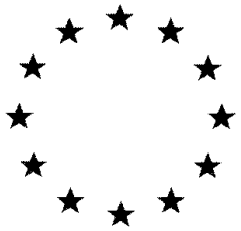
European Commission



VOLUME 3 – Annex B (AS)

- FLUTOLANIL -

B.7 Residue

Rapporteur Member State: The Netherlands

August 2018

Draft Assessment Report and Proposed decision of the Netherlands prepared in the context of the possible approval of flutolanil under Regulation (EC) 1107/2009

Version history page

Date	Version history
August 2018	Initial RAR

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B.7 Residue data

Flutolanil has been approved as a fungicide in potatoes. The representative use supported for the initial peer review process was the outdoor seed treatment of potatoes prior to planting at a rate of 92 g as/ton of tubers in northern and southern Europe.

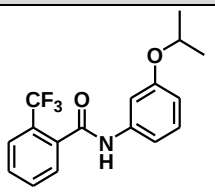
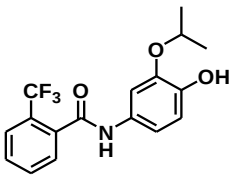
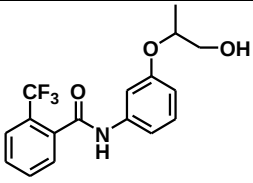
For the current renewal representative uses include: potato seed tuber treatment (ware, seed and starch) before and at planting (in store treatment, on/in planter treatment, application rate of 0.368 kg as/ha (92 g as/tonne potatoes).

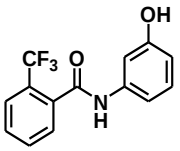
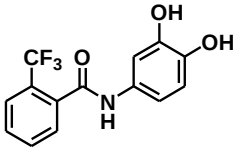
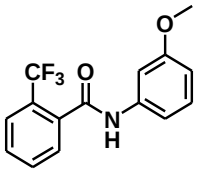
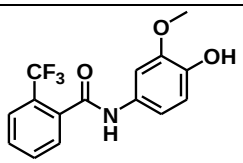
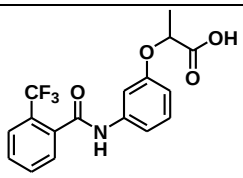
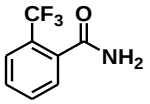
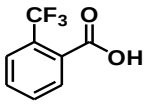
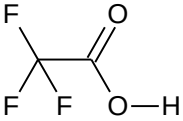
The use of flutolanil as in-furrow treatement on potatoes at planting has been submitted in the framework of MRL setting for flutolanil in potatoes.

Every study summary in this RAR starts with a box, stating whether the study has already been evaluated elsewhere. When a study has already been evaluated for the original peer review (i.e. there is a box in which is mentioned 'previous evaluation: in DAR'), the study summary/evaluation is copied as such into this RAR

To improve the readability of the evaluation on the residue behaviour of flutolanil, the following table (Table B.7-1) can be used, which contains the metabolites with their accompanying codes, numbers or synonyms, relevant for the residues evaluation.

Table B.7-1: Metabolites, relevant for the residues evaluation

Code	Chemical name	Compound structure	Description	Crop/Commodity
Flutolanil SN 84364 NNF-136 S-837	α,α,α-trifluoro-3'-isopropoxy-*o*-toluanilide		Parent compound	Primary crops: potato, peanut, rice, cabbage Rotational crops: lettuce, spinach, wheat, radish Livestock: goat, hens
M-2 HFT	α,α,α-trifluoro-4'-hydroxy-3'-isopropoxy-*o*-toluanilide		Metabolite	Primary crops : potato, peanut, cabbage, rice Rotational crops: Livestock: goat, hens
M-3 HIP	α,α,α-trifluoro-3'-(2-hydroxy-1-methylethoxy)-*o*-toluanilide		Metabolite	Primary crops : potato, rice, cabbage Rotational crops :wheat Livestock : goat, hens

Code	Chemical name	Compound structure	Description	Crop/Commodity
M-4	α,α,α-trifluoro-3'-hydroxy-DIP*o*-toluanilide		Metabolite	Primary crop : potato, peanut, rice, cabbage Rotational crops: lettuce, spinach, wheat, radish; barley Livestock: goat, hens
M-5 HDP	α,α,α-trifluoro-3', 4'-dihydroxy-*o*-toluanilide		Metabolite	Primary crops :cabbage Rotational crops: wheat; radish Livestock: hens
M-6 MDP	α,α,α-trifluoro-3'-methoxy-*o*-toluanilide		Metabolite	Primary crops: potato, rice, cabbage, Rotational crops: radish, wheat Livestock: goat
M-7 HMD	α,α,α-trifluoro-4'-hydroxy-3'-methoxy-*o*-toluanilide		Metabolite	Primary crops: potato, rice, cabbage Rotational crops: radish, Livestock: hens, goat
M-11	2-[3-(α,α,α-trifluoro-*o*-toluoylamino) phenoxy]propionic acid		Metabolite	Primary crops: rice, cabbage, Rotational crops: wheat Livestock: goat
M-101	2-(trifluoromethyl) benzamide		Metabolite	Primary crops: potato, rice, cabbage Rotational crops: lettuce, spinach, radish, wheat, barley Livestock: goat, hens
M-102	2-(trifluoromethyl) benzoic acid		Metabolite	Primary crops: potato, rice, cabbage Rotational crops: lettuce, spinach, radish, wheat, barley Livestock: hens
TFA	Trifluoroacetic acid		Metabolite	Rotational crops: lettuce, radish, wheat

B.7.1 Storage stability of residues

During the initial EU peer review, data on the storage stability of flutolanil was evaluated in potatoes, wheat (grain and straw) and rape (grain). Storage stability of metabolite M-4 was evaluated in potatoes.

For the purpose of the renewal, the notifier submitted new storage stability studies of flutolanil and its metabolites M-1, M-4, M-101 and M-102 in potato and spinach.

B.7.1.1 Storage stability of flutolanil and its metabolite M-4 in potatoes

Previous evaluation	In the DAR
RMS remark	RMS considers the study as acceptable

Report: Williams, L.E. (1996): Stability of flutolanil and its metabolite, M-4, in potatoes during frozen storage, USA, 1990. Nihon Nohyaku, Unpublished Report No.: A-3024.

Guidelines: EPA Pesticide Assessment Guidelines Subdivision O, Guideline 171-4(e)

GLP: Yes

Material and methods

Stability of flutolanil and its metabolite M-4 was tested in potatoes in frozen storage. Untreated potatoes obtained from the local super market were composited to provide a bulk matrix. Representative sub samples (20 g of each) of the bulk matrix were individually fortified with either flutolanil or its metabolite M-4 at a concentration of 1.0 mg/kg and were stored at – 15 ºC prior to analysis. After 54 and 67 months storage samples were analysed for flutolanil and M-4 by using a method that converts flutolanil and all known-metabolites to a common-moiety, 2-trifluoromethyl benzoic acid (2-TFBA). All residue results were expressed in terms of flutolanil. The 2-TFBA was methylated and quantified by gas chromatography with mass selective detection (GC/MSD). The LOQ was 0.05 mg/kg.

At each time point, two untreated control samples, two freshly and separately spiked samples with flutolanil and M-4 at 0.50 µg/l were analysed together with three replicate of stored samples previously fortified with flutolanil only and three replicate fortified with M-4 only. Therefore, each time point a total of twelve samples was analysed. The recovery rates of stored samples were corrected on the basis of freshly fortified samples.

Findings

At the time when this study was initiated, no method of analysis for potatoes was available. Consequently, it was not possible to perform a true zero time (day 0) analysis of the fortified samples. The samples were fortified and placed directly into frozen storage where they remained until an analytical method was available. The method ultimately adapted was similar to that used for other plant and animal matrices. The stability of flutolanil and its metabolite M-4 after frozen storage of 54 and 67 months was evaluated by comparing the recoveries of aged samples to the initial concentration of fortified samples (1.0 mg/kg). The values were corrected for the mean recovery of the appropriate analyte from freshly fortified samples. The mean procedural recovery of the method was for flutolanil (90 % ± 14 %, n=4) and for M-4 (82 % ± 14 %, n=4). The recoveries of aged samples were within acceptable criteria thereby

demonstrating that the storage of the samples did not adversely affect the residues determined. The frozen storage stability data of flutolanil and its metabolite M-4 in potatoes is presented in Table B.7.1.1.

Table B.7.1.1 Stability of flutolanil and M-4 in frozen in potatoes

Matrix	Residue	Fortification level (mg/kg)	Storage duration (months)	Stability of residues		Procedural recovery (%)	Std deviation (%)
				residue level after storage* (%)	% initial level**		
Potato	Flutolanil	1	0	-	-	-	-
	M-4	1	0	-	-	-	-
	Flutolanil		54	81.6	90.3	90.6 (n=2)	-
	M-4		54	80.2	93.3	72.8 (n=2)	-
	Flutolanil		67	78.3	103.5	89.5 (n=2)	-
	M-4		67	75.5	94.3	92 (n=2)	-

M-4 = α,α,α-trifluoro-3'-hydroxy-o-tolyanilide
* Residue level at day 0 was not measured since no analytical method for flutolanil was available at that time. Residue levels after storage was compared to the initial fortification level of 1 mg/kg. Not corrected for procedural recovery
** Corrected with procedural recovery

Conclusions

The frozen storage stability (at – 10 ºC to – 20 ºC) of flutolanil and its metabolite M-4 was tested in potatoes. No true zero time (day 0) analysis of the fortified samples was performed. Thus, it was not possible to calculate the actual degradation percentage of the analytes. However, it was considered that the study sufficiently demonstrated the frozen storage stability of the flutolanil and its M-4 metabolite as the recoveries of the aged samples of the initial fortification level were in acceptable level after 54 and 67 months frozen storage.

B.7.1.2 Storage stability of flutolanil in potatoes, wheat (grain and straw) and rape (grain)

Previous evaluation	In the Addendum 1A to the DAR
RMS remark	Acceptable

Report: Ricau H. (2004) Stability study of Flutolanil in potato, wheat (grain and straw) and rape (grain) after storage in a congelator at a temperature under minus 18 °C. Bayer Crop Science study no. 02-80 (R-3209).

Guidelines: None

GLP: Yes

The objective of this study was to investigate the frozen storage stability of flutolanil in potato, wheat (grain and straw) and rape (grain) over an eighteen months period.

Materials and methods:

Accurately 10 g (5 g of wheat straw) of a grinded potato/wheat grain/rape grain samples were fortified with flutolanil at ten times of the limit of quantification (0.1 mg/kg) and were stored at – 18 ºC. Samples were analysed immediately (at day 0) and at 1, 2, 3, 6, 12 and 18 months. At each time point, one untreated control sample, one freshly spiked sample with flutolanil at 0.10 mg/kg were analysed together with two replicate of stored samples previously fortified with flutolanil.

Flutolanil was extracted from the sample material with acetone. The acetone was evaporated and the water phase back-extracted with petroleum ether. The petroleum ether extract was purified on a neutral aluminium oxide column with diethyl ether as eluent. The residues of flutolanil were quantified by a gas chromatography (GC) equipped with a thermionic detector (TID) or an electron capture detector (ECD). The quantification was carried out by external standardisation. The limit of quantification (LOQ) was 0.01 mg/kg.

Method was validated for each matrix by analysing three specimens of potato, wheat grain and straw and rape grain fortified with flutolanil at LOQ or at 10 times LOQ.

Findings

Procedural recoveries were in acceptable range for each matrix. The validation data are given in Table B.7.1.2-1

Frozen storage stability data are given in Table B.7.1.2-2. The results indicate that flutolanil stays stable under frozen storage conditions in potato, wheat (grain and straw) and rape for up to 18 months.

Table B.7.1.2-1 Validation data for each matrix

Matrix	Fortification level (mg/kg)	Recovery*	STD (%) (n=3)
Potato	0.010	81	12
	0.10	86	13
Rape	0.010	86	20
	0.10	90	19
Wheat grain	0.010	80	6
	0.10	73	0
Wheat straw	0.010	74	5
	0.10	75	3

* Mean of three replicates

Table B.7.1.2-2 Frozen storage stability of flutolanil in potato, wheat (grain and straw) and rape (grain)

Matrix	Fortification level (mg/kg)	Storage duration		Stability of residues		Concurrent recovery (%)
		Nominal interval (month)	Actual Storage Time (days)	Residue level after storage* (mg/kg)	% initial level	
Potato	0.10	0	0	0.086	86	-
		1	34	0.105	105	104
		3	94	0.105	105	103
		6	191	0.078	78	107
		9	273	0.091	91	84
		12	364	0.073	73	76
		18	548	0.103	103	99
Rape	0.10	0	0	0.090	90	-
		1	34	0.085	85	75
		3	94	0.073	73	109
		6	191	0.079	79	70
		9	273	0.091	91	80
		12	364	0.072	72	100
		18	548	0.089	89	80
Wheat grain	0.10	0	0	0.073	73	-
		1	34	0.088	88	85
		3	94	0.107	107	71
		6	191	0.091	91	76
		9	273	0.090	90	101
		12	364	0.078	78	74
		18	548	0.085	85	87
Wheat straw	0.10	0	0	0.075	75	-
		1	34	0.072	72	85
		3	94	0.105	105	102
		6	191	0.098	98	107
		9	273	0.107	107	89
		12	364	0.091	91	91
		18	548	0.076	76	90

Conclusions

The frozen storage stability (at – 18 ºC) of flutolanil was tested in various raw agricultural commodities. The study indicated that flutolanil is stable under frozen storage conditions for at least 1.5 years (18 months) in potato, wheat (grain and straw) and rape (grain).

B.7.1.3 Storage stability of flutolanil, metabolites M-2, M-4, M-101, M-102 and their conjugates in potato and spinach

Previous evaluation	Submitted for the purpose of renewal
RMS remark	Acceptable

Reference:	**CA 6.1/03: Merdian H. (2017)**
Title:	Storage Stability of Flutolanil and its Metabolites (M-2, M-4, M-101, M-102) in 2 Crops under Deep Frozen Conditions
Document No.:	S16-00671 (3rd Interim Report) (R-3411)
Guidelines:	OECD 506, 2007
GLP:	Yes
Comment	The final report of this study was not available in time to be included in this dossier, however, the interim report, given the results on 12 months of storage stability, has been included for reference. The final report will be available for May 2018

Executive Summary

The objective of the study was to obtain data about the storage stability of flutolanil and its metabolites (M-2, M-4, M-101 and M-102) in spinach and potato tuber under deep frozen conditions (≤ -18 °C) over a storage period up to 24 months. The current interim report reflects the results obtained after 12 months of storage. Stability was demonstrated for flutolanil, M-2, M-4, M-101 and M-102 in homogenates of spinach and potato tuber upon storage at ≤ -18 °C for 12 months, since the degradation per analyte and matrix is below <30% (for M-2 after taking into account the detected values of procedural recoveries).

1. MATERIALS AND METHODS

- Test Materials: Flutolanil

  Batch No.: 3710900
  Purity: 99.5%
  CAS No.: 66332-96-5
  Manufacturer: Chemservice
  Spiking levels: 1.0 mg/kg
- Test Materials: M-2 (α,α,α-trifluoro-4'-hydroxy-3'-isopropoxy-*o*-toluanilide)

  Batch No.: 2AE0202P
  Purity: 99.8%
  CAS No.: -
  Manufacturer: Nihon Nohyaku
  Spiking levels: 1.0 mg/kg
- Test Materials: M-4 (α,α,α-trifluoro-3'-hydroxy-*o*-toluanilide)

  Batch No.: 5AE0404S
  Purity: 100%
  CAS No.: -
  Manufacturer: Nihon Nohyaku
  Spiking levels: 1.0 mg/kg
- Test Materials: M-101 (2-(Trifluoromethyl)benzamide)

Batch No.: 1444422V
Purity: 100%
CAS No.: 360-64-5
Manufacturer: Sigma Aldrich
Spiking levels: 1.0 mg/kg

- Test Materials: M-102 (2-(Trifluoromethyl)benzoic acid)

Batch No.: MKBQ8335V
Purity: 99.4%
CAS No.: 433-97-6
Manufacturer: Sigma Aldrich
Spiking levels: 1.0 mg/kg

- Test commodity

Crop: Potato and spinach
Sample size: 20g

1. Study Design:

I. Test Procedure:

All samples for storage were fortified at the beginning of the experimental phase. For storage samples the analytes were fortified as follows:

- Flutolanil
- M-2 and M-4 as a mix
- M-101 and M-102 as a mix

Analysis of storage samples was done directly at day 0 and after each storage interval each time accompanied by analysis of a control sample and procedural recovery samples.

The fortification level was at 1.0 mg/kg (100xLOQ) of the method on aliquots of homogenized control sample material.

Storage samples allow assessment of storage stability, while procedural recoveries demonstrate the analytical performance of the method.

II. Description of analytical procedures:

Residues of flutolanil and its metabolites (M-2, M-4, M-101, M-102 and their conjugates, expressed as flutolanil) were determined following the validated analytical method “Validation of the Analytical Method for the Determination of Flutolanil and its Metabolites M-2, M-4, M-101 and M-102 in Potato after Hydrolysis”, S16-00710 (A-3081). Summary of the validation data is presented in Volume 3, B.5.1.2.5, Methods used in support of residues studies.

Quantification was performed by use of LC-MS/MS detection. The limit of quantification (LOQ) of the analytical method was 0.01 mg/kg per analyte and matrix with a limit of detection (LOD) set at 0.003 mg/kg (30 % of the LOQ).

2. RESULTS AND DISCUSSION

The current interim report reflects the results obtained after 12 months of storage. Stability was demonstrated for flutolanil, M-2, M-4, M-101 and M-102 (including their conjugates) in homogenates of spinach and potato tuber upon storage at ≤ -18 °C for 12 months.

Table B.7.1.3-1: Frozen storage stability of flutolanil and its metabolites (including conjugates) in potato and spinach

		Time of storage in months							
		Storage samples Mean (%) [RSD(%)], n=3				Procedural Recoveries Mean (%) [RSD(%)]			
		0		3	9	0		3	9
		Fortification level (mg/kg)							
	Matrix	**0.01**	**1.0**	**1.0**	**1.0**	**0.01**	**1.0**	**1.0**	**1.0**
Flutolanil	Spinach	85 [3.8]	85 [6.2]	78 [3.2]	84 [2.1]	-	-	80 [2.9]	84 [1.8]
	Potato	85 [1.8]	79 [7.7]	83 [2.5]	87 [13]	-	-	86 [3.3]	88 [0.7]
M-2	Spinach	78 [2.7]	83 [3.2]	73 [11]	**67** [3.1]	-	-	73 [11]	74 [2.7]
	Potato	94 [4.4]	88 [4.5]	73 [2.4]	**69** [3.0]	-	-	87 [2.9]	93 [2.2]
M-4	Spinach	85 [6.5]	89 [3.6]	76 [1.3]	75 [1.3]	-	-	76 [7.9]	74 [2.7]
	Potato	93 [5.0]	90 [2.8]	78 [2.7]	84 [1.2]	-	-	80 [4.0]	92 [4.3]
M-101	Spinach	85 [6.5]	89 [5.9]	84 [1.2]	90 [5.3]	-	-	87 [0.7]	79 [13]
	Potato	87 [4.8]	95 [2.7]	90 [2.3]	84 [1.4]	-	-	85 [2.5]	84 [2.5]
M-102	Spinach	75 [2.0]	86 [0]	85 [3.1]	104 [5.6]	-	-	84 [4.2]	85 [11]
	Potato	86 [7.6]	93 [2.2]	81 [8.8]	87 [5.2]	-	-	80 [6.1]	83 [3.7]

		12 months	
		Storage samples Mean (%) [RSD(%)]	Procedural Recoveries Mean (%) [RSD(%)]
		Fortification level (mg/kg)	
	Matrix	**1.0**	**1.0**
Flutolanil	Spinach	85 [1.8]	87 [5.8]
	Potato	79 [6.7]	88 [2.6]
M-2	Spinach [1]	**69** [4.3]	86 [2.3]
	Potato [2]	**63** [2.7]	90 [2.6]
M-4	Spinach	76 [4.6]	83 [3.2]
	Potato	78 [2.0]	87 [0.7]
M-101	Spinach	87 [4.6]	82 [4.9]
	Potato	82 [1.4]	78 [2.0]
M-102	Spinach	84 [3.4]	82 [3.1]
	Potato	95 [1.1]	97 [1.2]

Levels in bold display recoveries outside the acceptable range of 70-120% recovery.

[1] The stability of M-2 in spinach is confirmed as the low recoveries obtained in the storage samples are in correlation with the low recoveries of the fresh procedural recoveries. So, considering the results of the corresponding fresh fortification, it is demonstrated that the degradation of M-2 in spinach is below <30% (for RMS conclusion see Remark RMS below).

[2] The stability of M-2 in potato is also confirmed so far as the recoveries seems to have reached a plateau around 70% from 9 months and the lower results at 12 months seems to be more related to analytical variations. Obviously, the results of the next storage point will provide essential additional data allowing us to finalise the conclusion on the stability of M-2 in potato. So, taking into account the detected values of procedural recoveries, the stability of M-2 in potato is confirmed so far.

3. CONCLUSION

Stability was demonstrated, so far, for flutolanil, M-2, M-4, M-101 and M-102 (including the conjugates) in homogenates of spinach and potato tuber upon storage at ≤ -18 °C for 12 months. This study is still on-going and the storage stability in spinach and potato tuber is going to be investigate up to 24 months.

Remark RMS:

The applicant correlates lower recovery results for metabolite M-2 in spinach and potatoes to low recoveries of the fresh samples (procedural recoveries). However, RMS if of the opinion that the reported procedural recoveries for metabolite M-2 are not considered low at 12 months timepoint.
RMS concludes that based on the reported results, storage stability of flutolanil and its metabolites M-4, M-101 and M-102 has been demonstrated up to 12 months, however, for the final conclusion on metabolite M-2 the final report should be awaited, since a steady decline of stability of M-2 can be observed.

B.7.1.4 Storage stability of flutolanil and its metabolites M-2, M-4 and M-7 in whole milk

Previous evaluation	Submitted for the purpose of renewal
RMS remark	Not acceptable, no method validation submitted in Volume 3, B.5: Methods of analysis. Method validation should be submitted.

Reference:	**CA 6.1/04: Neal J. L. (1994)**
Title:	Storage stability of flutolanil residues in whole milk USA, 1993
Document No.:	AU-93R-11 (PC-3110)
Guidelines:	EPA Pesticide Assessment Guidelines Subdivision E, Part 72-4
GLP:	Yes

Executive Summary

The objective of the study was to obtain data about the storage stability of flutolanil and its metabolites (M-2, M-4 and M-7) in whole milk under deep frozen conditions (-20 °C) over a storage period up to 115 days. Sample sets were analysed at 0 and 115 days storage for each of the four analytes in whole milk. The samples were analysed by a method which hydrolyses all compounds of concern to 2-(trifluoromethyl)-benzoic acid (2-TFBA). Subsequent methylation of 2-TFBA yielded its methyl ester which was quantified using a gas chromatograph equipped with a mass selective detector. Stability was demonstrated for flutolanil, M-2, M-4 and M-7 in whole milk upon storage at -20 °C for 115 days.

1. MATERIALS AND METHODS

I. Materials:

A. Test Materials: Flutolanil

Batch No.: AU/29/03
Purity: 100%
CAS No.: 66332-96-5
Spiking levels: 0.10 mg/kg

B. Test Materials: M-2 (α,α,α-trifluoro-4'-hydroxy-3'-isopropoxy-*o*-toluanilide)

Batch No.: AU/30/03
Purity: 98.2%

CAS No.: -
Spiking levels: 0.098 mg/kg

C. Test Materials: M-4 (α,α,α-trifluoro-3'-hydroxy-*o*-toluanilide)

Batch No.: AU/31/03
Purity: 99.9%
CAS No.: -
Spiking levels: 0.10 mg/kg

D. Test Materials: M-7 (N-(4-hydroxy-3-methoxyphenyl)-2-(trifluoromethyl)benzamide)

Batch No.: AU/32/03
Purity: 96.9%
CAS No.: -
Spiking levels: 0.097 mg/kg

E. Test Materials: 2-TFBA Me-ester

Batch No.: AU/28
Purity: 98.3%
CAS No.: -

F. Test commodity

Crop: whole milk (from local store)
Sample size: 10 grams

II. Study Design:

III. Test Procedure:

Sixty 10 gram samples of whole milk were weighted into screw capped Pyrex tubes. Ten tubes were fortified with flutolanil, ten with M-2, ten with M-4 and ten with M-7 each at a nominal concentration of 0.10 mg/kg. The remaining were to serve as control samples. All samples were kept inside closed cardboard boxes stored in laboratory freezers maintained at -20°C.

Sample sets were analysed at 0 and 115 days storage for each of the four analytes in whole milk. Storage samples allow assessment of storage stability, while procedural recoveries demonstrate the analytical performance of the method.

IV. Description of analytical procedures:

The samples were analysed by a method which hydrolyses all compounds of concern to 2-(trifluoromethyl)-benzoic acid (2-TFBA). Subsequent methylation of 2-TFBA yielded its methyl ester which was quantified using a gas chromatograph equipped with a mass selective detector (GC-MS).

2. RESULTS AND DISCUSSION

Stability was demonstrated for flutolanil, M-2, M-4 and M-7 in whole milk upon storage at -20 °C for 115 days.

Table B.7.1.4-1: Frozen storage stability of flutolanil and its metabolites in whole milk

Compound	Matrix	Storage Interval (days)	Fortification level (mg/kg)	Percentage recovery (%) (n=2)
Flutolanil	Whole milk	0	0.10	110
	Whole milk	115	0.10	88
M-2	Whole milk	0	0.098	101
	Whole milk	115	0.098	86
M-4	Whole milk	0	0.10	110
	Whole milk	115	0.10	100
M-7	Whole milk	0	0.097	100

	Whole milk	115	0.097	94

3. CONCLUSION

Stability was demonstrated for flutolanil, M-2, M-4 and M-7 in whole milk upon storage at -20 °C for 115 days.

Remark RMS: No method validation has been submitted by the applicant to support the study. Method validation is required in Volume 3, B.5.

B.7.1.5 Storage stability of flutolanil and its metabolites M-2, M-4 and M-7 in animal products

Previous evaluation	Submitted for the purpose of renewal
RMS remark	Not acceptable, no method validation submitted in Volume 3, B.5: Methods of analysis. Method validation should be submitted.

Reference:	**CA 6.1/05: Dacus S. C. (1994)**
Title:	Stability of Flutolanil and its metabolites M-2, M-4 and M-7 in animal products during frozen storage, USA 1993
Document No.:	AU-93R-12 (PC-3111)
Guidelines:	EPA Pesticide Assessment Guideline Subdivision O, Part 171-4
GLP:	Yes

Executive Summary

The objective of the study was to obtain data about the storage stability of flutolanil and its metabolites (M-2, M-4 and M-7) in beef muscle, beef fat, chicken liver and chicken eggs under deep frozen conditions (-20 °C) over a storage period up to 92 - 127 days (depending on the matrix). Sample sets were analysed at 0, 79-90 days and 92-127 days storage for each of the four analytes in each matrices. The samples were analysed by a method which hydrolyses all compounds of concern to 2-(trifluoromethyl)-benzoic acid (2-TFBA). Subsequent methylation of 2-TFBA yielded its methyl ester which was quantified using a gas chromatograph equipped with a mass selective detector. Stability was demonstrated for flutolanil, M-2, M-4 and M-7 upon storage at -20 °C for about 120 days in beef muscle, chicken liver and eggs under frozen storage conditions. Degradation of these analytes may occur at an appreciable rate in fat over an approximate period of 90 days under frozen stored conditions. However, it is suspected that the method is not extracting the residues from that fat tissues after frozen storage.

1. MATERIALS AND METHODS

I. Materials:

A. Test Materials: Flutolanil

Batch No.: AU/29/03
Purity: 100%
CAS No.: 66332-96-5
Spiking levels: ca. 0.10 mg/kg

B. Test Materials: M-2 (α,α,α-trifluoro-4'-hydroxy-3'-isopropoxy-*o*-toluanilide)

Batch No.: AU/30/03
Purity: 98.2%
Spiking levels: ca. 0.10 mg/kg

C. Test Materials: M-4 (α,α,α-trifluoro-3'-hydroxy-*o*-toluanilide)

Batch No.: AU/31/03
Purity: 99.9%
Spiking levels: ca. 0.10 mg/kg

D. Test Materials: M-7 (N-(4-hydroxy-3-methoxyphenyl)-2-(trifluoromethyl)benzamide)

Batch No.: AU/32/03
Purity: 96.9%
Spiking levels: ca. 0.10 mg/kg

E. Test Materials: 2-TFBA Me-ester

Batch No.: AU/28
Purity: 98.3%

F. Test commodity

Crop: Beef muscle, beef fat, chicken liver and chicken eggs (from local store)
Sample size: 5 grams for muscle, liver, and eggs and 10 grams for fat

II. Study Design:

V. Test Procedure:

Thirty-three 5 gram samples of muscle, liver, and eggs were weighted into screw capped teflon tubes. Thirty-three 10 gram samples of fat were weighted into glass jars. Six samples of each matrix were fortified with flutolanil, M-2, M-4 and M-7 each at a nominal concentration of 0.10 mg/kg. The remaining were to serve as control samples. All samples were stored in laboratory freezers maintained at -20°C.

Sample sets were analysed at 0, 79-90 days and 92-127 days storage for each of the four analytes in each matrices.

Storage samples allow assessment of storage stability, while procedural recoveries (in range of 71-103% in all matrices) demonstrate the analytical performance of the method.

VI. Description of analytical procedures:

The samples were analysed by a method which hydrolyses all compounds of concern to 2-(trifluoromethyl)-benzoic acid (2-TFBA). Subsequent methylation of 2-TFBA yielded its methyl ester which was quantified using a gas chromatograph equipped with a mass selective detector (GC-MS).

2. RESULTS AND DISCUSSION

Data from the table below show that no appreciable degradation occurs over the course of this study (approximately 120 days) in the muscle, liver or eggs. However, data indicates that either the compounds are breaking down in fat or the method does not perform well on stored fat samples

Table B.7.1.5-1: Frozen storage stability of flutolanil and its metabolites in muscle, fat, liver and egg

Matrix	Storage Interval (days)	Spike level (mg/kg)	Flutolanil (%)	M-2 (%)	M-4 (%)	M-7 (%)
Muscle	0	0.10	110	100	99	100

Matrix	Storage Interval (days)	Spike level (mg/kg)	Flutolanil (%)	M-2 (%)	M-4 (%)	M-7 (%)
	90		97	100	97	98
	127		110	100	100	100
Fat	0	0.10	84	86	78	77
	88		100	**42**	**39**	**39**
	92		**50**	**32**	**69**	**28**
Liver	0	0.10	110	97	88	96
	85		110	110	110	97
	122		100	100	88	100
Egg	0	0.10	97	110	97	99
	79		95	99	97	100
	125		95	84	98	92

Levels in bold display recoveries outside the acceptable range of 70-120% recovery.

3. CONCLUSION

The results indicate that residues of flutolanil, M-2, M-4 and M-7 show no appreciable degradation over an approximate period of 120 days in beef muscle, chicken liver and eggs under frozen storage conditions. Degradation of these analytes may occur at an appreciable rate in beef fat over an approximate period of 90 days under frozen stored conditions. However since these analytes have proven stable in many other matrices (soil, water, rice grain and straw and milk) and the data are somewhat erratic for this one matrix, it is justified to suspect that the method is not extracting the residues from that fat tissues after frozen storage.

Remark RMS:

The method performance has been reported in the study. Reported recoveries (concurrent and procedural) have been within acceptable ranges (74-91%). It suggest that the method is acceptable for the fat extraction from the fresh fortifies samples. It can be concluded based on the studies that flutolanil and its metabolites M-2, M-4 and M-7 are stable in muscle, liver and eggs for 4 months and are not stable in fat during the investigated timelines.

However, no method validation has been submitted for the renewal: Volume 3, B.5: Methods of analysis. Method validation should be submitted.

B.7.1.6 Storage stability of flutolanil and its metabolites M-2, M-4, M-7, M-101 and M-102 in animal products

Previous evaluation	Submitted for the purpose of renewal
RMS remark	Acceptable

Reference:	**CA 6.1/06: Dias N., 2016a**
Title:	Flutolanil: Residues of Flutolanil and its Metabolites in Eggs and Tissues of Laying Hens
Document No.:	LMS0104 (A-3075)
Guidelines:	OECD 505: Residues in Livestock (2007) OCSPP 860.1480 (1996)

GLP:	Yes

Executive Summary

A livestock feeding study was performed to quantify levels of flutolanil residues in eggs and tissues of laying hens after dietary inclusion of flutolanil for 28 to 29 days. Through the conduct of this study, a storage stability was conducted for flutolanil and 5 of its metabolites (M-2, M-4, M-7, M-101 and M-102) in chicken liver, muscle, fat and eggs under deep frozen conditions (-20 °C) over a storage period up to 4 days. Additional storage stability data was required for M-101 in eggs and M-2 and M-4 in liver and muscle as some samples were analysed more than 30 days after sampling. Therefore, a further stability time point (436 days) was performed for these analytes only.

The results from the storage stability investigations demonstrated that flutolanil, M-2, M-4, M-7, M-101 and M-102 were stable in chicken eggs, liver, muscle and fat for a period of 4 days. It was also demonstrated that M-101 in eggs and M-2 and M-4 in liver and muscle were stable for a period of 436 days.

1. MATERIALS AND METHODS

- **Materials:**

- Test Materials: Flutolanil

  Batch No.: 1AE0012P
  Purity: 99.6%
  CAS No.: 66332-96-5
  Spiking levels: 0.1 mg/kg
- Test Materials: M-2 (α,α,α-trifluoro-4'-hydroxy-3'-isopropoxy-o-toluanilide)

  Batch No.: 2AE0202P
  Purity: 99.8%
  CAS No.: -
  Spiking levels: 0.1 mg/kg
- Test Materials: M-4 (α,α,α-trifluoro-3'-hydroxy-o-toluanilide)

  Batch No.: 4AE0403P
  Purity: 99.4%
  CAS No.: -
  Spiking levels: 0.1 mg/kg
- Test Materials: M-7 (α,α,α-trifluoro-4'-hydroxy-3'methoxy-o-toluanilide)

  Batch No.: 4AE0702P
  Purity: 99.6%
  CAS No.: 360-64-5
- Test Materials: M-101 (2-(Trifluoromethyl)benzamide)

  Batch No.: 1440587V
  Purity: 100%
  CAS No.: 360-64-5
  Spiking levels: 0.1 mg/kg
- Test Materials: M-102 (2-(Trifluoromethyl)benzoic acid)

  Batch No.: MKBP3429V
  Purity: 99.0%
  CAS No.: 433-97-6
  Spiking levels: 0.1 mg/kg
- Test commodity

  Crop: eggs, liver, muscle and fat

- **Study Design:**

- **Test Procedure:**

Eleven control sub-samples of each of the four matrices (eggs, liver, muscle and fat) were weighted into a suitable vessel and fortified according to the following regime:

- 5 remained untreated sub-samples
- 6 sub-samples fortified at 0.1 mg/kg with flutolanil and 5 metabolites

All the above sub-samples were then stored in a freezer at app. -20°C. At each stability time points (0 and 4 days), one untreated and two fortified samples were analysed along to a freshly fortified sub-samples at 0.1 mg/kg with flutolanil and 5 metabolites.
Additional storage stability data was required for M-101 in eggs and M-2 and M-4 in liver and muscle as some samples were analysed more than 30 days after sampling. Therefore a further stability time point (436 days) was performed for these analytes only.

- **Analytical method:**

Original method:
Samples (except fat) were extracted with acetonitrile and clean-up with a C18 solid phase extraction (SPE) cartridge. Fat samples were extracted with acetonitrile/hexane (50/50, v/v) and cleaned-up by liquid-liquid partition. Quantification was performed using liquid chromatography with tandem mass spectrometric detection (LC-MS/MS).

Modified method (with acid extraction and de-conjugation step):
Samples (except fat) were extracted with acetonitrile and acidic acetonitrile and clean-up with a C18 solid phase extraction (SPE) cartridge. Fat samples were extracted with acetonitrile/hexane (50/50, v/v) and acidic acetonitrile/hexane (50/50, v/v) and cleaned-up by liquid-liquid partition. An enzyme hydrolysis step with ß-glucorinidase was also included for M-2 and M-4 for all matrices except fat, followed by a clean-up with a C_{18} solid phase extraction (SPE) cartridge. Quantification was performed using liquid chromatography with tandem mass spectrometric detection (LC-MS/MS).
The original method was used to analyse the samples at the Day 0 and Day 4 stability time points and the modified method was used to analyse the samples at the Day 436 stability time point.

2. RESULTS AND DISCUSSION

Method (original and modified) validation was reported within the study report. Linearity of the methods was shown for each metabolite with r ≥ 0.997. Recovery experiments at 0.01 and 0.1 mg/kg (n=5 per level) were performed for each matrix for the original method (table B.7.1.6-1) and for the modified method which included a deconjugation step with ß-glucorinidase and clean-up with a C18 solid phase extraction (SPE) cartridge (table B.7.1.6-2). Recovery was always between 70-110% with RSD<20%.

Table B.7.1.6-1: Recovery experiments (n=5 per matrix per level) for the method validation for the original method

analyte	Fortification level (mg/kg)	Recovery % (mean ± rsd)			
		Liver	Muscle	Fat	Eggs
Flutolanil	0.01	80±11	84±10	77±5	88±11
	0.1	93±3	92±2	89±7	85±7
M-2	0.01	89±12	89±10	77±8	95±4
	0.1	102±4	96±6	91±9	89±4
M-4	0.01	99±12	90±8	89±7	82±5
	0.1	104±5	95±5	91±9	85±9
M-7	0.01	99±14	87±8	75±8	99±8
	0.1	101±4	96±4	91±8	102±6
M-101	0.01	83±6	92±7	81±20	86±8
	0.1	92±2	92±4	85±6	94±2
M-102	0.01	99±16	93±18	79±7	75±6
	0.1	105±3	94±5	88±5	93±4

Table B.7.1.6-2: Recovery experiments (n=5 per matrix per level) for the method validation for the modified method (acid hydrolysis, applied to samples after 436 days storage)

analyte	Fortification level (mg/kg)	Recovery % (mean ± rsd)			
		Liver	Muscle	Fat	Eggs
Flutolanil	0.01	99±8	97±3	79±13	99±5
	0.1	95±3	92±6	86±13	105±7
M-2	0.01	82±7	107±4	90±13	87±8
	0.1	97±3	98±6	92±9	99±6
M-2 after deconjugation	0.01	98±13	82±8	-	-
	0.1	100±10	85±9	-	-
M-4	0.01	95±4	90±8	101±8	95±6
	0.1	102±2	102±5	109±8	102±6
M-4 after deconjugation	0.01	82±2	91±9	-	-
	0.1	95±8	94±9	-	-
M-7	0.01	101±5	97±6	80±10	83±13
	0.1	93±4	103±4	96±5	95±5
M-101	0.01	74±6	96±6	76±9	96±7
	0.1	71±3	103±4	78±9	99±3
M-102	0.01	99±16	93±18	79±7	75±6
	0.1	105±3	94±5	88±5	93±4

Procedural recoveries were also determined during the analysis (t=4 days) of the stored samples and were 76-105% for flutolanil, 84-106% for M-2, 81-107 (M-4), 86-108% (M-7), 82-94% (M-101) and 66-83% (M-102). With respect to M-102, procedural recovery was only 66% in muscle and 70% in liver. All other procedural recoveries determined for M-102 were >70%.

The results from the storage stability investigations demonstrated that Flutolanil, M-2, M-4, M-7, M-101 and M-102 were stable in chicken eggs, liver, muscle and fat for a period of 4 days. It was also demonstrated that M-101 in eggs and M-2 and M-4 in liver and muscle were stable for a period of 436 days. The results are presented in Table B.7.1.6-3.

Table B.7.1.6-3: Frozen storage stability of flutolanil and its metabolites in liver, muscle, fat and egg

Matrix	**Storage Interval (days)**	**Fortification level**	**Flutolanil (%)**	**M-2 (%)**	**M-4 (%)**	**M-7 (%)**	**M-101 (%)**	**M-102 (%)**
Liver	0	0.1 mg/kg	78	89	102	90	76	81

Matrix	Storage Interval (days)	Fortification level	Flutolanil (%)	M-2 (%)	M-4 (%)	M-7 (%)	M-101 (%)	M-102 (%)
	4		84	86	99	90	92	74
	436		-	78	87	-	NR	-
Muscle	0		82	76	82	82	79	72
	4		73	**67**	72	71	88	**68**
	436		-	96	90	-	NR	-
Fat	0		90	97	94	100	88	88
	4		81	78	87	77	93	89
	436		-	NR	NR	-	NR	-
Egg	0		97	105	94	105	84	92
	4		93	98	97	100	97	74
	436		-	NR	NR	-	90	-

NR : not required
Levels in bold display recoveries outside the acceptable range of 70-120% recovery.

3. CONCLUSION

The results from the storage stability investigations demonstrated that Flutolanil, M-2, M-4, M-7, M-101 and M-102 were stable in chicken eggs, liver, muscle and fat for a period of 4 days. It was also demonstrated that M-101 in eggs and M-2 and M-4 in liver and muscle were stable for a period of 436 days.

Remarks RMS:

Procedural recovery of M-102 in liver was only 66% at t= 4 days and recovery of the stored sample at this time interval was only 68%. All other procedural recoveries determined for M-102 were >70%. Therefore it is concluded that the low stability of M-102 in muscle (68%) is due to the incidental low procedural recovery (66%). In conclusion, M-102 can be assumed stable in muscle tissue for 4 days.

B.7.1.7 Storage stability of flutolanil and its metabolites in animal products

Previous evaluation	Submitted for the purpose of renewal
RMS remark	Acceptable

Reference:	**CA 6.1/07: Yoshizane T. (2017)**
Title:	Flutolanil: Storage stability for Flutolanil and its Metabolites in Foodstuffs of Animal Origin (Cow)
Document No.:	GE-03, 16-0103 (R-3403)
Guidelines:	OECD Guidelines for the Testing of Chemicals, Section 5: Test No. 506: Stability of Pesticide Residues in Stored Commodities (2007)
GLP:	Yes

Executive Summary

A storage stability was conducted for flutolanil and 4 of its metabolites (M-2, M-4, M-7 and M-101) in cow muscle, liver, kidney, milk and fat under deep frozen conditions (-20 °C) over a storage period up to 73-89 days.

The results from the storage stability investigations demonstrated that flutolanil, M-2, M-4, M-7 and M-101 were stable in cow muscle, liver, kidney, milk and fat for 74, 87, 73, 89 and 77 days respectively, except for M-2 and M-7 in cow muscle, where only 12% and 9% of the nominal spiking level, respectively, were detected.

4. MATERIALS AND METHODS

- **Materials:**

- Test Materials: Flutolanil

  Batch No.: 1AE0012P
  Purity: 99.6%
  CAS No.: 66332-96-5
  Spiking levels: 0.1 mg/kg
- Test Materials: M-2 (α,α,α-trifluoro-4'-hydroxy-3'-isopropoxy-o-toluanilide)

  Batch No.: 2AE0202P
  Purity: 99.8%
  CAS No.: -
  Spiking levels: 0.1 mg/kg
- Test Materials: M-4 (α,α,α-trifluoro-3'-hydroxy-o-toluanilide)

  Batch No.: 5AE0404S
  Purity: 100.0%
  CAS No.: -
  Spiking levels: 0.1 mg/kg
- Test Materials: M-7 (α,α,α-trifluoro-4'-hydroxy-3'methoxy-o-toluanilide)

  Batch No.: 4AE0702P
  Purity: 99.6%
  CAS No.: 360-64-5
  Spiking levels: 0.1 mg/kg
- Test Materials: M-101 (2-(Trifluoromethyl)benzamide)

  Batch No.: 336876
  Purity: 100%
  CAS No.: 360-64-5
  Spiking levels: 0.1 mg/kg
- Test commodity

  Crop: cow muscle, liver, kidney, milk and fat

- **Study Design:**

- **Test Procedure:**

Foodstuffs of animal origin (Cow muscle, liver, kidney, milk, fat) were purchased from local market. These matrices except for milk were stored frozen (<-20°C) until use. Milk was stored in refrigerator (<10°C) until use.

Nine control sub-samples of matrices (cow muscle, liver, kidney, milk and fat) were weighted into a suitable vessel and fortified according to the following regime:

- 5 remained untreated sub-samples
- 4 sub-samples fortified at 0.1 mg/kg with flutolanil and 4 metabolites (M-2, M-4, M-7 and M-101)

All the above sub-samples were then stored in a freezer at app. -20°C. At each stability time points (0 and 73-89 days), one untreated and two fortified samples were analysed along to one or two freshly fortified

sub-samples (two fortifications at 0 days and only one at the last stability time point) at 0.1 mg/kg with flutolanil and 4 metabolites.

- **Analytical method:**

Residues of flutolanil and its metabolites (M-2, M-4, M-7 and M-101) were determined following the validated analytical method “Flutolanil: Validation of methodology for the determination of residues of flutolanil and metabolites in bovine liver, kidney, muscle, fat, whole milk, skimmed milk and cream”, LMS0125 (A-3073). Summary of the validation data is presented in Volume 3, B.5.1.2.5: Methods used in support of residues study.

Samples (except fat) were extracted with acetonitrile and acidic acetonitrile and clean-up with a C18 solid phase extraction (SPE) cartridge. Fat samples were extracted with acetonitrile/hexane (50/50, v/v) and acidic acetonitrile/hexane (50/50, v/v) and cleaned-up by liquid-liquid partition. Quantification was performed using liquid chromatography with tandem mass spectrometric detection (LC-MS/MS).

5. RESULTS AND DISCUSSION

Procedural recoveries were also determined along with the analysis of the samples (n=1 at t=0 and n=2 at t=74d). Procedural recoveries were al between 83-117% and method performance was considered acceptable. The results from the storage stability investigations demonstrated that flutolanil, M-2, M-4, M-7 and M-101 were stable in cow muscle, liver, kidney, milk and fat for 74, 87, 73, 89 and 77 days, except for M-2 and M-7 in cow muscle, where only 12% and 9% of the nominal spiking level, respectively were detected. The results are presented in the table below.

Table B.7.1.7-1: Frozen storage stability of flutolanil and its metabolites in cow muscle, liver, kidney, milk and fat

Matrix	Analyte	Storage Period, days	Residue level in Freezer storage Stability sample, (% of nominal spiking level)	Mean Residue level in Freezer storage Stability sample, (% of nominal spiking level)	Procedural Recovery %
Muscle	Flutolanil	0	92, 94	93	92, 94
		74	79, 86	83	90
	M-2	0	89, 100	95	89, 100
		74	**13, 11**	**12**	96
	M-4	0	100, 99	100	100, 99
		74	70, 72	71	90
	M-7	0	94, 94	94	94, 94
		74	**10, 7**	**9**	97
	M-101	0	94, 95	94	94, 95
		74	90, 94	92	87
Liver	Flutolanil	0	92, 93	92	92, 93
		87	84, 83	84	87
	M-2	0	108, 99	104	108, 99
		87	93, 94	94	92
	M-4	0	117, 100	108	117, 100
		87	95, 95	95	93
	M-7	0	112, 98	105	112, 98
		87	93, 90	92	90
	M-101	0	89, 89	89	89, 89

		87	80, 87	83	89
Kidney	Flutolanil	0	90, 90	90	90, 90
		73	94, 93	93	96
	M-2	0	96, 102	99	96, 102
		73	77, 81	79	94
	M-4	0	105, 111	108	105, 111
		73	83, 81	82	93
	M-7	0	88, 91	89	88, 91
		73	76, 72	74	91
	M-101	0	87, 91	89	87, 91
		73	97, 90	94	93
Milk	Flutolanil	0	93, 95	94	93, 95
		89	79, 85	82	90
	M-2	0	85, 94	89	85, 94
		89	85, 91	88	91
	M-4	0	107, 107	107	107, 107
		89	92, 93	93	95
	M-7	0	91, 99	95	91, 99
		89	101, 105	103	92
	M-101	0	89, 91	90	89, 91
		89	84, 90	87	89
Fat	Flutolanil	0	104, 103	103	104, 103
		77	98, 101	99	92
	M-2	0	83, 86	85	83, 86
		77	105, 108	106	96
	M-4	0	109, 107	108	109, 107
		77	100, 103	101	97
	M-7	0	105, 109	107	105, 109
		77	94, 96	95	100
	M-101	0	101, 106	104	101, 106
		77	87, 88	87	95

Levels in bold display recoveries outside the acceptable range of 70-120% recovery.

6. CONCLUSION

The results from the storage stability investigations demonstrated that flutolanil, M-2, M-4, M-7 and M-101 were stable in cow muscle, liver, kidney, milk and fat for 74, 87, 73, 89 and 77 days respectively, except for M-2 and M-7 in cow muscle. In cow muscle, only 12% of the nominal spiking level for M-2 and 9% of the nominal spiking level for M-7 were detected.

B.7.1.8 Storage stability of flutolanil in soil

Previous evaluation	in DAR
RMS remark	Supplementary information only

Report: Wouters, G.A.J.M. (1999): Storage stability for flutolanil in soil, Analytico Research B.V., Breda, The Netherlands, Unpublished report No.: A-3026.

Guidelines: Directive 96/68EC, Document 7028/VI/95, Deviation : None
GLP: Yes (OECD)

Material and methods

Storage stability of flutolanil in four different European soils was tested at concentration levels of ca. 0.050 mg/kg and 0.50 mg/kg. Samples were stored frozen approximately at –18° C and analysed by GC/MS at each time intervals (0, ½, 1, 2, 4, 6, and 12 months).

Results

In general a recovery of >90% was observed after 6 months of storage. Though slightly decreased recovery, 80-90%, was observed after 12 months, this value could be adjusted to >90% when recoveries of flutolanil from freshly prepared addition samples (around 80%) were taken into consideration for correction.

Conclusion

During storage at -18° C flutolanil loss in spiked soil samples was not more than 10% per year. The study is supplementary to studies on rotational crops. Soil types were not characterised.

Remark RMS:

The study has been copied from the original DAR. It should be noted that storage stability in soil samples is not a data requirement. The study has limited data and it was not evaluated in the details by RMS.

B.7.2 Metabolism, distribution and expression of residues

During the initial EU review of the active substance flutolanil, the metabolism in plant has been studied in potatoes, peanut and rice. In animal matrices, metabolism has been studied in goats and laying hens.
For the purpose of the renewal new metabolism studies have been submitted: in plants in leafy crops (cabbage), miscellaneous crops (paddy rice) and root crops (potatoes). New metabolism studies have also been submitted in animal matrices: in laying hens and lactating ruminants.

B.7.2.1 Plants

B.7.2.1.1 Metabolism in potatoes

Previous evaluation	in DAR , with additional analytical information included during the renewal process
RMS remark	Acceptable

Report: Lewis, C.J. (1999) ^{14}C-Flutolanil: Metabolism in potatoes, Covance Laboratories Ltd., North Yorkshire, England, Unpublished report No.: R-3025.

Guideline: Directive 96/68EC, Document 7028/VI/95, USA EPA/1996, JPN MAFF/1985.

GLP: Compliance with UK/1997, OECD/1982, USA EPA/1989, and JPN MAFF/1984.

Test formulation:	In seed treatments: solution of a.s. in acetonitrile corresponding to a dose level 120 – 360 mg/kg in tuber. In row treatment: as Moncut 40SC formulation, containing 45% w/v of a.s. at a dose rate of 4.5 kg/ha
Radioactive probe:	[aniline ring -^{14}C(U)] flutolanil; specific activity 2.76 MBq/mg; radiochemical purity ≥ 99 %.
Test site:	Outside enclosure.
Soil type:	Sandy loam.

Material and methods

The metabolism of flutolanil in potatoes (Solanum tuberosum, var. Estima) was studied by pre-planting administration of ^{14}C-flutolanil formulation (aniline labeled) either by topical application of 27-86 µl volumes onto surface of seed potatoes as a single dose corresponding to 120 mg/kg or onto soil by single row treatment at dose level of 4.5 kg/ha on the day of planting. Although not intended for row treatments, this dose rate would correspond to 225 g a.s. /ha. Concentration of a.s. in the formulation (45% w/v) used in these settings was the same as described for intended use (460 g/l). In these two experiments mature potatoes were harvested after 131 days from planting.

In a third experimental setting a.s. (360 mg/kg) was administered by seed treatment and the potatoes were harvested already at day 52 from planting in order to facilitate identification of metabolites in immature tubers.

Biodistribution was further investigated by additional analyses of foliage, peel and flesh samples obtained from each experiment.
Adhering soil was removed from the tubers by brushing. The foliage and tuber samples were chopped and frozen at -10 C on the day of collection.
Prior to chromatographic analyses, homogenised tuber samples were extracted for two minutes sequentially with acetonitrile, acetonitrile:water and water. Extracts were pooled, concentrated and the processed using a variety of techniques including portioning with dichloromethane under neutral, acidic and basic conditions: precipitation with ice cold water, elution from C18 solid phase extraction (SPE) cartridges. Final extracts were analysed by reverse-phase HPLC and normal-phase TLC was used to confirm indentification of analytes. Samples were also analysed to reveal conjugation of the parent and its metabolites. A modified sample extraction procedure involving solid-phase extraction was employed for analyses of residues in foliage. Radioactivity remaining in PES samples of mature tubers after extraction with neutral solvents were characterised by additional extractions as follows: incubation in 4N HCl for 16h at 50°C and incubaction in 2N NaOH for 8h at 50°C. As before, following extraction the supernatant was separated by centrifugation and radioactivity present in extracts quantified by liquid scintillation counting (LSC) and radioactive residues in the post extraction solids (PES) quantified by combustion. The radioactivity in the acidic/basic extracts was further characterised by partitioning with dichloromethane at acid (pH 2 with hydrochloric acid), neutral and basic pHs.

Extracts originating from the combined acetonitrile/water and water extracts of foliage and mature tubers were subject to the following hydrolysis step prior to partitioning with dichloromethane and re-analysis by HPLC and TLC: Incubation in 4N HCl for 16 hours at 50°C

The peaks referred to Metabolite A and B were identified as a conjugates of M-4 and M-2 by identification of the aglycones by HPLC and confirmed by TLC post hydrolysis.

Organic extracts were analysed within 3 months and aqueous exctracts within 6 months from collection of samples. In order to investigate residue stability one aqueous extract was analysed within one month and reanalysed after 5 months.

Metabolic profile was obtained from the collected extracts corresponding to >10% of total radioactive residue (TRR) by reversed-phase radio-HPLC with gradient elution and straight-phase TLC and radioluminescence imaging. The identity of each radioactive metabolite was relied on HPLC and TLC co-chromatography with reference to synthetic putative metabolites.

Results

Total Radioactive Residues

In the table below Total radioactive residues (TRR) in potato crops following treatment with [aniline-U-^{14}C]-flutolanil are presented:

Application	Sample	Days after treatment	TRR (mg as-eq/kg)
Seed treatment (360 mg/kg)	Immature potatoes	52	0.029
	Foliage (Subsample A)		0.295
	Foliage (Subsample A)		0.327
Seed treatment (120 mg/kg)	Mature tubers	131	0.014
	Peel		0.044
	Flesh		0.009
In-furrow application (4.5 kg/ha)	Mature tubers	132	0.119

At mature harvest highest residues were found in tubers following soil furrow application as expected based on the higher application rate. At immature harvest the radioactive residues measured in tubers was more than 10 times lower than the residues measured in foliage.

Extractability and Characterisation of residues

A summary of the extractability of the radioactive residues for tubers and foliage is presented below:

Addiational distribution of radioactive residues in potato tuber after application of [aniline-U-^{14}C]-flutolanil**,** submitted by the applicant in the renewal process:

Radiolabel	[Aniline-U-^{14}C]-Flutolanil					
Application	Seed treatment (360 mg/kg)		Seed treatment (120 mg/kg)		In furrow treatment (4.5 kg/ha)	
Sample	Immature tuber		Mature tuber		Mature tuber	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
TRR		0.029		0.014		0.119
Acetonitrile	72.4	0.021	54.3	0.008	62.5	0.074
Acetonitrile:Water	2.2	0.0006	5.7	<0.001	6.1	0.006
Water	2.6	0.0008	1.4	<0.001	ND	ND
PES	22.9	0.007	38.5	0.005	31.3	0.037

Addiational distribution of radioactive residues in potato foliage after application of [aniline-U-^{14}C]-flutolanil, submitted by the applicant in the renewal process:

Radiolabel	[Aniline-U-^{14}C]-Flutolanil			
Application	Seed treatment (360 mg/kg)			
Sample	Foliage (Subsample A)		Foliage (Subsample B)	
	%TRR	mg/kg	%TRR	mg/kg
TRR		0.295		0.327
Acetonitrile	69.2	0.204	55.1	0.180
Acetonitrile:Water	4.7	0.014	4.9	0.016
Water	ND	ND	ND	ND
PES	26.1	0.077	38.9	0.131

Residues ranging from 23 to 39 % of TRR in tubers were non-extractable into acetonitrile or aqueous acetonitrile mixtures in all experiments. In immature potato tubers, seed-treated at an exaggerated rate, 50% of the extractable residue was soluble in dichloromethane.

In mature, 1.3 N seed-treated tubers, the most abundant extractable residues were Met A, Met B and untransformed flutolanil. In immature tubers residue levels were higher and the main residue was untransformed flutolanil. Row treatment, at the selected dose rate, resulted in highest residue levels most of which comprised of untransformed flutolanil and Met A (Table B.7.2.1.1-1)

Samples were kept up to 6 months frozen prior analyses of Met A and Met B. Reanalysis of one sample after 5 month storage indicated that the ratios of these metabolites were similar: 0.42, initially and 0.52 at reanalysis.

On basis of these non-validated analyses it was estimated that the recoveries in the reanalysis for Met A and Met B were in the range 82-126% and consequently indicate that degradation during storage for these metabolites were not more than 30%.

In addition, small amounts of α, α, α -trifluoro-3'-hydroxy-o-toluanilide (M-4/DIP) and α, α, α -trifluoro-3',4'-dihydroxy-o-toluanilide (M-5/HDP) were detected in the extract of immature tubers. Hydrolysis of

extracted radioactivity liberated M-4 from Met A and α,α ,α -trifluoro-4'-hydroxy-3'-isopropoxy-o-toluanilide (M-2/HFT) from Met B. This indicated that Met A was mostly comprised of conjugated M-4 and Met B of conjugated M-2 (Table B.7.2.1.1-2)

In the extracts of immature foliage, flutolanil, Met A, Met B and α, α, α-trifluoro-4'-hydroxy-3'-methoxy-o-toluanilide (M-7/HMD) were detected.

The non-extractable radioactivity in mature tuber fiber (39% of TRR by seed treatment and 31% of TRR by row treatment) was first subjected to acid and then base catysed hydrolysis. At each hydrolysis step only 5-7% of TRR was released from the tuber fiber. Residue levels in peel and flesh separated from an at 1.3 N seed-treated mature tuber were 44 µg/kg and 8 µg/kg, expressed as flutolanil equivalents respectively, i.e. peel having aproximately 5 times higher concentration as compared with flesh.

In immature potatoes, seed-treated with exaggerated application rate and with reduced PHI, residue levels in foliage were 10 times higher as compared to tubers and consisted mainly of Met A, Met B and M-7.

Row treatment at exaggerated dose level resulted in higher residue levels, which were mainly due to untransformed flutolanil (35%) and to fiber-incorporated, non-extractable fraction (31%). The higher residue levels were most likely arising from the difference in application rate. The 120 mg/kg corresponds to 0.6 kg a.s./ha, which is markedly lower than the rate employed in row treatment 4,5 kg a.s./ha.

Table B.7.2.1.1-1a Identity of flutolanil residues in potato tuber as % of TRR or as flutolanil equivalents µg/kg in tubers, either treated indirectly by row treatment or directly by seed treatment prior to planting.

	Row Treatment		Seed treatment			
	Mature tuber (4.5 kg/ha)		Mature tuber (120 mg/kg)		Immature tuber (360 mg/kg)	
	PHI 131 days		PHI 131 days		PHI 52 days	
Metabolite #	% TRR	µg/kg equiv	% TRR	µg/kg equiv	% TRR	µg/kg equiv
ERR	69	73	62	8	77	23
Flutolanil	35	42	16	2	57	16
M-4	-	-	-	-	2	1
M-5	-	-	-	-	2	1
Met A	21	24	23	3	6	2
Met B	6	7	14	2	3	1
Other	-	-	-	-	1	<1
Not analysed	7	9	9	1	6	2
URR	31	37	39	5	23	7
Accountabilty	100	119	100	14	100	29

TRR, total radioactive residues; ERR, extracted radioactive residues; URR, unextracted radioactive residues**;** NA, not analysed; accountability, sum of ERR and URR

Table B.7.2.1.1-1b: Addiational identification of radioactive residues in potato tuber after application of [aniline-U-^{14}C]-flutolanil, submitted by the applicant in the renewal process:

Radiolabel	[Aniline-U-^{14}C]-Flutolanil					
Application	Seed treatment (360 mg/kg)		Seed treatment (120 mg/kg)		In-furrow treatment (4.5 kg/ha)	
Sample	Immature tuber		Mature tuber		Mature tuber	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
Flutolanil	57	0.016	16	0.002	35	0.042
M-2	ND	ND	ND	ND	ND	ND
M-4	2	0.001	ND	ND	ND	ND
M-5	2	0.001	ND	ND	ND	ND
M-7	ND	ND	ND	ND	ND	ND
Metabolite A (M-4 conjugate)	6	0.002	23	0.003	21	0.024
Metabolite B (M-2 conjugate)	3	0.001	14	0.002	6	0.007
Others	1	<0.001	ND	ND	ND	ND
Not analysed	6	0.002	9	0.001	7	0.009
PES Characterisation						
PES after neutral extraction	23	0.007	39	0.005	31	0.037
Further PES extractions						
4N HCl Reflux	-	-	7	0.001	6	0.007
2N NaOH Reflux	-	-	5	0.0007	5	0.006
PES after strong basic extraction	-	-	27	0.004	20	0.024

Table B.7.2.1.1-1c: Addiational identification of radioactive residues in potato foliage after application of [aniline-U-^{14}C]-flutolanil, submitted by the applicant in the renewal process:

Radiolabel	[Aniline-U-^{14}C]-Flutolanil					
Application	Seed treatment (360 mg/kg)					
Sample	Foliage (Subsample A)		Foliage (Subsample B before fractionation)		Foliage (Subsample B after fractionation)	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
Flutolanil	4	0.010	3	0.010	3	0.009
M-2	ND	ND	ND	ND	ND	ND
M-4	ND	ND	ND	ND	ND	ND
M-5	ND	ND	ND	ND	ND	ND
M-7	6	0.017	7	0.022	7	0.024
Metabolite A (M-4 conjugate)	13	0.038	10	0.034	11	0.036
Metabolite B (M-2 conjugate)	44	0.131	21	0.069	28	0.093
Others	4	0.013	19	0.061	10	0.033
Not analysed	3	0.008	-	-	<1	0.001
PES	26	0.077	40	0.131	40	0.131

Table B.7.2.1.1-2 Degree of conjugation among flutolanil and its metabolites in row-treated and seed-treated (120 mg/kg) mature potatoes after a PHI of 131 days as % TRR or as flutolanil equivalents µg/kg.

	Row Treatment (4.5 kg/ha)		Seed treatment (120 mg/kg)	
Metabolite #	% TRR	µg/kg equiv	% TRR	µg/kg equiv
Flutolanil	30	35	13	2
M-2			8	1
M-4	9	11	11	2
Unknown	11	13	11	1
Total	50	59	41	6

Conclusions

This study indicates that in seed-treated potato tubers, at 1.3N, the extractable residue is mainly comprised of untransformed flutolanil (16%), Met A (23%) and Met B -fraction (14%). The two latter metabolites mainly represent conjugates of M-4 (desisopropyl flutolanil) and M-2 (hydroxyl-flutolanil), respectively. Of total radioactive residue, 39 % was incorporated into non-extractable fraction, from which relatively small amounts of residual radioactivity could be liberated by acid or base hydrolysis, or by extraction.

The chosen formulation (a.s. in acetonitrile) is not exactly the same as the one disclosed for intended use, but has comparable a.s. concentration. The rationale behind using as low volumes as possible for application of a.s. in seed treatments represents another slight deviation from intended use, since surface

area is one of the factors determining absorption rate and subsequent residue levels. Study protocol included also an experiment in which dose rate was comparable to intended use Degradation during 5 month storage was estimated not to exceed 30% as judged from two submitted chromatograms showing Met A and Met B peaks.

The study fulfills its main objective to elucidate metabolic pathways of flutolanil and hence is acceptable.

B.7.2.1.2 Metabolism in potatoes

Previous evaluation	Submitted for the purpose of the renewal
RMS remark	Acceptable

Report:	**CA 6.2.1/06. Ki Chang Ahn (2016)**
Title:	A metabolism study with [trifluoromethyl ring-U-^{14}C]flutolanil (1 radiolabel) in potatoes
Document No:	2556W-1 (R-3381)
Guidelines:	OECD 501 (2007)
Deviations:	None
Testing laboratory:	PTRL West, Hercules, California, USA
GLP:	Yes

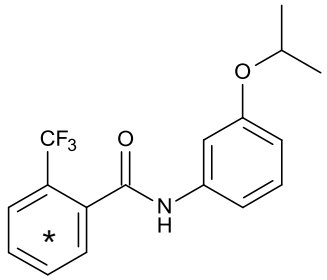
Executive Summary

The metabolism of flutolanil was investigated in potatoes (variety Red Lasoda). A simulated 40SC formulation of [phenyl-U-^{14}C]-flutolanil (called [trifluoromethyl ring-U-^{14}C]-flutolanil in the report) was applied to seed potatoes by uniformly painting tubers at a rate of 123.1 mg a.s./kg seed potatoes (equivalent to 553.1 g a.s./ha) or to soil at a rate of 2530.2 g a.s./ha prior to planting.
The potato crop was grown to maturity under glasshouse conditions in California, USA. Immature harvest samples were taken as foliage and new potatoes 89-90 days after application. Final mature harvest potatoes were taken on 121-122 days after application (BBCH 92).
In tubers, flutolanil accounted for between 9.9% to 47.8% TRR. The other main components of the tuber residue were M-102 (16.4% to 38.3% TRR), M-101 (6.2% to 11.9% TRR) and M-4, both as the free metabolite and as a glycoside conjugate (<1.5% to 10.5% TRR). M-4 was formed as a major metabolite (>10% TRR) only in seed treated mature tubers where it was present in trace amounts < 0.01 mg eq./kg (maximum 0.007 mg eq./kg). In other potato RACs it was a minor metabolite present at <10% TRR (maximum 8.2% TRR). In addition, M-2 and M-3, both as free metabolites and as glycoside conjugates, M-6 and M-7, were detected as minor metabolites (≤ 2.1% TRR) in tubers. A similar metabolic pattern was seen in foliage.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material: [Phenyl-U-^{14}C]-flutolanil



* Denotes position of [^{14}C]-radiolabel

Chemical name (IUPAC)	α, α, α-trifluoro-3'-isopropoxy-*o*-toluanilide
CA registry number:	66332-96-5
Lot or batch number:	CFQ42127
Specific activity:	Original 118 mCi/mmol
Radiochemical purity:	≥ 98.0 %

III. STUDY DESIGN AND METHODS

1. In-life dates:

14 November 2014 to 28 April 2016

2. Experimental design

Test System:

Potato tubers (*Solanum tuberosum*, variety Red Lasoda) were sown into boxes (24 x 36 inches, 18 inches deep). In total two treated plots and one control plot were prepared. Each plot consisted of 2 boxes, each with 5 potatoes approximately 7 inches apart, for a total of 10 plants per plot. Control plants were grown in a separate glasshouse from the treated plants.

