081
Forages / Fodders	Oat	straw	1-03-283	R	HR	90	0.110
Forages / Fodders	Rye	forage	2-04-018	R	HR	30	0.081
Forages / Fodders	Rye	straw	1-04-007	R	HR	88	0.110
Forages / Fodders	Triticale	forage	2-02-647	R	HR	30	0.081
Forages / Fodders	Triticale	straw	NA	R	HR	90	0.110
Forages / Fodders	Wheat	forage	2-08-078	R	HR	25	0.081
Forages / Fodders	Wheat	straw	1-05-175	R	HR	88	0.110
Roots & Tubers	Potato	culls	4-03-787	CC	HR	20	0.110
Cereal grains / Crop Seeds	Barley	grain	4-00-549	CC	STMR	88	0.050
Cereal grains / Crop Seeds	Corn, field	grain	4-20-698	CC	STMR	88	0.050
Cereal grains / Crop Seeds	Oat	grain	4-03-309	CC	STMR	89	0.050
Cereal grains / Crop Seeds	Rye	grain	4-04-047	CC	STMR	88	0.050
Cereal grains / Crop Seeds	Triticale	grain	4-20-362	CC	STMR	89	0.050
Cereal grains / Crop Seeds	Wheat	grain	4-05-211	CC	STMR	89	0.050
By-products	Wheat gluten	meal	5-05-221	CC	STMR	40	0.050[a]
By-products	Wheat	milled by products	4-06-749	CC	STMR	88	0.22[a]

[a] for derivation of processing factors, please refer to CA 6.5.3.
For wheat gluten meal a processing factor < 1 was derived. For the calculation of the dietary burden a processing factor of 1 has been used.
For milled by-products from wheat a worst case processing factor (mean) of 4.4 derived from bran and shorts has been used.

Table 6.4- 5: Results of the dietary burden calculation for flufenacet in Europe (OECD guidance, 2013)

	Feedstuff type	Crop	Feedstuff	Maximum Percent of Diet	Dietary burden (mg/kg bw/d) in EU
Cattle - Beef	Roots & Tubers	Potato	culls	30	0.0040
	Forages	Barley	forage	30	0.0019
	By-products	Wheat	Milled by-products	30	0.0018
	Cereal Grains/Crops Seeds	Corn, field	grain	10	0.0001
	Total			**100**	**0.008**
Cattle – Dairy	Roots & Tubers	Potato	culls	30	0.0063
	Forages	Barley	forage	30	0.0031
	By-products	Wheat	Milled by-products	30	0.0029
	Cereal Grains/Crops Seeds	Barley	grain	10	0.0002
	Total			**100**	**0.013**
Sheep - Rams/Ewes	Roots & Tubers	Potato	culls	50	0.0055
	Forages	Barley	forage	50	0.0045
	By-products	Wheat	Milled by-products	20	0.0017
	Total			**100**	**0.012**
Sheep - Lambs	Roots & Tubers	Potato	culls	20	0.0047
	Forages	Barley	forage	50	0.0057
	By-products	Wheat	Milled by-products	30	0.0032
	Total			**100**	**0.014**
Swine - Breeding	Roots & Tubers	Potato	culls	50	0.0063
	Forages	Wheat	forage	20	0.0015
	By-products	Wheat	Milled by-products	30	0.0017
	Total			**100**	**0.010**
Swine - Finishing	Roots & Tubers	Potato	culls	50	0.0083
	By-products	Wheat	Milled by-products	50	0.0038
	Total			**100**	**0.012**
Poultry - Broiler	Roots & Tubers	Potato	culls	10	0.0039
	By-products	Wheat	Milled by-products	20	0.0035
	Cereal Grains/Crops Seeds	Barley	grain	70	0.0028
	Total			**100**	**0.010**
Poultry - Layer	Roots & Tubers	Potato	culls	10	0.0038
	Forages	Wheat	forage	10	0.0022
	By-products	Wheat	Milled by-products	20	0.0034
	Cereal Grains/Crops Seeds	Barley	grain	60	0.0023
	Total			**100**	**0.012**
Poultry - Turkey	Roots & Tubers	Potato	culls	20	0.0079
	Cereal Grains/Crops Seeds	Rye	grain	60	0.0024
	By-products	Wheat	Milled by-products	20	0.0036
	Total			**100**	**0.014**

Conclusion

As outlined above, during the EU peer review process and recently concluded in the EFSA Reasoned Opinion on existing MRLs (2012) the transfer of flufenacet derived residues into animal tissues, milk and eggs is very low and no residues above the respective LOQs can be expected based on the evaluated GAPs. The representative uses on cereals supported in the present dossier are shown not to produce higher residues than those previously evaluated.

The conclusion was drawn based on the available metabolism data obtained after a dose 200times the maximum dietary burden for ruminants and 350times the maximum dietary burden for poultry and using 3 different labels (fluorophenyl-U-^{14}C label, thiadiazole-2-^{14}C label and fluorophenyl-U-^{14}C flufenacet oxalate (main plant metabolite)). The metabolism studies with flurophenyl-UL-^{14}C flufenacet oxalate - showing by far the lowest transfer into animal tissues, milk and eggs - are considered to provide the most relevant information because the parent compound is rapidly metabolized and no parent is found in plant commodities. Taking into account the findings from the ruminant feeding study, the dose of flufenacet oxalate fed to cows amounted to 0.955 mg/kg bw/d corresponding to 23times the maximum dietary burden calculated for meat cattle.

In addition to the European methodology applied in the EU peer review process and by EFSA, the dietary burden was calculated according to the OECD guidance document (2013) taking into account the most recent feeding tables. Based on the feeding items cereals (including by-products), potatoes and corn (maize) which are the crops intended to be supported in the future, the dietary burden calculated for livestock was up to a maximum of 0.014 mg/kg bw/d (sheep, lambs and poultry, turkey).

According to the OECD methodology livestock exposure is expected to be comparable for dairy cattle and poultry and less for meat cattle and pig compared to the previous calculations. Thus, the conclusions drawn for Annex I inclusion and in the EFSA Reasoned Opinion on existing MRLs are considerd to be sull valid and no further data are considered necessary for this submission.

CA 6.4.1 Poultry

No supplementary study has been generated following the inclusion of flufenacet in Annex I of Directive 91/414/EEC. Please refer to CA 6.4

CA 6.4.2 Ruminants

No supplementary study has been generated following the inclusion of flufenacet in Annex I of Directive 91/414/EEC. Please refer to CA 6.4

CA 6.4.3 Pigs

The metabolic pathway of flufenacet was similar in rats, poultry (laying hens), and ruminants (goat). Therefore it can be expected that the metabolism in other farm animals does not differ, and thus for the active substance studies in pigs are not required.

CA 6.4.4 Fish

The nature of the residue in fish was addressed in chapter 6.2.5 above based on an available bio-concentration study with bluegill sunfish also reporting metabolism data in fish.

No final test guideline or feeding tables are currently available which detail how the dietary burden has to be calculated and which provide an agreed test methodology. Therefore it is the opinion of the applicant that it is not appropriate to address this issue until such guidance is available. Therefore the risk assessment should be conducted in accordance with the current published guidelines.

This opinion is in agreement with a publication of European Commission Health & Consumer Protection Directorate-General published as SANCO/10181/2013-rev. 2 of 2-May-2013 on "Guidance Document for Applicants on Preparing Dossiers for the Approval of a Chemical New Active Substance and For the Renewal of Approval of a Chemical Active Substance According to Regulation (EU) No 283/2013 and Regulation (EU) No 284/2013".

This SANCO document notes in Section 4. "Documents to be included in a submission" under the Subsection "Special cases":
"*In some cases, agreed test methods of guidance documents are not yet available for particular data requirements. In these cases, waving of these particular data requirement points is considered acceptable as long as no test methods or guidance documents are published in form of an update of the Commission Communications 2013/C 95/01 and 2013/C95/02.*"

CA 6.5 Effects of processing

CA 6.5.1 Nature of the residue

Evaluation during the EU peer review process

Excerpt from Monograph B.6.7.1:
"The parameter which is most likely to affect the nature of residue during processing operations is hydrolysis, because processes like heating would generally inactivate enzymes present in the substrate, leaving primarily simple hydrolysis as degradation mechanism.
Experiments conducted to study the hydrolytic degradation of FOE 5043 [flufenacet] *at pH values 5, 7 and 9 showed that the parent compound is not significantly affected by this process (see chapter B.7.4* [hydrolytic behaviour]*). It is therefore unlikely that processing will affect the nature of FOE 5043 residue. In addition, the analytical method used for raw and processed commodities determines the total residue of FOE 5043 by converting the relevant residue into a common derivate. Therefore, any minor changes of the molecule would not influence the residue determined. Due to these facts, it is not considered necessary to conduct special radioactive studies on the nature of FOE 5043 residues in processed products."*

A detailed justification is provided in the following position paper.

Report:	**KCA 6.5.1/01, [redacted]; 2011; M-409521-01**
Title:	Flufenacet – Waiving of the high temperature hydrolysis study for the determination of the nature of pesticide residues in processed commodities
Document No Report No	**M-409521-01-1** MEF-11/482 dated 2011-06-10
Guidelines:	Not applicable (position paper)
GLP	No (position paper)

The relevant residues of flufenacet in raw agricultural commodities are determined by means of a common moiety method capturing the parent substance and all metabolites that contain the N-fluorophenyl-N-isopropyl functional group according to the residue definition in plants. This residue analytical method for risk assessment and enforcement involves a hydrolysis at conditions that are much harsher than those used to investigate the nature of processed residues according to OECD Test Guideline 507.
Half-concentrated sulfuric acid used in the flufenacet residue method is significantly more acidic than pH 6, 5 and 4 as requested in the processing hydrolysis study. The temperature used for the hydrolysis step of the residue method reaches 115°C. This temperature is only slightly below the highest temperature used for high temperature processing hydrolysis. However, the duration of the hydrolysis applied in the residue analytical method is by far longer (≥ 20 hours) when compared to the processing hydrolysis (0.3 – 1 hour).

The residue definition in plants consists of parent flufenacet and all its derivatives and metabolites which comprise the N-fluorophenyl-N-isopropyl functional group. These residues are determined by means of the common moiety method covering all the metabolites derived from the fluorophenyl acetamide moiety.

All incurred residues containing the N-fluorophenyl-N-isopropyl group in the RACs as well as each potential breakdown product containing this moiety resulting from processing of these RACs are captured by the residue analytical methods for determination of flufenacet residues. By application of these residue methods all N-fluorophenyl-N-isopropyl containing residues are hydrolysed to the analytical target 4-fluoro-N-isopropylaniline that is quantified by GC-MS after derivatization with TFAA or directly by HPLC-MS/MS determination.
Therefore, a study on the nature of processed residues (high temperature hydrolysis according to OECD 507) resulting from use of flufenacet in crops does not provide any new information and can thus be omitted.

CA 6.5.2 Distribution of the residue in peel and pulp

The distribution of the residue in peel and pulp is not relevant for the small grain cereals.

CA 6.5.3 Magnitude of residues in processed commodities

Evaluation during the EU peer review process

Based on European residue data, processing studies were not considered necessary since residue levels for all edible commodities were less than the LOQ of 0.05 mg/kg and thus below the threshold of

0.1 mg/kg. However, processing studies on soybean and maize available from the US were submitted and evaluated. The active substance was applied at higher rates than in Europe (8N rate). Although residues in the raw agricultural commodities were still below the validated LOQ of 0.05 g/kg in maize, it could be shown that no concentration of residues in any of the tested commodities occurs. The tested pocedures included wet and dry milling (tested commodities starch, crude oil and refined-bleached-deodorized oil for wet milling and germs, grits, meal, flour, crude oil and refined-bleached-deodorized oil for dry milling. In soybean at the 8N rate, residues were obtained in seed. It was demonstrated that no concentration occurs in the investigated commodities meal, hulls, crude oil and refined-bleached deodorized oil (see also Monograph B.6.7.2).

Supplementary studies

Within the present dossier supplementary processing studies on wheat and barley are submitted.

Wheat

Report:	**KCA 6.5.3/04,** [redacted], [redacted], 2013, M-457286-01
Title:	Determination of the residues of flufenacet in/on wheat and the processed fractions (white flour, white flour bran, white bread, whole meal, whole meal bread, middlings, shorts, gluten, gluten feed meal, starch) after spraying of Flufenacet WG 60 in the United Kingdom and the Netherlands
Document No Report No	**M-457286-01-1** 11-3401 dated 2013-06-20
Guidelines:	• EU-Ref: Council Directive 91/414/EEC of July 15, 1991 • EC Guidance working document 7029/VI/95 rev.5 (1997-07-22) • EC guidance working document 7035/VI/95 rev.5 (1997-07-22) • OECD Guideline for the Testing of Chemicals, Magnitude of the Pesticide Residues in Processed Commodities, 508 (2008-10-03), • US EPA OCSPP Guideline No. 860.1520
GLP	Yes ; Deviations : none

Materials and methods

Two studies were performed in 2011 on wheat in the Netherlands and the United Kingdom in order to to collect sample material for processing studies. The samples of wheat (grain) to be processed were obtained after one post-emergence spray application (BBCH 25) at exaggerated rate (2N = 0.48 kg as/ha) with Flufenacet WG 60, an WG formulation containing 60 % flufenacet. The higher rate was used in order to obtain appropriate residue levels in the raw agricultural commodity for derivation of transfer factors.

Wheat grain samples to be processed were sampled 120-135 days after treatment, at growth stage BBCH 89.

The processing of the wheat samples into the processed fractions bran, gluten, gluten feed meal, middlings, shorts, starch A and B, wheat germ, white bread, white flour, whole meal and wholemeal bread was performed in a specialized pilot plant to simulate industrial procedures at a laboratory scale.

Residues of the raw agricultural commodity and the processed fractions were analysed using method 01100/M001 ([redacted], S., [redacted], J.; [redacted], S.; 2012; M-433720-01) with an LOQ of 0.01 mg/kg which

yields the combined level of the parent compound and all its metabolites containing the N-fluorophenyl-N-isopropyl functional group. Residues are expressed as parent flufenacet.

Processing Procedures

Drying / Cleaning / Conditioning of the Grain

Frozen field samples for processing were defrosted and cleaned. The grain samples were conditioned until an optimum moisture content between 14.6 to 16.6 % was reached.

Milling of White Flour (Type 550) and Baking of White Bread

In a closed system with different pairs of smooth rollers and sifter passages the grain was milled to straight flour, bran and middlings. Samples of bran and middlings were collected.

In a further processing step the low grade meal (toppings) were separated from the bran and middlings using a centrifuge/scouring machine. This process resulted in shorts and low grade meal. A sample of shorts was collected.

After determination of the mineral content of straight flour and low grade meal, both fractions were mixed (if necessary) to the final product white flour type 550 until a mineral content of 510-630 g/100 kg flour was reached. A sample of white flour type 550 was taken. The white flour was used to prepare white bread.

Milling of Whole Meal and Baking of Whole-Meal Bread

For the preparation of whole-meal and whole-meal bread the same milling procedure as used for the production of flour type 550 was used. After milling the coarse bran and middlings were cracked with an impact mill to smaller pieces. All milling products of the process were mixed homogeneously in a special flour mixer. A sample of the whole meal was collected. The whole meal flour was used to prepare whole meal bread.

Production of Wheat Germ

First the grain was broken to bruised grain in a special mill. The fraction 400-1000 µm, a mixture of bran, middlings and germs was put in a special separator. Due to the different specific weights of the bran, middlings and germs, the middlings/germ mixture was separated from most parts of the bran.

Subsequently, the middlings/germ mixture was milled to flour and small wheat germ discs incl. parts of bran in a mill with a pair of smooth rollers. The wheat germ with parts of bran was then sieved to separate the various fractions (germs with small parts of bran (germ fraction) and bran). A sample of wheat germ was taken.

Production of Starch and Gluten

The first step of the production of starch and gluten was milling the grain to straight flour, bran and middlings. Straight flour and water were mixed to obtain a hydrated dough. The dough was separated by centrifugation into wet starch, process water and gluten (containing starch). Subsequently, the starch was washed out with water 3times and separated by centrifugation into starch A, process water and gluten. Starch A was dried at 60°C and milled yielding the sample material starch A.

The gluten (containing starch) was washed several times out with water and resulted in gluten and process water (containing starch B and fibre). Gluten was dried by freeze drying, milled and sampled.

Remaining process water was separated by centrifugation into starch B, fibre and water. The sample materials starch B and fibre were dried at 60 °C, milled and collected. Milled starch B, gluten and fibre were combined to the sample material gluten feed meal.

