Draft Assessment Report

Evaluation of Active Substances

Plant Protection Products

Prepared according to **assimilated Regulation No 1107/2009**
as it applies in Great Britain

Inpyrfluxam

Volume 3 – B.7 (AS)

Residue Data

Great Britain

March 2026

Version History

When	What
November 2025	Initial DAR
March 2026	Updates made after ECP

Contents

B.7. Residue Data

B.7.1. Storage stability of residues

B.7.1.1 Plant matrices

As samples in the residue studies on primary crops (wheat and barley), rotational crops (carrot, lettuce, barley and wheat) and processed commodities (wheat and barley) were routinely stored frozen prior to their analysis, the effects of frozen storage on the residue levels has been investigated.

Five studies reports have been evaluated and these investigated the stability of inpyrfluxam and its metabolites *N*-des-Me-S-2840, *N*-des-Me-1'-CH_2OH-S-2840-A, *N*-des-Me-1'-CH_2OH-S-2840-B, *N*-des-Me-DFPA, DFPA, 3'-OH-S-2840, DFPA-$CONH_2$, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B, 1'-COOH-S-2840-A and 1'-COOH-S-2840-B in five different commodity groups (high water, high acid, high oil, high starch and high protein). Inpyrfluxam contains a chiral centre and therefore exists as both R- and S- isomers. The active substance is identified as the R-isomer. Some of the metabolites contain a chiral centre and are also present as R and S isomers, these are referred to as 'A' and 'B' throughout this document. For the metabolites which are also isomers, the A&B isomers were fortified in the same samples in the study and are therefore listed together below.

Given analytes were investigated in five different commodity groups (high water, high acid, high oil, high starch and high protein), the following analytes can be considered stable in all matrix types for at least 22 months:

- Inpyrfluxam
- *N*-des-Me-DFPA
- DFPA, 3'-OH-S-2840
- DFPA-$CONH_2$
- 1'-COOH-S-2840-A & 1'-COOH-S-2840-B
- 1'-CH_2OH-S-2840-A & 1'-CH_2OH-S-2840-B

The following analytes can be considered stable in all matrix types for at least 12 months:

- *N*-des-Me-S-2840
- *N*-des-Me-1'-CH_2OH-S-2840-A & *N*-des-Me-1'-CH_2OH-S-2840-B

The storage stability studies are summarised in the Table 7.1.1-1.

Table 7.1.1-1: Supported storage stability lengths of inpyrfluxam and its metabolites *N*-des-Me-S-2840, *N*-des-Me-1'-CH_2OH-S-2840-A, *N*-des-Me-1'-CH_2OH-S-2840-B, *N*-des-Me-DFPA, DFPA, 3'-OH-S-2840, DFPA-$CONH_2$, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B, 1'-COOH-S-2840-A and 1'-COOH-S-2840-B in plant samples

Sample material		Frozen storage stability (months)	Report number	Reference
Commodity group	Crop matrix			
Analyte: Inpyrfluxam				
High water content	Cucumber	22	S16-05016 (TES-1614L)	KCA 6.1/02
	Apples	16	VP-39115 (MRID 50312811)	KCA 6.1/04
	Maize/corn Forage	19	VP-39115 (MRID 50312811)	KCA 6.1/04
	Wet apple pomace	5	VP-40066 (MRID 50312804)	KCA 6.1/05
High acid content	Grape	22	S16-05016 (TES-1614L)	KCA 6.1/02
High oil content	Soyabean seed	19 22	VP-39115 (MRID 50312811) S16-05016 (TES-1614L)	KCA 6.1/04 KCA 6.1/02
	Maize/corn oil	3	VP-40066 (MRID 50312804)	KCA 6.1/05
Dry / High starch content	Wheat grain	22	S16-05016 (TES-1614L)	KCA 6.1/02
	Potatoes	20	VP-39115 (MRID 50312811)	KCA 6.1/04
	Maize/corn Grain	20	VP-39115 (MRID 50312811)	KCA 6.1/04
	Potato starch	14	S18-03229 (TES-1816L)	KCA 6.1/03
	Polished rice	5	VP-40066 (MRID 50312804)	KCA 6.1/05
	Maize/corn starch	3	VP-40066 (MRID 50312804)	KCA 6.1/05
Dry / High protein content	Field bean	22	S16-05016 (TES-1614L)	KCA 6.1/02
Miscellaneous	Potato crisps	14	S18-03229 (TES-1816L)	KCA 6.1/03
	Wheat flour	17	S18-03229 (TES-1816L)	KCA 6.1/03
	Wheat bread	17	S18-03229 (TES-1816L)	KCA 6.1/03
	Maize/corn Stover	19	VP-39115 (MRID 50312811)	KCA 6.1/04
	Soyabean hulls	2	VP-40066 (MRID 50312804)	KCA 6.1/05
	Rice hulls	5	VP-40066 (MRID 50312804)	KCA 6.1/05
	Rice bran	5	VP-40066 (MRID 50312804)	KCA 6.1/05
	Peanut meal	3	VP-40066 (MRID 50312804)	KCA 6.1/05
	Wheat germ	10	VP-40066 (MRID 50312804)	KCA 6.1/05
	Sugar beet dried pulp	8	VP-40066 (MRID 50312804)	KCA 6.1/05
	Sugar beet sugar	2	VP-40066 (MRID 50312804)	KCA 6.1/05
	Sugar beet molasses	2	VP-40066 (MRID 50312804)	KCA 6.1/05
	Potato flakes	8	VP-40066 (MRID 50312804)	KCA 6.1/05
	Potato chips	8	VP-40066 (MRID 50312804)	KCA 6.1/05