Test Soil:

Each box was filled with soil classified as a loamy sand. Further details are tabulated below.

Parameter	
Texture Class (USDA)	Loamy sand
pH (water)	5.8
Organic matter (%)	0.65
Cation exchange capacity (meq/100 g)	11.9
Bulk density (gm/cc)	1.44
USDA classification Sand (>50 µm) % Silt (2 - 50 µm) % Clay (< 2 µm) %	 81 13 6
Moisture content 1/3 bar (%)	8.9

Experimental Conditions:

[^{14}C]-Flutolanil was prepared as Moncut 40SC using blank formulation. The [^{14}C]-formulation was applied to seed potatoes by uniformly painting the surface on 9 December 2014 and the tubers planted the following day in Plot 2 on 10 December 2014. On 10 December 2014 [^{14}C]- formulation was applied to soil by hand operated sprayer in Plot 3 and untreated seed potatoes planted on the same day. The potato crop was grown to maturity under glasshouse conditions at the field site in Tulare County, California, USA. Immature harvest samples were taken as foliage and new potatoes on 9 March 2015, 89-90 days after application. Final mature harvest samples were taken on 10 April 2015, 121-122 days after application.

	Seed treatment	In furrow treatment
Nominal application rate	120 mg a.s./kg seed potatoes	2100 g a.s./ha
Number of applications	1	1
Target seasonal application rate	120 mg a.s./kg seed potatoes	2100 g a.s./ha
Achieved seasonal application rate	123.1 mg a.s./kg seed potatoes (equivalent to 553.1 g a.s./ha) [A]	2530.2 g a.s./ha
Application date	9 December 2014	10 December 2014
Sowing date	10 December 2014	10 December 2014
Formulation code	Moncut 40SC	Moncut 40SC
Method of application	Uniformly painted onto tuber prior to planting following day	Hand held sprayer to soil prior to planting untreated tuber same day
Growth stage at application	BBCH 0	BBCH 0
Environmental conditions	Glasshouse conditions	Glasshouse conditions

[A] Based on weight of treated seed potatoes (494.249 g) in the plot (2 boxes of surface area 24 x 36 inches, 1.1 m2 in total)

Test Samples

Samples (both treated and control) were taken for analysis at the following times:

Sample	Date	Days after tuber treatment	Days after soil treatment
Foliage	9 March 2015	89	90
New potatoes	9 March 2015	89	90
Mature tubers (BBCH 92)	10 April 2015	121	122

Sample Preparation

Foliage and immature tubers were collected at the interim harvest. At final harvest mature tubers were collected (BBCH 92). No foliage was collected at maturity. Adhering soil was removed from the tubers by brushing. Tubers were washed first with distilled water and then with acetonitrile. Samples of foliage, immature tubers, mature tubers, distilled water washes and acetonitrile washes were stored frozen until shipment to the analytical laboratory in Hercules, California, USA.

Rinsed tubers were homogenised by macerator with dry ice, which was then allowed to sublimate in a freezer overnight. Total radioactive residues (TRR) were determined by combustion. Homogenised samples were stored at -20°C until required for analysis.

Extraction and Fractionation of Residues

Samples of homogenised plant samples were sequentially extracted by wrist action shaker for 60 minutes using the following sequence of solvents:

Acetonitrile : water (1:1, v/v), 2 times
Acetonitrile, once

Following each extraction, the supernatant separated by centrifugation. Radioactivity present in extracts was quantified by liquid scintillation counting (LSC).

Extracts were pooled, concentrated and analysed by reverse phase HPLC and normal phase TLC. Following extraction, remaining radioactive residues in plant matrices were quantified by combustion.

As levels of unextracted residues exceeded 10% TRR all sample were subject to further investigation using the following sequence of extraction:

0.1 N HCl : acetonitrile (1:4, v/v)
1 N HCl : acetonitrile (1:4, v/v)
0.1 N NaOH : acetonitrile (1:4, v/v)
1 N NaOH : acetonitrile (1:4, v/v)

As before each extraction was for 60 minutes using a wrist action shaker and following extraction, the supernatant was separated by centrifugation. Radioactivity present in extracts was quantified by liquid scintillation counting (LSC). Combined weak acidic/basic extracts containing > 0.01 mg eq./kg were analysed by HPLC and TLC.

The radioactive residues in the post extraction solids (PES) were quantified by combustion.

Combined acetonitrile/water and combined weak acidic/basic extracts were analysed for recovery of radioactivity following concentration. Average recoveries were 98.1 ± 6.9%. The residues were finally dissolved in a mixture of acetonitrile : water (generally 1:4, v/v) prior to HPLC analysis. For TLC analysis concentrates were mixed with analytical standards and then further concentrated under a stream of N2 gas prior to analysis.

Radioactivity remaining in PES samples after weak acidic/basic extractions from immature and mature tubers after furrow soil application were characterized by additional extractions as follows:

Incubation in 200 mM acetate buffer (pH 5) containing cellulase for 24 hours at 37°C
Reflux with 6N HCl for 2 hours
Reflux with 10 M NaOH for 2 hours

As before, following extraction the supernatant was separated by centrifugation and radioactivity present in extracts quantified by liquid scintillation counting (LSC) and radioactive residues in the post extraction solids (PES) quantified by combustion. The radioactivity in the cellulose and strong acidic/basic extracts was further characterised by partitioning with ethyl acetate and butanol. The organic layers were analysed by TLC.

Combined acetonitrile/water extracts of foliage, immature tubers (furrow treatment only) and mature tubers were partitioned with ethyl acetate under acidic conditions. An unidentified polar region was

isolated from the origin site of preparative TLC plates following normal phase 2D TLC analysis of the organic phases and subject to the following hydrolysis prior to re-analysis by HPLC and TLC:

Incubation in 2N HCl for 24 hours at 50 or 60°C

Incubation in 100 mM acetate buffer (pH 5) containing β-glucosidase for 48 hours at 37°C

The polar region was identified as glycoside conjugates of M-2, M-3, M-4 and in one case (furrow treated immature tubers) trace levels of glycoside conjugate of M-7 by identification of the aglycones by HPLC and confirmed by 2D TLC post hydrolysis.

Radioactivity in the aqueous phase was shown to be polar consisting of origin material in the standard 2D TLC system which was separated by developing the plates in a further 1D TLC system. The plates were sprayed with 0.2% ninhydrin solution in ethanol to detected the presence of ammonia, primary or secondary amines to characterize any residues related to proteins, peptides and amino acids.

II. RESULTS AND DISCUSSION

Total Radioactive Residues

The total radioactive residues (TRR) measured by combustion and by summing the residues measured in extracts and post extraction solids are summarised below in Table B.7.2.1.2-1B.7.2.1.2-1. TRR values obtained by both methods are in good agreement. Highest residues were found following soil furrow application as expected based on the higher application rate. For both treatments the radioactive residues measured in tubers was on average 5 times lower than the residues measured in foliage.

Table B.7.2.1.2-1: Total radioactive residues (TRRs) in potato crops following treatment with [phenyl-U-^{14}C]-Flutolanil

Matrix	Application	Days after treatment	TRR by combustion (mg as-eq/kg) [A]	TRR by extraction (mg as-eq/kg) [A]
Interim - Foliage	Seed tuber	89	0.382	0.362
Interim – Immature tuber		89	0.062	0.065
Mature tuber		121	0.063	0.067
Interim - Foliage	Soil furrow	90	2.459	2.713
Interim – Immature tuber		90	0.726	0.680
Mature tuber		122	0.492	0.486

[A] TRR includes radioactive residues measured in water and acetonitrile rinses of tubers

Extraction and Characterisation of Residues

A summary of the extractability of the radioactive residues is provided in table B.7.2.1.2-2.
Foliage samples were collected at harvest as a potential aid to metabolite identification and are not a raw agricultural commodity of potatoes. In foliage 55.1 to 60.8% of the TRR was extracted with neutral solvents i.e. acetonitrile : water (1:1, v/v) and acetonitrile, with a further 3.2 to 4.4% TRR released with weak acidic and basic extractions i.e. mixtures of acetonitrile with dilute (0.1 N and 1 N) acid and base. Unextracted bound residues in post-extracted solids (PES) accounted for 34.8% to 41.7% TRR.

Tubers were rinsed first with distilled water and then with acetonitrile, prior to extraction with neutral solvents and a series of weak acidic and basic solvent mixtures. In total the % TRR removed by rinsing ranged from 2.1% in mature furrow treated tubers to 12% in mature seed treated tubers, with the exception of treated immature seed treated tubers where rinses removed 41.6 % of the TRR. The proportion of radioactivity extracted from tubers with neutral solvents ranged from 43.1 to 67.7% TRR and with weak acidic and basic extractions from <1.5 to 6.8% TRR. Unextracted bound residues in PES accounted for 15.4% to 26.7% TRR. In seed treated tubers residues in PES represented < 0.05 mg eq./kg (maximum 0.017 mg eq./kg) and were not characterised further. In furrow treated tubers PES were further characterised by incubating with cellulase, followed by refluxing with 6N HCl and 10 N NaOH. The proportion of radioactivity extracted was 26.3 to 26.8% TRR. Unextracted bound residues in PES after strong basic extraction in furrow treated tubers accounted for 10.1% to 11.3% TRR.

Table B.7.2.1.2-2 : Distribution of radioactive residues in potatoes after application of [phenyl-U-^{14}C]-Flutolanil

Radiolabel	[Phenyl-U-^{14}C]-Flutolanil					
	Immature Foliage		Immature tuber		Mature tuber	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
Seed treatment						
Water rinse	-	-	18.5	0.012	3.0	0.002
Acetonitrile rinse	-	-	23.1	0.015	9.0	0.006
ACN/Water	60.8	0.220	43.1	0.028	61.2	0.041
0.1 N HCl/ACN	0.6	0.002	0.0	0.0	0.0	0.0
1N HCl/ACN	0.8	0.003	0.0	0.0	0.0	0.0
0.1N NaOH/ACN	0.8	0.003	0.0	0.0	0.0	0.0
1N NaOH/ACN	2.2	0.008	0.0	0.0	1.5	0.001
PES	34.8	0.126	15.4	0.010	25.4	0.017
In furrow treatment						
Water rinse	-	-	1.5	0.010	1.2	0.006
Acetonitrile rinse	-	-	5.3	0.036	1.9	0.009
ACN/Water	55.1	1.496	60.0	0.408	67.7	0.329
0.1 N HCl/ACN	0.6	0.017	2.1	0.014	0.6	0.003
1N HCl/ACN	0.7	0.019	0.7	0.005	0.4	0.002
0.1N NaOH/ACN	0.7	0.018	0.9	0.006	0.2	0.001
1N NaOH/ACN	1.2	0.033	3.1	0.021	1.2	0.006
PES	41.7	1.130	26.5	0.180	26.7	0.130

The identification and characterisation of radioactive residues in potatoes following seed treatment are summarised in Table 3 and in Table 4 following in furrow application.

In foliage samples, flutolanil was detected at a maximum of 2.2% TRR (0.008 mg/kg) and 1.1% TRR (0.031 mg/kg) in seed and furrow treated samples respectively. Two major metabolites (>10% TRR) were observed in foliage; M-101 and M-2. The latter was present largely as a glycoside conjugate with small amounts of the free metabolite also detected. In addition M-4, both as the free metabolite and as a glycoside conjugate, M-6 and M-102 were observed as minor metabolites (<10% TRR in total).

In tubers, flutolanil was the only significant residue identified in rinses. Overall flutolanil accounted for 9.9% to 47.8% TRR (0.013 to 0.146 mg/kg) in tuber samples. The other main components of the tuber residue were M-102 (in total 16.4% to 31.3% TRR; 0.011 to 0.1852 mg eq./kg), M-101 (in total 6.2% to 11.9% TRR; 0.004 to 0.008 mg eq./kg) and M-4, both as the free metabolite and as a glycoside conjugate (in total 1.5% to 10.5% TRR; 0.001 to 0.007 mg eq./kg). The levels of M-102 and M-101 include additional amounts of the metabolites released by weak acidic/basic extractions, cellulase incubations and strong acidic/basic extractions in furrow treated tubers. M-4 was formed as a major metabolite (>10% TRR) only in seed treated mature tubers where it was present in trace amounts < 0.01 mg eq./kg (maximum 0.007 mg eq./kg). In other potato RACs it was a minor metabolite present at <10% TRR (maximum 8.2% TRR). In addition, M-2 and M-3, both as free metabolites and as glycoside conjugates, M-6 and M-7, were detected as minor metabolites (≤ 2.1% TRR per metabolite) in tubers.
In foliage and furrow treated tubers additional unidentified polar residues were characterised as proteins/peptides residues by their reaction with ninhydrin.

Table B.7.2.1.2-3: Characterisation and identification of radioactive residues in potatoes after seed treatment with [phenyl-U-^{14}C]-Flutolanil

Radiolabel	[Phenyl-U-^{14}C]-Flutolanil					
Seed treatment	Immature Foliage		Immature tuber		Mature tuber	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
Flutolanil	2.2	0.008	47.8	0.031	19.4	0.013
M-2	1.9	0.007	ND	ND	<1.5	<0.001
M-3	ND	ND	ND	ND	ND	ND
M-4	1.7	0.006	<1.5	<0.001	1.5	0.001
M-5	ND	ND	ND	ND	ND	ND
M-6	ND	ND	ND	ND	ND	ND
M-7	ND	ND	ND	ND	ND	ND
M-11	ND	ND	ND	ND	ND	ND
M-101	17.9	0.065	6.2	0.004	11.9	0.008
M-102	0.8	0.003	20.0	0.013	16.4	0.011
Glycoside conjugate of M-2	10.2	0.037	ND	ND	ND	ND
Glycoside conjugate of M-3	ND	ND	ND	ND	ND	ND
Glycoside conjugate of M-4	7.5	0.027	ND	ND	9.0	0.006
Ninhydrin +ve protein residue	7.7	0.028	ND	ND	NA	NA
Non-ninhydrin +ve protein residue	5.0	0.018	ND	ND	NA	NA
Unassigned others	10.2	0.037	10.8	0.007	11.9	0.008
Maximum other single	2.5	0.009	9.2	0.006	11.9	0.008
PES	34.8	0.126	15.4	0.010	24.4	0.017

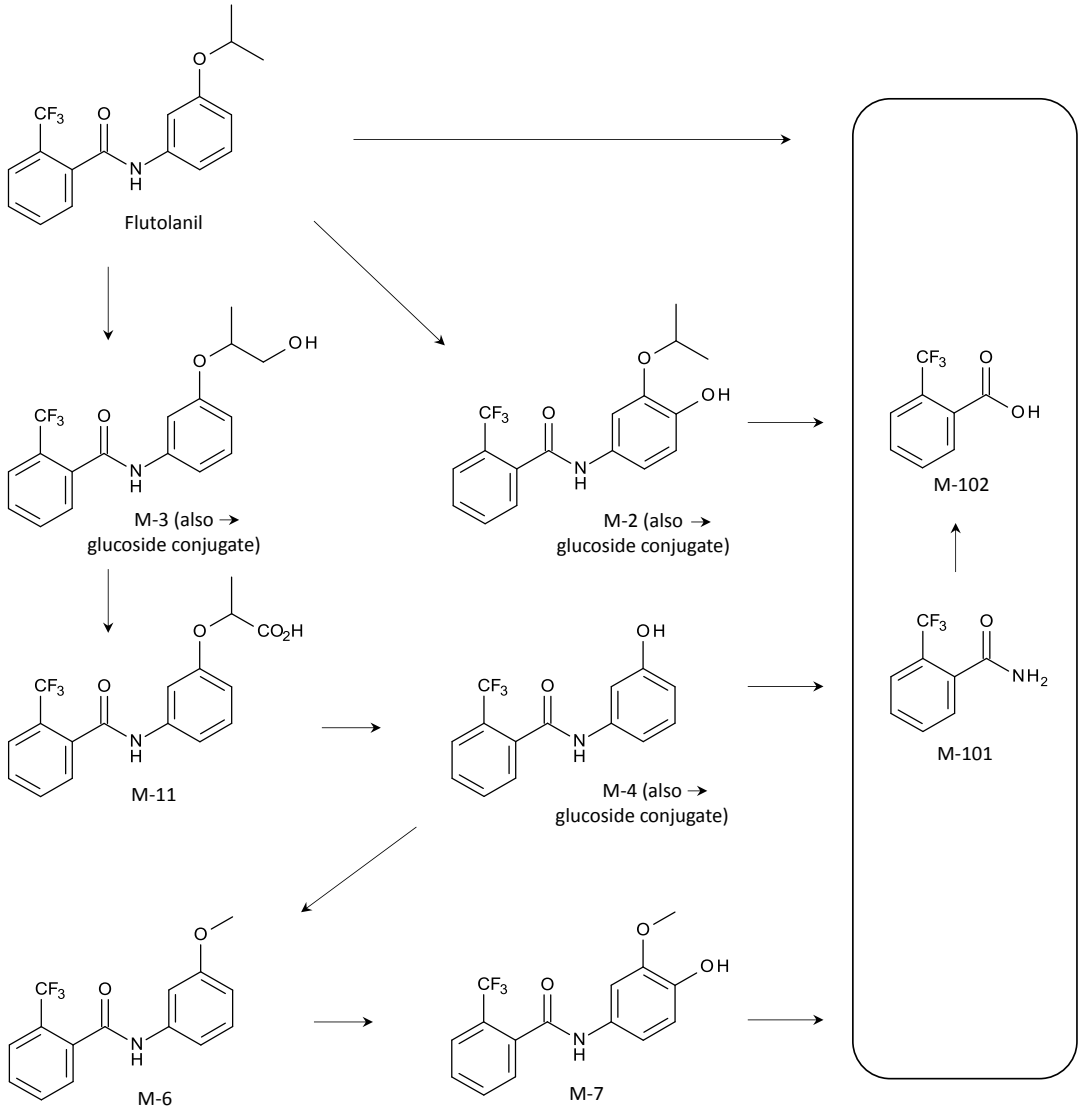
Table B.7.2.1.2-4: Characterisation and identification of radioactive residues in potatoes after in furrow treatment with [phenyl-U-^{14}C]-Flutolanil

Radiolabel	[Phenyl-U-^{14}C]-Flutolanil					
In furrow treatment	Immature Foliage		Immature tuber		Mature tuber	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
Flutolanil	1.1	0.031	21.5	0.146	9.9	0.048
M-2	0.9	0.025	0.6	0.004	0.4	0.002
M-3	ND	ND	0.3	0.002	ND	ND
M-4	0.7	0.019	2.2	0.015	1.4	0.007
M-5	ND	ND	ND	ND	ND	ND
M-6	0.4	0.010	0.1	0.001	ND	ND
M-7	ND	ND	0.1	0.001	ND	ND
M-11	ND	ND	ND	ND	ND	ND
M-101	14.2	0.384	9.7	0.066	8.5	0.041
M-102	2.0	0.055	18.8	0.128	31.3	0.152
Glycoside conjugate of M-2	14.3	0.388	1.5	0.010	1.0	0.005
Glycoside conjugate of M-3	ND	ND	0.9	0.006	ND	ND
Glycoside conjugate of M-4	7.2	0.194	6.0	0.041	6.2	0.030
Ninhydrin +ve protein residue	8.3	0.224	4.0	0.027	8.4	0.041
Non-ninhydrin +ve protein residue	3.4	0.091	3.4	0.023	3.9	0.019
Unassigned others	6.0	0.162	4.4	0.030	2.2	0.011
Maximum other single	1.7	0.046	1.5	0.010	1.0	0.005
PES	41.7	1.130	26.5	0.180	26.7	0.130
Further PES characterisation						
Cellulase	-	-	3.8	0.026	3.9	0.019
Partition of Cellulase Extracts						
Aqueous layer	-	-	0.7	0.005	0.6	0.003
Biosolids	-	-	0.6	0.004	1.0	0.005
Organic layer	-	-	2.4	0.016	2.3	0.011
Analysis of organic layer						
M-101	-	-	1.2	0.008	1.0	0.005
Others	-	-	1.2	0.008	1.2	0.006
Maximum other single	-	-	0.7	0.005	0.4	0.002
6N HCl Reflux	-	-	4.1	0.028	3.5	0.017
10N NaOH Reflux	-	-	7.1	0.048	9.3	0.045
Partition of Strong Acidic/Basic Extracts	-	-	11.2	0.076	12.8	0.062
Aqueous layer	-	-	1.2	0.008	0.6	0.003
Organic layer	-	-	10.0	0.068	12.1	0.059
Analysis of organic layer						
M-102	-	-	5.7	0.039	7.0	0.034
Others	-	-	4.3	0.029	5.1	0.025
Maximum other single	-	-	3.2	0.022	3.9	0.019
PES after strong basic extraction	-	-	11.3	0.077	10.1	0.049

Metabolic pathway

A metabolic pathway for [phenyl-U-^{14}C]-flutolanil in potatoes is proposed in Figure B.7.2.1.2-5.

Figure B.7.2.1.2-5: Proposed metabolic profile of [phenyl-U-^{14}C]-flutolanil in potatoes



4. CONCLUSIONS

At harvest seed treated new potatoes and mature tuber samples contained TRRs of 0.065 and 0.067 mg eq./kg, while furrow treated samples contained TRRs of 0.680 mg eq./kg and 0.486 mg eq./kg, respectively.

The major components detected in potatoes grown from seed treated tubers were:

- Immature tubers: Flutolanil (47.8% TRR, 0.031 mg/kg) and M-102 (20.0% TRR, 0.013 mg eq./kg)
- Mature tubers: Flutolanil (19.4% TRR, 0.013 mg/kg), M-102 (16.4% TRR, 0.011 mg eq./kg), M-101 (11.9% TRR, 0.008 mg eq./kg) and M-4 both as the free metabolite and as a glycoside conjugate (in total < 10.5% TRR; 0.007 mg eq./kg).

The major components detected in potatoes grown form in furrow treated tubers, treated at an exaggerated rate, were:

- Immature tubers: Flutolanil (21.5% TRR, 0.146 mg/kg), M-102 (24.5% TRR, 0.167 mg eq./kg) and M-101 (10.9% TRR, 0.074 mg eq./kg).

- Mature tubers: Flutolanil (9.9% TRR, 0.048 mg/kg) and M-102 (38.3% TRR, 0.186 mg eq./kg).

B.7.2.1.3 Metabolism in peanut

Previous evaluation	DAR, with additional analytical information included during the renewal process
RMS remark	Acceptable

Report: Downey, S.S., Meyer, B.N. and Rupprecht, J.K. (1993) Metabolism of ^{14}C-flutolanil in peanuts, NOR_AM Chemical Co., North Carolina, USA, Unpublished report No.: R-3015.

Guideline: Directive 96/68EC, Document 1607/VI/97, USA EPA/1996.

GLP: Compliance with USA EPA/1989.

Test formulation: Suspension with a final concentration of 6.05 g a.s./l.

Radioactive probe: [aniline ring -^{14}C(U)] flutolanil; specific activity 63,8 µCi/mg MBq/mg; radiochemical purity ≥ 99.3%

Test site: Covered outside enclosure, North Carolina, USA

Material and methods

The metabolism of [aniline ring-U-^{14}C] flutolanil was investigated in peanuts grown in an outside enclosure. After 64 days from planting, peanut plants (var. Florigiant) were treated with ^{14}C-flutolanil by spray application at a rate of 2.24 kg/ha (2 lb/A). Of the two possible methods, broadcast and banded application, the latter was selected as to give higher residue levels. Mature plants were harvested 84 days after treatment and separated into vines, hulls and nuts and kept frozen until analysed.

Peanut plants were separated into nuts and vines in the field. Adhering soil was removed from the nuts by hand before separating into hulls and nutmeats. Samples were frozen prior to milling.

Hull samples were sonicated briefly in cold water and filtered on cheese cloth to remove any remaining soil. Hulls, nuts and vines (with dry ice) were ground in a Hobart food cutter and / or a Glen Mills Disc Mill. Total radioactive residues (TRR) were determined by combustion.

Extraction and Fractionation of Resiudes

Samples of homogenised vines were first washed with dichloromethane and filtered. Filtered vine residues and homogenized hull samples were sequentially extracted by Waring blender using the following sequence of solvents:

Acetonitrile, 4 or 5 times
Acetonitrile : water (1:1, v/v), 4 times

Following each extraction, the extract was separated from the plant residue by vacuum filtration. The plant material was finally extracted with water in a Soxhlet apparatus for approximately 18 hours. Radioactivity present in extracts was quantified by liquid scintillation counting (LSC).

Samples of homogenised nuts were sequentially extracted by Waring blender using the following solvent mixture:

Hexane and acetonitrile (1:1, v/v), 3 times

The combined filtrates were separated into upper phase (hexane) and lower phase (acetonitrile). The plant material was further extracted with:

Acetonitrile : water (1:1, v/v), 3 times

Following each extraction, the extract was separated from the plant residue by vacuum filtration. The remaining plant material was further extracted with water in a Soxhlet apparatus for approximately 18 hours. Radioactivity present in extracts was quantified by liquid scintillation counting (LSC).

For vine extracts, the dichloromethane wash was concentrated and analysed by TLC and HPLC directly. The acetonitrile extract was concentrated, triturated with dichloromethane and the remainder solubilized in water/methanol prior to combining with the acetonitrile/water (1:1, v/v) extract. The dichloromethane portion was concentrated and analysed by TLC and HPLC. A combined water/methanol and acetonitrile/water portion was concentrated and partitioned with dichloromethane under neutral, acidic and basic conditions. The dichloromethane phases were combined and concentrated for chromatographic analysis. The aqueous residue was subject to the following hydrolysis step prior to partitioning with ethyl acetate under unchanged, neutral and basic conditions to yield the post-hydrolysis organic phases (which were concentrated for analysis by TLC and HPLC) and a residual aqueous phase.

Incubation in 2N H_2SO_4 for 24 hours at 50°C

For hull extracts, acetonitrile, acetonitrile/water and water extracts were combined, concentrated and partitioned with dichloromethane under unchanged, acidic and basic conditions. The dichloromethane phases were combined and concentrated for chromatographic analysis. The aqueous residue was acid hydrolysed and partitioned with ethyl acetate as described before for the aqueous residue from vine extracts.

The hexane extract of nuts (which contained the majority of the peanut lipids) could not be analysed without further concentration due to the high levels of oil present. The extract was concentrated to an oily residue and subjected to a wide range of fractionation and analysis techniques. Physical methods included solvent partitioning and cold precipitation. Chromatographic methods included column chromatography on florisil, silica gel, silicic acid, and alumina, preparative TLC on silica gel, gel filtration (several gel types), ion exchange and liquid-liquid counter-current chromatography. Hydrolysis with KOH/ethanol, Raney nickel, and lipase enzyme were tried, as was trans-esterification to methyl and ethyl esters followed by chromatographic separation. Hydrolytic extraction of the whole nut tissue with KOH/ethanol and HCl/tetrahydrofuran were also investigated. None of these methods were successful and it was concluded the hexane soluble residue consisted of metabolic products very similar in physical properties to the triglycerides which comprised the oil. The radioactive residue may have been associated, either physically or chemically with oil components and thus not released by the hydrolysis methods tried although they were extensive.

Other nut extracts of acetonitrile, acetonitrile/water and water extracts were further processed to provide a combined polar extract which was applied to a reverse phase chromatography column and ultimately divided into two samples, column fraction 1 and 2 on the basis of mass remaining after evaporation of solvent. Fraction 1 contained the majority of the mass (plant natural products) and minimal radioactivity (9% TRR). Fraction 2 contained very little mass and the majority of the radioactivity (32.9% TRR). Each was concentrated and then hydrolysed in 2M H_2SO_4 as described for the aqueous residue from vine extracts, prior to partitioning with dichloromethane and ethyl acetate under unchanged, neutral and basic conditions. Attempts to isolate the radioactivity were unsuccessful in the case of Fraction 1 but were successful with Fraction 2 and the dichloromethane and ethyl acetate extracts were concentrated and analysed by HPLC and TLC.
Following extraction, remaining radioactive residues in plant matrices were quantified by combustion.

Radioactivity remaining in PES samples of hull after extraction with neutral solvents were characterized by additional incubations in parallel as follows:

1M phosphate buffer containing protease for 24 hours at 37°C
1M phosphate buffer containing lipase for 24 hours at 37°C
10% HNO_3 for 24 hours at 37°C
HCl/dioxane (1:9) for 24 hours at 37°C
2M H_2SO_4 for 24 hours at 37°C
0.2M acetate buffer containing Viscozyme 120L for 24 hours at 42°C
0.2M acetate buffer containing Celluclast 1.5L for 24 hours at 42°C

As before, following extraction the supernatant was separated by filtration, the residue washed with ethyl acetate and radioactivity present in extracts quantified by liquid scintillation counting (LSC) and radioactive residues in the post extraction solids (PES) quantified by combustion.

Residue levels were also compared with those obtained by a multi-residue method in which flutolanil related metabolites are converted to trifluorobenzoic acid and analysed by GC-MS. The total extractable residue in each tissue (including aqueous soluble or unidentified radioactivity by HPLC/TLC) showed excellent accountability (89-106%) with the multi-residue method of analysis.

Samples of two peaks referred to a Metabolite A (60 µg) and Metabolite B (25 µg) were isolated from a bulk acetonitrile extraction of vines. Structural analysis by mass spectrometry (MS), including chemical ionization (CI), electron impact (EI), fast atom bombardment (FAB) and liquid chromatography-MS (LC-MS), and by proton nuclear magnetic resonance (1H-NMR), including 2D COSY.

Chemical and physical data led to the conclusion that Metabolites A and B were structurally related to each other and M-4, and were likely to be stable conjugates of M-4 or another aniline ring substituted metabolite. It was established that both Metabolite A and Metabolite B were accountable for by the residue method again confirming that both metabolites contained the phenyl (i.e trifluoromethylbenzoyl) ring moiety.

Extractable residue was analysed by gradient high performance liquid chromatography (HPLC) equipped with UV, mass-selective and radio detector for identification of the metabolites. Subsequent structural analyses of candidate metabolites were carried out employing CIMS, EIMS and FAB mass spectrometry and ^{1}H-NMR methods allowing positive confirmation of the metabolites.

Results

Total Radioactive Residues

TRR levels were 11.92 mg eq/kg in vines, 3.01 mg eq/kg in hulls and 0.39 mg eq/kg in nuts. TRR values obtained by summing the residues measured in extracts and post extraction solids were in good agreement for hulls and nuts but were higher for vines.

Sample	Days after treatment	TRR (mg as-eq/kg)
Vines	84	11.92
Hulls		3.01
Nuts		0.39

In each plant tissue (vines, nuts, hulls) , 57-91 % of TRR was recovered as extractable residue. Unextracted bound residues in PES accounted for 9.5% TRR (1.94 mg eq/kg) in vines, 27.1% TRR (0.08 mg eq/kg) in nuts and 42.9% TRR (1.30 mg eq/kg) in hull samples (see Table below).

Radiolabel	[Aniline-U-^{14}C]-Flutolanil					
Sample	Vines		Hulls		Nuts	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
TRR		20.41		3.03		0.31
Hexane	-	-	-	-	28.7	0.09
Dichloromethane	4.8	0.98	-	-	-	-
Acetonitrile	69.5	14.17	48.9	1.48	21.9	0.07
Acetonitrile : Water	15.4	3.14	5.1	0.15	12.8	0.04
Water Soxhlet	0.9	0.18	3.2	0.10	9.4	0.03
PES	9.5	1.94	42.9	1.30	27.1	0.08

Identification and characterisation of flutolanil residues in peanuts are presented in the Table B.7.2.1.3-1a and 1b.

In peanuts vines, flutolanil, M-4, Metabolite A and Metabolite B represented in total 18.5%, 13.6%, 17.4% and 19.9% TRR respectively, including both free components and conjugated material released by hydrolysis. Metabolite A and B were characterised as stable conjugates of M-4 or closely related aniline ring substituted metabolite. M-11 was detected as minor metabolite.

In nuts flutolanil was detected at 1% TRR. The only major residue identified was M-4 (10.2% TRR). In addition M-3, M-11, Metabolite A, Metabolite B and Metabolite C, which was not identified, were detected as minor metabolites (maximum ≤ 3.3% TRR). Despite extensive and well planned efforts, the remainder was unidentifiable. Indications were that these fractions may have resulted from a strong associacion of the flutolanil residue with peanut lipids.

In hulls flutolanil and M-4 represented in total 11,2% and 16,7% TRR, including free and conjugated material, accounting for 27,9% of the TRR. Metabolite A and B, presumed to be stable conjugates of M-4 of closely related metabolites were detected as minor metabolites (5.5 and 2.2% TRR, respectively). Extensive efforts were made to characterise the bound residues in hulls but this material proved to be highly intractable and none of the methods solubilised more than 6.2% TRR. Moreover, the natural plant products underwent considerable charring which precluded any further analysis of the residue.

Conclusions

The main observations in this well-conducted study were that in peanut wines and hulls parent flutolanil and its metabolites M-4, Met A and Met B were principal residue components. In nuts major part of radioactivity was localised into a fraction consisting of unidentified conjugated or unconjugated metabolites (49 %). Despite of extensive and well-planned efforts, oily component present in nuts hampered determination of the low levels of unconjugated flutolanil and its unconjugated metabolites in this matrix and therefore the data were only related to conjugated metabolites. Among these M-4 was quantitatively most important.

While the intended use refers to seed-treatment of potato only, the study on peanut is supplementary and lends further support on the proposed metabolic pathways that was observed in potato.

Table B.7.2.1.3-1a Identity and distribution of flutolanil residues in peanut as % of TRR and as mg flutolanil equivalents /kg.

Metabolite #	Vines		Hulls		Nuts	
	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
ERR	90.6	10.78	57.2	1.72	72.8	0.29
Flutolanil, total	18.5	2.20	11.2	0.34	NA	NA
- unconjugated	17.1	2.03	3.4	0.10	NA	NA
- conjugated	1.4	0.17	7.8	0.23	1.0	0.004
M-4, total	13.6	1.62	16.7	0.50	NA	NA
- unconjugated	3.0	0.36	11.7	0.35	NA	NA
- conjugated	10.6	1.26	5.0	0.15	10.2	0.04
Met A, total	17.4	2.07	5.5	0.17	NA	NA
- unconjugated	13.8	1.64	0.7	0.02	NA	NA
- conjugated	3.6	0.43	4.8	0.14	3.3	0.01
Met B, total	19.9	2.37	2.2	0.07	NA	NA
- unconjugated	7.1	0.84	0.3	0.01	NA	NA
- conjugated	12.8	1.52	1.9	0.06	0.9	0.004
Others, total	20.2	2.40	21.6	0.65	NA	NA
- unconjugated	7.2	0.86	1.0	0.03	NA	NA
- conjugated	13.0	1.55	20.6	0.62	49.4	0.20
URR	9.4	1.12	42.8	1.28	27.2	0.11
- unconjugated	47.9	5.70	17.1	0.51	28.7	0.11
- conjugated	38.8	4.62	37.2	1.12	30.3	0.12
- water soluble unidentified	3.9	0.46	2.9	0.09	13.8	0.06
Accountability/TRR	100	11,9	100	3,0	100	0,4

ERR, Extracted Radioactive Residues; URR, Unextracted Radioactive, TRR, Total Radioactive Residues Residues**;** NA, not analysed; accountability, sum of ERR and URR.

Table B.7.2.1.3-1b Identity and distribution of flutolanil residues in peanut as % of TRR and as mg flutolanil equivalents /kg.

Radiolabel		[Aniline-U-^{14}C]-Flutolanil					
Sample		Vines		Hulls		Nuts	
		%TRR	mg/kg [A]	%TRR	mg/kg [A]	%TRR	mg/kg [A]
Flutolanil	Total	18.5	3.78	11.2	0.34	1.0	0.004
	Free	17.1	3.49	3.4	0.10	NA	NA
	Conjugate	1.4	0.29	7.8	0.24	1.0	0.004
M-3	Total	ND	ND	ND	ND	3.3	0.01
	Free	ND	ND	ND	ND	NA	NA
	Conjugate	ND	ND	ND	ND	3.3	0.01
M-4	Total	13.6	2.78	16.7	0.51	10.2	0.04
	Free	3.0	0.61	11.7	0.35	NA	NA
	Conjugate	10.6	2.16	5.0	0.15	10.2	0.04
M-11	Total	1.0	0.20	ND	ND	2.0	0.008
	Free	ND	ND	ND	ND	NA	NA
	Conjugate	1.0	0.20	ND	ND	2.0	0.008
Metabolite A (Conjugate of M-4)	Total	17.4	3.55	5.5	0.17	3.3	0.01
	Free	13.8	2.82	0.7	0.02	NA	NA
	Conjugate	3.6	0.73	4.8	0.15	3.3	0.01
Metabolite B (Conjugate of M-4)	Total	19.9	4.06	2.2	0.07	0.9	0.004
	Free	7.1	1.45	0.3	0.01	NA	NA
	Conjugate	12.8	2.61	1.9	0.06	0.9	0.004
Metabolite C	Total	ND	ND	ND	ND	2.7	0.01
	Free	ND	ND	ND	ND	NA	NA
	Conjugate	ND	ND	ND	ND	2.7	0.01
Not analysed	Free	7.2	1.47	1.0	0.03	NA	NA
	Conjugate	13.0 [B]	2.65	20.6 [C]	0.62	49.4 [D]	0.20
PES after neutral extraction		9.5	1.94	42.9	1.30	27.1	0.08
Further PES extractions							
	Protease	-	-	2.1	0.06	-	-
	Lipase	-	-	1.6	0.05	-	-
	10% HNO_3	-	-	6.0	0.18	-	-
	HCl / Dioxane (1:9)	-	-	6.2	0.19	-	-
	2M H_2SO_4	-	-	0.7	0.02	-	-
	Viscozyme	-	-	0.8	0.02	-	-
	Celluclast	-	-	0.9	0.03	-	-

B.7.2.1.4 Metabolism in rice

Previous evaluation	in DAR, with additional analytical information included during the renewal process
RMS remark	Acceptable

Report: Smith, S., Shelley, M. and O'Neal S. (1994) Metabolic fate and distribution of ^{14}C-flutolanil in rice, NOR_AM Chemical Co., North Carolina, USA, Unpublished report No.: R-3016.

Guideline: Directive 96/68EC, Document 1607/VI/97, USA EPA/1996.

GLP: Compliance with USA EPA/1989.

Test formulation: Suspension formulated as MONCUT 50WP with a final a.s. concentration 5.4 g /l for 10 times field-rate experiments and 0.6 g/l for normal field-rate experients.

Radioactive probe: [aniline ring -^{14}C(U)] flutolanil; specific activity 73.5 µCi/mg MBq/mg; radiochemical purity ≥ 98 %.

Test site: Greenhouse facility, Richmond, Kentucky, USA

Material and methods

The metabolism of [aniline ring-U-^{14}C] flutolanil was studied in rice plants grown in the greenhouse. Rice plants were treated twice with ^{14}C -flutolanil at 92 and 106 days after planting by spray application at an overall rate of 1.06 kg/ha. Another plot was concomitantly treated twice by spray application at an overall rate of 10.5 kg/ha.

Mature plants were harvested 30 days after treatment and separated into foliage below the water-line, foliage above the water-line, hulk and grain. The samples were kept frozen until analysed.

The total residue in each tissue was determined by combustion followed by LSC. Prior to analyses by HPLC, homogenised samples were extracted sequentially with acetonitrile, acetonitrile/water and water and partitioned with dichloromethane at different pH. Degree of conjugation was studied subjecting the extractable residue to acid hydrolysis and in the case of the fiber also to base-catalyzed hydrolysis.

Extractable residue was analysed by reversed-phase gradient high performance liquid chromatography (HPLC) and by straight-phase TLC. Radioactive residues were quantified from HPLC eluent fractions or fractions produced by scraping off radioactive areas from TLC plates. The identity of each radioactive metabolite relied on HPLC and TLC cochromatography with authentic reference standards of synthetic putative metabolites.

Results

Total Radioactive Residues

RAC	TRR by combustion (mg eq./kg)	TRR by extraction (mg eq./kg)	Recovery [A]
Immature Harvest			
Foliage (below water)	0.79	0.83	105.1
Foliage (above water)	6.05	5.40	89.2
Seed head	0.37	0.40	108.1
Mature Harvest			
Foliage (below water)	11.92	10.54	88.4
Foliage (above water)	20.56	21.63	105.2
Husk	7.19	7.41	103.1
Grain	0.32	0.29	90.5

[A] TRR by extraction expressed as a percentage of TRR by combustion.

Distribution of radioactive residues after foliar application of [aniline-U-^{14}C]-flutolanil.

Harvest Interval	**Immature Harvest**		**Mature Harvest**	
RAC	%TRR	mg/kg	%TRR	mg/kg
Foliage (below water)				
ACN extract	83.8	0.70	68.0	7.17
Acetonitrile : water (1:1, v/v) extract	6.2	0.05	15.7	1.65
Water extract	2.7	0.02	0.9	0.09
PES	7.3	0.06	15.4	1.62
Foliage (above water)				
ACN extract	94.0	5.08	91.4	19.77
Acetonitrile : water (1:1, v/v) extract	3.2	0.17	5.1	1.10
Water extract	0.03	0.60	0.3	0.07
PES	2.2	0.12	3.2	0.69
Seed head			-	-
ACN extract	94.5	0.38	-	-
Acetonitrile : water (1:1, v/v) extract	2.0	0.01	-	-
Water extract	0.4	<0.01	-	-
PES	3.0	0.01	-	-
Husk	-	-		
ACN extract	-	-	77.2	5.72
Acetonitrile : water (1:1, v/v) extract	-	-	9.9	0.73
Water extract	-	-	1.1	0.08
PES	-	-	11.8	0.88
Grain	-	-		
ACN extract	-	-	47.2	0.14
Acetonitrile : water (1:1, v/v) extract	-	-	27.6	0.08
Water extract	-	-	1.2	<0.01
PES	-	-	24.1	0.07

Following foliar application of [aniline-U-^{14}C]-flutolanil the radioactive residue in rice grain was 0.29 mg eq./kg (treated 30 days before harvest) with 7.41 mg eq./kg found in the husk. The concentration of radioactivity in foliage below the water line and above the water line at harvest was 10.54 and 21.63 mg eq./kg, respectively. For foliage taken below the water line and above the water line at the immature harvest the radioactive residue was 0.83 and 5.40 mg eq./kg, respectively.

All samples were extracted and analysed, with virtually all of the radioactivity (≥ 93% TRR) extracted from the immature plants with minimal radioactivity remaining in the PES (maximum 7.3% TRR).
At mature harvest rice grain and foliage, which are RACs, and also husk samples were extracted and analysed, with between 76.0 to 96.8% of the TRR released by neutral solvent extraction i.e extraction with acetonitrile, acetonitrile/water (1:1) and water. According to OECD guidance document on residue chemistry studies (OECD 2009) husks are not considered a raw agricultural commodity of rice for crop metabolism studies. Virtually all of the radioactivity (>96% TRR) was extracted from foliage collected from above the water line with neutral extractions but unextracted bound residues in PES accounted for 15.4%

TRR in foliage collected from below the water line, 11.8% TRR in husks and 24.1% TRR in grain. Unextracted residues in PES were further characterised by strong acidic/basic extractions.

After 30 days of treatment flutolanil, when applied to rice at the maximum field rate under a normal field regime, was localised mainly in the foliage (33 % of TRR) and to a lesser extent in the hulk (7 %). In each plant tissue, more than 75% of TRR was recovered as extracted residue. Major part of radioactivity was recovered as untransformed flutolanil. While extent of metabolism was slightly higher in the grain (64 % remained unchanged), the uptake of radioactivity was low (0.3 % of TRR).

Identified metabolites were unchanged flutolanil and α,α,α -trifluoro-3'-hydroxy-o-toluanilide (M-4/DIP). These components were principally present in their free form, but were also found as acid-labile conjugates in limited quantities. No other unidentified components exceeded 10% of TRR. Consecutive hydrolysis of fiber residue by acid and base released radioactivity into organic and aqueous soluble fraction. Amount of radioactivity in each fraction was less than 10% of TRR.

Table B.7.2.1.4-1a Identity and distribution of flutolanil residues in rice at 30 days after last of two treatments applied after 92 and 106 days from planting.

	Foliage below water line		Foliage above water line		Hulk		Grain	
Metabolite #	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
ERR	84.7	10.10	96.8	19.90	88.1	6.33	76.0	0.24
Flutolanil	80.9	9.64	93.2	19.16	78.3	5.63	64.1	0.21
M-4	0.2	0.02	0.0	0.00	5.3	0.38	2.3	0,01
Other	3.5	0,42	3.6	0,74	4.2	0,30	9.6	0,03
URR	15.4	1,84	3.2	0,66	11.8	0,85	24.1	0,08
Accountability /TRR	100	11.92	100	20.56	99,6	7.19	100,1	0.32

TRR, total radioactive residues; ERR, extracted radioactive residues; URR, unextracted radioactive residues; accountability, sum of ERR and URR

Table B.7.2.1.4-1b Identity and distribution of flutolanil residues inimmature harvest rice, additional information submitted during renewal proces.

Radiolabel	[Aniline-U-^{14}C]-Flutolanil					
Immature Harvest	Foliage (below water)		Foliage (above water)		Seed head	
	%TRR	mg/kg [A]	%TRR	mg/kg [A]	%TRR	mg/kg [A]
Flutolanil	81.9	0.680	94.1	5.081	93.4	0.374
M-4	2.1	0.017	2.5	0.135	0.9	0.004
Remainder	8.6 [B]	0.071	1.3 [C]	0.070	1.4 [D]	0.006
PES Characterisation						
PES after neutral extraction	7.3	0.06	2.2	0.12	3.0	0.01
Further PES extraction						
4N H_2SO_4 extract	1.8	0.01	-	-	-	-
Partition of strong acidic extracts						
Aqueous layer	1.1	0.01	-	-	-	-
Organic layer	0.7	<0.01	-	-	-	-
PES after strong acidic extraction	5.5	0.05	-	-	-	-

[A] Calculated from the TRR measured by summing radioactivity in extracts and post extraction solids

[B] Includes water soxhlet extract (2.7%), residual aqueous (4.1%) and minor components of organic soluble (from HPLC, 1.8%)

[C] Includes water soxhlet extract (0.6%) and residual aqueous (0.7%)

[D] Includes water soxhlet extract (0.4%) and residual aqueous (1.0%)

Table B.7.2.1.4-1c Identity and distribution of flutolanil residues mmature harvest rice, additional information submitted during renewal proces.

Radiolabel	[Aniline-U-^{14}C]-Flutolanil							
Mature Harvest	Foliage (below water)		Foliage (above water)		Husk		Grain	
	%TRR	mg/kg [A]	%TRR	mg/kg [A]	%TRR	mg/kg [A]	%TRR	mg/kg [A]
Flutolanil	80.9	8.527	93.2	20.159	78.3	5.802	64.1	0.186
M-4	0.2	0.021	ND	ND	5.3	0.393	2.3	0.007
Remainder	2.7 [B]	0.285	3.6 [C]	0.779	4.4 [D]	0.326	9.6 [E]	0.028
PES Characterisation								
PES after neutral extraction	15.4	1.62	3.2	0.69	11.8	0.88	24.1	0.07
Further PES extraction								
4N H_2SO_4 extract	1.7	0.18	0.5	0.10	0.6	0.04	13.9	0.04
Partition of strong acidic extracts								
Aqueous layer	1.0 [F]	0.10 [F]	0.2	0.05	0.3	0.02	12.2 [F]	0.04 [F]
Organic layer	0.7	0.08	0.3	0.05	0.3	0.02	1.7	<0.01
2N NaOH extract	11.7	1.23	-	-	10.0	0.75	8.2	0.02
Partition of strong basic extracts								
Aqueous layer	9.1	0.96	-	-	7.2	0.54	3.4	0.01
Organic layer	2.6	0.27	-	-	2.8	0.21	4.8	0.01
PES after strong basic extraction	2.0	0.21	2.7	0.59	1.2	0.09	2.0	0.01

[A] Calculated from the TRR measured by summing radioactivity in extracts and post extraction solids

[B] Includes post-hydrolysis organic soluble (0.2%) & residual aqueous (0.1%) of soxhlet extract, post-hydrolysis residual aqueous (0.8%) and minor components of post hydrolysis organic soluble (from HPLC, 1.6%)
[C] Includes water soxhlet extract (0.3%) and residual aqueous (3.3%)
[D] Includes water soxhlet extract (1.1%), post-hydrolysis residual aqueous (0.7%) and minor components of pre- and post-hydrolysis organic soluble (from HPLC, 1.5% and 1.1%)
[E] Includes water soxhlet extract (1.2%), pre-hydrolysis residual aqueous (8.2%) and minor components of pre-hydrolysis organic soluble (from HPLC, 0.2%)
[F] Further partitioned at pH 7 and pH 10

Conclusion

Flutolanil, when applied to rice twice at 0.50-0.56 kg/ha rate (overall 1.06 kg/ha) under a normal field regime with a 30-day PHI, underwent only limited metabolism, principally to M-4 and other metabolites. The results are in line with metabolic studies in other crops employing comparable PHI. Significant fraction of radioactivity, in grain samples up to 24% of TRR, was non-extractable. After two applications of flutolanil to rice plants at an overall rate of 1.06 kg/ha, TRR in foliage, hulk and grain were 11.9–20.6, 7.2 and 0.3 mg/kg as flutolanil equivalents, respectively.

B.7.2.1.5 Metabolism in rice

Previous evaluation	Submitted for the purpose of the renewal
RMS remark	Acceptable

Report:	**CA 6.2.1/05. Yoshizane. T. (2013b)**
Title:	Plant Metabolism of ^{14}C-Flutolanil in Rice
Document No:	LSRC-M12-129A (R-3342)
Guidelines:	OECD 501 (2007) JMAFF (2-4-1), 12-Nousan-No.8147 (2000), Revised 19 Shouan-No.14966 (2008)
Deviations:	None
Testing laboratory:	Nihon Nohyaku Co. Ltd
GLP:	Yes

Executive Summary

The metabolism of [phenyl ^{14}C]- and [aniline ^{14}C]-labelled flutolanil was investigated in rice plants (*Oryza sativa L., var., japonica, cv, Nihonbare*). Two experiments were performed: plants treated in the irrigation water (‘paddy application’) and plants treated by foliar application.

A simulated 7% granular formulation of Moncut GR was prepared with both radiolabels and mixed into paddy water at a rate equivalent to 8.4 kg a.s./ha approximately 2 weeks before the ear emergence. A second formulation of Moncut WP was prepared with [phenyl-U-^{14}C]-flutolanil and applied to rice plants, first approximately 2 weeks before the ear emergence and again 14 days before harvest, at a rate equivalent to 0.75 kg a.s./ha (1.5 kg a.s./ha in total).

The rice crop was grown under glasshouse conditions in Osaka, Japan. Rice treated by paddy application was harvested at maturity 47 days after application. Rice treated by foliar application was harvested at

maturity 14 days after the second application. Brown rice, straw and hull samples were processed separately. The latter is not considered a RAC for rice.

In paddy treated plants, flutolanil accounted for between 47.36 to 82.95% TRR in brown rice. The other main components of the grain residue were M-4, both as the free metabolite and as a glucoside conjugate representing 9.78 to 10.02% TRR (0.21 to 0.30 mg eq./kg) and 1.82 to 2.94% TRR (0.04 to 0.09 mg eq./kg), respectively and the phenyl ring metabolites M-101 (23.23% TRR, 0.71 mg eq./kg) and M-102 (10.80% TRR, 0.33 mg eq./kg). In straw, flutolanil accounted for between 40.98 to 43.61% TRR. The other main component of the straw residue was M-4, both free metabolite and more significantly as a glucoside conjugate representing 6.24 to 7.41% TRR (11.20 to 11.77 mg eq./kg) and 21.54 to 29.30% TRR (40.65 to 44.32 mg eq./kg). Additional amounts of M-4 were released from straw bound residues in post extraction solids by refluxing with 10 N NaOH. In hulls, flutolanil and M-4, both free and conjugated were the only significant residues.

The only significant residue detected in foliar treated rice plants was flutolanil which formed >95% of the TRR at harvest in brown rice, straw and hulls. M-4, both free and as a glucose conjugate, M-101 and M-102 were observed as minor metabolites (≤ 4% TRR).

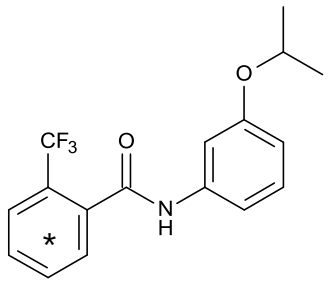
In addition, a number of minor metabolites were observed in rice plants treated by either paddy or foliar application; M-2, M-3 (both as the free metabolite and as a glucose conjugate), M-6, M-7 and M-11 (maximum 4.33% TRR). Throughout the study no specific aniline ring metabolites were detected in rice treated either by paddy or foliar application.

In soil flutolanil was the only major residue found (87.54 to 91.26% TRR) along with trace amounts of the metabolites M-4, M-11 and the phenyl ring metabolites M-101 and M-102 (maximum 1.28%) in systems treated with [^{14}C]-phenyl labelled flutolanil.

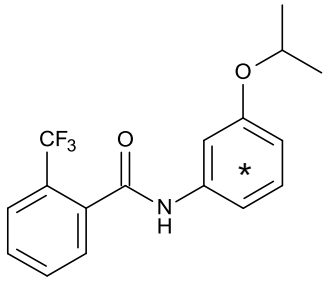
I. MATERIALS AND METHODS

A. MATERIALS

Test Material: Label 1: [Phenyl-U-^{14}C]-flutolanil



Label 2: [Aniline-U-^{14}C]-flutolanil



* Denotes position of [^{14}C]-radiolabel

Chemical name (IUPAC)	α, α, α-trifluoro-3'-isopropoxy-*o*-toluanilide
CA registry number:	66332-96-5
Label 1:	
Lot or batch number:	0AE0002S-R
Specific activity:	2.37 GBq / mmol
Radiochemical purity:	99.75 %
Label 2:	
Lot or batch number:	CP-3778
Specific activity:	3.23 GBq / mmol
Radiochemical purity:	99.86 %

B. STUDY DESIGN AND METHODS

1. In-life dates:

11 March 2011 to 27 November 2013

2. Experimental design

Test System:

Rice (*Oryza sativa L., var., japonica, cv, Nihonbare*) were transplanted as small plants into pots set up as paddy systems and grown to maturity under glasshouse conditions.

Experimental Conditions:

[Phenyl-U-^{14}C]-flutolanil was prepared as both Moncut GR (7% granular formulation) for paddy application and as Moncut WP (25% water dispersible powder formulation) for foliar application using blank formulations. [Aniline-U-^{14}C]-flutolanil was prepared as Moncut GR for paddy application using blank formulation.

The [^{14}C]- Moncut GR formulations were applied to paddy water approximately 2 weeks before ear emergence (47 days before harvest) on 30 March 2011. The [^{14}C]-Moncut WP formulation was applied to rice plants on 30 March 2011 approximately 2 weeks before ear emergence (47 days before harvest) and again on 2 May 2011, 14 days before harvest.

The crop was grown under glasshouse conditions at the test site in Osaka, Japan and plants were harvested at maturity on 17 May 2011.

	Paddy application	Foliar application
Nominal application rate	840 g a.s./10 a (equivalent to 8.4 kg a.s./ha)	75 g a.s./10 a (equivalent to 0.75 kg a.s./ha)
Number of applications	1	2
Target seasonal application rate	840 g a.s./10 a (equivalent to 8.4 kg a.s./ha)	150 g a.s./10 a (equivalent to 1.5 kg a.s./ha)
Application date	30 March 2011	30 March 2011 & 2 May 2011
Application timing (after sowing)	104 days	104 and 137 days
Application timing (before harvest)	47 days	47 and 14 days
Formulation type	7% granular	25% water dispersible powder
Formulation code	Moncut GR, Lot 20110221	Moncut WP, Lot 20110221
Method of application	Applied to paddy water *ca.* 2 weeks before ear emergence	Sprayed on rice plants *ca.* 2 weeks before ear emergence & 14 days before harvest
Radiolabel	[^{14}C]-phenyl & [^{14}C]-aniline	[^{14}C]-phenyl
Environmental conditions	Glasshouse conditions	Glasshouse conditions

Test Samples

The following samples were taken for analysis:

Test system	Sample	Date	Days after last treatment
Paddy application	Brown rice Straw Hull Roots Soil	17 May 2011	47
Foliar application	Brown rice Straw Hull Roots Soil	17 May 2011	14

Sample Preparation

Two individual plants were collected at harvest for each group. The aerial portion (straw and ear), roots and soil were sampled. The aerial portion was dried for approximately 2 weeks in the greenhouse.

After drying the ear was divided into hull and brown rice. Ear axes were combined with the straw. The straw and hull of rice treated by foliar application was rinsed in acetonitrile. Plant samples were cut into small pieces with scissors and then homogenized. Total radioactive residues (TRR) in root samples were determined by combustion.

Extraction and Fractionation of Residues

Samples of homogenised plant samples were sequentially extracted by shaker using the following sequence of solvents. Brown rice was soaked for 20 minutes in distilled water and straw was soaked for 2 hours prior to extraction.

Acetonitrile : water (4:1, v/v), 3 times
Acetonitrile : 0.1 N HCl (4:1, v/v)
Acetonitrile : 1 N HCl (4:1, v/v)
Acetonitrile : 0.1 N NaOH (4:1, v/v)
Acetonitrile : 1 N NaOH (4:1, v/v)

Soil samples were extracted with acetonitrile : 1 N HCl (4:1, v/v).

Following each extraction, the supernatant separated by centrifugation. Radioactivity present in extracts was quantified by liquid scintillation counting (LSC).

Plant and soil extracts containing significant radioactivity were concentrated to remove acetonitrile, partitioned with ethyl acetate and butanol and the organic layers mixed. Extracts were analysed by normal phase 2D TLC and reverse phase HPLC. The identity of metabolites was confirmed by co-chromatography with reference standards. The radioactive residues in the post extraction solids (PES) were quantified by combustion.

Radioactivity remaining in PES samples after weak acidic/basic extractions of straw samples were characterized by additional extractions as follows:

Incubation in 100 mM acetate buffer (pH 5) containing cellulase for 20-21 hours at 37°C
Reflux with 6N HCl for 2 hours at 110 °C
Reflux with 10 M NaOH for 2 hours at 110 °C

As before, following extraction the supernatant was separated by centrifugation and radioactivity present in extracts quantified by liquid scintillation counting (LSC) and radioactive residues in the post extraction solids (PES) quantified by combustion. The radioactivity in the strong basic extracts of straw was further characterised by partitioning with ethyl acetate and butanol. The organic layers were analysed by TLC and the radioactivity released with alkaline hydrolysis treatment identified as M-4.

Radioactivity remaining in PES samples after weak acidic/basic extractions of hull samples were characterized by additional extractions as follows:

Incubation in 100 mM acetate buffer (pH 5) containing cellulase for 20-21 hours at 37°C
Incubation with 2N HCL for 2 hours at 40 °C
Incubation with 2N NaOH for 2 hours at 40 °C
Reflux with 6N HCl for 2 hours at 110 °C
Reflux with 10 M NaOH for 2 hours at 110 °C

The radioactive residues in the post extraction solids (PES) quantified by combustion.

An unidentified polar region was observed on TLC plates following normal phase 2D TLC analysis. For straw, combined acetonitrile/water, acetonitrile/0.1N HCl and acetonitrile/1N HCl extracts were concentrated, redissolved in water and partitioned with hexane/ethyl acetate (2:1, v/v). The aqueous

phase was then partitioned with ethyl acetate and subject to the following hydrolysis steps prior to extraction with methanol and re-analysis by TLC:

Taken to dryness and redissolved in water, prior to incubation with an equal volume of 4N HCl for 24 hours at 50 °C.

Incubation in 100 mM acetate buffer (pH 5) containing β-glucosidase and cellulase for 48 hours at 37°C.

Grains were extracted as follows: a mixed extract consisting of combined acetonitrile/water and acetonitrile/0.1N HCl extracts for rice and all initial extracts (acetonitrile/water and weak acidic/basic extractions) for hull was developed on TLC. The radioactivity retained on the origin collected and extracted with methanol. The methanol extracts were taken to dryness and subject to the following hydrolysis steps prior to extraction with ethyl acetate or acetone/methanol followed by re-analysis by HPLC and/or TLC.

Redissolved and incubated in 4N HCl for 24 hours at 50 °C.

Incubation in 100 mM acetate buffer (pH 5) containing β-glucosidase for 18 hours at 37°C

The polar material was identified as glycoside conjugates of M-3 and M-4 by identification of the aglycones by HPLC and TLC post hydrolysis

II. RESULTS AND DISCUSSION

Total Radioactive Residues

The total radioactive residues (TRR) in rice are summarised below in Table for paddy applications and in **Fout! Verwijzingsbron niet gevonden.**B.7.2.1.5-2 for foliar application.

Following paddy application of [^{14}C]-flutolanil, radioactive residues in brown rice ranged from 3.06 mg eq./kg in plants treated with [phenyl-U-^{14}C]-flutolanil to 2.12 mg eq./kg in plants treated with [aniline-U-^{14}C]-flutolanil (approximately 14 days before the ear emergence). The concentration of radioactivity in straw and hull at harvest ranged from 151.23 to 188.70 mg eq./kg and 49.83 to 59.46 mg eq./kg, respectively. Residues detected in root ranged from 9.83 to 19.57 mg eq./kg.

Brown rice and straw, which are RACs, and also hull samples were extracted and analysed, with between 56.3 to 100% TRR extracted. According to OECD guidance document on residue chemistry studies (OECD 2009) hulls are not considered a raw agricultural commodity of rice for crop metabolism studies. Virtually all of the radioactivity (>99% TRR) was extracted from brown rice with acetonitrile/water and weak acidic (0.1 & 1N HCl) .Unextracted bound residues in PES accounted for 11.10 to 19.27% TRR in straw and 43.71 to 43.75% TRR in hull samples. These residues were further characterised by cellulase and strong acidic/basic extractions.

Table B.7.2.1.5-1: Total radioactive residues (TRRs) in rice following paddy application of [^{14}C]-Flutolanil

Radiolabel	[Phenyl-U-^{14}C]-Flutolanil		[Aniline-U-^{14}C]-Flutolanil	
RAC	%TRR	mg/kg	%TRR	mg/kg
Brown rice	100	3.06	100	2.12
Surface rinse	-	-	-	-
ACN/Water	66.55	2.04	54.35	1.15
ACN/0.1N HCl extract	13.48	0.41	12.29	0.26
ACN/1N HCl extract	19.67	0.60	33.36	0.71
ACN/0.1N NaOH extract	ND	ND	ND	ND
ACN/1N NaOH extract	ND	ND	ND	ND
PES	0.30	0.01	ND	ND
Hull	100	49.83	100	59.46
Surface rinse	-	-	-	-
ACN/Water	45.85	22.84	38.96	23.16
ACN/0.1N HCl extract	3.44	1.71	4.04	2.40
ACN/1N HCl extract	1.79	0.89	2.09	1.24
ACN/0.1N NaOH extract	2.75	1.37	9.18	5.46
ACN/1N NaOH extract	2.43	1.21	2.03	1.20
PES	43.75	21.80	43.71	25.99
Straw	100	188.70	100	151.23
Surface rinse	-	-	-	-
ACN/Water	65.15	122.93	70.44	106.53
ACN/0.1N HCl extract	6.64	12.53	8.69	13.15
ACN/1N HCl extract	3.76	7.09	2.10	3.17
ACN/0.1N NaOH extract	0.47	0.88	2.76	4.17
ACN/1N NaOH extract	4.72	8.90	4.90	7.41
PES	19.27	36.37	11.10	16.79
Root	100	9.83	100	19.57

- = Not sampled ND = Not detected

Following foliar application of [phenyl-U-^{14}C]-flutolanil the radioactive residue in brown rice was 0.09 mg eq./kg (treated approximately 14 days before the ear emergence and 14 days before harvest). The concentration of radioactivity in straw and hull at harvest was 18.07 and 61.80 mg eq./kg, respectively. Residues detected in roots were 0.07 mg eq./kg.

Brown rice and straw, which are RACs, and also hull samples were extracted and analysed, with virtually all of the radioactivity (≥ 99% TRR) extracted with minimal radioactivity remaining in the PES (maximum 0.39% TRR).

Table B.7.2.1.5-2 Total radioactive residues (TRRs) in rice following foliar application of [^{14}C]-Flutolanil

Radiolabel	[Phenyl-U-^{14}C]-Flutolanil	
RAC	%TRR	mg/kg
Brown rice	100	0.09
Surface rinse	-	-
ACN/Water	89.42	0.08
ACN/0.1N HCl extract	ND	ND
ACN/1N HCl extract	10.48	0.01
ACN/0.1N NaOH extract	ND	ND
ACN/1N NaOH extract	ND	ND
PES	0.10	<0.01
Hull	100	61.80
Surface rinse	92.39	57.10
ACN/Water	6.20	3.83
ACN/0.1N HCl extract	0.96	0.56
ACN/1N HCl extract	0.04	0.02
ACN/0.1N NaOH extract	0.02	0.01
ACN/1N NaOH extract	ND	ND
PES	0.39	0.24
Straw	100	18.07
Surface rinse	61.88	11.18
ACN/Water	34.96	6.32
ACN/0.1N HCl extract	2.10	0.38
ACN/1N HCl extract	0.72	0.13
ACN/0.1N NaOH extract	0.14	0.03
ACN/1N NaOH extract	0.20	0.01
PES	ND	ND
Root	100	0.07

- = Not sampled ND = Not detected

Characterisation and Identification of Residues

The identification and characterisation of radioactive residues in rice are summarised below in Table 3 to Table 4 for paddy applications and in Table 5 and Table 6 for foliar application.

In rice treated by paddy application, flutolanil, M-4, and M-4 glycoside conjugate, represented 47.36 to 82.95% TRR, 9.78 to 10.02% TRR and 1.82 to 2.94% TRR, respectively, in brown rice. Overall these three compounds accounted for between 60.08 to 94.79% TRR in rice. In rice from plants treated with [^{14}C]-phenyl labelled flutolanil, the phenyl ring metabolites M-101 and M-102 also formed a significant part of the residue at 23.23% TRR (0.71 mg eq./kg) and 10.80% TRR (0.33 mg eq./kg), respectively.

Straw and hulls after paddy application

In straw and hulls from rice treated by paddy application, flutolanil, M-4 and M-4 glucose conjugate represented 40.98 to 43.61% TRR, 6.24 to 7.41% TRR and 21.54 to 29.30% TRR in straw and 31.30 to 32.61% TRR, 9.04 to 10.46% TRR and 6.90 to 8.73% TRR in hulls. Overall these three compounds

accounted for between 71.39 to 77.69% TRR in rice straw and 47.24 to 51.8% TRR in hulls. Levels of M-101 and M-102 in plants treated with [^{14}C]-phenyl labelled flutolanil by paddy application were much less significant in rice straw (1.17% TRR in total) and hulls (4.10% TRR in total) than in rice (34.03% TRR in total).

The most prominent residue in plants treated by foliar application was flutolanil which formed 95.81% TRR (0.09 mg/kg) in brown rice, 95.11% TRR (17.18 mg/kg) in straw and 99.49% TRR (61.49 mg/kg) in hulls. M-4, both free and as a glucose conjugate, was observed as a minor metabolite (≤ 4% TRR in total). Levels of M-101 and M-102 in plants treated with [^{14}C]-phenyl labelled flutolanil by foliar application were much less significant in brown rice in particular (2.26% TRR in total) and also somewhat lower in rice straw (0.44% TRR in total) and hulls (0.07% TRR in total) compared to rice treated by paddy application.