Flow Charts

Fig. 1: Milling of White Flour (Type 550) and Baking of White Bread

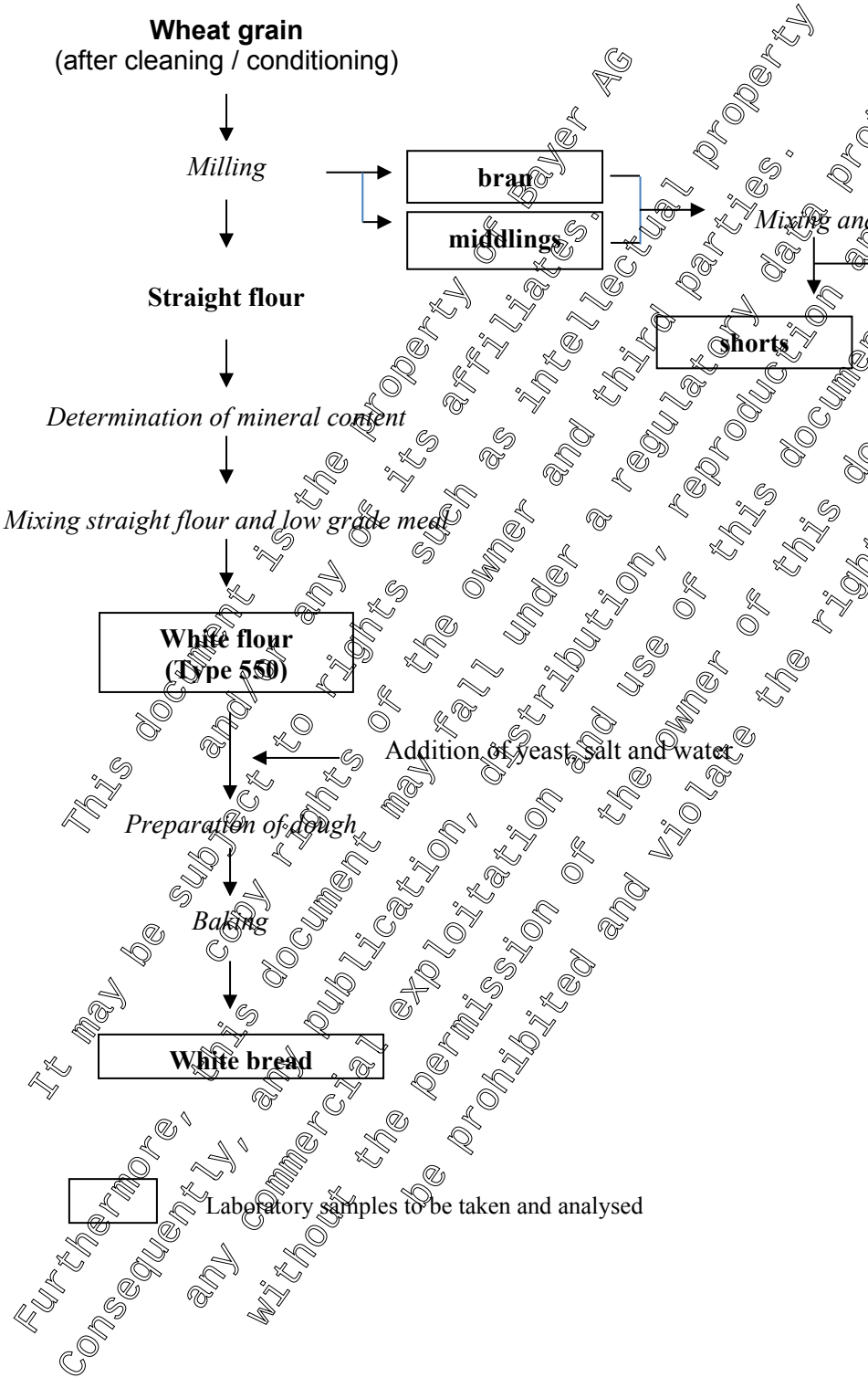


Fig. 2: Milling of Whole Meal and Baking of Whole-meal Bread

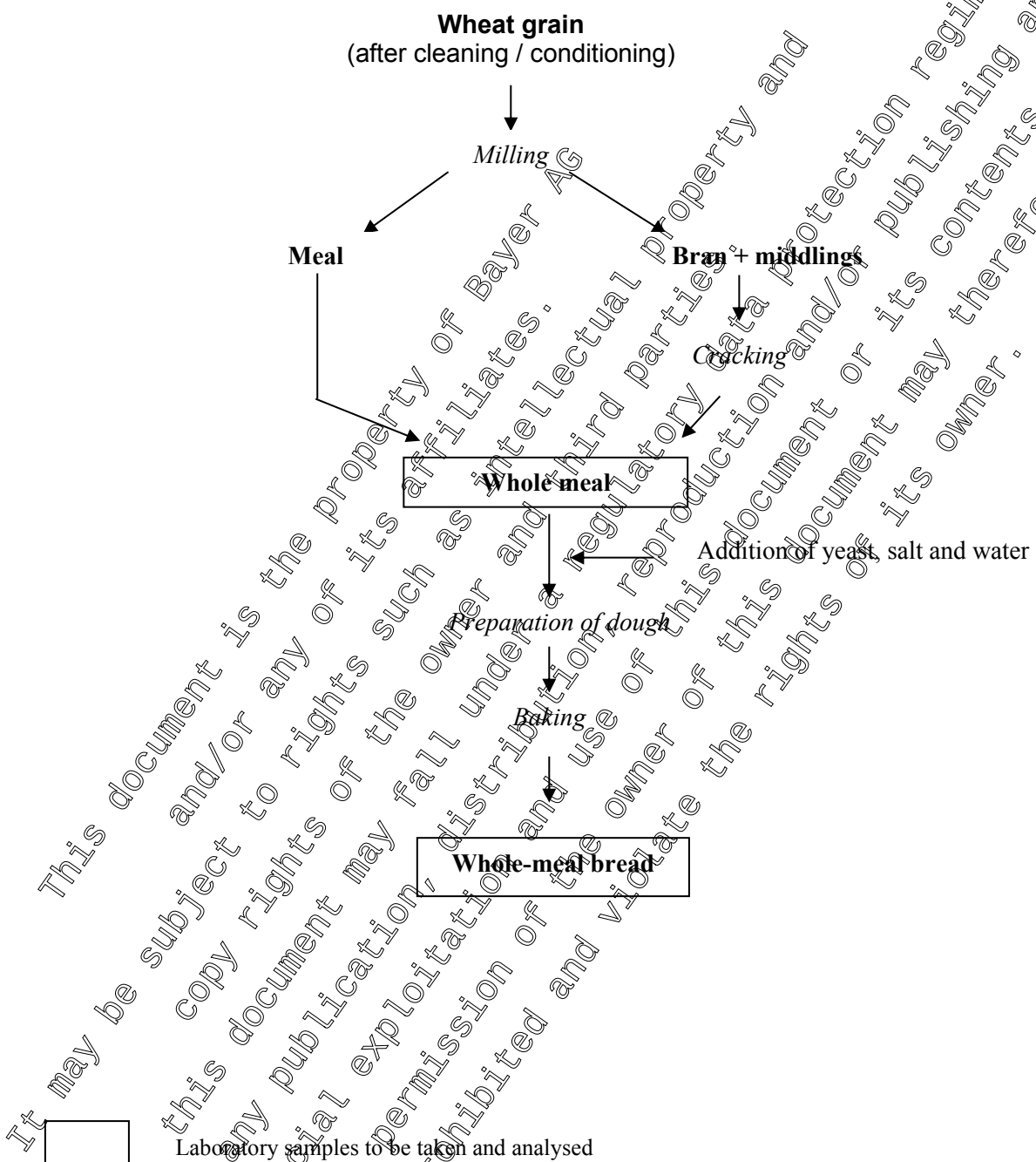


Fig. 3: Production of Wheat germ

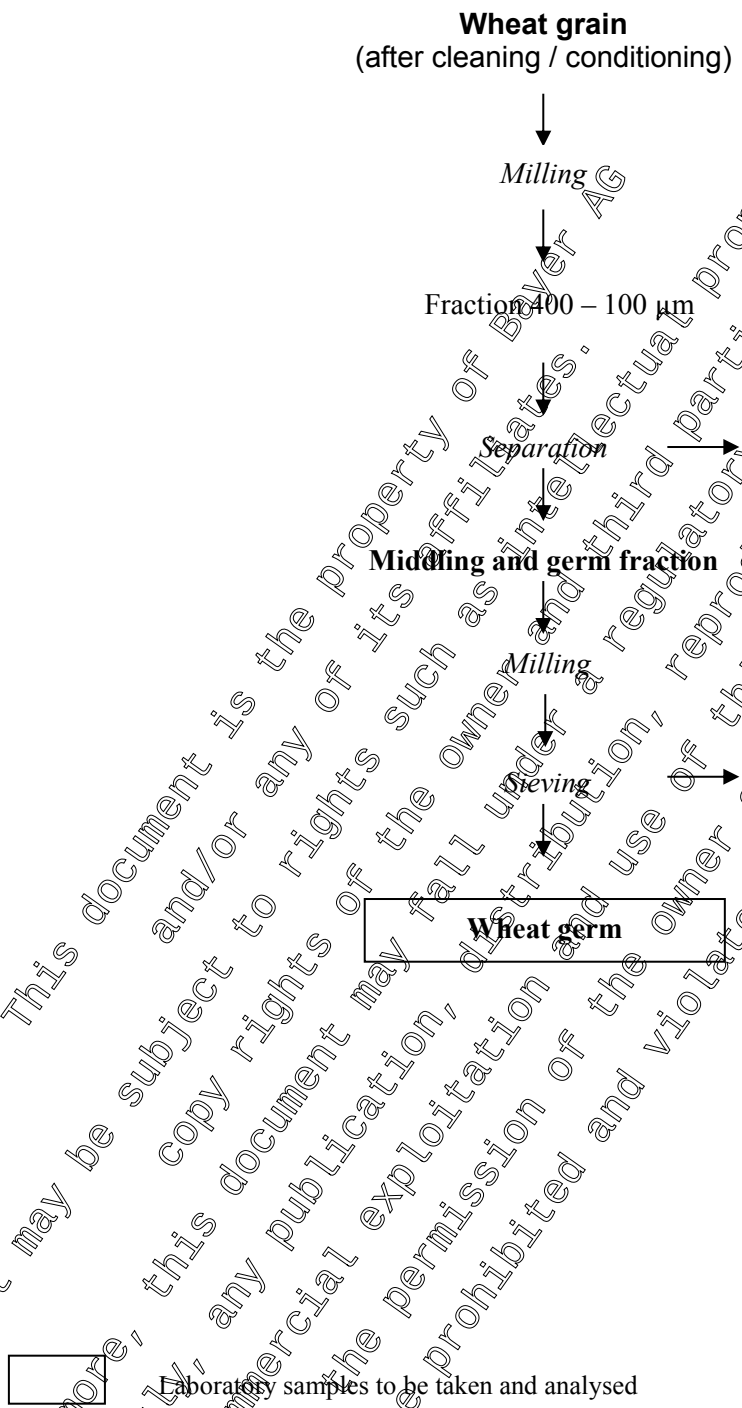


Fig. 4: Production of Starch and Gluten (general)

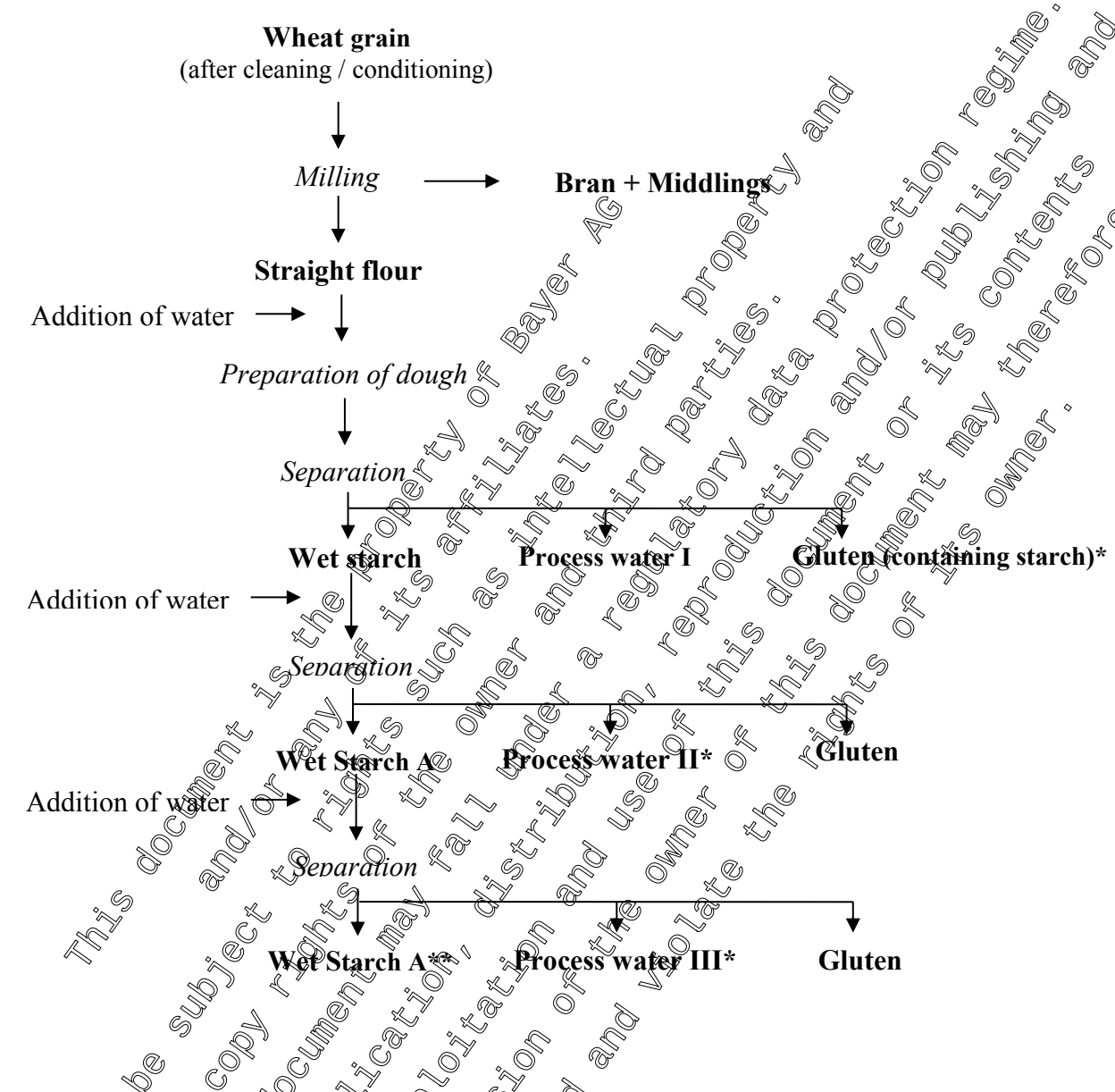


Fig. 5: Production of Starch A

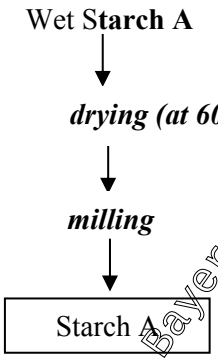
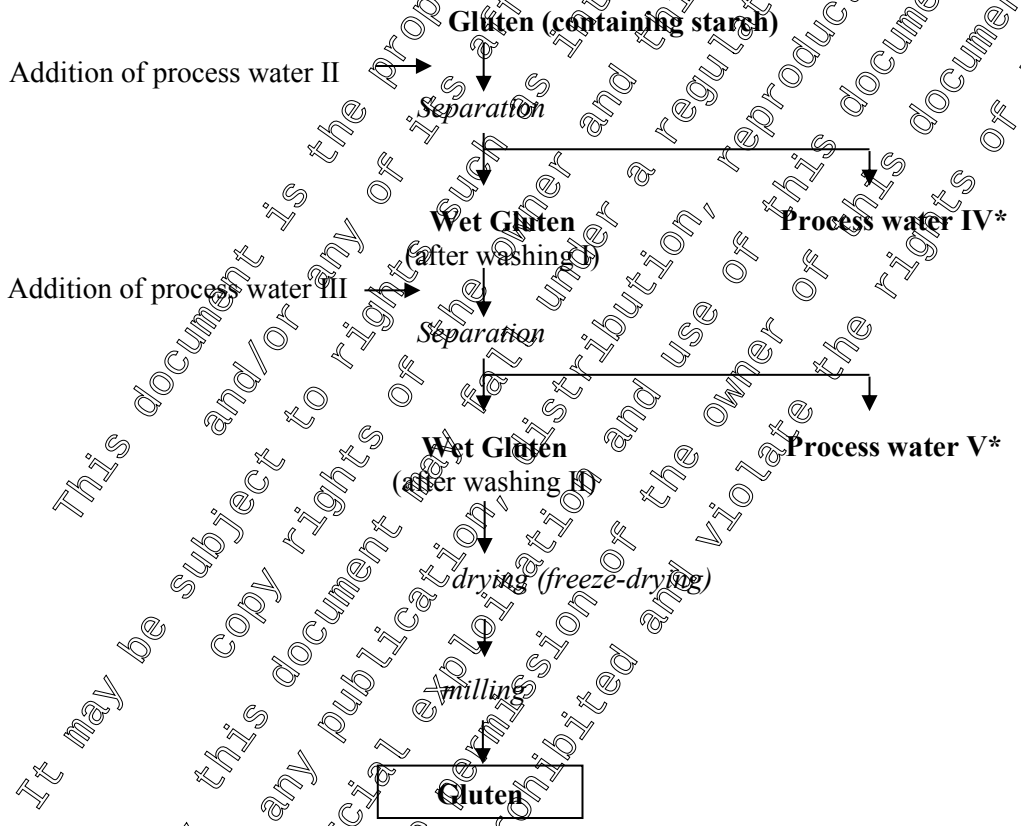


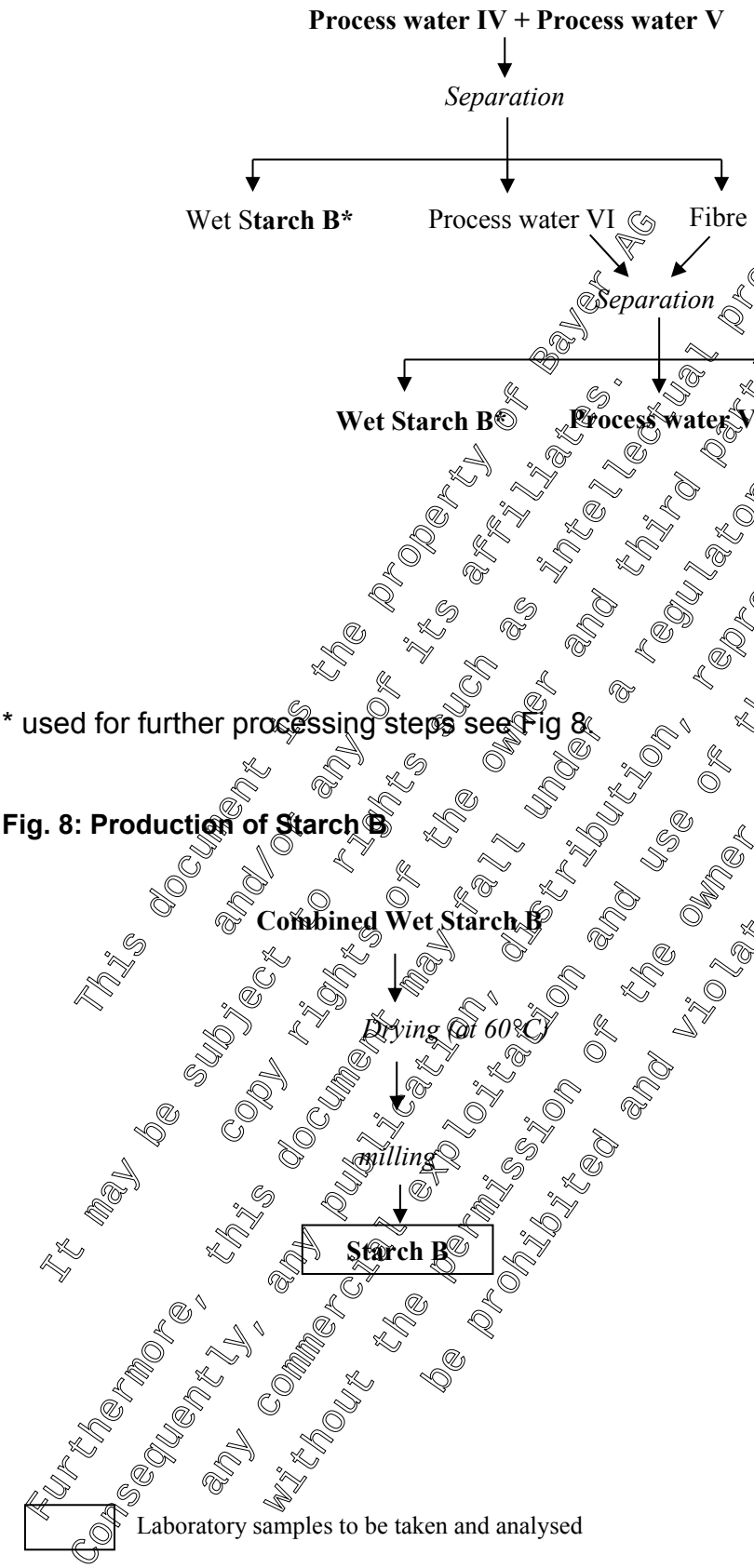
Fig.6: Production of Gluten



* used for further processing steps see Fig 7.

Laboratory samples to be taken and analysed

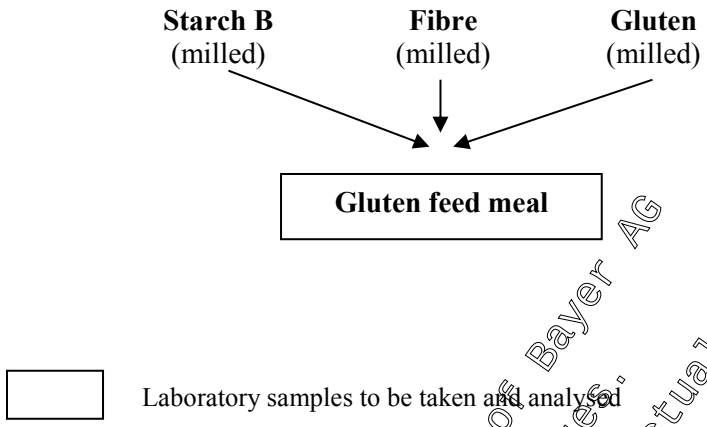
Fig.7: Production of Wet Starch B and Dry fibre



* used for further processing steps see Fig 8

Fig. 8: Production of Starch B

Fig.9: Production of Gluten feed meal



Findings

Recovery rates were determined prior to analysis in order to validate the method and concurrently with the sample analysis in order to check the accuracy of the residue analysis. The sample materials chosen served to represent all relevant sample materials collected in this study. The data demonstrate acceptable method performance during sample analysis. The summaries of recoveries are provided in Table 6.5.3-2. No residues were determined in the control samples.

Residues in wheat grain and the processed fractions are summarised in Table 6.5.3-3 and in more detail in the Tier 1 summary forms.

In the grain samples taken at harvest, flufenacet residues amounted to 0.1/0.085 mg/kg and 0.011/0.015 mg/kg (double sampling) for both trials. Processing factors were calculated based on the mean values from the individual studies.

For all processed commodities, transfer factors were calculated since residue levels above the LOQ were measured in the raw agricultural commodities, even though for some processed commodities the residue levels were less than the LOQ. In such cases the residue level was set at the LOQ in order to calculate a transfer factor. However, only cases in which both the raw agricultural commodity and the processed fraction show measurable residues are considered to truly indicate a processing factor (see Table 6.5.3-3). Processing factors are compiled in table 6.5.3-1 for both trials.

Storage period for samples:

The storage period of grain field samples and processed samples ranged between 243 and 338 days. Samples were kept deep frozen at -18°C or below before processing starts and were returned to the freezer (-18°C) after termination of the processing until analysis.

All storage intervals are covered by the storage stability testing.

Table 6.5.3-1: Summary of processing factors for flufenacet in wheat processed fractions

Commodity	Trial 11-3401-01 United Kingdom	Trial 11-3401-02 The Netherlands
Bran	4.4	5.2
Middlings	3.0	3.2
Shorts	4.4	5.3
White flour	0.1	< 0.8*
White bread	0.5	0.8
Whole meal	1.1	1.3
Wholemeal bread	0.9	1.2
Wheat germ	1.2	1.6
Starch A	< 0.2*	< 0.8*
Gluten	1.0	1.2
Starch B	0.2	< 0.8*
Gluten feed meal	0.6	< 0.8*

For the calculation of the processing factors the mean value of the residues in two RAC samples was used.
* In case the residue level in the processed fraction was less than the LOQ, the LOQ was used for calculation of the transfer factor.

Table 6.5.3-2: Recovery data for Flufenacet
The LOQ is marked in bold

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s. + metabolite	n	Fortification level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
11-3401	Wheat, winter	grain	total residue flufenacet	1	**0.01**	89	89	89	89	--
11-3401-01 and 11-3401-02 GLP: yes 2011				1	0.10	81	81	81	81	--
				2	overall		81	89	85	--
		white flour/ middlings [a)]	total residue flufenacet	3	**0.01**	107;111; 111	107	111	110	2.1
				4	0.10	95;109; 111;113	95	113	107	7.6
				7	overall		95	113	108	5.6
		white bread/ wholemeal bread	total residue flufenacet	2	0.10	86;87	86	87	87	
				2	overall		86	87	87	
		wheat germ	total residue flufenacet	3	**0.01**	90;95; 112	90	112	99	11.6
				3	0.10	88;90; 94	88	94	91	3.4
				6	overall		88	112	95	9.3
		gluten feed meal/ starch A [b)]	total residue flufenacet	3	**0.01**	80;89; 93	80	93	87	7.6
				4	0.10	86;87; 91;88	86	91	88	2.5
				7	overall		80	93	88	4.7

Fortified with flufenacet, determined as 4-fluoro-N-isopropylaniline and calculated as flufenacet.

a) recoveries for white flour and middlings are also representative for shorts, whole meal flour and bran
b) recoveries for gluten feed meal and starch A are also representative for starch B and gluten

Table 6.5.3-3: Results of processing trials conducted with Flufenacet WG 60 (containing (60 % flufenacet) on wheat

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues		
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Total residue flufenacet*: (mg/kg)
11-3401 11-3401-01 GLP: yes 2011	Wheat, winter Robigus (Winter wheat nabim Gp 3)	United Kingdom [redacted] Europe, North	60 WG	1	0.48	0.24	25	grain	135 135	0.10 0.085
								bran	135	0.41
								middlings	135	0.28
								shorts	135	0.41
								white flour	135	0.012
								white bread	135	0.046
								whole meal	135	0.10
								wholemeal bread	135	0.086
								wheat germ	135	0.11
								starch A	135	<0.01
								gluten	135	0.091
								starch B	135	0.020
								gluten feed meal	135	0.053
11-3401 11-3401-02 GLP: yes 2011	Wheat, winter Tabasco	Nether-lands [redacted] Europe, North	60 WG	1	0.48	0.16	25	grain	120 120	0.011 0.015
								bran	120	0.067
								middlings	120	0.042
								shorts	120	0.069
								white flour	120	<0.01
								white bread	120	0.011
								whole meal	120	0.017
								wholemeal bread	120	0.016
								wheat germ	120	0.021
								starch A	120	<0.01
								gluten	120	0.015
								starch B	120	<0.01
								gluten feed meal	120	<0.01

*Residues for flufenacet determined as 4-fluoro-N-isopropylaniline and calculated as flufenacet

Report:	**KCA 6.5.3/03, [redacted]; 1997; M-002403-01**
Title:	FOE 5043 60 DF - Magnitude of the residue in wheat processed commodities and aspirated grain fractions
Document No Report No	**M-002403-01-1** 107840 dated 1997-12-03
Guidelines:	EPA Ref.: 860.1500, 860.1520
GLP	Yes ; Deviations : none

Materials and methods

In one trial conducted in Stilwell, Kansas (NAFTA Region 5) during the 1995 growing season, flufenacet was applied as FOE 5043 60 DF (60% DF flufenacet formulation) once with an early post-emergence foliar application (BBCH 14) to winter wheat at 2017 g ai/ha (8.4N). One treated and one untreated control bulk wheat grain sample was harvested at normal maturity, 115 days after treatment. The wheat grain samples were processed into bran, flour, shorts, middlings, and germ; aspirated grain fractions were also collected. All of the procedures simulated commercial wheat processing practices.

Residues of the raw agricultural commodity and the processed fractions were analysed using method 00346 ([redacted], M.; 1995; M-018864-02) reported previously (Annex II dossier) which yields the combined level of the parent compound and all its metabolites containing the N-fluorophenyl-N-isopropyl functional group. Residues were determined using GC-MSD with an LOQ of 0.05 mg/kg and are expressed as parent flufenacet.

Processing procedures

Aspirated grain fractions

After determining the moisture content of the grain, the sample was dried in an oven until the moisture content was 10-13%. After drying, the sample was placed in a dust generation room containing holding bins, drag conveyors, and a bucket conveyor. As the sample was moved in the system, aspiration was used to remove light impurities (grain dust). The light impurities were then classified by sieving. Drying used for dust generation took precedence over drying before processing, and the light impurities collected during generation were kept separately from those collected during cleaning before processing.

Preparation of germs

The samples were aspirated and screened to separate light impurities and screenings (small and large) from the wheat. For wheat germ recovery, the cleaned wheat was moisture adjusted to 16% (1 to 1.5 hours), milled, and sifted to separate the bran from the germ fraction. The germ (with endosperm) was then passed through a reduction mill and sifted to separate the germ from the endosperm.

Preparation of bran, flour shorts and middlings

For flour, the cleaned wheat grain was moisture adjusted to 16% and broken four times in corrugated roller mills and sieved. After four breaks, material on top of the 730 µm sieve was collected as bran, material on top of the 390 and 240 µm screens was combined as middlings, material on top of the 132 µm screen was considered low grade flour, and material through the 132 µ:m screen was patent flour. After bran separation, the middlings were reduced four times into flour with a smooth roller mill and sieved. After the fourth reduction, material again was separated corresponding to particle seize into

shorts, low grade flour, and patent flour. Low grade flour and patent flour from the reducing steps were combined with the flours from the break steps.

Flow Charts

Fig. 1: Generation of aspirated grain fraction

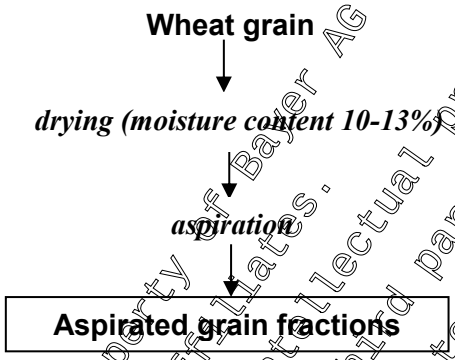
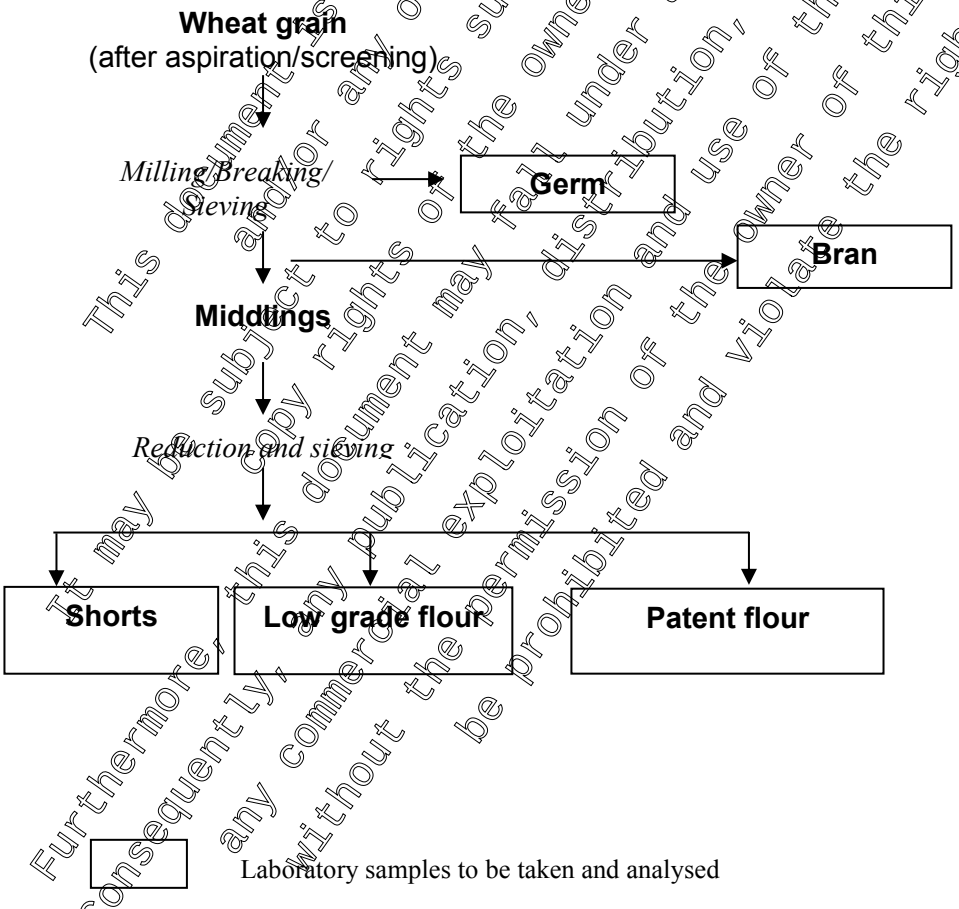


Fig. 2: Generation of germ, bran, middlings, shorts, low grade flour and patent flour



Laboratory samples to be taken and analysed

Findings

Recovery rates were determined prior to analysis and concurrently with the sample analysis in order to check the accuracy of the residue analysis. Validation of processed fractions was conducted using the parent compound and metabolites containing the N-fluophenyl-N-isopropyl functional group. For flufenacet, fortification was performed by spiking control samples with one of the following compounds or a mixture thereof: parent flufenacet, flufenacet oxalate hydrate, flufenacet sulfonic acid sodium salt, flufenacet thioglycolate sulfoxide. The recovery-rates and corresponding relative standard deviations (RSD) in grain and processed fractions were satisfactory, as shown in Table 6.5.3-5. The sample materials chosen served to represent all relevant sample materials collected in this study. No residues were determined in the control samples.

Residues in wheat grain and the processed fractions are summarised in Table 6.5.3-6 and in more detail in the Tier 1 summary forms.

In the grain samples taken at harvest, flufenacet residues amounted to 1.76 mg/kg (mean of 3 individual samples). For all processed commodities 3 individual samples were analysed and processing factors were calculated based on the mean per commodity. Processing factors are compiled in table 6.5.3-4.

Storage interval of samples

Grain field samples were stored frozen up to 25 months (766 days) and less than 1 months for processed fractions. The storage intervals are covered by the storage stability data.

Table 6.5.3-4: Summary of processing factors for flufenacet in wheat processed fractions

Commodity	Average residue from 3 samples (mg/kg)	Processing factor (as given in the report)	Processing factor (calculated)
Bran	3.61	2.1	2.1
Flour	0.78	< 1	0.44
Shorts	1.56	< 1	0.89
Middlings	1.41	< 1	0.80
Germ	2.28	1.3	1.3
Aspirated grain fractions	0.86	< 1	0.49

The processing factor was calculated based on the mean value of 3 individual samples for the RAC and the processed fractions.

Table 6.5.3-5: Recovery data for Flufenacet

The LOQ is marked in bold

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./ metabolite*	n	Fortification level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
107840 STF-F3082-94P GLP: yes 1995	Wheat winter	grain	total residue flufenacet	2	**0.05**	108;96	96	108	102	
				3	2.0	73;76;82	73	82	77	6.0
				5	overall		73	108	87	16.9
		bran	total residue flufenacet	13	**0.05**	108;112;116;106;110;102;88;86;86;98;98;100;84	84	116	100	10.9
				3	5.0	84;86;94	84	94	88	6.0
				16	overall		84	116	97	11.2
		flour	total residue flufenacet	13	**0.05**	102;96;100;100;114;92;92;84;90;80;86;88;88	80	114	93	9.7
				3	1.0	115;89;90	89	115	98	15.0
				16	overall		80	115	94	10.5
		shorts	total residue flufenacet	13	**0.05**	88;94;102;86;114;114;90;76;86;82;84;80;90	76	114	91	13.1
				3	2.0	70;80;71	70	80	74	7.5
				16	overall		70	114	88	14.8
		middlings	total residue flufenacet	13	**0.05**	108;98;108;98;104;110;90;98;96;96;98;90;88	88	110	99	7.2
				3	2.0	93;83;70	70	93	82	14.1
				16	overall		70	110	96	10.6
		germ	total residue flufenacet	13	**0.05**	85;82;104;102;106;118;78;104;98;86;92;114;72	72	118	95	14.8
				3	5.0	83;78;91	78	91	84	7.8
				16	overall		72	118	93	14.7
		aspirated grain fractions	total residue flufenacet	6	**0.05**	74;76;98;98;82;108	74	108	89	15.6
				3	1.0	87;100;98	87	100	95	7.4
				9	overall		74	108	91	13.0

* Spiking with one of the following compounds or a mixture thereof: parent flufenacet, flufenacet oxalate hydrate, flufenacet sulfonic acid sodium salt, flufenacet thioglycolate sulfoxide. Residues for flufenacet determined as FOE 5043 trifluoroacetamide and calculated as flufenacet.

Table 6.5.3-6: Results of processing trials conducted with Flufenacet 60 DF (containing 60 % flufenacet) on wheat

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues		
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Total residue flufenacet* (mg/kg)
107840 STF-F3082-94P GLP: yes 1995	Wheat, winter Karl 92	USA Stilwell, Kansas America, North	60 WG	1	2.016	2.1	4-leaf	grain	115	1.76
								bran	115	3.61
								flour	115	0.78
								shorts	115	1.56
								middlings	115	1.41
								germ	115	2.28
								aspirated grain fractions	115	0.86

*Residues for flufenacet determined as FOE-3043 trifluoroacetamide and calculated as flufenacet
For grain and the processed fractions the mean of 3 individual samples was calculated

Conclusion

Three processing trials on wheat are reported, two available from Europe and one study was performed in the US. Flufenacet was applied to the crop at exaggerated rates (2N and 8N) with a WG or DF formulation. Wheat samples were processed into commercially representative fractions and aspirated grain fractions were obtained from the US trial. Residues of flufenacet were found to concentrate in bran (median 4.4X), middlings (median 3.0X), shorts (median 4.4X), whole meal (mean 1.2X) and whole meal bread (mean 1.1X), germ (median 1.3X) and gluten (mean 1.1X). No concentration of flufenacet residues were seen in white flour, white bread, starch, gluten feed meal and aspirated grain fractions.

Table 6.5.3-7: Summary of processing factors for flufenacet in wheat processed fractions

Commodity	Report no 11-3401 Trial 11-3401-01 United Kingdom	Report no 11-3401 Trial 11-3401-02 The Netherlands	Report No 107840 Stilwell, Kansas (US)	Mean / Median processing factor*
Bran	4.4	5.2	2.1	4.4
Middlings	3.0	3.2	0.80	3.0
Shorts	4.4	5.3	0.89	4.4
White flour	0.1	< 0.8	0.44	0.3
White bread	0.5	0.8	--	0.7
Whole meal	1.1	1.3	--	1.2
Wholemeal bread	0.9	1.2	--	1.1
Wheat germ	1.2	1.6	1.3	1.3
Starch A	< 0.1	< 0.8	--	--
Gluten	1.0	1.2	--	1.1
Starch B	0.2	< 0.8	--	--
Gluten feed meal	0.6	< 0.8	--	--
Aspirated grain fractions	--	--	0.49	--

*The median is given in case more than 2 individual results are available; in case of two individual results > LOQ the mean value is calculated.

Barley

Report:	**KCA 6.5.3/05,** [redacted]; 2014; M-468736-02-1
Title:	Determination of the residues of flufenacet in/on barley and the processed fractions from pearl barley processing and preparation of alcoholic beverages (malting, brewing, distillation) after spray application of Flufenacet WG 60 in Germany and Belgium
Document No Report No	**M-468736-02-1** 11-3400 dated 2014-01-07
Guidelines:	• Regulation (EC) No 1107/2009 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), • EC guidance working document 7035/VI/95 rev.5 (1997-07-22) • OECD Guideline for the Testing of Chemicals, Magnitude of the Pesticide Residues in Processed Commodities, 508 (2008-10-03), • US EPA OCSPP Guideline No. 860.1520
GLP	Yes ; Deviations: none

Materials and methods

Two studies were performed in 2011 on spring barley in Germany and Belgium in order to to collect sample material for processing studies. The samples of barley (grain) to be processed were obtained after one post emergence spray application (BBCH 23/25) at exaggerated rate (2N = 0.48 kg as/ha) with Flufenacet WG 60, an WG formulation containing 60 % flufenacet. The higher rate was used in order to obtain appropriate residue levels in the raw agricultural commodity for derivation of processing factors.

Barley grain samples to be processed were sampled 114-116 days after treatment at growth stage BBCH 89.

The processing of the barley samples into the processed fractions was representative for production of beverages i.e. malting, brewing, distillation (beer, brewer's grain, brewer's yeast, brewer's malt, dried distillers grain, fresh distillers grain, malt sprouts, hops draff)) and production of pearl barley (pearl barley rub off, pearl barley). Processing was performed simulating industrial processes at a laboratory scale.

Residues of the raw agricultural commodity and the processed fractions were analysed using method 01100/M002 ([redacted], S.; [redacted], L., 2013; M-448503-01) with an LOQ of 0.01 mg/kg which yields the combined level of the parent compound and all its metabolites containing the N-fluorophenyl-N-isopropyl functional group. Residues are expressed as parent flufenacet.

Processing Procedures

Malting

After cleaning and sieving the grain the steeping process was conducted as a combined wet and dry steeping in a special steeping vessel activating enzymes until germination begins. The final steeping degree was in the range of 43.2 to 43.5 %

During the intensive respiration of the germinating grain the steeped good was turned over continuously.

After germination, the life processes are terminated by kilning. Kiln-drying was conducted in a dry chamber. The maximum temperature during the kiln-drying process was 80.0°C. After kiln-drying the germs were removed mechanically by a trimmer. Brewer's malt and malt sprouts were sampled immediately after end of malting. Until brewing (approx. 4 weeks malt rest) the malt was stored at room temperature.

Brewing

Mashing

Before mashing, the brewer's malt was dry milled in a special malt mill. The crushed malt was mixed with brew water according to a definite temperature time regime (mash program) in order to obtain the extract of good quality.

Lautering: Wort extraction and separation

After mash boiling, the wort was separated from the insoluble malt components (brewer's grain). The extract remaining in the brewer's grain was extracted by washing with hot water (first filter runnings). The wort separation was done using a refining vat. After separation, the brewer's grain was sampled.

Wort boiling and conditioning

After addition of hop pellets, the separated wort was boiled (about 90 min at normal pressure). In order to deactivate the malt enzymes, sterilize the wort, extract essential components of the hops, precipitate high molecular proteins and expel unwanted aromatic substances.
After boiling, the flocs (hops draff) were separated in a whirlpool causing the sludge to deposit on the bottom. For cooling and ventilating the wort, an intra-plant circulation was used. By adding oxygen (intra-plant circulation) the conditions for the start of the fermentation were prepared.

Fermentation and maturation

In the pilot plant the classical primary fermentation (low fermentation) was carried out in bottom fermentation containers. The fermentation temperature was 9 °C.

As soon as the extract content of the fermented young beer was 2 % higher than the final attenuation, the storing time began. Before maturation the young beer was cooled down. During the main fermentation the yeast deposits on the tank bottom and was sampled as brewer's yeast.

At the beginning of maturation the young beer was stored at room temperature (warm maturation to break down the diacetyl) in casks. Then the young beer was stored under pressure (approx. 0.7 - 1.8 bar) at 2 °C (cold maturation) for approx. 4 weeks.

The rack beer was filtered using a special filter combination. The final product beer was sampled.

Distillers grain production

Mashing

Barley grain was cleaned and subsequently milled into coarse meal. The coarse meal was homogeneously mixed with water according to a definite temperature time regime (mash program).

Fermentation

For the fermentation yeast was added to the produced mash. The fermentation duration was 4 days (23 - 25 °C) and was stopped at reaching of the final attenuation. The alcohol content was 5.0 – 7.7 %vol

Distillation

The fermented mash was transferred in a distillation vessel and slowly heated up until the distillation temperature was reached. After reaching of 80 °C the temperature was very slowly increased to 100 °C. Alcohol distillation was done until the alcohol content in the distillate decreased to approx. 3 %vol. The remaining distillers wash was separated into thin distillers wash and into thick distillers wash (distillers grain, fresh) by using a centrifuge or a press. Distillers grain, fresh was sampled. Subsequently the remaining thick distillers wash was dried at 38 °C until moisture content < 10 % was reached. Distillers grain, dried was sampled.

Pearl barley production

Cleaning and Conditioning

After cleaning barley grain was conditioned until an optimal moisture content of approx. 14 % was achieved.

Hulling

The corresponding samples were hulled until the stipulated abrasion for pearl barley (30 - 35 %) was reached. Pearl barley and pearl barley rub off were sampled.

Flow Charts

Fig. 1: Flow Chart of the Processing of Grain to Malt.

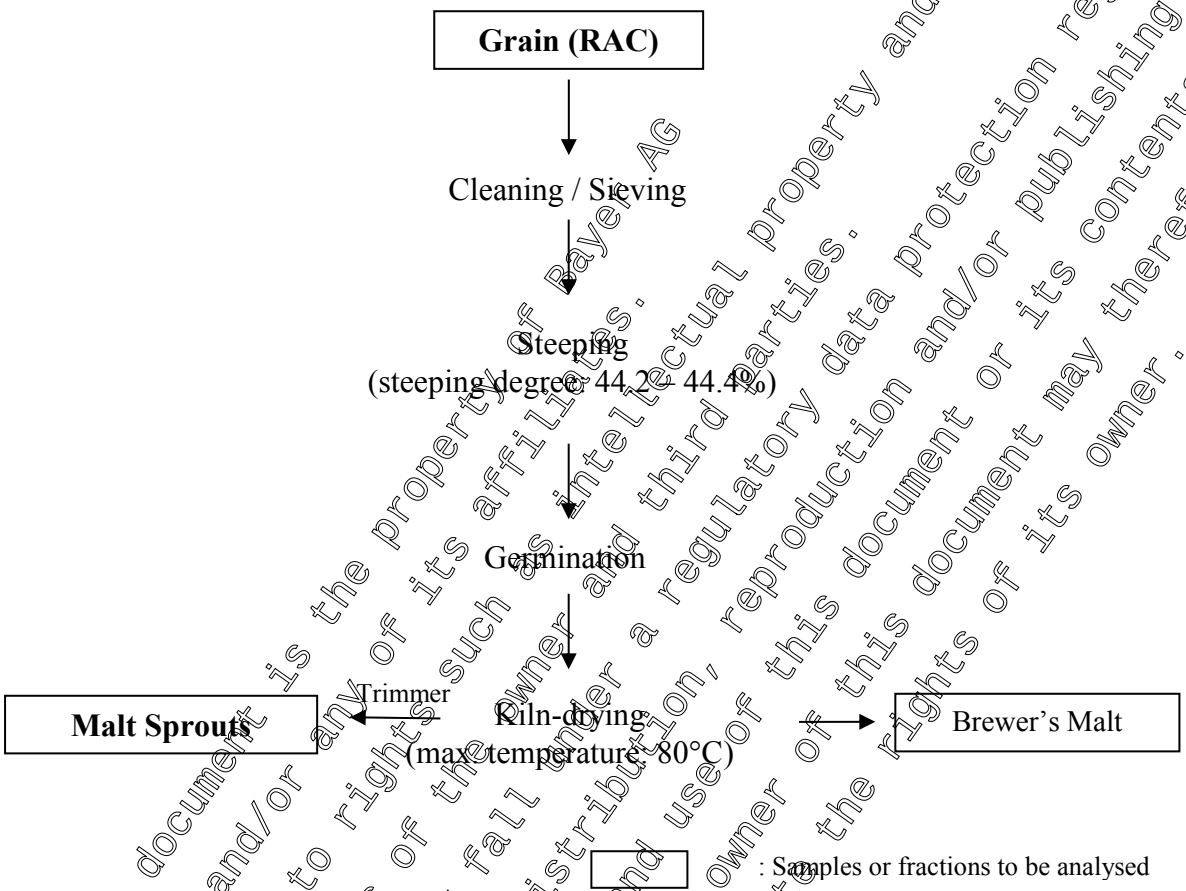


Fig. 2: Flow Chart of the Processing of Malt to Beer.