In addition a number of minor metabolites were observed in rice plants M-2, M-3 (both as the free metabolite and as a glucose conjugate), M-6, M-7 and M-11 (maximum 4.33% TRR per metabolite). Throughout the study no specific aniline ring metabolites were detected in rice treated either by paddy or foliar application.

Significant residues remained in post extraction solids (PES) in straw and hull samples from rice treated by paddy application. The majority of radioactivity was not solubilized with either cellulase or hydrolytic treatment with acid and alkaline. Radioactivity released with alkaline hydrolysis treatment in straw (10 N NaOH, 110°C) was identified as M-4, assumed to be released from residues strongly bound to biomolecules.

The identification and characterisation of radioactive residues in soil following paddy application are summarised below (Table B.7,2.1.5-3). In soil extracts 87.54 to 91.26% TRR was identified as flutolanil and trace amounts of the metabolites M-4 and M-11 (maximum 1.28%) were observed in both [^{14}C phenyl]- and [^{14}C aniline]- treated soil. Trace amounts of the phenyl ring metabolites M-101 and M-102 (maximum 0.76%) were also observed in phenyl-label treated soil.

Table B.7.2.1.5-3: Summary of identification and characterisation of residues in brown rice following paddy application of [^{14}C]-Flutolanil

Radiolabel	[Phenyl-U-^{14}C]-Flutolanil		[Aniline-U-^{14}C]-Flutolanil	
	%TRR	mg/kg	%TRR	mg/kg
TRR Brown rice	100	3.06	100	2.12
Flutolanil	47.36	1.45	82.95	1.76
M-2	ND	ND	ND	ND
M-3	0.92	0.03	0.80	0.02
M-4	9.78	0.30	10.02	0.21
M-5	ND	ND	ND	ND
M-6	4.33	0.13	4.16	0.09
M-7	ND	ND	ND	ND
M-11	ND	ND	ND	ND
M-101	23.23	0.71	-	-
M-102	10.80	0.33	-	-
M-3 glucoside	0.35	0.01	0.25	<0.01
M-4 glucoside	2.94	0.09	1.82	0.04
PES	0.30	<0.01	ND	ND

Table B.7.2.1.5-4: Summary of identification and characterisation of residues in rice straw following paddy application of [^{14}C]-Flutolanil

Radiolabel	[Phenyl-U-^{14}C]-Flutolanil		[Aniline-U-^{14}C]-Flutolanil	
	%TRR	mg/kg	%TRR	mg/kg
TRR Straw	100	188.70	100	151.23
Flutolanil	43.61	82.30	40.98	61.98
M-2	0.15	0.29	0.12	0.18
M-3	ND	ND	ND	ND
M-4	6.24	11.77	7.41	11.20
M-5	ND	ND	ND	ND
M-6	0.74	1.40	0.81	1.23
M-7	0.42	0.78	0.60	0.91
M-11	0.20	0.37	0.32	0.49
M-101	0.78	1.47	-	-
M-102	0.39	0.73	-	-
M-3 glucoside	1.49	2.81	1.68	2.54
M-4 glucoside	21.54	40.65	29.30	44.32
Others	5.18	9.78	7.66	11.58
PES characterisation				
PES after weak acidic extraction	19.27	36.37	11.10	16.79
Further PES extractions				
Cellulase	0.56	1.05	0.96	1.45
6N HCl Reflux	0.43	0.82	0.68	1.02
10N NaOH Reflux	7.94	14.99	5.55	8.39
PES after strong basic extraction	10.34	19.51	3.92	5.93

Table B.7.2.1.5-5: Summary of identification and characterisation of residues in rice hull following paddy application of [^{14}C]-Flutolanil

Radiolabel	[Phenyl-U-^{14}C]-Flutolanil		[Aniline-U-^{14}C]-Flutolanil	
	%TRR	mg/kg	%TRR	mg/kg
TRR Hull	100	49.83	100	59.46
Flutolanil	31.30	15.60	32.61	19.39
M-2	ND	ND	ND	ND
M-3	ND	ND	ND	ND
M-4	9.04	4.50	10.46	6.22
M-5	ND	ND	ND	ND
M-6	2.46	1.23	2.08	1.24
M-7	0.78	0.39	1.01	0.60
M-11	ND	ND	ND	ND
M-101	3.18	1.59	-	-
M-102	0.92	0.46	-	-
M-3 glucoside	ND	ND	ND	ND
M-4 glucoside	6.90	3.44	8.73	5.19
Others	1.66	0.83	1.40	0.83
PES characterisation				
PES after weak acidic extraction	43.75	21.80	43.71	25.99
Further PES extractions				
Cellulase	0.56	0.28	2.26	1.35
2N HCl Reflux	0.27	0.13	0.15	0.09
2N NaOH Reflux	3.43	1.71	1.05	0.63
6N HCl Reflux	1.12	0.56	0.78	0.46
10N NaOH Reflux	2.66	1.33	1.85	1.10
PES after strong basic extraction	35.72	17.80	37.61	22.37

Table B.7.2.1.5-6: Summary of identification and characterisation of residues in rice following foliar application with [Phenyl-U-^{14}C]-Flutolanil

Radiolabel	[Phenyl-U-^{14}C]-Flutolanil					
Sample	Brown rice		Straw		Hull	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
TRR		0.09		18.07		61.80
Flutolanil	95.81	0.09	95.11	17.18	99.49	61.49
M-2	ND	ND	ND	ND	ND	ND
M-3	ND	ND	ND	ND	ND	ND
M-4	1.82	<0.01	1.01	0.18	0.03	0.02
M-5	ND	ND	ND	ND	ND	ND
M-6	ND	ND	ND	ND	ND	ND
M-7	ND	ND	ND	ND	ND	ND
M-11	ND	ND	ND	ND	ND	ND
M-101	1.24	<0.01	0.44	0.08	0.07	0.04
M-102	1.02	<0.01	ND	ND	ND	ND
M-3 glucoside	N.D	N.D	0.15	0.03	ND	ND
M-4 glucoside	ND	N.D	2.96	0.53	ND	ND
Others	ND	ND	0.34	0.06	ND	ND
TLC Origin	ND	ND	ND	ND	0.02	0.01
PES	0.10	<0.01	ND	ND	0.39	0.24

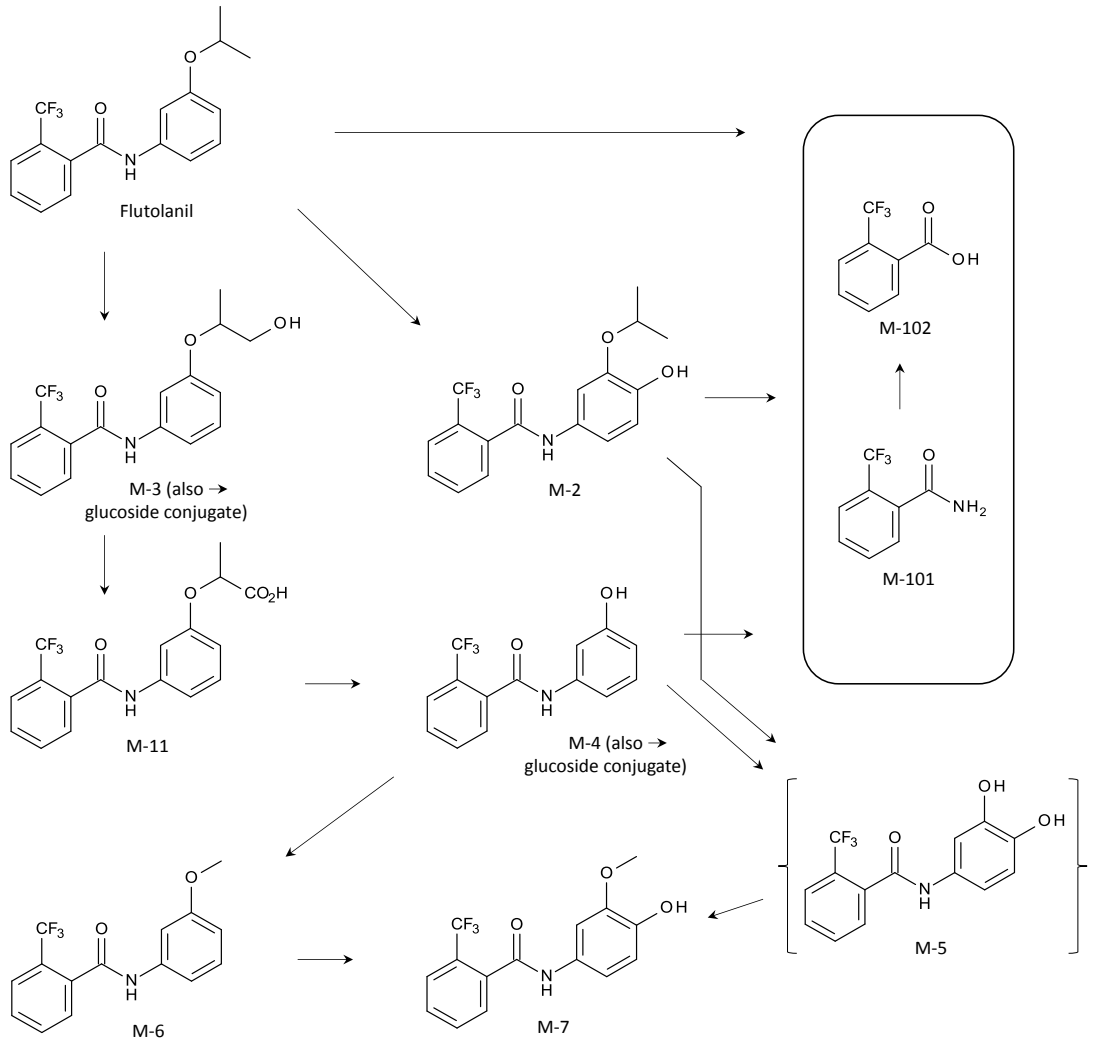
Table B.7.2.1.5-7: Summary of identification and characterisation of soil residues following paddy application with [^{14}C]-Flutolanil

Radiolabel	[Phenyl-U-^{14}C]-Flutolanil		[Aniline-U-^{14}C]-Flutolanil	
Harvest	Mature		Mature	
	%TRR	mg/kg	%TRR	mg/kg
TRR Soil		6.51		5.10
Flutolanil	87.54	5.70	91.26	4.65
M-2	ND	ND	ND	ND
M-3	ND	ND	ND	ND
M-4	1.28	0.08	1.19	0.06
M-5	ND	ND	ND	ND
M-6	ND	ND	ND	ND
M-7	ND	ND	ND	ND
M-11	1.23	0.08	1.03	0.05
M-101	0.57	0.04	-	-
M-102	0.76	0.05	-	-
TLC Origin	1.57	0.10	0.75	0.04
PES	7.04	0.46	5.78	0.29

Metabolic pathway

A metabolic pathway for flutolanil in rice is proposed in Figure B.7.2.1.5-8

Figure B.7.2.1.5-8: Proposed metabolic profile of flutolanil in rice



IV. CONCLUSIONS

At maturity, paddy treated brown rice contained TRRs of 3.06 and 2.12 mg eq./kg, while the straw contained TRRs of 188.70 mg eq./kg and 151.23 mg eq./kg for [^{14}C phenyl]- and [^{14}C aniline]- treated plants, respectively.

The major components detected in paddy treated rice were:

- Flutolanil (47.36 to 82.95% TRR, 1.45 to 1.76 mg/kg), M-4 both as the free metabolite and as a glucoside conjugate (in total 12.72 to 11.84% TRR; 0.25 to 0.39 mg eq./kg) and the phenyl ring metabolites M-101 (23.23% TRR, 0.71 mg eq./kg) and M-102 (10.80% TRR, 0.33 mg eq./kg) in brown rice.
- Flutolanil (40.98 to 43.61% TRR, 61.9 to 82.3 mg/kg) and M-4 both free and more significantly as a glucoside conjugate (in total 27.78 to 36.71% TRR; 52.42 to 55.52 mg eq./kg) in straw.
- Additional amounts of M-4 were released from bound plant residues in straw after refluxing in strong base.

At maturity, foliar treated brown rice contained a TRR of 0.09 mg eq./kg, while the straw contained a TRR of 18.07 mg eq./kg following application of [^{14}C phenyl]- flutolanil. The only significant residue detected in foliar treated rice plants was flutolanil which formed >95% of the TRR at harvest.

B.7.2.1.6 Metabolism in cabbage

Previous evaluation	Submitted for the purpose of the renewal
RMS remark	Acceptable

Report:	**CA 6.2.1/04. Yoshizane, T. (2013a)**
Title:	Plant Metabolism of ^{14}C-Flutolanil in Cabbage
Document No:	LSRC-M12-085A (R-3341)
Guidelines:	OECD 501 (2007) JMAFF (2-4-1), 12-Nousan-No.8147 (2000), Revised 19 Shouan-No.14966 (2008)
Deviations:	None
Testing laboratory:	Nihon Nohyaku Co. Ltd
GLP:	Yes

Executive Summary

The metabolism of [phenyl ^{14}C]- and [aniline ^{14}C]-labelled flutolanil was investigated in cabbage plants (variety YR Seitoku). Two experimetns were performd: one with flutolanil applied to soil and one with foliar applied flutolanil. A simulated 2% dust formulation of Moncut 40DL was prepared with both radiolabels and mixed into soil at a rate equivalent to 8 kg a.s./ha prior to transplanting cabbage seedlings. A second formulation of Moncut Flowable 40FL was prepared with [phenyl-U-^{14}C]-flutolanil and applied to foliage of cabbage plants, first as heads began to form and again 7 days before harvest, at a rate equivalent to 0.9 kg a.s./ha (1.8 kg a.s./ha in total).

The cabbage crop was grown to maturity under glasshouse conditions in Japan. Cabbages treated by soil application were harvested as immature plants 56 days after application and at maturity 97 days after application. Cabbages treated by foliar application were harvested at maturity 7 days after the second application. Heads and outer leaves were processed separately.

In cabbages treated by soil application, flutolanil accounted for between 49.31% to 69.17% TRR. The other main component of the residue was M-4, both as the free metabolite and as a glucoside conjugate representing 5.19 to 8.51% TRR (0.01 to 0.26 mg eq./kg) and 13.84 to 25.14% TRR (0.04 to 0.79 mg eq./kg), respectively. Additional amounts of M-4 were released from bound residues in post extraction solids by refluxing with 10 N NaOH.

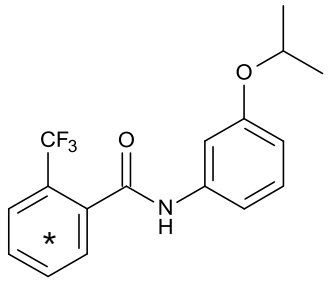
The only significant residue in cabbages treated by foliar application was flutolanil which formed 90.41 to 98.54% TRR at harvest. M-4, both free and as a glucose conjugate, was observed as a minor metabolite (≤ 5% TRR).

In addition M-2, M-3, M-5, M-6, M-7, M-11 and in phenyl-label treated cabbage, M-101 and M-102 were observed as minor metabolites (maximum 1.19% TRR). No specific aniline ring metabolites were detected. A similar metabolic pattern was seen in soil.

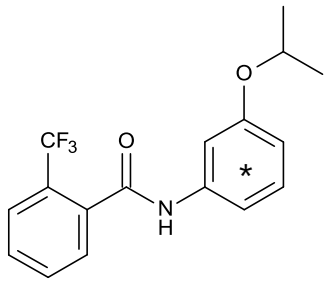
I. MATERIALS AND METHODS

A. MATERIALS

Test Material: Label 1: [Phenyl-U-^{14}C]-flutolanil



Label 2: [Aniline-U-^{14}C]-flutolanil



* Denotes position of [^{14}C]-radiolabel

Chemical name (IUPAC)	α, α, α-trifluoro-3'-isopropoxy-*o*-toluanilide
CA registry number:	66332-96-5
Label 1:	
Lot or batch number:	0AE0002S-R
Specific activity:	2.37 GBq / mmol
Radiochemical purity:	99.27 %
Label 2:	
Lot or batch number:	CP-3778
Specific activity:	3.23 GBq / mmol
Radiochemical purity:	99.60 %

B. STUDY DESIGN AND METHODS

1. In-life dates:

10 April 2011 to 16 January 2013

2. Experimental design

Test System:

Cabbage (variety YR Seitoku) were transplanted as small plants and grown to maturity in pots (diameter 30 cm) under glasshouse conditions. Each pot contained approximately 13 kg of soil (Soil type: Tsuchitarou).

Experimental Conditions:

[Phenyl-U-^{14}C]-flutolanil was prepared as both Moncut 40DL (2% dust formulation) for soil application and as Moncut Flowable 40FL (40% flowable formulation) for foliar application using blank formulations. [Aniline-U-^{14}C]-flutolanil was prepared as Moncut 40DL for soil application using blank formulation.

The [^{14}C]-Moncut 40DL formulations were mixed into soil prior to transplanting cabbage seedlings on 19 October 2011. The [^{14}C]-Moncut Flowable 40FL formulation was applied to cabbage plants on 21 December 2011 as heads began to form and again on 23 January 2012, 7 days before harvest. The crops were grown to maturity under glasshouse conditions at the test site in Japan.

For soil application, cabbages were taken as immature plants as heads began to form on 14 December 2011 and again at maturity once heads were fully formed on 24 January 2012. Soil samples were also taken at mature harvest.

For foliar application, cabbages were taken at maturity once heads were fully formed on 30 January 2012.

	Soil application	Foliar application
Nominal application rate	800 g a.s./10 a (equivalent to 8 kg a.s./ha)	90 g a.s./10 a (equivalent to 0.9 kg a.s./ha)
Number of applications	1	2
Target seasonal application rate	800 g a.s./10 a (equivalent to 8 kg a.s./ha)	180 g a.s./10 a (equivalent to 1.8 kg a.s./ha)
Application date	19 October 2011	21 December 2011 & 23 January 2012
Application timing (after sowing)	43 days	106 and 139 days
Application timing (after transplanting)	At transplanting	63 and 96 days
Formulation type	2% fine dust formulation	40% flowable
Formulation code	Moncut 40DL, Lot 110922	Moncut Flowable 40FL, Lot 220015B
Method of application	Uniformly mixed with soil prior to transplanting cabbage seedling	Sprayed on cabbage plants at start of head formation & 7 days before harvest
Radiolabel	[^{14}C]-phenyl & [^{14}C]-aniline	[^{14}C]-phenyl
Environmental conditions	Glasshouse conditions	Glasshouse conditions

Test Samples

Samples were taken for analysis at the following times:

Test system	Sample	Date	Days after last treatment
Soil application	Head formation	14 December 2011	56
Soil application	Maturity	24 January 2012	97
Foliar application	Maturity	30 January 2012	7

Sample Preparation

Immature and mature plants were collected following soil application and mature plants were collected after foliar applications. At each harvest one plant was taken for extraction and analysis. Heads, outer leaves, stems and roots were sampled. Soil was also collected.

Head and outer leaves of cabbages treated by foliar application were rinsed 3 times in acetonitrile. Plant samples were cut into small pieces with scissors and then homogenized. Total radioactive residues (TRR) in stem and root samples were determined by combustion.

Extraction and Fractionation of Residues

Samples of homogenised plant and soil samples were sequentially extracted by shaker using the following sequence of solvents:

Acetonitrile : water (4:1, v/v), 3 times
Acetonitrile : 0.1 N HCl (4:1, v/v)
Acetonitrile : 1 N HCl (4:1, v/v)
Acetonitrile : 0.1 N NaOH (4:1, v/v)
Acetonitrile : 1 N NaOH (4:1, v/v)

Following each extraction, the supernatant separated by centrifugation. Radioactivity present in extracts was quantified by liquid scintillation counting (LSC).

Plant extracts containing significant radioactivity were concentrated to remove acetonitrile, partitioned with ethyl acetate and butanol and the organic layers mixed. Soil extracts containing significant radioactivity were treated in the same manner but were partitioned with ethyl acetate/hexane (1/1, v/v) and the organic layers mixed. Extracts were analysed by normal phase 2D TLC and reverse phase HPLC. The identity of metabolites was confirmed by co-chromatography with reference standards. The radioactive residues in the post extraction solids (PES) were quantified by combustion.

Radioactivity remaining in PES samples after weak acidic/basic extractions in cabbages treated by soil application were characterized by additional extractions as follows:

Incubation in 100 mM acetate buffer (pH 5) containing cellulase for 24 hours at 37°C
Reflux with 6N HCl for 2 hours at 110 °C
Reflux with 10 M NaOH for 2 hours at 110 °C

As before, following extraction the supernatant was separated by centrifugation and radioactivity present in extracts quantified by liquid scintillation counting (LSC) and radioactive residues in the post extraction solids (PES) quantified by combustion. The radioactivity in the strong basic extracts was further

characterised by partitioning with ethyl acetate and butanol. The organic layers were analysed by TLC and the radioactivity released with alkaline hydrolysis treatment identified as M-4.

An unidentified polar region was observed on TLC plates following normal phase 2D TLC analysis. Combined acetonitrile/water, acetonitrile/0.1N HCl and acetonitrile/1N HCl extracts were partitioned with hexane/ethyl acetate (2:1, v/v). The aqueous phase was then partitioned with ethyl acetate. Aqueous phases were subject to the following hydrolysis prior to partitioning with ethyl acetate followed by re-analysis by HPLC and/or TLC:

Taken to dryness and redissolved in water, prior to incubation with an equal volume of 8N HCl for 24 hours at 50 °C.

Incubation in 100 mM acetate buffer (pH 5) containing β-glucosidase and cellulase for 24 hours at 37°C

The polar region was identified as a glycoside conjugate of M-4 by identification of the aglycone by HPLC and TLC post hydrolysis.

II. RESULTS AND DISCUSSION

Total Radioactive Residues

The total radioactive residues (TRR) in cabbages are summarised below in Table B.7.2.1.6-1 for soil applications and in Table B.7.2.1.6-2 for foliar application.

Following soil application of [^{14}C]-flutolanil radioactive residues in cabbage heads ranged from 1.34 mg eq./kg in plants treated with [phenyl-U-^{14}C]-flutolanil to 1.40 mg eq./kg in plants treated with [aniline-U-^{14}C]-flutolanil at the start of head formation (56 days after application), but decreased to 0.21 to 0.26 mg eq./kg at mature harvest (97 days after treatment). The concentration of radioactivity in the outer leaves was similar at immature and mature harvest ranging from 2.92 to 3.37 mg eq./kg. Residues detected in stem and root at the time of harvest ranged from 2.71 to 3.61 mg eq./kg and 7.49 to 11.91 mg eq./kg, respectively.

Cabbage heads and outer leaves were extracted and analysed, with between 79.2 to 97.0% TRR extracted. Unextracted bound residues in PES accounted for 9.93 to 20.81% TRR in cabbage heads and 2.97 to 6.12% TRR in outer leaves. PES residues were further characterised by cellulase and strong acidic/basic extractions.

Table B.7.2.1.6-1 : Total radioactive residues (TRRs) in cabbage following soil application with [^{14}C]-Flutolanil

Radiolabel	[Phenyl-U-^{14}C]-Flutolanil				[Aniline-U-^{14}C]-Flutolanil			
Harvest	Head formation		Mature		Head formation		Mature	
RAC	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
Head	100	1.34	100	0.21	100	1.40	100	0.26
Surface rinse	-	-	-	-	-	-	-	-
ACN/Water	26.96	0.36	28.44	0.06	28.02	0.39	68.18	0.18
ACN/0.1N HCl extract	47.78	0.64	50.75	0.11	51.79	0.72	20.94	0.05
ACN/1N HCl extract	8.35	0.11	ND	ND	10.26	0.15	ND	ND
PES	16.92	0.23	20.81	0.04	9.93	0.14	10.87	0.03
Outer leaf	100	3.37	100	3.03	100	2.92	100	3.34
Surface rinse	-	-	-	-	-	-	-	-
ACN/Water	54.10	1.82	64.82	1.97	67.45	1.97	46.14	1.54
ACN/0.1N HCl extract	37.75	1.27	32.13	0.97	22.35	0.65	50.89	1.7
ACN/1N HCl extract	3.67	0.13	ND	ND	4.08	0.12	ND	ND
PES	4.48	0.15	3.04	0.09	6.12	0.18	2.97	0.1
Stem	100	1.28	100	3.61	100	1.34	100	2.71
Root	100	11.85	100	7.49	100	11.91	100	14.19

Following foliar application of [phenyl-U-^{14}C]-flutolanil radioactive residues in the head and outer leaves of cabbage were 0.09 mg eq./kg and 90.89 mg eq./kg, respectively (7 days after last application) with most of the radioactivity recovered from the outer leaf. The radioactivity remaining in the stem and root at the time of harvest was 0.54 mg eq./kg and 0.53 mg eq./kg, respectively.

Cabbage heads and outer leaves were extracted and analysed, with virtually all of the radioactivity (≥ 99% TRR) extracted with minimal radioactivity remaining in the PES (maximum 1.1% TRR).

Table B.7.2.1.6-2: Total radioactive residues (TRRs) in cabbage following foliar application with [^{14}C]-Flutolanil

Radiolabel	[Phenyl-U-^{14}C]-Flutolanil	
Harvest	Mature	
RAC	%TRR	mg/kg
Head	100	0.09
Surface rinse	71.54	0.06
ACN/Water	16.36	0.02
ACN/0.1N HCl extract	11.00	0.02
ACN/1N HCl extract	ND	ND
PES	1.10	<0.01
Outer leaf	100	90.89
Surface rinse	93.74	84.53
ACN/Water	2.94	2.98
ACN/0.1N HCl extract	3.18	3.23
ACN/1N HCl extract	0.04	0.04
PES	0.10	0.10
Stem	100	0.54
Root	100	0.53

Characterisation and Identification of Residues

The identification and characterisation of radioactive residues in cabbages are summarised below in Table B.7.2.1.6-3 for soil applications and in Table B.7.2.1.6-4 for foliar application.

In cabbages treated by soil application, the most prominent residues in both the head and outer leaves were flutolanil,M-4, and M-4 glucose conjugate, representing 49.31 to 69.17% TRR (0.10 to 2.31 mg/kg), 5.19 to 8.51% TRR (0.01 to 0.26 mg eq./kg) and 13.84 to 25.14% TRR (0.04 to 0.79 mg eq./kg), respectively.

The most prominent residue in cabbages treated by foliar application was flutolanil which formed 90.41 to 98.54% TRR at harvest. M-4, sum of free and glucose conjugate, was observed as a minor metabolite (≤ 7.57% TRR).

In phenyl-label treated cabbage, the phenyl ring metabolites M-101 and M-102 produced by cleavage of the amide bond were found in trace amounts (≤ 1% TRR). No specific aniline ring metabolites were detected. In addition M-2, M-3, M-5, M-6, M-7, M-11 were observed as minor metabolites (maximum 1.19% TRR).

The majority of post extraction solids (PES) in cabbage samples were solubilized with alkaline hydrolysis treatment (10 N NaOH, 110°C). Radioactivity released was identified as M-4, assumed to be released from residues strongly bound to biomolecules.

The identification and characterisation of radioactive residues in soil following soil application are summarised below in Table B.7.2.1.6-5. A similar metabolic profile was observed in soil extracts with 85.60 to 86.21% TRR identified as flutolanil and trace amounts of the metabolites M-2, M-3, M-4 , M-11 (maximum 1.25%) observed in both [^{14}C phenyl]- and [^{14}C aniline]- treated soil. Trace amounts of the phenyl ring metabolites M-101 and M-102 (maximum 0.17%) were also observed in phenyl-label treated soil.

Table B.7.2.1.6-3: Summary of identification and characterisation of residues in cabbage following soil application with [^{14}C]-Flutolanil

Radiolabel	[Phenyl-U-^{14}C]-Flutolanil				[Aniline-U-^{14}C]-Flutolanil			
Harvest	Head formation		Mature		Head formation		Mature	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
TRR Head		1.34		0.21		1.40		0.26
Flutolanil	61.62	0.83	49.31	0.10	66.25	0.93	55.70	0.15
M-2	0.16	0.01	ND	ND	0.04	<0.01	ND	ND
M-3	0.45	0.01	0.48	<0.01	0.43	<0.01	0.62	<0.01
M-4	5.19	0.07	6.60	0.01	5.36	0.07	7.50	0.02
M-5	0.16	0.01	ND	ND	0.14	<0.01	ND	ND
M-6	0.52	0.01	0.74	<0.01	0.47	<0.01	0.73	<0.01
M-7	ND	ND	ND	ND	0.08	<0.01	ND	ND
M-11	ND	ND	0.13	<0.01	ND	ND	ND	ND
M-101	0.85	0.01	0.99	<0.01	-	-	-	-
M-102	0.31	0.01	0.40	<0.01	-	-	-	-
M-4 glucoside	13.84	0.19	20.53	0.04	17.33	0.24	24.57	0.06
PES characterisation								
PES after weak acidic extraction	16.92	0.23	20.81	0.04	9.93	0.14	10.87	0.03
Further PES extractions								
Cellulase	ND	ND	ND	ND	ND	ND	ND	ND
6N HCl Reflux	ND	ND	ND	ND	ND	ND	ND	ND
10N NaOH Reflux	15.69	0.21	14.56	0.03	9.26	0.13	6.20	0.02
PES after strong basic extraction	1.23	0.02	6.25	0.01	0.67	<0.01	4.67	0.01
TRR Outer leaf		3.37		3.03		2.92		3.34
Flutolanil	62.31	2.10	60.01	1.82	62.88	1.84	69.17	2.31
M-2	0.07	0.01	ND	ND	0.11	<0.01	ND	ND
M-3	0.50	0.02	0.58	0.02	0.52	0.02	0.60	0.02
M-4	6.59	0.22	8.51	0.26	0.45	0.19	7.31	0.24
M-5	0.28	0.01	ND	ND	0.23	<0.01	ND	ND
M-6	0.62	0.02	0.82	0.03	0.77	0.02	1.19	0.04
M-7	<0.01	0.01	ND	ND	0.16	<0.01	ND	ND
M-11	0.09	0.01	0.60	0.02	0.07	<0.01	ND	ND
M-101	1.00	0.03	0.68	0.02	-	-	-	-
M-102	0.55	0.02	0.62	0.02	-	-	-	-
M-4 glucoside	23.51	0.79	25.14	0.76	22.71	0.66	18.75	0.63
PES characterisation								
PES after weak acidic extraction	4.48	0.15	3.04	0.09	6.12	0.18	2.97	0.10
Further PES extractions								
Cellulase	ND	ND	ND	ND	ND	ND	ND	ND
6N HCl Reflux	0.94	0.03	ND	ND	0.86	0.03	ND	ND
10N NaOH Reflux	2.77	0.09	2.01	0.06	4.87	0.14	2.39	0.08
PES after strong basic extraction	0.78	0.03	1.03	0.03	0.39	0.01	0.59	0.02

Table B.7.2.1.6-4: Summary of identification and characterisation of residues in cabbage following foliar application with [Phenyl-U-^{14}C]-Flutolanil

Radiolabel	[Phenyl-U-^{14}C]-Flutolanil			
Sample	Head		Outer leaf	
	%TRR	mg/kg	%TRR	mg/kg
TRR		0.10		90.89
Flutolanil	90.41	0.09	98.54	89.40
M-2	ND	ND	ND	ND
M-3	0.19	<0.01	ND	ND
M-4	2.48	<0.01	0.12	0.12
M-5	ND	ND	ND	ND
M-6	0.27	<0.01	0.03	0.03
M-7	ND	ND	ND	ND
M-11	0.04	<0.01	ND	ND
M-101	0.29	<0.01	0.07	0.08
M-102	0.14	<0.01	ND	ND
M-4 glucoside	5.09	<0.01	1.14	1.15
PES	1.10	<0.01	0.10	0.10

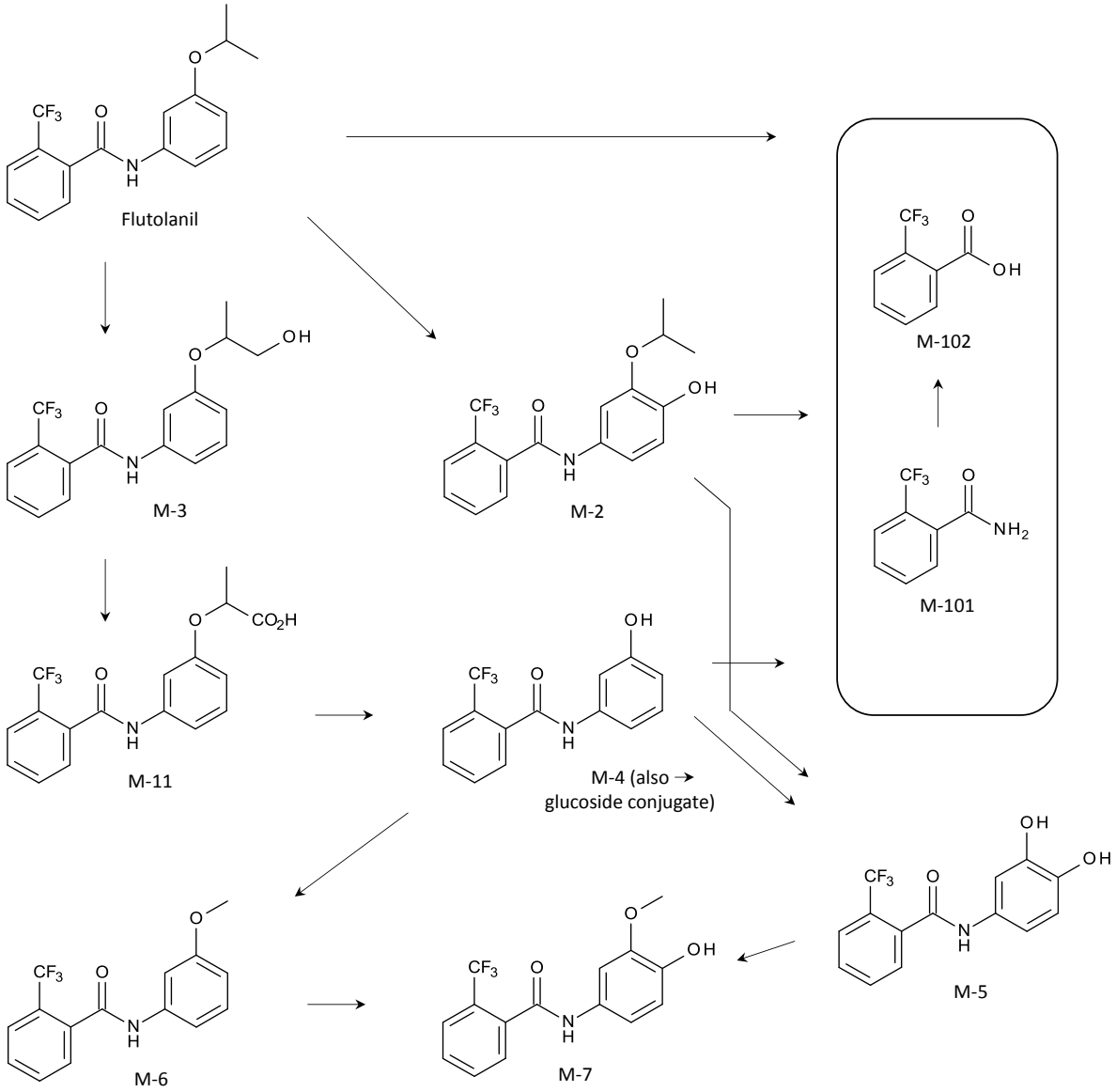
Table B.7.2.1.6-5: Summary of identification and characterisation of soil residues following soil application with [^{14}C]-Flutolanil

Radiolabel	[Phenyl-U-^{14}C]-Flutolanil		[Aniline-U-^{14}C]-Flutolanil	
Harvest	Mature		Mature	
	%TRR	mg/kg	%TRR	mg/kg
TRR Soil		24.28		20.80
Flutolanil	85.60	20.79	86.21	17.93
M-2	0.15	0.04	0.17	0.03
M-3	0.09	0.02	0.10	0.02
M-4	0.91	0.22	0.71	0.15
M-5	ND	ND	ND	ND
M-6	ND	ND	ND	ND
M-7	ND	ND	ND	ND
M-11	1.25	0.30	1.12	0.23
M-101	0.17	0.04	-	-
M-102	0.06	0.01	-	-
PES	11.77	2.86	11.70	2.43

Metabolic pathway

A metabolic pathway for flutolanil in cabbage is proposed in Figure B.7.2.1.6-1 below:

Figure B.7.2.1.6-1: Proposed metabolic profile of flutolanil in cabbage



III. CONCLUSIONS

At maturity, soil treated cabbages contained TRRs of 0.21 and 0.26 mg eq./kg, while the outer leaves contained TRRs of 3.03 mg eq./kg and 3.34 mg eq./kg for [^{14}C phenyl]- and [^{14}C aniline]- treated plants, respectively.

The major components detected in soil treated cabbages were:

- Flutolanil (49.31 to 69.17% TRR, 0.10 to 2.31 mg/kg) and M-4 both as the free metabolite and as a glucoside conjugate (in total 19.03 to 33.65% TRR; 0.05 to 1.02 mg eq./kg).
- Additional amounts of M-4 were released from bound plant residues after refluxing in strong base

At maturity, foliar treated cabbages contained a TRR of 0.09 mg eq./kg, while the outer leaves contained a TRR of 90.89 mg eq./kg following application of [^{14}C phenyl]- flutolanil. The only significant residue detected in foliar treated cabbages was flutolanil which formed 90.41 to 98.54% of the TRR at harvest.

B.7.2.2 Poultry

B.7.2.2.1 Metabolism in laying hens

Previous evaluation	in the DAR, with additional information over methods and extractions, submitted within renewal proces.
RMS remark	Acceptable

Report

[redacted] 1989. The metabolism of ^{14}C-flutolanil in laying hens, [redacted]. Report No. R-3012.

Test guideline and GLP

The study was performed using EPA guideline 171/4 and it was done under GLP.

Materials and methods

[Aniline ring-U-14C] flutolanil in gelatine capsule was orally administered to egg-laying hens (Gallus gallus domesticus) at doses of 0.035 and 1.0 mg/kg bw/day for 4 days (10 birds/dose). Radioactivity in excreta and produced eggs were measured during and after administration. Eggs were collected daily from each bird for 24 hours prior to dosing measured at 6 and 24 hr after and up until the time of sacrifice. Concentrations of radioactivity in tissues were the last dose. Obtained samples were also subjected to metabolite analysis by thin-layer chromatography.
Sample Preparation and Extraction
Excreta samples were extracted by maceration with methanol. Eggs were removed from the shells, weighted and individually homogenised. Liver, kidneys and samples of muscles were cut up with scissors and homogenised. Samples of bile were diluted with distilled water to 4.5 - 5.0 mL.

Samples of methanol extracts of excreta, aqueous and methanolic cage washes and diluted bile were mixed with MI-31 scintillator. Samples of whole-blood, tissue homogenates, egg homogenates and skin/fat were combusted in oxygen using an Automatic Sample Oxidiser. The products of combustion were absorded into Optisorb ‘I’ and mixed with Optisorb ‘S’ scintillator for measurement of radioactivity. Radioactivity was measured using an Automatic Liquid Scintillation Analyser or Counter.

The only tissues containing sufficient radioactivity for investigation of metabolites were liver and kidneys from birds sacrificed at 6 hours. No eggs contained sufficient radioactivity for investigation of metabolites. The measurement of the proportions of radioactive components in excreta and tissues (liver and kidney) were examined by TLC.

Table B.7.2.2.1-1 Radioactivity in hens fed radiolabelled flutolanil

Dose (mg/kg bw)	Sacrificed Time*	Radioactivity concentration (µg/g)				
		Eggs	Kidneys	Liver	Muscle	Skin and fat
0.035	6	<0.0010	0.006-0.012	0.011-0.014	<0.001	0.002-0.004
0.035	24		0.001	0.004-0.012	<0.001	<0.002
1.0	6	<0.0074	0.040-0.082	0.081-0.205	<0.005	0.012-0.024
1.0	24		0.007-0.014	0.035-0.096	<0.005	<0.016

At 24 hr after the last dose of 14C-flutolanil, more than 85% of the cumulative radioactive dose was excreted via urine and faeces. Radioactivity concentrations in most eggs from hens dosed at 0.035 mg/kg bw/day were below the detection limit (0.0007 µg/g) except for 3 eggs, which had radioactivity concentrations at 0.0008-0.0010 µg/g. With 1 mg/kg bw dosing, 20 eggs out of a total of 27 had radioactivity concentrations below the detection limit (0.0044 µg/g). The remaining 7 eggs had concentrations at 0.0044-0.0074 µg/g. Among tissues examined, liver and kidney had the highest radioactivities. Whereas in muscle, skin and fat, radioactivity concentrations were below the detection limit 24 hr after the last dose (TableB. 7.2.2.1-1).

In excreta (urine and faeces), major metabolites found were unchanged flutolanil, α,α,α-trifluoro-3'-hydroxy-o-toluanilide (M-4/DIP) and the glucuronide conjugate of M-4. There was also a small portion of the conjugate of α, α, α -trifluoro-4'-hydroxy-3'-methoxy-o-toluanilide (M-7/HMD). In kidney and liver extracts, radioactivity was almost completely associated with glucuronide/sulphate conjugates of M-4.

Conclusion

Radioactivity concentrations in eggs produced from hens administered with ^{14}C-flutolanil at doses of 0.035 and 1.0 mg/kg bw/day were less than 0.0010 and 0.0074 µg/g, respectively. Liver and kidney contained higher radioactivity concentrations than muscle, skin and fat. At 24 hr after the last dose, concentrations in the latter tissues were below the detection limit. In liver and kidneys, glucuronide and/or sulphate conjugates of M-4 were found as major radioactive component. The study is acceptable.

B.7.2.2.2 Metabolism in laying hens

Previous evaluation	Submitted for the purpose of renewal
RMS remark	Acceptable

Reference:	[redacted], **2016a**
Title:	Flutolanil: Metabolism in Laying Hens
Document No.:	LMS0102 (R-3386)
Guidelines:	OECD 503: Metabolism in Livestock (2007) US EPA OPPTS 860, 1300
Deviations:	None
Testing laboratory:	[redacted]
GLP:	Yes

Executive Summary

A livestock metabolism study was performed to assess the excretion, distribution and metabolism of [phenyl-U-^{14}C]-flutolanil in the laying hen after 14 consecutive daily oral doses at a rate of 13.7 mg/kg in the diet, equivalent to 0.78 mg/kg bw/d.

The concentration profiles of radioactivity in eggs and excretion patterns were followed. Hens were sacrificed 2 hours after the last dose and liver, muscle, fat, skin and partially formed eggs collected. Residual radioactivity in all tissues and metabolite patterns in liver, muscle, fat and eggs were determined.

Approximately 94% of the dose was recovered with radioactivity in excreta and cage washes accounting for 90.4% and 3% of the dose. A low amount of radioactivity was detected in eggs (<0.1% dose) and 0.1% of the dose was detected in tissues.

Total radioactive residues detected in animal matrices are summarised below.

Tissue	**Total radioactive residues (mg eq./kg)**
Eggs	Range < LOD – 0.063, Pool 0.051
Liver	0.518
Fat	0.127
Muscle	0.034

Residues in eggs reached a plateau of 0.04 - 0.06 mg/kg during Days 7 to 14.

In eggs the only significant residue (> 0.01 mg eq./kg) was M-101 (in total 51.8% TRR, 0.026 mg eq./kg) including amounts released by further harsh extraction of PES samples, accompanied by smaller amounts of flutolanil and M-4 (maximum 5.4% TRR, 0.003 mg eq./kg).

In liver flutolanil was detected at 2.6% TRR (0.014 mg/kg). The major components identified in liver were M-4, both free and conjugated, and M-101 which overall accounted for a total of 10.3% and 45.2% TRR respectively (0.055 and 0.234 mg eq./kg), including amounts released by further harsh extraction of PES samples.

Flutolanil was identified as the only significant residue in fat (42.8% TRR, 0.054 mg/kg).

The low residues in muscle was composed of mainly M-101 (45.8% TRR, 0.016 mg eq./kg) with small amounts of flutolanil (6.3% TRR, 0.002 mg/kg).

No other major metabolites (>10% TRR) were formed in livestock tissues, but M-2, both free and conjugated, M-3, M-5, M-7 and M-102 were detected as minor metabolites (maximum 7.0% TRR).

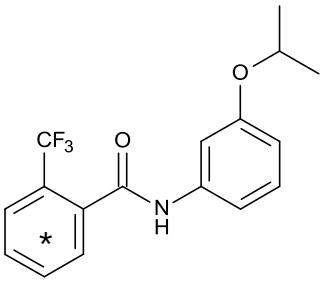
In excreta flutolanil, M-2 and M-4 were the main components identified (representing 10.3, 7.8 and 14.6% of the dose). Smaller amounts of M-3, M-5, M-11, M-101 and M-102 was observed (maximum 3.3% dose).

It was concluded that flutolanil and its metabolites were not retained in the laying hen after repeated oral administration at a highly exaggerated dose.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: [Phenyl-U-^{14}C]-flutolanil



* Denotes position of [^{14}C]-radiolabel

Chemical name (IUPAC)	α, α, α-trifluoro-3'-isopropoxy-*o*-toluanilide
CA registry number:	66332-96-5
Lot or batch number:	LMS0105/OOE01/01
Specific activity:	13.348 MBq/mg
Radiochemical purity:	>97%

2. Test animals

Species:	Laying hen
Strain:	Bovan Brown
Gender:	Female
Age:	28 - 30 weeks
Weight at dosing:	Average 1.7983 kg, Range 1.562 – 1.958 kg
Number of animals:	Ten
Acclimatisation period:	14 days
Diet:	Pellets (Special Diet Services Ltd.,Witham, Essex)
Water:	Mains water, *ad libitum*
Housing:	Stainless steel cage fitted with removable tray to collect excreta
Photoperiod:	17 hours light / 7 hours dark cycles

B. STUDY DESIGN AND METHODS

1. In-life dates:

11 December 2014 to 2 August 2016

2. Experimental design

1.	Dosing Regime:	Oral
	Amount of dose:	13.7 mg/kg in diet, equivalent to 0.78 mg/kg bw/d
	Food consumption:	Average 1467.4 g, Range 1125 - 1931 g over 14 days (experimental phase)
	Vehicle:	Gelatin capsules
	Timing:	Once daily in the morning (at *ca* 09:00 hours)
	Duration:	14 days
2.	Sample Collection	
	Egg collection:	Twice daily
	Excreta collection:	Twice daily (0-6 and 6-24 hour)
	Cage wash:	Once daily & after sacrifice
	Interval from last dose to sacrifice:	2 hours
	Tissues harvested and analysed:	Blood samples were taken at 0.5, 1, 2, 4, 6, 8, 10, 12 & 24 hours following the first dose. The C_{max} was subsequently measured as 0.095 mg eq./kg 1 hour after dosing and on this basis, the hens were sacrificed 2 hours after the final dose. Immediately prior to sacrifice a sample of blood was collected and a portion used to prepare plasma. Liver, muscle (leg & breast), fat (abdominal, omental & subcutaneous), skin, partially formed eggs and GI tract (the latter was not analysed).

Sample Preparation

Eggs from each collection period were pooled, weighed and homogenised (yolk and white combined). Excreta from individual hens was pooled to provide a bulk excreta sample per day and collection period. Skin, liver, muscle and fat samples were combined from all ten treated hens. All pooled tissues and partially formed eggs from each hen were homogenised using a commercial food blender. As the concentration of radioactivity from leg and breast muscle and from omental, subcutaneous and abdominal fat was similar, combined samples were prepared. Pooled fat, muscle and skin were scissor-minced prior to homogenisation.

TRRs were determined by LSC either directly (plasma and cage washings) or by combustion followed by LSC (liver, excreta and whole blood). TRRs in eggs, fat, muscle and skin were measured by LSC following the addition of a solubilising agent or digestion.

Extraction and Fractionation of Residues

A pool of homogenised eggs from Day 14 was created and sequentially extracted with a rotary shaker for 45-60 minutes with the following sequence of solvents:

Acetonitrile, 3 times

Acetonitrile : 0.1N HCl (4:1, v/v), 2 times

Samples of liver and muscle were sequentially extracted with a rotary shaker for 45 minutes with the following sequence of solvents:

Acetonitrile, 3 times

Acetonitrile : water (1:1, v/v), 1 times

Acetonitrile : 0.1N HCl (4:1, v/v), 2 times

A pooled fat sample (mixture of abdominal, omental and subcutaneous) was extracted 3 times with acetonitrile on a rotary shaker for 45 minutes.

Following each extraction, the supernatant separated by centrifugation. Radioactivity present in extracts was quantified by liquid scintillation counting (LSC). Acetonitrile and acetonitrile/water extracts were combined and concentrated prior to analysis by reverse phase HPLC and 2-D normal phase TLC, as were the acidic acetonitrile extracts.

The egg, liver and muscle residues were then subjected to protease digestion, and egg and liver samples to further acid or base extraction as follows:

Incubation in 0.1M phosphate buffer (pH 7) with protease enzymes (Type 1 from Bovine pancreas) for *ca.* 18 hours at 37°C (egg, liver and muscle)

Incubation with 1M HCl for *ca.* 18 hours at 37°C (egg & liver only)

Incubation with 1M NaOH for *ca.* 18 hours at 37°C (liver only)

Following each incubation, the sample was extracted with acetonitrile by vigorous shaking (45 minutes), the supernatant separated by centrifugation and partitioned with dichloromethane. After the final basic extraction of liver the aqueous fraction was neutralised by the addition of acid analysis by reverse phase HPLC and 2-D normal phase TLC.

Radioactive residues in post extraction solids (PES) was quantified by solubilisation.

Extraction and analysis of excreta was undertaken to aid metabolite identification. A combined excreta pool was prepared by combining aliquots (5% by weight) of samples collected from Day 1 to 14. Samples were extracted 4 times with acetonitrile on a rotary shaker (45 minutes). Extracts were pooled and concentrated prior to analysis by reverse phase HPLC.

Neutral liver extracts were incubated in 0.2M acetate buffer (pH 5) as follows:

Buffer only (control)

Buffer + β-glucuronidase/sulfatase (Type H-1, *Helix pomatia*)

Buffer + mixture of β-glucuronidase/sulfatase and a β-glucuronidase specific inhibitor (D-saccharic acid-1,4-lactone)

Samples were incubated at 37°C for 24 hours and analysed by reverse phase HPLC and 2-D normal phase TLC. Peaks corresponding to M-2 and M-4 glucuronide conjugates were identified by chromatographic analysis of samples before and after each incubation.

II. RESULTS AND DISCUSSION

Identification of metabolites occurred based on (1D or 2D) TLC. Confirmation of identity and quantity occurred by HPLC-MS/MS. Examples of TLC plates and HPLC chromatograms showed that metabolites were well separated and quantified.

The overall recovery was 93.5% of the cumulative dose. Radioactivity recovered in excreta and cage washes accounted for 90.4% and 3.0% of the total cumulative dose respectively. Recovery of radioactivity from eggs was <0.1% and radioactivity remaining in tissues at sacrifice accounted for 0.1% of the total dose.

The total radioactive residues measured in livestock matrices following administration of [phenyl-U-^{14}C]-flutolanil in the diet are summarised in Table B.7.2.2.2- 1.

Table B.7.2.2.2- 2: Total radioactive residues in eggs, tissues and excreta after administration of [phenyl-U-^{14}C]-flutolanil

Matrix	Collection timing	% AR	TRR (mg/kg)	Pooled TRR for analysis (mg/kg)
Excreta	Day 1-14	90.4	-	-
Cage wash	Day 1-14	3.0	-	-
Eggs	Day 1-14	<0.1	< LOD – 0.063	-
Eggs (pooled)	Day 14	<0.1	-	0.051
Tissues	Day 14	0.1	-	-
Liver	Day 14	0.1	0.518	0.518
Fat (Abdominal)	Day 14	<0.1	0.114	-
Fat (Omental)	Day 14	<0.1	0.193	-
Fat (Subcutaneous)	Day 14	<0.1	0.074	-
Fat (Pooled)	Day 14	<0.1	-	0.127
Muscle (Leg)	Day 14	<0.1	0.037	-
Muscle (Breast)	Day 14	<0.1	0.031	-
Muscle (Pooled)	Day 14	<0.1	-	0.034
Skin	Day 14	<0.1	0.085	-
Whole blood	Day 14	<0.1	0.068	-
Plasma	Day 14	<0.1	0.078	-
Partially formed eggs	Day 14	<0.1	0.114	-
Accountability		93.5		

Total radioactive residues (TRR) were measured twice-daily in pooled eggs and found to increase gradually, reaching a plateau level of 0.043 - 0.063 mg/kg during Days 7 to 14 (see Table B.7.2.2.2-2).

Table B.7.2.2.2-2: TRR in eggs with time after administration of [phenyl-U-^{14}C]-flutolanil

Time (Hours after first dose)	Day	TRR in eggs (mg/kg)	
		Afternoon collection	Morning collection
Predose	-1 / 1	-	ND
0 - 24	1 / 2	ND	0.006
24 - 48	2 / 3	0.008	0.01
48 - 72	3 / 4	0.015	NS
72 - 96	4 / 5	0.023	NS
96 - 120	5 / 6	0.030	0.04
120 - 144	6 / 7	0.027	0.036
144 - 168	7 / 8	0.045	NS
168 - 192	8 / 9	0.054	NS
192 - 216	9 / 10	0.043	0.045
216 - 240	10 / 11	0.045	0.063
240 - 264	11 / 12	0.044	0.047
264 - 288	12 / 13	0.053	NS
288 - 312	13 / 14	0.056	NS
312 - 314	14	0.051	-

ND = Not detected NS = No sample

Edible tissues containing significant levels of radioactivity (liver, fat, muscle and eggs) were extracted with solvent and solvent/water mixtures to characterise the nature of the residue (see Table B.7.2.2.2-3). Homogenised samples were extracted with solvent/water mixtures, followed by treatment with protease enzymes (liver, muscle and eggs) and acid/base extraction (liver and eggs).

In all cases the majority of the radioactivity was successfully extracted (liver 55%, fat 95%, muscle 87% and eggs 66%). Radioactivity released from post extraction solids (PES) with protease enzymes, acid and base accounted for 37% of the TRR in liver, 4% in muscle and 29% in eggs.

Table B.7.2.2.2-3: Distribution of radioactivity in extracts of livestock matrices after administration of [phenyl-U-^{14}C]-flutolanil

Fraction	Liver		Fat		Muscle		Eggs	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
TRR		0.518		0.127		0.034		0.051
Extracts	54.7	0.283	94.8	0.120	86.7	0.029	65.8	0.033
Neutral solvents	52.3	0.271	94.8	0.120	82.3	0.028	59.0	0.030
MeCN / 0.1N HCl	2.4	0.012	NA	NA	4.4	0.001	6.8	0.003
PES	45.4	0.235	5.2	0.007	13.3	0.004	34.3	0.018
PES characterisation								
Protease enzyme	12.7	0.066	NA	NA	3.9	0.001	15.3	0.008
1N HCl	9.3	0.048	NA	NA	NA	NA	13.2	0.007
1N NaOH	15.4	0.080	NA	NA	NA	NA	NA	NA
PES after further extraction	8.0	0.041	NA	NA	9.4	0.003	5.8	0.003

NA = Not applicable

The identification and characterisation of radioactive residues in the hen tissues, eggs and excreta are summarised below in Table B.7.2.2.2-4 and Table B.7.2.2.2-5.

In liver flutolanil was detected at 2.6% TRR. The major component identified in neutral and weak acidic extracts was the phenyl ring metabolite M-101 (16.6% TRR, 0.086 mg eq./kg) produced by cleavage of the amide bond. M-2 and M-4, both as the free metabolite and as glucuronide conjugates, were detected as minor metabolites in neutral and weak acidic extracts of liver (in total 7.0 and 9.0% TRR, 0.036 and 0.047 mg eq./kg). In addition, small amounts of M-3, M-5, M-7 and the phenyl ring metabolite M-102 were found (maximum 3.1% TRR, 0.006 mg eq./kg). No flutolanil was released from liver by further extraction of PES samples with protease enzymes and 1N acid/base extractions. The major component identified was M-101 (28.6% TRR, 0.148 mg eq./kg) along with smaller amounts of M-4, M-5 and M-102 (maximum 1.5% TRR, 0.008 mg eq./kg). Overall M-101 accounted for a total of 45.2% TRR in the liver. The total levels of M-4, including those released by further extraction of PES samples, represented 10.3%. No other major metabolites (>10% TRR) were formed.

Flutolanil was identified as the major component in fat (42.8% TRR, 0.054 mg/kg). In addition, M-2, M-4, M-5, M-101 and M-102 were identified as minor metabolites (maximum 6.1% TRR, 0.008 mg eq./kg).

In muscle flutolanil was detected at 6.3% TRR (0.002 mg/kg). The low residues in muscle was composed of mainly M-101 (45.8% TRR, 0.016 mg eq./kg) with trace amounts of M-2, M-4, M-5 and M-102 (maximum 1.6% TRR, 0.001 mg eq./kg).

A similar pattern was seen in eggs, where flutolanil was detected at 5.4% TRR (0.003 mg/kg). The major component identified in neutral extracts of eggs was composed of mainly M-101 (36.5% TRR, 0.019 mg eq./kg) with trace amounts of M-4 (2.2% TRR, 0.001 mg eq./kg). No flutolanil was released from eggs by further extraction of PES samples with protease enzymes and 1N acid extraction. The major component identified was M-101 (15.3% TRR, 0.007 mg eq./kg) along with smaller amounts of M-4 (0.7% TRR, <0.001 mg eq./kg). Overall M-101 accounted for a total of 51.8% TRR in eggs (0.026 mg eq./kg). No other major metabolites (>10% TRR) were formed.

In excreta flutolanil, M-2 and M-4 were the main components identified (10.3, 7.8 and 14.6% of the dose). No other components exceeded 5% dose. Smaller amounts of M-3, M-5, M-11, M-101 and M-102 was observed (maximum 3.3% dose).

Table B.7.2.2.2-4: Summary of characterisation and identification in livestock tissues after administration of [phenyl-U-^{14}C]-flutolanil

Metabolites	Liver		Fat		Muscle		Eggs	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
TRR		0.518		0.127		0.034		0.051
Extracted	54.7	0.283	94.8	0.120	86.7	0.029	65.8	0.033
Flutolanil	2.6	0.014	42.8	0.054	6.3	0.002	5.4	0.003
M-2	2.5	0.013	5.7	0.007	1.6	0.001	-	-
M-3	2.4	0.012	-	-	-	-	-	-
M-4	0.1	0.001	3.3	0.004	1.6	0.001	2.2	0.001
M-5	2.2	0.011	2.5	0.003	0.7	<0.001	-	-
M-7	3.1	0.016	-	-	-	-	-	-
M-101	16.6	0.086	6.1	0.008	45.8	0.016	36.5	0.019
M-102	2.3	0.012	2.1	0.003	0.8	<0.001	-	-
M-2 glucuronide	4.5	0.023	-	-	-	-	-	-
M-2 sulfate	-	-	-	-	-	-	-	-
M-4 glucuronide	8.9	0.046	-	-	-	-	-	-
M-4 sulfate	-	-	-	-	-	-	-	-
Others [A]	9.5	0.049	32.3	0.041	29.9 [B]	0.010 [B]	21.7 [C]	0.011 [C]
PES	45.4	0.235	5.2	0.007	13.3	0.004	34.3	0.018
PES characterisation								
Protease enzyme	12.7	0.066	NA	NA	3.9	0.001	15.3	0.008
1N HCl	9.3	0.048	NA	NA	NA	NA	13.2	0.007
1N NaOH	15.4	0.080	NA	NA	NA	NA	NA	NA
PES Extracts	37.4	0.194	NA	NA	3.9	0.001	28.5	0.015
M-4	1.3	0.008					0.7	<0.001
M-5	0.6	0.004					-	-
M-101	28.6	0.148					15.3	0.007
M-102	1.5	0.008					-	-
Others	5.4	0.026					12.5	0.007
PES after further extraction	8.0	0.041	NA	NA	9.4	0.003	5.8	0.003

NA = Not applicable - = Not detected

[A] Multiple components of which no individual component exceeded 0.039, 0.019, 0.002 and 0.002 mg eq./kg liver, fat, muscle and egg, respectively

[B] Includes 4.4% TRR, 0.001 mg eq./kg in acetonitrile / 0.1N HCl extract not analysed.

[C] Includes 6.8% TRR, 0.003 mg eq./kg in acetonitrile / 0.1N HCl extract not analysed.

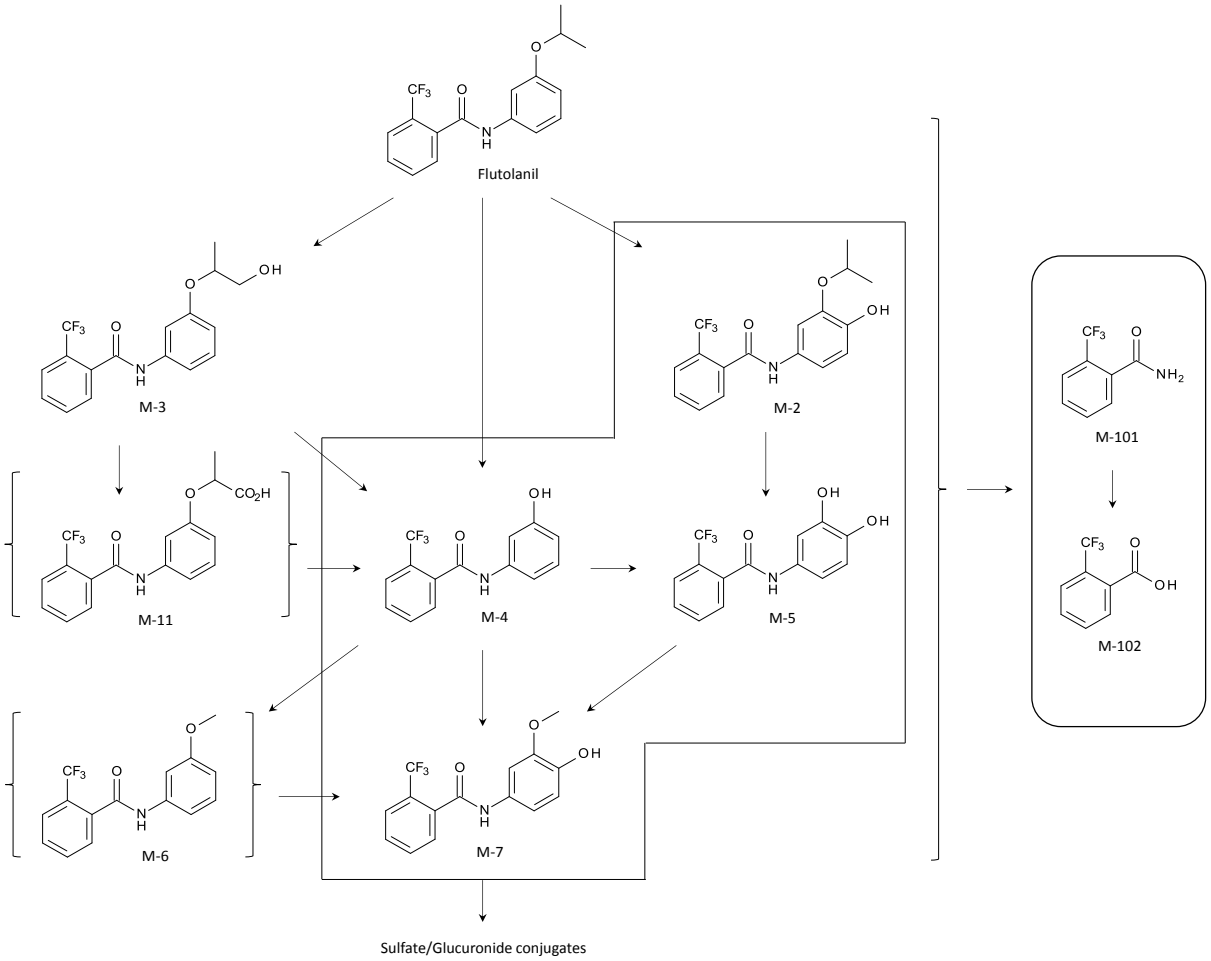
Table B.7.2.2.2-5: Summary of characterisation and identification in livestock excreta after administration of [phenyl-U-^{14}C]-flutolanil

Metabolites	Urine (% Dose)
Flutolanil	10.3
M-2	7.8
M-3	1.4
M-4	14.6
M-5	3.3
M-11	1.0
M-101	0.9
M-102	3.7
Others	47.4
Total	90.4

Metabolic pathway

A metabolic pathway for [phenyl-U-^{14}C]-flutolanil in hens is proposed in Figure B.7.2.2.2-1. The metabolic profile is similar to that seen in rats treated with flutolanil. All of the identified metabolites were observed in the rat.

Figure B.7.2.2.2-1: Proposed metabolic profile of [phenyl-U-^{14}C]-flutolanil in hen. Metabolites in parenthesis (M6, M11) are proposed intermediates not identified. Blocked metabolites (M2, M4, M5 and M7) were found free and as conjugated glucuronides and/or sulfates.



Conclusions

Flutolanil and its metabolites were rapidly excreted by the laying hen with 93.4% of the administered dose recovered in excreta and cage wash (> 99.9% of the recovered dose). Tissues (liver, fat, muscle, and skin) retained only low levels of radioactivity (0.1%). The TRR values for eggs ranged from < LOD – 0.063 mg eq./kg which represented <0.1% of the administered dose.

Metabolism of [phenyl-U-^{14}C]-flutolanil was extensive. The major residues (> 10% TRR) in livestock tissues were flutolanil and its metabolites M-4 (including the free metabolite, conjugates and amounts released by harsh extraction) and the phenyl ring metabolite M-101.

In eggs the only significant residue (> 0.01 mg eq./kg) was the metabolite M-101.

In conclusion, if hens were exposed to significant flutolanil residues through the diet, the residues are rapidly metabolised and excreted with low transfer of residues to eggs or edible tissues. However, the dose level in the study represents more than 200 times the calculated maximum dietary burden for flutolanil residues in poultry feed (based on residues in potatoes in this dossier). Consequently, no residues of flutolanil or its metabolites are anticipated in eggs or tissues of laying hens.

B.7.2.3 Lactating ruminants

B.7.2.3.1 Metabolism in lactating goats

Previous evaluation	in Addendum 1A to the DAR
RMS remark	Not accepted during the initial peer review

Report: [redacted] 1991. The metabolism of ^{14}C-flutolanil in goats, [redacted] Report No. R-3013.

Guidelines: The study was performed using EPA guideline 171/4 and it was done under GLP.

GLP: Yes.

Radioactive probe: Aniline ring-U-^{14}C] flutolanil 22.4 mCi/mmol (69.1 µCi/mg)

Materials and methods

Test animals: Two female lactating goats were administered single [aniline ring-U-^{14}C] flutolanil daily bolus in gelatine capsules for 4 days at a dose level of 0.61 mg/kg bw/day.

Goats were milked twice a day, immediately before and 6 hrs after the bolus and aliquots were stored frozen. Excreta and gage wash were collected on 24hr basis. At sacrifice the animals were desanguinated in anesthesia and tissue samples were excised. Aliquots of plasma and whole blood were stored. Goat 1 was sacrificed at 6 hours after the last dose and goat 2 at 24 hours.

Total radioactive residue was determined in each sample type by liquid scintillation after combustion of the samples. Concentrations of radioactivity in tissues were measured 6 and 24 hr after the last dose. Excretion of radioactivity in urine, faeces and milk was investigated during and after administration.

Milk, excreta and tissues were subjected to metabolite analysis by thin-layer chromatography using two developing systems separately on a one-dimensional setting and two other systems in a two-dimensional setting. Identification was based on cochromatography of putative synthetic metabolites. Some of the metabolites were identified on structural information provided by mass spectrometry (electron impact spectra of TMS-derivatives) and also by proton magnetic resonance studies.

Sample preparation and extraction

Total radioactive residue was determined in each sample type by liquid scintillation after combustion of the samples. Concentrations of radioactivity in tissues were measured 6 and 24 hr after the last dose. Excretion of radioactivity in urine, faeces and milk was investigated during and after administration.

Milk, excreta and tissues were subjected to metabolite analysis by thin-layer chromatography using two developing systems separately on a one-dimensional setting and two other systems in a two-dimensional setting. Identification was based on chromatography of putative synthetic metabolites. Some of the metabolites were identified on structural information provided by mass spectrometry (electron impact spectra of TMS-derivatives) and also by proton magnetic resonance studies.

Results

Feed consumption was not recordedprior to or during the experiment. Body weigths decreased, which may indicate e.g. loss of apetite or changes in hydration status. The animals maintained normal milk production. No feed refusals were reported.

Body weight of goat #1: 51.5 kg (before administration), 45.4 kg (at Day 4).

Body weight of goat #2: 62.5 kg (before administration), 56.5 kg (at Day 5).

Animal husbandry personel did not report of any abnormalities, but the physical status was not recorded.

Urinary data indicated that steady-state kinetics were not reached during the 4-day dose regimen. Totally 64.8% of 14C-flutolanil dose was excreted during the experiment. Aproximately one third of the dose was found in the urine.

After 4 daily oral doses of ^{14}C-flutolanil, 52.2% of the administered radioactivity was eliminated in the excreta and milk of Goat 1 sacrificed at 6 hr after the last dose. The corresponding result for Goat 2 sacrificed at 24 hr after the last dose was 64.8%.

Concentrations of radioactivity in milk collected 6 hr after dosing were in the range of 0.021-0.040 µg/ml. Concentrations of radioactivity in milk collected immediately before dosing each day were in the range of 0.018-0.032 µg/ml and were always lower than the corresponding 6 hr sample. As shown in the table B.7.2.3-1-1, radioactivity concentrations were higher in tissues of the goat sacrificed at 6 hr after the last dose.

Table B.7.2.3-1-1 Radioactivity in goats fed radiolabelled flutolanil. The time after last dose and sacrifice (wash-out period) was 6 hr for first of the goats and 24 hr for the other.

	Goat #1, 6-hr wash-out	Goat #2, 24-hr wash-out
	mg equiv./kg	mg equiv./kg
Liver	0.302	0.113
Kidney	0.365	0.087
Muscle	0.004	<0.004
Fat	0.043	<0.013
Bile	20.949	6.674
Whole-blood	0.033	0.012
Plasma	0.052	0.015

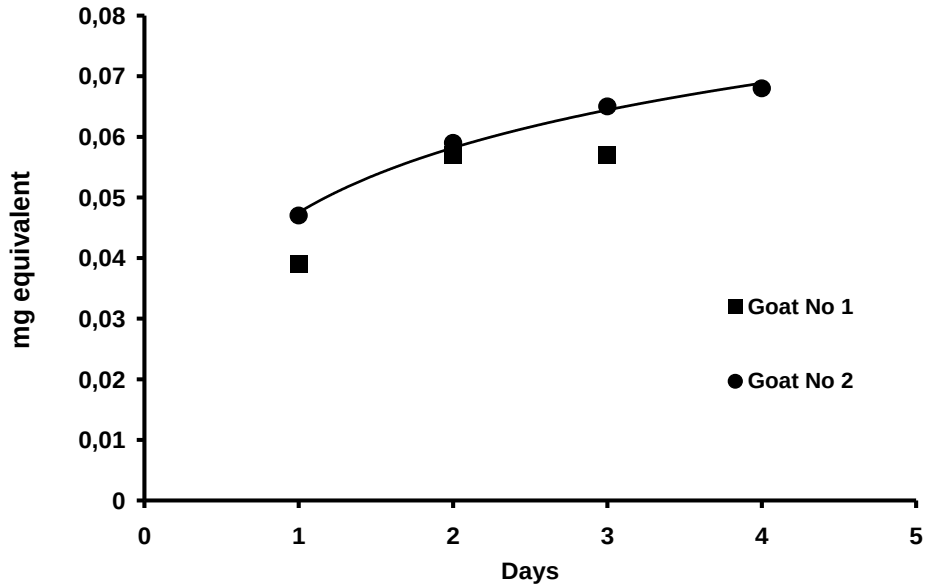
In urine, the major metabolite was glucuronide and sulphate conjugates of α,α,α-trifluoro-3'-hydroxy-o-toluanilide (M-4/DIP). Other components identified were the glucuronide conjugates of α,α,α-trifluoro-4'-hydroxy-3'-isopropoxy-o-toluanilide (M-2/HFT) and α,α,α-trifluoro-4'-hydroxy-3'-methoxy-o-toluanilide (M-7/HMD). Unchanged flutolanil and M-4 were found in fecal extracts.

Table B.7.2.3.1-2 Total mg equivalents (TRR) excreted in separated milkings.

Time	Goat #1		Goat #2	
	mg equiv.	Milk vol. L	mg equiv.	Milk vol. L
1 a.m.	NS	1.54	NS	1.62
1 p.m.	0.021	0.64	0.023	0.69
2 a.m.	0.018	1.54	0.024	1.70
2 p.m.	0.037	0.68	0.027	0.65
3 a.m.	0.020	1.66	0.032	1.72
3. p.m.	0.032	0.49	0.039	0.67
4 a.m.	0.025	1.57	0.026	1.66
4 p.m.	0.034	0.37	0.040	0.62
5 a.m.	NS	NS	0.028	1.57

NS no sample taken

Figure B.7.2.3.1-1 Total mg equivalents (TRR) excreted per day in milk is shown for each day. Steady-state kinetics in milk was essentially reached at the end of the experiment.



Consentration of total radioactive residue (TRR) in milk was at the end of the experiment approximately 0.035 mg equivalents / kg.

Of extracted radioactivity 83.8 - 85.8% was identified as M4 after treatment with β-gluronidase/suphatase. The rest, i.e. 14.2 - 14.7 %TRR comprised of unidentified components.

Table B.7.2.3.1-3 Residue speciation in milk.

Milk day 4 p.m.	Goat #1		Goat #2	
	%TRR	mg eq./kg	%TRR	mg eq./kg
ERR	**N/A**	**N/A**	**N/A**	**N/A**
Component 1	2.4	N/A	ND	N/A
M4	ND	N/A	ND	N/A
- M4 conjugates	81.4 (% applied on TLC1)	N/A	86.5 (% applied on TLC1)	N/A
Component 3 conj.	1.1 (% applied on TLC1)	N/A	2.8 (% applied on TLC1)	N/A
Component 4 conj.	14.3 (% applied on TLC1)	N/A	9.5 (% applied on TLC1)	N/A
Others	3.8 (% applied on TLC1)	N/A	1.2 (% applied on TLC1)	N/A
Unidentified	**19.2 % of appied on TLC1**		**13.5% of appied on TLC1**	
ERR	**N/A**	**N/A**	**N/A**	**N/A**
URR	**N/A**	**N/A**	**N/A**	**N/A**
TRR	**N/A**	**0.034**	**N/A**	**0.040**

The levels of URR and ERR in milk were unavailable.

Table B.7.2.3.1-4. Tables A.C showing residue speciation in the liver and the kidney. Two chromatographic systems were employed for metabolite identification. Data from both systems are given for the kidney.

A. Liver, TLC system 1	Goat #1 (6 hrs wash-out)		Goat #2 (24 hrs wash-out)	
	% of radioactivity applied on a TLC plate	mg eq./kg	% of radioactivity applied on a TLC plate	mg eq./kg
Component 1 conj.	47.8	N/A	27.3	N/A
Component 2 conj.		N/A	28.8	N/A
M4	ND	N/A	ND	N/A
- M4 conjugates	25.6	N/A	15.5	N/A
Component 4 conj.	2.4	N/A	4.9	N/A
Component 5 conj.	12.9	N/A	12.5	N/A
Others	11.3	N/A	11.0	N/A
Unidentified	**74.4**		**84.5**	
ERR	**N/A**	**N/A**	**N/A**	**N/A**
URR	**N/A**	**N/A**	**N/A**	**N/A**
TRR	**N/A**	**0.302**	**N/A**	**0.113**

B. Kidney, TLC system 1	Goat #1 (6 hrs wash-out)		Goat #2 (24 hrs wash-out)	
	% of radioactivity applied on a TLC plate	mg eq./kg	% of radioactivity applied on a TLC plate	mg eq./kg
Component 1 conj.				
M7	4.9		ND	
- M7 conjugates	6.4		6.2	
M4	ND	N/A	ND	N/A
- M4 conjugates	32.8	N/A	21.5	N/A
Component 4 conj.	6.5	N/A	4.8	N/A
Component 5 conj.	9.5	N/A	24.0	N/A
Others	1.2	N/A	6.7	N/A
Unidentified	**17.2 %**		**35.5**	
ERR	**N/A**	**N/A**	**N/A**	**N/A**
URR	**N/A**	**N/A**	**N/A**	**N/A**
TRR	**N/A**	**0.365**	**N/A**	**0.087**

C. Kidney, TLC system2	Goat #1 (6 hrs wash-out)		Goat #2 (24 hrs wash-out)	
	% of radioactivity applied on a TLC plate	mg eq./kg	% of radioactivity applied on a TLC plate	mg eq./kg
Component 1 conj.	16.0		24.5	
M2	ND		ND	
- M2 conj	22.7		5.2	
M4	ND	N/A	ND	N/A
- M4 conjugates	37.6	N/A	22.8	N/A
Component 4 conj.	2.2	N/A	7.3	N/A
Component 5 conj.	17.6	N/A	30.8	N/A
Others	3.9	N/A	9.4	N/A
Unidentified	**39.7%**		**72 %**	
ERR	**N/A**	**N/A**	**N/A**	**N/A**
URR	**N/A**	**N/A**	**N/A**	**N/A**
TRR	**N/A**	**0.365**	**N/A**	**0.087**

In faeces 52 -66 % of radioactivity applied on a TLC plate was identified as flutolanil by cochromatography. The proportiion of M2 on same system was 24-39%. M4 accounted for 2.3 - 5.7 of the radioactivity applied on a TLC plate.

Conclusion

* The feed intake was not recorded, but administered Flutolanil doses (0.61 mg/kg bw/d) in this experiment are estimated to correspond to approximately 2.25 mg/kg wet weight of feed.

For cattle in general, daily dry-weight feed-intake levels are approximately 4% of body weight per day as calculated on gounds of the data given in the Lundehn document. Using this estimation the corresponding feed intakes for these two goats range from 2.1 to 2.5 kg/d. These assumptions and the actual dose are consistent with an intakelevel of 15 mg/kg dry weight. Dry matter content in potatoes is 15%. For green forage dry matter content ranges from 14% to 20%.

* The doses used in the present study are approximately 70 fold compared with estimated dietary intake of Flutolanil was 0.004 - 0.009 mg/kg bw/d, which results from GAP-accordant use.

* The data show that elimination half-life in tissues is less than 24 hrs and consequently there is no accumulation potential.

* Steady-state in milk will be reached approximately within one week.

Administration of ^{14}C-flutolanil to lactating goats at a dose of 0.61 mg/kg bw/day showed that radioactivity was excreted in milk at plateued concentrations of approx. 0.040 mg/L. Exctractable residue in milk comprised mainly (ca. 85%) of conjugated M-4. The concentrations in milk were not available as %TRR. M-4 was also the major residue species in tissues. The levels were expressed only as percentage of applied on a TLC plate.

* Detectable residue levels are possible in ruminant tissues.

At 6 hr after the last dose, concentrations in liver, kidney, muscle and fat were 0.302, 0.365, 0.034 and 0.004 µg/g, respectively. Data obtained with one excessive dose does not allow precise extrapolation to real-life levels. Division renal levels by 70 gives 0.005 mg/kg, but as kinetics may be non-linear, e.g.

absorption may decrease by increasing dose etc, real-life total residue levels in the kidney and the liver may be significantly higher than this estimate.

* The study shows distribution and amounts excreted. Extraction efficiencies from tissues have not been demonstrated. The amount of unextracted material has not been given or studied further.

* The study does not fulfil all of its objectives as to identification of metabolites in tissues for the purposes of residue definition in products derived from ruminants. Unidentified residue species accounted up to 85% of characterized species. The levels of residue species were not given as %TRR or mg eq./kg. In this respect the study does not comply with guidelines and is unacceptable. The study points towards that in the milk, total residue levels resulting from GAP-accordant use, is estimated be less than 0.05 mg/kg.

B.7.2.3.2 Metabolism in lactating goats

Previous evaluation	Submitted for the purpose of renewal
RMS remark	Acceptable

Reference:	**CA 6.2.3/01: [redacted], 2011**
Title:	[^{14}C]-Flutolanil – Absorption, distribution, metabolism and excretion following repeated oral administration to the lactating ruminant
Document No.:	Report No. 8241196 (R-3304)
Guidelines:	OECD 503: Metabolism in Livestock (2007) US EPA OPPTS 860, 1300
Deviations:	None
Testing laboratory:	[redacted]
GLP:	Yes

Executive Summary

A livestock metabolism study was performed to assess the excretion, distribution and metabolism of [aniline-U-^{14}C]-flutolanil in a lactating goat after five consecutive daily oral doses at a rate of 13.26 mg/kg in the diet, equivalent to 0.27 mg/kg bw/d.

The concentration profiles of radioactivity in milk and excretion patterns via urine and faeces were followed. The goat was sacrificed *ca.* 6-7 hours after the last dose and kidney, liver, muscle and fat were collected. Residual radioactivity and metabolite patterns in these organs were determined.

Approximately 74% of the dose was recovered. Excretion of the test item proceeded mainly via urine (55% dose). In faeces, excreted radioactivity accounted for 16% of the dose. A low amount was excreted in milk (<0.02% dose in milk fat and 0.3% in the aqueous milk fraction) and 0.4% of the dose was detected in tissues.

Total radioactive residues detected in animal matrices are summarised below.

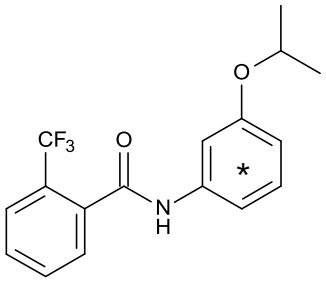
Tissue	Total radioactive residues (mg eq./kg)
Milk fat fraction	0.027 - 0.089
Milk aqueous fraction	0.021 - 0.098
Liver	0.230
Kidney	0.377
Muscle	0.007
Fat	0.013

Residues in milk reached a plateau within one day of dosing. Levels of radioactivity detected in both flank and loin muscle was < 0.01 mg/kg (maximum 0.007 mg/kg) and these samples were not extracted.
In milk fat, flutolanil, M-4 and its sulfate ester and glucuronide conjugate were found at 13.8%, 2.6%, 12.3% and 15.9% TRR respectively (0.012, 0.002, 0.011 and 0.014 mg eq./kg). In the aqueous fraction of milk the only major residues were M-4 sulfate ester and glucuronide conjugate which represented 21.1% and 26.9% of the TRR (0.021 and 0.026 mg eq./kg).
M-4 with its sulfate and glucuronide conjugates were the most abundant components found in liver and kidney at 18% TRR (0.041 mg eq./kg) in total, and 46% TRR (0.173 mg eq./kg) in total, respectively. The low residues in fat was composed of mainly flutolanil (44.7% TRR, 0.006 mg/kg).
It was concluded that flutolanil and its metabolites did not accumulate in the lactating goat after repeated oral administration at an exaggerated dose.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: [Aniline-U-^{14}C]-flutolanil (called [U-^{14}C-aminophenoxy]-flutolanil in report)



* Denotes position of [^{14}C]-radiolabel

Chemical name (IUPAC): α, α, α-trifluoro-3'-isopropoxy-*o*-toluanilide
CA registry number: 66332-96-5
Lot or batch number: CP-3778
Specific activity: 10 MBq/mg
Radiochemical purity: 98.9

2. Test animals
Species: Goat
Strain: British Alpine cross
Gender: Female

Age:	ca. 2 years
Weight at dosing:	60.5 kg
Number of animals:	One study goat and one companion goat
Acclimatisation period:	30 days. During the last 3 days the goat was acclimatised to the metabolism cage for 2 to 4 hours per day.
Diet:	Hay and concentrate twice daily
Water:	Mains water, *ad libitum*
Housing:	Metabolism cage
Environmental conditions	
Temperature:	18 to 21°C
Humidity:	24 to 62%
Air changes:	15 to 20 changes/hour
Photoperiod:	16 hours light / 8 hours dark cycles

B. STUDY DESIGN AND METHODS

1. In-life dates:

20 January 2011 to 21 December 2011

2. Experimental design

1.	Dosing Regime:	Oral
	Amount of dose:	13.26 mg/kg in diet, equivalent to 0.27 mg/kg bw/d
	Food consumption:	1.244 Kg dry diet/day (experimental phase)
	Vehicle:	Gelatin capsules
	Timing:	Once daily in the morning
	Duration:	Five days
2.	Sample Collection	
	Milk collection:	Twice daily
	Urine and faeces collection:	24 hour intervals (including cage washes)
	Interval from last dose to sacrifice:	ca. 6-7 hours
	Tissues harvested and analysed:	Kidneys, liver, fat (omental, subcutaneous & renal) and muscle (flank & loin). A terminal sample of blood was collected and a portion used to prepare plasma.

Sample Preparation

Milk was separated into the fat and aqueous fractions by centrifugation on the day of collection. Tissues were macerated on the day of collection. Combined samples of fat and muscle were prepared. Faeces were homogenised.

TRRs were determined by LSC either directly (urine, milk fractions, plasma and cage washings) or following the addition of a solubilising agent (tissues, faeces and blood).

Extraction and Fractionation of Residues

Samples of milk fat, liver, kidney and fat were sequentially extracted by homogenisation with the following sequence of solvents:

Milk fat, Liver, Kidney and Fat
Hexane
Ethyl acetate
Acetonitrile
1% Formic acid in acetonitrile

In addition, liver and kidney samples were further extracted with the following additional solvents

Liver and Kidney only
Water,
1M Hydrochloric acid
1M Ammonia solution

Following each extraction, the supernatant was separated by centrifugation. Radioactivity present in extracts was quantified by liquid scintillation counting (LSC).

The milk aqueous fraction was partitioned against hexane and the aqueous residue freeze dried, reconstituted in organic solvents and water, then concentrated prior to analysis by HPLC and TLC.
The hexane extracts of the milk fat fraction, liver and fat were each partitioned against acetonitrile leaving very little radioactivity in the hexane phase. The acetonitrile phase and ethyl acetate, acetonitrile and acidic acetonitrile extracts were pooled and concentrated prior to analysis by HPLC and TLC. For kidney, in which very little radioactivity was extracted by hexane, only the ethyl acetate, acetonitrile and acidic acetonitrile extracts were pooled prior to chromatographic analysis.
Portions of concentrated organic extracts from milk aqueous fraction, milk fat fraction, liver, kidney and fat and concentrated organic extracts from milk aqueous fraction were incubated in 0.2M acetate buffer (pH 5) as follows:

β-glucuronidase which exhibits both glucuronidase and sulphatase activity
β-glucuronidase and saccharolactone (*ca* 100 mg/mL), which inhibits glucuronidase activity

Samples were incubated at 37°C for 18 hours and analysed by HPLC and TLC. Peaks corresponding to M-4 sulfate ester and M-4 glucuronide conjugate were identified by chromatographic analysis of samples before and after each incubation.

The further extracts of liver and kidney with water, 1M HCl and 1M NH_3 were combined, freeze dried and reconstituted in organic solvents and water, then concentrated prior to analysis by HPLC. The kidney and liver residues were then subjected to protease digestion and acid/base reflux as follows:

Incubation in 0.1M phosphate buffer with protease solution (*Subtilisin Carlsberg* Type VIII bacterial) for *ca.* 18 hours at 37°C
Strong acid reflux with 10M HCl
Strong basic reflux with 10M NaOH (kidney only)

The protease digests were partitioned against ethyl acetate and in the liver the aqueous phase was analysed by HPLC.

II. RESULTS AND DISCUSSION

Identification of metabolites occurred based on (1D or 2D) TLC-LCD. Confirmation of identity and quantity occurred by HPLC-UV. Examples of TLC plates and HPLC chromatograms showed that metabolites were well separated and quantified.

The overall recovery was 74% of the cumulative dose. Radioactivity was excreted principally via the urine which accounted for 55% of the administered dose. Radioactivity eliminated in faeces accounted for a further 16%. Cage washings accounted for another 3% and were presumably due to urinary contamination of the metabolism cage. Recovery of radioactivity from milk fat was <0.02% and <0.4% was recovered from the aqueous fraction of milk. Radioactivity remaining in tissues at sacrifice accounted for 0.4% of the total dose.

The total radioactive residues measured in livestock matrices following administration of [aniline-U-^{14}C]-flutolanil in the diet are summarised in Table B.7.2.3.2-1.

Table B.7.2.3.2-1: Total radioactive residues in milk, tissues and excreta after administration of [aniline-U-14C]-flutolanil

Matrix	Collection timing	% AR	TRR (mg/kg)	Pooled TRR for analysis (mg/kg)
Urine	Day 1-5	54.87	-	-
Faeces	Day 1-5	15.51	-	-
Cage wash	Day 1-5	2.941	-	-
Milk fat fraction	Day 1-5	0.019	0.027 - 0.089	0.0887
Milk aqueous fraction	Day 1-5	0.311	0.021 - 0.098	0.0975
Tissues	Day 5	0.402	-	-
Liver	Day 5	0.296	0.230	0.230
Kidney	Day 5	0.074	0.377	0.3769
Fat (Omental)	Day 5	0.019	0.014	-
Fat (Renal)	Day 5	0.006	0.015	-
Fat (Subcutaneous)	Day 5	0.001	0.011	-
Fat (Pooled)	Day 5	-	-	0.0132
Muscle (Flank)	Day 5	0.005	0.007	-
Muscle (Loin)	Day 5	0.001	0.007	-
Blood	Day 5	< 0.001	0.031	-
Plasma	Day 5	< 0.001	0.039	-
Accountability		74.05		

Milk was collected in the morning prior to dose administration and again in the afternoon. Total radioactive residues (TRR) were measured separately in the aqueous and fat fractions. Low levels of radioactivity were detected in both fractions. Steady state conditions were achieved within one day of the first dose administration in both aqueous and fat fractions of milk (see Table B.7.2.3.2-2).

Table Table B.7.2.3.2-2: TRR in milk with time after administration of [aniline-U-14C]-flutolanil

Timepoint	Aqueous fraction	Fat fraction
	TRR (mg/kg)	TRR (mg/kg)

	am	pm	am	pm
Day 1	NA	0.021	NA	0.027
Day 2	0.047	0.089	0.039	0.083
Day 3	0.042	0.098	0.034	0.089
Day 4	0.037	0.079	0.032	0.076
Day 5	0.035	0.069	0.030	0.068

Radioactivity was detected in all tissue samples with the greater concentrations detected in kidneys and liver. Levels of radioactivity detected in both flank and loin muscle was < 0.01 mg/kg (maximum 0.007 mg/kg) and these samples were not extracted. Low levels of radioactivity were also detected in fat (range 0.011 to 0.015 mg/kg, 0.0132 mg/kg in pooled sample taken for extraction).

Edible tissues containing significant levels of radioactivity (milk, liver, kidney and fat) were extracted with solvent and solvent/water mixtures to characterise the nature of the residue (see Table B.7.2.3.2-3). In all cases the majority of the radioactivity was successfully extracted (milk fat fraction 92%, milk aqueous fraction >100%, liver 70%, kidney 73% and fat 100% TRR). Aqueous residues accounted for an additional 12% of the TRR in liver and 14% in kidney. Post extraction solids (PES) in liver and kidneys were further extracted following sequential treatment with protease enzyme, strong acid and strong base (only kidneys). The protease digests were characterised by partitioning with organic solvent.

Table B.7.2.3.2-3: Distribution of radioactivity in extracts of livestock matrices after administration of [aniline-U-^{14}C]-flutolanil

Fraction	Milk Fat		Milk Aqueous		Liver		Kidney		Fat	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
TRR		0.0887		0.0975		0.230		0.3769		0.0132
Hexane extract	31.4	0.0279	NA	NA	5.0	0.0116	0.5	0.002	75.6	0.010
Acetonitrile partition	*24.7*	*0.0247*			*4.7*	*0.0108*	-	-	*75.0*	*0.0099*
Hexane residue	*4.7*	*0.0042*			*<0.1*	*0.0001*	-	-	*0.1*	*<0.0001*
Aqueous partition	NA	NA			NA	NA	NA	NA	NA	NA
Aqueous partition			99.4	0.097						
Hexane residue			0.6	0.005						
Ethyl Acetate	5.1	0.0045	NA	NA	28.5	0.0657	10.3	0.0389	15.1	0.002
Acetonitrile	52.8	0.0469	NA	NA	28.3	0.0652	54.2	0.2044	8.2	0.0011
1% Formic in acetonitrile	2.3	0.002	NA	NA	8.6	0.0197	8.2	0.0309	4.7	0.0006
Organic pool	91.6	0.0812	NA	NA	70.1	0.1613	72.7	0.2741	103	0.0136
Water	NA	NA	NA	NA	3.5	0.0082	3.4	0.0129	NA	NA
1M HCl	NA	NA	NA	NA	5.1	0.0112	5.3	0.020	NA	NA
1M NH_3 solution	NA	NA	NA	NA	3.8	0.0087	4.9	0.0184	NA	NA
Aqueous pool	NA	NA	NA	NA	12.5	0.0286	13.6	0.0513	NA	NA
PES characterisation	8.4	0.0075	-	-	17.4	0.0401	13.2	0.049	-	-

								5		
Protease digest	NA	NA								
Organic phase					0.3	0.0006	0.5	0.0018		
Aqueous phase					10.4	0.0238	2.6	0.0099		
Acetonitrile	NA	NA			0.8	0.0018	0.3	0.0013		
10M HCl	NA	NA			2.8	0.0064	0.2	0.0007		
10M NaOH	NA	NA			NA	NA	0.2	0.0009		
Not characterised (Final PES)	8.4	0.0075	-	-	3.1	0.0080	9.4	0.0349	-	-

- No residue remained NA = Not applicable

Pooled extracts from milk aqueous fraction, milk fat, liver, kidney and fat were concentrated prior to analysis by reverse phase HPLC using authentic reference substances as chromatographic markers, with confirmation by normal phase TLC. The aqueous phase of protease digest which contained significant residues (> 0.01 mg/kg) was analysed by HPLC. The identification and characterisation of radioactive residues in the goat are summarised below in Table B.7.2.3.2-4.

In the milk fat fraction, flutolanil, M-4 and its sulfate ester and glucuronide conjugate represented in 13.8%, 2.6%, 12.3% and 15.9% TRR respectively (0.012, 0.002, 0.011 and 0.014 mg eq./kg) and overall accounted for 44.5% of the TRR. In the aqueous fraction of milk the only major residues were M-4 sulfate ester and glucuronide conjugate which represented 21.1% and 26.9% of the TRR (0.021 and 0.026 mg eq./kg) and overall accounted for 48% of the TRR.

In liver flutolanil was detected at 4.9% TRR (0.011 mg/kg). M-4 with its sulfate and glucuronide conjugates accounted for 17.7%, 4.2% and 13.1% TRR respectively (0.041, 0.010 and 0.030 mg eq./kg) . M-2, M-3, M-6 and M-11 were detected as minor metabolites in liver (maximum 2.9% TRR each) along with multiple minor unidentified metabolites (maximum 3.6% each).

Flutolanil was found in trace amounts in the kidney (0.3%TRR, 0.001 mg/kg), where the major residues identified were M-4 sulfate ester (22.4% TRR, 0.085 mg eq./kg) and glucuronide conjugate (23.4% TRR, 0.088 mg eq./kg) along with small amounts of 'free' M-4 (5.7% TRR, 0.022 mg eq./kg). M-2 and M-11 were detected as minor metabolites in kidney (maximum each 6.1% TRR, 0.023 mg eq./kg).

The low residues in fat was composed of mainly flutolanil (44.7% TRR), which was accompanied by small amounts of M-3 (5.3% TRR) and M-4 (9.1% TRR), none of which exceeded 0.01 mg/kg (maximum 0.006 mg/kg).

In all tissues a further portion of the residue was characterised but not identified, being composed of multiple minor unidentified metabolites. Regions eluting late in the chromatograph as the mobile phase became increasing composed of organic solvent were >10% TRR in milk fat, milk aqueous fraction and kidney but shown to be multi-component regions.

Table B.7.2.3.2-4: Summary of characterisation and identification in livestock matrices after administration of [aniline-U-^{14}C]-flutolanil

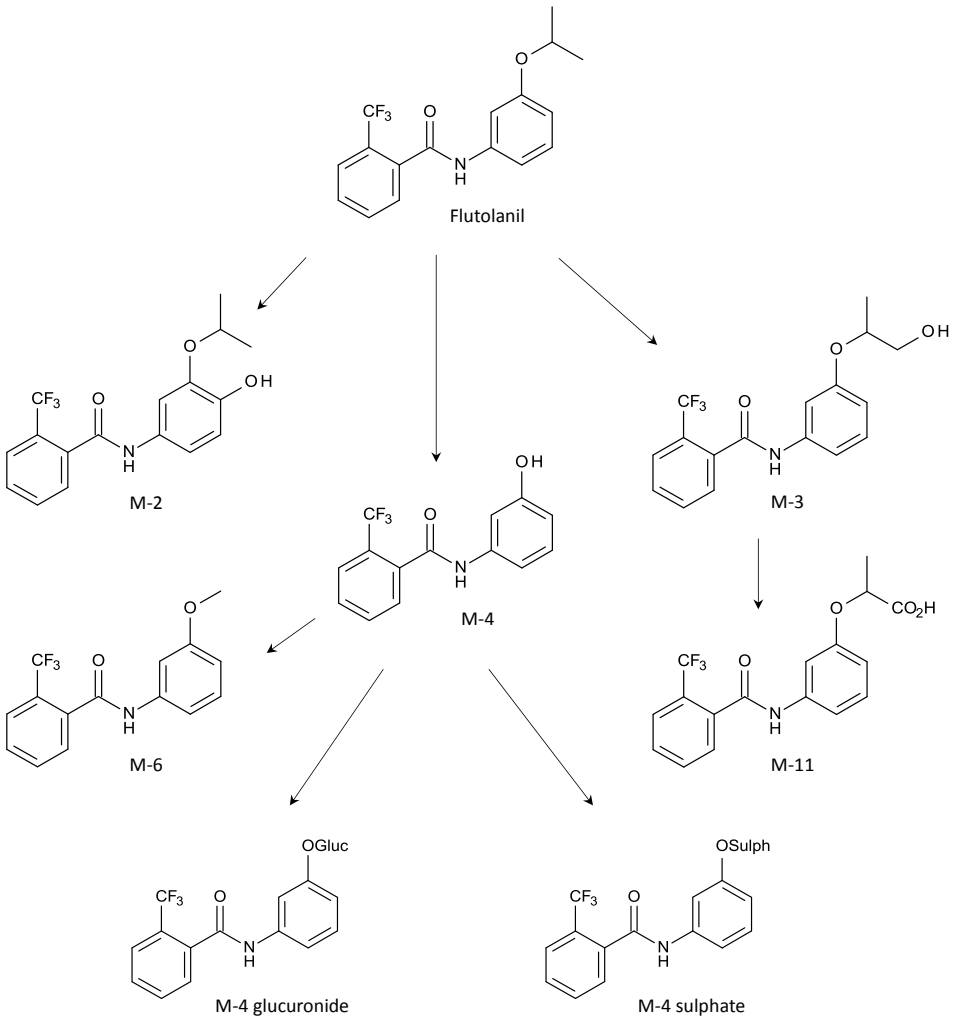
Fraction	Milk Fat		Milk Aqueous		Liver		Kidney		Fat	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
Total chromatographed	77.0	0.0683	81.8	0.0798	68.2	0.157	73.8	0.2784	97.4	0.0128
Identified metabolites										
Flutolanil	13.8	0.0122	-	-	4.9	0.0112	0.3	0.0013	44.7	0.0059
M-2	-	-	-	-	0.6	0.0014	0.4	0.0014	-	-
M-3	-	-	-	-	2.0	0.0045	-	-	5.3	0.0007
M-4	2.6	0.0023	-	-	17.7	0.0407	5.7	0.0215	9.1	0.0012
M-6	-	-	-	-	0.9	0.002	-	-	-	-
M-11	-	-	-	-	2.9	0.0067	6.1	0.023	-	-
M-4 Sulfate	12.3	0.0109	21.1	0.0206	4.2	0.0097	22.4	0.0845	-	-
M-4 Glucuronide	15.9	0.0141	26.9	0.0262	13.1	0.0302	23.4	0.0881	-	-
Total identified	44.5	0.0395	48.0	0.0468	46.2	0.1064	58.3	0.2198	59.1	0.0078
Characterised metabolites	32.5	0.0288	33.8	0.033	22.0	0.0506	15.5	0.0586	38.3	0.005
No. of unidentified peaks	4		3		17		18		8	
Maximum	16.2 [A]	0.0144	22.4 [A]	0.0218	3.6	0.0083	10.2 [A]	0.0383	7.6	0.001
Not chromatographed										
Hexane residue	4.7	0.0042	0.6	0.0005	<0.1	0.0001	0.5	0.002	0.1	<0.0001
PES	8.4	0.0075	-	-	17.4	0.0401	13.2	0.0495	-	-
Procedural losses [B]	11.0	0.0097	17.6	0.0172	14.4	0.0329	12.5	0.047	2.6	0.0003
Additional characterisation of liver and kidney residue										
Total released by protease, acid and base treatments					14.3	0.0326	3.8	0.0146	NA	NA
- of which characterised by chromatography					10.4	0.0238	-	-		
- of which no. of unidentified peaks					5		-			
- of which maximum					1.4	0.0032	-	-		
TRR	100	0.0887	100	0.0975	100	0.230	100	0.3769	100	0.0132

- No residue remained NA = Not applicable
[A] Shown to be multi-component regions [B] Losses on concentration were considered to be non-specific.

Metabolic pathway

A metabolic pathway for [aniline-U-^{14}C]-flutolanil in goats is proposed in Figure B.7.2.3.2-1. The metabolic profile is similar to that seen in rats treated with flutolanil. All of the identified metabolites were common to the rat with the exception of M-6, observed in trace amounts in liver (0.6% TRR, 0.002 mg eq./kg). No evidence of cleavage of the molecule was seen as no metabolites with only the aniline ring were observed.

Figure B.7.2.3.2-1 Proposed metabolic profile of [aniline-U-^{14}C]-flutolanil in goat



III. CONCLUSIONS

Flutolanil and its metabolites were rapidly excreted by lactating goats with 74% of the administered dose recovered in excreta (> 99% of the recovered dose). Tissues (kidney, liver, fat and muscle) retained only low levels of radioactivity (0.4%). The TRR values for milk ranged from 0.027 - 0.089 mg eq./kg in fat and 0.021 - 0.098 mg eq./kg in the aqueous fraction, which represented 0.019 and 0.311% of the administered dose.

Thus there was no evidence of bioaccumulation of flutolanil residues in the goat. Metabolism of [aniline-U-^{14}C]-flutolanil was extensive, but the only major residues (> 10% TRR) were flutolanil and its metabolite M-4 plus the sulfate ester and glucuronide conjugate of M-4.

In conclusion, if goats were exposed to flutolanil residues through the diet, the residues are rapidly metabolised and excreted with low transfer of residues to milk or edible tissues.

B.7.2.3.3 Metabolism in lactating goats

Previous evaluation	Submitted for the purpose of renewal

RMS remark	Acceptable

Reference:	**CA 6.2.3/02: [redacted], 2016b**
Title:	Flutolanil: Metabolism in the Lactating Goat
Document No.:	LMS0101 (R-3387)
Guidelines:	OECD 503: Metabolism in Livestock (2007) US EPA OPPTS 860, 1300
Deviations:	None
Testing laboratory:	[redacted]
GLP:	Yes

Executive Summary

A livestock metabolism study was performed to assess the excretion, distribution and metabolism of [phenyl-U-^{14}C]-flutolanil in a lactating goat after five consecutive daily oral doses at a rate of 34.7 mg/kg in the diet, equivalent to 0.95 mg/kg bw/d.

The concentration profiles of radioactivity in milk and excretion patterns via urine and faeces were followed. The goat was sacrificed 8 hours after the last dose and kidney, liver, muscle and fat were collected. Residual radioactivity and metabolite patterns in these organs were determined.

Approximately 78% of the dose was recovered. Excretion of the test item proceeded mainly via urine (50% dose). In faeces, excreted radioactivity accounted for 19% of the dose. A further 8% was recovered in the gastro-intestinal tract. A low amount was excreted in milk (<0.1% dose) and 0.3% of the dose was detected in tissues.

Total radioactive residues detected in animal matrices are summarised below.

Tissue	**Total radioactive residues (mg eq./kg)**
Whole milk	0.013 – 0.035
Milk fat fraction	0.016 – 0.054
Milk aqueous fraction	0.011 – 0.031
Liver	0.392
Kidney	0.256
Muscle	0.005
Fat	0.012

Residues in milk reached a plateau within 2 to 3 days of dosing. Levels of radioactivity detected in rump, foreleg and loin muscle was < 0.01 mg/kg (maximum 0.005 mg/kg) and these samples were not extracted.

In whole milk and the aqueous fraction, the only significant residue (> 0.01 mg eq./kg) was the M-4 glucuronide conjugate (45.6% TRR, 0.013 mg eq./kg & 46.5% TRR, 0.011 mg eq./kg), accompanied by smaller amounts of flutolanil (maximum 6.0% TRR, 0.002 mg/kg), M-4 (maximum 2.2% TRR, 0.001 mg eq./kg) and its sulfate ester (maximum 8.2% TRR, 0.002 mg eq./kg). In milk fat, flutolanil and M-7 were

detected as major components of the residue at ≤ 15% TRR but never exceeded 0.01 mg/kg (maximum 0.007 mg eq./kg).

Overall M-2, both free and conjugated, accounted for a total of 50.1% TRR (0.198 mg eq./kg) in the liver. The total levels of M-4 and M-101, including those released by further extraction of PES samples, represented 10% and 17.2% of the TRR in the liver, respectively (0.038 and 0.067 mg eq./kg).

In the kidney, M-2 and M-4, both free and conjugated, accounted for a total of 63.8% and 23.3% TRR (0.163 and 0.06 mg eq./kg).

The low residues in fat was composed of mainly flutolanil (47.6% TRR) and M-2 (25.3% TRR), neither of which exceeded 0.01 mg/kg (maximum 0.006 mg/kg).

No other major metabolites (>10% TRR) were formed in livestock tissues.

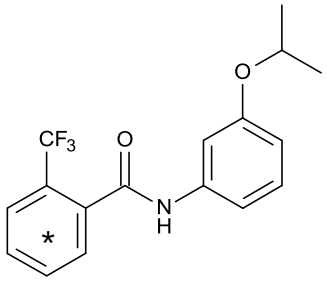
The metabolic profile in urine was very similar to the kidney, with the largest components identified as the M-2 glucuronide conjugate (representing 25.4% of the dose), M-4 glucuronide conjugate (11.1%) and M-4 sulfate ester (5.6%). In faeces flutolanil and M-2 were the major components identified (6.6 and 11.2% of the dose).

It was concluded that flutolanil and its metabolites did not accumulate in the lactating goat after repeated oral administration at a highly exaggerated dose.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: [Phenyl-U-^{14}C]-flutolanil



* Denotes position of [^{14}C]-radiolabel

Chemical name (IUPAC)	α, α, α-trifluoro-3'-isopropoxy-*o*-toluanilide
CA registry number:	66332-96-5
Lot or batch number:	LMS0105/OOE01/01
Specific activity:	13.348 MBq/mg
Radiochemical purity:	>97%

2. Test animals

Species:	Goat
Strain:	British Saanen
Gender:	Female
Age:	1 - 5 years
Weight at dosing:	66.3 kg
Number of animals:	One

Acclimatisation period:	At least 14 days. During the last 3 days the goat was housed in a stainless steel cage fitted with steel mesh floor
Diet:	Concentrate and dried grass pellets twice daily
Water:	Mains water, *ad libitum*
Housing:	Stainless steel cage fitted with steel mesh floor

B. STUDY DESIGN AND METHODS

1. In-life dates:

24 November 2014 to 3 August 2016

2. Experimental design

1.	Dosing Regime:	Oral
	Amount of dose:	34.7 mg/kg in diet, equivalent to 0.95 mg/kg bw/d
	Food consumption:	9076 g over 5 days (experimental phase)
	Vehicle:	Gelatin capsules
	Timing:	Once daily in the morning (at *ca* 09:00 hours)
	Duration:	Five days
2.	Sample Collection	
	Milk collection:	Twice daily
	Urine and faeces collection:	24 hour intervals (including cage washes)
	Interval from last dose to sacrifice:	8 hours
	Tissues harvested and analysed:	A blood sample was taken before each dose and at 0.5, 1, 2, 4, 6, 8, 10, 12 & 24 hours following the first dose. The C_{max} was subsequently measured as 0.043 mg eq./kg 6 hours after dosing and on this basis, the goat was sacrificed 8 hours after the final dose. Liver, kidneys, fat (omental, subcutaneous & perirenal), muscle (foreleg, rump & loin), bile and GI tract (plus contents). In addition, the uterus, placenta & foetuses were also taken for radioactivity measurement when the animal was unexpectedly found to be pregnant at necropsy. A terminal sample of blood was collected and a portion used to prepare plasma.

Sample Preparation

Whole milk was separated into the fat and aqueous fraction by centrifugation. Tissues were homogenised using a commercial food blender. As the concentration of radioactivity from omental, subcutaneous and perirenal fat was similar, a combined sample was prepared for extraction. Faeces were homogenised. TRRs were determined by LSC either directly (urine, milk fractions, plasma, cage washings and bile) or by combustion followed by LSC (muscle, liver, kidney, placenta, uterus, faeces and blood). TRRs in fat and foetuses were measured by LSC following the addition of a solubilising agent or digestion.
The contents of the rumen and reticulum, small intestine and large intestine were separated from the gastrointestinal tract walls and mixed with water in a blender prior to combustion followed by LSC.

Extraction and Fractionation of Residues

A pool of milk from Day 2 was used to generate milk fat and aqueous fraction by centrifugation. Whole milk, the aqueous milk fraction and milk fat were extracted twice with acetonitrile. The extracts were concentrated prior to analysis by reverse phase HPLC and 2-D normal phase TLC. Radioactive residues in post extraction solids (PES) was quantified by combustion.
Samples of liver and kidney were sequentially extracted with a rotary shaker for 45-60 minutes with the following sequence of solvents:

Acetonitrile, 3 times
Acetonitrile : water (1:1, v/v), 1 to 2 times
Acetonitrile : 0.1N HCl (4:1, v/v), 2 times

Following each extraction, the supernatant separated by centrifugation. Radioactivity present in extracts was quantified by liquid scintillation counting (LSC). Acetonitrile and acetonitrile/water extracts were combined and concentrated prior to analysis by reverse phase HPLC and 2-D normal phase TLC, as were the acidic acetonitrile extracts.
A pooled fat sample (mixture of perirenal, subcutaneous and omental) was sequentially extracted with a rotary shaker for 45-60 minutes with the following sequence of solvents:

Acetonitrile, 3 times
Acetonitrile : 0.1N HCl (4:1, v/v), 2 times

The liver and fat residues were then subjected to protease digestion and liver to further acid/base extraction as follows:

Incubation in 0.01M phosphate buffer with protease enzymes (Type 1 from Bovine pancreas) for *ca.* 18 hours at 37°C
Incubation with 1M HCl for *ca.* 18 hours at 37°C (liver only)
Incubation with 1M NaOH for *ca.* 18 hours at 37°C (liver only)

Following each incubation, the sample was extracted with acetonitrile by vigorous shaking (45 minutes), the supernatant was separated by filtration and radioactivity present in extracts quantified by LSC. After the final basic extraction of liver the aqueous fraction was neutralised by the addition of acid. Extracts were concentrated prior to analysis by HPLC. Radioactive residues in liver and fat PES was quantified by combustion.

A pooled sample of faeces (Day 1-5) was sequentially extracted with a rotary shaker for 45 minutes with the following sequence of solvents:

Acetonitrile, 3 times

Acetonitrile : water (1:1, v/v), 2 times

Extracts were pooled and concentrated prior to analysis by reverse phase HPLC.

A pooled sample of urine (Day 1-5) was analysed by reverse phase HPLC and 2-D normal phase TLC.

Portions of pooled urine and tissue extracts from liver, kidney, whole milk and aqueous milk were incubated in 0.2M acetate buffer as follows:

Buffer only (control)

Buffer + β-glucuronidase/sulfatase (Type H-1, *Helix pomatia*)

Buffer + mixture of β-glucuronidase/sulfatase and a β-glucuronidase specific inhibitor (D-saccharic acid-1,4-lactone)

Samples were incubated at 37°C for 18 hours and analysed by reverse phase HPLC and 2-D normal phase TLC. Peaks corresponding to M-4 sulfate ester and M-4 glucuronide conjugate were identified by chromatographic analysis of samples before and after each incubation.

Storage stability

Samples and extracts were stored at ≤ -18°C, apart from whole blood and subsamples of whole milk which were stored at about 4°C until measurement of radioactivity was completed and then subsequently at ≤-18°C. Storage stability was confirmed for liver, kidney, milk fat and aqueous fractions extracts.

II. RESULTS AND DISCUSSION

Identification of metabolites occurred based on (1D or 2D) TLC. Confirmation of identity and quantity occurred by HPLC (UV and radio detection). Examples of TLC plates and HPLC chromatograms showed that metabolites were well separated and quantified.

The overall recovery was 78.1% of the cumulative dose. Radioactivity was excreted principally via the urine which accounted for 49.6% of the administered dose. Radioactivity eliminated in faeces accounted for a further 19.4%. Cage washings accounted for another 0.9% and were presumably due to urinary contamination of the metabolism cage. A further 7.9% of the dose was recovered in the gastro-intestinal tract (including rumen). Recovery of radioactivity from milk was <0.1% and radioactivity remaining in tissues at sacrifice accounted for 0.3% of the total dose.

The total radioactive residues measured in livestock matrices following administration of [phenyl-U-^{14}C]-flutolanil in the diet are summarised in Table B.7.2.3.3-1.

Table Table B.7.2.3.3-1: Total radioactive residues in milk, tissues and excreta after administration of [phenyl-U-^{14}C]-flutolanil

Matrix	Collection timing	% AR	TRR (mg/kg)	Pooled TRR for analysis (mg/kg)
Urine	Day 1-5	49.6	-	-
Faeces	Day 1-5	19.4	-	-
Cage wash	Day 1-5	0.9	-	-
GI tract	Day 5	7.9	-	-
Milk	Day 1-5	<0.1	0.013 – 0.035	0.028
Milk fat fraction	Day 1-5	-	0.016 – 0.054	0.046
Milk aqueous fraction	Day 1-5	-	0.011 – 0.031	0.025
Tissues	Day 5	0.3	-	-
Liver	Day 5	0.2	0.392	0.392
Kidney	Day 5	<0.1	0.256	0.256
Fat (Omental)	Day 5	<0.1	0.014	-
Fat (Perirenal)	Day 5	<0.1	0.011	-
Fat (Subcutaneous)	Day 5	<0.1	0.012	-
Fat (Pooled)	Day 5	-	-	0.012
Muscle (Rump)	Day 5	<0.1	0.005	-
Muscle (Foreleg)	Day 5	<0.1	0.005	-
Muscle (Loin)	Day 5	<0.1	0.005	-
Bile	Day 5	0.1	25.7	-
Blood	Day 5	<0.1	0.032	-
Plasma	Day 5	<0.1	0.041	-
Foetuses	Day 5	<0.1	0.002	-
Placenta	Day 5	<0.1	0.016	-
Uterus	Day 5	<0.1	0.019	-
Accountability		78.1		

Milk was collected in the morning prior to dose administration and again in the afternoon. Total radioactive residues (TRR) were measured separately in whole milk and in the aqueous and fat fractions. Steady state conditions were achieved within 2 to 3 days of the first dose administration in milk (see Table B.7.2.3.3-2).

Table B.7.2.3.3-2: TRR in milk with time after administration of [phenyl-U-^{14}C]-flutolanil

Time (Hours after first dose)	Whole milk			Aqueous fraction			Fat fraction		
	TRR (mg/kg)			TRR (mg/kg)			TRR (mg/kg)		
	pm	am	Pooled	pm	am	Pooled	pm	am	Pooled
Pre dose	ND	ND	ND	ND	ND	ND	ND	ND	ND
0-24	0.024	0.018	0.020	0.021	0.017	0.019	0.031	0.029	0.029
24-48	0.035	0.023	0.028	0.031	0.022	0.025	0.054	0.033	0.046
48-72	0.031	0.023	0.026	0.029	0.022	0.024	0.047	0.032	0.036
72-96	0.033	0.013	0.018	0.031	0.011	0.017	0.042	0.016	0.024
96-104	0.025	-	-	0.023	-	-	0.031	-	-

Radioactivity was detected in all tissue samples with the greater concentrations detected in kidneys and liver. Levels of radioactivity detected in rump, foreleg and loin muscle was < 0.01 mg/kg (maximum 0.005 mg/kg) and these samples were not extracted. Low levels of radioactivity were also detected in fat (range 0.012 to 0.014 mg/kg, 0.012 mg/kg in pooled sample taken for extraction).

Edible tissues containing significant levels of radioactivity (milk, liver, kidney and fat) were extracted with solvent and solvent/water mixtures to characterise the nature of the residue (see Table B.7.2.3.3-3). In all cases the majority of the radioactivity was successfully extracted (liver 70%, kidney 99%, fat 88%, whole milk 94%, milk aqueous fraction 96% and milk fat fraction 95% TRR). Radioactivity released from post extraction solids (PES) following sequential treatment with protease enzyme, acid and base accounted for 19% of the TRR in liver and 3% in fat.

Table B.7.2.3.3-3: Distribution of radioactivity in extracts of livestock matrices after 5 daily administrations of [phenyl-U-^{14}C]-flutolanil

Fraction	Liver		Kidney		Fat	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
TRR		0.392		0.256		0.012
Extracts	70.0	0.275	99.1	0.254	87.9	0.011
Neutral solvents	64.0	0.251	80.1	0.205	80.7	0.010
Acetonitrile / 0.1N HCl	6.0	0.024	19.0	0.049	7.2	0.001
PES	30.0	0.118	0.9	0.002	12.1	0.004
PES characterisation						
Protease enzyme	3.6	0.014	NA	NA	2.7	<0.001
1N HCl	3.7	0.015	NA	NA	NA	NA
1N NaOH	11.8	0.046	NA	NA	NA	NA
PES after further extraction	10.9	0.043	0.9	0.002	9.4	0.004

Fraction	Whole Milk		Milk Aqueous		Milk Fat	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
TRR		0.028		0.025		0.046
Acetonitrile	94.1	0.026	95.7	0.024	94.7	0.045
PES	5.9	0.002	4.3	0.001	2.6	0.001

NA = Not applicable

The identification and characterisation of radioactive residues in the goat tissues, milk, urine and faeces are summarised below in Table B.7.2.3.3-4 and -5 and -6.

In liver no flutolanil was detected in neutral and weak acidic extracts and the major residue identified was M-2 and its glucuronide conjugate which accounted for 11.9% and 37.4% TRR respectively (0.047 and 0.147 mg eq./kg). M-4, M-7 and M-11, the former two both as the free metabolite and as sulfate and glucuronide conjugates, were detected as minor metabolites in neutral and weak acidic extracts of liver (maximum 7.9% TRR, 0.03 mg eq./kg). In addition, small amounts of the phenyl ring metabolites M-101 and M-102 produced by cleavage of the amide bond were found (maximum 6.5% TRR, 0.025 mg eq./kg).

Trace amounts of flutolanil (0.4% TRR, 0.002 mg/kg) were released from liver by further extraction of PES samples with protease enzymes and 1N acid/base extractions, along with trace amounts of M-2, M-4, M-11, M-102 and larger amounts of M-101.

Overall M-2, both free and conjugated, accounted for a total of 50.1% TRR in the liver. The total levels of M-4 and M-101, including those released by further extraction of PES samples, represented 10% and 17.2% of the TRR in the liver, respectively. No other major metabolites (>10% TRR) were formed.

No flutolanil was found in the kidney, where the major residues identified were M-2 and M-4 glucuronide conjugates (59.0% and 15.2% TRR, 0.151 and 0.039 mg eq./kg) accompanied by smaller amounts of free M-2 and M-4 (4.8% and 1.5% TRR, 0.012 and 0.004 mg eq./kg), plus the M-4 sulfate conjugate (6.6% TRR, 0.017 mg eq./kg). Overall M-2 and M-4, both free and conjugated, accounted for a total of 63.8% and 23.3% TRR in kidney. M-7 and its glucuronide conjugate, M-11, M-101 and M-102 were detected as minor metabolites in kidney (maximum 2.4% TRR, 0.006 mg eq./kg).

The low residues in fat was composed of mainly flutolanil (47.6% TRR) and M-2 (25.3% TRR), neither of which exceeded 0.01 mg/kg (maximum 0.006 mg/kg).

Small amounts of flutolanil (6.0% TRR, 0.002 mg/kg) were found in whole milk. M-4 glucuronide conjugate (45.6% TRR, 0.013 mg/kg) was identified as a major metabolite accompanied by smaller amounts of the M-4 sulfate ester (8.2% TRR, 0.002 mg eq./kg), overall accounting for 53.8% TRR. M-2 glucuronide conjugate was detected as a minor metabolite (2.3% TRR).

In the aqueous milk fraction the profile was very similar to whole milk, M-4 and its glucuronide and sulfate conjugates accounted for 2.2%, 46.5% and 1.6% TRR respectively (0.0001, 0.011 and <0.001 mg eq./kg), overall accounting for 50.3% TRR. Minor amounts of M-7 and M-101 were identified (maximum 5.6% TRR).

In the milk fat fraction, flutolanil (13.6% TRR) and M-7 (14.8% TRR) were detected as major components of the residue, accompanied by M-101 (9.2% TRR), but none exceeded 0.01 mg/kg (maximum 0.007 mg/kg).

The metabolic profile in urine was very similar to the kidney. No flutolanil was detected and the largest components identified in the urine were the M-2 glucuronide conjugate (representing 25.4% of the cumulative applied dose), M-4 glucuronide conjugate (11.1%) and M-4 sulfate ester (5.6%). Overall M-2 and M-4 with their conjugates accounted for 26.4 and 16.9% of the dose in urine. M-7 and M-11, along with their sulfate and glucuronide conjugates were found as minor metabolites (maximum 2.4%) and no phenyl ring metabolites were detected in urine.

In faeces flutolanil and M-2 were the major components identified (6.6 and 11.2% of the dose). Trace amounts of M-4, M-7 and M-101 was observed (<1%).

Table Table B.7.2.3.3-4 Summary of characterisation and identification in livestock tissues after administration of [phenyl-U-^{14}C]-flutolanil

Metabolites	Liver		Kidney		Fat	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
TRR		0.392		0.256		0.012
Extracted	70.0	0.275	99.1	0.254	87.9	0.011
Flutolanil	-	-	-	-	47.6	0.006
M-2	11.9	0.047	4.8	0.012	25.3	0.003
M-4	2.3	0.009	1.5	0.004	-	-
M-7	0.9	0.004	0.7	0.002	-	-
M-11	0.6	0.002	2.4	0.006	-	-
M-101	6.5	0.025	2.2	0.006	-	-
M-102	1.2	0.005	0.2	0.001	-	-
M-2 glucuronide	37.4	0.147	59.0	0.151	-	-
M-2 sulfate	-	-	-	-	-	-
M-4 glucuronide	5.5	0.021	15.2	0.039	-	-
M-4 sulfate	0.1	<0.001	6.6	0.017	-	-
M-7 glucuronide	0.1	<0.001	1.1	0.003	-	-
M-7 sulfate	0.3	0.001	-	-	-	-
Others	3.2	0.012	5.4	0.014	15.0	0.002
PES	30.0	0.118	0.9	0.002	12.1	0.004
PES characterisation						
Protease enzyme	3.6	0.014	NA	NA	2.7	<0.001
1N HCl	3.7	0.015	NA	NA	NA	NA
1N NaOH	11.8	0.046	NA	NA	NA	NA
PES Extracts	19.1	0.075	NA	NA	NA	NA
Flutolanil	0.4	0.002				
M-2	0.8	0.004				
M-4	2.1	0.008				
M-7	-	-				
M-11	0.4	0.001				
M-101	10.7	0.042				
M-102	2.0	0.009				
Others	2.7	0.010				
PES after further extraction	10.9	0.043	NA	NA	9.4	0.004

NA = Not applicable - = Not detected

Table B.7.2.3.3-5: Summary of characterisation and identification in milk after administration of [phenyl-U-^{14}C]-flutolanil

Metabolites	Whole Milk		Milk Aqueous		Milk Fat	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
		0.028		0.025		0.046
Extracted	94.1	0.026	95.7	0.024	97.4	0.045
Flutolanil	6.0	0.002	-	-	13.6	0.006
M-2	-	-	-	-	-	-
M-4	-	-	2.2	0.001	-	-
M-7	-	-	4.0	0.001	14.8	0.007
M-11	-	-	-	-	-	-
M-101	-	-	5.6	0.001	9.2	0.004
M-102	-	-	-	-	-	-
M-2 glucuronide	2.3	0.001	-	-	-	-
M-2 sulfate	-	-	-	-	-	-
M-4 glucuronide	45.6	0.013	46.5	0.011	-	-
M-4 sulfate	8.2	0.002	1.6	<0.001	-	-
M-7 glucuronide	-	-	-	-	-	-
M-7 sulfate	-	-	-	-	-	-
Others [A]	38.0	0.011	34.9	0.008	59.8	0.027
PES	5.9	0.002	4.3	0.001	2.6	0.001

[A] Multiple components of which no individual component exceeded 0.002, 0.001 and 0.013 mg eq./kg whole milk, aqueous and milk fat fractions, respectively - = Not detected

Table B.7.2.3.3-6: Summary of characterisation and identification in livestock urine and faeces after administration of [phenyl-U-^{14}C]-flutolanil

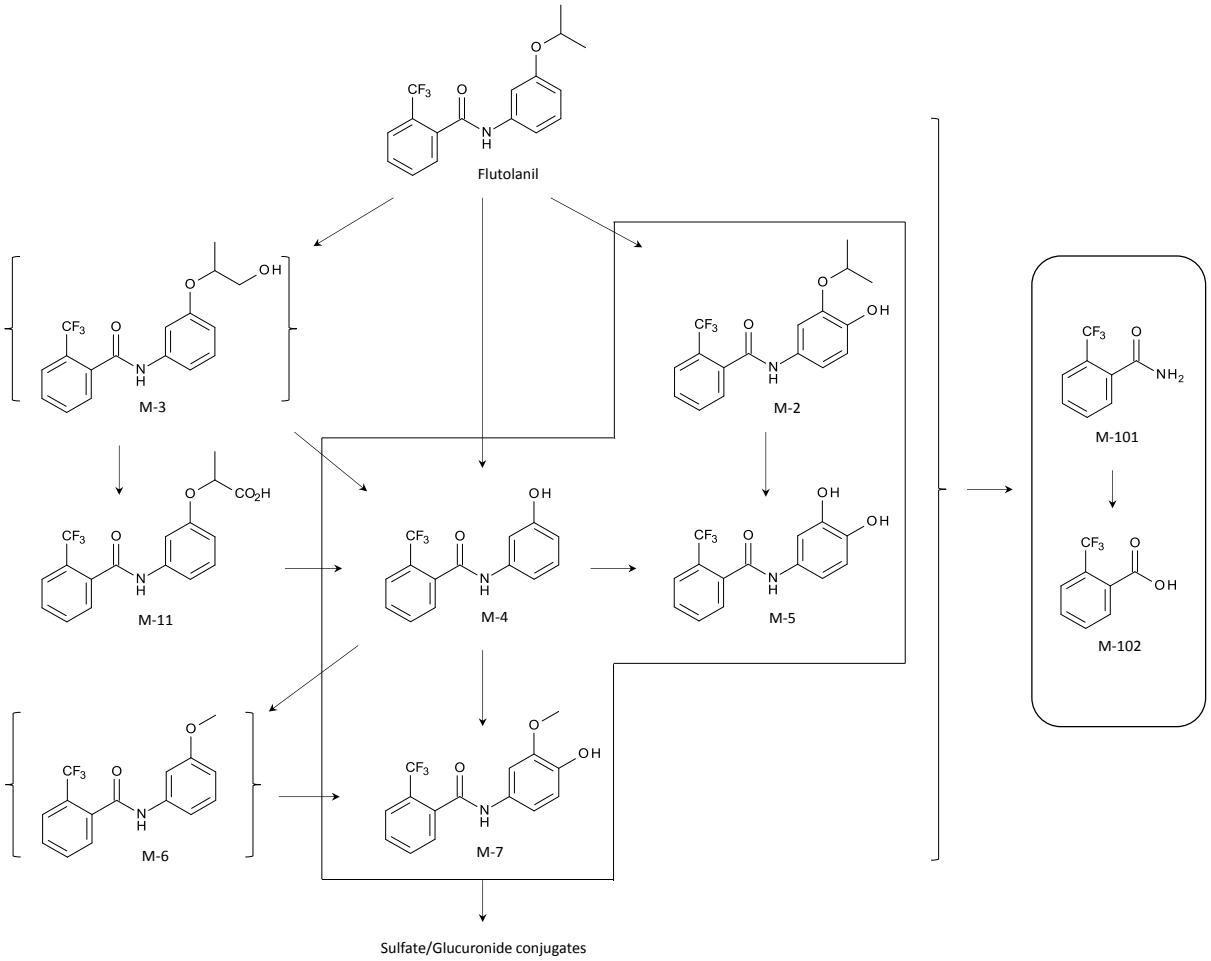
Metabolites	Urine	Faeces
	% Dose	
Flutolanil	-	6.6
M-2	0.7	11.2
M-4	0.2	0.9
M-7	-	0.2
M-11	1.2	-
M-101	-	0.2
M-102	-	-
M-2 glucuronide	25.4	-
M-2 sulfate	0.3	-
M-4 glucuronide	11.1	-
M-4 sulfate	5.6	-
M-7 glucuronide	2.1	-
M-7 sulfate	0.3	-
M-11 glucuronide	-	-
M-11 sulfate	0.2	-
Others	2.5	0.3
Total	49.6	19.4

- = Not detected

Metabolic pathway

A metabolic pathway for [phenyl-U-^{14}C]-flutolanil in goats is proposed in Figure B.7.2.3.3-1. The metabolic profile is similar to that seen in rats treated with flutolanil. All of the identified metabolites were observed in the rat.

Figure B.7.2.3.3.-1 Proposed metabolic profile of [phenyl-U-^{14}C]-flutolanil in goat. Metabolites in parenthesis are proposed intermediates. Metabolites M-2, M-4, M-5 and M-7 were found free, or conjugated as glucuronide and/or sulfate**.**



III. CONCLUSIONS

Flutolanil and its metabolites were rapidly excreted by lactating goats with 70% of the administered dose recovered in excreta (> 89% of the recovered dose) and a further 8% in the contents of the gastrointestinal tract. Tissues (kidney, liver, fat and muscle) retained only low levels of radioactivity (0.3% in total). The TRR values for milk ranged from 0.013 – 0.035 mg eq./kg in whole milk, 0.016 – 0.054 mg eq./kg in fat and 0.011 – 0.031 mg eq./kg in the aqueous fraction, which represented <0.1% of the administered dose. Total residues in muscle, fat, kidney and liver were 0.005 mg/kg, 0.014 mg/kg, 0.256 mg/kg and 0.392 mg/kg, respectively, at a >300N dose, compared to the actual expected exposure (via residues in potatoes).

Thus there was no evidence of bioaccumulation of flutolanil residues in the goat. Metabolism of [phenyl-U-^{14}C]-flutolanil was extensive. The major residues (> 10% TRR) in livestock tissues were flutolanil and its metabolites M-2 and M-4 plus the sulfate ester and glucuronide conjugates of these metabolites.

In whole milk and the aqueous fraction the only significant residue (> 0.01 mg eq./kg) was the M-4 glucuronide conjugate. In milk fat, flutolanil and M-7 were detected as major components of the residue at < 15% TRR but never exceeded 0.01 mg/kg.
In conclusion, if goats were exposed to flutolanil residues through the diet, the residues are rapidly metabolised and excreted with low transfer of residues to milk or edible tissues.

B.7.2.4 Pigs

The major metabolic pathways for flutolanil in goats were the same as those in rats. Hence, a metabolism study in pigs is considered not required.

B.7.2.5 Fish

No metabolism study in fish has been submitted for the renewal process. Potato protein can be used as a part of fish diet. Flutolanil has been recovered in fat tissue in poultry (0.127 mg eq./kg) and in goat (0.012-0.043 mg equi./kg), which suggest that flutolanil is fat soluble and can be recovered in animal tissues. Further investigation of metabolism of flutolanil in fish might be required, when agreed EU methodology will be available to fully address this data requirement .

B.7.3 Magnitude of residue trials in plants

The following cGAPs are proposed:
For potatoes (EU):
Potato seed treatment (ware, seed, starch potatoes):
In store treatment (indoor/outdoor): 1x 0.368 kg as/ha (based on a planting rate of 4 ton tubers/ha), BBCH 00-03 (before planting).
On planter treatment as tuber falls into furrow (outdoor): 1x 0.368 kg as/ha (based on a planting rate of 4 ton tubers/ha), BBCH 00-03 (at planting)
In planter treatment before catching up by planting chains (outdoor): 1x 0.368 kg as/ha (based on a planting rate of 4 ton tubers/ha), BBCH 00-03 (at planting)

Ornamental crops:
Tulip, iris: 1x 2.76 kg as/ha, incorporation into the soil (10-15 cm).

For the MRL application, the following cGAP has been submitted:
Potato in furrow treatment at planting (EU):
1x 2.10 kg as/ha, BBCH 00-03 (at planting), in furrow application, directed at soil.

B.7.3.1 Potato

B.7.3.1.1 Potato, seed treatment, NEU

Previous evaluation	DAR
RMS remark	Accetable. The cGAP requested within the renewal framework is potato seed treatement: 1x 0.368 kg as/ha, which is equivalent to 92 g/ ton potatoes. In the original DAR there are six residue trials, which have been performed with cGAP within the 25% of the requested cGAP and therefore, considered acceptable. It should be noted that in those trials only parent compound flutolanil has been measured. However, since it is in accordance with the proposed residue definition for monitoring, it is considered acceptable for MRL calculations only. Those data has not been used for the risk assessment calculations.