Fg. 3: Flow Chart of the Processing of Spring Barley to Distillers grain.

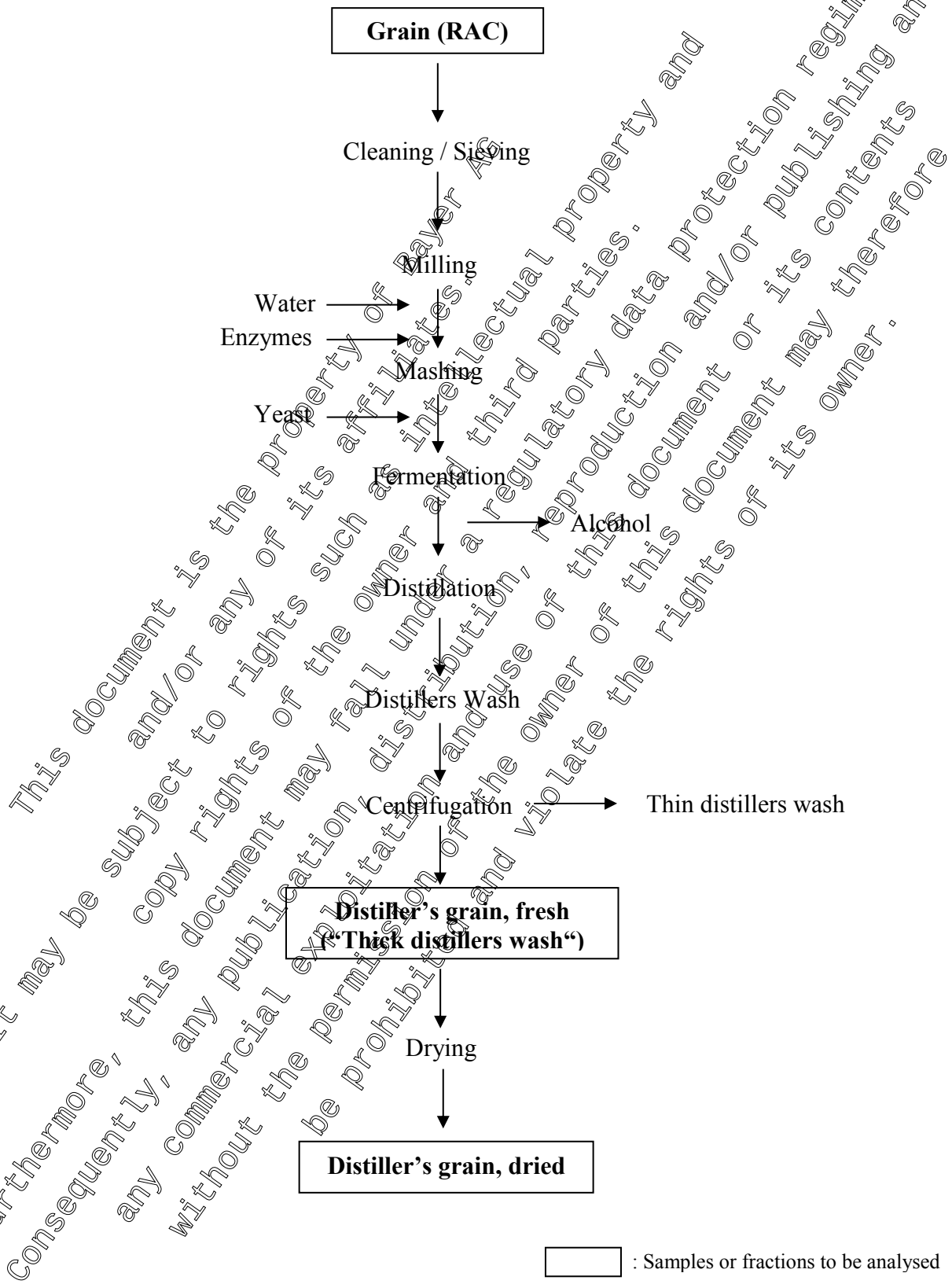
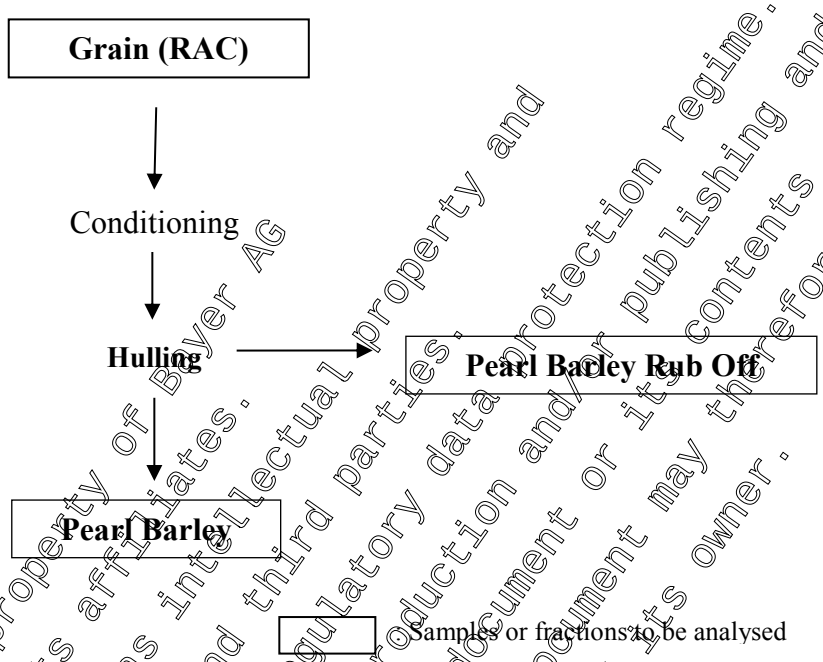


Fig. 4: Flow Chart of the Processing of Spring Barley to Pearl Barley.



Findings

Recovery rates were determined prior to residue analysis in order to validate the method and concurrently with the sample analysis in order to check the accuracy of the residue analysis. Control material was fortified with flufenacet, FOE5043 oxalate hydrate, FOE5043 sulfonic acid and FOE5043 thioglycolate sulfoxide as a mixture (1/1/1/1). The sample materials chosen served to represent all relevant sample materials collected in this study. The data demonstrate acceptable method performance during sample analysis. The summaries of recoveries and corresponding relative standard deviations (RSD) are provided in Table 6.5.3-9. No residues were determined in the control samples.

Residues in barley grain and the processed fractions are summarised in Table 6.5.3-10 and in more detail in the Tier 1 summary forms.
In spite of the exaggerated rate used and late application during tillering in spring (BBCH 23/25) no flufenacet residues were determined in the raw agricultural commodity in both trials. Nevertheless grain was processed in order to investigate possible concentration. Except for the by-products destiller's grain (dried) and pearl barley rub off, residues were less than the LOQ also in processed fractions of barley. Thus, processing factors were calculated only for these commodities using the LOQ as residue level for the RAC.
An overview on processing factors is compiled in table 6.5.3-8 for both trials.

Storage period for samples:
Barley grain was stored at ambient temperature for 6-8 weeks until processing according to industrial practice in order not to compromise the germination processes by freezing the raw agricultural commodity. The storage period of deep-frozen laboratory samples intended for the analysis of flufenacet ranged between 12 and 21 months (350 - 619 days). The storage period is covered by the storage stability data.

Table 6.5.3-8: Summary of processing factors for flufenacet, in barley processed fractions

Commodity	Trial: 11-3400-01, Germany	Trial: 11-3400-02, Belgium
Processing into beer		
malt sprouts	n.c.	n.c.
brewer's malt	n.c.	n.c.
brewer's grain	n.c.	n.c.
hops draff	n.c.	n.c.
brewer's yeast	n.c.	n.c.
beer	n.c.	n.c.
Processing into distillers grain		
Distillers grain, fresh	n.c.	n.c.
Distillers grain dried	3.2*	3.3*
Processing into pearl barley		
pearl barley rub off	>1.8*	>2.1*
pearl barley	n.c.	n.c.

n.c. = not calculated because residues in the raw agricultural commodity and the processed fraction were < LOQ.
*In case residues in the processed fraction were >LOQ the LOQ of the RAC was used to calculated the transfer factor.

Table 6.5.3-9: Recovery data for Flufenacet

The LOQ is marked in bold

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
11-3400MAN 11-3400-01 and 11-3400-02 GLP: yes 2011	Barley, spring	brewer's malt [a)]	Total residue flufenacet	4	**0.010**	91; 101; 107; 113	91	113	103	9.1
				3	0.10	72; 74; 74	72	74	73	1.6
				7	overall		72	113	90	19.1
		brewer's grain [b)]	Total residue flufenacet	5	**0.010**	75; 78; 79; 87; 105	75	105	85	14.3
				4	0.10	70; 73; 78; 100	70	100	80	16.9
				9	overall		70	105	83	14.7
		hops draff	Total residue flufenacet	3	**0.010**	89; 109; 117	89	117	105	13.7
				3	0.10	81; 99; 101	81	101	94	11.8
				6	overall		81	117	99	13.1
		brewer's yeast	Total residue flufenacet	4	**0.010**	86; 99; 106; 113	86	113	101	11.4
				3	0.10	75; 79; 94	75	94	83	12.1
				7	overall		75	113	93	15.0
		beer	Total residue flufenacet	4	**0.010**	63; 66; 68; 81	63	81	70	11.4
				3	0.10	64; 76; 77	64	77	72	10.0

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
				7	overall		63	81	71	10.1
		pearl barley [c]	Total residue flufenacet	4	**0.010**	85; 91; 92; 97	85	97	91	5.4
				3	0.10	85; 86; 89	85	89	87	2.4
				7	overall		85	97	89	5.0
		grain, stored	Total residue flufenacet	5	**0.010**	62; 83; 89; 93; 101	62	101	86	17.2
				4	0.10	85; 87; 89; 91	85	91	88	2.9
				9	overall		62	101	87	12.2

FL = Fortification level, RSD = Relative standard deviation, LOQ = Practical limit of quantification
Fortified with flufenacet, FOE5043 oxalate hydrate, FOE5043 sulfonic acid and FOE5043 thioglycolate sulfoxide (1/1/1/1), determined as 4-fluoro-N-isopropylaniline and calculated as flufenacet

a) Recoveries for brewer's malt are also representative for malt sprouts
b) Recoveries for brewer's grain are also representative for distiller's grain fresh and dried
c) Recoveries for pearl barley are also representative for pearl barley rub-off

Table 6.5.3-10: Results of processing trials conducted with Flufenacet WG 60 (containing 60% flufenacet) on barley

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues		
			FL	No	kg/ha (a.s.)	kg/hl (a.s.)	GS	Portion analysed	DALT (days)	Total residue flufenacet* (mg/kg)
11-3400MAN 11-3400-01 GLP: yes 2011	Barley, spring Simba	Germany [redacted] Europe, North	60 WG	1	0.48	0.16	25	malt sprouts	116	<0.01
								brewer's malt	116	<0.01
								brewer's grain	116	<0.01
								hops draff	116	<0.01
								brewer's yeast	116	<0.01
								beer	116	<0.01
								distillers grain, fresh	116	<0.01
								distillers grain, dried	116	0.012
								pearl barley rub off	116	0.018

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Residues Portion analysed	DALT (days)	Total residue flufenacet* (mg/kg)
								pearl barley	116	<0.01
								grain, stored	116	<0.01
									116	<0.01
									116	<0.01
									116	<0.01
									116	<0.01
11-3400MAN 11-3400-02 GLP: yes 2011	Barley, spring Quench	Belgium [redacted] Europe, North	60 WG	1	0.480	0.16	23	malt sprouts	114	<0.01
								brewer's malt	114	<0.01
								brewer's grain	114	<0.01
								hops draff	114	<0.01
								brewer's yeast	114	<0.01
								beer	114	<0.01
								distillers grain, fresh	114	<0.01
								distillers grain, dried	114	0.013
								pearl barley rub off	114	0.021
								pearl barley	114	<0.01
								grain, stored	114	<0.01
									114	<0.01
									114	<0.01
									114	<0.01
									114	<0.01
									114	<0.01

*Residues for flufenacet determined as 4-fluoro-N-isopropylaniline and calculated as flufenacet

Conclusion

Two processing trials were conducted in Europe on spring barley at exaggerated rates (2N) in order to obtain processing factors for sample materials representative for production of alcoholic beverages (malting, brewing, distillation) and production of pearl barley. The final consumable products as well as a number of by-products were analysed for residues of flufenacet. In spite of using an exaggerated application rate and application in spring no flufenacet residues could be determined in the RAC. No residues were determined in the processed fractions either, except for the by-products dried distiller's

grain and pearl barley rub-off. The processing factor is considered to be indicative because the LOQ of the RAC has been used for calculation of the processing factor.
Following harvest, barley grain was stored at ambient temperature for a short time frame until processing according to industrial practice. However, it is not appropriate to store the grain deep-frozen since this would adversely affect the germination of the grain. Handling of the harvested produce truly reflects commercial processes and is therefore considered adequate. It is concluded that processing of barley when treated with flufenacet has no relevance for the consumer risk assessment.

CA 6.6 Residues in rotational crops

Confined rotational crop studies with flufenacet were conducted using the ^{14}C-labelled test substance, the radiolabel being in the [fluorophenyl-UL-^{14}C][20] and in the [thiadiazole-2-^{14}C][21] -position. These studies were already included in the submission for Annex I inclusion. However, labeling in the thiadiazole-5-position is still missing. This study is now added to complete the nature of residue constituents originating from flufenacet in succeeding crops.
Table 6.6-1 gives an overview on the metabolism studies in rotational crops.

Table 6.6- 16: Overview of all plant metabolism studies with ^{14}C-labeled flufenacet in succeeding crops

Study type	Crop	Application scenario	Label	Report	Submission	
					EU dossier, Annex II, Section 4, Point 6	Presented in supplementary dossier Section 4, Point 6
Confined rotational crop	Wheat, Kale, Turnips	soil application 900 g as/ha	[Fluorophenyl-UL-^{14}C	[redacted], M.F. [redacted], M.K. (1994) M-002369-01-1	KCA 6.6.1/02	-
	Wheat, Kale, Turnips	soil application 900 g as/ha	[Thiadiazole-2-^{14}C]	[redacted], P.P., [redacted], E.J. (1995) M-002368-01-1	KCA 6.6.1/01	-
	Wheat, Swiss chard, Turnips	soil application 900 g as/ha	[Thiadiazole-5-^{14}C]	[redacted], R., [redacted], M. (2012) M-443538-01-1		KCA 6.6.1/03

[20] [redacted], M. F. [redacted], M. K., (1994): Accumulation of [Phenyl-^{14}C]FOE 5043 Residues in Confined Rotational Crops; unpublished report 106768 of Miles Inc. Kansas, USA, now Bayer CropScience AG, Comp. No. M-002369-01-1

[21] [redacted], P. P, [redacted], E. J. (1995): Accumulation of [Thiadiazole-2-^{14}C]FOE 5043 Residues in Confined Rotational Crops; unpublished report 106639 of Bayer Cor. Kansas, USA, now Bayer CropScience AG, Comp. No. M-002368-01-1

Evaluation in the EU peer review process

Excerpt from Monograph:

The results of the confined rotational crop studies demonstrate that the metabolic pattern after application of FOE 5043 (flufenacet) is similar in target crops and crops grown in rotation. No active ingredient was found and all metabolites are derived by the same metabolic pathway via glutathione and homoglutathione, which is common to all plant species. Although several additional compounds were only observed in rotational crops, they are considered as products of further metabolism of known metabolites. Most of them should be detectable with the total residue method developed for plant residue analysis and/or are considered of being of no relevance because they are not expected to appear in significant amounts.

After normal agricultural use of FOE 5043 no significant residues are to be expected in leafy or root crops grown in rotation with the target crops, even at rates which are considerably higher than the highest recommended field application in Europe. According to the above mentioned studies the only exception would be wheat (which at the same time is also a target crop). However, a comparison with the results from field trials in cereals and maize at recommended application rates of 240 ai/ha and 600 g a.i./ha (see Chapter 6.3 [of the AII dossier] *reveals that no residues were detected. Therefore, it is concluded, that the high residue levels in the confined rotational crop study are a consequence of the experimental design and do not reflect normal practice relevant conditions. Consequently, a field rotational crop study is considered as not being necessary.*

Evaluation in EFSA Reasoned Opinion on existing MRLs (EFSA Journal 2012;10(4):2689)

Excerpt from the EFSA Reasoned Opinion which makes reference to the Monograph

In the DAR it was concluded that after use of flufenacet according to the GAPs (…), no significant residues are expected in leafy or root crops grown in rotation with the primary crops. According to the confined rotational crop metabolism studies the only exception to this would be wheat. However an assessment of the results from field trials in cereals and maize (…) shows that no residues are detected in any trial, except in green material sampled within 40 days of application and therefore it was concluded in the DAR that the high residue levels seen in wheat were a consequence of the experimental design and do not reflect normal practice. Considering, also, that the application rate of flufenacet within the EU ranges between 0.15-0.6 kg a.s./ha it can be concluded that flufenacet residue levels in rotational commodities are not expected to exceed 0.01 mg/kg, provided flufenacet is applied in compliance with the GAPs reported in Appendix A.

Since the highest supported application rates evaluated for Annex I inclusion and particularly the critical GAP for cereals did not change the conclusions drawn in the Monograph and in the EFSA reasoned opinion are still considered valid.

CA 6.6.1 Metabolism in rotational crops

Additional confined rotational crop study with [thiadiazole-5-^{14}C]flufenacet

Report	**KCA 6.6.1/03,** [redacted] C; [redacted]; 2012; M-443538-01
Title:	Metabolism of [thiadiazole-5-^{14}C]Flufenacet in Confined Rotational Crops
Document No:	M-443538-01-1
Report No:	EnSa-12-0535 dated 2012-11-29
Guidelines:	OECD guideline 502: Metabolism in Rotational Crops, adopted 8-January-2007 US EPA OCSPP Residue Chemistry Guideline OPPTS 860.1850
GLP	Yes; Deviations: none

Executive Summary

Following confined rotational crop studies with [trifluorophenyl-UL-^{14}C] and [thiadiazole-2-^{14}C]flufenacet a respective study was conducted with flufenacet radiolabelled in the C-5 position of the thiadiazole ring to complete the picture of all potential metabolic pathways in rotated crops. Therefore, [thiadiazole-5-^{14}C]flufenacet was applied to bare soil at a use rate of approximately 900 g as/ha and wheat (cereal crop), turnip (root crop) and Swiss chard (leafy crop) were sown 30 days (1st rotation), 142 days (2nd rotation) and 317 days (3rd rotation) after application. The crops were cultivated and harvested according to agricultural practice.

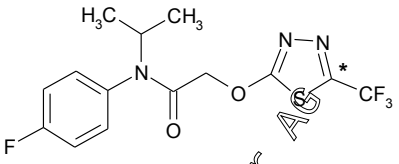
The total radioactive residues (TRR) increased in wheat from the 1st to the 2nd rotation and followed by a decrease at the 3rd rotation, whereas TRR continually decreased in turnip and Swiss chard from the 1st to the 3rd rotation. Extraction of harvested crops with acetonitrile/water (8/2, v/v) was almost complete, amounting to more than 93% of TRR. Radio-HPLC and radio-TLC of the extracts revealed that more than 80% of TRR consisted of radiolabelled trifluoroacetate (TFA, M45) in all crops accompanied by minor amounts of FOE-thiadone-glycoside (M25) and trifluoroethane sulfonic acid (M44).

Soil core samples were taken shortly before each sowing. The residues in soil consisted mainly of the parent substance and TFA. FOE-thiadone (M9) was found at a minor extent. All residues in soil decreased with time.

These results indicated an initial cleavage of the thiadiazole ring from the parent substance in soil. Lower portions of the split-off thiadiazole ring were taken up by rotated crops and conjugated as glycoside. The main metabolic pathway proceeded via complete degradation of the thiadiazole ring in soil to form TFA (M45). On a short-term period, a low amount of trifluoroethane sulfonic acid (M44) was also formed in soil. The major portion of TFA and a small amount of the sulfonic acid obviously were taken up by the rotated crops since their concentration in the crops was higher than in the soil.
The proposed metabolic pathway of [thiadiazole-5-^{14}C] in rotated crops is shown in Figure 6.6.1- 1.