Table B.7.3.1.1.-1 Summary of residue trials conducted with flutolanil on potato in Northern Europe

Country/ Year	Application					Residues		Reference
	Form	No.	Method	Rate (g as/t)	Concentration (g/kg)	Day	Flutolanil	Report No/ Trial No
Potatoes								
Netherlands, 1991	DS	1	Seed tuber	120	60	132	0.03	R-3028/ NZ191S02
Netherlands, 1991	DS	1	Seed tuber	120	60	136	< 0.01	R-3028/ NN391S02
Netherlands, 1991	DS	1	Seed tuber	120	60	163	< 0.01	R-3028/ NN291S02
France, 1990	DS	1	Seed tuber	120	60	175	0.026	R-3060
France, 1993	DS	1	Seed tuber	120	60	122	< 0.020	R-3029/ R93585D1
France, 1993	DS	1	Seed tuber	180	60	122	0.026	R-3029/ R93585D1
France, 1993	DS	1	Seed tuber	120	60	138	< 0.020	R-3029/ R93585C1
France, 1993	DS	1	Seed tuber	180	60	138	< 0.020	R-3029/ R935871
France, 1993	DS	1	Seed tuber	180	60	69	< 0.020	R-3030/ R93587D1
France, 1993	DS	1	Seed tuber	180	60	92	< 0.020	R-3030/ R93587C1
France, 1993	FS	1	Seed tuber	108	40	70	0.022	R-3031/ R93588D1
France, 1993	FS	1	Seed tuber	108	40	92	< 0.022	R-3031/ R93588C
France, 1994	DS	1	Seed tuber	120	60	81	< 0.01	R-3021/ 94571RN1
France, 1994	DS	1	Seed tuber	180	60	81	< 0.01	R-3021/ 94571RN1
France, 1994	DS	1	Seed tuber	120	60	84	0.077	R-3021/ 94571AM1
France, 1994	DS	1	Seed tuber	180	60	84	0.096	R-3021/ 94571AM1
France, 1994	DS	1	Seed tuber	120	60	113	0.025	R-3022/ 9457AM1
France, 1994	DS	1	Seed tuber	180	60	113	0.027	R-3022/ 9457AM1
France, 1994	DS	1	Seed tuber	120	60	125	0.022	R-3022/ 94572RN1
France, 1994	DS	1	Seed tuber	180	60	125	0.039	R-3022/ 94572RN1

Country/ Year	Application					Residues		Reference
	Form	No.	Method	Rate (g as/t)	Concentration (g/hl)	Day	Flutolanil (mg/kg)	Report No/ Trial No
Potatoes								
France, 1994	FS	1	Seed tuber	36.65	44.89	81	0.014	R-3023/ 94569RN1
France, 1994	FS	1	Seed tuber	87.7	44.89	81	0.014	R-3023/ 94569RN1
France, 1994	FS	1	Seed tuber	36.65	44.80	84	<0.010-0.073	R-3023/ 94569AM1
France, 1994	FS	1	Seed tuber	87.7	44.89	84	0.035	R-3023/ 94569AM1
France, 1994	FS	1	Seed tuber	94.5	44.89	125	0.014	R-3024/ 9470RN1
France, 1994	FS	1	Seed tuber	140.6	44.89	125	0.032	R-3024/ 9470RN1
France, 1994	FS	1	Seed tuber	94.5	44.89	122	0.030	R-3024/ 9470AM1
France, 1994	FS	1	Seed tuber	140.6	44.89	122	0.073	R-3024/ 9470AM1
Germany, 1997	FS	1	Seed tuber	131.5	44.9	78 105 140	< 0.010 < 0.010 < 0.010	R-3058/ 97747DGR1
Germany, 1997	FS	1	Seed tuber	117.6	44.9	76 99 128	0.025 0.020 < 0.010	R-3058/ 97747DAS1

DS = Powder for dry seed treatment, FS = Flowable concentrate for seed treatment

B.7.3.1.2 Potato, seed treatment, SEU

Previous evaluation	in DAR
RMS remark	Not acceptable. The cGAP requested within the renewal framework is potato seed treatement: 1x 0.368 kg as/ha, which is equivalent to 92 g/ ton potatoes. In the original DAR there are trials available, which were performed with much more critical GAP. Moreover, a zero-residue situation is not demonstrated in all the trials. Hence, the trials are considered not acceptable.

Table B.7.3.1.1.-2 Summary of residue trials conducted with flutolanil on potato in Southern Europe.

Country/ Year	Application					Residues		Reference
	Form	No.	Method	Rate (g as/ha)	Concentration (kg/hl)	Day	Flutolanil	Report No/ Trial No
Potatoes								
Spain, 2001	FS	1 1	Seed tuber Soil application	500 750	40	78	< 0.01	R-3054/ AF/6012/NN/3
Spain, 2001	FS	1 1	Seed tuber Soil application	500 750	40	82	< 0.01	R-3054/ AF/6012/NN/4
Spain, 2001	FS	1 1	Seed tuber Soil application	500 750	40	90	< 0.01	R-3054/ AF/6012/NN/2
Spain, 2001	FS	1 1	Seed tuber Soil application	500 750	40	90	0.03	R-3054/ AF/6012/NN/1
Spain, 2002	FS	1 1	Seed tuber Soil application	500 750	40	120	< 0.01	R-3057/ AF/6279/NN/3
Spain, 2002	FS	1 1	Seed tuber Soil application	500 750	40	120	< 0.01	R-3057/ AF/6279/NN/4
Spain, 2002	FS	1 1	Seed tuber Soil application	500 750	40	124	< 0.01	R-3057/ AF/6279/NN/2
Spain, 2002	FS	1 1	Seed tuber Soil application	500 750	40	135	< 0.01	R-3057/ AF/6279/NN/1
Spain, 2002	FS	1 1	Seed tuber Soil application	500 750	40	164	< 0.01	R-3057/ AF/6279/NN/5

DS = Powder for dry seed treatment, FS = Flowable concentrate for seed treatment

B.7.3.1.3 Potato, seed treatment, EU

Previous evaluation	Submitted for the purpose of renewal
RMS remark	Acceptable. It should be noted that the RMS does not consider the residue value for flutolanil of 0.09 mg/kg as an outlier as proposed by the notifier (trial S16-02157-02) . This

	value has been included in the further assessment.

Studies from the field program 2014:

Report	**CA 6.3.1/01: Sutherland J. (2015a)**
Title:	Flutolanil 40SC / Rhino DS: Study to generate seed potato treated with flutolanil for use in subsequent residue studies
Document No:	S14-02899 (R-3373)
Guidelines:	OECD (2011) Guidance Document on Crop Field Trials (Series on Testing and Assessment No. 164 and Series on Pesticides No. 66)
GLP	Yes
Report:	**CA 6.3.1/02: Sutherland J. (2015b)**
Title:	Flutolanil 40SC / Rhino DS treated seed: Determination of residues of flutolanil following a seed treatment application of Flutolanil (MONCUT) 40SC and Rhino DS in potato at 3 sites in northern Europe, 2014
Document No:	S14-02900 (R-3374), Residue trials
Guidelines:	OECD (2011) Guidance Document on Crop Field Trials (Series on Testing and Assessment No. 164 and Series on Pesticides No. 66)
GLP	Yes
Report:	**CA 6.3.1/03: Merdian H. (2016a)**
Title:	Determination of residues of flutolanil following a seed treatment application of Flutolanil (MONCUT) 40SC and Rhino DS in potato at 3 sites in northern Europe, 2014
Document No:	S16-03442 (R-3382), analytical report for study S14-02900
Guidelines:	Not stated
GLP	Yes
Report:	**CA 6.3.1/04: Sutherland J. (2015c)**
Title:	Flutolanil 40SC / Rhino DS treated seed: Determination of residues of flutolanil following a seed treatment application of Flutolanil (MONCUT) 40SC and Rhino DS in potato at 4 sites in southern Europe, 2014
Document No:	S14-02901 (R-3375), Residue trials
Guidelines:	OECD (2011) Guidance Document on Crop Field Trials (Series on Testing and Assessment No. 164 and Series on Pesticides No. 66)
GLP	Yes
Report	**CA 6.3.1/05: Merdian H. (2016b)**
Title:	Determination of residues of flutolanil following a seed treatment application of Flutolanil (MONCUT) 40SC and Rhino DS in potato at 3 sites in southern Europe, 2014
Document No:	S16-03453 (R-3383), analytical report for study S14-02901
Guidelines:	Not stated
GLP	Yes

Studies from the field program 2015:

Report:	**CA 6.3.1/06: Lines J. (2016a)**
Title:	Moncut 40SC / Flutolanil 40SC: Study to generate seed potato treated with flutolanil for use in subsequent residue studies
Document No:	S15-00013 (R-3388)
Guidelines:	OECD (2011) Guidance Document on Crop Field Trials (Series on Testing and Assessment No. 164 and Series on Pesticides No. 66)
GLP	Yes

Report:	**CA 6.3.1/07: Lines J. (2016b)**
Title:	Flutolanil (Moncut) 40SC / Rhino DS Treated Seed: Determination of residues of flutolanil following a seed treatment application of Flutolanil (MONCUT) 40SC and Rhino DS in potato at 5 sites in northern Europe, 2015
Document No:	S15-00014 (R-3391), residue trials
Guidelines:	OECD (2011) Guidance Document on Crop Field Trials (Series on Testing and Assessment No. 164 and Series on Pesticides No. 66)
GLP	Yes
Report:	**CA 6.3.1/08: Lines J. (2016c)**
Title:	Flutolanil (Moncut) 40SC / Rhino DS Treated Seed: Determination of residues of flutolanil following a seed treatment application of Flutolanil (MONCUT) 40SC and Rhino DS in potato at 4 sites in southern Europe, 2015
Document No:	S15-00016 (R-3393), residue trials
Guidelines:	OECD (2011) Guidance Document on Crop Field Trials (Series on Testing and Assessment No. 164 and Series on Pesticides No. 66)
GLP	Yes

Studies from the field program 2016

Report:	**CA 6.3.1/09: Sutherland J. (2016a)**
Title:	Study to generate seed potato treated with flutolanil for use in subsequent residue studies
Document No:	S16-02156 (R-3402)
Guidelines:	OECD (2011) Guidance Document on Crop Field Trials (Series on Testing and Assessment No. 164 and Series on Pesticides No. 66)
GLP	Yes
Report:	**CA 6.3.1/10: Sutherland J. (2017a)**
Title:	Determination of residues of flutolanil following a seed treatment application of Flutolanil (MONCUT) 40SC and Rhino DS in potato at 3 sites in northern Europe, 2016
Document No:	S16-02157 (R-3406), residue trials
Guidelines:	OECD (2011) Guidance Document on Crop Field Trials (Series on Testing and Assessment No. 164 and Series on Pesticides No. 66)
GLP	Yes
Report:	**CA 6.3.1/11: Sutherland J. (2017b)**
Title:	Determination of residues of flutolanil following a seed treatment application of Flutolanil (MONCUT) 40SC and Rhino DS in potato at 4 sites in southern Europe, 2016
Document No:	S16-02159 (R-3408), residue trials
Guidelines:	OECD (2011) Guidance Document on Crop Field Trials (Series on Testing and Assessment No. 164 and Series on Pesticides No. 66)
GLP	Yes

Studies set up

The first field program was conducted in 2014 at two locations in the United Kingdom and in Spain.

The study S14-02899 generated seed potato, treated with flutolanil for use in subsequent residue studies: S14-02900 and S14-02901. Analytical phase of the studies S14-02900 has been reported in a separate study S16-03442 and analytical phase from the studies S14-02901 has been reported in a separate study S16-03453.

To generate seed potatoes, flutolanil 40 SC was applied, as seed treatment, using roller table spray application, at a rate of 91 g a.i/ t of potato tuber. Considering a planting rate of 4 ton of tuber / ha, the

application rate was 0.368 kg ai/ha, which is according to the proposed cGAP for the active substance renewal.
The objective of the studies S14-02900 and S14-02901 was to determine residue levels of flutolanil and its metabolites (M-2, M-4, M-101 and M-102 and their conjugates) in raw agricultural commodity potato, following seed treatment. Potato seeds treated with flutolanil were planted in three residue trials in NEU. Specimens of potatoes (untreated and treated) were taken by hands 101 and 102 days after planting. Potato seeds treated with flutolanil were planted in four residue trials in SEU. Specimens of potatoes (untreated and treated) were taken by hands 101 and 102 days after planting.

A second field program was conducted in 2015 to the same principle, to have a set of trials conducted in northern Europe and trials conducted in southern Europe. The objective of the study S15-00013 was to generate seed potato treated with flutolanil for use in subsequent residue trials from the studies S15-00014 and S15-00016. The seed treatment was conducted on potato tubers in United Kingdom. One roller table spray application of Flutolanil 40 SC was applied at a target rate of 92 g/ tonne potatoes. Considering a planting rate of 4 ton of tuber / ha, the application rate was 0.368 kg ai/ha, which is according to the proposed cGAP for the substance renewal.
The objective of the study S15-00014 was to determine residue levels of flutolaniol and its metabolites (M-2, M-4, M-101 and M-102 and their conjugates) in raw agricultural commodity potato, following seed treatment in northern Europe. Potato seeds treated with flutolanil were planted in five residue trials in NEU. Specimens of potatoes (untreated and treated) were taken by hands 97 to 102 days after planting.
The objective of the study S15-00016 was to determine residue levels of flutolaniol and its metabolites (M-2, M-4, M-101 and M-102 and their conjugates) in raw agricultural commodity potato, following seed treatment in southern Europe. Potato seeds treated with flutolanil were planted in four residue trials in SEU. Specimens of potatoes (untreated and treated) were taken by hands 99 to 104 days after planting.

A third field program was conducted in 2016 at two locations (United Kingdom and France).
The objective of the study S16-02156 was to generate seed potato treated with flutolanil for use in subsequent residue trials (S16-02157 and S16-02159) and processing studies (S16-02690). One roller table spray application of flutolanil 40SC was applied at a target rate of 92 g/ tonne potatoes. Considering a planting rate of 4 ton of tuber / ha, the application rate was 0.368 kg ai/ha, which is according to the proposed cGAP for the substance renewal.
The objective of the studies S16-02157 and S16-02159 was to determine residue levels of flutolanil and its metabolites (M-2, M-4, M-101 and M-102 and their conjugates) in raw agricultural commodity potato, after treatment of potato seeds.
Potato seeds treated with flutolanil were planted in three residue trials in NEU.
Specimens of potatoes (untreated and treated) were taken by hand 98 and 100 days after planting.
Potato seeds treated with flutolanil) were planted in four residue trials in SEU.
Specimens of potatoes (untreated and treated) were taken by hand 75 and 97 days after planting
Analytical method:

In the residue trials, residues of flutolanil and its metabolites (M-2, M-4, M-101, M-102 and their conjugates, expressed as flutolanil) were determined following the validated analytical method (AGR/MOA/FLUTO-4) described in “Validation of the Analytical Method for the Determination of Flutolanil and its Metabolites M-2, M-4, M-101 and M-102 in Potato after Hydrolysis”, S16-00710 (A-3081). Summary of the validation data is presented in Volume 3, B.5.1.2.5: Methods used in support of residues studies.

The method achieves an LOQ of 0.01 mg/kg per analyte. No residues in the untreated samples have been measured in all the residue trials. The amount of M-2, M-4, M-101, M-102 and their conjugates, is reported as mg/kg flutolanil equivalents in the study reports.

Results:
An overview of maximal storage period of potato samples is given in Table B.7.3.1.3 -1 and the procedural recovery during analysis of the samples is given in Table B.7.3.1.3-2 . An overview of relevant residue data is given in Table B.7.1.3.1-3.
The flutolanil result obtained in the trial S16-02157-02 is not consistent with the results of the other trials. Therefore, the Dixon’s Q test needs to be conducted to determine if the result of this trial is an outlier or not. All calculations were done based on the results of flutolanil only as no metabolites were detected above the limit of quantification, except for one trial where the results of M-102 and its conjugates were detected at the LOQ. It is considered that the impact of this metabolite result would be negligible for the calculation. As the trials from Northern and Southern Europe are considered to have similar distribution from Mann Whitney U-Test (see table below), the results of all trials (Northern and Southern Europe) are considered in the Dixon’s Q test: Mann-Whitney U-Test (α: 0.005) (FAO manual 197, p. 87-88)

Data set	Potato NEU	Potato SEU	Rank Set 1	Rank Set 2
1	0.05	0.00	21.5	5
2	0.04	0.00	19.5	5
3	0.00	0.05	5	21.5
4	0.00	0.04	5	19.5
5	0.01	0.01	12.5	12.5
6	0.00	0.01	5	12.5
7	0.02	0.01	16.5	12.5
8	0.00	0.00	5	5
9	0.00	0.00	5	5
10	0.09	0.01	23	12.5
11	0.02	0.01	16.5	12.5
12		0.030		18
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				
Mean	0.02	0.02		
STMR	0.01	0.01		
	Number of values:		11	12
	Sum Rank:		135	142
	U_1 and U_2 values:		63.5	68.5
	Critical value:		33	(α = 0.05)
	n_a = 11		n_b = 12	
	Result:		**Populations similar**	

Dixon's Q-test for rejection of HIGH outliers:

Q_{table}

	Total number of points in sample set											
	3	4	5	6	7	8	9	10	15	20	25	30
$Q_{90\%}$:	0.941	0.765	0.642	0.560	0.507	0.468	0.437	0.412	0.338	0.300	0.277	0.26
$Q_{95\%}$:	0.970	0.829	0.710	0.625	0.568	0.526	0.493	0.466	0.384	0.342	0.317	0.298
$Q_{99\%}$:	0.994	0.926	0.821	0.740	0.680	0.634	0.598	0.568	0.475	0.425	0.393	0.372

$$Q_{calc} = \frac{\text{gap}}{\text{range}} = \frac{\text{highest point} - \text{2nd highest}}{\text{highest point} - \text{lowest point}}$$

	Data set	
1	0.09	
2	0.05	
3	0.05	
4	0.04	
5	0.04	
6	0.03	
7	0.02	
8	0.02	
9	0.01	
10	0.01	
11	0.01	
12	0.005	<0.01
13	0.005	<0.01
14	0.005	<0.01
15	0.003	<0.003
16	0.003	<0.003
17	0.003	<0.003
18	0.003	<0.003
19	0.003	<0.003
20	0.003	<0.003
21	0.003	<0.003
22	0.003	<0.003
23	0.003	<0.003

Highest point = 0.09
Second highest point = 0.05
Lowest point = 0.003
Total number of points = 23

$$Q_{calc} = \frac{0.09 - 0.05}{0.09 - 0.003} = \frac{0.04}{0.087} = 0.460$$

Instructions

1. List the data set in Column B.
2. Sort the data in descending order.
3. The highest and second highest data are added automatically to column F. Also add the lowest data point to Column F.
4. The Q_{calc} result is displayed in Column P.
5. Compare Q_{calc} with the Q_{table} column data for the same total number of points in your data set.
6. If $Q_{calc} > Q_{table}$ then the highest point can be rejected with 90%, 95% or 99% confidence for rows 4, 5 and 6 , respectively.

Following the Dixon's Q test, the results from the Trial S16-02157-02 are considered as outlier and therefore the trial is considered as not acceptable. The results from this trial are not considered in the risk assessment.
So, in total, 22 residue trials were carried out with flutolanil (10 in Northern and 12 in Southern Europe). All reported trials were conducted following the proposed good agricultural practice (GAP).
In summary, residues of flutolanil found in potato tubers at harvest ranged from <0.003 mg/kg (30% of the LOQ) to 0.05 mg/kg. The results for the metabolites were expressed as flutolanil equivalent. No residue of M-2 and its conjugates were detected above <0.003 mg/kg (30% of the LOQ). No residue of M-4 and its conjugates, and M-101 were detected above <0.01 mg/kg (LOQ). No residue of M-102 and its conjugates were detected above <0.01 mg/kg (LOQ), except for 1 trial (S15-00016-02) in Spain, where the residue of M-102 and its conjugates was detected at 0.01 mg/kg. The sum of flutolanil, M-2, M-4, M-101, M-102 and their conjugates, expressed as flutolanil, ranged from <0.015 to <0.08 mg/kg**.
No residues of flutolanil, M-2, M-4, M-101, M-102 and their conjugates were detected above 30% of the LOQ (0.003 mg/kg) in any control samples.

** RMS does not agree with the residue definition proposed by the notifier. All calculations performed by the RMS are indicated below.

Table B.7.3.1.3-1 Storage period flutolanil and metabolites in the supervised residue trials with potatoes (seed treatment)

Report	study type/trial	Storage time (days)
S14-02899	seed treatment	175d*
S14-02900 trial 01-03	supervised residue trial	103
S14-02901		99
S15-00013	seed treatment	375*
S15-00014	supervised residue trial	259
S15-00016		262
S16-02156	seed treatment	57
S16-02157	supervised residue trial	106
S16-02159		75

* Time between treatment and analysis

Table B.7.3.1.3-2 Reported recoveries of flutolanil and its metabolites in the submitted residue studies (seed treatment)

Report	Validation (% at spiking met 0.01, 1, 10, or 30 mg/kg)											
	Flutolanil				M2		M4		M101		M102	
	0.01	1.0	10	30	0.01	1.0	0.01	1.0	0.01	1.0	0.01	1.0
S14-02899	77, 87	-	83	84	-	-	-	-	-	-	-	-
S14-02900	93	91	-	-	94	90	99	88	96	93	109	93
S14-02901	93	91	-	-	94	90	99	88	96	93	109	93
S15-00013	82, 81, 85	-	95, 101, 91	83, 68, 72	-	-	-	-	-		-	-
S15-00014	79	89	-	-	83	101	77	89	90	98	83	89
S15-00016	86	88	-	-	77	90	79	90	88	93	87	84
S16-02156	87	-	-	-	-	-	-	-	-	-	-	-
S16-02157	98, 88	92, 82	-	-	92, 95	90, 83	96, 93	91, 80	101, 96	99, 90	89, 97	90, 80
S16-02159	97	87	-	-	89	82	91	87	99	94	93	87

Table B.7.1.3.1-3 Summary table with the available supervised residue trials with flutolanil in potatoes (seed treatment) in Northern and Southern Europe

Trial No	Year	Country	Application Rate (kg as/ha)	DAA	Portion analysed	Flutolanil	M-2*	M-4*	M-101**	M-102**	Total[(a)]
Northern European Trials							Residues (mg/kg)				
S14-02900-01 DE73 8HJ Derbyshire, UK	2014	UK	0.368	102	Potato tuber	0.05	<0.01	<0.01	0.006	0.006	0.06
S14-02900-02 B76 0DF, Warwickshire, UK	2014	UK	0.368	101	Potato tuber	0.03	<0.01	<0.01	0.006	0.006	0.04
S14-02900-03 L39 9EE	2014	UK	0.368	101	Potato tuber	<0.01	<0.01	<0.01	0.006	0.006	<0.02

Trial No	Year	Country	Application Rate (kg as/ha)	DAA	Portion analysed	Flutolanil	M-2*	M-4*	M-101**	M-102**	Total[(a)]
Lancashire, UK											
S15-00014-01 YO43 4HB, Arglam East Yorkshire, UK	2015	UK	0.368	102	Potato tuber	<0.01	<0.01	<0.01	0.006	0.006	<0.02
S15-00014-02 IP26 4QT, Feltwell, Norfolk, UK	2015	UK	0.368	100	Potato tuber	0.01	<0.01	<0.01	0.006	0.006	0.02
S15-00014-03 L39 8SU, Southport, Lancashire, UK	2015	UK	0.368	101	Potato tuber	<0.01	<0.01	<0.01	0.006	0.006	<0.02
S15-00014-04 B78 2AB, Staffordshire, UK	2015	UK	0.368	98	Potato tuber	0.02	<0.01	<0.01	0.006	0.006	0.03
S15-00014-05 DE73 8BR, Melbourne, Derbyshire, UK	2015	UK	0.368	97	Potato tuber	<0.01	<0.01	<0.01	0.006	0.006	<0.02
S16-02157-01 DE73 8AG Melbourne, Derbyshire, UK	2016	UK	0.368	98	Potato tuber	<0.01	<0.01	<0.01	0.006	0.006	<0.02
S16-02157-02 B78 2AA. Staffordshire, UK	2016	UK	0.368	100	Potato tuber	0.09	<0.01	<0.01	0.006	0.006	0.10
S16-02157-03 PE7 3SA, Cambridgeshire, UK	2016	UK	0.368	98	Potato tuber	0.02	<0.01	<0.01	0.006	0.006	0.03

Trial No	Year	Country	Application Rate (kg as/ha)	DAA	Portion analysed	Flutolanil	M-2*	M-4*	M-101**	M-102**	Total[(a)]
Southern European trials											
S14-02901-01 50297 Grisen, Alagon, Spain	2014	Spain	0.368	102	Potato tuber	<0.01	<0.01	<0.01	0.006	0.006	<0.02
S14-02901-02 50669, Santa Engracia, Aragon, Spain	2014	Spain	0.368	102	Potato tuber	<0.01	<0.01	<0.01	0.006	0.006	<0.02
S14-02901-03 50591, Alcala de Moncayo, Aragon, Spain	2014	Spain	0.368	99	Potato tuber	0.03	<0.01	<0.01	0.006	0.006	0.04
S14-02901-04 50059, Montana, Aragon, Spain	2014	Spain	0.368	100	Potato tuber	0.04	<0.01	<0.01	0.006	0.006	0.05
S15-00016-01 50630, Alagon, Spain	2015	Spain	0.368	99	Potato tuber	0.01	<0.01	<0.01	0.006	0.006	0.02
S15-00016-02 50591, Alcala de Moncayo, Aragon, Spain	2015	Spain	0.368	100	Potato tuber	0.01	<0.01	<0.01	0.006	0.006	0.02
S15-00016-03 50669, Santa Engracia, Spain	2015	Spain	0.368	100	Potato tuber	<0.01	<0.01	<0.01	0.006	0.006	<0.02
S15-00016-04 50059, Montana, Aragon, Spain	2015	Spain	0.368	104	Potato tuber	<0.01	<0.01	<0.01	0.006	0.006	<0.02
S16-02159-01 82440 Realville France	2016	S. France	0.368	97	Potato tuber	<0.01	<0.01	<0.01	0.006	0.006	<0.02

Trial No	Year	Country	Application Rate (kg as/ha)	DAA	Portion analysed	Flutolanil	M-2*	M-4*	M-101**	M-102**	Total[(a)]
S16-02159-02 81630 Salvagnac, France	2016	S. France	0.368	97	Potato tuber	<0.01	<0.01	<0.01	0.006	0.006	<0.02
S16-02159-03 82100 Castelsarrasin, Tarn-et-Garonne, France	2016	S. France	0.368	97	Potato tuber	<0.01	<0.01	<0.01	0.006	0.006	<0.02
S16-02159-04 66600 Rivesaltes, Pyrenees-Orientales, France	2016	S. France	0.368	75	Potato tuber	0.03	<0.01	<0.01	0.006	0.006	0.04

* including conjugates and expressed as flutolanil

** expressed as flutolanil

(a): Sum of Flutolanil and M-4 (free and conjugated), expressed as flutolanil, calculated by RMS

Remarks RMS:

1. In the submitted residue data, seed potatoes were generated in a separate location, following shipment to the supervised residue trials locations (both northern and southern Europe). Frozen storage stability data demonstrates stability of flutolanil in potatoes up to 5 years. Frozen stability of metabolites M-4, M-101 and M-102 was demonstrated in potatoes up to 12 months. Stability of metabolite M-2 was demonstrated up to 9 months. Except of metabolite M-2 for which storage stability in some samples is not covered by the available data (report S15-00013), stability data in potatoes is covered by the available data.
It should be noted that the storage during shipment time has not been included in the stability studies. Stability of flutolanil and its metabolites in ambient/transport conditions might be required.

2.Reported procedural recoveries are within the acceptable ranges. Linearity of the methods used in the residue trials have been reported and considered acceptable.

3. In the study evaluation, the notifier stated that the result of residue trial S16-02157-02 is not consistent with the results of the other trials and it should be described as an outlier (Dixon's Q test has been performed, see calcuations above). RMS does not agree with this conclusion. Residues of flutolanil in the other available trials in northern Europe were: 5x <0.01; 0.01; 0.02; 0.03; 0.05 mg/kg. The measured value of 0.09 mg/kg does not appear as an outlier when compared to the whole data set and it is considered relevant for further assessment.

4.In the northern European trials, in potato tubers, flutolanil was measured in a range from <0.01 mg/kg to 0.09 mg/kg. In southern European trials, flutolanil residues were measured from <0.01 mg/kg to 0.04 mg/kg. Metabolites M-2 and M-4 (including conjugates and expressed as flutolanil) were below the LOQ in all the trials in North and South Europe.

5.It should be noted that metabolite M-101 and M-102 have separate toxicological characteristics and are not covered by the toxicological reference values of the parent compound flutolanil. In the trials, residues of metabolites M-101 and M-102 were expressed as parent flutolanil. To convert the residue levels found to M-101 and M-102, the residue level should be corrected using the molecular weight of flutolanil (323.34 g/mol), M-101 (189.13 g/mol) and M-102 (190.12 g/mol), which results in a conversion factor (CF) of 0.585 for M-101 and 0.588 for M-102. However, since in all residue trials residues of those metabolites, expressed as flutolanil, were below the LOQ, it is concluded that the metabolites itself were also below the LOQ of 0.01 mg/kg (0.006 mg/kg) .

B.7.3.1.4 Potato, in-furrow treatment, EU (submitted in the framework of the MRL application)

Previous evaluation	Submitted for the purpose of renewal
RMS remark	Acceptable. RMS concluded that residue trial S16-02160-05 is acceptable and the mean

	value (0.13 mg/kg) of the analysed sample (0.18 mg/kg) and the retained sample (0.08 mg/kg) should be taken for further assessment.

Report	**CA 6.3.1/12: Sutherland J. (2015d)**
Title:	Flutolanil 70DF: Determination of residues of flutolanil following in-furrow application of Flutolanil 70DF in potato at 4 sites in northern Europe, 2014
Document No:	S14-03028 (R-3376)
Guidelines:	OECD (2011) Guidance Document on Crop Field Trials (Series on Testing and Assessment No. 164 and Series on Pesticides No. 66)
GLP	Yes
Report:	**CA 6.3.1/13: Merdian H. (2016c)**
Title:	Determination of residues of flutolanil following in-furrow application of Flutolanil 70DF in potato at 4 sites in northern Europe, 2014
Document No:	S16-03454 (R-3384), Analytical part of study S14-03028
Guidelines:	SANCO/3023/99 REv.4- EU Guidance document generating and reporting methods of analysis in support of pre-registration data requirements.
GLP	Yes

Report:	**CA 6.3.1/14: Sutherland J. (2015e)**
Title:	Flutolanil 70DF: Determination of residues of flutolanil following in-furrow application of Flutolanil 70DF in potato at 4 sites in southern Europe, 2014
Document No:	S14-03029 (R-3377)
Guidelines:	OECD (2011) Guidance Document on Crop Field Trials (Series on Testing and Assessment No. 164 and Series on Pesticides No. 66)
GLP	Yes
Report:	**CA 6.3.1/15: Merdian H. (2016d)**
Title:	Determination of residues of flutolanil following in-furrow application of Flutolanil 70DF in potato at 4 sites in southern Europe, 2014
Document No:	S16-03455 (R-3385), Analytical part of the study S14-03029
Guidelines:	SANCO/3023/99 REv.4- EU Guidance document generating and reporting methods of analysis in support of pre-registration data requirements.
GLP	Yes

Report	**CA 6.3.1/16: Lines J. (2016d)**
Title:	Flutolanil 70DF: Determination of residues of flutolanil following in-furrow application of Flutolanil 70DF in potato at 4 sites in northern Europe, 2015
Document No:	S15-00015 (R-3392)
Guidelines:	OECD (2011) Guidance Document on Crop Field Trials (Series on Testing and Assessment No. 164 and Series on Pesticides No. 66)
GLP	Yes

Report:	**CA 6.3.1/17: Martin C. (2016)**
Title:	Flutolanil 70DF: Determination of residues of flutolanil following in-furrow application of Flutolanil 70DF in potato at 4 sites in southern Europe, 2015
Document No:	S15-00017 (R-3394) Final Report Amendment 1
Guidelines:	OECD (2011) Guidance Document on Crop Field Trials (Series on Testing and Assessment No. 164 and Series on Pesticides No. 66)
GLP	Yes

Report:	**CA 6.3.1/18: Sutherland J. (2017c)**
Title:	Determination of residues of flutolanil following in-furrow application of Flutolanil 70DF in potato at 4 sites in northern Europe, 2016
Document No:	S16-02158 (R-3407)

Guidelines:	OECD (2011) Guidance Document on Crop Field Trials (Series on Testing and Assessment No. 164 and Series on Pesticides No. 66)
GLP	Yes

Report:	**CA 6.3.1/19: Sutherland J. (2017d)**
Title:	Determination of residues of flutolanil following in-furrow application of Flutolanil 70DF in potato at 5 sites in southern Europe, 2016
Document No:	S16-02160 (R-3409)
Guidelines:	OECD (2011) Guidance Document on Crop Field Trials (Series on Testing and Assessment No. 164 and Series on Pesticides No. 66)
GLP	Yes

Study set up:

The first field program was conducted in 2014 at two locations in the United Kingdom and in Spain. Flutolanil 70DF was applied in-furrow at a rate of 2.1 - 2.3 kg ai/ha (water volume 100-250 L/ha), which is according to the proposed cGAP in the renewal process. Specimens (treated and untreated) were collected 101-103 days after the single application.

A second field program was conducted 2015 to the same principle, in order to have a full set of 8 trials conducted in northern Europe and 8 trials conducted in southern Europe. Specimens (treated and untreated) were collected 98-103 days after the single application.

A third field program was conducted in 2016 at two locations (United Kingdom and France).

Treated samples received an in-furrow application of flutolanil at an application rate: 2.0- 2.4 kg as/ha, which is according to the proposed cGAP. Specimens (treated and untreated) were collected 75-102 days after the single application.

Analytical method:

Residues of flutolanil and its metabolites were determined following the validated analytical method "Validation of the Analytical Method for the Determination of Flutolanil and its Metabolites M-2, M-4, M-101 and M-102 in Potato after Hydrolysis", S16-00710 (A-3081). Summary of the validation data is presented in Volume 3, B.5.1.2.4: Methods used in support of residues studies.

The method achieves an LOQ of 0.01 mg/kg per analyte. Reported recoveries are within the acceptable ranges. No residues in untreated commodities were detected in the residue trials.

The method achieves an LOQ of 0.01 mg/kg per analyte. The amount of M-2, M-4, M-101, M-102 and their conjugates, is reported as mg/kg flutolanil equivalents in the study reports.

Results:

Due to high residue observed in the trial S16-02160-05, retained sample was analysed. Unfortunately, as the samples were not analysed in duplicate, it is no possible to determine which results are correct. Therefore, this trial S16-02160-05 is considered not acceptable and the results won't be considered for the calculation of the MRL.

So in total, 24 residue trials were carried out with Flutolanil 70DF (12 in Northern and 12 in Southern Europe). All reported trials are supporting the proposed good agricultural practice (GAP) for in-furrow application.

In summary, residues of flutolanil found in potato tubers at harvest ranged from <0.003 mg/kg (30% of the LOQ) to 0.11 mg/kg. The results for the metabolites were expressed as flutolanil equivalent. No residue of M-2 and its conjugates were detected above <0.003 mg/kg (30% of the LOQ). Residue of M-4 and its conjugates and M-101 ranged from <0.003 mg/kg (30% of the LOQ) to 0.03 mg/kg. Residue of M-102 and its conjugates ranged from <0.003 mg/kg (30% of the LOQ) to 0.07 mg/kg. The sum of flutolanil, M-2, M-4, M-101, M-102 and their conjugates, expressed as flutolanil, ranged from <0.015 to <0.17 mg/kg.
No residues of flutolanil, M-2, M-4, M-101, M-102 and their conjugates were detected above 30% of the LOQ (0.003 mg/kg) in any control samples.

An overview of maximal storage period of potato samples and the procedural recovery during analysis of the samples is given in Table B.7.3.1.4.1-1. An overview of relevant residue data is given in B.7.3.1.4.1-2

Table B.7.3.1.4-1 Storage period and procedural recovery of flutolanil and metabolites in the supervised residue trials with potatoes (in furrow treatment)

Report	Max storage between harvest and analysis	Validation (% at spiking with 0.01 and 1 mg/kg)									
		Flutolanil		M2		M4		M101		M102	
		0.01	1.0	0.01	1.0	0.01	1.0	0.01	1.0	0.01	1.0
S14-03028	104	103	92	101	89	107	91	109	89	101	86
S16-02160	105	88, 72	89, 70	87, 80	89, 74	91, 79	90, 77	89, 90	97, 88	99, 76	86, 89
S14-03029	100	103	92	101	89	107	91	109	89	101	86
S14-03455	612	92	78	90	93	78	86	84	86	94	87
S14-00015	255	99	93	96	93	97	92	103	97	88	86
S15-00017	264	75	82, 84, 85	77	88, 85, 87	73	84, 84, 84	82	81, 82, 85	76	85, 81, 84

Table B.7.3.1.4-1 Summary table with the available supervised residue trials with flutolanil in potatoes (in-furrow treatment) in Northern and Southern Europe

Trial No	Application Rate (kg as/ha)	DAA	Portion analysed	Flutolanil	M-2*	M-4*	M-101**	M-102**	Total$_{(a)}$
Northern Europe									
S14-03028-01 DE73 8HJ Melbourne, UK	2.2	102	Potato tuber	0.08	<0.01	0.02	0.006	0.006	0.1
S14-03028-02 B76 0DF Curdworth, UK	2.3	101	Potato tuber	0.09	<0.01	0.02	0.006	0.012	0.11
S14-03028-03 L39 9EE Southport, UK	2.1	101	Potato tuber	<0.01	<0.01	<0.01	0.006	0.006	<0.02
S14-03028-04 B75 6LG Sutton Coldfield, UK	2.3	103	Potato tuber	0.04	<0.01	<0.01	0.006	0.006	0.05
S15-00015-01 YO43 4HB Arglam, UK	2.1	103	Potato tuber	0.03	<0.01	<0.01	0.006	0.006	0.04
S15-00015-02 IP26 4QT Feltwell, UK	2.45	100	Potato tuber	0.10	<0.01	0.03	0.006	0.018	0.13
S15-00015-03 L39 8SE Southport, UK	2.45	101	Potato tuber	<0.01	<0.01	<0.01	0.006	0.006	<0.02
S15-00015-04 B78 2AB, Drayton Basset, UK	2.1	98	Potato tuber	0.11	<0.01	0.02	0.006	0.006	0.13
S16-02158-01 DE73 8AG Derbyshire, UK	2.3	98	Potato tuber	<0.01	<0.01	<0.01	0.006	0.012	<0.02
S16-02158-02 B78 2AA	2.4	100	Potato tuber	<0.01	<0.01	<0.01	0.006	0.006	<0.02

Trial No	Application Rate (kg as/ha)	DAA	Portion analysed	Flutolanil	M-2*	M-4*	M-101**	M-102**	Total$_{(a)}$
Staffordshire, UK									
S16-02158-03 PE7 3SA Cambridgeshire UK	2.3	98	Potato tuber	<0.01	<0.01	<0.01	0.006	0.006	<0.02
S16-02158-04 l39, 9EE, Lancashire, UK	2.0	99	Potato tuber	<0.01	<0.01	<0.01	0.006	0.006	<0.02

Trial No	Application Rate (kg as/ha)	DAA	Portion analysed	Flutolanil	M-2*	M-4*	M-101**	M-102**	Total$_{(a)}$
Southern Europe									
S14-03029-01 50297 Grisen, Spain	2.1	102	Potato tuber	0.02	<0.01	0.01	0.006	0.012	0.03
S14-03029-02 50669 Santa Engracia, Spain	2.2	102	Potato tuber	0.02	<0.01	<0.01	0.006	0.006	0.03
S14-03029-03 50591 Alcala de Moncayo, Spain	2.1	99	Potato tuber	0.01	<0.01	<0.01	0.006	0.006	0.02
S14-03029-04 50059 Montanana, Spain	2.1	100	Potato tuber	0.03	<0.01	<0.01	0.006	0.006	0.04
S15-00017-01 50630 Alagon, Spain	2.3	99	Potato tuber	0.02	<0.01	<0.01	0.018	0.006	0.03
S15-00017-02 50591 Alcala de Moncayo, Spain	2.21	100	Potato tuber	<0.01	<0.01	<0.01	0.006	0.012	<0.02
S15-00017-03 50669 Santa Engracia, Spain	**2.9**	99	Potato tuber	<0.01	<0.01	<0.01	0.006	0.006	<0.02
S15-00017-04 50669 Santa Engracia, Spain	**3.47**	104	Potato tuber	0.03	<0.01	<0.01	0.006	0.024	0.04
S16-02160-01 82440 Réalville, Tarn-et-Garonne, France	2.1	97	Potato tuber	<0.01	<0.01	<0.01	0.006	0.006	<0.02
S16-02160-02 81630 Salvagnac, France	2.3	97	Potato tuber	0.02	<0.01	0.01	0.006	0.041	0.03
S16-02160-03	2.2	97	Potato tuber	0.04	<0.01	0.02	0.006	0.012	0.06

Trial No	Application Rate (kg as/ha)	DAA	Portion analysed	Flutolanil	M-2*	M-4*	M-101**	M-102**	Total$_{(a)}$
82100 Castelsarrasin, Tarn-et-Garonne, France									
S16-02160-04 66600 Rivesaltes, Pyrénées-Orientales France	2.2	75	Potato tuber	0.09	<0.01	0.03	0.006	0.012	0.12
S16-02160-05 66200 Elne, Pyrénées-Orientales France	2.3	75	Potato tuber [#]	0.13 (0.18; 0.08)	<0.01 (<0.01; <0.01)	0.05 (0.06; 0.04)	0.012	0.024	0.18

[#] retained sample was analysed and the mean results are presented (mean from treated sample 002A and retained sample 002R1)

* including conjugates and expressed as flutolanil

** recalculated by RMS (metabolite M-101 and M-102 have different toxicological references than the parent compound and therefore, should not be expressed as parent compound flutolanil).

(a) Total: sum of flutolanil and metabolite M-4 (free and conjugated), expressed as flutolanil

Remarks RMS:

- In the submitted residue data, seed potatoes were generated in a separate location, following shipment to the supervised residue trials locations (both northern and southern Europe). Frozen storage stability data demonstrates stability of flutolanil in potatoes up to 5 years. Frozen stability of metabolites M-4, M-101 and M-102 was demonstrated in potatoes up to 12 months. Stability of metabolite M-2 was demonstrated up to 9 months. Except of metabolite M-2 for which storage stability in some samples is not covered by the available data (report S14-03455), stability data in potatoes is covered by the available data.
- It should be noted that the storage during shipment time has not been included in the stability studies. The applicant states that after sampling potato specimens were frozen within 5 hours and transported under deep frozen conditions.

- In six trials, from the second field program, treated samples received an in-furrow application of flutolanil at an application rate of 2.1-2.45 kg as/ha, which is within the 25% of the proposed cGAP. In two trials in Southern Europe; S15- 00017-03 and S15-00017-04, samples received an application at a rate higher than 25% of the proposed cGAP, hence those trials are not taken into further assessmet.
- The applicant states that in trial S16-02160-05 a high level of flutolanil residues have been measured (0.18 mg/kg). A retained sample was analysed, giving residues levels of 0.08 mg/kg. The applicant concluded that since the retained sample was not analysed in duplicate, the results of those analysis cannot be acceptable and the result from the whole trial was considered not acceptable by the applicant. RMS is of the opinion that the trial is acceptable, since there are no other reasons to withdraw the result. RMS proposes to use the mean value of 0.13 mg/kg from the analysed sample (0.18 mg/kg) and retained sample (0.08 mg/kg) for further assessment.
- It should be noted, that metabolite M-101 and M-102 have separate toxicological characteristics and are not covered by the toxicological reference values of the parent compound flutolanil. In the trials, residues of metabolites M-101 and M-102 were expressed as parent flutolanil. To convert the residue levels found, expressed as M-101 and M-102, the residue levels should be corrected using the molecular weight of flutolanil (323.34 g/mol), M-101 (189.13 g/mol) and M-102 (190.12 g/mol), which results in a conversion factor (CF) of 0.585 for M-101 and 0.588 for M-102. RMS corrected the residue values (see Table B.7.3.1.4-1).

B.7.4 Feeding studies

B.7.4.1 Poultry

Previous evaluation	Submitted for the purpose of renewal
RMS remark	Acceptable

Reference:	**CA 6.4.1/01: [redacted], 2016a**
Title:	Flutolanil: Residues of Flutolanil and its Metabolites in Eggs and Tissues of Laying Hens
Document No.:	LMS0104 (A-3075)
Guidelines:	OECD 505: Residues in Livestock (2007) OCSPP 860.1480 (1996)
Deviations:	None
Testing laboratory:	[redacted]
GLP:	Yes

Executive Summary

A livestock feeding study was performed to quantify levels of flutolanil residues in eggs and tissues of laying hens after dietary inclusion of flutolanil for 28 to 29 days. Four groups of laying hens received flutolanil incorporated in the diet as the only feed source at dose levels equivalent to 1, 10, 30 and 100 mg/kg. A control group received untreated basal diet throughout the treatment period.
Half of the hens in the high dose group (Group 5) were maintained for up to 2 weeks after cessation of treatment in order to provide data on the decline of residues.
The study design was as follows:

Group	Treatment	Dose (mg/kg)	Number of replicate subgroups	Number of birds	
				per subgroup	per group
1	Control	0	3	4	12
2	Flutolanil	1	3	4	12
3	Flutolanil	10	3	4	12
4	Flutolanil	30	3	4	12
5	Flutolanil	100	6	4	24*

* Twelve birds killed at zero withdrawal; 12 birds included for depuration

Eggs were collected daily and at termination of the experimental period, all hens were sacrificed and residue concentrations measured in selected tissues.
Eggs: Quantifiable residues occurred primarily as the metabolite M-101 in all subgroups treated at 30 and 100 ppm from Day 4 of the study. Levels generally increased over the treatment period and reached a plateau concentration during the third week of dose administration.

Liver: Quantifiable residues in liver occurred mainly as M-101 at 10 ppm and above. Quantifiable residues flutolanil and M-2, conjugated M-2 and conjugated M-4 occurred at 30 ppm and above, and the metabolites M-4 and M-7 at 100 ppm. There were no quantifiable residues of the metabolite M-102.
Skin and subcutaneous fat: Quantifiable residues in skin and subcutaneous fat occurred as flutolanil and its metabolite M-101 at 30 ppm and above. There were no quantifiable residues of the metabolites M-2, M-4, M-7 or M-102.
Abdominal fat: Quantifiable residues in abdominal fat occurred mainly as flutolanil at 10 ppm and above and its metabolites M-2 and M-101 at 30 ppm and above. There were no quantifiable residues of the metabolites M-4, M-7 or M-102.
Muscle: Residues in muscle above the LOQ (0.01 mg/kg) occurred as the metabolite M-101 only at 30 ppm and above. There were no quantifiable residues of flutolanil, or its metabolites M-2, conjugated M-2, M-4, conjugated M-4, M-7 or M-102.
Overall flutolanil derived residues were highest in liver, with lower residues in abdominal and skin/subcutaneous fat and the lowest residue concentrations were found in muscle. Residues showed direct correlation with exposure levels.
In the depuration groups, quantifiable residues in eggs occurred as the metabolite M-101 only at 3 and 7 days withdrawal and were below the limit of quantitation at 14 days withdrawal. In tissues, quantifiable residues similarly occurred as the metabolite M-101 but were no longer quantifiable at 14 days withdrawal with the exception of liver, where residues were close to the LOQ.

Residue data are summarised in the following table:

Sum of overall residues in hen matrices (flutolanil equivalents, mg/kg)**								
Group	Treatment	Dose (ppm)	Withdrawal (days)	Eggs	Liver	Muscle	Skin / subcutaneous fat	Abdominal fat
1	Control	0	0	ND	<LOQ	<LOQ	<LOQ	<LOQ
2	Flutolanil	1	0	<LOQ	<0.013 [A]	<0.010 [A]	<0.014 [A]	<0.017 [A]
3	Flutolanil	10	0	0.014	0.066	<0.011 [A]	<0.014 [A]	0.024
4	Flutolanil	30	0	0.046	0.205	0.049	0.063	0.092
5	Flutolanil	100	0	0.148	0.751	0.155	0.205	0.226
			3	0.103	0.328	0.084	0.068	0.031
			7	0.063	0.077	0.032	0.031	<0.029 [A]
			14	<LOQ	0.036	<LOQ	<0.014 [A]	<0.021 [A]

ND Not detected (< LOD of 0.002 mg/kg) LOQ = 0.01 mg/kg

[A] No quantifiable residues found in any individual analysis (all <LOQ or ND). Where the result for an analyte was below the LOQ, i.e. <0.01 mg/kg, and during the conversion to flutolanil equivalents this gave a numerical value above 0.010 then this is expressed as less than the numerical value.

** For conversion of metabolites to flutolanil equivalents, the following converiosn factors were used: M-2: 0.953, M-4: 1.15, M-7: 1.04, M-101: 1.71, M-102: 1.70

In the group of hens dosed at 1 mg/kg (equivalent to 0.076 mg/kg bw/d) no quantifiable residues were found in any individual analysis. All values were <LOQ (<0.01 mg/kg) or not detected (<0.002 mg/kg). Consequently no detectable residues of flutolanil and its metabolites are anticipated in eggs or tissues of laying hens following use of Moncut 40SC at the proposed GAP.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Flutolanil

   Chemical name (IUPAC): α, α, α-trifluoro-3'-isopropoxy-*o*-toluanilide

   CA registry number: 66332-96-5

   Lot or batch number: 101141

   Purity: 99.1%

2. Test animals

   Species: Laying hen (*Gallus gallus domesticus*)

   Strain: Bovan Brown

   Gender: Female

   Age: 26 - 28 weeks

   Weight at dosing: Range 1.337 to 1.980 kg

   Number of animals: 12 per treatment group and control, except top dose where additional 12 birds included for depuration phase (24 in total)

   Diet: Meal (HRC Avian Layer), *ad libitum* as only feed source

   Water: Mains water, *ad libitum*

Housing: Group housed in pens (4 hens per pen) made of galvanised steel and concrete in a purpose-built facility. Wood shavings provided as litter, replenished as necessary.

Photoperiod: 17 hours light / 7 hours dark cycles

B. STUDY DESIGN AND METHODS

1. In-life dates:

14 January 2015 to 11 August 2016

2. Experimental design

1. Dosing Regime: Oral

Amount of dose:
Group 1: 0 mg/kg in diet
Group 2: 1 mg/kg in diet, equivalent to 0.076 mg/kg bw/d
Group 3: 10 mg/kg in diet, equivalent to 0.692 mg/kg bw/d
Group 4: 30 mg/kg in diet, equivalent to 2.354 mg/kg bw/d
Group 5: 100 mg/kg in diet, equivalent to 7.632 mg/kg bw/d

Mean food consumption per treatment group (g per bird per day)

Treatment Group		Week						
		-2	-1	1	2	3	4	5
1	Mean	119	109	79	122	133	133	-
	SD	23.3	21.1	31.9	3.2	17.5	9.9	-
	n	3	3	3	3	3	3	-
2	Mean	96	107	118	122	129	137	-
	SD	46.6	23.1	11.7	7.1	12	12.5	-
	n	3	3	3	3	3	3	-
3	Mean	109	106	115	125	127	115	-
	SD	13.1	4.2	10.3	9.3	2	4.2	-
	n	3	3	3	3	3	3	-
4	Mean	111	108	118	139	136	138	-
	SD	9.2	10.5	3	25.3	25.4	26	-
	n	3	3	3	3	3	3	-
5	Mean	121	124	122	127	132	139	128#
	SD	17.1	6.8	12.8	12.9	7.8	8.5	4.2
	n	6	6	6	6	6	6	2

Untreated basal diet

Vehicle: Avian layer diet formulations

Timing: Fed continuously

Duration: Treatment phase: 28 to 29 days

Group 5 Depuration phase: 3, 7 and 14 days

2.	Sample Collection	
	Egg collection:	Daily, pooled to give 3 composite samples per treatment group (6 samples Group 5)
	Tissues harvested and analysed:	Skin & subcutaneous fat, muscle (leg & breast pooled), liver, and abdominal fat
3.	Storage of samples	Flutolanil, M-2, M-4, M-7, M-101 and M-102 were stable in eggs, liver, muscle and fat for a period of 4 days at -20 °C. M-101 in eggs and M-2 and M-4 in liver and muscle were stable for a period of 436 days at -20 °C.
4.	Analytical method	Samples (except fat) were extracted with acetonitrile and acetonitrile : 0.1N HCl (4:1, v/v) and cleaned-up with a C18 solid phase extraction (SPE) cartridge. Fat samples were extracted with acetonitrile : hexane (50:50, v/v) and acidic acetonitrile : hexane (50:50 v/v) and cleaned-up by liquid-liquid partition. Quantitation was performed using LC-MS/MS. A de-conjugation step required enzyme hydrolysis with ß-glucorinidase of extracted samples and clean-up with a SPE cartridge.

Samples of eggs and tissues were analysed to determine the concentrations of flutolanil and its significant metabolites which were M-2, M-4, M-7 and M-101 for eggs and M-2, M-4, M-7, M-101 and M-102 for tissues, with additionally conjugated M-2 and conjugated M-4 for liver and muscle.
Full details of the analytical method used in the study are summarised in Volume 3, B.5.1.2.5: Methods used in support of residues studies. The limit of quantification (LOQ) was 0.01 mg/kg and the limit of detection (LOD) was 0.002 mg/kg.

II. RESULTS AND DISCUSSION

Analtytical method

Matrix-matched recoveries were run along with the analysis of the hen tissue and egg samples and were between 65 and 119% individual values and between 71 and 109% for mean values per analyte per level per matrix (n=5) for both the original method and modified method with acid extraction and deconjugation. Linearity of the detector response was shown for standard solutions for each analyte (Table B.7.4.1-1).

Table B.7.4.1-1 **Recovery of the original analytical method during analysis of the hen samples (n=5 per analyre per matrix)**

Analyte	Fortification	Liver		Muscle		Fat		Eggs	
	Mg/kg	Mean (range)	RSD	Mean (range)	RSD	Mean (range)	RSD	Mean (range)	RSD
Flutolanil	0.01	80 (72-95)	11	84 (76-99)	10	77 (82-92)	5	88 (84-104)	11
	0.1	93 (89-96)	3	92 (89-95)	2	89 (83-97)	7	85 (95-98)	7
M-2	0.01	89 (75-105)	12	89 80-98)	10	77 (68-84)	8	95 (90-98)	4
	0.1	102 (98-106)	4	96 (87-100)	6	91 (83-101)	9	98 (91-101)	4
M-4	0.01	99 (80-120)	12	90 (83-97)	8	89 (79-94)	7	82 (77-87)	5
	0.1	104 (97-109)	5	95 (89-99)	5	91 84-105)	9	85 (72-91)	9
M-7	0.01	99 (86-120)	14	87 (79-96)	8	75 65-81)	8	99 (91-112)	8
	0.1	101 (96-107)	4	96 (91-99)	4	91 85-101)	8	102 (92-109)	6
M-101	0.01	83 (78-89)	6	92 (82-98)	7	81 (69-108)	20	86 (80-98)	8
	0.1	92 (89-94)	2	92 (87-95)	4	85 (78-89)	6	94 (92-97)	2
M-102	0.01	99 (77-119)	16	93 (74-119)	18	79 (74-85)	7	75 (70-81)	6
	0.1	105 (101-110)	3	94 (87-99)	5	88 (84-94)	5	93 (89-99)	4

Table B.7.4.1-1 **(continued) Recovery of the modified analytical method (with acid extraction and with and without deconjugation) during analysis of the hen samples (n=5 per analyre per matrix)**

Analyte	Fortification	Liver		Muscle		Fat		Eggs	
	Mg/kg	Mean (range)	RSD	Mean (range)	RSD	Mean (range)	RSD	Mean (range)	RSD
Flutolanil	0.01	99 (91-111)	8	97 (93-102)	3	79 (71-79)	13	99 (93-105)	5
	0.1	95 (90-97)	3	92 (85-99)	6	86 (75-105)	13	105 (94-113)	7
M-2	0.01	82 (77-91)	7	107 (101-112)	4	90 (80-103)	13	87 (76-95)	8
	0.1	97 (92-100)	3	98 (89-104)	6	92 (84-106)	10	99 (93-109)	6
M-2 with deconjugation	0.01	98 (82-109)	13	82 (74-90)	8	-	-	-	
	0.1	100 (85-108)	10	85 (78-98)	9	-	-	-	6
M 4	0.01	95 (91-99)	4	90 (82-100)	8	101 (89-111)	8	95 (86-101)	6
	0.1	102 (100-105)	2	102 (94-108)	5	109 (99-117)	8	102 (94-109)	-
M-4 with deconjugation	0.01	82 (81-85)	2	91 (82-100)	9	-	-	-	
	0.1	95 (86-106)	8	94 (82-106)	9	-	-	-	13
M-7	0.01	101 (94-106)	5	97 (92-106)	6	80 (75-94)	10	83 (66-93)	5
	0.1	93 (90-97)	3	103 (98-108)	5	96 (92-104)	5	95 (89-103)	7
M-101	0.01	74 (67-79)	6	96 (87-103)	6	76 (69-87))	9	96 (89-106)	3
	0.1	71 (68-73)	3	103 (98-109)	4	78 (68-85)	9	99 (93-101)	-

Storage stability

Storage stability data were generated within the study. They are separately reported in CA 6.1/06 (Dias N., 2016a). Flutolanil, M-2, M-4, M-7, M-101 and M-102 were stable in chicken eggs, liver, muscle and fat for a period of 4 days. It was also demonstrated that M-101 in eggs and M-2 and M-4 in liver and muscle were stable for a period of 436 days. This covers the storage period of analytical samples of the hen in the study.

Stability of standards of flutolanil, M-2, M-4, M-7, M-101 and M-102 in buffer were found to be stable (Recovery of 102-116%) for 132 days).

Eggs

Flutolanil derived residues in eggs were measured as the parent compound and its metabolites M-2, M-4 and M-7 and M-101. A summary of the concentrations expressed as flutolanil equivalents in eggs is presented in Table B.7.4.1-2a. Residues above the LOQ occurred mainly as the metabolite M-101 and were demonstrable in egg samples from all replicates in Groups 4 and 5 from Day 4 and showed a dose-related trend. Overall mean values from Days 1-28 were 0.027 and 0.082 mg/kg in those groups receiving 30 and 100 ppm respectively and residues attained plateau concentrations during the third week of dosing. In the groups receiving 1 or 10 ppm (equivalent to 0.076 and 0.692 mg/kg bw/d) levels were generally not detected or below the LOQ.

In the depuration groups, depletion of residues occurred after cessation of dosing, with no quantifiable residues present at 14 days withdrawal.

No residues of flutolanil or its metabolites were detected in control birds (Group 1) during the treatment period.

Table B.7.4.1-2a: Summary of residues of flutolanil and metabolites in eggs at day -1 and day 1

		Day -1				Day 1			
Tissue	Sub-group	Flutolanil	M-2	M-4	M-7	Flutolanil	M-2	M-4	M-7
0 mg/kg diet	1A	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	1B	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	1C	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
1 mg/kg in diet	2A	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	2B	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	3C	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
3 mg/kg in diet	3A	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	3B	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	3C	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
10 mg/kg in diet	4A	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	4B	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	4C	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
30 mg/kg in diet	5A	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	5B	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	5C	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table B.7.4.1-2**b: Summary of residues of flutolanil and metabolites in eggs at day 4 and day 7**

Tissue	Sub-group	Day 4				Day 7			
		Flutolanil	M-2	M-4	M-7	Flutolanil	M-2	M-4	M-7
0 mg/kg diet	1A	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	1B	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	1C	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
1 mg/kg in diet	2A	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	2B	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	3C	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
3 mg/kg in diet	3A	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	3B	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	3C	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
10 mg/kg in diet	4A	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	4B	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	4C	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
30 mg/kg in diet	5A	<0.01	<0.01	<0.01	<0.01	<0.01	0.011	0.013	0.011
	5B	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	5C	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table B.7.4.1-2-c**: Summary of residues of flutolanil and metabolites in eggs at day 10 and day 13**

Tissue	Sub-group	Day 10				Day 13			
		Flutolanil	M-2	M-4	M-7	Flutolanil	M-2	M-4	M-7
0 mg/kg diet	1A	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	1B	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	1C	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
1 mg/kg in diet	2A	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	2B	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	3C	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
3 mg/kg in diet	3A	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	3B	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	3C	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
10 mg/kg in diet	4A	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	4B	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	4C	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
30 mg/kg in diet	5A	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	5B	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	5C	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table B.7.4.1-2-d**: Summary of residues of flutolanil and metabolites in eggs at day 16 and day 19**

Tissue	Sub-group	Day 16				Day 19			
		Flutolanil	M-2	M-4	M-7	Flutolanil	M-2	M-4	M-7
0 mg/kg diet	1A	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	1B	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	1C	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
1 mg/kg in diet	2A	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	2B	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	3C	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
3 mg/kg in diet	3A	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	3B	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	3C	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
10 mg/kg in diet	4A	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	4B	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	4C	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
30 mg/kg in diet	5A	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	5B	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	5C	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table B.7.4.1-2-e**: Summary of residues of flutolanil and metabolites in eggs at day 22 and day 25**

Tissue	Sub-group	Day 22				Day 25			
		Flutolanil	M-2	M-4	M-7	Flutolanil	M-2	M-4	M-7
0 mg/kg diet	1A	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	1B	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	1C	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
1 mg/kg in diet	2A	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	2B	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	3C	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
3 mg/kg in diet	3A	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	3B	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	3C	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
10 mg/kg in diet	4A	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	4B	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	4C	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
30 mg/kg in diet	5A	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	5B	<0.01	<0.01	<0.01	<0.01	0.0027	<0.01	<0.01	<0.01
	5C	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table B.7.4.1-2f: Summary of residues of flutolanil and metabolites in eggs at day 28 and day 31

Tissue	Sub-group	Day 28				Day 31			
		Flutolanil	M-2	M-4	M-7	Flutolanil	M-2	M-4	M-7
0 mg/kg diet	1A	<0.01	<0.01	<0.01	<0.01	-	-	-	-
	1B	<0.01	<0.01	<0.01	<0.01	-	-	-	-
	1C	<0.01	<0.01	<0.01	<0.01	-	-	-	-
1 mg/kg in diet	2A	<0.01	<0.01	<0.01	<0.01	-	-	-	-
	2B	<0.01	<0.01	<0.01	<0.01	-	-	-	-
	3C	<0.01	<0.01	<0.01	<0.01	-	-	-	-
3 mg/kg in diet	3A	<0.01	<0.01	<0.01	<0.01	-	-	-	-
	3B	<0.01	<0.01	<0.01	<0.01	-	-	-	-
	3C	<0.01	<0.01	<0.01	<0.01	-	-	-	-
10 mg/kg in diet	4A	<0.01	<0.01	<0.01	<0.01	-	-	-	-
	4B	<0.01	<0.01	<0.01	<0.01	-	-	-	-
	4C	<0.01	<0.01	<0.01	<0.01	-	-	-	-
30 mg/kg in diet	5A	0.011	<0.01	<0.01	<0.01	-	-	-	-
	5B	<0.01	<0.01	<0.01	<0.01	-	-	-	-
	5C	<0.01	<0.01	<0.01	<0.01	-	-	-	-
30 mg/kg in diet (stopped at day 28)	5D	-	-	-	-	-	<0.01	<0.01	<0.01
	5E	-	-	-	-	-	<0.01	<0.01	<0.01
	5F	-	-	-	-	-	<0.01	<0.01	<0.01

Poultry Tissues

Residues in poultry tissues were measured as flutolanil and its metabolites M-2 (and conjugated M-2 for liver and muscle), M-4 (and conjugated M-4 for liver and muscle), M-7, M-101 and M-102.

In treated birds, the highest overall flutolanil derived residues occurred in the liver; lower residues were found in abdominal and skin/subcutaneous fat and the lowest residue concentrations occurred in muscle.

In the group of hens receiving 1 ppm flutolanil in the diet (equivalent to 0.076 mg/kg bw/d), no quantifiable residues were found in any individual analysis. All values were <LOQ (<0.01 mg/kg). The results are presented in Table B.7.4.1-3a

Measurable residues were detected in liver and abdominal fat in birds receiving 10 ppm flutolanil in the diet, and in all tissues in birds receiving 30 and100 ppm flutolanil in the diet. No residues of flutolanil or its metabolites above the LOQ (0.01 mg/kg) were detected any tissue in control birds (Group 1).

Liver

Residues in the liver occurred mainly as M-101 (mean values of <LOQ, 0.027, 0.068 and 0.214 mg/kg for Groups 2, 3, 4 and 5 respectively). Lower concentrations of flutolanil and M-2, both free and conjugated, were detected (mean values of <LOQ, <LOQ, <LOQ and 0.037 mg/kg for flutolanil, <LOQ, <LOQ, 0.023 and 0.045 mg/kg for M-2 and <LOQ, <LOQ, 0.034 and 0.209 mg/kg for conjugated M-2 for Groups 2, 3, 4 and 5 respectively). The metabolite M-4, free and conjugated were the lowest residues detected (mean values of <LOQ, <LOQ, <LOQ and 0.012 mg/kg for M-4 and <LOQ, <LOQ, 0.012 and 0.072 mg/kg for conjugated M-4 for Groups 2, 3, 4 and 5 respectively). Mean residues of the metabolite M-7 were below the LOQ, and no quantifiable residues of M-102 were found in any liver samples analysed.

Results for subgroups 5D, 5E and 5F included for depuration data, indicated depletion of residues in liver after cessation of dosing, with only M-101 residues remaining, close to the LOQ (0.016 mg/kg), at 14 days withdrawal.

Muscle

Quantifiable residues in muscle samples occurred only as the metabolite M-101 (mean values of <LOQ, <LOQ, 0.022 and 0.076 mg/kg for Groups 2, 3, 4 and 5 respectively).

No quantifiable residues of flutolanil, M-2, conjugated M-2, M-4, conjugated M-4, M-7 or M-102 were found in any muscle samples analysed.

In the depuration groups, depletion of residues in muscle occurred after cessation of dosing, with no quantifiable residues present at 14 days withdrawal.

Skin and subcutaneous fat

Residues in skin and subcutaneous fat occurred mainly as flutolanil (mean values of <LOQ, <LOQ, 0.026 and 0.077 mg/kg for Groups 2, 3, 4 and 5 respectively) and M-101 (mean values of <LOQ, <LOQ, 0.018 and 0.069 mg/kg for Groups 2, 3, 4 and 5 respectively).

No quantifiable residues of M-2, M-4, M-7 or M-102 were found in any skin and subcutaneous fat samples analysed.

In the depuration groups, depletion of residues in skin and subcutaneous fat occurred after cessation of dosing, with no quantifiable residues present at 14 days withdrawal.

Abdominal fat

Residues in abdominal fat occurred mainly as flutolanil (mean values of <LOQ, 0.014, 0.056 and 0.146 mg/kg for Groups 2, 3, 4 and 5 respectively) and M-2 and M-101 (mean values <LOQ, <LOQ, 0.011 and 0.019 mg/kg for M-2 and <LOQ, <LOQ, <LOQ, 0.034 mg/kg for M-101 for Groups 2, 3, 4 and 5 respectively).

No quantifiable residues of M-4, M-7 or M-102 were found in any abdominal fat samples analysed.

In the depuration groups, depletion of residues in abdominal fat occurred after cessation of dosing, with no quantifiable residues present at either 7 or 14 days withdrawal.