Material and methods

Test Material

Structural formula	 * denotes the ^{14}C label
Chemical name	N-(4-Fluoro-phenyl)-N-isopropyl-2-(5-trifluoromethyl-[1,3,4]thiadiazol-2-yloxy)-acetamide (IUPAC); Acetamide, N-(4-Fluorophenyl)-N-(1-methylethyl)-2-[[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]oxy]- (9Cl; CAS)
Common name	Flufenacet
CAS RN	142459-58-3
Empirical formula	$C_{14}H_{13}F_4N_3O_2S$
Company code	FOE 5043
Molar mass (non-labelled)	363.34 g/mol
Label	[thiadiazole-5-^{14}C]Flufenacet
Specific radioactivity	1.9 MBq/mg (used in the study; the original test substance had a specific radioactivity of 3.81 MBq/mg or 103.04 µCi/mg)
Radiochemical purity	>99% by TLC and HPLC (radio-detection)
Chemical purity	>99% by HPLC (UV detection at 210 nm)

Test Plants

1st Species (small grain)	Spring wheat
Variety	Thasos
Harvested commodities	Forage (BBCH 29), Hay (BBCH 75-83), Grain and straw (BBCH 89-92, maturity)
2nd Species (root crops)	Turnip
Variety	Rondo
Harvested commodities	Roots and leaves (BBCH 45-49, maturity)
3rd Species (leafy crops)	Swiss chard
Variety	Lukullus
Harvested commodities	Top plant (BBCH 45, intermediate and BBCH 49, maturity)

Preparation of the spray mixture and application

The original radiolabelled test substance was diluted with non-labelled flufenacet resulting in a specific radioactivity of 1.9 MBq/mg. Addition of a blank formulation yielded a SC 500 formulation with a concentration of the active substance of 42.4% (w/w). Addition of water finally resulted in the spray mixture of a volume of 104.5 mL.

A plant container (surface area 1 m^2) was filled with a sandy loam soil (67% sand, 18% silt, 15% clay, 1.2% organic carbon, pH 6.9 ($CaCl_2$)). During the first rotation, the plant container was placed in an

open vegetation hall with natural temperatures and sunlight conditions, but protected from rain by a glass roof. The glass roof was opened during the sunshine periods and automatically closed during rainfall. During the second and third rotation, the container was moved into a greenhouse.

The spray solution was applied to the bare soil surface of the prepared plant container using a computer controlled track sprayer fitted with a flat jet nozzle. The actual application rate amounted to 903 g as/ha; it was higher by 7.5% than the maximum annual application rate of 840 g as/ha. The homogeneity of spray was proven by ten round filter papers (1.5 cm diameter) randomly placed onto the surface before application. The stability the test substance in the spray mixture was demonstrated by radio-HPLC before and after application. After spraying the soil remained undisturbed until sowing for the first rotation (30 days). The soil was watered to maintain adequate soil moisture.

Sowing and cultivation of rotated crops

The rotated crops were sown at three intervals after application (plant back intervals, PBI):

First rotation:	PBI = 30 days
Second rotation:	PBI = 142 days
Third rotation:	PBI = 317 days

Shortly before each sowing the upper soil layer (10 cm) was loosed and intensively mixed. Soil cores to a depth of 15 cm were sampled to investigate additionally the degradation of flufenacet in soil. Wheat was sown in 5 rows over 0.5 m^2, turnip was sown in 1 row over 0.25 m^2 and Swiss chard in 2 rows also over 0.25 m^2. The crops were grown to maturity. After harvest of the previous set of crops the crops for next rotation were sown.

Fertilizing, watering and plant protection measures were performed according to agricultural practice. During the outdoor season (first rotation, April – September 2011) the mean temperatures amounted to 16 – 22°C and the mean sunshine periods to 83 – 291 hours/month. During the greenhouse season (second and third rotation, September 2011 – June 2012) the mean temperatures were 17 – 22°C. The crops were artificially irradiated with greenhouse lamps at 35 kLux during the day period (6.00 – 20.00 h).

Harvesting and processing of rotated crops

Wheat samples were taken at forage stage (BBCH 29, end of tillering), at hay stage (BBCH 75 – 83, grain content milky – early dough) and straw and grain at maturity (BBCH 89 – 92, grain hard to very hard). Immature top wheat plants were cut above the soil surface (roots remained in the soil), cut in small pieces and homogenized in liquid nitrogen with aid of a high speed stirrer (Polytron). Mature plants were manually separated in grain and straw (empty ears and chaff were added to the straw) before homogenization in liquid nitrogen. The homogenized samples were stored in freezers an approximately -18°C until analysis.

Turnips were completely sampled in the interval shortly before maturity (BBCH 45, 50% of expected root diameter reached) and full maturity (BBCH 49, expansion complete) and separated into roots and leaves. Roots and leaves were cut into slices and pieces, homogenized in liquid nitrogen and stored at approximately -18°C until analysis.

The green parts of Swiss chard were harvested as intermediated commodity (BBCH 45, 50% of leaf mass reached) and at maturity (BBCH 49, typical leaf mass reached). The roots remained in the soil. The sampled foliage was cut into pieces, homogenized in liquid nitrogen and stored at approximately -18°C.

Radioassaying, extraction and analysis of the plant samples

Radioassaying (measurement of the radioactivity) was conducted by liquid scintillation counting (LSC). The counting was repeated three times. Quenching was automatically compensated using an external standard. Solid samples were firstly combusted and the formed $^{14}CO_2$ absorbed in an alkaline scintillation liquid. The limit of quantification (LOQ) was set to twice the background radioactivity for radioassaying of solid samples. Given the aliquot amount of combustion and the specific radioactivity used in this study the LOQ for radioassaying was 0.002 mg parent equivalents/kg (0.002 mg equ/kg).

Homogenized plant samples were extracted with acetonitrile/water (8/2, v/v, 3x) using a high speed stirrer (Polytron) followed by one extraction with pure acetonitrile. The radioactivity contents of the extracts and the remaining solids were numerically summarized to yield the total radioactive residues (TRR) of the original sample. The extracts were combined, concentrated and analyzed for the metabolite profile by radio-HPLC and radio-TLC (TLC only done for polar HPLC fractions).

Radio-HPLC was conducted on a RP18 column (250 x 4.6 mm, 5 µm particle size) operated with a gradient mixture of water/formic acid (99/1, v/v) and acetonitrile/formic acid (99/1, v/v) at 40°C. The HPLC system was equipped with a UV detector (254 nm) and a radiomonitor with a glass scintillator (cell size 370 µL). Column recovery (98 – 101%) was proven by comparison of the eluted and injected radioactivity. The LOQ for HPLC determination was derived from the background noise and the smallest radio-peak of the respective sample. HPLC-LOQs for samples of the first and second rotation were in the range of 0.005 – 0.05 mg equ/kg.

One-dimensional radio-TLC was conducted on a silica gel TLC plates (20 x 20 cm, layer thickness 0.25 mm). Development of the spotted plates was performed with a solvent mixture consisted of ethyl acetate/2-propanol/water/acetic acid (65/24/10/1, v/v/v/v) after chamber saturation. The radioactive spots on developed plates were visualized and quantified using a Bio-Imaging Analyzer. Non-labelled FOE-5043-sulfonic acid (2,2,2-trifluoroethane sulfonic acid) used as reference standard was stained with aqueous 0.1% 'Pinacryptol yellow' and visualized by extinction of the fluorescence dye of the plate under UV light.

The radioactivity in the isolated polar HPLC fraction of the wheat forage sample of the first rotation was identified by LC-MS as TFA-trifluoroacetate and was later used as radiolabelled reference standard in co-chromatography of the other samples. LC-MS was conducted on a combination of RP18-HPLC and an Orbitrap mass spectrometer using electro-spray for ionization. Non-labelled FOE 5043-sulfonic acid and FOE-thiadone (5-trifluoromethyl-1,2,4-thiadiazol-2(3*H*)-one) were used as additional reference standards for co-chromatography.

Extraction of soil samples

The soil core samples (0 – 15 cm layer) of each sampling interval (shortly before sowing of rotated crops) were mixed, homogenized and extracted with acetonitrile/water (1/1, v/v, 3x) using a high-speed stirrer. The combined extracts were concentrated and analyzed by radio-HPLC and radio-TLC together with the parent substance and the mentioned reference standards for co-chromatography.

Findings

Total radioactive residues in rotated crops and soil

Total radioactive residues (TRR) in the agricultural commodities of the three rotated crop species are presented in Table 6.6.1- 1. They increased in wheat from the first to second rotation following by a decrease at the third rotation. In contrast, TRR continuously decreased in turnip and Swiss chard from the first to the third rotation (except Swiss chard of intermediate growth stage).

For comparison, TRR in soil samples taken shortly before each sowing steadily decreased from the first to the last rotation: TRR in soil: 1st rotation: 0.638 mg equ/kg; 2nd rotation: 0.239 mg equ/kg; 3rd rotation: 0.104 mg equ/kg.

Extractability and identification of the extracted residues in rotated crops

The extraction of rotated crops with acetonitrile/water (4/1, v/v) and pure acetonitrile was almost complete accounting to 93.1 - 100% of TRR. In turn, the non-extractable residues ("post extraction solids", PES) ranged from 0 to maximum 6.0% of TRR (wheat grain of the 3rd rotation).

Reversed-phase radio-HPLC profiles of the extracts were performed immediately after extraction. Radiolabelled trifluoroacetate (M45, isolated from wheat forage of the first rotation and identified by HPLC-MS), radiolabelled FOE-thiadone-glycoside (M25, isolated in wheat metabolism study of [thiadiazole-5-^{14}C]flufenacet) and non-labelled FOE-trifluoroethane sulfonic acid served as reference standards for co-chromatography.

The predominant portion of the radioactive residues extracted from all crops proved to be very polar as it was eluted in reversed phase HPLC as a radio-peak close to the dead volume. This peak showed sometimes a shoulder and seems to represent more than one metabolite. Therefore, the respective fraction was collected and additionally analyzed by radio-TLC on a straight phase silica gel plate. The mentioned radiolabelled reference standards were used for co-chromatography.

It turned out that nearly the complete portion of polar radioactive residues (83.6 - 99.9% of TRR) consisted of ^{14}C-trifluoroacetate (M45). FOE-thiadone-glycoside (M25) and FOE 5043-trifluoroethane sulfonic acid (M44) were detected at minor amounts (< 10% of TRR). The rate of identification of the radioactive residues in all rotated crops was very high accounting for ≥ 92.5% of TRR. The composition of the radioactive residues in crops rotated after application of [thiadiazole-5-^{14}C]flufenacet to bare soil at a rate of approximately 900 g as/ha is presented in Table 6.6.1- 2 (first rotation), Table 6.6.1- 3 (second rotation) and Table 6.6.1- 4 (third rotation). Metabolic pathway of [thiadiazole-5-^{14}C]flufenacet in rotated crops is shown in Figure 6.6.1- 1.

Storage stability of the radioactive residues of flufenacet on rotated crops

All crop samples were stored at temperature ≤ -18 °C immediately after sampling until extraction and analysis.

The samples of the first rotation were extracted within 12 days after sampling at maximum, those of the second rotation within 8 days and those of the third rotation within one month after sampling. The earliest metabolite profiles (used for quantitation of metabolites) were obtained by radio-HPLC analysis within 4 days after extraction.

Approximately one year after sampling, repeated extraction and profiling of metabolites were performed from wheat straw, wheat grain and Swiss chard (at maturity) of the first rotation using identical analytical conditions. There were no differences between the metabolite profiles of the initial and repeated analysis. Therefore, it is concluded that the residues of flufenacet in the samples of rotated crops were stable for at least one year.

Extraction and identification of extracted residues in soil

Soil core samples taken shortly before sowing of rotated crops (days 30, 142 and 317 days after application of the radiolabelled substance) were analyzed for the composition of residues. These analyzes revealed a continuous decrease of the parent substance from 0.459 to 0.043 mg/kg and a similar decrease of the major metabolite trifluoroacetate (M45) from 0.162 mg equ/kg to 0.034 mg equ/kg. The minor metabolite FOE-thiadone (M9) was only detected in the first soil sample (30 days after application) amounting to 4.5% of TRR corresponding to 0.030 mg equ/kg. The non-extractable residues increased from 23.9% to 58.9% of TRR. The composition of residues in soil is presented in Table 6.6.1- 5.

Conclusion

Following application of [thiadiazole-5-^{14}C]flufenacet to soil at a use rate of approximately 900 g as/ha wheat (cereal crop), turnip (root crop) and Swiss chard (leafy crop) were sown and rotated 30 days (1st rotation), 142 days (2nd rotation) and 317 days (3rd rotation). Extraction of rotated crops with acetonitrile/water (8/2, v/v) was almost complete amounting to more than 93% of TRR. Radio-HPLC and radio-TLC of the extracts revealed that more than 80% of TRR consisted of radiolabelled trifluoroacetate (TFA, M45) in all crops accompanied with minor amounts of FOE-thiadone-glycoside (M25) and trifluoroethane sulfonic acid (M44).

These results indicated an initial cleavage of the thiadiazole ring from the parent substance in soil. Low amounts of the split-off thiadiazole ring were taken up by rotated crops and conjugated as glycoside. The main metabolic pathway proceeded via complete degradation of this ring in soil to form TFA (M45). On a short-term period, low amounts of trifluoroethane sulfonic acid (M44) were also found in soil. The major portion of TFA (M45) and a small amount of the sulfonic acid (M44) obviously was taken up by the rotated crops since their concentration in the crops were higher than in the soil.

The proposed metabolic pathway of [thiadiazole-5-^{14}C] in rotated crops is shown in Figure 6.6.1- 1.

Table 6.6.1- 1: Total radioactive residues (TRR) in rotated crops following application of [thiadiazole-5-^{14}C]flufenacet at a use rate of 900 g as/ha to bare soil

TRR in rotated crops	1st rotation	2nd rotation	3rd rotation
PBI (days)	30	142	317
Crop commodity	[mg equ/kg]		
wheat forage	1.543	2.318	1.441
wheat hay	3.755	8.225	3.740
wheat straw	4.376	9.335	4.035
wheat grain	3.024	7.673	1.371
turnip leaves	6.792	3.536	0.993
turnip roots	0.601	0.197	0.087
Swiss chard (intermediate)	6.117	1.951	4.784
Swiss chard (at maturity)	3.386	2.950	1.973

Table 6.6.1- 2: Composition of the radioactive residues in crops of the 1st rotation after application of [thiadiazole-5-^{14}C]flufenacet at a use rate of 900 g as/ha to bare soil

Wheat, 1st rotation	Forage		Hay		Straw		Grain	
	%TRR	mg/kg#	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
TFA (trifluoroacetic acid)	95.2	1.469	90.7	3.404	92.6	4.054	95.9	2.899
FOE 5043-trifluoroethane sulfonic acid	3.3	0.50	2.1	0.079	0.5	0.021	n.d.	n.d.
FOE-thiadone-glycoside	1.3	0.019	3.8	0.142	4.5	0.198	n.d.	n.d.
Total identified	**99.7**	**1.538**	**96.6**	**3.625**	**97.7**	**4.274**	**95.9**	**2.899**
unknown	n.d.***	n.d.	0.4	0.016	0.6	0.027	n.d.	n.d.
Total characterized	n.d.	n.d.	0.4	0.016	0.6	0.027	n.d.	n.d.
Procedural loss	--	--	---	---	0.2	0.008	3.5	0.106
Total extractable	**99.7**	**1.538**	**97.0**	**3.641**	**98.5**	**4.309**	**99.4**	**3.006**
Non-extractable (PES) **	0.3	0.004	3.0	0.113	1.5	0.067	0.6	0.018
Accountability	**100.0**	**1.543**	**100.0**	**3.755**	**100.0**	**4.376**	**100.0**	**3.024**

Turnip and Swiss chard 1st rotation	Turnip leaves		Turnip roots		Swiss chard		Swiss chard	
	mature		mature		intermediate		mature	
	%TRR	mg/kg#	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
TFA (trifluoroacetic acid)	94.7	6.432	83.6	0.503	92.0	5.625	93.4	3.162
FOE 5043-trifluoroethane sulfonic acid	2.2	0.146	n.d.	n.d.	3.2	0.193	2.2	0.076
FOE-thiadone-glycoside	2.4	0.163	8.9	0.054	0.8	0.051	1.6	0.053
Total identified	**99.3**	**6.741**	**92.5**	**0.556**	**95.9**	**5.870**	**97.2**	**3.291**
unknown	0.5	0.031	5.2	0.031	3.5	0.217	2.5	0.083
Total characterized*	0.5	0.031	5.2	0.031	3.5	0.217	2.5	0.083
Procedural loss	-	-	-	-	0.2	0.010	-	
Total extractable	**99.7**	**6.772**	**97.8**	**0.588**	**99.7**	**6.096**	**99.7**	**3.374**
Non-extractable (PES) **	0.3	0.019	2.2	0.013	0.3	0.021	0.3	0.011
Accountability	**100.0**	**6.792**	**100.0**	**0.601**	**100.0**	**6.117**	**100.0**	**3.386**

* unidentified compounds are characterized by their extraction and chromatographic behaviour.
** PES = post extraction solids
*** n.d. = not detected
# mg/kg means mg parent equivalents/kg

Table 6.6.1- 3: Composition of the radioactive residues in crops of the 2nd rotation after application of [thiadiazole-5-^{14}C]flufenacet at a use rate of 900 g as/ha to bare soil

Wheat, 2nd rotation	Forage		Hay		Straw		Grain	
	%TRR	mg/kg#	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
TFA (trifluoroacetic acid)	99.3	2.302	99.7	8.198	98.6	9.205	99.4	7.624
FOE-thiadone-glycoside	0.6	0.014	n.d.**	n.d.	0.5	0.051	n.d.	n.d.
Total identified	99.9	2.316	99.7	8.198	99.2	9.256	99.4	7.624
Procedural loss	-	-	-	-	-	-	0.3	0.020
Total extractable	**99.9**	**2.316**	**99.7**	**8.198**	**99.2**	**9.256**	**99.6**	**7.643**
Non-extractable (PES)*	0.1	0.002	0.3	0.028	0.8	0.078	0.4	0.029
Accountability	**100.0**	**2.318**	**100.0**	**8.225**	**100.0**	**9.335**	**100.0**	**7.673**

Turnip and Swiss chard, 2nd rotation	Turnip leaves		Turnip roots		Swiss chard		Swiss chard	
	mature		mature		intermediate		mature	
	%TRR	mg/kg#	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
TFA (trifluoroacetic acid)	99.9	3.534	97.0	0.191	100.0	1.951	99.8	2.946
FOE-thiadone-glycoside	n.d.**	n.d.	2.6	0.005	n.d.	n.d.	n.d.	n.d.
Total identified	**99.9**	**3.534**	**99.5**	**0.196**	**100.0**	**1.951**	**99.8**	**2.946**
Procedural loss	-	-	-	-	-	-	-	-
Total extractable	**99.9**	**3.534**	**99.5**	**0.196**	**100.0**	**1.951**	**99.8**	**2.946**
Non-extractable (PES) *	0.1	0.003	0.5	0.001	<0.1	0.001	0.2	0.005
Accountability	**100.0**	**3.536**	**100.0**	**0.197**	**100.0**	**1.951**	**100.0**	**2.950**

* PES = post extraction solids
** n.d. = not detected
# mg/kg means mg parent equivalents/kg

Table 6.6.1- 4: Composition of the radioactive residues in crops of the 3rd rotation after application of [thiadiazole-5-^{14}C]flufenacet at a use rate of 900 g as/ha to bare soil

Wheat, 3rd rotation	Forage		Hay		Straw		Grain	
	%TRR	mg/kg#	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
TFA (trifluoroacetic acid)	99.9	1.440	99.7	3.729	99.2	4.004	93.1	1.277
FOE-thiadone-glycoside	n.d.**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total identified	**99.9**	**1.440**	**99.7**	**3.729**	**99.2**	**4.004**	**93.1**	**1.277**
Procedural loss	-	-	-	-	-	-		
Total extractable	**99.9**	**1.440**	**99.7**	**3.729**	**99.2**	**4.004**	**93.1**	**1.277**
Non-extractable (PES) *	0.1	0.001	0.3	0.011	0.8	0.031	6.9	0.094
Accountability	**100.0**	**1.441**	**100.0**	**3.740**	**100.0**	**4.035**	**100.0**	**1.371**

Turnip and Swiss chard, 3rd rotation	Turnip leaves		Turnip roots		Swiss chard		Swiss chard	
	mature		mature		intermediate		mature	
	%TRR	mg/kg#	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
TFA (trifluoroacetic acid)	99.9	0.992	99.8	0.086	99.7	4.769	99.7	1.967
FOE-thiadone-glycoside	n.d.**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total identified	**99.9**	**0.992**	**99.8**	**0.086**	**99.7**	**4.769**	**99.7**	**1.967**
Procedural loss	-	-		-	0.3	0.013	0.3	0.005
Total extractable	**99.9**	**0.992**	**99.8**	**0.086**	**100.0**	**4.782**	**100.0**	**1.972**
Non-extractable (PES) *	0.1	0.001	0.2	<0.00	<0.1	0.002	<0.1	0.001
Accountability	**100.0**	**0.993**	**100.0**	**0.087**	**100.0**	**4.784**	**100.0**	**1.973**

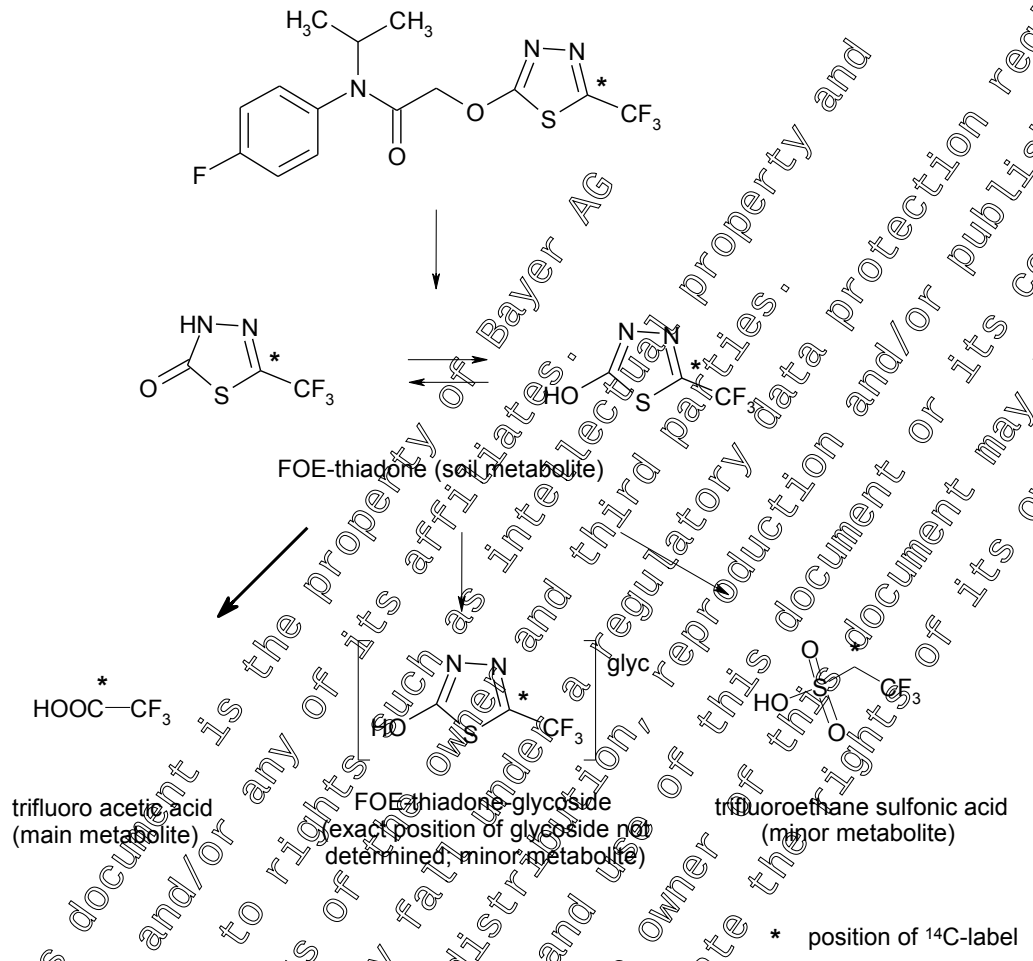
* PES = post extraction solids
** n.d. = not detected
# mg/kg means mg parent equivalents/kg

Table 6.6.1-5: Composition of the radioactive residues in soil after application of [thiadiazole-5-^{14}C]flufenacet at a use rate of 900 g as/ha

Soil (0 – 15 cm)	Day 30		Day 142		Day 317	
Days after application	30		142		317	
TRR (mg equ/kg)	0.638		0.232		0.104	
	%TRR	mg/kg#	%TRR	mg/kg	%TRR	mg/kg
Flufenacet	41.9	0.267	29.0	0.069	8.6	0.009
TFA (trifluoroacetic acid)	25.4	0.162	24.6	0.059	32.5	0.034
FOE-thiadone	4.7	0.030	n.d.	n.d.	n.d.	n.d.
Total identified	**72.0**	**0.459**	**53.7**	**0.128**	**41.1**	**0.043**
Procedural loss	4.1	0.026	3.8	0.009	n.d.	n.d.
Total extractable	**76.1**	**0.486**	**57.5**	**0.137**	**41.1**	**0.043**
Non-extractable (PES) *	23.9	0.152	42.5	0.102	58.9	0.061
Accountability	**100.0**	**0.638**	**100.0**	**0.239**	**100.0**	**0.104**

* PES = post extraction solids
** n.d. = not detected
# mg/kg means mg parent equivalents/kg

Figure 6.6.1- 1: Metabolic pathway of [thiadiazole-5-^{14}C]flufenacet in rotated crops after application to bare soil at a use rate of approximately 900 g as/ha



Summary of the levels of the major metabolite trifluoroacetate (TFA) in primary and rotated crops following application of [thiadiazole-5-^{14}C]flufenacet

TFA (M45) has frequently been found as the main metabolite in primary and rotated crops following application of flufenacet radiolabelled in the [thiadiazole-5-^{14}C]-position that enables the detection of TFA via radioassaying. TFA is addressed by a separate dietary risk assessment in document N4 and in a position paper (Flufenacet - Toxicological profile and exposure assessment of the plant metabolites, M-476535-01-1), using a compound specific toxicological endpoint. In the corresponding toxicity studies TFA-Na is the relevant compound which has been dosed. Therefore, the respective residue levels of TFA from the different plant metabolism studies described above are compiled in the Table 6.6.1-6. In this table TFA residues are still given as parent equivalents. Transformation of these TFA residues to the sodium salt TFA-Na via the molar ratio of TFA-Na (136.01 g/mol)/flufenacet (363.34 g/mol) = 0.3743 results in Table 6.6.1-7.

Table 6.6.1-6: Summary of TFA residues in primary and rotated crops resulting from of [thiadiazole-5-^{14}C]flufenacet (given in flufenacet equivalents)

Crop	Appl. Type	Actual Appl. Rate [g as/ha]	Commodity	TFA Residue [mg flufenacet equivalents/kg]		
Primary Crops						
Potato	Pre-emerg.	630	Tuber	0.801		
			Foliage	36.450		
Wheat	Post-emerg.	270	Grain	0.698		
			Straw	1.836		
			Hay	1.697		
			Forage	-		
Rotated Crops				PBI 30 days#	PBI 142 days	PBI 317 days
Wheat	Pre-plant.	903	Grain	2.899	7.624	1.277
			Straw	4.054	9.205	4.004
			Hay	3.404	8.198	3.729
			Forage	1.469	2.302	1.440
Turnip	Pre-plant.	903	Root	0.503	0.192	0.086
			Leaves	6.432	3.534	0.992
Swiss chard	Pre-plant.	903	Leaves, mat.*	3.162	2.946	1.967
			Leaves, int*	5.625	1.951	4.769

PBI: plant back interval, interval between application of a.s. to soil and sowing of rotated crop

* mat.: mature
int.: intermediate growth stage (50% of final leaf mass)

Table 6.6.1-7: Summary of TFA residues in primary and rotated crops resulting from of [thiadiazole-5-^{14}C]flufenacet (given in equivalents of TFA-Na)

Crop	Appl. Type	Actual Appl. Rate [g as/ha]	Commodity	TFA Residue [mg TFA-Na/kg]		
Primary Crops						
Potato	Pre-emerg.	630	Tuber	0.300		
			Foliage	13.644		
Wheat	Post-emerg.	270	Grain	0.261		
			Straw	0.687		
			Hay	0.635		
			Forage	-		
Rotated Crops				PBI 30 days#	PBI 142 days	PBI 313 days
Wheat	Pre-plant.	903	Grain	1.085	2.854	0.478
			Straw	1.518	3.446	1.499
			Hay	1.274	3.069	1.396
			Forage	0.550	0.862	0.539
Turnip	Pre-plant.	903	Root	0.188	0.072	0.032
			Leaves	2.408	1.323	0.371
Swiss chard	Pre-plant.	903	Leaves, mat.*	1.184	1.103	0.736
			Leaves, int*	2.106	0.730	1.785

PBI: plant back interval, interval between application of a.s. to soil and sowing of rotated crop

* mat.: mature

int.: intermediate growth stage (50% of final leaf mass)

In the context of TFA findings in primary and rotated crops following application of flufenacet it is kindly recommended to refer to the previous note in this dossier (chapter 6.2.1) with an explanation that TFA is formed as trifluoroacetate salt, but denoted as trifluoroacetic acid. This note is provided under the title:

"Remark about formation of trifluoroacetate TFA under environmental and physiological conditions"

CA 6.6.2 Magnitude of residues in rotational crops

According to the evaluation in the Monograph and by EFSA, in principle, no field rotational crop trials with flufenacet are deemed necessary to support the representative uses of flufenacet in cereals. However, field rotational crop studies were conducted at four different locations in northern Europe (northern France, Germany and the United Kingdom) on request of UK CRD to investigate the residues in treated winter cereals which are sown after the preceding crop potatoes which also received an application of a flufenacet containing product within the same calendar year. The potato crop can

be considered as a representative for any possible spring crop that might be grown as a preceding crop to winter cereals. The highest registered application rates for any spring crop is 600 g as/ha.
This study has already been evaluated by UK CRD in support of flufenacet containing products to be used in cereals.

Report:	**KIIIA 6.6.2/04, [redacted]; [redacted]; 2008; M-306269-01**
Title:	Determination of the residues of FOE 5043 in/on the rotational crops cereals after spraying of Artist (41.5 WG) and Liberator (500 SC) in the field in the United Kingdom, Germany and Northern France
Document No: Study no.	M-306269-01-1 Study No. RA-2020/06 dated 2008-08-22
Guidelines:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, EC guidance working document 7029/VI/95 rev. 5 (1997-07-22) EC guidance document on rotational crop studies 7524/VI/95 rev 2 (1997-07-22)
GLP	Yes; Deviations: none

The purpose of this study was to determine the magnitude of flufenacet residues in cereals (winter wheat and winter barley) grown as rotational crops following the preceding crop potato. Potatoes and cereals were both treated with one spray application with a flufenacet containing product within the same calendar year. The study objective was to investigate whether treatment of the preceding crop with a flufenacet containing product has an impact on the residue levels determined in cereals grown as the following crop. The application rates for flufenacet correspond to the maximum registered rates for a spring crop (potatoes, maize) and cereals. The trials were performed in northern Europe (the United Kingdom, Germany and Northern France).

Material and methods

This study comprises four supervised residue trials with potatoes followed by cereals (2 trials on barley and wheat, each). All plots received the application of 'Flufenacet + Metribuzin 41.5 WG' to potato plants pre emergence with an application rate of 2.5 kg/ha of test item, corresponding to 600 g flufenacet /ha (and 440 g metribuzin/ha). The water rate was 300 L/ha. After harvesting potatoes, the aerial parts of the plants were incorporated into soil in order not to remove potential residues from the plot. Cereals were sown 133 - 158 days after application on potatoes. The application of 'Flufenacet + Diflufenican 500 SC' on cereals (wheat or barley) was performed between growth stages BBCH 12-22 but not later than November. The application rate was 0.6 L/ha of test item, corresponding to 240 g flufenacet /ha (and 60 g diflufenican /ha). The water rate was also 300 L/ha.
For residue analysis, samples were taken from the treated and the control plots. Only the rotational crops (cereals) were sampled for analysis and the samples were analysed only for flufenacet. Samples were collected at growth stage BBCH 30 (green material) and at harvest (BBCH 89, grain and straw)

The residues of flufenacet in/on the collected samples were determined according to the method 00346 which yields the combined level of the parent compound and all its metabolites containing the N-fluorophenyl-N-isopropyl functional group. Residues are expressed as parent flufenacet. For grain, supplement E004 (Rzepka, S.; 2006; M-277805-01) was applied which provides a lower LOQ for grain than the basic method. The method was modified for the clean-up of grain samples since SPE clean-up was not necessary.

The Limit of Quantification (LOQ) was 0.01 mg/kg for grain, 0.05 mg/kg for green material and 0.1 mg/kg for straw.

Findings

Recovery rates were determined prior to analysing the samples in order to validate the method and concurrently with the sample analysis in order to check the accuracy of the residue analysis. Fortification was performed by spiking control samples with one of the following compounds or a mixture thereof : parent flufenacet, flufenacet oxalate, flufenacet sulfonic acid, flufenacet thioglycolate sulfoxide. The recovery-rates and corresponding relative standard deviations (RSD) were satisfactory, as shown in Table 6.6.2-1 for pre-validation recoveries and Table 6.6.2-2 for concurrent recoveries.

Before the analyses, samples were stored deep frozen for a maximum storage period of 12 months (371 days). The storage period is covered by the storage stability studies conducted with flufenacet.

No flufenacet residues were found in any of the untreated samples. Table 6.6.2-3 compiles the residue levels found in samples of treated cereals sown after a normal re-planting interval following potatoes which were also treated with a flufenacet containing product. The total residue of flufenacet was found to be less than the limit of quantification in green material (< 0.05 mg/kg), grain (< 0.01 mg/kg) and straw (< 0.1 mg/kg) in all treated samples.

Table 6.6.2- 1: Pre validation data for flufenacet and its metabolites on wheat grain

Analyte	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
Flufenacet (FOE 5043)	0.01	107; 102; 99; 90; 70	94	16	0.01
FOE 5043 Oxalate Hydrate	0.01	70; 90; 78; 61	75	16	0.01
FOE 5043 Sulfonic Acid Sodium Salt	0.01	71; 67; 64; 74	69	6	0.01
FOE 5043 Thioglycolate Sulfoxide	0.01	70; 78; 71; 74	73	5	0.01

FL = Fortification Level RSD = Relative Standard Deviation LOQ = Practical Limit of Quantification
Residues were determined as FOE 5043 trifluoroacetamide and expressed as flufenacet (FOE 5043) equivalents

Table 6.6.2-2. Recovery data for flufenacet
The LOQ is marked in bold

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%) Individual recoveries	Min	Max	Mean	RSD
RA-2020/06 R 2006 0420/3 0420-06/01	Barley, winter Wheat, winter (R1)	green material	Total residue flufenacet	3	**0.050**	107; 93; 82	82	107	94	13.3
				3	overall mg/kg		82	107	94	13.3
R 2006 0418/1 0418-06/01		straw	Total residue flufenacet	2	**0.10**	113; 113	113	113	113	
				2	1.00	101; 87	87	101	94	
R 2006 0003/8 0003-06/01				4	overall mg/kg		87	113	104	12.0
		grain	Total residue flufenacet	2	**0.010**	87; 91	87	91	89	
R 2006 0046/1 0046-06/01				2	0.40	84; 81	81	84	83	
				4	overall mg/kg		81	91	86	5.0
GLP: yes 2006										

FL = Fortification Level RSD = Relative Standard Deviation LOQ = Practical Limit of Quantification
Residues were determined as FOE 5043 trifluoroacetamide and expressed as flufenacet (FOE 5043) equivalents
FOE 5043 Mix : ¼ of FOE 5043, ¼ FOE 5043 Oxalate Hydrate, ¼ of FOE 5043 Sulfonic Acid Sodium Salt, ¼ of FOE 5043 Thioglycolate Sulfoxide

Table 6.6.2-3: Residues of flufenacet in wheat and barley after post-emergence application of 240 g flufenacet/ha succeeding potatoes (treated with 600 g flufenacet/ha)

Study Trial No. Trial SubID GLP Year	Crop Variety	Country	Application FL	No	kg/ha (a.s.) FFA	kg/hL (a.s.) FFA	GS	Residues Portion analysed	DALT (days)	total residue flufenacet * (mg/kg)
RA-2020/06 R 2006 0418 1 0418-06 GLP yes 2006	Potato Cilena	Germany [redacted]	41.5 WG	1	0.6	0.2	00	--	--	--
	Barley, winter Franzis-ka	Europe, North	500 SC	1	0.24	0.08	13	green material grain straw	164 255 255	<0.05 <0.01 <0.1
RA-2020/06 R 2006 0420 3 0420-06 GLP yes 2006	Potato Pomme Fine	France [redacted]	41.5 WG	1	0.6	0.2	00	--	--	--
	Barley, winter Colibri	Europe, North	500 SC	1	0.24	0.08	12	green material grain straw	97 218 218	<0.05 <0.01 <0.1
RA-2020/06 R 2006 0003 8 0003-06 GLP yes 2006	Potato Maris Peer	United Kingdom [redacted]	41.5 WG	1	0.6	0.2	00	--	--	--
	Wheat, winter Consort	Europe, North	500 SC	1	0.24	0.08	13	green material grain straw	179 294 294	<0.05 <0.01 <0.10

RA-2020/06 R 2006 0046 1 0046-06 GLP yes 2006	Potato Cilena	Germany [redacted]	41.5 WG	1	0.6	0.2	00	--	--	--
	Wheat, winter Limes	Europe, North	500 SC	1	0.24	0.08	21	green material grain straw	147 277 277	0.05 <0.01 <0.1

*Residues for total residue flufenacet determined as FOE 5043 Trifluoro acetamide and calculated as flufenacet
DALT : Days after last treatment
FFA Flufenacet

Conclusion

Four field residue trials were conducted in northern Europe (the United Kingdom, Germany and France) in order to determine the magnitude of flufenacet derived residues in/on cereals (winter wheat and winter barley) grown as suceeding crops following the preceding crop potatoes. Potatoes and cereals were both treated with one spray application of a flufenacet containing product (at the maximum rates of 600 g as/ha for potatoes and 240 g as/ha for cereals). No residues were apparent in green material of cereals collected at growth stage BBCH 29 – 30 or grain and straw sampled at harvest (BBCH 89). The findings show that treatment of the preceding crop with a flufenacet containing product at the maximum field rate does not impact residue levels in/on cereals grown as succeeding crops. No uptake from the soil into the following crop has been observed. This scenario reflects a worst case rotation with regard to potential uptake from soil. Shorter plant back intervals (e.g. 30 days) were not investigated since the time for sowing spring cereals has already passed in case of failure of other spring crops (i.e. potatoes, maize) that may have received a treatment with a flufenacet. The absence of residues in cereals when sown as following crop is considered to be representative for all other rotational crop situations where the preceding crop is treated with application rates up to 600 g as/ha.
Flufenacet residues were found to be less than the limit of quantification of 0.01 mg/kg in grain, 0.05 mg/kg in green material and 0.1 mg/kg in straw.

CA 6.7 Proposed residue definitions and maximum residue levels

CA 6.7.1 Proposed residue definitions

Evaluation in the EU peer review process

Primary (corn, cotton and soyabean) and rotational crop metabolism of flufenacet was investigated using [fluorophenyl-UL-^{14}C] and [thiadiazole-2-^{14}C]flufenacet. The studies were evaluated in the Monograph. In all plant species flufenacet was rapidly and extensively metabolized so that no parent compound was detected even at early sampling dates. The metabolism of the fluorophenyl-isopropyl acetamide moiety of flufenacet results in a number of metabolites which all contain the N-fluorophenyl-N-isopropyl amine moiety. It was concluded that the metabolites containing the thiadiazole moiety are not relevant and should not be included in the residue definition.
From the available metabolism data FOE 5043 oxalate, FOE 5043 sulfonic acid and FOE 5043 thioglycolate sulfoxide were considered to be of quantitative relevance. A ‘total residue’ approach was established for risk assessment and monitoring including the sum of all compounds containing

this moiety. The same residue definition was established for animal matrices. Although flufenacet is extensively metabolized in all animal species investigated only the metabolites found in animal feed might be expected as residues in animal tissues, milk and eggs, as the parent compound itself has never been detected in any feed item.

The Review Report for flufenacet (7469/VI/98-Final – 3rd July 2003) does not contain information on the residue definition. The relevant information can be taken from the Complete List of Endpoints, Report of ECCO 73, Annex 2, 5 Residue Section. The following table summarises the endpoints used in the evaluation.

Matrices	Residue definition		Reference
Food of plant origin	Risk assessment Monitoring	Flufenacet including all metabolites containing the N-fluorophenyl-N-isopropyl moiety, expressed as flufenacet	Report of ECCO 73, Complete list of endpoints, Annex 2, 5 Residue section:
Food of animal origin	Risk assessment Monitoring	Flufenacet including all metabolites containing the N-fluorophenyl-N-isopropyl moiety, expressed as flufenacet	

Evaluation in EFSA Reasoned Opinion on existing MRLs (EFSA Journal 2012;10(4):2689)

In addition to the metabolism studies available at the time of Annex I inclusion studies on pre-emergence and foliar treatment on root vegetables and cereals (foliar treatment) using fluorophenyl-U-^{14}C labeled flufenacet were evaluated after the peer review was completed. The metabolic pattern after post-emergence treatment showed further metabolites at significant amounts also containing the common moiety (FOE sulfinyl lalactic acid glycoside, FOE cysteine and sulfanyl lactic acid glucoside).

The evaluation conducted by the RMS and EFSA, in principle, is in line with the evaluation in the Monograph. However, EFSA also mentioned that the 'common moiety residue definition' might not be the most adequate for enforcement purposes and therefore proposed to investigate the option to include six individual metabolites in a multi-residue method. It is concluded that new residue trials would not be needed as the current common moiety method includes all of these metabolites.

In presentations held at the 9th European Pesticide Residue Workshop in Vienna (Austria) on 27-June-2012 and at the 7th International Fresenius Conference (Düsseldorf, 16 May 2013) a representative of the EFSA Pesticide Unit outlined EFSA's role and view concerning the setting of enforcement residue definitions.

Since flufenacet is included in the presentation as a case study this reference is considered to provide relevant information. In the presentation on 'Potential and possible solutions for simplifying complex residue definitions' it is concluded that the marker concept would not be an appropriate solution for deriving a residue method for enforcement of flufenacet residues. Instead of a marker concept it is concluded that the common moiety approach would be more appropriate in this case and need to be maintained.

The applicant's position concerning the residue definition for enforcement in plants is also addressed in chapters CA 6.2.1 and CA 4.2.
Please refer also to the Bayer CropScience position paper ([redacted]; 2013; M-457898-01) and the EFSA presentation at 7th Fresenius Conference ([redacted]; 2013; M-459903-01-1) reported in chapter CA 4.2.

The applicant concludes that the established residue definitions are still adequate and shall be maintained.