Table B.7.4.1-3a: Residues of flutolanil and its metabolites in livestock tissues (as mg/kg) for hens dosed at 1 mg/kg in diet, equivalent to 0.076 mg/kg bw/d (Group 2)

Tissue	Sub-group	Flutolanil	M-2	M-2 conj.	M-4	M-4 conj.	M-7	M-101	M-102
Liver	2A	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	2B	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	2C	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Muscle	2A	<0.01	<0.01	[A]	<0.01	[A]	<0.01	<0.01	<0.01
	2B	<0.01	<0.01	[A]	<0.01	[A]	<0.01	<0.01	<0.01
	2C	<0.01	<0.01	[A]	<0.01	[A]	<0.01	<0.01	<0.01
Skin & Subcutaneous Fat	2A	<0.01	<0.01	-	<0.01	-	<0.01	<0.01	<0.01
	2B	<0.01	<0.01	-	<0.01	-	<0.01	<0.01	<0.01
	2C	<0.01	<0.01	-	<0.01	-	<0.01	<0.01	<0.01
Abdominal Fat	2A	<0.01	<0.01	-	<0.01	-	<0.01	<0.01	<0.01
	2B	<0.01	<0.01	-	<0.01	-	<0.01	<0.01	<0.01
	2C	<0.01	<0.01	-	<0.01	-	<0.01	<0.01	<0.01

<0.01 = None detected (less than the LOD of 0.002 mg/kg)

[A] Analysis not required as no residues > LOQ detected at top dose level

Table B.7.4.1-3b: Residues of flutolanil and its metabolites in livestock tissues (as mg/kg) for hens dosed at 10 mg/kg in diet, equivalent to 0.692 mg/kg bw/d (Group 3)

Tissue	Sub-group	Flutolanil	M-2	M-2 conj.	M-4	M-4 conj.	M-7	M-101	M-102
Liver	3A	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.028	<0.01
	3B	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.025	<0.01
	3C	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.028	<0.01
Muscle	3A	<0.01	<0.01	[A]	<0.01	[A]	<0.01	<0.01	<0.01
	3B	<0.01	<0.01	[A]	<0.01	[A]	<0.01	<0.01	<0.01
	3C	<0.01	<0.01	[A]	<0.01	[A]	<0.01	<0.01	<0.01
Skin & Subcutaneous Fat	3A	<0.01	<0.01	-	<0.01	-	<0.01	<0.01	<0.01
	3B	<0.01	<0.01	-	<0.01	-	<0.01	<0.01	<0.01
	3C	<0.01	<0.01	-	<0.01	-	<0.01	<0.01	<0.01
Abdominal Fat	3A	0.017	<0.01	-	<0.01	-	<0.01	<0.01	<0.01
	3B	0.013	<0.01	-	<0.01	-	<0.01	<0.01	<0.01
	3C	0.013	<0.01	-	<0.01	-	<0.01	<0.01	<0.01

<0.01 = None detected (less than the LOD of 0.002 mg/kg)

[A] Analysis not required as no residues > LOQ detected at top dose level

Table B.7.4.1-3c**: Residues of flutolanil and its metabolites in livestock tissues (as mg/kg) for hens dosed at 30 mg/kg in diet, equivalent to 2.35 mg/kg bw/d (Group 4)**

Tissue	Sub-group	Flutolanil	M-2	M-2 conj.	M-4	M-4 conj.	M-7	M-101	M-102
Liver	4A	0.010	0.025	0.041	<0.01	0.010	<0.01	0.072	<0.01
	4B	0.013	0.020	0.028	<0.01	0.013	<0.01	0.060	<0.01
	4C	<0.01	0.024	0.033	<0.01	0.013	<0.01	0.073	<0.01
Muscle	4A	<0.01	<0.01	[A]	<0.01	[A]	<0.01	0.022	<0.01
	4B	<0.01	<0.01	[A]	<0.01	[A]	<0.01	0.022	<0.01
	4C	<0.01	<0.01	[A]	<0.01	[A]	<0.01	0.022	<0.01
Skin & Subcutaneous Fat	4A	0.036	<0.01	-	<0.01	-	<0.01	0.021	<0.01
	4B	0.020	<0.01	-	<0.01	-	<0.01	0.017	<0.01
	4C	0.021	<0.01	-	<0.01	-	<0.01	0.016	<0.01
Abdominal Fat	4A	0.073	0.017	-	<0.01	-	<0.01	0.013	<0.01
	4B	0.049	0.012	-	<0.01	-	<0.01	0.010	<0.01
	4C	0.047	<0.01	-	<0.01	-	<0.01	<0.01	<0.01

<0.01 = None detected (less than the LOD of 0.002 mg/kg)

Table B.7.4.1-3d: Residues of flutolanil and its metabolites in livestock tissues (as mg/kg) for hens dosed at 100 mg/kg in diet, equivalent to 7.63 mg/kg bw/d (Group 5)

Tissue	Sub-group	Flutolanil	M-2	M-2 conj.	M-4	M-4 conj.	M-7	M-101	M-102
Liver	5A	0.035	0.040	0.212	0.012	0.071	<0.01	0.179	<0.01
	5B	0.038	0.057	0.249	0.011	0.071	0.012	0.226	<0.01
	5C	0.037	0.049	0.165	0.014	0.073	0.011	0.237	<0.01
	5D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.175	<0.01
	5E	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.04	<0.01
	5F	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.016	<0.01
Muscle	5A	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.072	<0.01
	5B	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.082	<0.01
	5C	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.075	<0.01
	5D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.049	<0.01
	5E	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.014	<0.01
	5F	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Skin & Subcutaneous Fat	5A	0.070	<0.01	-	<0.01	-	<0.01	0.068	<0.01
	5B	0.090	<0.01	-	<0.01	-	<0.01	0.072	<0.01
	5C	0.072	<0.01	-	<0.01	-	<0.01	0.068	<0.01
	5D	<0.01	<0.01	-	<0.01	-	<0.01	0.037	<0.01
	5E	<0.01	<0.01	-	<0.01	-	<0.01	0.015	<0.01
	5F	<0.01	<0.01	-	<0.01	-	<0.01	<0.01	<0.01
Abdominal Fat	5A	0.136	0.019	-	<0.01	-	<0.01	0.030	<0.01
	5B	0.136	0.020	-	<0.01	-	<0.01	0.035	<0.01
	5C	0.166	0.018	-	<0.01	-	<0.01	0.036	<0.01
	5D	<0.01	<0.01	-	<0.01		<0.01	-	<0.01
	5E	<0.01	<0.01	-	<0.01	-	<0.01	<0.01	<0.01
	5F	<0.01	<0.01	-	<0.01	-	<0.01	<0.01	<0.01

<0.01 = None detected (less than the LOD of 0.002 mg/kg)
LOQ = 0.01 mg/kg

III. CONCLUSIONS

The study is acceptable with regard to number of animals, dose groups, performance of the analytical method and maximal storage period of analytical samples. No detectable residues of flutolanil and its metabolites are anticipated eggs or tissues (liver, muscle, skin plus subcutaneous fat and abdominal fat) of laying hens at the comparable maximum dietary burden.

B.7.4.2 Ruminants

Previous evaluation	Submitted for the purpose of renewal
RMS remark	Acceptable

Report:	**CA 6.4.2/01: [redacted], 2016b**
Title:	Flutolanil: Residues of Flutolanil and its metabolites in Milk and Tissues of Dairy Cows
Document No:	LMS0103 (R-3396)
Guidelines:	OECD 505: Residues in Livestock
GLP	Yes

Executive Summary

A livestock feeding study was performed to quantify levels of flutolanil residues and its significant metabolites in milk and tissues of lactating dairy cows following oral administration of Flutolanil for a minimum period of 28 days. Four groups of lactating cows received flutolanil incorporated in the diet by dispensing individual doses of liquid formulation onto individual concentrate feed rations at dose levels equivalent to 3, 30, 90 and 300 mg/kg. A control group received untreated basal diet throughout the treatment period.

Half of the cows in the high dose group (Group 5) were maintained for up to 2 weeks after cessation of treatment in order to provide data on the decline of residues.

The study design was as follows:

Group	Treatment	Nominal Dose Level	Achieved Dose Level		Number of animal
		(mg/kg in total diet)	(mg/kg in total diet)	(mg a.i./kg bw/day)	
1	Control	0	0	0	3
2	Flutolanil	3	3	0.120	3
3	Flutolanil	30	28	1.186	3
4	Flutolanil	90	81	3.376	3
5	Flutolanil	300	287	11.952	6*

* Three cows terminated at zero withdrawal; 3 cows included for depuration

Milk samples were taken from all cows daily throughout the test period and were submitted for assay or stored frozen. At termination of the experimental period, all cows were sacrificed and residue concentrations measured in selected tissues.

Whole milk: Quantifiable residues in whole milk occurred primarily as the metabolites M-2/conjugated M-2 and M-4/conjugated M-4 in Group 3 (1.186 mg a.i./kg bw/day), Group 4 (3.376 mg a.i./kg bw/day) and Group 5 (11.952 mg a.i./kg bw/day).

Skimmed milk: Quantifiable residues in skimmed milk occurred only as the metabolites conjugated M-2 and M-4/conjugated M-4 in Group 3 (1.186 mg a.i./kg bw/day), Group 4 (3.376 mg a.i./kg bw/day) and Group 5 (11.952 mg a.i./kg bw/day). There was a correlation between treatment levels and residues found.

Cream: Quantifiable residues in cream occurred as the parent compound, Flutolanil and the metabolites M-2/conjugated M-2 and M-4/conjugated M-4 in Group 3 (1.186 mg a.i./kg bw/day), Group 4 (3.376 mg a.i./kg bw/day) and Group 5 (11.952 mg a.i./kg bw/day). There was a correlation between treatment levels and residues found.

Liver: Quantifiable residues in liver occurred as the parent compound, Flutolanil and the metabolites M-2/conjugated M-2 and M-4/conjugated M-4 in Group 2 (0.120 mg a.i./kg bw/day), Group 3 (1.186 mg a.i./kg bw/day), Group 4 (3.376 mg a.i./kg bw/day) and Group 5 (11.952 mg a.i./kg bw/day). The Flutolanil metabolite M-101 was also present in Group 3 (1.186 mg a.i./kg bw/day), Group 4 (3.376 mg a.i./kg bw/day) and Group 5 (11.952 mg a.i./kg bw/day).

Kidney: Quantifiable residues in kidney occurred only as the metabolites M-2/conjugated M-2 and M-4/conjugated M-4 in Group 2 (0.120 mg a.i./kg bw/day), Group 3 (1.186 mg a.i./kg bw/day), Group 4 (3.376 mg a.i./kg bw/day) and Group 5 (11.952 mg a.i./kg bw/day).

Muscle: Residues in muscle above the LOQ (0.01 mg/kg) occurred as the metabolite conjugated M-2 in Group 5 (11.952 mg a.i./kg bw/day) only. In addition, there was only one quantifiable level of the metabolite conjugated M-4 in a single animal from this group.

Fat: Residues in subcutaneous, perirenal and omental fat occurred as Flutolanil and M-2 in Group 4 (3.376 mg a.i./kg bw/day) and Group 5 (11.952 mg a.i./kg bw/day), and in Group 3 (1.186 mg a.i./kg bw/day) for omental fat only. In addition, there was one quantifiable residue of the metabolite M-4 in a single animal from Group 5 (11.952 mg a.i./kg bw/day) for omental and subcutaneous fat.

Overall Flutolanil-derived residues were highest in kidney and liver. Lower residues occurred in whole milk, omental, subcutaneous and perirenal fat, and the lowest residue concentrations were found in muscle. Residues showed direct correlation with exposure levels.

In the depuration animals, quantifiable residues in milk occurred as the metabolite conjugated M-4 only at 4 days withdrawal and were below the limit of quantification at 7 and 14 days withdrawal. In tissues, quantifiable residues occurred primarily as the metabolite M-101 in liver, where residues were close to the limit of quantification at 14 days withdrawal. Lower residues of Flutolanil, M2/conjugated M-2 and M-4/conjugated M-4 were observed in subcutaneous and omental fat and kidney at 4 days with drawal but were either not detected or below the limit of quantification at 7 and 14 days withdrawal.

In the lowest dose group of lactating cows (Group 2) dosed at 3 mg/kg (equivalent to 0.120 mg/kg bw/d), no quantifiable residues were found in any individual analysis, excepted for liver which showed M-2 (conjugate) at 0.01 mg/kg in 1 out of 3 animals and for kidney which showed M-2 (conjugate) at 0.028-0.054 mg/kg and M-4 (conjugate) at 0.013-0.019 mg/kg All other values in Group 2 were <LOQ (<0.01 mg/kg) or not detected (<0.002 mg/kg). Consequently no detectable residues of flutolanil and its metabolites are anticipated in milk or tissues of lactating cows following use of Moncut 40SC at the proposed GAP.

Residue data are summarised in the following table:

Overall mean flutolanil derived residues in bovine matrices (flutolanil equivalents, mg/kg)**										
Group	Treatment	Dose (ppm)	Withdrawal (days)	Whole milk	Liver	Muscle	Kidney	Subcut. fat	Perineral fat	Omental fat
1	Control		0	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
2	Flutolanil		0	<0.01	<0.017 [A]	<0.01	0.056	<0.01	<0.01	<0.01
3	Flutolanil		0	0.033	0.217	<0.01	0.930	<0.01	<0.01	<0.018 [A]
4	Flutolanil		0	0.077	0.687	<0.019 [A]	1.498	0.024	<0.012 [A]	0.045
5	Flutolanil		0	0.222 [b]	2.564	<0.038 [A]	4.225	0.046	0.041	0.139
			4#	0.023	0.154	<0.019 [A]	0.056	0.07	<0.01	0.05
			7#	<0.01	0.089	<0.01	<0.01	<0.011 [A]	<0.01	<0.013 [A]
			14#	<0.01	0.035	<0.01	<0.01	<0.01	<0.01	<0.01

ND Not detected (< LOD of 0.002 mg/kg) LOQ = 0.01 mg/kg

[A] No quantifiable residues found in any individual analysis (all <LOQ or ND). Where the result for an analyte was below the LOQ, i.e. <0.01 mg/kg, and during the conversion to flutolanil equivalents this gave a numerical value above 0.010 then this is expressed as less than the numerical value.

B: includes depuration animals over Days 1-28 inclusive

one animal

** For conversion of metabolites to flutolanil equivalents, the following converiosn factors were used: M-2: 0.953, M-4: 1.15, M-7: 1.04, M-101: 1.71, M-102: 1.70

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Flutolanil

Chemical name (IUPAC)	α, α, α-trifluoro-3'-isopropoxy-*o*-toluanilide
CA registry number:	66332-96-5
Lot or batch number:	101141
Purity:	99.1%
Expiry date:	28 June 2017

2. Test animals

Species:	dairy cow, lactating females
Strain:	Friesian
Gender:	Female
Age:	5 to 11 years
Weight at dosing:	Range 543.0 to 689.0 kg
Milk yield at start of baseline period	≥12 kg per day
Number of animals:	3 per treatment group and control, except top dose where additional 3 lactating dairy cows included for depuration phase (6 in total)

Diet:	Pelleted concentrate ration offered at a rate of 4.0 kg (fresh weight) per cow per day. Good quality meadow hay was offered after each milking at a rate of 22-24 kg (fresh weight) per cow per day. The overall feed allowance (Concentrates + hay) was approximately equivalent to 22 to 24 kg dry matter intake. The diet consisted of barley, wheatfeed, maize gluten, rapeseed meal, molassed sugar beet pulp, molasses, dehulled extracted toasted soya, minerals, MEGALAC (rumen protected fat) and vitamins.
Water:	Fresh drinking water from the public supply
Housing:	Group housed in pens made of galvanised steel with free-draining floors. The cows were moved twice daily to a separate milking area, each cow being allocated a permanent stall position for the duration of the test period. Individual concentrate feeds were given at each milking and hay was offered on a group basis in the home pens. Straw was provided as bedding material and was replenished as necessary to maintain a deep straw litter system.
Photoperiod:	Natural light, supplemented as necessary with overhead fluorescent tube lighting.

B. STUDY DESIGN AND METHODS

1. In-life dates:

15 September 2015 to 17 August 2016

1. Experimental design

1.	Dosing Regime:	Oral by dispensing individual doses of liquid formulation onto individual concentrate feed rations	
	Amount of dose:	Group 1:	0 mg/kg in diet
		Group 2:	3 mg/kg in diet, equivalent to 0.120 mg/kg bw/d
		Group 3:	30 mg/kg in diet, equivalent to 1.186 mg/kg bw/d
		Group 4:	90 mg/kg in diet, equivalent to 3.376 mg/kg bw/d
		Group 5:	300 mg/kg in diet, equivalent to 11.952 mg/kg bw/d
		Food consumption is presented in Table	
	Vehicle:	Corn oil	
	Timing:	Twice daily	

	Duration:	Acclimatisation: 14 days before commencement of treatment Treatment phase: 29 to 32 days Group 5 Depuration phase: 4, 7 and 14 days
2.	Sample Collection	
	Milk collection:	All cows were machine-milked twice daily using a six-abreast milking parlour. The morning milk production of each cow was retained in a separate, closed polypropylene container at +4ºC until the afternoon milking on the same day was then combined and mixed thoroughly.
	Tissues harvested and analysed:	Skeletal muscle (loin/adductor muscle of thigh), fat (subcutaneous and perirenal and omental), liver (with gall bladder removed) and kidney
3.	Analytical method	Samples (except fat) were extracted with acetonitrile and acetonitrile : 0.1N HCl (4:1, v/v) and cleaned-up with a C18 solid phase extraction (SPE) cartridge. Fat samples were extracted with acetonitrile : hexane (50:50, v/v) and acidic acetonitrile : hexane (50:50 v/v) and cleaned-up by liquid-liquid partition. Quantitation was performed using LC-MS/MS. A de-conjugation step required enzyme hydrolysis with ß-glucorinidase of extracted samples and clean-up with a SPE cartridge.

Samples of whole milk, cream, skimmed milk and tissues (except fat) were analysed to determine the concentrations of flutolanil and its significant metabolites which were M-2, M-4, M-7, M-101, M-2/conjugated M-2 and M-4/conjugated M-4. Samples of fat tissues were analysed to determine the concentrations of flutolanil and its significant metabolites which were M-2, M-4, M-7, M-101,
Full details of the analytical method used in the study are summarised in Volume 3, section B.5.1.2.5. The limit of quantification (LOQ) was 0.01 mg/kg and the limit of detection (LOD) was 0.002 mg/kg.

Table B.7.4.2-1: Mean concentrate consumption per treatment group (kg per cow per day)

Group	Day	-7	-6	-5	-4	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	Mean	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	SD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	n	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
2	Mean	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	SD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	n	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
3	Mean	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	SD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	n	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
4	Mean	4	4	4	4	4	4	4	4	4	3.677	3.013	3.757	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	SD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	560.0	1709.0	421.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	n	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
5	Mean	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	SD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	n	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6

Group	Day	22	23	24	25	26	27	28	29	30	31	32
1	Mean	4	4	4	4	4	4	4	-	-	-	-
	SD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-
	n	3	3	3	3	3	3	3	-	-	-	-
2	Mean	4	4	4	4	4	4	4	4	-	-	-
	SD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-
	n	3	3	3	3	3	3	3	3	-	-	-
3	Mean	4	4	4	4	4	4	4	4	-	-	-
	SD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-
	n	3	3	3	3	3	3	3	3	-	-	-
4	Mean	4	4	4	4	4	4	4	4	-	-	-
	SD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-

	n	3	3	3	3	3	3	3	3	-	-	-
5	Mean	4	4	4	4	4	4	4	4	4	4	4
	SD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	n	6	6	6	6	6	6	6	6	5	4	3

II. RESULTS AND DISCUSSION

Procedural Recovery

Matrix-matched recoveries were run along with the analysis of the cow tissue and milk samples.
In cow tissues, individual values were between 70-110% except for on recovery of M4 in subcutaneous fat (63%, Second recovery 97%), mean 80%). For muscle and omental fat 3 recoveries were determined per concentration per analyte per matrix and relative standard deviations have been calculated. They were below the required 20%, except for M-4 conjugates in muscle (21%).
In milk, recoveries were determined at n=2 per level per analyte. Some values were outside the 70-110% range, however, mean values were acceptable except for M-4 conjugates in skimmed milk at day 21 where 62 and 70% (mean 66%) was found.

Storage stability

The storage period of analytical samples of cow tissue and milk samples in the study is maximal 85 days. The results from the storage stability investigations reported in CA 6.1/07 (Yoshizane, 2017) demonstrated that flutolanil, M-2, M-4, M-7 and M-101 were stable in cow muscle, liver, kidney, milk and fat for 74, 87, 73, 89 and 77 days, except for M-2 and M-7 in cow muscle, where only 12% and 9% of the nominal spiking level, respectively, were detected.
However, muscle samples were stored for a shorter time than 74 days: 8-15 days only,except for the withdraw period at day14 . From the goat metabolism study it was found that goats fed for 5 days 13 mg flutolanil/kg dry feed (KCA 6.2.3/01, Hardwick, 2017) did not show accumulation of flutolanil or its metabolites (TRR_{muscle} = 0.007 mg eq./kg). Overall, instability of M-2 and M-7 in ruminant muscle was considered a minor issue for evaluation or risk assessment.

Tissue	Animal no.	Day -1							Day 1						
		Flutolanil	M-2	M-2 conjugates	M-4	M-4 conjugates	M-7	M101	Flutolanil	M-2	M-2 conjugates	M-4	M-4 conjugates	M-7	M101
0 mg/kg in diet	1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	2	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	3	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
3 mg/kg in diet	4	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	5	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	6	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
30 mg/kg in diet	7	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	9	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
90 mg/kg in diet	10	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	11	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	12	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
300 mg/kg in diet	13	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.018	<0.01	<0.01
	14	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	16	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.017	<0.01	<0.01
	17	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	18	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.014	<0.01	<0.01

Table B.7.4.2-2a: Summary of residues of flutolanil and metabolites in milk at day -1 and day 1

Table **B.7.4.2-2b: Summary of residues of flutolanil and metabolites in milk at day 4 and day 7**

Tissue	Animal no.	Day 4							Day 7						
		Flutolanil	M-2	M-2 conjugates	M-4	M-4 conjugates	M-7	M101	Flutolanil	M-2	M-2 conjugates	M-4	M-4 conjugates	M-7	M101
0 mg/kg in diet	1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	2	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	3	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
3 mg/kg in diet	4	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	5	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	6	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
30 mg/kg in diet	7	<0.01	<0.01	<0.01	<0.01	0.022	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.023	<0.01	<0.01
	8	<0.01	<0.01	<0.01	<0.01	0.25	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.016	<0.01	<0.01
	9	<0.01	<0.01	<0.01	<0.01	0.013	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.029	<0.01	<0.01
90 mg/kg in diet	10	<0.01	<0.01	<0.01	<0.01	0.034	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.038	<0.01	<0.01
	11	<0.01	<0.01	<0.01	<0.01	0.019	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.015	<0.01	<0.01
	12	<0.01	<0.01	<0.01	<0.01	0.029	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.035	<0.01	<0.01
300 mg/kg in diet	13	<0.01	0.013	0.032	<0.01	0.207	<0.01	<0.01	<0.01	0.011	0.030	0.011	0.214	<0.01	<0.01
	14	0.010	0.011	0.024	<0.01	0.092	<0.01	<0.01	<0.01	0.011	0.016	<0.01	0.078	<0.01	<0.01
	15	<0.01	0.010	0.023	0.011	0.102	<0.01	<0.01	<0.01	0.010	0.018	0.011	0.086	<0.01	<0.01
	16	0.012	0.013	0.030	0.017	0.194	<0.01	<0.01	<0.01	<0.01	0.010	0.010	0.125	<0.01	<0.01
	17	<0.01	<0.01	0.033	0.015	0.169	<0.01	<0.01	<0.01	<0.01	0.026	0.015	0.211	<0.01	<0.01
	18	<0.01	<0.01	0.028	0.016	0.219	<0.01	<0.01	<0.01	<0.01	0.023	0.015	0.221	<0.01	<0.01

Table B.7.4.2-2c: Summary of residues of flutolanil and metabolites in milk at day 10 and day 13

Tissue	Animal no.	Day 10							Day 13						
		Flutolanil	M-2	M-2 conjugates	M-4	M-4 conjugates	M-7	M101	Flutolanil	M-2	M-2 conjugates	M-4	M-4 conjugates	M-7	M101
0 mg/kg in diet	1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	2	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	3	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
3 mg/kg in diet	4	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	5	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	6	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
30 mg/kg in diet	7	<0.01	<0.01	<0.01	<0.01	0.019	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.031	<0.01	<0.01
	8	<0.01	<0.01	<0.01	<0.01	0.036	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.043	<0.01	<0.01
	9	<0.01	<0.01	<0.01	<0.01	0.016	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.023	<0.01	<0.01
90 mg/kg in diet	10	<0.01	<0.01	<0.01	0.011	0.134	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.112	<0.01	<0.01
	11	<0.01	<0.01	<0.01	<0.01	0.033	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.040	<0.01	<0.01
	12	<0.01	<0.01	0.013	<0.01	0.056	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.092	<0.01	<0.01
300 mg/kg in diet	13	<0.01	0.013	0.036	0.010	0.271	<0.01	<0.01	<0.01	0.018	0.059	0.015	0.384	<0.01	<0.01
	14	0.010	0.011	0.024	<0.01	0.109	<0.01	<0.01	<0.01	<0.01	0.036	0.013	0.135	<0.01	<0.01
	15	<0.01	0.010	0.026	0.010	0.118	<0.01	<0.01	<0.01	0.012	0.018	<0.01	0.075	<0.01	<0.01
	16	0.012	0.011	0.026	0.017	0.166	<0.01	<0.01	<0.01	<0.01	0.024	<0.01	0.107	<0.01	<0.01
	17	<0.01	<0.01	0.019	0.011	0.131	<0.01	<0.01	<0.01	<0.01	0.028	<0.01	0.132	<0.01	<0.01
	18	<0.01	<0.01	0.026	0.014	0.269	<0.01	<0.01	<0.01	<0.01	0.044	0.024	0.344	<0.01	<0.01

Table B.7.4.2-2**d: Summary of residues of flutolanil and metabolites in milk at day 16 and day 19**

Tissue	Animal no.	Day 16							Day 19						
		Flutolanil	M-2	M-2 conjugates	M-4	M-4 conjugates	M-7	M101	Flutolanil	M-2	M-2 conjugates	M-4	M-4 conjugates	M-7	M101
0 mg/kg in diet	1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	2	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	3	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
3 mg/kg in diet	4	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	5	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	6	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
30 mg/kg in diet	7	<0.01	<0.01	<0.01	<0.01	0.026	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.017	<0.01	<0.01
	8	<0.01	<0.01	<0.01	<0.01	0.027	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.031	<0.01	<0.01
	9	<0.01	<0.01	<0.01	<0.01	0.013	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.014	<0.01	<0.01
90 mg/kg in diet	10	<0.01	<0.01	<0.01	<0.01	0.069	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.053	<0.01	<0.01
	11	<0.01	<0.01	<0.01	<0.01	0.020	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.016	<0.01	<0.01
	12	<0.01	<0.01	<0.01	<0.01	0.069	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.048	<0.01	<0.01
300 mg/kg in diet	13	<0.01	0.010	0.034	<0.01	0.233	<0.01	<0.01	0.013	0.014	0.023	0.017	0.143	<0.01	<0.01
	14	<0.01	<0.01	0.011	<0.01	0.075	<0.01	<0.01	<0.01	<0.01	0.019	0.013	0.079	<0.01	<0.01
	15	<0.01	<0.01	0.024	<0.01	0.120	<0.01	<0.01	<0.01	<0.01	0.019	0.014	0.107	<0.01	<0.01
	16	<0.01	<0.01	0.018	<0.01	0.132	<0.01	<0.01	<0.01	<0.01	0.016	0.019	0.146	<0.01	<0.01
	17	<0.01	<0.01	0.045	<0.01	0.232	<0.01	<0.01	<0.01	<0.01	0.018	0.023	0.143	<0.01	<0.01
	18	<0.01	<0.01	0.013	<0.01	0.154	<0.01	<0.01	<0.01	<0.01	<0.01	0.024	0.159	<0.01	<0.01

Table B.7.4.2-2**e: Summary of residues of flutolanil and metabolites in milk at day 22 and day 25**

Tissue	Animal no.	Day 22							Day 25						
		Flutolanil	M-2	M-2 conjugates	M-4	M-4 conjugates	M-7	M101	Flutolanil	M-2	M-2 conjugates	M-4	M-4 conjugates	M-7	M101
0 mg/kg in diet	1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	2	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	3	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
3 mg/kg in diet	4	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	5	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	6	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
30 mg/kg in diet	7	<0.01	<0.01	<0.01	<0.01	0.044	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.024	<0.01	<0.01
	8	<0.01	<0.01	<0.01	<0.01	0.033	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.024	<0.01	<0.01
	9	<0.01	<0.01	<0.01	<0.01	0.013	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.012	<0.01	<0.01
90 mg/kg in diet	10	<0.01	<0.01	<0.01	<0.01	0.065	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.050	<0.01	<0.01
	11	<0.01	<0.01	<0.01	<0.01	0.036	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.023	<0.01	<0.01
	12	<0.01	<0.01	<0.01	<0.01	0.072	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.048	<0.01	<0.01
300 mg/kg in diet	13	<0.01	<0.01	0.028	<0.01	0.214	<0.01	<0.01	<0.01	<0.01	0.021	<0.01	0.233	<0.01	<0.01
	14	<0.01	<0.01	0.017	<0.01	0.127	<0.01	<0.01	<0.01	<0.01	0.012	<0.01	0.076	<0.01	<0.01
	15	<0.01	<0.01	0.019	<0.01	0.123	<0.01	<0.01	<0.01	<0.01	0.013	<0.01	0.072	<0.01	<0.01
	16	<0.01	<0.01	0.023	<0.01	0.206	<0.01	<0.01	<0.01	<0.01	0.012	<0.01	0.163	<0.01	<0.01
	17	<0.01	<0.01	0.023	<0.01	0.185	<0.01	<0.01	<0.01	<0.01	0.013	<0.01	0.133	<0.01	<0.01
	18	<0.01	<0.01	0.023	<0.01	0.297	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.080	<0.01	<0.01

Table B.7.4.2-2f**:** **Summary of residues of flutolanil and metabolites in milk at day 28 and day 31**

Tissue	Animal no.	Day 28							Day 31						
		Flutolanil	M-2	M-2 conjugates	M-4	M4 conjugates	M-7	M101	Flutolanil	M-2	M-2 conjugates	M-4	M-4 conjugates	M-7	M101
0 mg/kg in diet	1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01							
	2	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01							
	3	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01							
3 mg/kg in diet	4	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01							
	5	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01							
	6	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01							
30 mg/kg in diet	7	<0.01	<0.01	<0.01	<0.01	0.034	<0.01	<0.01							
	8	<0.01	<0.01	<0.01	<0.01	0.043	<0.01	<0.01							
	9	<0.01	<0.01	<0.01	<0.01	0.017	<0.01	<0.01							
90 mg/kg in diet	10	<0.01	<0.01	<0.01	<0.01	0.060	<0.01	<0.01							
	11	<0.01	<0.01	<0.01	<0.01	0.037	<0.01	<0.01							
	12	<0.01	<0.01	<0.01	<0.01	0.073	<0.01	<0.01							
300 mg/kg in diet	13	<0.01	<0.01	0.027	<0.01	0.160	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.016	<0.01	<0.01
	14	<0.01	<0.01	0.021	<0.01	0.119	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15	<0.01	<0.01	0.018	<0.01	0.079	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	16	<0.01	<0.01	0.019	<0.01	0.276	<0.01	<0.01							
	17	<0.01	<0.01	0.035	<0.01	0.216	<0.01	<0.01							
	18	<0.01	<0.01	0.030	<0.01	0.307	<0.01	<0.01							

Table B.7.4.2-2**g: Summary of residues of flutolanil and metabolites in skimmed milk and milk fat at day 21**

Tissue	Animal no.	Skimmed milk							Milk fat						
		Flutolanil	M-2	M2 conjugates	M-4	M-4 conjugates	M-7	M101	Flutolanil	M-2	M-2 conjugates	M-4	M-4 conjugates	M-7	M101
0 mg/kg in diet	1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	2	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	3	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
3 mg/kg in diet	4	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	5	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	6	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
30 mg/kg in diet	7	<0.01	<0.01	<0.01	<0.01	0.016	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.013	<0.01	<0.01
	8	<0.01	<0.01	<0.01	<0.01	0.018	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.012	<0.01	<0.01
	9	<0.01	<0.01	<0.01	<0.01	0.013	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
90 mg/kg in diet	10	<0.01	<0.01	<0.01	0.011	0.086	<0.01	<0.01	0.014	<0.01	<0.01	<0.01	0.039	<0.01	<0.01
	11	<0.01	<0.01	0.013	<0.01	0.025	<0.01	<0.01	0.018	0.016	<0.01	<0.01	0.014	<0.01	<0.01
	12	<0.01	<0.01	<0.01	<0.01	0.035	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.032	<0.01	<0.01
300 mg/kg in diet	13	<0.01	<0.01	0.033	0.011	0.326	<0.01	<0.01	0.046	0.038	0.016	0.016	0.125	<0.01	<0.01
	14	<0.01	<0.01	0.019	<0.01	0.075	<0.01	<0.01	0.019	0.025	0.011	<0.01	0.051	<0.01	<0.01
	15	<0.01	<0.01	0.020	<0.01	0.096	<0.01	<0.01	0.048	0.029	0.013	<0.01	0.055	<0.01	<0.01
	16	<0.01	<0.01	0.012	<0.01	0.141	<0.01	<0.01	0.057	0.038	0.012	<0.01	0.101	<0.01	<0.01
	17	<0.01	<0.01	0.017	<0.01	0.134	<0.01	<0.01	0.042	0.028	0.010	0.013	0.086	<0.01	<0.01
	18	<0.01	<0.01	0.011	<0.01	0.275	<0.01	<0.01	0.038	0.037	0.011	0.012	0.108	<0.01	<0.01

Table B.7.4.2-2**h** **Recovery of the original analytical method during analysis of the cow tissue samples**

Analyte	Fortification	Liver	Kidney	Muscle		Omental Fat		Subcutaneous fat	Perirenal fat
	Mg/kg	Individual values	values	Mean (Individual values)	RSD	Mean (Individual values)	RSD	Individual values	Individual values
Flutolanil	0.01	81, 96	89, 95	106 (99, 113, 107)	7	106 (89, 119, 109)	15	103,103	100, 98
	0.1	95, 103	92, 106	90 (81, 85, 105)	13	98 (96, 98, 99)	2	97, 108	91, 98
M-2	0.01	104, 111	84, 108	102 (103, 106, 98)	4	102 (88, 104, 114)	13	110, 112	102, 103
	0.1	100, 110	96, 100	87 (81, 82, 98)	10	97 (95, 93, 102)	5	93, 107	93, 100
M-2 (conjugates)	0.01	99, 104	109, 115	86 (76, 88, 94)	9	-	-	-	-
	0.1	98, 101	100, 102	81 (69, 73, 102)	18	-	-	-	-
M-4	0.01	91, 106	83, 94	96 (90, 84, 115)	16	100 (98, 99, 103)	3	97, 63**	104, 90
	0.1	103, 105	92, 104	90 (82, 90, 98)	8	103 (110, 100, 98)	6	93, 108	89, 104
M-4 (conjugates)	0.01	101, 95	89, 91	85 (76, 89, 91)	5	-	-	-	-
	0.1	92, 75	92, 77	80 (66, 70, 104)	21*	-	-	-	-
M-7	0.01	106, 106	88, 102	105 (100, 102, 112)	6	95 (82, 95, 107)	13	103, 103	100, 95
	0.1	99, 107	101, 106	88 (75, 92, 97)	12	96 (93, 96, 100)	4	91, 101	93, 104
M-101	0.01	106, 104	97, 93	89 (77, 97, 93)	11	102 (93, 107, 105)	8	89, 94	76, 87
	0.1	108, 108	95, 104	96 (93, 93, 102)	5	96 (88, 101, 100)	7	88, 86	84, 85

* RSD exceeding 20%

** value below 70%, however, mean value of 2 recoveries (97, 63) = 80

Table B.7.4.2-2i **Recovery of the original analytical method during analysis of the cow milk samples**

Analyte	Fortification	Milk D-1, D1	Milk D4, D7	Milk D10, D13	Milk D16	Milk D19	Milk D22	Milk D25	Milk D28	Milk D31, D34	Skimmed milk D21	Milk fat D21
	Mg/kg	Individual	Individual	Individual	Individual	Individual	Individual	Individual	Individual	Individual	Individual	Individual
Flutolanil	0.01	102, 89	83, 100	69, 78	88, 105	75	95, 110	79, 81	84, 112	-	102, 120**	119
	0.1	81, 86	101, 91	61, 73*	91, 92	76, 80	106, 103	89, 92	85, 95	96	91, 103	98, 108
M-2	0.01	101, 90	94, 93	71, 116	104, 89	85, 90	77, 120**	74, 81	83, 111	102	92, 90	75, 87
	0.1	75, 76	106, 90	92, 110	91, 101	93, 94	116, 110	89, 92	94, 106	94	98, 102	90, 109
M-2 (conjugates)	0.01	106, 92	99, 104	86, 115	105, 88	89, 93	84, 98	88, 89	73, 81	116	102, 88	99, 106
	0.1	96, 100	115, 96	86, 106	89, 84	89, 99	100, 101	71, 81	97, 102	114	89, 79	79, 105
M-4	0.01	94, 82	88, 103	79, 84	84, 83	79, 105	100, 113	86, 89	88, 115	113	94, 109	101, 110
	0.1	83, 92	105, 102	84, 86	91, 100	90, 102	110, 109	91, 88	93, 101	102	88, 101	91, 98
M-4 (conjugates)	0.01	85, 98	81, 96	66, 108	72, 87	78, 83	77, 105	83, 96	90, 102	116	84, 97	85, 90
	0.1	77, 94	104, 94	66, 80	80, 70	81, 89	100, 103	62	85, 95	113	70, 62*	83, 100
M-7	0.01	107, 109	106, 116**	96, 120**	97, 107	74, 92	82, 115**	97, 93	94, 96	107	74, 92	104, 107
	0.1	95, 101	113	120	88, 108	91, 99	107, 110	94, 96	96, 99	105	95, 99	95, 107
M-101	0.01	87, 85	78, 85	77, 86	75, 84	75, 83	87, 92	69, 86	82, 93	90	68, 91	75, 91
	0.1	76, 80	79, 93	82, 86	75, 82	71, 83	93, 91	80, 74	82, 88	110	90, 76	93, 94

* Recovery mean value (n=2) below 70%

** Recovery of 1 sample above 110%

Milk

Flutolanil derived residues in whole milk, cream and skimmed milk were measured as the parent compound and its metabolites M-2/conjugated M-2, M-4/conjugated M-4 and M-7 and M-101.

Whole milk:

No residues of Flutolanil or its metabolites were detected above the limit of detection in any samples from control animals (Group 1) apart from a single occasion on Day 7, where the concentration of the metabolites, conjugated M-4 in two animals, and conjugated M-2 in one of these animals, was below the limit of quantitation (0.01 mg/kg).

No Flutolanil-derived residues above the limit of quantitation were detected in samples from animals in Group 2.

There were only a few occasions of low but quantifiable residues of the parent compound Flutolanil on Days 4, 7, and 19 in Group 5 (mean values of 0.010, 0.011 and 0.011 mg/kg, respectively. Individual residues occurred primarily as the metabolites conjugated M-2 in Group 5 only (maximum mean value of 0.035 mg/kg) and conjugated M-4 (maximum mean values of 0.032, 0.081 and 0.196 mg/kg for Groups 3, 4 and 5 respectively). No quantifiable residues of the metabolites M-7 and M-101 were found in any whole milk samples analysed, with the exception of a low but quantifiable residue of M-101 on Days 22 and 28 in a single animal in Group 5.

Residues in whole milk samples were no longer detectable by seven days after withdrawal of treatment.

Skimmed milk:

No Flutolanil-derived residues were detected in any skimmed milk samples from the control animals (Group 1) and those in Group 2.

Individuals residues occurred primarily as the metabolites conjugated M-2 (mean values of <LOQ, 0.011 and 0.019 mg/kg for Groups 3, 4 and 5 respectively) and conjugated M-4 (mean values of 0.016, 0.049 and 0.175 mg/kg for Groups 3, 4 and 5 respectively). No quantifiable residues of Flutolanil or the metabolites M-7 and M-101 were found in any skimmed milk samples analysed.

Cream:

No Flutolanil-derived residues were detected in any cream samples from the control animals (Group 1) and those in Group 2.

. Individuals residues occurred primarily as the parent compound Flutolanil (mean values of <LOQ, 0.014 and 0.042 mg/kg for Groups 3, 4 and 5 respectively) and the metabolites M-2 (mean values of <LOQ, 0.012,and 0.033 mg/kg for Groups 3, 4 and 5 respectively) and conjugated M-4 (mean values of 0.012, 0.028 and 0.088 mg/kg for Groups 3, 4 and 5 respectively). No quantifiable residues of the metabolites M-7 or M-101 were found in any cream samples analysed.

Tissues

Flutolanil-derived residues were measured as the parent compound Flutolanil and its significant metabolites M-2 (and conjugated M-2 for kidney, liver and muscle), M-4 (and conjugated M-4 for kidney, liver and muscle), M-7 and M-101.

No Flutolanil-derived residues above the limit of quantitation were found in any tissue samples from control animals (Group 1).

In treated animals, the highest overall Flutolanil-derived residues occurred in the kidney; lower residues were found in liver, omental, subcutaneous and perirenal fat, and the lowest residue concentrations occurred in muscle.

A summary of the individual and group mean concentrations expressed as Flutolanil equivalents in tissues is presented in Table B.7.4.2-3a-d.

Liver:

Individual residues occurred predominantly as Flutolanil (mean values of <0.01, 0.015, 0.023 and 0.095 mg/kg for Groups 2, 3, 4 and 5 respectively) and the metabolites M-2/ conjugated M-2 (mean values of <LOQ/<LOQ, 0.024/0.123, 0.090/0.458 and 0.418/1.823 mg/kg for Groups 2, 3, 4 and 5 respectively), M-4/conjugated M-4 (mean values of <LOQ/<LOQ, <LOQ/0.023, 0.014/0.064 and 0.033/0.147 mg/kg for Groups 2, 3, 4 and 5 respectively) and M-101 (mean values of <LOQ, 0.019, 0.032 and 0.072 mg/kg for Groups 2, 3, 4 and 5 respectively). The magnitude of the residues of all three metabolites was greater than that of the parent, Flutolanil (up to 21x in the case of conjugated M-2). No quantifiable residues of the metabolite M-7 were found in any liver samples analysed.

In the depuration animals, there was still a low, but quantifiable residue of M-101 at 14 days withdrawal (results not tabulated but can be found in the study report).

Kidney:

Individual residues occurred predominantly as the metabolites M-2/conjugated M-2 and M-4/conjugated M-4 (mean values of <LOQ/0.040, 0.047/0.592, 0.026/1.033 and 0.084/2.570 mg/kg for Groups 2, 3, 4 and 5 respectively for M-2/conjugated M-2 and <LOQ/0.016, 0.020/0.257, 0.019/0.426 and 0.070/1.390 mg/kg for Groups 2, 3, 4 and 5 respectively for M-4/conjugated M-4). No quantifiable residues of Flutolanil or the metabolites M-7 and M-101 were found in any kidney samples analysed.

Results for Cows 13, 14 and 15, included for depuration data, indicated depletion of residues in kidney after cessation of dosing, with no quantifiable residues present at 7 days withdrawal (results not tabulated but can be found in the study report).

Muscle:

Individual residues occurred predominantly as the metabolite conjugated M-2 (mean values of <LOQ, <LOQ, <LOQ and 0.013 mg/kg for Groups 2, 3, 4 and 5 respectively). In addition, there was a single low but quantifiable residue of conjugated M-4 in one animal from Group 5 (0.012 mg/kg). No quantifiable residues of Flutolanil or the metabolites M-7 and M-101 were found in any muscle samples analysed.

In the depuration animals, no quantifiable residues were detectable after 4 days withdrawal with none detected after 7 days withdrawal (results not tabulated but can be found in the study report).

Omental fat:
Individual residues occurred as the parent compound Flutolanil (mean values of <LOQ, 0.014, 0.019 and 0.071 mg/kg for Groups 2, 3, 4 and 5 respectively) and as the metabolite M-2 (mean values of <LOQ, 0.011, 0.015 and 0.059 for Groups 2, 3, 4 and 5 respectively). In addition, there was a single low but quantifiable residue of M-4 in one animal from Group 5 (0.011 mg/kg). No quantifiable residues of the metabolites M-7 or M-101 were found in any omental fat samples analysed.
In the depuration animals, quantifiable residues were present after 4 days withdrawal, but were less than the limit of quantitation by 7 days withdrawal (results not tabulated but can be found in the study report).
Subcutaneous fat:
Individual residues occurred as the parent compound Flutolanil in Group 5 only (mean value of 0.016 mg/kg) and as the metabolite M-2 in Groups 4 and 5 (mean values of 0.014 and 0.021 respectively). No quantifiable residues of the metabolites M-7 or M-101 were found in any subcutaneous fat samples analysed. In the depuration animals, quantifiable residues were present after 4 days withdrawal (0.011 mg/kg M-4 in 1 animal), but were less than the limit of quantitation by 7 days withdrawal.

Perirenal fat:
Individual residues occurred as the parent compound Flutolanil in Group 5 only (mean value of 0.024 mg/kg) and as the metabolite M-2 in Groups 4 and 5 (mean values of 0.011 and 0.017 respectively). There was also an anomalous finding in Group 3, whereby quantifiable residues of M-2 and M-4 were detected in one animal; these results are excluded for MRL setting and risk assessment. No quantifiable residues of the metabolites M-4, M-7 or M-101 were found in any other perirenal fat samples analysed.
In the depuration animals, quantifiable residues were not detectable by 4 days withdrawal.

Table B.7.4.2-3a: Residues of flutolanil and its metabolites in livestock tissues (as mg/kg) for cows dosed at 3 mg/kg in diet, equivalent to 0.120 mg/kg bw/d (Group 2)

Tissue	Cow No.	Flutolanil	M-2	M-2 conj. [a]	M-4	M-4 conj. [a]	M-7	M-101
Kidney	4	ND	ND	0.054	ND	0.015	ND	ND
	5	ND	ND	0.039	ND	0.019	ND	ND
	6	ND	ND	0.028	ND	0.013	ND	ND
Liver	4	ND	<LOQ	<LOQ	ND	<LOQ	ND	ND
	5	ND	<LOQ	0.010	ND	<LOQ	ND	ND
	6	ND	<LOQ	<LOQ	ND	<LOQ	ND	ND
Muscle	4	ND	ND	ND	ND	ND	ND	ND
	5	ND	ND	ND	ND	ND	ND	ND
	6	ND	ND	ND	ND	ND	ND	ND
Subcutaneous Fat	4	ND	ND	ND	ND	ND	ND	ND
	5	ND	ND	ND	ND	ND	ND	ND
	6	ND	ND	ND	ND	ND	ND	ND
Omental Fat	4	ND	ND	ND	ND	ND	ND	ND
	5	ND	ND	ND	ND	ND	ND	ND
	6	ND	ND	ND	ND	ND	ND	ND
Perirenal Fat	4	ND	ND	ND	ND	ND	ND	ND
	5	ND	ND	ND	ND	ND	ND	ND
	6	ND	ND	ND	ND	ND	ND	ND

ND = None detected (less than the LOD of 0.002 mg/kg)
LOQ = 0.01 mg/kg
[a] results corrected for un-conjugated residues (for calculation purposes, <LOQ was assigned a value of 0.01 mg/kg)

Table B.7.4.2-3b: Residues of flutolanil and its metabolites in livestock tissues (as mg/kg) for cows dosed at 30 mg/kg in diet, equivalent to 1.186 mg/kg bw/d (Group 3)

Tissue	Cow No.	Flutolanil	M-2	M-2 conj. [a]	M-4	M-4 conj. [a]	M-7	M-101
Kidney	7	<0.01	0.087	0.510	0.030	0.262	ND	ND
	8	<0.01	0.038	0.657	0.019	0.258	ND	ND
	9	<0.01	0.017	0.610	0.010	0.252	ND	ND
Liver	7	0.014	0.015	0.134	<0.01	0.023	ND	0.013
	8	0.020	0.030	0.124	<0.01	0.034	ND	0.013
	9	<0.01	0.028	0.110	<0.01	0.012	ND	0.030
Muscle	7	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	9	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Subcutaneous Fat	7	<0.01	<0.01	-	<0.01	-	<0.01	<0.01
	8	<0.01	<0.01	-	<0.01	-	<0.01	<0.01
	9	<0.01	<0.01	-	<0.01	-	<0.01	<0.01
Omental Fat	7	0.021	0.014	-	<0.01	-	<0.01	<0.01
	8	<0.01	<0.01	-	<0.01	-	<0.01	<0.01
	9	<0.01	<0.01	-	<0.01	-	<0.01	<0.01
Perirenal Fat	7	<0.01	0.195*	-	0.062*	-	<0.01	<0.01
	8	<0.01	<0.01	-	<0.01	-	<0.01	<0.01
	9	<0.01	<0.01	-	<0.01	-	<0.01	<0.01

[a] results confirmed by reanalysis of extract (highest value)

Table B.7.4.2-3c: Residues of flutolanil and its metabolites in livestock tissues (as mg/kg) for cows dosed at 90 mg/kg in diet, equivalent to 3.37 mg/kg bw/d (Group 4)

Tissue	Cow No.	Flutolanil	M-2	M-2 conj. [a]	M-4	M-4 conj. [a]	M-7	M-101
Kidney	10	<0.01	0.031	1.06	0.020	0.422	<0.01	<0.01
	11	<0.01	0.028	1.03	0.013	0.260	<0.01	<0.01
	12	<0.01	0.020	0.92	0.024	0.595	<0.01	<0.01
Liver	10	0.034	0.159	0.575	0.019	0.084	ND	0.019
	11	<0.01	0.048	0.483	<0.01	0.040	ND	0.062
	12	0.026	0.062	0.316	0.012	0.067	ND	0.015
Muscle	10	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	11	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	12	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Subcutaneous Fat	10	<0.01	0.013	-	<0.01	-	<0.01	<0.01
	11	<0.01	0.018	-	<0.01	-	<0.01	<0.01
	12	<0.01	0.010	-	<0.01	-	<0.01	<0.01
Omental Fat	10	0.014	0.017	-	<0.01	-	<0.01	<0.01
	11	0.033	0.018	-	<0.01	-	<0.01	<0.01
	12	<0.01	0.010	-	<0.01	-	<0.01	<0.01
Perirenal Fat	10	<0.01	<0.01	-	<0.01	-	<0.01	<0.01
	11	<0.01	0.012	-	<0.01	-	<0.01	<0.01
	12	<0.01	<0.01	-	<0.01	-	<0.01	<0.01

Table B.7.4.2-3d: Residues of flutolanil and its metabolites in livestock tissues (as mg/kg) for cows dosed at 300 mg/kg in diet, equivalent to 11.95 mg/kg bw/d (Group 5)

Tissue	Cow No.	Flutolanil	M-2	M-2 conj. [a]	M-4	M-4 conj. [a]	M-7	M-101
Kidney	16	<0.01	0.103	3.29	0.096	1.85	<0.01	<0.01
	17	<0.01	0.104	2.92	0.086	1.56	<0.01	<0.01
	18	<0.01	0.044	1.50	0.027	0.761	<0.01	<0.01
	13*	<0.01	<0.01	0.029	<0.01	0.017	<0.01	<0.01
	14*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Liver	16	0.110	0.406	2.10	0.034	0.157	<0.01	0.110
	17	0.107	0.528	1.51	0.041	0.131	<0.01	0.029
	18	0.058	0.321	1.86	0.024	0.154	<0.01	0.077
	13*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.081
	14*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.052
	15*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.017
Muscle	16	<0.01	<0.01	0.012	<0.01	<0.01	<0.01	<0.01
	17	<0.01	<0.01	0.018	<0.01	0.012	<0.01	<0.01
	18	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	13*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	14*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Subcutaneous Fat	16	<0.01	0.023	-	<0.01	-	<0.01	<0.01
	17	0.028	0.019	-	<0.01	-	<0.01	<0.01
	18	0.012	0.021	-	<0.01	-	<0.01	<0.01
	13*	0.011	0.04	-	0.011	-	<0.01	<0.01
	14*	<0.01	<0.01	-	<0.01	-	<0.01	<0.01
	15*	<0.01	<0.01	-	<0.01	-	<0.01	<0.01
Omental Fat	16	<0.01	0.065	-	<0.01	-	<0.01	<0.01
	17	<0.01	0.023	-	<0.01	-	<0.01	<0.01
	18	<0.01	0.089	-	<0.01	-	<0.01	<0.01
	13*	<0.01	0.025	-	0.011	-	<0.01	<0.01
	14*	<0.01	<0.01	-	<0.01	-	<0.01	<0.01
	15*	<0.01	<0.01	-	<0.01	-	<0.01	<0.01
Perirenal Fat	16	0.021	0.017	-	<0.01	-	<0.01	<0.01
	17	0.032	<0.01	-	<0.01	-	<0.01	<0.01
	18	0.019	0.014	-	<0.01	-	<0.01	<0.01
	13*	<0.01	<0.01	-	<0.01	-	<0.01	<0.01
	14*	<0.01	<0.01	-	<0.01	-	<0.01	<0.01
	15*	<0.01	<0.01	-	<0.01	-	<0.01	<0.01

* Cow 13: withdrawal time 4 days, cow 14: withdrawal time 7 days, cow 14: withdrawal time 14 days

III. CONCLUSIONS

The study is acceptable with regard to number of animals, dose groups, performance of the analytical method and maximal storage period of analytical samples.

B.7.4.3 Pigs

No feeding study in pigs is necessary since the metabolic pathways in rat and livestock are similar. Residue levels in pig tissues can thus be anticipated based on the available residue data generated in cattle.

B.7.4.4 Fish

No metabolism study in fish has been submitted for the renewal process. Potato protein can be used as a part of fish diet. Flutolanil has been recovered in fat tissue in poultry (0.127 mg eq./kg) and in goat (0.012-0.043 mg equi./kg), which suggest that flutolanil is fat soluble and can be recovered in animal tissues. Further investigation of metabolism of flutolanil in fish might be required, when agreed EU methodology will be available to fully address this data requirement .

B.7.5 Effects of processing

B.7.5.1 Nature of the residue

Previous evaluation	Submitted for the purpouse of the renewal
RMS remark	Acceptable

Report:	**CA 6.5.1/01. O'Connell., C & Pratt., E, (2015)**
Title:	[^{14}C]-Flutolanil: High Temperature Hydrolysis
Document No.:	XG/15/020
Guidelines:	OECD Guideline 507, Guideline 7035/VI/95 Revision 5, Appendix E
Testing Laboratory	Battelle UK Ltd., Chelmsford, Essex, UK.
GLP:	Yes

Executive summary

The hydrolytic degradation of flutolanil was investigated at elevated temperatures of:

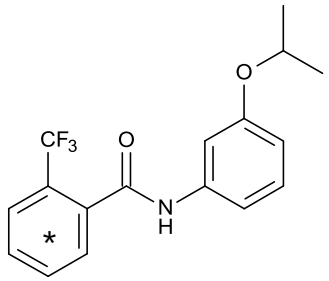
- 90°C and pH 4 buffer solution for 20 minutes, simulating pasteurisation.
- 100°C and pH 5 buffer solution for 60 minutes, simulating baking, brewing and boiling.
- 120°C and pH 6 buffer solution for 20 minutes, simulating sterilisation.

The nominal concentration was 0.5 mg/L. The concentration of acetonitrile as a co-solvent was <1 %. The total mean recovery of radioactivity was in the range 101.9 – 106.7 %. Flutolanil was found to be stable at each pH and temperature.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material: [Phenyl-U-^{14}C]-flutolanil



* Denotes position of [^{14}C]-radiolabel

Chemical name (IUPAC)	α, α, α-trifluoro-3'-isopropoxy-*o*-toluanilide
CA registry number:	66332-96-5
Lot or batch number:	2747W
Specific activity:	13.3 MBq/mg
Radiochemical purity:	>99.0
Stability of test compound:	Shown to be stable under the conditions of the test
Application vehicle:	Acetonitrile

B. STUDY DESIGN AND METHODS

1. In-life dates:

30 April 2015 – 22 June 2015

2. Test System

This study was performed in 0.05M pH 4, 5 and 6 acetate buffer. Ammonium acetate was dissolved in de-ionised water and portions of this buffer were then adjusted to pH 4, 5 or 6 by the addition of acetic acid. All buffers were sterilised by filtration using 0.22 µm sterile filter.

Experimental design

Parameter		**Description**
Duration of test	Pasteurisation	20 minutes
	Baking, Brewing, Boiling	60 minutes
	Sterilisation	20 minutes
Test concentration (Nominal)		0.5 mg/L
Number of replicates		Six treated with [^{14}C]test-item (two zero-time, two heated, two ambient controls) One treated with non-labelled test item to monitor pH Two with untreated buffer (to monitor temperature and sterility)
Preparation of test medium	Volume of treatment solution per vessel	5 mL
	Test medium	0.05 M Ammonium acetate buffer solution
Test material application	Co-solvent	Acetonitrile, final concentration <1.0% (v/v)
	Volume of test solution	0.025 mL of stock solution into 5 mL buffer at each pH

	Application method		Pipette
Test apparatus			8 mL amber glass tubes
Traps for CO_2 and organic volatiles			None
Is there any indication of the test material absorbing to the walls of the test apparatus			No
Experimental conditions	Pasteurisation	Temperature	90°C ± 3.0°C
		Lighting	Dark
		pH	4
	Baking, Brewing, Boiling	Temperature	100°C ± 3.0°C
		Lighting	Dark
		pH	5
	Sterilisation	Temperature	120°C ± 0.5°C in an autoclave
		Lighting	Dark
		pH	6

Sampling

Parameter	**Details**
Sampling intervals for the parent/transformation products	At end of test
Sampling procedure	Entire vessel
Measurements at sampling: pH measurement	Prior to, and on completion of test
Sample storage before analysis	Ambient temperature, <12 hours
Other observations	None

The processes at pH 4 and pH 5 were stopped by cooling the samples at *ca* 4°C for approximately 15 minutes. For the pH 6 process, which was conducted at 120°C in an autoclave, access to the autoclave chamber was only possible once safe handling conditions had been reached (as determined by temperature and pressure safety features, which took approximately 30 minutes). Samples were then cooled further at *ca* 4°C for approximately 15 minutes.

Description of analytical procedures

The pH value of each buffer solution was measured using a pH meter prior to use. The pH values for the dosed controls (treated with unlabelled flutolanil) were measured after processing and cooling to ambient temperature and were within the acceptable limits.

Aqueous samples were radioassayed using LSC and analysed by HPLC (co-chromatography with unlabelled compounds to determine the levels of parent and significant degradates in each sample. The presence of flutolanil in selected samples was confirmed by LC/MS.

II. RESULTS AND DISCUSSION

A. Mass balance:

The mean recovery of applied radioactivity was in the range of 101.8 – 106.7% for both treated and control samples (Table B.7.5.1-1).

B. Findings:

Following incubation under each set of hydrolysis conditions, analysis of the buffer solutions showed the major component present to be flutolanil, accounting for 101.9% AR (0.52 mg/L), 106.7% AR (0.54 mg/L) and 102.4% AR (0.54 mg/L) under pasteurisation, baking/brewing/boiling and sterilisation conditions respectively. One minor component accounting for 0.1% AR (0.001 mg/L) was observed in one replicate incubated under conditions simulating sterilisation. No further components were observed.
In the zero-time and 20 minute ambient control samples, Flutolanil accounted for 101.2–105.1% AR (0.52–0.54 mg/L). No further components were observed. (Table B.7.5.1-2).

Table B.7.5.1-1: Recovery of radioactivity (results expressed as % applied radioactivity)

Process	System	Vessel ID	% Recovery
Pasteurisation	T0	D1	100.0
		D2	102.3
		Mean	**101.2**
	Heated	D3	103.1
		D4	100.6
		Mean	**101.9**
	Control	D5	105.5
		D6	104.6
		Mean	**105.1**
Baking, Brewing, Boiling	T0	D10	104.8
		D11	104.0
		Mean	**104.4**
	Heated	D12	106.0
		D13	107.4
		Mean	**106.7**
	Control	D14	104.3
		D15	104.0
		Mean	**104.2**
Sterilisation	T0	D19	101.8
		D20	101.8
		Mean	**101.8**
	Heated	D21	103.1
		D22	101.9
		Mean	**102.5**
	Control	D23	103.2
		D24	102.9
		Mean	**103.1**

Table B.7.5.1-2: Proportions of radioactive components (results expressed as % applied radioactivity)

Components	% Applied Radioactivity								
	Control (T0)			Heated			Control (ambient)		
	D1	D2	Mean	D3	D4	Mean	D5	D6	Mean
Pasteurisation									
Flutolanil	100.0	102.3	101.2	103.1	100.6	101.9	105.5	104.6	105.1
Degradate 1	ND	ND	ND	ND	ND	ND	ND	ND	ND
Total Recovery	100.0	102.3	101.2	103.1	100.6	101.9	105.5	104.6	105.1
Baking, brewing, boiling									
	D10	D11	Mean	D12	D13	Mean	D14	D15	Mean
Flutolanil	104.8	104.0	104.4	106.0	107.4	106.7	104.3	104.0	104.2
Degradate 1	ND	ND	ND	ND	ND	ND	ND	ND	ND
Total Recovery	104.8	104.0	104.4	106.0	107.4	106.7	104.3	104.0	104.2
Sterilisation									
	D19	D20	Mean	D21	D22	Mean	D23	D24	Mean
Flutolanil	101.8	101.8	101.8	102.8	101.9	102.4	103.2	102.9	103.1
Degradate 1	ND	ND	ND	0.3	ND	0.1	ND	ND	ND
Total Recovery	101.8	101.8	101.8	103.1	101.9	102.5	103.2	102.9	103.1

ND = Not detected

III. CONCLUSIONS

The potential of flutolanil to undergo hydrolysis, under conditions simulating pasteurisation, baking, brewing and boiling and sterilisation, was studied at a nominal concentration of 0.5 mg L^{-1}. Radioactive recoveries of all samples were acceptable (range 100.0–107.4% AR). Flutolanil was stable under all processing conditions and was the only component detected during HPLC analysis of the study solutions (100.0–107.4% AR), with the exception of one minor component equivalent to 0.3% AR, which was observed in one replicate incubated under conditions simulating sterilisation.

B.7.5.2 Distribution of the residue in peel and pulp

Studies investigating distribution of the residues in peel and pulp are not relevant for the defended uses since potatoe is not separated this way. However, one study investigating distribution of residues in potato, peeled potato and peel has been conducted in terms of processing studies.

B.7.5.2.1 Residues in potato tuber, peel and peeled potatoes

Previous evaluation	in the DAR
RMS remark	Acceptable

Report: Souvignet, I. (1999): Flutolanil: Formulation EXP10066A or RPA10066F (FS). Trials Germany 1997. Residues in potato. Decline study. Rhône-Poulenc Agro, Lyon, France, Unpublished report No.: R-3058.

Guidelines: BBA-guideline part IV, 3-1, 3.3, 3.-3.1.1 and 3-8

GLP: Yes

Material and methods

Residue trials were conducted in two different locations in Germany. A FS-formulation containing 449 g/l flutolanil was applied on potato seeds at a rate of 131.5 or 117.6 g as/t potatoes (Ref. R-3058). The first sampling took place as soon as the tubers were available. The second sampling was carried out between the first sampling date and harvest time. The third sampling was conducted at normal harvest time.

The collected samples were subjected for direct analysis of the active ingredient flutolanil in potatoes. Additionally, samples collected at harvest time (3 rd sampling) were processed before analysis. Samples were processed by separating skin and tuber as usual in household. All potato samples were homogenised and subjected for a series of extraction procedures involving acetone, saturated aqueous solution of NaCl and petroleum-ether. The petroleum-ether phase was then dehydrated with Na_2SO_4 and samples were concentrated by using solid phase extraction (aluminium oxide column, diethyl ether eluent). The diethylether was evaporated and the residue was redissolved in methanol. GC/MS method was then employed with a LOQ of 0.010 mg/kg.

Findings

Summary of the results is shown in Table B.7.5.2.1-1. All samples obtained from one trial site (Grossenaspe) contained flutolanil at levels below the LOQ (0.010 mg/kg). In peel sample from another site (Bad Sooden-Allendorf) flutolanil was detected at 0.035 mg/kg, but in potato and peeled potato samples the levels were below the LOQ.

Table B.7.5.2.1-1 Residues of flutolanil in potato, peeled potato and peel

Location,Year	Reference	Application					PHI	Flutolanil residue, mg/kg		
		Form	No	Method	Rate (g as/t)	Conc. (kg/hL)	(days)	Tuber	Peeled potato	Peel
Grossenaspe, Germany,1997	R-3058/ 97747DGR1	FS	1	Seed tuber	131.5	44.9	78	< 0.010	NR	NR
							105	< 0.010	NR	NR
							140	< 0.010	< 0.010	< 0.010
Bad Sooden-Allendorf, Germany,1997	R-3058/ 97747DAS1	FS	1	Seed tuber	117.6	44.9	76	0.025	NR	NR
							99	0.020	NR	NR
							128	< 0.010	< 0.010	0.035

FS = Flowable concentrate for seed treatment, NR = Not Reported

Conclusions

Processing studies were not required as no significant (> 0.1 mg/kg) residues were found in the raw commodity and the TMDI was clearly less than 10 % of the ADI. However, a study investigating distribution of residues in potato, peeled potato and peel has been submitted. According to this study peel is the main part of translocation of flutolanil in potato. No detectable residues were found in potato tuber and peeled potato.

B.7.5.3 Magnitude of residues in processed commodities

No processing studies were conducted with potato treated with flutolanil.

Total residues of flutolanil and metabolite M-4, expressed as flutolanil, after potatoes seed treatment were in Europe up to 0.1 mg/kg. According to the current OECD Guidance No 508, processing studies are triggered. On the other hand, estimated TMDI is lower than 10% of the ADI. Hence, no processing studies are further required.

B.7.6 Residues in rotational crops

B.7.6.1 Metabolism in rotational crops

B.7.6.1.1 Study 1

Previous evaluation	in the DAR
RMS remark	Acceptable

Report: Downey, S.S. (1992): Uptake of [^{14}C]-Flutolanil residues in soil by rotational crops under confined conditions, NOR-AM Chemical Company, North Carolina, USA, Unpublished report No.: Aventis ref: B002405. E-3021.

Guideline: Studies conducted in three sites having site-specific protocols. Directive 96/68EC, Document 7028/VI/95, USA EPA/1996, Japanese MAFF/1985.

GLP: Study was started prior to USA EPA/1989 (FIFRA). Field-trials were conducted using separate site specific protocols and thus multiple study directors. Residue analyses were carried out in two different laboratories.

Test formulation: Equivalent to Moncut 50WP.

Radioactive probe: [Aniline ring -14C(U)] flutolanil; specific activity 69.1 µCi/mg, radiochemical purity ≥ 98%, diluted to 10 µCi/mg for application.

Test site: Outdoor facility at Southern Agricultural Research Inc., Donalsonville, Georgia, USA.

Soil type: Sandy loam soil.

Material and methods

A study was conducted to provide information of the extent of any accumulation into rotational crops after various aging periods in soil. A field plot of sandy loam soil was treated in 1988 with [aniline ring-U-14C] labelled flutolanil at a rate of 2.69 kg/ha (2.4 lb/A). Soil samples were taken from different plots at 2-hours, and at 30, 91, 120 and 186 days from treatment corresponding to significant time points such as treatment, planting, harvest.

The soil samples were divided into two fractions representing 0-15 cm (0-6") and 15-30 cm (6-12") parts before being combusted to determine TRR. Extraction and Fracination of Residues
Samples of homogenised plant samples were sequentially extracted by Waring blender. Samples were extracted using the following sequence of solvents:

Acetonitrile, 3 times
Acetonitrile : water (1:1, v/v), 3 times

Following each extraction, the extract was separated from the plant residue by vacuum filtration. The plant material was finally extracted with water in a Soxhlet apparatus for approximately 18 hours. Radioactivity present in extracts was quantified by liquid scintillation counting (LSC) and the radioactive residues in the post extraction solids (PES) were quantified by combustion.