CA 6.7.2 Proposed MRLs and justification of the acceptability of the levels proposed

Established EU MRLs
The EU MRLs for flufenacet in all types of small grain cereals (wheat, rye, triticale, barley, oats) were set at the limit of quantification of 0.05 mg/kg in Annex II of Commission Regulation No 149/2008 of 29 January 2008 amending Regulation (EC) 396/2005. Initially flufenacet MRLs were set with Commission Directive 2005/48/EC of 23 August 2005 amending Council Directives 86/362/EEC, 86/363/EEC and 90/642/EEC.
MRLs were supported by 17 field trials on wheat, rye and barley for the northern European region which were submitted in the Annex II dossier and evaluated in the EU peer review process (study nos. RA-2008/94 and RA-2054/93).
Based on the conclusions in the EU peer review process MRLs were not considerd necessary for commodities of animal origin and thus were not established.

EFSA Reasoned Opinion on the review of existing MRLs according to Article 12 of Regulation (EC) No 396/2005 (EFSA Journal 2012;10(4):2689
During the review of existing MRLs additional and more recent data were reviewed by the RMS (France) and provided to EFSA with the Resticde Residues Overview File (PROFile). These data concern also uses in cereals (wheat, barley) in the southern European region. All residue data supplementary to those evaluated in the EU review process are reported in chapter 6.3.1
All MRLs on cereals (wheat, barley, rye and oats) were 'recommended' in the EFSA reasoned opinion and thus were considered to be sufficiently supported by data.

The residue data referred to in the EFSA evaluation are summarized in the table below.

Table 6.7.2-1: Overview of the residue trials data relevant for MRL setting as evaluated by EFSA (EFSA Journal 2012;10(4):2689

Commo-dity	Residue region [a]	Individual trial results for enforcement and risk assessment (mg/kg)	Median residue (mg/kg)	Highest residue (mg/kg)	MRL proposal (mg/kg)	Median CF [d]	Comments
Wheat grain, Barley grain	NEU	24** x <0.05	0.05	0.05	0.05*	1	Combined dataset on barley (8), rye (3) and wheat (13) supporting the GAP for all small grain cereals
	SEU	Barley: 3 x <0.01; <0.05 Wheat: 2 x <0.01; 0.01; <0.05; 0.05	0.01	0.05	0.1	1	Combined dataset on barley (4) and wheat (5)
Oats grain, rye grain	NEU	24** x 0.05	0.05 [b]	0.05	0.05*	1	Extrapolation from northern GAPs on barley and wheat is possible
Barley straw; wheat straw	NEU	<0.01; 0.011; 18**x <0.1 [c]	0.1	0.1	0.1	1	Combined dataset on barley (8), rye (3) and wheat (9) [c]
	SEU	Barley: <0.05; 2x 0.06; 0.10 Wheat: 3x <0.05; 0.09; < 0.10	0.06	0.11	0.2	1	Combined dataset on barley (4) and wheat (5)
Oats straw, rye straw	NEU	<0.01; 0.011; 18** x <0.1 [c]	0.1	0.1	0.1	1	Combined dataset on barley (8), rye (3) and wheat (9) [c]

* indicates the MRL is set at the LOQ

** one trial was erroneously harvested before sampling of grain and straw

[a] NEU = northern Europe, SEU = southern Europe

[b] in EFSA Table 0.01 mg/kg

[c] according to applicant's information 23 x < 0.1 mg/kg for the critical GAP of 240 g as/ha (combined dataset on barley (8), rye (3) and wheat (12)) corresponding to the data set for wheat and barley grain in northern Europe

[d] conversion factor from for enforcement to risk assessment residue definition

In the following tables MRL calculations for cereal grain are performed for the critical GAP (southern Europe) using the EU methodologies and the OECD MRL calculator.

For both methodologies used the proposed MRL results in 0.1 mg/kg and is in line with the proposal in the EFSA document.

No calculations are performed for the critical GAP from the northern region since residues in cereal grain were less than the LOQ (0.05 mg/kg) in all trials.

Table 6.7.2-2: Calculation of MRL proposal for flufenacet according to EU guideline 7039/VI/95 of 22 July 1997 based on the data set from southern Europe

No.	Crop	Days after application	Residue value (mg/kg)	Plot No. [1]/ Study No.	No. of applic.	FL-Type	Product	Country
1	Wheat, winter	153	<0.01	09-2052-02 / 09-2052MAN	1	SC 600	Flufenacet & Diflufenican SC 600	France
2	Wheat, winter	220	<0.01	09-2052-04 / 09-2052MAN	1	SC 600	Flufenacet & Diflufenican SC 600	France
3	Barley, winter	148	<0.05	0570-00 / RA-2144/00	1	SC 600	Flufenacet & Diflufenican SC 600	Spain
4	Barley, winter	203	<0.01	09-2048-03 / 09-2048MAN	1	SC 600	Flufenacet & Diflufenican SC 600	France
5	Wheat, winter	196	0.05	09-2052-03 / 09-2052MAN	1	SC 600	Flufenacet & Diflufenican SC 600	France
6	Wheat, winter	202	0.01	09-2052-01 / 09-2052MAN	1	SC 600	Flufenacet & Diflufenican SC 600	France
7	Barley, winter	197	<0.01	09-2048-01 / 09-2048MAN	1	SC 600	Flufenacet & Diflufenican SC 600	France
8	Wheat, winter	196	<0.05	0567-00 / RA-2144/00	1	SC 600	Flufenacet & Diflufenican SC 600	France
9	Barley, winter	188	<0.01	09-2048-02 / 09-2048MAN	1	SC 600	Flufenacet & Diflufenican SC 600	France

[1] as given in the Tier 1 summaries

Results (Wheat, winter;Barley, winter)

Method I (Weimmann/Nolting) (all values)	R	0.023
	s	0.020
	k	3.032
	Rmax=R+k*s	**0.084**
Method II (Wilkening) (75 % quantile)	R (0.75)	0.050
	Rber=2*R(0.75)	**0.100**

STMR: <0.01;<0.01;<0.01;<0.01;**<0.01**;0.01;<0.05;<0.05;0.05

Table 6.7.2-3: Calculation of MRL proposal for flufenacet according to OECD Calculator based on the data set from southern Europe

No.	Crop	Days after application	Residue value (mg/kg)	Plot No. [1]/ Study No.	No. of applic.	FL-Type	Product	Country
1	Wheat, winter	153	<0.01	09-2052-02 / 09-2052MAN	1	SC 600	Flufenacet & Diflufenican SC 600	France
2	Wheat, winter	220	<0.01	09-2052-04 / 09-2052MAN	1	SC 600	Flufenacet & Diflufenican SC 600	France
3	Barley, winter	148	<0.05	0570-00 / RA-2144/00	1	SC 600	Flufenacet & Diflufenican SC 600	Spain
4	Barley, winter	203	<0.01	09-2048-03 / 09-2048MAN	1	SC 600	Flufenacet & Diflufenican SC 600	France
5	Wheat, winter	196	0.05	09-2052-03 / 09-2052MAN	1	SC 600	Flufenacet & Diflufenican SC 600	France
6	Wheat, winter	202	0.01	09-2052-01 / 09-2052MAN	1	SC 600	Flufenacet & Diflufenican SC 600	France
7	Barley, winter	197	<0.01	09-2048-01 / 09-2048MAN	1	SC 600	Flufenacet & Diflufenican SC 600	France
8	Wheat, winter	196	<0.05	0567-00 / RA-2144/00	1	SC 600	Flufenacet & Diflufenican SC 600	France
9	Barley, winter	188	<0.01	09-2048-02 / 09-2048MAN	1	SC 600	Flufenacet & Diflufenican SC 600	France

[1] as given in the Tier 1 summaries

Results (Wheat, winter; Barley, winter)

Total number of data (n)	9	Standard deviation (SD)	0.020
Lowest residue	0.01	Percentage of censored data	78
Highest residue	0.05	Number of non-censored data	2
Median residue	0.000	Correction factor for censoring (CF)	0.481
Mean	0.023		

Proposed MRL estimate

Highest residue	0.05
Mean + 4 SD	0.103
CF x 3 mean	0.034
Unrounded MRL	0.103
Rounded MRL	0.1

MRLs in products of animal origin

No MRLs are currently set for products of animal origin in Regulation (EC) 396/2005.

As outlined above in the chapters on animal metabolism and livestock feeding studies residues in animal matrices, milk and eggs are unlikely to occur. The representative uses supported within the present dossier correspond to the frame which was evaluated by EFSA when reviewing the MRLs. The calculation of the dietary burden based on the OECD feeding tables (cf. chapter 6.4) does not result in a more unfavourable situation. Thus, the EFSA conclusion to set the MRLs at the LOQ for the individual matrices is still considered to be appropriate for the representative uses of flufenacet and MRLs do not need to be modified.

Table 8.7.2-4: Existing and anticipated EU MRLs for flufenacet

Crop/animal commodities	Existing EU MRL (mg/kg) Regulation (EC) No. 149/2008, (Annex II)	EU MRL proposed by EFSA (mg/kg) (EFSA Journal 2012; 10(4):2689)
Wheat, Barley	0.05*	0.1
Rye, Oats [a]	0.05*	0.05*
Products of animal origin		Meat: 0.05* Fat: 0.05* Liver: 0.02* Kidney (excl. poultry): 0.05* milk: 0.01* Eggs: 0.05*

* indicates that the MRL is set at the LOQ

[a] Uses in rye and oats were only reported for the northern region and thus included in EFSA's evaluation in the framework of the MRL review according to Art. 12 of (EC) 396/2005. Thus, MRLs for rye and oats were derived from the northern European data set by means of extrapolation from wheat and barley.

CA 6.7.3 Proposed MRLs and justification of the acceptability of the levels proposed for imported products (import tolerance)

There are no relevant import tolerances established at EU level; and no CXLs are set.

CA 6.8 Proposed safety intervals

Proposed pre-harvest intervals for envisaged uses, or withholding periods and justification

The intervals and waiting periods proposed all pertain to the herein supported representative uses, namely pre- to early post-emergence applications in cereals (wheat, rye and barley) at the maximum rate of 240 g as/ha.

Pre-harvest interval for each relevant crop

Setting a pre-harvest interval (PHI) is not needed since according to the representative uses flufenacet is applied to cereals (wheat, rye and barley) either in autumn or very early in the growing season (maximum at BBCH 22). The PHI is covered by the vegetation period from application until the crop is mature for harvest.

Re-entry period for livestock to areas to be grazed

Cereals (barley, oats, rye, triticale and wheat) are normally not grazed by livestock. It is, therefore, not necessary to define a re-entry period for livestock after use of flufenacet in cereals.

Re-entry period for man to crops, buildings or spaces treated

Flufenacet is used in cereals at early growth stages, when there is no need to enter the crop shortly after spraying. It is therefore not necessary to define particular re-entry times for workers. As a general rule, however, treated fields should not be re-entered until the spray deposit is completely dry.

Withholding period for animal feedingstuffs

According to EU guidance document 7031/VI/95-rev.4 the cereal commodities fed to livestock consist of grain and straw harvested at normal maturity. According to the OECD guidance document on residues in livestock (no. 73, dated 04 Sep 2013) relevant feeding items are grain, straw and cereal forages and silage. The highest levels of flufenacet residues likely to be present in these commodities were taken into account, as appropriate, to evaluate the dietary burden of livestock (refer to point 6.4) and when considering the need for MRLs in food of animal origin (refer to point 6.7.2). It is not necessary to define a withholding period for animal feeding stuff.

Waiting period between last application and sowing or planting the crops to be protected

Flufenacet is always applied after sowing the cereals to be protected (pre-or early post-emergence). Therefore, there is no need to define a waiting period between application and sowing the crops to be protected.

Waiting period between last application and handling treated products

Handling of treated cereals is generally not required before harvest and the supported representative uses result in low residues in mature grain and straw. Furthermore, harvest of cereals is always done mechanically. Thus, there is no need to define a waiting period between application of flufenacet to cereals and handling treated products.

Waiting period between last application and sowing or planting succeeding crops

As demonstrated in the confined crop rotational studies (cf. chapter 6.6.1) and field rotational crop trials (cf. chapter 6.6.2), the uptake of flufenacet residues from treated soil is low. The use of flufenacet in cereals is not likely to result in significant residues of flufenacet in succeeding crops. Therefore, it is not necessary to set a waiting period before sowing or planting succeeding crops for the purpose of limiting the residue levels in these crops.
Waiting periods that may be required to avoid phytotoxicity to succeeding crops are dealt with in the efficacy summaries of the formulated product.

CA 6.9 Estimation of the potential and actual exposure through diet and other sources

Evaluation in the EU peer review process

The toxicological reference values (ADI, ARfD) as published in the Review Report (7469/VI/98-Final – 3rd July 2003) are summarized in the table below.

Table 6.9- 1: Toxicological endpoints for flufenacet

Endpoint	Value (mg/kg bw/day)	Study	Safety factor	Reference
Acceptable Daily Intake (ADI)	0.005	2 year rat study (LOEL)	250	Review Report (7469/VI/98-Final – 3rd July 2003)
Acute Reference Dose (ARfD)	0.017	90 day, 1 year dog study	100	

The review has established that the residues arising from the proposed uses (cereals, maize, soybean and sunflower), following application consistent with good plant protection practice, have no harmful effects on human or animal health.

Acceptable Daily Intake (ADI) and Dietary Exposure Calculation

TMDI calculation

The Theoretical Maximum Daily Intake (TMDI) was calculated using the EFSA PRIMo rev. 2 and compared with the toxicological reference value. The calculation of the chronic exposure is based on the mean consumption data representative for 22 national diets collected from MS surveys plus 1 regional and 4 cluster diets from the WHO GEMS Food database. Table 6.9-2 compiles the input data for the calculation: all MRLs as established in Regulation (EC) 396/2005 with the exception of wheat, barley and potatoes where the new MRLs were used as proposed in the EFSA reasoned opinion (EFSA Journal 2012;10(4):2689). Also, for commodities of animal origin for which MRLs were not established in Regulation (EC) 396/2005, the proposed MRLs as included in the EFSA reasoned opinion were used.
Table 6.9-3 summarises the results of the TMDI calculation. The total calculated intake values accounted up to 59.4 % of the ADI (NL child). The PRIMo output template is included in the appendix (Table 1).

Table 6.9-2: TMDI calculation: Input values for the chronic consumer risk assessment

Commodity	Input value (mg/kg)	Comment
Commodities of plant origin		
Potatoes	0.15	Proposed MRL (EFSA , 2012)
Wheat	0.1	
Barley	0.1	
Other commodities of plant origin	0.05*	MRL; Regulation (EC) 396/2005
Commodities of animal origin		
Meat (swine, cattle, sheep, goat, poultry)	0.05*	Proposed MRL (EFSA, 2012)
Fat (swine, cattle, sheep, goat, poultry)	0.05*	
Liver (swine, cattle, sheep, goat, poultry)	0.02*	
Kidney (swine, cattle, sheep, goat)	0.05*	
Milk (cattle, sheep, goat)	0.01*	
Birds' eggs	0.05*	

*indicates that MRL is set at the LOQ of the analytical method

Table 6.9-3: TMDI calculation for flufenacet according to the EFSA PRIMo model (rev.2.0)

TMDI (% of ADI)	MS Diet	Highest contributor to MS diet	
		% of ADI	Commodity
59.4	NL child	17.7	Potatoes
57.4	WHO Cluster diet B	17.1	Wheat
55.4	UK Toddler	22.9	Sugar plants
51.0	DE child	23.0	Fruit (fresh or frozen)
44.9	FR toddler	15.2	Potatoes
43.1	FR infant	16.2	Fruit (fresh or frozen)
41.9	IE adult	10.7	Fruit (fresh or frozen)
39.8	WHO cluster diet E	11.8	Potatoes
39.2	WHO cluster diet D	13.0	Wheat
39.1	UK Infant	10.1	Sugar plants
38.2	PT General population	16.0	Potatoes
34.8	SE general population 90th percentile	12.5	Potatoes
34.1	DK child	11.0	Wheat
34.0	WHO regional European diet	12.0	Potatoes
33.3	ES child	8.9	Wheat
33.1	WHO Cluster diet F	10.2	Potatoes
25.4	NL general	8.2	Potatoes
24.9	IT kids/toddler	13.3	Wheat
21.8	FR all population	6.6	Wheat
21.0	UK vegetarian	4.1	Potatoes
20.7	ES adult	4.7	Wheat
20.5	LT adult	9.5	Potatoes
18.4	UK Adult	4.2	Potatoes

TMDI (% of ADI)	MS Diet	Highest contributor to MS diet	
		% of ADI	Commodity
17.5	IT adult	8.3	Wheat
16.6	PL general population	10.3	Potatoes
15.7	DK adult	4.4	Potatoes
11.1	FI adult	3.7	Potatoes

Evaluation in EFSA Reasoned Opinion on existing MRLs (EFSA Journal, 2012;10(4):2689)
Chronic consumer exposure resulting from all the authorised uses reported in the framework of the MRL review was calculated using revision 2 of the EFSA PRIMo and compared with the toxicological reference value derived for flufenacet.

The input values used by EFSA for the exposure calculations are summarised in Table 6.9-4 (corresponds to Table 4-1 of the RO).
The median residue values selected for chronic intake calculations are based on the residue levels in the raw agricultural commodities reported in section 3 of the Reasoned Opinion reflecting all crops where authorized uses are granted. The contributions of other commodities, for which no GAP was reported in the framework of this review, were not included in the calculation, *i.e.* no default values have been considered for the calculation. Detailed results of the calculations using the input values of Table 6.9-4 are presented in Table 6.9-5. The calculations reflect those presented in Appendix B of the EFSA Reasoned Opinion. The PRIMo output template is included in the appendix (Table 2).

No long-term consumer intake concerns were identified for any of the European diets incorporated in the EFSA PRIMo. The total calculated intake values accounted up to 24.7 % of the ADI (WHO cluster diet B).

It can be concluded that the existing uses of flufenacet do not result in a consumer exposure exceeding the toxicological reference value and therefore flufenacet is unlikely to pose a consumer health risk.

The applicant intends to limit the uses to cereals, maize, and potato in the future. A modified calculation is also presented below taking into account only the limited number of crops and in addition commodities of animal origin (cf. Table 6.9-6).
For commodities of animal origin, MRL values (set at the LOQ of the analytical method) are used as input data. The limitation of uses thus results in a slightly lower usage of the ADI (21.2%, NL child). The PRIMo output template is included in the appendix (Table 3).

Table 6.9-4: Input values for the chronic consumer risk assessment (corresponding to EFSA Reasoned Opinion), (EFSA Journal, 2012;10(4):2689)

Commodity	Input value (mg/kg)	Comment
Strawberries	0.05	Median residue (tentative) (a)
Blueberries	0.05	Median residue (tentative) (a)
Cranberries	0.05	Median residue (tentative) (a)
Currants (red, black and white)	0.05	Median residue (tentative) (a)
Gooseberries	0.05	Median residue (tentative) (a)
Potatoes	**0.05**	**Median residue (b)**
Celeriac	0.02	Median residue (b)
Onions	0.02	Median residue (b)
Tomatoes	0.05	Median residue (b)
Cucumbers	0.05	Median residue (b)
Courgettes	0.05	Median residue (b)
Pumpkins	0.05	Median residue (b)
Sweetcorn	0.05	Median residue (b)
Lettuce	0.01	Median residue (b)
Scarole (broad-leaf endive)	0.01	Median residue (b)
Beans (with pods)	0.05	Median residue (b)
Asparagus	0.05	Median residue (b)
Leek	0.01	Median residue (b)
Sunflower seed	0.05	Median residue (b)
Soya bean	0.05	Median residue (b)
Barley	**0.05**	**Median residue (b)**
Maize	**0.05**	**Median residue (b)**
Rice	0.05	Median residue (b)
Oats	**0.05**	**Median residue (b)**
Rye	**0.05**	**Median residue (b)**
Wheat	**0.05**	**Median residue (b)**
Meat (swine, cattle, sheep, goat, poultry)	0.05	Median residue (=LOQ) (c)
Fat (swine, cattle, sheep, goat, poultry)	0.05	Median residue (=LOQ) (c)
Liver (swine, cattle, sheep, goat, poultry)	0.02	Median residue (=LOQ) (c)
Kidney (swine, cattle, sheep, goat)	0.05	Median residue (=LOQ) (c)
Milk (cattle, sheep, goat)	0.01	Median residue (=LOQ) (c)
Birds' eggs	0.05	Median residue (=LOQ) (c)

(a): Use reported by the RMS is not fully supported by data but the risk assessment values derived in section 3 of the RO are used for indicative exposure calculations. The data gap was related to missing method validation data for matrices of high acid content. However, the data are available but were not considered for the evaluation.
(b): At least one relevant GAP reported by the RMS is fully supported by data for this commodity; the risk assessment values derived in section 3 of the RO are used for the exposure calculations.
(c): Dietary burden relevant to this commodity of animal origin, resulting from the GAPs reported by the RMS, is fully supported by data, the risk assessment values derived in section 3 of the RO are used for the exposure calculations.

Table 6.9-5: IEDI/NEDI calculation for flufenacet according to the EFSA PRIMo model (rev.2.0), (EFSA Journal, 2012;10(4):2689)

IEDI/NEDI (% of ADI)	MS Diet	Highest contributor to MS diet	
		% of ADI	Commodity
24.7	WHO Cluster diet B	8.5	Wheat
23.6	NL child	5.9	Potatoes
17.9	WHO cluster diet D	6.5	Wheat
16.3	DK child	5.5	Wheat
16.2	ES child	4.4	Wheat
16.1	WHO cluster diet E	3.9	Wheat
16.1	DE child	4.1	Wheat
15.1	WHO regional European diet	4.0	Potatoes
15.1	FR toddler	5.1	Potatoes
14.7	WHO Cluster diet F	3.6	Wheat
14.3	FR infant	5.1	Milk and milk products: Cattle
13.4	SE general population 90th percentile	4.2	Potatoes
12.9	IE adult	2.3	Maize
12.4	PT General population	5.3	Potatoes
10.3	NL general	2.7	Potatoes
10.3	UK Toddler	3.9	Wheat
10.0	UK Infant	3.3	Potatoes
9.8	IT kids/toddler	6.6	Wheat
9.6	LT adult	3.2	Potatoes
9.4	ES adult	2.3	Wheat
8.1	FR all population	3.3	Wheat
6.7	IT adult	4.1	Wheat
6.2	DK adult	2.0	Wheat
5.4	UK vegetarian	2.0	Wheat
4.7	PL general population	3.4	Potatoes
4.6	UK Adult	1.7	Wheat
4.4	FI adult	1.2	Potatoes

Table 6.9-6: IEDI/NEDI calculation for flufenacet according to the EFSA PRIMo model (rev.2.0)

(limited to cereals, potatoes and maize, including commodities of animal origin)

TMDI (% of ADI)	MS Diet	Highest contributor to MS diet	
		% of ADI	Commodity
21.2	NL child	5.,9	Potatoes
19.1	FR toddler	7.9	Milk *
18.2	WHO Cluster diet B	8.5	Wheat
16.3	UK Infant	7.7	Milk *
16.2	DK child	5.5	Wheat
14.6	WHO cluster diet D	6.5	Wheat
14.0	ES child	4.4	Wheat
13.5	WHO cluster diet E	3.9	Wheat
13.2	DE child	4.1	Wheat
12.7	WHO regional European diet	4.0	Potatoes
12.5	WHO Cluster diet F	3.6	Wheat
12.5	UK Toddler	4.1	Milk *
11.8	FR infant	5.1	Milk *
11.0	SE general population 90th percentile	4.2	Potatoes
10.9	IE adult	2.3	Maize
9.9	PT General population	5.3	Potatoes
9.0	NL general	3.7	Potatoes
8.3	LT adult	3.2	Potatoes
7.6	ES adult	2.3	Wheat
7.6	IT kids/toddler	6.6	Wheat
6.7	FR all population	3.3	Wheat
6.3	DK adult	2.0	Wheat
4.8	IT adult	4.1	Wheat
4.6	UK vegetarian	2.0	Wheat
4.4	FI adult	1.2	Potatoes
4.0	UK Adult	1.7	Wheat
3.4	PL general population	3.4	Potatoes

*Milk and cream, not concentrated, nor containing added sugar or sweetening matter, butter and other fats derived from milk, cheese and curd.

Acute Reference Dose (ARfD) and Dietary Exposure Calculation

In order to evaluate the potential acute exposure to flufenacet residues through the diet, the National Estimated Short Term Intakes (NESTI)/International Estimated Short Term Intakes (IESTI) are estimated using the EFSA PRIMo model (revision 2).

According to the Review Report (7469/VI/98-Final – 3rd July 2003), an ARfD of 0.017 mg/kg bw/d was established based on the 90d and 1year dog study.

In the EFSA Reasoned Opinion (2012) the acute consumer exposure was calculated for all types of cereals (wheat, rye, barley and oats) using the highest residue level found in cereal grain (0.05 mg/kg). This value corresponds to the currently established MRLs for these crops.
The input values for cereal grain and commodities of animal origin were considered to be adequately supported by data. As evident from the supplementary trials reported in section 6.3.1, the residue level used by EFSA remains the highest value over all available residue data and GAPs and is considered adequate to be used for the short-term risk assessment calculation.

The results of the acute exposure calculations are compiled in Table 6.9-7.

Taking into account the ARfD of 0.017 mg/kg, the highest NESTI was estimated at **7.3% of ARfD** for children due to consumption of milk and **2.3% of ARfD** for adults due to consumption of wheat. It is concluded that the herein supported uses in cereals do not result in unacceptable health risks to European consumers.

Table 6.9-7: NESTI calculation for flufenacet according to the EFSA PRIMo model (rev 2)

Commodity	Input value (mg/kg)	Maximum food intake reported (g/kg bw/d)	Percentile	MS diet	Body weight (kg)	IESTI 1 (mg/kg bw/d)	% ARfD
Children							
Barley	0.05	1.77	97.5	UK 4-6 yrs.	20.5	0.0001	0.5
Oats	0.05	3.98	97.5	DE	16.15	0.0002	1.2
Rye	0.05	6.32	97.5	UK Infant	8.7	0.0003	1.9
Wheat	0.05	14.45	97.5	UK 4-6 yrs	20.5	0.0007	4.2
Meat (bovine)	0.05	12.78	95	DE	16.15	0.0006	3.8
Fat (bovine)	0.05	2.07	97.5	UK Infant	8.70	0.0001	0.6
Liver (bovine)	0.02	8.07	97.5	UK Infant	8.70	0.0002	0.9
Kidney (bovine)	0.05	3.77	97.5	UK Toddler	14.50	0.0002	1.1
Milk (cattle)	0.01	124.22	97.5	UK Infant	8.70	0.0012	**7.3**
Eggs	0.05	12.41	97.5	UK Infant	8.7000	0.0006	3.7
Adults							
Barley	0.05	7.24	97.5	NL	63	0.0004	2.1
Oats	0.05	1.43	97.5	LT	70	0.0001	0.4
Rye	0.05	4.85	97.5	LT	70	0.0002	1.4
Wheat	0.05	7.82	97.5	UK vegetarian	66.7	0.0004	**2.3**
Meat (bovine)	0.05	5.95	97.5	NL	63.00	0.0003	1.8
Fat (bovine)	0.05	0.67	97.5	UK Adult	76.00	0.0000	0.2
Liver (bovine)	0.02	2.70	97.5	UK Adult	76.00	0.0001	0.3
Kidney (bovine)	0.05	1.70	97.5	UK Adult	76.00	0.0001	0.5
Milk (cattle)	0.01	17.24	97.5	NL	63.00	0.0002	1.0
Eggs	0.05	3.79	97.5	UK Vegetarian	66.70	0.0002	1.1

CA 6.10 Other studies

The toxicological profile and exposure assessment of metabolites in food of plant origin is addressed in a position paper also provided in section 5 (toxicological and metabolism studies).

Report:	**KCA 6.10/01** [redacted]; 2014; M-476535-01
Title:	Flufenacet - Toxicological profile and exposure assessment of the plant metabolites
Document No: Study no.	M-476535-01-1
Guidelines:	Not applicable (position paper)
GLP	Not applicable (position paper)

Flufenacet is both rapidly and extensively metabolised such that even at early sampling dates no parent compound is detected in plant commodities. A detailed comparison of plant and rat metabolism reveals that several plant metabolites were not detected as systemic metabolites in rat ADME studies.

For flufenacet (including its metabolites) a comprehensive toxicological database exists which was already evaluated during the peer review under Directive 91/414/EEC to a great extent. In the context of the application for renewal of approval of the active substance flufenacet according to Regulation (EC) 1107/2009 the toxicological data base has even been extented by several new toxicological studies.

The toxicological characterization of several plant metabolites containing either the fluorophenyl isopropyl amine moiety or the thiadone moiety shows that an additional toxicological impact from these compounds is not expected.
Due to structural similarity considerations, the absence of a genotoxicity potential, some further toxicity studies and supplementary information from metabolism studies with FOE sulfonic acid (M02), FOE oxalate (M01), thiadone-N-glucoside (M25) and trifluoroacetate (M45) the plant metabolites containing the fluorophenyl isopropyl amine moiety as well as the metabolites derived from the thiadone moiety are not expected to exert higher toxicity or additional hazards beyond those identified for flufenacet.

The metabolites derived from the fluorophenyl isopropyl acetamide moiety are included in the established residue definition by means of a common moiety approach. For two metabolites (FOE sulfonic acid and FOE oxalate) supplementary information is available from metabolism studies in rats and ruminants and feeding studies with FOE oxalate in cattle and poultry. These studies show their metabolic stability and low bio-availability. Thus, it deems to be justified to use the toxicological endpoints of the parent compound for the risk assessments.
From the long-term and short-term consumer exposure calculations for the metabolites containing the fluorophenyl isopropyl amine moiety it can be concluded that possible intakes do not present a consumer health concern.
The risk assessments performed for FOE oxalate and FOE sulfonic acid which may contribute through possible occurrence in food of plant origin and in drinking water demonstrate that the toxicological reference values are not exhausted also when combining both sources of exposure.

Thiadone-N-glucoside (M25) is a plant metabolite originating from the thiadiazole part of the parent compound. Thiadone-N-glucoside is a polar metabolite which is excreted in rats without undergoing further metabolisation or cleavage of glucose as evident from a supplementary goat metabolism study. The experiment showed low a bio-availability and the metabolic stability of thiadone-N-glucoside. However, free thiadone may be formed in ruminants after ingesting feeding items containing thiadone-N-glucoside. Taking into account the findings from the supplementary goat metabolism study with overdosed thiadone-N-glucoside the human dietary burden of free thiadone anticipated in food of animal origin is considered to be minimal.
Considering that thiadone is a major rat metabolite, the toxicological properties can be considered to be co-tested with the parent compound flufenacet.

Trifluoroacetate (TFA, M45) is a major plant metabolite observed in primary and rotational crops. Based on the toxicological information is deems justified to conduct the dietary risk assessment using a specific toxicological endpoint for this metabolite. From the risk assessment presented in the position paper considering food of plant and animal origin it can be concluded that exposure arising from uses of flufenacet does not result in a consumer health concern, also when taking into account possible contributions from drinking water as an additional source. The TFA concentrations used in the risk assessment are considered to be sufficiently conservative and, in practice, the actual intake is likely to be much lower than the calculated values. When applying several worst case assumptions the calculations indicate that the intended use of flufenacet containing products does not pose a risk to consumers as a result of exposure to TFA.

Some of the plant metabolites dealt with in this position paper are prone to reach groundwater at levels exceeding 0.1 µg/L. Their toxicological profiles and exposure assessments are addressed in documernt N4 following the stepwise approach required in SANCO 221/2000 rev 10 taking into account the routes of exposure through drinking water and food.

CA 6.10.1 Effect on the residue level in pollen and bee products

The objective of such studies would be to determine the residues in pollen and bee products for human consumption resulting from residues taken up by honeybees from crops at blossom.

No final test guideline is currently available which provides an agreed test methodology. Therefore it is the opinion of the applicant that it is not appropriate to address this issue until such guidance is available (cf. also: "Guidance Document for Applicants on Preparing Dossiers for the Approval of a Chemical New Active Substance and For the Renewal of Approval of a Chemical Active Substance According to Regulation (EU) No 283/2013 and Regulation (EU) No 284/2013").

Flufenacet is applied to cereals pre-emergence or at early stages of plant development during leaf development or tillering before blossom. Furthermore, residues are very low in all plant commodities investigated. Also, cereals are typically no feeding item for bees. Therefore, any studies to investigate residues in pollen and bee products as a result of flufenacet uses in cereals are not considered necessary.

Appendix

Table 1: Flufenacet EFSA PRIMo (2.0), TMDI calculation)

Flufenacet			
Status of the active substance:		Code no.	
LOQ (mg/kg bw):		proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	0,005	ARfD (mg/kg bw):	
Source of ADI:		Source of ARfD:	
Year of evaluation:		Year of evaluation:	

Chronic risk assessment

TMDI (range) in % of ADI minimum - maximum	
11	59
No of diets exceeding ADI:	---

Highest calculated TMDI values in % of ADI	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities
59,4	NL child	17,7	Potatoes	15,0	FRUIT (FRESH OR FROZEN)	9,6	Wheat
57,4	WHO Cluster diet B	17	Wheat	8,0	Potatoes	7,2	FRUIT (FRESH OR FROZEN)
55,4	UK Toddler	22,9	SUGAR PLANTS	10,5	Potatoes	7,8	Wheat
51,0	DE child	23,0	FRUIT (FRESH OR FROZEN)	8,2	Wheat	7,7	Potatoes
44,9	FR toddler	15,2	Potatoes	11,8	FRUIT (FRESH OR FROZEN)	5,5	Wheat
43,1	FR infant	15,2	FRUIT (FRESH OR FROZEN)	12,4	Potatoes	5,1	Milk and milk products: Cattle
41,9	IE adult	10,7	FRUIT (FRESH OR FROZEN)	6,9	Potatoes	4,6	Wheat
39,8	WHO cluster diet E	11,5	Potatoes	7,9	Wheat	5,8	FRUIT (FRESH OR FROZEN)
39,2	WHO cluster diet D	13,0	Wheat	12,2	Potatoes	2,8	FRUIT (FRESH OR FROZEN)
39,1	UK Infant	10,1	SUGAR PLANTS	9,8	Potatoes	5,5	FRUIT (FRESH OR FROZEN)
38,2	PT General population	16,0	Potatoes	7,8	Wheat	6,4	FRUIT (FRESH OR FROZEN)
34,8	SE general population 90th percentile	12,5	Potatoes	6,4	Wheat	5,8	FRUIT (FRESH OR FROZEN)
34,1	DK child	11,0	Wheat	7,3	Potatoes	5,1	FRUIT (FRESH OR FROZEN)
34,0	WHO regional European diet	12,0	Potatoes	5,9	Wheat	3,5	FRUIT (FRESH OR FROZEN)
33,3	ES child	8,9	Wheat	5,6	FRUIT (FRESH OR FROZEN)	5,5	Potatoes
33,1	WHO Cluster diet F	10,2	Potatoes	7,2	Wheat	3,8	FRUIT (FRESH OR FROZEN)
25,4	NL general	8,2	Potatoes	4,9	FRUIT (FRESH OR FROZEN)	4,1	Wheat
24,9	IT kids/toddler	12,3	Wheat	3,5	FRUIT (FRESH OR FROZEN)	2,7	Potatoes
21,8	FR all population	6,6	Wheat	6,2	FRUIT (FRESH OR FROZEN)	3,4	Potatoes
21,0	UK vegetarian	4,1	Potatoes	4,1	Wheat	3,8	SUGAR PLANTS
20,7	ES adult	4,7	Wheat	4,1	FRUIT (FRESH OR FROZEN)	2,8	Potatoes
20,5	LT adult	4,5	Potatoes	2,3	FRUIT (FRESH OR FROZEN)	2,1	Wheat
18,4	UK Adult	4,2	Potatoes	4,0	SUGAR PLANTS	3,4	Wheat
17,5	IT adult	8,3	Wheat	2,8	FRUIT (FRESH OR FROZEN)	1,8	Fruiting vegetables
16,6	PL general population	10,2	Potatoes	3,4	FRUIT (FRESH OR FROZEN)	1,1	Fruiting vegetables
15,7	DK adult	4,4	Potatoes	4,0	Wheat	3,3	FRUIT (FRESH OR FROZEN)
11,1	FI adult	3,7	Potatoes	2,5	FRUIT (FRESH OR FROZEN)	2,0	Wheat

Table 2: Flufenacet EFSA PRIMo (2.0), long-term consumer risk assessment (based on EFSA Reasoned Opinion; EFSA Journal, 2012;10(4):2689)

Flufenacet			
Status of the active substance:		Code no.	
LOQ (mg/kg bw):		proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	0,005	ARfD (mg/kg bw):	
Source of ADI:		Source of ARfD:	
Year of evaluation:		Year of evaluation:	

Chronic risk assessment

	TMDI (range) in % of ADI minimum - maximum
	4 25
No of diets exceeding ADI:	---

Highest calculated TMDI values in % of ADI	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities
24,7	WHO Cluster diet B	8,5	Wheat	8,1	Tomatoes	2,7	Potatoes
23,6	NL child	5,9	Potatoes	5,9	Milk and milk products: Cattle	1,7	Wheat
17,9	WHO cluster diet D	6,5	Wheat	4,1	Potatoes	1,0	Tomatoes
16,3	DK child	6,5	Wheat	4,4	Rye	2,4	Potatoes
16,2	ES child	4,4	Wheat	2,6	Milk and milk products: Cattle	1,8	Potatoes
16,1	WHO cluster diet E	3,9	Wheat	3,8	Potatoes	1,0	Poultry: Meat
16,1	DE child	4,1	Wheat	2,9	Milk and milk products: Cattle	2,6	Potatoes
15,1	WHO regional European diet	4,0	Potatoes	3,0	Wheat	1,3	Swine: Meat
15,1	FR toddler	5,1	Potatoes	2,6	Wheat	1,3	Bovine: Meat
14,7	WHO Cluster diet F	3,6	Wheat	3,4	Potatoes	1,2	Swine: Meat
14,3	FR infant	5,1	Milk and milk products: Cattle	4,1	Potatoes	0,8	Beans (with pods)
13,4	SE general population 90th percentile	4,2	Potatoes	3,2	Wheat	2,5	Milk and milk products: Cattle
12,9	IE adult	2,3	Maize	2,3	Maize	2,3	Potatoes
12,4	PT General population	5,5	Potatoes	3,9	Wheat	0,9	Tomatoes
10,3	NL general	4,7	Potatoes	2,1	Wheat	1,3	Milk and milk products: Cattle
10,3	UK Toddler	3,9	Wheat	3,5	Potatoes	0,9	Birds' eggs
10,0	UK Infant	3,3	Potatoes	2,6	Wheat	1,3	Birds' eggs
9,8	IT kids/toddler	6,6	Wheat	1,4	Tomatoes	0,9	Potatoes
9,6	LT adult	3,2	Potatoes	1,1	Rye	1,1	Wheat
9,4	ES adult	2,3	Wheat	1,0	Milk and milk products: Cattle	0,9	Potatoes
8,1	FR all population	3,3	Wheat	1,1	Potatoes	0,6	Poultry: Meat
6,7	IT adult	4,1	Wheat	1,2	Tomatoes	0,6	Potatoes
6,2	DK adult	2,0	Wheat	1,5	Potatoes	0,7	Rye
5,4	UK vegetarian	2,0	Wheat	1,4	Potatoes	0,6	Tomatoes
4,7	PL general population	3,4	Potatoes	0,9	Tomatoes	0,1	Onions
4,6	UK Adult	2,1	Wheat	1,4	Potatoes	0,4	Tomatoes
4,4	FI adult	1,2	Potatoes	1,0	Wheat	0,7	Rye

Table 3: Flufenacet EFSA PRIMo (2.0), long-term consumer risk assessment (uses limited to cereals, potatoes and maize)

Flufenacet			
Status of the active substance:		Code no.	
LOQ (mg/kg bw):		proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	0,005	ARfD (mg/kg bw):	
Source of ADI:		Source of ARfD:	
Year of evaluation:		Year of evaluation:	

Chronic risk assessment

	TMDI (range) in % of ADI minimum	maximum
	3	21
No of diets exceeding ADI:	---	

Highest calculated TMDI values in % of ADI	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities
21,2	NL child	5,9	Potatoes	5,9	Milk and cream,	4,7	Wheat
19,1	FR toddler	7,9	Milk and cream,	5,1	Potatoes	2,6	Wheat
18,2	WHO Cluster diet B	8,5	Wheat	2,7	Potatoes	2,5	Maize
16,3	UK Infant	7,7	Milk and cream,	3,3	Potatoes	2,6	Wheat
16,2	DK child	5,5	Wheat	4,4	Rye	2,5	Milk and cream,
14,6	WHO cluster diet D	6,6	Wheat	4,1	Potatoes	1,0	Milk and cream,
14,0	ES child	4,4	Wheat	2,5	Milk and cream,	1,8	Potatoes
13,5	WHO cluster diet E	3,9	Wheat	3,8	Potatoes	1,0	Poultry: Meat
13,2	DE child	4,1	Wheat	2,9	Milk and cream,	2,6	Potatoes
12,7	WHO regional European diet	4,0	Potatoes	3,0	Wheat	1,3	Swine: Meat
12,5	WHO Cluster diet F	3,6	Wheat	3,4	Potatoes	1,2	Swine: Meat
12,5	UK Toddler	4,1	Milk and cream,	3,9	Wheat	3,5	Potatoes
11,8	FR infant	5,1	Milk and cream,	4,1	Potatoes	0,8	Wheat
11,0	SE general population 90th percentile	4,2	Potatoes	3,2	Wheat	2,5	Milk and cream,
10,9	IE adult	2,3	Maize	2,3	Maize	2,3	Potatoes
9,9	PT General population	5,3	Potatoes	3,9	Wheat	0,5	Maize
9,0	NL general	3,7	Potatoes	2,1	Wheat	1,3	Milk and cream,
8,3	LT adult	3,2	Potatoes	1,1	Rye	1,1	Wheat
7,6	ES adult	2,3	Wheat	1,0	Milk and cream,	0,9	Potatoes
7,6	IT kids/toddler	6,6	Wheat	0,9	Potatoes	0,0	Maize
6,7	FR all population	3,3	Wheat	1,1	Potatoes	0,6	Poultry: Meat
6,3	DK adult	2,0	Wheat	1,5	Potatoes	1,1	Milk and cream,
4,8	IT adult	4,1	Wheat	0,6	Potatoes	0,0	Maize
4,6	UK vegetarian	2,0	Wheat	1,4	Potatoes	0,7	Milk and cream,
4,4	FI adult	1,2	Potatoes	1,1	Milk and cream,	1,0	Wheat
4,0	UK Adult	1,7	Wheat	1,4	Potatoes	0,6	Milk and cream,
3,4	PL general population	3,4	Potatoes	0,0	Maize		FRUIT (FRESH OR FROZEN)