Acetonitrile, acetonitrile/water and water extracts were combined, concentrated and partitioned with dichloromethane at neutral, acidic and basic conditions (pH 7, pH 2 and pH 9). The dichloromethane phases were combined and concentrated for HPLC analysis. The aqueous residue was subject to the following hydrolysis step prior to partitioning with ethyl acetate under unchanged, neutral and basic conditions to yield the post-hydrolysis organic phases (which were concentrated for analysis by HPLC) and a residual aqueous phase.

Incubation in 2M H_2SO_4 for 24 hours at 50°C

Soil samples were extracted with one of two similar methods. Samples of homogenised soil cores were vigorously shaken for 2 minutes with either methanol : water (4:1, v/v), 5 times or shaken for 30 minutes with acetone : water (4:1, v/v), 3 times. Soil residues were then extracted with 2N NaOH : methanol (3:1, v/v) overnight. All extracts were partitioned with dichloromethane. Radioactivity present in extracts was quantified by LSC and the radioactive residues in the PES quantified by combustion.

Dichloromethane extracts were evaporated to dryness and reconstituted in acetonitrile prior to analysis by HPLC.
Major metabolites were confirmed by isolating radioactive HPLC peaks from selected samples and confirming their identity by TLC analysis.

Residue levels were also compared with those obtained by a multi-residue method in which flutolanil related metabolites are converted to trifluoromethyl benzoic acid and analysed by GC-MS. Eight crops from the 30 and 148 day soil aging periods, covering all the crop types, were analysed. The accountability of the method, compared to that determined by radioactive counting, ranged from 89 to 160% with a mean of 117%. The residue levels in the crop extracts were close to the limit of determination of the method (0.05 mg/kg). The total extractable residue in each tissue (including aqueous soluble or unidentified radioactivity by HPLC/TLC) showed good accountability with the multi-residue method of analysis.
Field trials were conducted to determine the presence of flutolanil residues in rotational crops including leafy vegetable (lettuce), root vegetable (raddish), and cereal (oat, sorgum) cultivated after aging periods of 30, 90, 148 and 366 days from flutolanil treatment. Crops were harvested at an immature stage and at maturity. In these trials soil samples representing 0-15 cm part of the soil surface were obtained.

Results

TRR and extractability

The total radioactive residues measured in rotational crops by combustion are summarised below:

Crop		Total Radioactive Residue (mg as-eq/kg)		
		30 DAT	148 DAT [A]	366 DAT
Lettuce	Immature	0.32	0.02	0.03
	Mature	0.18	0.03	0.01
Radish root [B]	Immature	0.80	0.21 [C]	0.08
	Mature	0.17	0.17	0.02
Radish top [B]	Immature	0.53	0.21 [C]	0.05
	Mature	0.36	0.14	0.03
Oat or sorghum [D]	Immature	0.31	0.05	0.04
	Straw	0.80	0.02	0.02
	Hull	0.30	0.02	0.01
	Grain	0.15	< 0.01	0.01

[A] The radish crop was planted on 120 DAT in 1991

[B] The 30 DAT and 120 DAT plots were replanted in 1991

[C] Radish roots and tops were analysed as a single sample

[D] The cereal crop for the 30 DAT and 148 DAT plots was oat. For the 366 DAT grain plot the crop was sorghum.

Residue levels in soil are presented in Table B.7.6.1.1-1. In theory, initial concentration in soil after a dosage of 2.69 kg/ha corresponds to 1.25 mg/kg, if evenly distributed in the top 15 cm of the soil. The initial mean value 0.62 was lower than the theoretical value, but standard deviation at this time point was rather high 0.32 mg/kg. Still after 90 days the total residue levels observed were not lower as compared to initial observed levels and, in fact, close to the theoretical level. At 293 days the observed levels were still 0.63 mg/kg indicating slow elimination of the residue in soil. The residue was localized in the same subfraction during the whole study period, indicating low mobility. TLC analyses and identification based on cochromatography indicated that more than 95% of the residue was untransformed flutolanil.
Transition factors (relative residue in the various parts of plant/relative residue in the soil) were at level of few percents indicating very little uptake from the soil.
The effect of aging period on total resiude levels has been given in Tables B.7.6.1.1-2, -3, and -4
The levels a.s. and its metabolites are shown in Table B.7.6.1.1-5. Metabolites in hulls and grain were not characterized by HPLC, since the residue level was considered low (0.10 and <0.05 mg/kg, respectively).

Table B.7.6.1.1-1 Average flutolanil equivalent residues in treated soil in two separate studies.

A 30-day plot	Total residue in soil (mg/kg) Soil aging period (days)				
	0	30	90	121	293
Soil fraction	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg

0-15 cm	0.62	1.04	1.15	0.74	0.63
15-30 cm	N.D.	<0.01	0.03	0.01	0.01

B	Total residue in soil (mg/kg) Soil aging period (days)				
	0	30	90	148	253
Soil fraction	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
0-15 cm	0.69	1.01	1.01	0,47	0,46

Table B.7.6.1.1-2 Average flutolanil equivalent residues in lettuce after different soil aging periods

Species/ season	Sample type	Days from treatment	Days from planting	Soil aging period (days)	Residue g/kg
Lettuce/ summer	immature	88	59	29	0.32
	mature	119	90	29	0.18
Lettuce/ winter	immature	248	102	146	0.02
	mature	287	141	148	0.03
Lettuce/ spring	immature	406	45	361	0.03
	mature	423	62	365	0.01

Table B.7.6.1.1-3 Average flutolanil equivalent residues in oat and sorghum after different soil aging periods

Species/ season	Sample type	Days from treatment	Days from planting	Soil aging period (days)	Residue mg/kg
Oat/ summer	immature foliage	88	59	29	0.31
	straw	119	90	29	0.80
	hull	119	90	29	0.30
	grain	119	90	29	0.15
Oat/ winter	immature foliage	248	102	146	0.05
	straw	361	215	146	0.02
	hull	361	215	146	0.02
	grain	361	215	146	<0.01
Sorghum/ summer	immature foliage	423	62	361	0.04
	straw	496	135	361	0.02
	hull	496	135	361	0.01
	grain	496	135	361	0.01

Table B.7.6.1.1-4 Average flutolanil equivalent residues in raddish roots and tops after different soil aging periods

Species/ season	Sample type	Days from treatment	Days from planting	Soil aging period (days)	Residue mg/kg
Raddish root	immature	60	31	31	0.80
	mature	89	60	31	0.17
Raddish root	immature	165	47	120	0.21
	mature	182	64	120	0.17
Raddish root	immature	385	24	361	0.08
	mature	406	45	361	0.02
Raddish tops	immature	60	31	31	0.53
	mature	89	60	31	0.36
Raddish tops	immature	165	47	120	0.21
	mature	182	64	120	0.14
Raddish tops	immature	385	24	361	0.05
	matur	406	45	361	0.03

Table B.7.6.1.1-5 Characterisation of residue as flutolanil equivalent residues or % of total radioactive residue in selected mature crops planted after 30 days soil aging.

	Cereal (oats) Forage		Straw		Hulls		Grain		Root (raddish) Tops		Root (raddish) Root		Leaf (lettuce)	
	mg/kg equiv	% TRR	mg/kg equiv	% TRR	mg/kg equiv	% TRR	mg/kg equiv	% TRR	mg/kg equiv	% TRR	mg/kg equiv	% TRR	mg/kg equiv	% TRR
TRR	0.26		0.88		0.24		0.14		0.31		0.20		0.11	
ERR	0.19	75.9	0.63	71.5	0.12	48.4	0.06	44.6	0.22	70.6	0.12	54.1	0.08	76.6
Organosoluble radioactivity	0.13	51.6	0.48	54.1	0.10	41.8	0.04	28.9	0.20	61.3	0.09	46.3	0.07	65.6
Watersoluble radioactivity	0.06	24.3	0.15	17.4	0.02	6.6	0.02	15.7	0.02	9.3	0.03	7.8	0.01	11.0
Flutolanil	0.015	5.6	0.134	15.2	NA	NA	NA	NA	0.05	17	0.03	15.9	0.04	31.3
- - unconjugated	<0.01	2.2	0.03	3.2	NA	NA	NA	NA	0.01	4.5	0.02	9.6	0.02	14.4
- conjugated	<0.01	3.4	0.11	12.0	NA	NA	NA	NA	0.04	12.5	0.01	6.3	0.02	16.9
M-3	0.013	5.0	0.036	4.1	NA	NA	NA	NA	<0.01	2.4	-	-	<0.01	4.10
M-4	0.018	6.9	0.047	5.3	NA	NA	NA	NA	0.03	12.4	0.01	6.0	0.01	4.10
- unconjugated	0.01	4.4	0.03	3.8	NA	NA	NA	NA	0.03	10.4	0.01	6.0	- <0.01	2.5
- conjugated	<0.01	2.5	0.01	1.5	NA	NA	NA	NA	<0.01	2.0	-	-	- <0.01	1.6
M-5	0.008	3.2	0.035	4.0	NA	NA	NA	NA	-	-	-	-	<0.01	3.0
M-6. unconjugated	-	-	-	-	NA	NA	NA	NA	0.01	6.0	<0.01		-	-
M-7	-	-	-	-	NA	NA	NA	NA	-	-	0.02		-	-
M11	0.004	1.4	0.024	2.7	NA	NA	NA	NA	-	-	-	-	<0.01	0.90
Other	-	-	0.03	4.1	NA	NA	NA	NA	0.01	2.8	0.02	7.8	0-01	2.90
Total identified		22.1		35.4						53.0		29.7	0.02	15.0
Unindentified		29.5		16.6						26.5		13.6	0.02	19.2
URR	0.06	24.1	0.24	28.6	0.12	51.7	0.08	55.4	0.09	29.3	0.09	45.8	0.03	23.5
Accountability	0.25	100	0.87	100.1	0.24	100.1	0.14	100	0.31	99.9	0.21	99.9	0.11	100.1

TRR, Total Radioactive Residues; ERR, Extracted Radioactive Residues; URR, Unextracted Radioactive Residues; accountability, sum of ERR and URR; NA, Not Analysed

Conclusions

The effects of aging of a.s. in soil on following crops were studied by applying the a.s. as a radioactive formulation directly on the soil.

The studies revealed that flutolanil residue is very stable in sandy loam soil. In line with these observations the residue was localised into the upper 15 cm bed in the soil. After 90 days aging the residue level in soil was still more than 90% of theoretical initial value. These results thus indicate that the subsequent studies on rotational crops carried out in the presented study were necessary.

Rotational crops planted in soil after treatment at 2.69 a.s./kg/ha rate showed a significant decline in TRR at harvest with increasing rotational interval. The total residue levels in various plant parts ranged from 0.11 to 0.88 mg/kg after in crops planted after 30 days aging, from <0.01 to 0.05 mg/kg with 146 days aging, and from 0.01 to 0.04 mg/kg with 361 days of aging.

The metabolic profiles of a.s. were similar for all crops. The residues were mainly comprised of conjugated flutolanil and M-4. Unextractable residues were within a range of 23.5 - 55.4 % of TRR. The metabolic profile of cereal grain and hulls were not studied.

While some deviations from GLP are evident, such as carrying the study out in different test sites employing different study protocols and carrying out the analyses in different laboratories, the study is regarded as acceptable.

B.7.6.1.2 Study 2

Previous evaluation	Submitted for the purpouse of renewal
RMS remark	Acceptable

Report:	**CA 6.6.1/02. Ki Chang Ahn (2016a)**
Title:	Confined rotational crop study with [trifluoromethyl ring-U-^{14}C]flutolanil applied at 480 g ai/ha (one radiolabel)
Document No:	2697W (R-3390)
Guidelines:	OECD 502 (2007) US EPA OSCPP 860.1850
Deviations:	None
Testing laboratory:	PTRL West, Hercules, California, USA
GLP:	Yes

Executive Summary

The extent and nature of residue uptake by crops grown in soil previously treated with [^{14}C]-flutolanil was investigated. Bare sandy loam soil was treated at a rate of 480 g a.s./ha with [phenyl -U-^{14}C] flutolanil. Rotational crops (lettuce, radish and wheat representing leafy crops, root crops and cereal crops respectively) were sown 30 days, 120 days, and 270 days after treatment. The crops were grown to maturity under outdoor conditions in CA, USA.

Residue levels were rather variable in the raw agricultural commodities with some increases seen between 30-day and 120-day plant-back intervals; however, residues levels in the RACs had declined substantially by the 270-day plant-back interval.

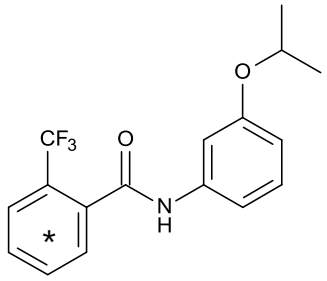
Crop		Total Radioactive Residue (mg a.s. eq./kg by combustion)		
		30-day plant-back	120-day plant-back	270-day plant-back
Lettuce	Immature	0.392	0.213	0.053
	Mature	0.232	0.173	0.033
Radish	Root	0.130	0.142	0.015
	Top	0.412	0.671	0.076
Wheat	Forage	0.934	1.237	0.112
	Hay	1.352	2.041	0.109
	Straw	1.971	2.457	0.110
	Grain	0.460	0.512	0.033

Immature and mature lettuce, radish roots and tops and wheat forage, hay, straw and grain were extracted and analysed from the 30-day, 120-day and 270-day plant back periods. Soil was not analysed. Flutolanil was identified as only a minor component of the residue in the rotational crops samples as were the metabolites M-3 and M-4 (both free and conjugated). Metabolite M-4 was observed as important plant metabolites in the primary crop metabolism studies. The main metabolites identified were M-101, M-102 and trifluoroacetic acid (TFA). Metabolites M-101 and M-102 appeared to decline in later plant-back intervals suggesting complete destruction of the trifluoromethyl-phenyl ring moiety. Other metabolites were present either as minor metabolites or in trace amounts.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material: [Phenyl -U-^{14}C]-flutolanil (called [trifluoromethyl ring-U-^{14}C]flutolanil in the report)



* Denotes position of [^{14}C]-radiolabel

Chemical name (IUPAC)	α, α, α-trifluoro-3'-isopropoxy-*o*-toluanilide
CA registry number:	66332-96-5
Lot or batch number:	CFQ42127
Specific activity:	118 mCi/mmol
Radiochemical purity:	>99.9%

B. STUDY DESIGN AND METHODS

1. In-life dates:

18 December 2014 to 27 May 2016

2. Experimental design

Test System:

[^{14}C]-Flutolanil was applied to bare soil at a nominal rate of 480 g a.s./ha (achieved rate 510 g a.s./ha, 1.4N with respect to the potato GAP) in 2014 and rotational crops (leafy vegetable, root crop, cereal) were sown after 30, 120 and 270 days.

In 2015, lettuce (*Lactuca sativa L.*, variety 'salad bowl', leafy vegetable), radish (*Raphanus sativus L.*, variety 'crimson giant', root crop), and wheat (*Triticum aestivum,* variety 'blanca royale', small grain) were sown for the 30-day, 120-day and 270-day plant-back periods. Crops were grown on outdoor plots (0.75 m^2 surface area and 35.6 cm column depth) in California, USA. Rainfall was supplemented by artificial irrigation where necessary.

Test Soil:

The soil in the plots was classified as a sandy loam. Further details are tabulated below.

Parameter	1988
Texture Class (USDA)	Sandy loam
pH	7.0
Organic matter (%)	0.51
Cation exchange capacity (meq/100 g)	11.4
USDA classification	
Sand % Silt % Clay %	63 21 16

Experimental Conditions:

[^{14}C]-flutolanil was prepared as a 40SC formulation. The [^{14}C]-formulation was applied to bare soil on 18 December 2014 (120 and 270-day plantback periods) and 18 March 2015 (30 plant back period) by a manual trigger-pulled pump sprayer. The achieved application rate was 510 g a.s./ha.

Crops were sown on 17 April 2015 (30-day and 120-day plant-back intervals) and 14 September 2015 (270-day plant back interval). Crops were grown to maturity outdoors at the field site in California, USA, harvested and shipped frozen to the analytical laboratory in California, USA.

Test Samples

Plant samples (both treated and control) were taken for analysis at the following times:

Plant-back interval	Plant sample	Harvest date	Days after sowing
30 days	Lettuce (immature)	01 Jun 2015	44
	Lettuce (mature)	15 Jun 2015	58
	Radish (roots and tops)	28 May 2015	41
	Wheat (forage)	27 May 2015	40
	Wheat (hay)	15 Jun 2015	58
	Wheat (straw and grain)	08 Jul 2015	81
120 days	Lettuce (immature)	01 Jun 2015	44

	Lettuce (mature)	15 Jun 2015	58
	Radish (roots and tops)	28 May 2015	41
	Wheat (forage)	27 May 2015	40
	Wheat (hay)	15 Jun 2015	58
	Wheat (straw and grain)	08 Jul 2015	81
270 days	Lettuce (immature)	23 Oct 2015	39
	Lettuce (mature)	11 Nov 2015	57
	Radish (roots and tops)	23 Oct 2015	39
	Wheat (forage)	23 Oct 2015	39
	Wheat (hay)	16 Nov 2015	62
	Wheat (straw and grain)	19 Apr 2016	215

Sample Preparation

Immature harvest consisting of all the crops was taken when the young plants required thinning to normal agricultural spacing. In cereals this was chosen to approximate the stage at which the crop might be used as forage.

Final harvest was taken when the plants reached maturity. Lettuce and the cereal crops were harvested by excising at soil level. The mature cereals were divided into straw, and grain. Root crops were harvested by pulling them from the soil before dividing them into aerial portion and roots. Soil adhering to the root crop was removed by water washing.

Crop samples were frozen immediately after sampling. Frozen crop samples were homogenised with dry ice or liquid nitrogen, which was then allowed to sublimate prior to further analysis. Total radioactive residues (TRR) were determined by combustion and liquid scintillation counting (LSC). Homogenised samples were stored at -20°C until required for analysis.

Extraction and Fractionation of Residues

Samples of homogenised plant samples were sequentially extracted by Waring blender. Samples were extracted using the following sequence of solvents:

2 – 3 × acetonitrile:water (1:1 v/v)

1 – 3 × acetonitrile

Following each extraction, the extract was separated from the plant solids by centrifugation. The radioactivity in each extract was determined by LSC and the combined extracts were then concentrated by rotary evaporation vacuum for HPLC and TLC analyses. Post extraction solids (PES) exceeding 10% TRR were further extracted sequentially as follows:

1 × 0.1N HCl:acetonitrile (1:4 v/v)

1 × 1N HCl:acetonitrile (1:4 v/v)

1 × 0.1N NaOH:acetonitrile (1:4 v/v)

1 × 1N NaOH:acetonitrile (1:4 v/v)

The remaining PES from the 30-day and 120-day radish root, wheat straw and wheat grain were additionally further extracted using the following steps:

1 × cellulase extraction: (EC 3.2.1.4 from *Aspergillus Niger* in 100 mM acetate buffer pH 5 at 37°C for 24 hours).

1 × 6 N HCl: Reflux for 2 hours.

1 x 10 N NaOH: 110°C for 2 hours

PES were subjected to combustion analysis to quantify any un-extracted bound radioactivity.

Combined weak acidic/basic extracts containing >0.05 mg eq./kg or >10% TRR were analysed by HPLC and TLC. Major metabolites were identified by retention time matching against reference standards by HPLC or by isolating radioactive HPLC peaks and confirming their identity by TLC analysis against reference standards. The polar solvent front isolate from the HPLC chromatograms was identified as TFA by LC-MS and confirmed by HPLC and TLC against a ^{14}C-trifluoroacetic sodium salt reference standard. Other unknown isolates from the chromatograms were subject to enzymatic hydrolysis (β-glucosidase (EC 3.2.1.21 from almonds, 100 mM acetate buffer (pH 5) at 37°C for 24 hours) or acidic hydrolysis (2N HCl at 55 – 60°C for 24 hours) followed by HPLC/TLC analysis.

II. RESULTS AND DISCUSSION

Total Radioactive Residues and Extractability

The total radioactive residues (TRR) measured in rotation crops by combustion are summarised below in Table B.7.6.1.2-1. Residue levels were rather variable in the raw agricultural commodities with some increases seen between the 30-day and 120-day plant-back intervals; however, residues levels in the RACs had declined substantially by the 270-day plant-back interval

Table B.7.6.1.2-1: Summary of radioactive residues in rotational crops following treatment of soil with 510 g a.s./ha [phenyl-U-^{14}C]-flutolanil

Crop		Total Radioactive Residue (mg a.s. eq./kg by combustion)		
		30-day plant-back	120-day plant-back	270-day plant-back
Lettuce	Immature	0.392	0.213	0.053
	Mature	0.232	0.173	0.033
Radish	Root	0.130	0.142	0.015
	Top	0.412	0.671	0.076
Wheat	Forage	0.934	1.237	0.112
	Hay	1.352	2.041	0.109
	Straw	1.971	2.457	0.110
	Grain	0.460	0.512	0.033

The distribution of crop radioactive residues amongst the acetonitrile:water extract; weak acidic/basic extract; cellulase extract and strong acidic/basic extract and the radioactive residue remaining bound are summarised in Table B.7.6.1.2-2. Residues in the acetonitrile:water and weak acidic/basic extract were identified by HPLC/TLC; residues in the cellulase and strong acidic/basic extracts were characterised into aqueous and organic soluble fractions and the residue in the organic-soluble fraction of the strong

acidic/basic extract was identified by HPLC/TLC. For all commodities, exhaustive extraction by cellulase and strong acid/base did not release a significant additional residue. The level of extraction achieved was in accordance with OECD 502 (2007).

The summed identification and characterisation of radioactive residues in the acetonitrile:water; weak acidic/basic extract and organic-soluble fraction of the strong acidic/basic extract are summarised below in Table B.7.6.1.2-3 (lettuce); Table B.7.6.1.2-4 (radish) and Table B.7.6.1.2-5 (wheat).

Table B.7.6.1.2-2: Summary of TRR and extractability in rotational crops following treatment of soil with 510 g a.s./ha [phenyl-U-^{14}C]-flutolanil

Plant-back interval / days	Crop		TRR by Extraction	Combined acetonitrile:water extract		Combined weak acidic/basic extract		Cellulase extract		Combined strong acidic/basic extract		Post extraction solids	
			mg/kg	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
30	Lettuce	Immature	0.374	0.348	93.0	-	-	-	-	-	-	0.026	7.0
		Mature	0.262	0.248	94.7	-	-	-	-	-	-	0.014	5.3
	Radish	Root	0.113	0.079	69.9	0.001	0.9	0.002	1.8	0.012	10.6	0.019	16.8
		Top	0.459	0.433	94.3	-	-	-	-	-	-	0.026	5.7
	Wheat	Forage	1.056	0.863	81.7	0.026	2.5	0.010	0.9	0.121	11.5	0.036	3.4
		Hay	1.416	1.214	85.7	0.063	4.4	-	-	-	-	0.139	9.8
		Straw	1.714	1.318	76.9	0.178	10.4	0.080	4.7	0.089	5.2	0.049	2.9
		Grain	0.465	0.352	75.7	0.019	4.1	0.034	7.3	0.049	10.5	0.011	2.4
120	Lettuce	Immature	0.194	0.181	93.3	-	-	-	-	-	-	0.013	6.7
		Mature	0.187	0.177	94.7	-	-	-	-	-	-	0.010	5.3
	Radish	Root	0.140	0.096	68.6	0.005	3.6	0.001	0.70	0.013	9.3	0.025	17.9
		Top	0.608	0.583	95.9	-	-	-	-	-	-	0.025	4.1
	Wheat	Forage	1.087	1.003	92.3	-	-	-	-	-	-	0.084	7.7
		Hay	1.883	1.659	88.1	0.056	3.0	-	-	-	-	0.167	8.9
		Straw	2.373	1.741	73.4	0.243	10.2	0.041	1.7	0.242	10.2	0.104	4.4
		Grain	0.612	0.469	76.8	0.030	4.9	0.040	6.5	0.061	10.0	0.012	2.0
270	Lettuce	Immature	0.063	0.052	82.5	0.005	7.9	-	-	-	-	0.008	12.7
		Mature	0.031	0.031	100	-	-	-	-	-	-	0.001	3.1
	Radish	Root	0.029	0.013	44.8	0.005	17.2	-	-	-	-	0.013	44.8
		Top	0.101	0.084	83.2	0.008	7.9	-	-	-	-	0.009	8.9
	Wheat	Forage	0.227	0.101	44.5	0.059	26.0	-	-	-	-	0.068	30.0
		Hay	0.108	0.095	88.0	0.005	4.6	-	-	-	-	0.008	7.4
		Straw	0.104	0.077	74.0	0.008	7.7	-	-	-	-	0.019	18.3

		Grain	0.033	0.028	84.8	0.000	0.0	-	-	-	-	0.005	15.2

Table B.7.6.1.2-3: Summary of the identification of the residue in rotational crops following treatment of soil with 510 g a.s./ha [phenyl-U-^{14}C]-flutolanil - lettuce

Identification of the residue	Lettuce (immature)						Lettuce (mature)					
	30 days plantback		120 days plantback		270 days plantback		30 days plantback		120 days plantback		270 days plantback	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Flutolanil	0.003	0.8	-	-	-	-	0.004	1.5	0.002	1.1	-	-
M-2	-	-	-	-	-	-	-	-	-	-	-	-
M-3	-	-	-	-	-	-	-	-	-	-	-	-
M-3 glycoside	-	-	-	-	-	-	-	-	-	-	-	-
M-4	0.003	0.8	-	-	-	-	0.004	1.5	-	-	-	-
M-4 glycoside	0.040	10.7	-	-	-	-	0.028	10.7	0.012	6.4	-	-
M-5	-	-	-	-	-	-	-	-	-	-	0.001	3.1
M-6	-	-	-	-	-	-	0.003	1.1	-	-	-	-
M-7	-	-	-	-	-	-	0.001	0.4	-	-	-	-
M-11	-	-	-	-	-	-	-	-	-	-	-	-
M-101	0.131	35.0	0.104	53.6	0.004	6.3	0.054	20.6	0.074	39.6	0.001	3.1
M-102	0.033	8.8	0.005	2.6	-	-	0.035	13.4	0.006	3.2	-	-
M-102 glycoside	-	-	-	-	-	-	-	-	-	-	-	-
TFA	0.074	19.8	0.042	21.6	0.042	66.6	0.057	21.8	0.034	18.2	0.028	87.5
Others	0.063	16.8	0.030	15.5	0.006	9.5	0.063	24.0	0.049	26.2	0.001	3.1
(Largest other)	0.010	2.7	0.006	3.1	0.001	1.6	0.008	2.1	0.008	4.3	0.001	3.1

Table B.7.6.1.2-4: Summary of the identification of the residue in rotational crops following treatment of soil with 510 g a.s./ha [phenyl-U-^{14}C]-flutolanil – radish

Identification of the residue	Radish (roots)						Radish (tops)					
	30 days plantback		120 days plantback		270 days plantback		30 days plantback		120 days plantback		270 days plantback	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Flutolanil	-	-	-	-	-	-	-	-	-	-	-	-
M-2	-	-	-	-	-	-	-	-	-	-	-	-
M-3	-	-	-	-	-	-	-	-	-	-	-	-
M-3 glycoside	-	-	-	-	-	-	-	-	-	-	-	-
M-4	0.002	1.8	-	-	-	-	-	-	-	-	-	-
M-4 glycoside			-	-	-	-	-	-	-	-	-	-
M-5	-	-	-	-	-	-	0.003	0.7	-	-	-	-
M-6	-	-	-	-	-	-	-	-	-	-	-	-
M-7	-	-	-	-	-	-	-	-	-	-	-	-
M-11	-	-	-	-	-	-	-	-	-	-	-	-
M-101	0.005	4.4	0.008	5.7	0.001	3.4	0.109	23.7	0.195	32.1	0.008	7.9
M-102	0.009	8.0	0.003	2.1	0.000	0.0	0.014	3.1	0.031	5.1	0.002	2.0
M-102 glycoside	0.014	12.4	0.020	14.3	-	-	0.048	10.5	0.089	14.6	0.008	7.9
TFA	0.033	29.2	0.019	13.6	0.006	20.7	0.174	37.9	0.205	33.7	0.058	57.4
Unknown RT 19.3 min	-	-	-	-	-	-	-	-	0.032	5.3	-	-
Others	0.017	15.0	0.044	31.4	0.005	17.2	0.084	18.3	0.031	5.1	0.008	7.9
(Largest other)	0.004	3.5	0.006	4.3	0.001	3.4	0.017	3.7	0.019	3.1	0.003	3.0

Table B.7.6.1.2-5: Summary of the identification of the residue in rotational crops following treatment of soil with 510 g a.s./ha [phenyl-U-^{14}C]-flutolanil – wheat

Identification of the residue	Wheat (forage)						Wheat (hay)					
	30 days plantback		120 days plantback		270 days plantback		30 days plantback		120 days plantback		270 days plantback	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Flutolanil	0.007	0.7	-	-	-	-	-	-	-	-	-	-
M-2	-	-	-	-	-	-	-	-	-	-	-	-
M-3	0.009	0.9	-	-	-	-	0.013	0.9	-	-	-	-
M-3-glycoside	0.064	6.1	-	-	-	-	0.151	10.7-	0.133	7.1	-	-
M-4	0.007	0.7	-	-	0.001	0.4	0.016	1.1	-	-	-	-
M-4 glycoside	-	-	-	-	-	-	0.011	0.8	-	-	-	-
M-5	0.007	0.7	-	-	0.001	0.4	-	-	-	-	-	-
M-6	-	-	-	-	-	-	-	-	-	-	-	-
M-7	0.010	0.9	-	-	-	-	-	-	-	-	-	-
M-11	-	-	-	-	-	-	-	-	-	-	-	-
M-101	0.155	14.7	0.346	31.8	0.003	1.3	0.056	4.0	0.264	14.0	0.002	1.9
M-102	0.059	5.6	0.162	14.9	0.012	5.3	0.192	13.6	0.307	16.3	0.048	44.4
M-102 glycoside	-	-	-	-	-	-	-	-	-	-	-	-
TFA	0.194	18.4	0.253	23.3	0.014	6.2	0.232	16.4	0.425	22.6	0.029	26.9
Unknown RT 20.0 min	-	-	-	-	0.015	6.6	-	-	-	-	-	-
Unknown RT 3.3 min	-	-	-	-	0.022	9.7	-	-	-	-	-	-
Unknown RT 5.1 min	-	-	-	-	-	-	-	-	0.096	5.1	-	-
Unknown RT 17.9 min	-	-	-	-	-	-	-	-	-	-	0.007	6.5
Unknown RT	-	-	-	-	-	-	-	-	-	-	-	-

Identification of the residue	Wheat (forage)						Wheat (hay)					
	30 days plantback		120 days plantback		270 days plantback		30 days plantback		120 days plantback		270 days plantback	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
17.7 min												
Others	0.351	33.2	0.242	22.3	0.032	14.1	0.532	37.6	0.435	23.1	0.009	8.3
(Largest other)	0.039	0.04	0.048	4.4	0.005	2.2	0.059	4.2	0.070	3.7	0.003	2.8

Identification of the residue	Wheat (straw)						Wheat (grain)					
	30 days plantback		120 days plantback		270 days plantback		30 days plantback		120 days plantback		270 days plantback	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Flutolanil	-	-	-	-	-	-	-	-	-	-	-	-
M-2	-	-	-	-	-	-	-	-	-	-	-	-
M-3	0.015	0.9	0.021	0.9	-	-	-	-	-	-	-	-
M-3 glycoside	0.215	12.5	0.193	8.1	-	-	-	-	-	-	-	-
M-4	0.020	1.2	-	-	-	-	-	-	0.006	1.0	-	-
M-4 glycoside	0.094	5.5	-	-	-	-	-	-			-	-
M-5	0.021	1.2	0.024	1.0	0.003	2.9	-	-	0.006	1.0	-	-
M-6	-	-	-	-	-	-	-	-	-	-	-	-
M-7	-	-	0.026	1.1	-	-	-	-	-	-	-	-
M-11	-	-	0.019	0.8	-	-	-	-	-	-	-	-
M-101	0.017	1.0	0.104	4.4	0.003	2.9	0.009	1.9	0.015	2.5	-	-
M-102	0.050	2.9	0.068	2.9	0.008	7.7	0.027	5.8	0.132	21.6	0.002	6.1
M-102 glycoside	-	-	-	-	-	-	-	-	-	-	-	-
TFA	0.222	13.0	0.322	13.6	0.039	37.5	0.067	14.4	0.045	7.4	0.015	45.5
Unknown RT 20.0 min	-	-	-	-	-	-	-	-	-	-	-	-
Unknown RT 3.3 min	-	-	-	-	-	-	-	-	-	-	-	-
Unknown RT 5.1 min	-	-	-	-	-	-	-	-	-	-	-	-
Unknown RT 17.9 min	-	-	-	-	-	-	-	-	-	-	-	-
Unknown RT 17.7 min	-	-	-	-	-	-	0.027	5.8	-	-	-	-
Others	0.666	38.9	0.963	40.6	0.025	24.0	0.223	48.0	0.266	43.5	0.011	33.3
(Largest	0.051	3.0	0.085	3.6	0.003	2.9	0.022	4.7	0.027	4.4	0.001	3.0

Identification of the residue	Wheat (straw)						Wheat (grain)					
	30 days plantback		120 days plantback		270 days plantback		30 days plantback		120 days plantback		270 days plantback	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
other)												

In rotational crops at the 30-day plant-back interval, the TRR varied between 0.130 mg/kg (radish root) and 1.971 mg/kg (wheat straw). The residue was readily extractable by acetonitrile:water with between 69.9% (radish root) and 94.7% (mature lettuce) of the TRR. Little further radioactivity was extracted by more exhaustive steps apart from in wheat straw where a further 10.4% was extracted by weak acid/base; in wheat grain where 7.3% was extracted by cellulase and in radish root, and in wheat forage and wheat grain where a further 10.6%, 11.5% and 10.5% respectively of the TRR was extracted by strong acid/base reflux. Bound residues were low in all commodities with only radish root containing >10% TRR in the post extraction solids (16.8%). Identification of the residue indicates that parent flutolanil is not a significant component of the residue and was only detected in immature lettuce, mature lettuce, and wheat forage at 0.003 mg/kg, 0.8%; 0.004 mg/kg, 1.5%; 0.007 mg/kg, 0.7% respectively. The known plant metabolites M-3, M-4, M-5, M-7, M-101 and M-102 were variously detected indicating a similar pattern of metabolism to primary crops. All of these metabolites apart from M-101 and M-102 were only present as very minor components. In addition to M-101 and M-102, TFA was present as a significant metabolite (0.057 mg/kg, 21.8% in mature lettuce, 0.033 mg/kg, 29.2% in radish roots, 0.222 mg/kg, 13.0% in wheat straw and 0.067 mg/kg, 14.4% in wheat grain).

In rotational crops at the 120-day plant-back interval, the TRR varied between 0.142 mg/kg (radish root) and 2.457 mg/kg (wheat straw), apparently generally increasing in magnitude in the various crop commodities compared to the 30-day plantback interval. The residue was readily extractable with between 68.6% (radish root) and 94.7% (mature lettuce) of the TRR extracted by acetonitrile:water. Little further radioactivity was extracted by more exhaustive steps apart from in wheat straw where a further 10.2% was extracted by weak acid/base; in wheat grain where 6.5% was extracted by cellulase and in radish root, wheat straw and wheat grain where a further 9.3%, 10.2% and 10.0% respectively of the TRR was extracted by strong acid/base reflux. Bound residues were low in all commodities with only radish root containing >10% TRR in the post extraction solids (17.9%). Identification of the residue indicates that parent flutolanil is not a significant component of the residue and was only detected in mature lettuce at 0.002 mg/kg, 1.1%. The known plant metabolites M-3, M-4, M-5, M-7, M-11, M-101 and M-102, were variously detected indicating a similar pattern of metabolism to primary crops. All of these metabolites apart from M-101 and M-102 were only present as very minor components. In addition to M-101 and M-102, TFA was present as a significant metabolite (0.034 mg/kg, 18.2% in mature lettuce, 0.019 mg/kg, 13.6% in radish roots, 0.322 mg/kg, 13.6% in wheat straw and 0.045 mg/kg, 7.4% in wheat grain).

In rotational crops at the 270-day plant-back interval, residues had substantially declined and the TRR varied between 0.015 mg/kg (radish root) and 0.112 mg/kg (wheat forage). Apart from in radish root and wheat forage, the residue was readily extractable with between 74.0% (wheat straw) and 100.0% (mature lettuce) of the TRR extracted by acetonitrile:water, the extractability in radish root and wheat forage was 44.8% and 44.5% respectively. Additional radioactivity was extracted by weak acid/base. Remaining bound residues were <0.01 mg/kg apart from in radish root (0.013 mg/kg), wheat forage (0.068 mg/kg) and wheat straw (0.019 mg/kg). The low residues levels meant that identification of the residue was limited and the metabolites M-3, M-4, M-5, M-101, M-102 were detected at trace levels. TFA was the most prominent metabolite (0.028 mg/kg, 87.5% in mature lettuce, 0.006 mg/kg, 13.6% in radish roots, 0.039 mg/kg, 37.5% in wheat straw and 0.015 mg/kg, 45.5% in wheat grain).

The levels of TFA reported in the rotational crop metabolism study are reported as parent equivalents and thus overestimate underestimate the absolute amount of TFA in the samples due to the respective molecular weights of flutolanil (323.31 g/mol) and TFA (114.02 g/mol). Additionally, since TFA contains only a single carbon atom derived from the [phenyl-U-^{14}C] ring a further 6-fold correction is applied in accordance with the precedent of the EFSA peer review of the pesticide risk assessment for the active substance flurtamone (EFSA Journal 2016;14(6):4498, 112 pp. doi:10.2903/j.efsa.2016.4498). This gives a correction factor of 0.3527 for the relative molecular weights and a further correction factor of 6 for the relative amount of label giving a net correction of 2.1162. The corrected concentration of TFA in the rotational crop commodities after application of this correction is summarised below.

Crop		Concentration of TFA (mg/kg)					
		30-day plantback		120-day plantback		270-day plantback	
		mg/kg parent equivalents	mg/kg TFA	mg/kg parent equivalents	mg/kg TFA	mg/kg parent equivalents	Corrected concentration
Lettuce	Immature	0.074	0.157	0.042	0.089	0.042	0.088
	Mature	0.057	0.121	0.034	0.072	0.028	0.059
Radish	Root	0.033	0.070	0.019	0.040	0.006	0.013
	Top	0.174	0.368	0.205	0.434	0.058	0.123
Wheat	Forage	0.194	0.411	0.253	0.535	0.014	0.030
	Hay	0.232	0.491	0.425	0.899	0.029	0.061
	Straw	0.222	0.470	0.322	0.681	0.039	0.083
	Grain	0.067	0.142	0.045	0.095	0.015	0.032

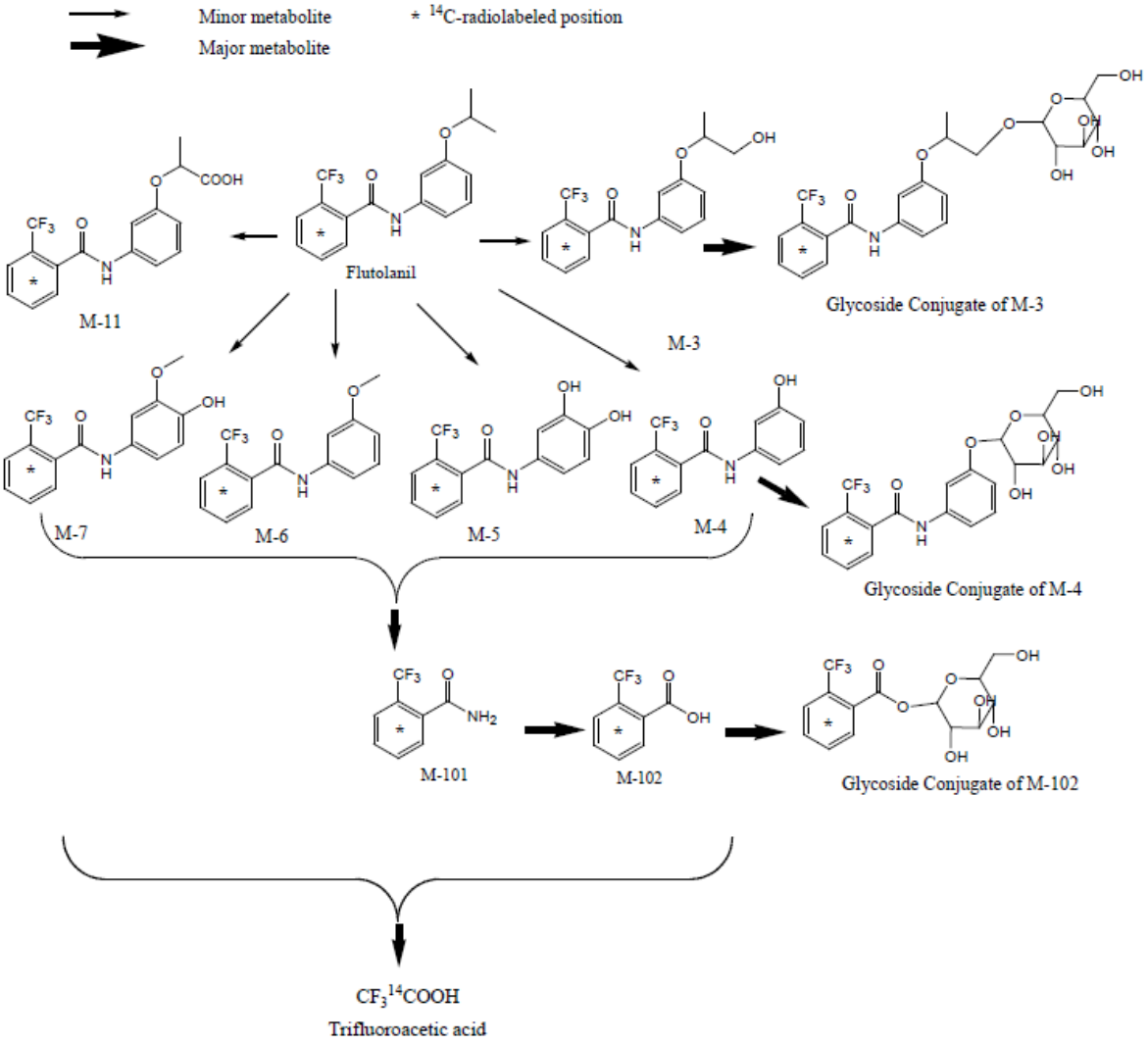
These calculations indicate that residues of TFA might be significant in rotational crops, and is included as an analytical target in the ongoing field rotational crop studies in order to provide more quantitative information. As TFA can be derived from a number of pesticide and non-pesticide sources from molecules containing a CF_3 moiety it is not a good marker for flutolanil residues and is not proposed to be included in the plant residue definition for risk assessment or monitoring.

The detection of radioactive residues in all plant commodities indicates that soil residues of flutolanil are taken up by growing plants and systemically transported and metabolised in all parts of the plant.

Metabolic pathway

A metabolic pathway for [phenyl-U-^{14}C]-flutolanil in rotational crops is proposed in Figure B.7.6.1.2-1. The metabolic profile was similar in all rotational crops and similar to that seen in primary crops treated with flutolanil apart from the additional identification of TFA. This is likely to arise following the complete degradation of the phenyl ring following the formation of the metabolites M-101 and M-102.

Figure B.7.6.1.2-1: Metabolic pathway of [phenyl-U-^{14}C]-flutolanil in rotational crops



III. CONCLUSIONS

Crop residue levels increased slightly between a 30-day plantback interval and a 120-day plantback interval but declined greatly as the soil aged at the 270-day plantback interval. TRRs measured by combustion ranged from 0.130 to 1.971 mg/kg in crops planted after 30 days aging, from 0.142 to 2.457 mg/kg with 120 days aging and from 0.015 to 0.112 mg/kg with 270 days aging.

The metabolic profile was similar in all rotational crops and similar to that seen in primary crops treated with flutolanil.

The major components detected in rotational crops were:

- M-101 (0.005 to 0.155 mg/kg, 1.0 to 35.0% TRR); M-102 and its glycoside (0.003 to 0.062 mg/kg, 2.1% to 20.4% TRR) and TFA (0.033 to 0.232 mg/kg, 13.0 to 37.9% TRR) from crops grown after a 30-day plant back-period (on a parent equivalent basis).
- M-101 (0.008 to 0.346 mg/kg, 5.7 to 53.6% TRR); M-102 and its glycoside (0.005 to 0.307 mg/kg, 2.6 to 21.6% TRR) and TFA (0.019 to 0.425 mg/kg, 7.4 to 33.7% TRR) from crops grown after a 120-day plant-back period.

- TFA was the most prominent metabolite in crops after a 270-day plantback interval (0.006 to 0.058 mg/kg, 6.2 to 87.5% TRR). Other metabolites were generally only found in trace amounts.
- When corrected for the relative molecular weight and labelling of TFA with respect to [phenyl-U-^{14}C]-flutolanil, the actual level of TFA in the crops was 0.070-0.491 mg/kg with a 30-day plant-back interval 0.040 – 0.899 mg/kg with a 120-day plant-back interval and 0.013 – 0.123 mg/kg with a 270-day plant-back interval.
- Flutolanil was not a significant component of the residue in any of the crops at any of the plantback intervals.

B.7.6.1.3 Study 3

Previous evaluation	Submitted for the purpouse of renewal
RMS remark	Acceptable

Report:	**CA 6.6.1/03. Ki Chang Ahn (2016b)**
Title:	Confined rotational crop study with [trifluoromethyl ring-U-^{14}C]Flutolanil applied at 2100 g ai/ha (one radiolabel)
Document No:	2698W (R-3389)
Guidelines:	OECD 502 (2007) US EPA OSCPP 860.1850
Deviations:	None
Testing laboratory:	PTRL West, Hercules, California, USA
GLP:	Yes

Executive Summary

The extent and nature of residue uptake by crops grown in soil previously treated with [^{14}C]-flutolanil was investigated. Bare sandy loam soil was treated at a rate of 2100 g a.s./ha with [phenyl -U-^{14}C] flutolanil). Rotational crops (lettuce, radish and wheat representing leafy crops, root crops and cereal crops respectively) were sown 30 days, 120 days, and 270 days after treatment. The crops were grown to maturity under outdoor conditions in CA, USA.

Residue levels were rather variable in the raw agricultural commodities with some increases seen between 30-day and 120-day plant-back intervals; however, residues levels in the RACs had declined substantially by the 270-day plant-back interval.

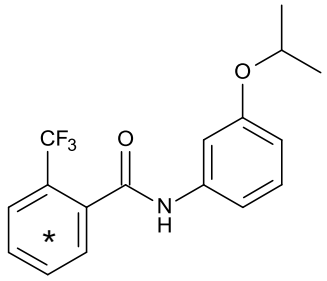
Crop		Total Radioactive Residue (mg a.s. eq./kg by combustion)		
		30-day plant-back	120-day plant-back	270-day plant-back
Lettuce	Immature	4.408	2.694	0.195
	Mature	2.083	1.838	0.233
Radish	Root	0.780	0.632	0.088
	Top	5.673	4.236	0.492
Wheat	Forage	6.521	12.605	1.444
	Hay	8.936	25.442	0.779
	Straw	11.288	14.578	0.667
	Grain	3.737	3.143	0.360

Immature and mature lettuce, radish roots and tops and wheat forage, hay, straw and grain were extracted and analysed from the 30-day, 120-day and 270-day plant back periods. Soil was not analysed. Flutolanil was identified as only a minor component of the residue in the rotational crops samples as were the metabolites M-3and M-4 (both free and conjugated) which were observed as important plant metabolites in the primary crop metabolism studies. The main metabolites identified were M-101, M-102 and trifluoroacetic acid (TFA). Metabolites M-101 and M-102 appeared to decline in later plant-back intervals suggesting complete destruction of the trifluoromethyl-phenyl ring moiety. Other metabolites were present either as minor metabolites or in trace amounts.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material: [Phenyl -U-^{14}C]-flutolanil (called [trifluoromethyl ring-U-^{14}C]flutolanil in the report)



* Denotes position of [^{14}C]-radiolabel

Chemical name (IUPAC)	α, α, α-trifluoro-3'-isopropoxy-*o*-toluanilide
CA registry number:	66332-96-5
Lot or batch number:	CFQ42127
Specific activity:	118 mCi/mmol
Radiochemical purity:	>99.9%

B. STUDY DESIGN AND METHODS

1. In-life dates:

18 December 2014 to 27 May 2016

2. Experimental design

Test System:

[^{14}C]-Flutolanil was applied to bare soil at a nominal rate of 2100 g a.s./ha (achieved rate 2140 g a.s./ha, 5.8N with respect to the potato GAP) in 2014 and rotational crops (leafy vegetable, root crop, cereal) were sown after 30, 120 and 270 days.

In 2015, lettuce (*Lactuca sativa L.*, variety 'salad bowl', leafy vegetable), radish (*Raphanus sativus L.*, variety 'crimson giant', root crop), and wheat (*Triticum aestivum,* variety 'blanca royale', small grain) were sown for the 30-day, 120-day and 270-day plant-back periods. Crops were grown on outdoor plots (0.75 m^2 surface area and 35.6 cm column depth) in California, USA. Rainfall was supplemented by artificial irrigation where necessary.

Test Soil:

The soil in the plots was classified as a sandy loam. Further details are tabulated below.

Parameter	1988
Texture Class (USDA)	Sandy loam
pH	7.0
Organic matter (%)	0.51
Cation exchange capacity (meq/100 g)	11.4
USDA classification	
Sand % Silt % Clay %	63 21 16

Experimental Conditions:

[^{14}C]-flutolanil was prepared as a 40SC formulation. The [^{14}C]-formulation was applied to bare soil on 18 December 2014 (120 and 270-day plantback periods) and 18 March 2015 (30 plant back period) by a manual trigger-pulled pump sprayer. The achieved application rate was 510 g a.s./ha.

Crops were sown on 17 April 2015 (30-day and 120-day plant-back intervals) and 14 September 2015 (270-day plant back interval). Crops were grown to maturity outdoors at the field site in California, USA, harvested and shipped frozen to the analytical laboratory California, USA.

Test Samples

Plant samples (both treated and control) were taken for analysis at the following times:

Plant-back interval	Plant sample	Harvest date	Days after sowing
30 days	Lettuce (immature)	01 Jun 2015	44
	Lettuce (mature)	15 Jun 2015	58
	Radish (roots and tops)	28 May 2015	41
	Wheat (forage)	27 May 2015	40
	Wheat (hay)	15 Jun 2015	58
	Wheat (straw and grain)	08 Jul 2015	81
120 days	Lettuce (immature)	01 Jun 2015	44
	Lettuce (mature)	15 Jun 2015	58
	Radish (roots and tops)	28 May 2015	41
	Wheat (forage)	27 May 2015	40
	Wheat (hay)	15 Jun 2015	58
	Wheat (straw and grain)	08 Jul 2015	81
270 days	Lettuce (immature)	23 Oct 2015	39
	Lettuce (mature)	11 Nov 2015	57
	Radish (roots and tops)	23 Oct 2015	39
	Wheat (forage)	23 Oct 2015	39
	Wheat (hay)	16 Nov 2015	62
	Wheat (straw and grain)	19 Apr 2016	215

Sample Preparation

Immature harvest consisting of all the crops was taken when the young plants required thinning to normal agricultural spacing. In cereals this was chosen to approximate the stage at which the crop might be used as forage.

Final harvest was taken when the plants reached maturity. Lettuce and the cereal crops were harvested by excising at soil level. The mature cereals were divided into straw, and grain. Root crops were harvested by pulling them from the soil before dividing them into aerial portion and roots. Soil adhering to the root crop was removed by water washing.

Crop samples were frozen immediately after sampling. Frozen crop samples were homogenised with dry ice or liquid nitrogen, which was then allowed to sublimate prior to further analysis. Total radioactive residues (TRR) were determined by combustion and liquid scintillation counting (LSC). Homogenised samples were stored at -20°C until required for analysis.

Extraction and Fractionation of Residues

Samples of homogenised plant samples were sequentially extracted by Waring blender. Samples were extracted using the following sequence of solvents:

2 – 3 × acetonitrile:water (1:1 v/v)

1 – 3 × acetonitrile

Following each extraction, the extract was separated from the plant solids by centrifugation. The radioactivity in each extract was determined by LSC and the combined extracts were then concentrated by rotary evaporation vacuum for HPLC and TLC analyses. Post extraction solids (PES) exceeding 10% TRR were further extracted sequentially as follows:

1 × 0.1N HCl:acetonitrile (1:4 v/v)

1 × 1N HCl:acetonitrile (1:4 v/v)

1 × 0.1N NaOH:acetonitrile (1:4 v/v)

1 × 1N NaOH:acetonitrile (1:4 v/v)

Remaining PES from the wheat straw and wheat grain were additionally further extracted using the following steps:

1 × cellulase extraction: (EC 3.2.1.4 from *Aspergillus Niger* in 100 mM acetate buffer pH 5 at 37°C for 24 hours).

1 × 6 N HCl: Reflux for 2 hours.

1 x 10 N NaOH: 110°C for 2 hours

PES were subjected to combustion analysis to quantify any un-extracted bound radioactivity.

Combined weak acidic/basic extracts containing >0.05 mg eq./kg (>10% TRR) were analysed by HPLC and TLC. Major metabolites were identified by retention time matching against reference standards by HPLC or by isolating radioactive HPLC peaks and confirming their identity by TLC analysis against reference standards. The polar solvent front isolate from the HPLC chromatograms was identified as TFA by LC-MS and confirmed by HPLC and TLC against a ^{14}C-trifluoroacetic sodium salt reference standard. Other unknown isolates from the chromatograms were subject to enzymatic hydrolysis (β-glucosidase (EC 3.2.1.21 from almonds, 100 mM acetate buffer (pH 5) at 37°C for 24 hours) or acidic hydrolysis (2N HCl at 55 – 60°C for 24 hours) followed by HPLC/TLC analysis.

II. RESULTS AND DISCUSSION

Total Radioactive Residues and Extractability

The total radioactive residues (TRR) measured in rotation crops by combustion are summarised below in Table B.7.6.1.3-1. Residue levels were rather variable in the raw agricultural commodities with some increases seen between the 30-day and 120-day plant-back intervals; however, residues levels in the RACs had declined substantially by the 270-day plant-back interval

Table B.7.6.1.3-1: Summary of radioactive residues in rotational crops following treatment of soil with 2140 g a.s./ha [phenyl-U-^{14}C]-flutolanil

Crop		Total Radioactive Residue (mg a.s. eq./kg by combustion)		
		30-day plant-back	120-day plant-back	270-day plant-back
Lettuce	Immature	4.408	2.694	0.195
	Mature	2.083	1.838	0.233
Radish	Root	0.780	0.632	0.088
	Top	5.673	4.236	0.492
Wheat	Forage	6.521	12.605	1.444
	Hay	8.936	25.442	0.779
	Straw	11.288	14.578	0.667
	Grain	3.737	3.143	0.360

The distribution of crop radioactive residues amongst the acetonitrile:water extract; weak acidic/basic extract; cellulase extract and strong acidic/basic extract and the radioactive residue remaining bound are summarised in table B.7.6.1.3-2. Residues in the acetonitrile:water and weak acidic/basic extract were identified by HPLC/TLC; residues in the cellulase and strong acidic/basic extracts were characterised into aqueous and organic soluble fractions and the residue in the organic-soluble fraction of the strong acidic/basic extract was identified by HPLC/TLC. For all commodities, exhaustive extraction by cellulase and strong acid/base did not release a significant additional residue. The level of extraction achieved was in accordance with OECD 502 (2007).

The summed identification and characterisation of radioactive residues in the acetonitrile:water; weak acidic/basic extract and organic-soluble fraction of the strong acidic/basic extract are summarised below in Table B.7.6.1.3-3 (lettuce); Table B.7.6.1.3-4 (radish) and Table B.7.6.1.3-5 (wheat). Identified metabolites were found only in the acetonitrile-water extracts, except for hay from wheat sown 30 DAA were also some small fractons were identified in the combined weak acid/alkaline extracts (weak acid/alkaline extracts are considered elevant for human exposure as well).

Table B.7.6.1.3-2: Summary of TRR and extractability in rotational crops following treatment of soil with 2140 g a.s./ha [phenyl-U-^{14}C]-flutolanil

Plant-back interval / days	Crop		TRR by Extraction	Combined acetonitrile:water extract		Combined weak acidic/basic extract		Cellulase extract		Combined strong acidic/basic extract		Post extraction solids	
			mg/kg	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
30	Lettuce	Immature	4.258	4.169	97.9	-	-	-	-	-	-	0.089	2.1
		Mature	2.195	2.087	95.1	-	-	-	-	-	-	0.108	4.9
	Radish	Root	0.719	0.525	73.0	0.016	2.2	0.013	1.8	0.068	9.4	0.178	24.8
		Top	5.695	5.513	96.8	-	-	-	-	-	-	0.182	3.2
	Wheat	Forage	6.550	5.955	90.9	-	-	-	-	-	-	0.595	9.1
		Hay	9.061	8.037	88.7	0.493	5.4	-	-	-	-	0.530	5.8
		Straw	9.669	7.885	81.5	0.468	4.8	0.072	0.7	0.829	8.5	1.318	13.6
		Grain	3.553	2.546	71.7	0.152	4.3	0.367	10.3	0.436	12.3	0.855	24.1
120	Lettuce	Immature	2.601	2.572	98.9	-	-	-	-	-	-	0.029	1.1
		Mature	2.033	1.976	97.2	-	-	-	-	-	-	0.057	2.8
	Radish	Root	0.662	0.528	79.8	0.016	2.2	0.005	0.8	0.051	7.7	0.121	18.3
		Top	4.090	3.968	97.0	-	-	-	-	-	-	0.122	3.0
	Wheat	Forage	11.073	10.475	94.6	-	-	-	-	-	-	0.595	9.1
		Hay	24.293	21.849	89.9	0.777	3.2	-	-	-	-	1.667	6.9
		Straw	12.462	10.076	80.9	0.899	7.2	0.158	1.3	0.911	7.3	1.486	11.9
		Grain	2.984	2.171	72.8	0.103	3.5	0.292	9.8	0.350	11.7	0.710	23.8
270	Lettuce	Immature	0.214	0.202	94.4	-	-	-	-	-	-	0.012	5.6
		Mature	0.204	0.194	95.1	0.003	1.5	-	-	-	-	0.007	3.4
	Radish	Root	0.107	0.087	81.3	0.003	2.8	-	-	-	-	0.017	15.9
		Top	0.480	0.463	96.5	-	-	-	-	-	-	0.017	3.5
	Wheat	Forage	1.512	1.375	90.9	-	-	-	-	-	-	0.137	9.1
		Hay	0.695	0.623	89.6	0.035	5.0	-	-	-	-	0.037	5.3
		Straw	0.660	0.462	70.0	0.069	10.5	-	-	-	-	0.129	19.5
		Grain	0.363	0.298	82.1	0.005	1.4	-	-	-	-	0.060	16.5

Table B.7.6.1.3-3 : Summary of the identification of the residue in rotational crops following treatment of soil with 2140 g a.s./ha [phenyl-U-^{14}C]-flutolanil - lettuce

Identification of the residue	Lettuce (immature)						Lettuce (mature)					
	30 days plantback		120 days plantback		270 days plantback		30 days plantback		120 days plantback		270 days plantback	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Flutolanil	0.042	1.0	-	-	-	-	-	-	0.024	1.2	-	-
M-2	-	-	-	-	-	-	-	-	-	-	-	-
M-3	-	-	-	-	-	-	-	-	-	-	-	-
M-3 glycoside	-	-	-	-	-	-	-	-	-	-	-	-
M-4	-	-	-	-	-	-	-	-	-	-	-	-
M-4 glycoside	0.417	9.8	0.183	7.0	0.012	5.6	0.154	7.0	0.115	5.7	-	-
M-5	-	-	-	-	-	-	-	-	-	-	-	-
M-6	-	-	-	-	-	-	-	-	-	-	-	-
M-7	-	-	-	-	-	-	-	-	-	-	-	-
M-11	-	-	-	-	-	-	-	-	-	-	-	-
M-101	2.253	52.9	1.615	62.1	0.029	13.6	0.639	29.1	0.909	44.7	0.008	3.9
M-102	0.484	11.4	0.267	10.3	0.040	18.7	0.445	20.3	0.397	19.5	0.062	30.4
M-102 glycoside	-	-	-	-	-	-	0.125	5.7	-	-	0.027	13.2
TFA	0.280	6.6	0.162	6.2	0.064	29.9	0.125	5.7	0.140	6.9	0.080	39.2
Unknown RT 15.6 min	-	-	-	-	-	-			-	-	-	-
Unknown RT 14.4 – 14.5 min	-	-	-	-	-	-					-	-
Others	0.697	16.4	0.345	13.3	0.057	26.6	0.599	27.3	0.391	19.2	0.016	7.8
(Largest other)	0.088	2.1	0.067	2.6	0.01	4.7	0.081	3.7	0.069	3.4	0.007	3.4

Table B.7.6.1.3-4: Summary of the identification of the residue in rotational crops following treatment of soil with 2140 g a.s./ha [phenyl-U-^{14}C]-flutolanil – radish

Identification of the residue	Radish (roots)						Radish (tops)					
	30 days plantback		120 days plantback		270 days plantback		30 days plantback		120 days plantback		270 days plantback	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Flutolanil	0.015	2.1	0.013	2.0	0.001	0.9	-	-	-	-	-	-
M-2	-	-	-		-	-	-	-	-	-	-	-
M-3	-	-	-		-		-	-	-	-	-	-
M-3 glycoside	-	-	-		-		-	-	-	-	-	-
M-4	0.016	2.2	0.008	1.2	0.003	2.8	-	-	-	-	-	-
M-4 glycoside	0.049	6.8	0.033	5.0	-		-	-	-	-	-	-
M-5	0.015	2.1	0.007	1.1	0.001	0.9	-	-	0.147	3.6		
M-6	-	-	-	-	0.001	0.9	-	-	-	-	-	-
M-7	-	-	-	-	0.001	0.9	-	-	0.020	0.5	-	-
M-11	-	-	-	-	-	-	-	-	-	-	-	-
M-101	0.089	12.4	0.131	19.8	0.006	5.6	3.076	54.0	2.595	63.4	0.039	8.1
M-102	0.022	3.1	0.108	16.3	0.020	18.7	0.259	4.5	0.139	3.4	0.035	7.3
M-102 glycoside	0.093	12.9	0.145	21.9	0.014	13.1	0.419	7.4	0.381	9.3	0.088	18.3
TFA	0.057	7.9	0.073	11.0	0.013	12.1	0.430	7.6	0.444	10.9	0.190	39.6
Unknown RT 21.8 min	0.049	6.8	-	-	-	-	-	-	-	-	-	-
Unknown RT 14.4 – 14.5 min					-	-	-	-	-	-	-	-
Others	0.120	16.7	0.010	1.5	0.027	25.2	1.329	23.3	0.242	5.9	0.110	22.9
(Largest other)	0.022	3.1	0.010	1.5	0.005	4.7	0.265	4.7	0.127	3.1	0.012	2.5

Table B.7.6.1.3-5: Summary of the identification of the residue in rotational crops following treatment of soil with 2140 g a.s./ha [phenyl-U-^{14}C]-flutolanil – wheat

Identification of the residue	Wheat (forage)						Wheat (hay)					
	30 days plantback		120 days plantback		270 days plantback		30 days plantback*		120 days plantback		270 days plantback	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Flutolanil	-	-	0.052	0.5	-	-	-	-	-	-	-	-
M-2	-	-	-	-	-	-	-	-	-	-	-	-
M-3	-	-	-	-	0.014	0.9	0.008	0.1	-	-	-	-
M-3-glycoside	-	-	-	-	-	-	-	-	-	-	-	-
M-4	-	-	-	-	-	-	0.012	0.1	-	-	-	-
M-4 glycoside	-	-	-	-	-	-	-	-	-	-	-	-
M-5	-	-	-	-	-	-	0.072	0.8	-	-	0.013	1.9
M-6	-	-	-	-	-	-	-	-	-	-	-	-
M-7	-	-	-	-	0.014	0.9	-	-	-	-	-	-
M-11	-	-	-	-	-	-	0.008	0.1	-	-	-	-
M-101	3.531	53.9	7.060	63.8	0.081	5.4	1.947	21.5	11.340	46.7	0.023	3.3
M-102	0.911	13.9	0.807	7.3	0.359	23.7	1.501	16.6	2.600	10.7	0.267	38.4
M-102 glycoside	-	-	-	-	0.106	7.0	-	-	-	-	-	-
TFA	0.828	12.6	0.869	7.8	0.157	10.4	0.876	9.7	1.464	6.0	0.121	17.4
Unknown RT 22.1 – 22.4 min	-	-	-	-	-	-	0.579	6.4	1.486	6.1	-	-
Unknown RT 17.6 min	-	-	-	-	-	-	-	-	-	-	0.054	7.8
Unknown RT 4.5 min	-	-	-	-	-	-	-	-	1.464	6.0	-	-
Others	0.685	10.5	1.686	15.2	0.645	42.7	3.387	37.4	0.777	3.2	0.145	20.9
(Largest other)	0.208	3.2	0.178	1.6	0.039	2.6	0.378	4.2	1.667	6.9	0.033	4.7

* After acetonitrile extraction, known metabolites (M-3, M-4, M-11, M010, M102) were also released form weak acid and alkaline extraction

Identification of the residue	Wheat (straw)						Wheat (grain)					
	30 days plantback		120 days plantback		270 days plantback		30 days plantback		120 days plantback		270 days plantback	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Flutolanil	-	-	-	-	-	-	-	-	-	-	-	-
M-2	-	-	-	-	-	-	-	-	-	-	-	-
M-3	0.189	2.0	0.081	0.6	-	-	-	-	-	-	-	-
M-3 glycoside	-	-	-	-	-	-	-	-	-	-	-	-
M-4	0.087	0.9	0.081	0.6	0.011	1.7	-	-	-	-	-	-
M-4 glycoside	-	-	-	-	-	-	-	-	-	-	0.018	5.0
M-5	0.008	0.1	0.060	0.5	-	-	0.048	1.4	0.061	2.0	-	-
M-6	-	-	-	-	-	-	-	-	-	-	-	-
M-7	0.024	0.2	-	-	-	-	-	-	-	-	0.003	0.8
M-11	-	-	-	-	0.017	2.6	-	-	-	-	-	-
M-101	0.213	2.2	1.118	9.0	0.016	2.4	0.023	0.6	0.039	1.3	0.020	5.5
M-102	1.230	12.7	0.463	3.7	0.083	12.6	0.535	15.1	0.362	12.2	0.120	33.0
M-102 glycoside	-	-	-	-	-	-	-	-	-	-	-	-
TFA	0.505	5.2	1.235	9.9	0.135	20.5	0.379	10.7	0.165	5.5	0.041	11.3
Unknown RT 3.7 – 4.8 min	0.678	7.0	0.665	5.3	-	-	-	-	-	-	-	-
Others	4.952	51.2	5.562	44.6	0.200	30.3	1.561	43.9	1.544	51.7	0.096	26.4
(Largest other)	0.315	3.3	0.363	2.9	0.032	4.8	0.109	3.1	0.115	3.9	0.015	4.1

In rotational crops at the 30-day plant-back interval, the TRR varied between 0.780 mg/kg (radish root) and 11.288 mg/kg (wheat straw). The residue was readily extractable with between 71.7% (wheat grain) and 97.7% (mature lettuce) of the TRR extracted by acetonitrile:water. Little further radioactivity was extracted by more exhaustive steps apart from in wheat grain where a further 10.3% was extracted by cellulase and 12.3% was extracted by strong acid/base reflux and in radish root where a further 9.4% was extracted by strong acid/base reflux. Bound residues were low (<25% TRR) with only radish root, wheat straw and wheat grain containing >10% TRR in the post extraction solids (24.8%, 13.6% and 24.1% respectively). Identification of the residue indicates that parent flutolanil is not a significant component of the residue and was only detected immature lettuce and radish root at 0.042 mg/kg, 1.0% and 0.015 mg/kg, 2.1% respectively. The known plant metabolites M-3, M-4, M-5, M-7, M-101 and M-102 were variously detected indicating a similar pattern of metabolism to primary crops. All of these metabolites apart from M-101 and M-102 were only present as very minor components. In addition to M-101 and M-102, TFA was present as a significant metabolite 0.125 mg/kg, 5.7% in mature lettuce, 0.057 mg/kg, 7.9% in radish roots, 0.505 mg/kg, 5.2% in wheat straw and 0.379 mg/kg, 10.7% in wheat grain).

In rotational crops at the 120-day plant-back interval, the TRR varied between 0.632 mg/kg (radish root) and 25.442 mg/kg (wheat hay), apparently generally increasing in magnitude in the various crop commodities compared to the 30-day plantback interval. The residue was readily extractable with between 72.8% (wheat grain) and 98.9% (mature lettuce) of the TRR extracted by acetonitrile:water. Little further radioactivity was extracted by more exhaustive steps apart from in wheat grain where a further 9.8% was extracted by cellulase and 11.7% was extracted by strong acid/base reflux. Bound residues were low (<25% TRR) with only radish root, wheat straw and wheat grain containing >10% TRR in the post extraction solids (18.3%, 11.9% and 23.8% respectively). Identification of the residue indicates that parent flutolanil is not a significant component of the residue and was only detected in mature lettuce, radish roots and wheat forage at 0.024 mg/kg (1.2%TRR), 0.013 mg/kg (2.0%TRR) and 0052 mg/kg (0.5%TRR), respectively. The known plant metabolites M-3, M-4, M-5, M-7, M-11, M-101 and M-102, were variously detected indicating a similar pattern of metabolism to primary crops. All of these metabolites apart from M-101 and M-102 were only present as very minor components. In addition to M-101 and M-102, TFA was present as a significant metabolite (0.140 mg/kg, 6.9% in mature lettuce, 0.073 mg/kg, 11.0% in radish roots, 1.245 mg/kg, 9.9% in wheat straw and 0.165 mg/kg, 5.5% in wheat grain).

In rotational crops at the 270-day plant-back interval, residues had substantially declined and the TRR varied between 0.088 mg/kg (radish root) and 1.444 mg/kg (wheat forage). The residue was readily extractable with between 70.0% (wheat straw) and 96.5% (radish tops) of the TRR extracted by acetonitrile:water. An additional 10.5% of the TRR was extracted from wheat straw by weak acidic/basic extraction. The low residues levels meant that identification of the residue was limited and the metabolites M-4, M-5, M-6, M-7, M-101 and M-102 were detected at trace levels. TFA was the most prominent metabolite (0080 mg/kg, 39.2% in mature lettuce, 0.013 mg/kg, 12.1% in radish roots, 0.135 mg/kg, 20.5% in wheat straw and 0.041 mg/kg, 11.3% in wheat grain).

The levels of TFA reported in the rotational crop metabolism study are reported as parent equivalents and thus overestimate underestimate the absolute amount of TFA in the samples due to the

respective molecular weights of flutolanil (323.31 g/mol) and TFA (114.02 g/mol). Additionally, since TFA contains only a single carbon atom derived from the [phenyl-U-^{14}C] ring a further 6-fold correction is applied in accordance with the precedent of the EFSA peer review of the pesticide risk assessment for the active substance flurtamone (EFSA Journal 2016;14(6):4498, 112 pp. doi:10.2903/j.efsa.2016.4498). This gives a correction factor of 0.3527 for the relative molecular weights and a further correction factor of 6 for the relative amount of label giving a net correction of 2.1162. The corrected concentration of TFA in the rotational crop commodities after application of this correction is summarised below.

Crop		Concentration of TFA (mg/kg)					
		30-day plantback		120-day plantback		270-day plantback	
		mg/kg parent equivalents	mg/kg TFA	mg/kg parent equivalents	mg/kg TFA	mg/kg parent equivalents	Corrected concentration
Lettuce	Immature	0.280	0.593	0.162	0.343	0.064	0.135
	Mature	0.125	0.265	0.140	0.296	0.080	0.169
Radish	Root	0.057	0.121	0.073	0.154	0.013	0.028
	Top	0.430	0.910	0.444	0.940	0.190	0.402
Wheat	Forage	0.828	1.752	0.869	1.839	0.157	0.332
	Hay	0.876	1.854	1.464	3.100	0.121	0.256
	Straw	0.505	1.069	1.235	2.614	0.135	0.286
	Grain	0.379	0.802	0.165	0.349	0.041	0.087

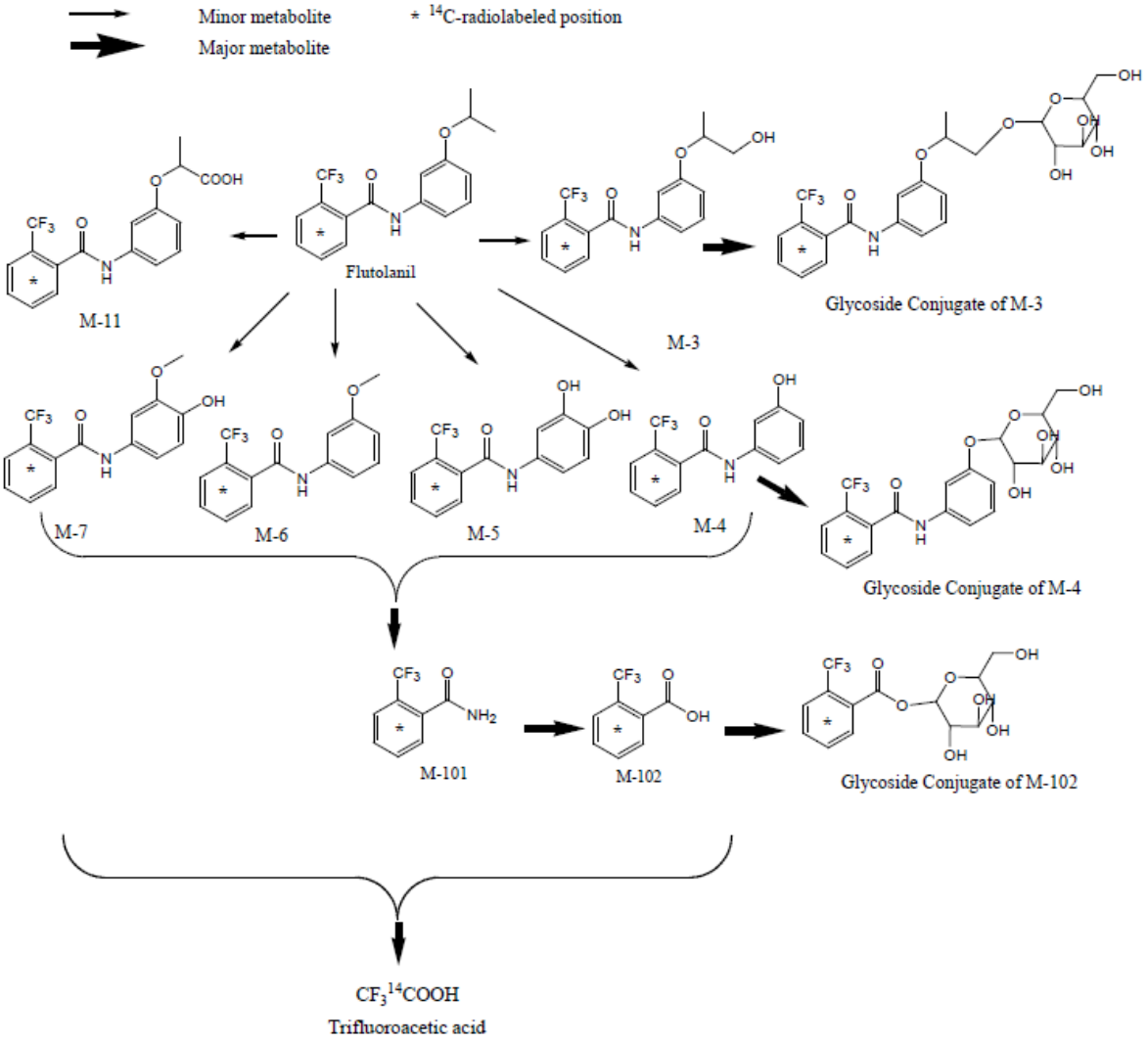
These calculations indicate that residues of TFA might be significant in rotational crops and is included as an analytical target in the ongoing field rotational crop studies in order to provide more quantitative information. As TFA can be derived from a number of pesticide and non-pesticide sources from molecules containing a CF_3 moiety it is not a good marker for flutolanil residues and is not proposed to be included in the plant residue definition for risk assessment or monitoring.

The detection of radioactive residues in all plant commodities indicates that soil residues of flutolanil are taken up by growing plants and systemically transported and metabolised in all parts of the plant.

Metabolic pathway

A metabolic pathway for [phenyl-U-^{14}C]-flutolanil in rotational crops is proposed in Figure B.7.6.1.3-1. The metabolic profile was similar in all rotational crops and similar to that seen in primary crops treated with flutolanil apart from the additional identification of TFA. This is likely to arise following the complete degradation of the phenyl ring following the formation of the metabolites M-101 and M-102.

Figure B.7.6.1.3-1: Metabolic pathway of [phenyl-U-^{14}C]-flutolanil in rotational crops



III. CONCLUSIONS

Crop residue levels increased slightly between a 30-day plantback interval and a 120-day plantback interval but declined greatly as the soil aged at the 270-day plantback interval. TRRs measured by combustion ranged from 0.780 to 11.288 mg/kg in crops planted after 30 days aging, from 0.632 to 25.442 mg/kg with 120 days aging and from 0.088 to 1.444 mg/kg with 270 days aging.

The metabolic profile was similar in all rotational crops and similar to that seen in primary crops treated with flutolanil.

The major components detected in rotational crops were:

- M-101 (0.023 to 3.531 mg/kg, 0.6 to 54.0% TRR); M-102 and its glycoside (0.115 to 1.501 mg/kg, 11.4% to 26.0% TRR) and TFA (0.057 to 0.876 mg/kg, 5.2 to 12.6% TRR from crops grown after a 30-day plant back-period (on a parent equivalent basis).
- M-101 (0.039 to 11.340 mg/kg, 1.3 to 63.8% TRR); M-102 and its glycoside (0.253 to 2.600 mg/kg, 3.7 to 38.2% TRR) and TFA (0.073 to 1.464 mg/kg, 5.5 to 11.0% TRR from crops grown after a 120-day plant-back period (on a parent equivalent basis).

- M-101 (0.006 to 0.081 mg/kg, 2.4 to 13.6% TRR); M-102 and its glycoside (0.034 to 0.465 mg/kg, 12.6 to 43.6% TRR)
- TFA was the most prominent metabolite in crops after a 270-day plantback interval (0.013 to 0.190 mg/kg, 10.4 to 39.6% TRR). Other metabolites were only found in trace amounts.
- When corrected for the relative molecular weight and labelling of TFA with respect to [phenyl-U-^{14}C]-flutolanil, the actual level of TFA in the crops was 0.121 – 1.854 mg/kg with a 30-day plant-back interval; 0.154 – 3.100 mg/kg with a 120-day plant-back interval and 0.028 – 0.402 mg/kg with a 270-day plant-back interval.
- Flutolanil was not a significant component of the residue in any of the crops at any of the plantback intervals.

B.7.6.2 Magnitude of residues in rotational crops

B.7.6.2.1 Study 1

Previous evaluation	in the DAR
RMS remark	Residues of parent compound flutolanil only have been measured in the study. The residues levels, representing unchanged fultolanil in the plant, grain and straw samples, were below the limit of quantification (0.01 mg/kg) in investigated plant matrices. No metabolites of flutolanil have been investigated in the study. From the available metabolism studies it is concluded, that flutolanil is almost completely degraded in rotational crops, hence the magnitude of the metabolites should also be determined. Moreover, no leafy crops have been included in the study. Therefore, this study is considered as supporting only.

STUDY I

Report:	Giraud, JP (2001): Flutolanil Formulation EXP10057A (DS), North/United Kingdom/1999-8 Decline study trials, Residue in winter wheat (soil, plant, grain and straw), rape (soil, plant and grain) Following crop study, Aventis CropScience, Lyon, France, Unpublished report No.: R-3059
Guideline:	Directive 96/68EC, no deviations.
GLP:	Yes (OECD/1998).
Test formulation:	Powder formulation EXP10057A (DS) with nominal a.s. concentration of 60 g/kg.
Test site:	Located at eastern United Kingdom.
Soil types:	Sandy, peaty clay loam, and sandy loam.

Material and methods

Field trials were conducted to determine the presence of flutolanil residues in winter wheat and rape (following crops) cultivated after a crop of flutolanil-treated potatoes. The field part consisted of eight trials conducted in United Kingdom (North).

One dusting application of the formulation EXP10057A (DS) was performed in each trial at a dose rate of 2.0 kg of formulation per tonne potato corresponding to 120 g a.s/tonne potato (120 mg/kg). For the intended use an application rate 92 g a.s./tonne potato (225 g/ha) is given and consequently in the present study the dose rate is 1.3 N.

Depending on trial soil samples were collected at 67-148 days after treatment. Sample plants were collected at 185-267 days after treatment. Grains were obtained for analysis at 330-448 days post-treatment.

Samples were analysed for unchanged flutolanil by GC-MS with a limit of quantification 0.010 mg/kg for plant, grain and straw matrix, and 0.005 mg/kg for soil. Flutolanil metabolite levels were not determined.

Results

The main findings of the study are presented in Table 7.9-1. While in grains, plants and straws residue levels were below LOD, 0.010 mg/kg, in soil the residue levels were in the range of 0.006 – 0.109 mg/kg.

The dosing rate 92 g a.s. /tonne potato corresponds to a initial concentration of 0.08 mg/kg in the soil given that the active substance is evenly distributed in the top 20 cm of the soil. The results show that after approximately 100 days there was more than 10% unchanged active substance left in the soil.

Table B.7.6.2.1-1 Flutolanil residues in succeeding crops, winter wheat and rape. In all experiments the main crop, potatoes, were seed-treated once at an application rate of 120 g a.s./t.

Location,		Application to main crop (Potato)		Succeeding crop			
Year	Study ID	Form	Soil type	Succeeding crop	Portion analysed	PHI days	Residue (mg/kg)
Brockdish, 1999-2000	99693GB1	DS	Sandy clay loam	Winter wheat var. Savannah	Soil Plant Grain Straw	118 238 447 447	0.059 <0.010 <0.010 <0.010
Ongar, 1999-2000	99693GB2	DS	Peaty clay loam	Winter wheat var. Savannah	Soil Plant Grain Straw	93 214 330 330	0.029 <0.010 <0.010 <0.010
Lawford, 1999-2000	99693GB3	DS	Sandy loam	Winter wheat var. Malacca	Soil Plant Grain Straw	148 267 447 447	0.013 <0.010 <0.010 <0.010
Ongar, 1999-2000	99693GB4	DS	Clay loam	Winter wheat var. Savannah	Soil Plant	132 253	0.021 <0.010
Brockdish, 1999-2000	99693GB5	DS	Sandy clay loam	Rape var. Escort	Soil Plant Grain	91 238 438	0.006 <0.010 <0.010
Ongar, 1999-2000	99693GB6	DS	Peaty clay loam	Rape var. Escort	Soil Plant Grain	67 185 401	0.010 <0.010 <0.010
Lawford, 1999-2000	99693GB7	DS	Sandy loam	Rape var. Alpine	Soil Plant Grain	105 245 440	0.109 <0.010 <0.010
Ongar, 1999-2000	99693GB8	DS	Clay loam	Rape var. Escort	Soil Plant Grain	106 224 448	0.010 <0.010 <0.010

Conclusion

In this study a powder formulation was used and, consequently, the study is at slight variance as compared with the intended use, in which the use of a suspension concentrate is disclosed.

The residues levels, representing unchanged fultolanil in the plant, grain and straw samples, were below limit of quantification (0.01 mg/kg). In soil residue level was 0.03 ± 0.04 mg/kg in average. The results indicate that there is little uptake of a.s. from the soil by the succeeding crops studied.

Consequently, as compared to criteria set in Lundehn study, the residue levels for unchanged flutolanil in succeeding wheat and rape grains did not exceed the trigger level of 0.01 mg eq/kg.

The present rotantional crop study did not address characterization of the chemical nature of the residue although this should have been one of the study objectives.

B.7.6.2.2 Study 2

Previous evaluation	Submitted for the purpous of the renewal

RMS remark	Acceptable

Reference:	CA 6.6.2/02: Raufer B., 2017
Title:	Determination of residues of flutolanil after one application of Moncut 40SC (EU) on bare soil rotational crops (radish, spinach and cereals) at 2 sites in Southern and Northern Europe 2014 / 2015
Document No.:	S14-04011(R-3405)
Guidelines:	7524/VI/95, rev. 2 and OECD guideline 504 covering the crop groups root and tuber vegetables (radish), leafy vegetables (spinach) and small grains (barley)
GLP:	Yes

Executive Summary

Field trials were conducted to determine residue levels of Flutolanil and its metabolites M-2, M-4, M-102 and their conjugates and M-101 in follow up crops (spinach, radish and barley) following a cultural practice typical for this crop protection.

Two trials were conducted during 2014 and 2015, one in Spain (S14-04011-01) and one in Germany (S14-04011-02).

To each treated plot one application of Moncut 40SC (SC formulation containing 460 g/L flutolanil, nominal content) was performed on bare soil at plant back intervals of nominal 30, 120 and 270 days at a nominal rate of either 480 g a.s./ha (supported rate) or 2100 g a.s/ha (an exaggerated rate). The product was diluted with water immediately prior to application to a nominal spray volume of 400 L/ha. Follow up crops (spinach, radish and barley) were drilled at 30, 120 and 270 days (nominal) after application of the test item.

Residue plant samples were taken from the treated and control plots:

- from spinach at earliest commercial harvest date (BBCH 19-43) and at harvest (BBCH-49);
- from radish at earliest commercial harvest date (BBCH 43-45) and at harvest (BBCH 49);
- from barley at BBCH 31-33 (whole plant without roots representative for forage) and at harvest (BBCH 89).

The plant specimens were analysed for residues of Flutolanil and its metabolites M-2, M-4, M-102 and their conjugates and M-101. The final determination of the analytes in the untreated and treated specimens was performed by extraction with acetonitrile/1 M hydrochloric acid (4/1 v/v), liquid-liquid partition by addition of sodium chloride and ethyl acetate followed by subsequent shaking and ultrasonication and SPE clean up, followed by liquid chromatography with mass spectrometric detection (LC-MS/MS).

While some residues of the metabolite M-4 were detected at the limit of quantification (0.01 mg/kg) in straw, no residues of Flutolanil, M-2, M-4, M-101 and M-102, was detected above the limit of quantification in the RAC commodities of the follow-up crops (barley grain, spinach and radish roots).

I. MATERIALS AND METHODS

A. MATERIALS

Test Material: Moncut 40SC

Active ingredient:	Flutolanil
CA registry number:	66332-96-5
Batch number:	A111019002
Content of a.s. analysed:	40.7% (w/w) or 462.3 g/L
Expiration date:	15.Oct.2017

B. STUDY DESIGN AND METHODS

Locations and Plot Design:

The residue trials are being carried out at 2 locations, one in Spain (S14-04011-01) and one in Germany (S14-04011-02). Regions of the trial sites are typical for the cultivation of potato. The Spanish trial comprised 11 treatments (3 untreated and 8 treated with Moncut 40SC) and the German trial comprised 8 treatments (2 untreated and 6 treated with Moncut 40SC).

Test System (Follow up crops):

- Radish (Raphanus sativus var. sativus), EPPO code RAPSR
- Spinach (Spinacia oleracea), EPPO code SPQOL
- Barley (Hordeum vulgare), EPPO code HORVS/HORVW

Application:

To each treated plot, one application of Moncut 40SC (SC formulation containing 460 g/L flutolanil, nominal content) was performed on bare soil at plant back intervals of nominal 30, 120 and 270 days at a nominal rate of either 480 g a.s./ha (supported GAP application rate) or 2100 g a.s/ha (exaggerated application rate) following the proposed application schedule presented in the table B.7.6.2.2-1 and Table B.7.6.2.2-2. The product was diluted with water immediately prior to application to a nominal spray volume of 400 L/ha. And after application the test item was incorporated mechanically into the soil with a plough or rotary harrow at 10 cm working depth.

Table B.7.6.2.2-1: Test Site description and Application details for the Spanish trial S14-04011-01

Appl' Code	Plot	Timing of Application	Interval until seeding	Application rate (g a.s/ha)	Cultivar (Variety)	Planting or seeding date
-	U1	-	PBI 28	-	Spinach (Gigante)	27.Oct.14
					Barley (Unia)	27.Oct.14
					Radish (Redondo)	Cancelled*
-	U2	-	PBI 121 and 270	-	Spinach (Gigante)	05.Apr.15
					Barley (Unia)	05.Apr.15
					Radish (Redondo)	05.Apr.15
-	U3**	-	PBI 28 (repeated radish)	-	Radish (Redondo)	30.Jul.15
A1	7	09.Jul.14	PBI 270	492	Spinach (Gigante)	05.Apr.15
					Barley (Unia)	05.Apr.15
					Radish (Redondo)	05.Apr.15

	8	09.Jul.14	PBI 270	2176	Spinach (Gigante)	05.Apr.15
					Barley (Unia)	05.Apr.15
					Radish (Redondo)	05.Apr.15
A2	3	29.Sep.14	PBI 28	484	Spinach (Gigante)	27.Oct.14
					Barley (Unia)	27.Oct.14
					Radish (Redondo)	Cancelled*
	4	29.Sep.14	PBI 28	2217	Spinach (Gigante)	27.Oct.14
					Barley (Unia)	27.Oct.14
					Radish (Redondo)	Cancelled*
A3	5	05.Dec.14	PBI 121	495	Spinach (Gigante)	05.Apr.15
					Barley (Unia)	05.Apr.15
					Radish (Redondo)	05.Apr.15
	6	05.Dec.14	PBI 121	2182	Spinach (Gigante)	05.Apr.15
					Barley (Unia)	05.Apr.15
					Radish (Redondo)	05.Apr.15
A4*	9	30.Jun.15	PBI 30	486	Radish (Redondo)	30.Jul.15
	10	30.Jun.15	PBI 30	2062	Radish (Redondo)	30.Jul.15

* Plot repeated for radish with a PBI 30 only.

Table B.7.6.2.2-2: Test Site description and Application details for the German trial S14-04011-02

Appl' Code	Plot	Timing of Application	Interval until seeding	Application rate (g a.s/ha)	Cultivar (Variety)	Planting or seeding date
-	U1	-	PBI 29	-	Spinach (Molokai)	27.Aug.14
			PBI 29		Radish (Alex)	27.Aug.14
			PBI 30		Barley (Naomie)	26.Sep.14
-	U2	-	PBI 120 and 273	-	Spinach (Molokai)	24.Mar.15
					Radish (Alex)	24.Mar.15
					Barley (Naomie)	24.Mar.15
A1	7	24.Jun.14	PBI 273	525	Spinach (Molokai)	24.Mar.15
					Radish (Alex)	24.Mar.15
					Barley (Naomie)	24.Mar.15
	8	24.Jun.14	PBI 273	2134	Spinach (Molokai)	24.Mar.15
					Radish (Alex)	24.Mar.15
					Barley (Naomie)	24.Mar.15
A2	3	29.Jul.14	PBI 29	497	Spinach (Molokai)	27.Aug.14
		29.Jul.14	PBI 29	497	Radish (Alex)	27.Aug.14
		27.Aug.14	PBI 30	513	Barley (Naomie)	26.Sep.14
	4	29.Jul.14	PBI 29	2153	Spinach (Molokai)	27.Aug.14
		29.Jul.14	PBI 29	2153	Radish (Alex)	27.Aug.14
		27.Aug.14	PBI 30	2053	Barley (Naomie)	26.Sep.14
A3	5	24.Nov.14	PBI 120	480	Spinach (Molokai)	24.Mar.15
					Radish (Alex)	24.Mar.15

					Barley (Naomie)	24.Mar.15
	6	24.Nov.14	PBI 120	2111	Spinach (Molokai)	24.Mar.15
					Radish (Alex)	24.Mar.15
					Barley (Naomie)	24.Mar.15

Test Samples:
Follow up crops (spinach, radish and barley) were drilled at 30, 120 and 270 days (nominal) after application of the test item.
Residue plant samples were taken from the treated and control plots:

- from spinach at earliest commercial harvest date (BBCH 19-43) and at harvest (BBCH-49);
- from radish at earliest commercial harvest date (BBCH 43-45) and at harvest (BBCH 49);
- from barley at BBCH 31-33 (whole plant without roots representative for forage) and at harvest (BBCH 89).

Extraction:
The analytical samples were stored deep frozen until shipment to the analytical laboratory.
The plant specimens were analysed for residues of Flutolanil and its metabolites M-2, M-4, M-101 and M-102, following an adopted method form the validated analytical method S16-00710 (A-3081) (Validation data are summarised in Document M-CA4 point CA 4.1.2/014). The final determination of the analytes in the untreated and treated specimens was performed by extraction with acetonitrile/1 M hydrochloric acid (4/1 v/v), liquid-liquid partition by addition of sodium chloride and ethyl acetate followed by subsequent shaking and ultrasonication and SPE clean up, followed by liquid chromatography with mass spectrometric detection (LC-MS/MS).

The limit of quantification (LOQ) was 0.01 mg/kg for all analytes and plant matrices. The limit of determination (LOD) was 0.003 mg/kg for all analytes and plant matrices. For analytes M-2, M-4, M-101 and M-102 the LOQ is expressed as parent equivalent.

II. RESULTS AND DISCUSSION

Method validation/Procedural recovery

Recovery experiments at 0.01 mg/kg (LOQ) and 1. 0 mg/kg (10*LOQ) were performed at n= 5 per level per matrix. The mean recovery at each fortification level was in the range of 70 - 110 % with a relative standard deviation of □□20 % for all analytes in all tested matrices except for flutolanil parent at 0.01 mg/kg in wheat straw were one recovery was only 44% (mean 66%) and M-4 in barley whole plant and barley straw at 1.0 mg/kg (mean values of 69% and 67%, respectively. Overall, the recoveries comply with the standard acceptance criteria.

Table B.7.6.2.2-3 Procedural recoveries for flutolanil parent at Mass Transition 324→242 m/z

Matrix	Fortification level (mg/kg)	Recovery		
		individual values	Mean	RSD

Radish (leaves)	0.01	95, 81, 106, 101, 70	91	16
	1.0	92, 87, 78, 90, 88	87	6
Radish (roots)	0.01	76, 93, 93, 92, 95	90	8
	1.0	87, 91, 91, 87, 93	90	3
Spinach (leaves)	0.01	84, 77, 78, 99, 90	86	11
	1.0	90, 89, 92, 76, 80	85	8
Barley (whole plant w/o roots)	0.01	79, 74, 72, 74, 75	75	4
	1.0	69, 72, 73, 75, 72,	72	3
Barley (grain)	0.01	92, 85, 82, 82, 93	87	6
	1.0	96, 92, 80, 87, 82	87	8
Barley (straw)	0.01	44, 72, 71, 72, 70 (when 44 excluded:)	66 71	12 1
	1.0	71, 78, 67, 68, 67	70	7

Table B.7.6.2.2-4 Procedural recoveries for M-2 at Mass Transition 340→258 m/z

Matrix	Fortification level (mg/kg)	Recovery		
		individual values	Mean	RSD
Radish (leaves)	0.01	109, 95, 86, 93, 74	91	14
	1.0	96, 89, 80, 85, 89	88	7
Radish (roots)	0.01	74, 96, 95, 92, 92	90	10
	1.0	84, 89, 92, 87, 96	90	5
Spinach (leaves)	0.01	79, 78, 77, 91, 89	83	8
	1.0	83, 86, 74, 77, 81	80	6
Barley (whole plant w/o roots)	0.01	87, 84, 81, 91, 86	86	4
	1.0	71, 79, 82, 84, 79	79	6
Barley (grain)	0.01	86, 83, 75, 80, 91	83	7
	1.0	94, 89, 79, 85, 80	85	7
Barley (straw)	0.01	75, 77, 78, 80, 80	78	3
	1.0	74, 83, 69, 66, 67	72	10

Table B.7.6.2.2-5 Procedural recoveries for M-4 at Mass Transition 282→262 m/z

Matrix	Fortification level (mg/kg)	Recovery		
		individual values	Mean	RSD
Radish (leaves)	0.01	96, 82, 79, 74, 68	80	13
	1.0	86, 81, 65, 76, 82	78	10
Radish (roots)	0.01	70, 85, 86, 90, 89	84	10
	1.0	75, 78, 89, 83, 87	82	7
Spinach (leaves)	0.01	80, 76, 68, 89, 82	79	10
	1.0	75, 77, 81, 67, 71	74	7
Barley (whole plant w/o roots)	0.01	76, 74, 70, 72, 70	72	4
	1.0	63, 67, 72, 72, 70	69	6
Barley (grain)	0.01	92, 76, 77, 78, 85	82	8
	1.0	87, 86, 75, 85, 75	82	7
Barley (straw)	0.01	79, 71, 64, 69, 74	71	8
	1.0	71, 76, 67, 61, 60	67	10

Table B.7.6.2.2-6 Procedural recoveries for M-101 at Mass Transition 190→170 m/z

Matrix	Fortification level (mg/kg)	Recovery		
		individual values	Mean	RSD
Radish (leaves)	0.01	96, 93, 81, 93, 83	89	8
	1.0	82, 80, 86, 96, 89	87	7
Radish (roots)	0.01	89, 95, 96, 89, 100	94	5

	1.0	91, 93, 94, 91, 95	93	2
Spinach (leaves)	0.01	90, 95, 81, 101, 99	93	9
	1.0	86, 87, 91, 79, 84	85	5
Barley (whole plant w/o roots)	0.01	92, 83, 96, 95, 91	91	6
	1.0	86, 93, 90, 97, 87	91	5
Barley (grain)	0.01	100, 90, 88, 85, 88	90	6
	1.0	89, 92, 85, 89, 84	88	4
Barley (straw)	0.01	89, 81, 74, 79, 65	78	11
	1.0	89, 95, 81, 67, 77	82	13

Table B.7.6.2.2-7 Procedural recoveries for M-102 at Mass Transition 189→145 m/z

Matrix	Fortification level (mg/kg)	Recovery		
		individual values	Mean	RSD
Radish (leaves)	0.01	92, 89, 92, 96, 73	88	10
	1.0	82, 84, 86, 85, 81	84	3
Radish (roots)	0.01	77, 103, 95, 94, 90	92	10
	1.0	79, 85, 84, 80, 83	82	3
Spinach (leaves)	0.01	85, 92, 80, 86, 90	87	5
	1.0	85, 86, 85, 99, 89	89	7
Barley (whole plant w/o roots)	0.01	83, 83, 77, 78, 83	81	4
	1.0	60, 71, 71, 67, 66, 87, 82	72	13
Barley (grain)	0.01	82, 83, 81, 81, 91	84	5
	1.0	81, 92, 83, 82, 83	84	5
Barley (straw)	0.01	83, 82, 80, 75, 78	80	4
	1.0	71, 70, 76, 76, 76	74	4

Outcome of the field trials

Due to the heat-damaged from the very high summer temperature, the PBI 120 and 270 of the Spanish spinach subplot of the trial S14-04011-01 have been cancelled. The subplot have repeated in a separate study.

All reported trials at the application rate of 0.480 kg a.s./ha were conducted following the proposed good agricultural practice (GAP). Results from a higher application rate of 2.1 kg a.s./ha are available and show some residue levels. However, this application rate is not considered relevant for the representative uses supported at renewal.

The main findings of the study are presented in the table B.7.6.2.2-8 and Table B.7.6.2.2-9. While some residues of the metabolite M-4 were detected at the limit of quantification (0.01 mg/kg) in straw, no residues of Flutolanil, M-2, M-4, M-101 and M-102, was detected above the limit of quantification in the RAC commodities of the follow-up crops (barley grain, spinach and radish roots).

Table **B.7.6.2.2-8**: Trial S14-04011-01 (Spain): Flutolanil residues in succeeding crops (spinach, radish and barley)

Applic. Rate (kg a.s./ha)	PBI (days)	Crop	BBCH	DAA	Residue levels (mg/kg)				
					Flutolanil	M-2	M-4	M-101	M-102
0.484	28	Spinach (leaves)	49	190	<0.01	<0.01	<0.01	<0.01	<0.01
2.217	28	Spinach (leaves)	49	190	<0.01	<0.01	<0.01	<0.01	0.02
-	120	Spinach (leaves)	-	-	-	-	-	-	-
-	273	Spinach (leaves)	-	-	-	-	-	-	-
0.486	30	Radish (Leaves)	49	108	<0.01	<0.01	<0.01	<0.01	<0.01
		Radish (Roots)	49	108	<0.01	<0.01	<0.01	<0.01	<0.01
2.062	30	Radish (Leaves)	49	108	<0.01	<0.01	<001	<001	0.01
		Radish (Roots)	49	108	<0.01	<0.01	<0.01	<0.01	<0.01
0.495	121	Radish (Leaves)	49	192	<0.01	<0.01	<0.01	<0.01	<0.01
		Radish (Roots)	49	192	<0.01	<0.01	<0.01	<0.01	<0.01
2.182	121	Radish (Leaves)	49	192	<0.01	<0.01	<0.01	<0.01	<0.01
		Radish (Roots)	49	192	<0.01	<0.01	<0.01	<0.01	<0.01
0.492	270	Radish (Leaves)	49	341	<0.01	<0.01	<0.01	<0.01	<0.01
		Radish (Roots)	49	341	<0.01	<0.01	<0.01	<0.01	<0.01
2.176	270	Radish (Leaves)	49	341	<0.01	<0.01	<0.01	<0.01	<0.01
		Radish (Roots)	49	341	<0.01	<0.01	<0.01	<0.01	<0.01
0.484	28	Whole plant	31	133	<0.01	<0.01	<0.01	<0.01	0.01
		Grain	89	247	<0.01	<0.01	<0.01	<0.01	<0.01
		Straw	89	247	<0.01	<0.01	0.01	<0.01	<0.01
2.217	28	Whole plant	31	133	0.02	<0.01	0.03	<0.01	<0.01
		Grain	89	247	<0.01	<0.01	<0.01	<0.01	0.03
		Straw	89	247	0.03	<0.01	0.1	0.03	0.02
0.495	121	Whole plant	31	180	<0.01	<0.01	<0.01	<0.01	<0.01
		Grain	89	220	<0.01	<0.01	<0.01	<0.01	<0.01
		Straw	89	220	<0.01	<0.01	<0.01	<0.01	<0.01
2.182	121	Whole plant	31	180	<0.01	<0.01	0.08	<0.01	0.02
		Grain	89	220	<0.01	<0.01	<0.01	<0.01	<0.01
		Straw	89	220	<0.01	<0.01	0.01	<0.01	<0.01
0.492	270	Whole plant	31	329	<0.01	<0.01	<0.01	<0.01	<0.01
		Grain	89	369	<0.01	<0.01	<0.01	<0.01	<0.01
		Straw	89	369	<0.01	<0.01	0.01	<0.01	<0.01
2.176	270	Whole plant	31	329	<0.01	<0.01	0.01	<0.01	<0.01
		Grain	89	369	<0.01	<0.01	<0.01	<0.01	<0.01
		Straw	89	369	<0.01	<0.01	0.01	<0.01	<0.01

Limit of quantification = 0.01 mg/kg (leaves); limit of detection = 0.003 mg/kg

Table B.7.6.2.2-9: Trial S14-04011-02 (Germany): Flutolanil residues in succeeding crops (spinach, radish and barley)

Applic. Rate (kg a.s./ha)	PBI (days)	Crop	BBCH	DAA	Flutolanil	M-2	M-4	M-101	M-102
0.497	30	Spinach (leaves)	49	69	<0.01	<0.01	<0.01	<0.01	<0.01
2.153	30	Spinach (leaves)	49	69	<0.01	<0.01	0.02	0.01	<0.01
0.480	120	Spinach (leaves)	49	211	<0.01	<0.01	<0.01	<0.01	<0.01
2.145	120	Spinach (leaves)	49	211	0.02	<0.01	0.02	0.03	0.03
0.525	273	Spinach (leaves)	49	364	<0.01	<0.01	<0.01	<0.01	<0.01
2.134	273	Spinach (leaves)	49	364	0.01	<0.01	0.01	0.02	0.02
0.497	29	Radish (Leaves)	49	69	<0.01	<0.01	<0.01	<0.01	<0.01
		Radish (Roots)	49	69	<0.01	<0.01	<0.01	<0.01	<0.01
2.153		Radish (Leaves)	49	69	0.01	<0.01	<0.01	<001	<0.01
		Radish (Roots)	49	69	<0.01	<0.01	<0.01	<0.01	<0.01
0.480	120	Radish (Leaves)	49	190	<0.01	<0.01	<0.01	<0.01	<0.01
		Radish (Roots)	49	190	<0.01	<0.01	<0.01	<0.01	<0.01
2.145	120	Radish (Leaves)	49	190	0.03	<0.01	0.02	0.02	<0.01
		Radish (Roots)	49	190	<0.01	<0.01	<0.01	<0.01	<0.01
0.525	273	Radish (Leaves)	49	343	<0.01	<0.01	<0.01	<0.01	<0.01
		Radish (Roots)	49	343	<0.01	<0.01	<0.01	<0.01	<0.01
2.134	273	Radish (Leaves)	49	343	0.02	<0.01	0.01	0.02	<0.01
		Radish (Roots)	49	343	<0.01	<0.01	<0.01	<0.01	<0.01
0.513	30	Whole plant	31	231	<0.01	<0.01	<0.01	<0.01	<0.01
		Grain	89	322	<0.01	<0.01	<0.01	<0.01	<0.01
		Straw	89	322	<0.01	<0.01	<0.01	<0.01	<0.01
0.2053	30	Whole plant	31	231	0.01	<0.01	0.02	<0.01	<0.01
		Grain	89	322	<0.01	<0.01	<0.01	<0.01	<0.01
		Straw	89	322	0.03	<0.01	0.03	<0.01	<0.01
0.480	120	Whole plant	31	199	<0.01	<0.01	<0.01	<0.01	<0.01
		Grain	89	261	<0.01	<0.01	<0.01	<0.01	<0.01
		Straw	89	261	<0.01	<0.01	<0.01	<0.01	<0.01
2.145	120	Whole plant	31	199	<0.01	<0.01	0.02	<0.01	<0.01
		Grain	89	261	<0.01	<0.01	<0.01	<0.01	0.02
		Straw	89	261	0.02	<0.01	0.03	0.01	<0.01
0.525	273	Whole plant	31	352	<0.01	<0.01	<0.01	<0.01	<0.01
		Grain	89	414	<0.01	<0.01	<0.01	<0.01	<0.01
		Straw	89	414	<0.01	<0.01	<0.01	<0.01	<0.01
2.134	273	Whole plant	31	352	<0.01	<0.01	0.02	<0.01	<0.01
		Grain	89	414	<0.01	<0.01	<0.01	<0.01	0.02
		Straw	89	414	0.01	<0.01	0.02	0.01	<0.01

Conclusion:
All reported trials at the application rate of 0.480 kg a.s./ha were conducted following the proposed good agricultural practice (GAP). While some residues of the metabolite M-4 were detected at the limit of quantification (0.01 mg/kg) in straw, no residues of Flutolanil, M-2, M-4, M-101 and M-102, was detected above the limit of quantification in the RAC commodities of the follow-up crops (barley grain, spinach and radish roots). This study shows that significant uptake of residues of Flutolanil, M-2, M-4, M-101 and M-102 is not expected on follow-up crops.
Due to the failure of the spinach subplot at PBI 120 and PBI 273 in the Spanish trial, a new study has been conducted to repeat this plot. The study (S16-01285) has been submitted separately and it is evaluated under point B.7.6.2.3 below.

Remark RMS:
The applicant draw the conclusions based on the residue trials with the application rate of 480 g as/ha, which corresponds to the proposed cGAP for potatoes seed treatment. The trials with the application rate of 2100 g as/ha have been reported as at exaggerated rate trials and the results have not been included in the summary tables.
RMS does not agree with the conclusion of the notifier that the application rate of 2100 g as/ha is exaggerated, since this application rate corresponds to the proposed cGAP for in-furrow treatment for the MRL application (1N).
Results of those trials have been included in the summary tables in this document by RMS and conclusions on those trials are included in the summary part in Volume 1.

B.7.6.2.3 Study 3

Previous evaluation	Submitted for the purpous of the renewal
RMS remark	Acceptable

Reference:	CA 6.6.2/03: Raufer B., 2017
Title:	Determination of residues of flutolanil and its metabolites M-101, M-102, M-2 and M-4 after one application of Moncut 40SC (EU) on bare soil rotational crop (spinach) at 1 site in Southern Europe 2016/2017
Document No.:	S16- 01285 (R-3418)
Guidelines:	7524/VI/95, rev. 2 and OECD guideline 504 covering the crop groups root and tuber vegetables (radish), leafy vegetables (spinach) and small grains (barley)
GLP:	Yes

Study summary
The objective of the study is to determine residue levels of flutolanil and its metabolites M-2, M-4, M-101 and M-102 in the follow up crop spinach . The follow up used in this rotational crop study are in accordance with the guideline 7524/VI/95 rev.2 and OECD Guideline 504 covering the crop group leafy vegetables (spinach).
One trial was conducted during 2017 and 2017 in Spain.

To each treated plot one application of Moncut 40SC (SG formulation containing 460 g/L flutolanil, nominal content) was performed on bare soil at plant back intervals of nominal 120 and 270 days at a nominal rate of either 480 g as/ha or 2100 g as/ha. The product was diluted with water immediately prior to application to a nominal spray volume of 400 L/ha.
Follow up crop spinach was drilled at 120 and 270 days (nominal) after application of the test item.

No	Treatm.No	Test item	Application date	PBI*	Crop	Water [L/ha]	Product [mL.ha]	Appl.rate [kg as/ha]
A1	4	Moncut 40 Sc	01.01.2016	270	Spinach	400	1043	0.480
	5					399	4549	2.092
A2	2		22.08.2016	120		384	1002	0.461
	3					399	4549	2.092

* actual 272/129 days

Sampling
Residue plant samples were taken from the treated and control plots from spinach at earliest commercial harvest date (BBCH 19-43) and at harvest (BBCH 49). Samples were taken when the crop was dry. Samples from untreated plots were collected first. Each sample was collected randomly from at least 12 areas distributed over the whole plot. Adhering soil was removed before deep freezing (≤-18°C).

Analytical method and recoveries
The analytical samples were stored deep frozen until shipment to the analytical laboratory.
The plant specimens were analysed for residues of Flutolanil and its metabolites M-2, M-4, M-102 and M-101 (method reference EAS Report S14-04011).
The final determination of the analytes in the untreated and treated specimens was performed by extraction with acetonitrile/1 M hydrochloric acid (4/1 v/v), hydrolysis, liquid-liquid partition by addition of sodium chloride and ethyl acetate followed by subsequent shaking and ultrasonication and SPE clean up, followed by liquid chromatography with mass spectrometric detection (LC-MS/MS).
The limit of quiantification (LOQ) was 0.01 mg/kg for all analytes. The limit of determination (LOD) was 0.003 mg/kg for all analytes. For analytes M-2, M-4, M-101, M-102, the LOQ is expressed as parent quivalent.
Procedural recoveries were determined upon applying the test method. Fortifications were performed at the level of 0.01 mg/kg and 1 mg/kg. The mean recovery at each fortification level was in the range of 70-110%, with exception of M-4 at the 0.01 mg/kg, where, fortification was 64%. However, all recoveries are considered acceptable, as the overall mean recovery was within the acceptable range, with RDS <20% at each level for all analytes. Linearity of the method has been reported, with R≥0.995.
No residues of flutolanil and its metabolites were detected in any of the untreated samples.

Table B.7.6.2.3-1: Procedural recoveries

Matrix	Fortfication level (mg/kg)	Procedural recovery (%)	Mean recovery (%)	RSD(%)	Replicates	Overal mean (%)	Overal RSD (%)
Flutolanil							
Spinach (leaf)	0.01	85; 84; 69	79	11.3	3	82	9.1
	0.1	81; 83; 92	85	6.9	3		
M-2							
Spinach (leaf)	0.01	82; 75; 71	76	7.3	3	79	7.1
	0.1	80; 81; 87	83	4.6	3		
M-4							
Spinach (leaf)	0.01	75; 62; 56	64	15.1	3	71	13.9
	0.1	77; 73; 82	77	5.8	3		
M-101							
Spinach (leaf)	0.01	91; 81; 84	85	6.0	3	88	6.0
	0.1	87; 88; 96	90	5.5	3		
M-102							
Spinach (leaf)	0.01	96; 90; 87	91	5.0	3	94	6.9
	0.1	98; 104; 89	97	7.8	3		

Results

In the treated commodities no residues of flutolanil and metabolites M-2, M-4, M-101 were detected in all the samples from both plant back intervals. Metabolite M-102 was detected at the value of 0.01 mg/kg in immature and mature spinach from the plot 3 with the application rate of 2.1 kg as/ha at PBI of 120 days. In all other samples, residues of the metabolite M-102 were below the LOQ. Results are presened in Table B.7.6.2.3-2 below.

Table B.7.6.2.3-2 **Trial S16-01285-01 (Spain): Flutolanil residues in succeeding crop spinach**

Applic. Rate (kg a.s./ha)	PBI (days)	Crop	BBCH	Residue levels (mg/kg)				
				Flutolanil	M-2	M-4	M-101	M-102
0.480 (plot 2)	120	Spinach (leaf)	19-43	<0.01	<0.01	<0.01	<0.01	<0.01
0.480 (plot 2)	120		49	<0.01	<0.01	<0.01	<0.01	<0.01
2.1 (plot 3)	120		19-43	<0.01	<0.01	<0.01	<0.01	0.01
2.1 (plot 3)	120		49	<0.01	<0.01	<0.01	<0.01	0.01
0.480 (plot 4)	270		19-43	<0.01	<0.01	<0.01	<0.01	<0.01
0.480 (plot 4)	270		49	<0.01	<0.01	<0.01	<0.01	<0.01
2.1 (plot 5)	270		19-43	<0.01	<0.01	<0.01	<0.01	<0.01
2.1 (plot 5)	270		49	<0.01	<0.01	<0.01	<0.01	<0.01

B.7.7 Other studies

B.7.7.1 Effect on the residue level in pollen and bee products

No studies investigating residues in honey and bee products are available. It is noted that currently no test method or guidance document is available for conducting a feeding study on bees. However, since potatoes are probably not relevant crops for producing honey from available nectar and/or honeydew and the proposed application time is not in any way related to the flowering stage of potatoes, such studies are considered not required.

References relied on

Public literature

A literature searach was carried out. The search was carried out with the active substance flutolanil and its metabolites. The search covered the period of January 2016 to January 2016 and returned 152 publications. After rapid screening assessment, 151 articles were considered not relevant and were excluded from the review.
After a second assessment involving more detailed review of abstracts and full documents, 1 publication was selected as relevant or unclear. After a detailed review of these article, no article was considered relevant and providing information that may establish or challenge the risk assessment of flutolanil and its relevant metabolites.

New studies submitted for the renewal of the active substance flutolanil

Data point	**Author(s)**	**Year**	**Title Company Report No. Source (where different from company) GLP or GEP status Published or not**	**Vertebrate study Y/N**	**Data protection claimed Y/N**	**Justification if data protection is claimed**	**Owner**
CA 6.1/03	Merdian, H	2017	Storage Stability of Flutolanil and its Metabolites (M-2, M-4, M-101, M-102) in 2 Crops under Deep Frozen Conditions. Eurofins Agrosciences Services Chem Ltd. Report numer: S16-00671 (3rd Interim Report) R-3411 GLP: Yes Published: No	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	Nihon-Nohyaku Cp. Ltd.
CA 6.1./06	Dias, N	2016a	Flutolanil: Residues of Flutolanil and its Metabolites in Eggs and Tissues of Laying Hens Envigo CRS Ltd Report number: LMS0104 (A-3075) GLP: Yes Published: No	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	Nihon-Nohyaku Cp. Ltd.
CA 6.1./07	Yoshizane, T	2017	Flutolanil: Storage stability for Flutolanil and its Metabolites in Foodstuffs of Animal Origin (Cow) Nihon-Nohyaku Co.	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	Nihon-Nohyaku Cp. Ltd.

			Ltd. Report number: GE-03, 16-0103 (R-3403) GLP: Yes Published: No				
CA 6.2.1/04	Yoshizane, T	2013a	Plant metabolism of 14-C Flutiolanil in cabbage Nihon-Nohyaku Co. Ltd. Report number: LSRC-M12-085A (R-3341)_ GLP: Yes Published: No	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	Nihon-Nohyaku Cp. Ltd.
CA 6.2.1./05	Yoshizane, T	2013b	Plant metabolism of 14-C Flutiolanil in Rice Nihon-Nohyaku Co. Ltd. Report number: LSRC-M12-129A (R-3342) GLP: Yes Published: No	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	Nihon-Nohyaku Cp. Ltd.
CA 6.2.1/06	Ki Chang Ahn	2016	A metabolism study with [Trifluoromethyl Ring-U-^{14}C] Flutolanil (1 Radiolabel) in Potatoes PTRL West Report number: 2556W-1 (R-3381) GLP: Yes Published: No	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	Nihon-Nohyaku Cp. Ltd.
CA 6.2.2/01	McDonald A	2016a	Flutolanil: Metabolism in laying hens Envigo CRS Ltd. Report number: LMS0102 (R-3386) GLP: Yes Published: No	Y	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	Nihon-Nohyaku Cp. Ltd.
CA 6.2.3/01	Hardwick T	2011	[^{14}C] Flutolanil – Absorption, distribution, metabolism and excretion following repeated oral administration to the lactating ruminant. Covance Labolatories Ltd Report number: 8241196 Nihon Nohyaky Co. Ltd code R-3304	Y	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	Nihon-Nohyaku Cp. Ltd.

			GLP: yes Published: No				
CA 6.2.3/02	[redacted]	2016b	Flutolanil: Metabolism in the Lactating Goat [redacted]. Report number: LMS0101 (R-3387) GLP: Yes Published: No	Y	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	Nihon-Nohyaku Cp. Ltd.
CA 6.3.1/01	Sutherland, J	2015a	Flutolanil 40SC/Rhino DS: Study to generate seed potato treated with flutolanil fore use in subsequent residue studies Europfins Agroscience Services Ltd Report number: S14-02899 (R-3373) GLP: Yes Published: No	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	Nihon-Nohyaku Cp. Ltd.
CA 6.3.1/02	Sutherland, J	2015b	Flutolanil 40SC/Rhino DS treated seed: Determination of residues of flutolanil following a seed treatment application of flutolanil (MONCUT) 40SC and Rhino DS in potato at 3 sites in northern Europe, 2014 Europfins Agroscience Services Ltd Report number: S14-02900 (R-3374) GLP: Yes Published: No	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	Nihon-Nohyaku Cp. Ltd.
CA 6.3.1/03	Merdian, H	2016a	Determination of resiudes of flutolanil following a seed treatment application of flutolanil (MONCUT) 40SC and Rhino DS in potato at 3 sites in northern Europe, 2014 Europfins Agroscience Services Ltd Report number:	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	Nihon-Nohyaku Cp. Ltd.

			S16-03442 (R-3382) GLP: Yes Published: No				
CA 6.3.1/04	Sutherland, J	2015c	Flutolanil 40SC/Rhino DS treated seed: Determination of residues of flutolanil following a seed treatment application of flutolanil (MONCUT) 40SC and Rhino DS in potato at 4 sites in southern Europe, 2014 Europfins Agroscience Services Ltd Report number: S14-02901 (R-3375) N GLP: Yes Published: No	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	Nihon-Nohyaku Cp. Ltd.
CA 6.3.1/05	Merdian, H	2016b	Determination of resiudes of flutolanil following a seed treatment application of flutolanil (MONCUT) 40SC and Rhino DS in potato at 3 sites in northern Europe, 2014 Europfins Agroscience Services Ltd Report number: S16-03453 (R-3383) GLP: Yes Published: No	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	Nihon-Nohyaku Cp. Ltd.
CA 6.3.1/06	Lines, J	2016a	Moncut 40SC/ Flutolanil 40SC: Study to generate seed potato treated with flutolanil fore use in subsequent residue studies Europfins Agroscience Services Ltd Report number: S15-00013 (R-3388) GLP: Yes	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	Nihon-Nohyaku Cp. Ltd.

			Published: No				
CA 6.3.1/07	Lines, J	2016b	Flutolanil (Moncut) 40SC/ Rhino DS treated seed: Determination of resiudes of flutolanil following a seed treatment application of flutolanil (MONCUT) 40SC and Rhino DS in potato at 5 sites in northern Europe, 2015 Europfins Agroscience Services Ltd Report number: S15-00014 (R-3391) GLP: Yes Published: No	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	Nihon-Nohyaku Cp. Ltd.
CA 6.3.1/08	Lines, J	2016c	Flutolanil (Moncut) 40SC/ Rhino DS treated seed: Determination of resiudes of flutolanil following a seed treatment application of flutolanil (MONCUT) 40SC and Rhino DS in potato at 4 sites in southern Europe, 2015 Europfins Agroscience Services Ltd Report number: S15-00016 (R-3393) GLP: Yes Published: No	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	Nihon-Nohyaku Cp. Ltd.
CA 6.3.1/09	Sutherland, J	2016	Study to generate seed potato treated with flutolanil fore use in subsequent residue studies Europfins Agroscience Services Ltd Report number: S16-02156 (R-3402) GLP: Yes Published: No	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	Nihon-Nohyaku Cp. Ltd.
CA6.3.1/10	Sutherland, J	2017a	Determination of resiudes of flutolanil	N	Y	Article 59(1) &(2) of	Nihon-Nohyaku

			following a seed treatment application of flutolanil (MONCUT) 40SC and Rhino DS in potato at 3 sites in northern Europe, 2016 Europfins Agroscience Services Ltd Report number: S16-02157 (R-3406) GLP: Yes Published: No			Regulation (EC) 1107/2009 applies	Cp. Ltd.
CA6.3.1/11	Sutherland, J	2017b	Determination of resiudes of flutolanil following a seed treatment application of flutolanil (MONCUT) 40SC and Rhino DS in potato at 4 sites in southern Europe, 2016 Europfins Agroscience Services Ltd Report number: S16-02159 (R-3408) GLP: Yes Published: No	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	Nihon-Nohyaku Cp. Ltd.
CA6.3.1/12	Sutherland, J	2015b	Determination of resiudes of flutolanil following in furrow application of Flutolanil 70DF in potato at 4 sites in northern Europe, 2014 Europfins Agroscience Services Ltd Report number: S14-03028 (R-3376) GLP: Yes Published: No	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	Nihon-Nohyaku Cp. Ltd.
CA 6.3.1/13	Merdian, H	2016c	Determination of resiudes of flutolanil following in furrow application of Flutolanil 70DF in potato at 4 sites in	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	Nihon-Nohyaku Cp. Ltd.

			northern Europe, 2014 Europfins Agroscience Services Ltd Report number: S16-03454 (R-3384) GLP: Yes Published: No				
CA6.3.1/14	Sutherland, J	2015e	Flutolanil 70DF: resiudes of flutolanil following in furrow application of Flutolanil 70DF in potato at 4 sites in southern Europe, 2014 Europfins Agroscience Services Ltd Report number: S14-03029 (R-3377) GLP: Yes Published: No	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	Nihon-Nohyaku Cp. Ltd.
CA 6.3.1/15	Merdian, H	2016d	Determination of resiudes of flutolanil following in furrow application of Flutolanil 70DF in potato at 4 sites in southern Europe, 2014 Europfins Agroscience Services Ltd Report number: S16-03455 (R-3385) GLP: Yes Published: No	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	Nihon-Nohyaku Cp. Ltd.
CA 6.3.1/16	Lines J	2016d	Flutolanil 70DF: resiudes of flutolanil following in furrow application of Flutolanil 70DF in potato at 4 sites in northern Europe, 2015 Europfins Agroscience Services Ltd Report number:	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	Nihon-Nohyaku Cp. Ltd.

			S15-00015 (R-3392) GLP: Yes Published: No				
CA 6.3.1/17	Martin C	2016	Flutolanil 70DF: resiudes of flutolanil following in furrow application of Flutolanil 70DF in potato at 4 sites in southern Europe, 2015 Europfins Agroscience Services Ltd Report number: S15-00017 (R-3394) GLP: Yes Published: No	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	Nihon-Nohyaku Cp. Ltd.
CA6.3.1/18	Sutherland, J	2017c	Determination of resiudes of flutolanil following in furrow application of Flutolanil 70DF in potato at 4 sites in northern Europe, 2016 Europfins Agroscience Services Ltd Report number: S16-02158 (R-3407) GLP: Yes Published: No	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	Nihon-Nohyaku Cp. Ltd.
CA6.3.1/19	Sutherland, J	2017d	Determination of resiudes of flutolanil following in furrow application of Flutolanil 70DF in potato at 5 sites in southern Europe, 2016 Europfins Agroscience Services Ltd Report number: S16-02160 (R-3409) GLP: Yes Published: No	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	Nihon-Nohyaku Cp. Ltd.
CA 6.4.1/01	[redacted]	2016a	Flutolanil: Residues of Flutolanil and its	Y	Y	Article 59(1) &(2) of	Nihon-Nohyaku

			Metabolites in Eggs and Tissues of Layng Hens [redacted] Report number LM0104 (A-3075) GLP: Yes Published: No			Regulation (EC) 1107/2009 applies	Cp. Ltd.
CA 6.4.2/02	Ross, V.A.	2016b	Flutolanil: Residues of Flutolanil and its Metabolites in Milk and Tissues of Dairy Cows Envigo CRS Ltd. Report number LM0103 (R-3396) GLP: Yes Published: No	Y	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	Nihon-Nohyaku Cp. Ltd.
CA 6.5.1/01	O'Connell, C; Pratt, E	2015	[^{14}C] flutolanil: high temperature hydrolysis Battelle UK Ltd Report number"XG/15/020 GLP: Yes Published:No	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	Nihon-Nohyaku Cp. Ltd.
CA 6.6.1/02	Ki Chang Ahn; Huang,J	2016a	Confined Rotational Crop Study with [Trifluoromethyl Ring-U-^{14}C] Flutolanil applied at 480 g ai/ha (one radiolabel) PTRL West Report number: 2697 W (R-3390) GLP: Yes Published:No	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	Nihon-Nohyaku Cp. Ltd.
CA 6.6.1/02	Ki Chang Ahn; Huang, J	2016b	Confined Rotational Crop Study with [Trifluoromethyl Ring-U-^{14}C] Flutolanil applied at 2100 g ai/ha (one radiolabel) PTRL West Report number: 2698 W (R-3389) GLP: Yes Published:No	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	Nihon-Nohyaku Cp. Ltd.
CA 6.6.2/02	Raufer, B	2017	Determination of residues of flutolanil after one appliacation of Moncut 40SC (EU) on bare soil in rotational crops (radish, spinach and cereals) at 2 sites in	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	Nihon-Nohyaku Cp. Ltd.

			Southern and Northern Europe 2014/2015 Europfins Agroscience Services Ltd Report number: S14-04011 (R-3405)				

Studies used for the initial approval of flutolanil

	Author(s)	**Year**	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Data protection claimed Y/N**	**Justification if data protection is claimed**	**Owner**
IIA 6.2.1/03	Lewis, C.J	1999	^{14}C-Flutolanil: Metabolism in potatoes, Covance Laboratories Ltd., North Yorkshire, England, Unpublished report No.: R-3025.	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	NIH
IIA 6.2.1/01	Downey, S.S, Meyer, B.N; Rupprecht, J.K	1993	Metabolism of ^{14}C-flutolanil in peanuts, NOR_AM Chemical Co., North Carolina, USA, Unpublished report No.: R-3015.	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	NIH
IIA 6.2.1/02	Smith, S.; Shelly, M; O'Neal S``	1994	Metabolic fate and distribution of ^{14}C-flutolanil in rice. NOR_AM Chemical Co., North Caroline, USA Unpublished Report No R-3016	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	NIH
IIA 6.2.2/02	[redacted]	1989	The metabolism of ^{14}C-flutolanil in laying hens, [redacted] Report No. R-3012.	Y	N		NIH
IIA 6.2.3/03	[redacted]	1989	The metabolism of ^{14}C-flutolanil in goats, [redacted] Report No. R-3013.	Y	N		NIH
IIA, 6.3/01	Messelink, H.J.	1992	Residues of flutolanil in ware-potatoes The Netherlands. 1991	N	Y	Article 59(1) &(2) of Regulatio	NIH

			Rhone-Poulenc Agro, B.V., Etten-Leur, The Netherlands Report No.: 92.005 Date: 31.3.1992 Nihon Nohyaku, Report No.: R-3028. GLP, Non published			n (EC) 1107/2009 applies	
IIA, 6.3/02	Dupont, C.	1990	Flutolanil formulation EXP 10057 (DS) Essai France 1990. Residus dans la pomme de terre Rhone-Poulenc Agro, Lyon, France Report No.: AG/CRDL/AN/9016657 Date: 27.11.1990 Nihon Nohyaku, Report No.: R-3060. GLP, Non published	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	NIH
IIA, 6.3/03	Richard, M.	1995a	Flutolanil and metabolite (M4). Formulation EXP 10057A (DS). Trials France 1994. Residues in early potato Rhone-Poulenc Agro, Lyon, France Report No.: R&D/CRLD/AN/fb/9515672 Date: 18.5.1995 Nihon Nohyaku, Report No.: R-3021 GLP, Non published	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	NIH
IIA, 6.3/04	Richard, M.	1995b	Flutolanil and metabolite (M4). Formulation EXP 10057A (DS). Trial France 1994. Residues in stored potato Rhone-Poulenc Agro, Lyon, France Report No.: R&D/CRLD/AN/bd/9515732 Date: 07.6.1995 Nihon Nohyaku, Report No.: R-3022 GLP, Non published	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	NIH
IIA, 6.3/05	Richard, M.	1994a	Flutolanil. Formulation EXP 10057A (DS). Essai France 1993. Residus dans la pomme de terre conservation Rhone-Poulenc Agro, Lyon, France Report No.: R&D/CRLD/AN/bd/9416369 Date: 21.9.1994 Nihon Nohyaku, Report No.: R-3029 GLP, Non published	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	NIH
IIA, 6.3/06	Richard, M	1994b	Flutolanil. Formulation EXP 10057A ou Iota P (DS). Essai France 1993. Residus dans la pomme de terre de primeur Rhone-Poulenc Agro, Lyon, France	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009	NIH

			Report No.: R&D/CRLD/AN/bd/9416370 Date: 20.9.1994 Nihon Nohyaku, Report No.: R-3030 GLP, Non published			applies	
IIA, 6.3/07	Richard, M.	1995c	Flutolanil and its metabolite (M4). Formulation EXP 10066 (SC). Essai France 1994. Residues in stored potato Rhone-Poulenc Agro, Lyon, France Report No.: R&D/CRLD/AN/bd/9515655 Date: 26.4.1995 Nihon Nohyaku, Report No.: R-3024 GLP, Non published	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	NIH
IIA, 6.3/08	Richard, M	1995d	Flutolanil and its metabolite (M4). Formulation EXP 10066A (SC). Trials France 1994. Residues in early potato Rhone-Poulenc Agro, Lyon, France Report No.: R&D/CRLD/AN/bd/9515654 Date: 27.4.1995 Nihon Nohyaku, Report No.: R-3023 GLP, Non published	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	NIH
IIA, 6.3/09	Richard, M.	1995c	Flutolanil. Formulation EXP 10066 (SC) OU IOTA L (SC). Essais France 1993. Residus dans la pomme de terre primeur Rhone-Poulenc Agro, Lyon, France Report No.: R&D/CRLD/AN/fd/9416371 Date: 20.9.1994 Nihon Nohyaku, Report No.: R-3031 GLP, Non published	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	NIH
IIA, 6.3/10	Souvignet, I.	1999	Flutolanil. Formulation EXP 10066A or RPA 10066F (FS). Trials Germany 1997. Residues in potatoes. Decline study, Rhone-Poulenc Agro, Lyon, France Report No.: R&D/CRLD/AN/msa /9816770 Date: 09.3.1999, Nihon Nohyaku, Report No.: R-3058 GLP, Non published	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	NIH
IIA, 6.3/11	Oxspring, S.	2003	Final Report on Project AF/6012/NN: To determine the magnitude of flutolanil residues at harvest in the raw agricultural commodity potatoes resulting from one dip	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009	NIH

			application and one overall application of Flutolanil 40 SC, in Spain, in 2001. AGRISEARCH UK LTD, Report No.: AF/6012/NN Date: 31.3.2003 Nihon Nohyaku, Report No.: R-3054 GLP, Non published			applies	
IIA, 6.3/12	Oxspring, S.	2003	Final Report on Project AF/6279/NN: To determine the magnitude of flutolanil residues at harvest in the raw agricultural commodity potatoes resulting from one dip application and one overall application of Flutolanil 40 SC, in Spain, in 2002. AGRISEARCH UK LTD, Report No.: AF/6279/NN Date:06.3.2003 Nihon Nohyaku, Report No.: R-3057	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	NIH
IIA, 6.6/01	Giraud, J.P.	2001	Flutolanil Formulation EXP10057A (DS), North/United Kingdom/1999-8 Decline study trials, Residue in winter wheat (soil, plant, grain and straw), rape (soil, plant and grain) Following crops study Aventis CropScience, Lyon, France Report No.: R&D/CRDL/AN/mr/0115271 Date:18.6.2001, Nihon Nohyaku, Report No.: R-3059 GLP, Non published	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	NIH
IIA 6.6/02 CA 6.6.1/01	Downey, S.S Meyer, BN	1992	Uptake of [^{14}C]-Flutolanil residues in soil by rotational crops under confined conditions, NOR-AM Chemical Company, North Carolina, USA, Unpublished report No.: Aventis ref: B002405. E-3021	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	NIH
IIA, 6.1/01	Williams, L.E.	1996	Stability of flutolanil and its metabolite, M-4, in potatoes during frozen storage AgrEvo USA Company, NC, USA Report No.:A55764 Date: 22.4.1996 Nihon Nohyaku, Report No.: A-3024 GLP: yes Non published	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	NIH
IIA 6.1/02	Ricau, H	2004	Stability study of Flutolanil in potato, wheat (grain and straw) and rape (grain) after storage in a congelator at a temperature under minus 18	N	Y	Article 59(1) &(2) of Regulation (EC)	NIH

			°C. Bayer Crop Science study no. 02-80 (R-3209). GLP: yes Non published			1107/2009 applies	
IIA 6 /03	Wouters, G.A.J.M	1999	Storage stability of flutolanil in soil, Analytico Research B.V., Breda, the Netherlands, Unpublished Report No A-3026	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	NIH
IIA 6.1/04	Neal, J.L	1994	Storage stability of flutolanil resiudes in Whole Milk, USA 1993 NOR-AM Chemical Company Report number: AU-93R-11 (PC-3110) GLP: yes Published: No	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	
Appendix IIA 6.1/05	Dacus, S.C	1994	Stability of flutolanil and ints metabolites M-2, M-4 and M-7 in animal products during frozen storage. USA, 1993 NOR-AM Chemical Company Report number: AU-93R-12 (PC-3111) GLP: yes Published: No	N	Y	Article 59(1) &(2) of Regulation (EC) 1107/2009 applies	