Analytes: *N*-des-Me-S-2840, *N*-des-Me-1'-CH_2OH-S-2840-A & *N*-des-Me-1'-CH_2OH-S-2840-B				
High water content	Cucumber	12	S17-01158 (TES-1702L)	KCA 6.1/01
High acid content	Grape	12	S17-01158 (TES-1702L)	KCA 6.1/01
High oil content	Soyabean seed	12	S17-01158 (TES-1702L)	KCA 6.1/01
High starch content	Wheat grain	12	S17-01158 (TES-1702L)	KCA 6.1/01
High protein content	Field bean	12	S17-01158 (TES-1702L)	KCA 6.1/01
Analytes: *N*-des-Me-DFPA & DFPA				
High water content	Cucumber	22	S16-05016 (TES-1614L)	KCA 6.1/02
High acid content	Grape	22	S16-05016 (TES-1614L)	KCA 6.1/02
High oil content	Soyabean seed	22	S16-05016 (TES-1614L)	KCA 6.1/02
High starch content	Wheat grain	22	S16-05016 (TES-1614L)	KCA 6.1/02
	Potato starch	14	S18-03229 (TES-1816L)	KCA 6.1/03
High protein content	Field bean	22	S16-05016 (TES-1614L)	KCA 6.1/02
Miscellaneous	Potato crisps	14	S18-03229 (TES-1816L)	KCA 6.1/03
	Wheat flour	17	S18-03229 (TES-1816L)	KCA 6.1/03
	Wheat bread	17	S18-03229 (TES-1816L)	KCA 6.1/03
Analytes: 3'-OH-S-2840 & DFPA-$CONH_2$				
High water content	Cucumber	22	S16-05016 (TES-1614L)	KCA 6.1/02
	Apples	16	VP-39115 (MRID 50312811)	KCA 6.1/04
	Maize/corn Forage	19	VP-39115 (MRID 50312811)	KCA 6.1/04
	Wet apple pomace	5	VP-40066 (MRID 50312804)	KCA 6.1/05
High acid content	Grape	22	S16-05016 (TES-1614L)	KCA 6.1/02
High oil content	Soyabean seed	19 22	VP-39115 (MRID 50312811) S16-05016 (TES-1614L)	KCA 6.1/04 KCA 6.1/02
	Maize/corn oil	3	VP-40066 (MRID 50312804)	KCA 6.1/05
High starch content	Wheat grain	22	S16-05016 (TES-1614L)	KCA 6.1/02
	Potatoes	20	VP-39115 (MRID 50312811)	KCA 6.1/04
	Maize/corn Grain	20	VP-39115 (MRID 50312811)	KCA 6.1/04
	Potato starch	14	S18-03229 (TES-1816L)	KCA 6.1/03
	Polished rice	5	VP-40066 (MRID 50312804)	KCA 6.1/05
	Maize/corn starch	3	VP-40066 (MRID 50312804)	KCA 6.1/05
High protein content	Field bean	22	S16-05016 (TES-1614L)	KCA 6.1/02
Miscellaneous	Potato crisps	14	S18-03229 (TES-1816L)	KCA 6.1/03
	Wheat flour	17	S18-03229 (TES-1816L)	KCA 6.1/03
	Wheat bread	17	S18-03229 (TES-1816L)	KCA 6.1/03
	Maize/corn Stover	19	VP-39115 (MRID 50312811)	KCA 6.1/04
	Soyabean hulls	2	VP-40066 (MRID 50312804)	KCA 6.1/05
	Rice hulls	5	VP-40066 (MRID 50312804)	KCA 6.1/05
	Rice bran	5	VP-40066 (MRID 50312804)	KCA 6.1/05
	Peanut meal	3	VP-40066 (MRID 50312804)	KCA 6.1/05
	Wheat germ	10	VP-40066 (MRID 50312804)	KCA 6.1/05

	Sugar beet dried pulp	8	VP-40066 (MRID 50312804)	KCA 6.1/05
	Sugar beet sugar	2	VP-40066 (MRID 50312804)	KCA 6.1/05
	Sugar beet molasses	2	VP-40066 (MRID 50312804)	KCA 6.1/05
	Potato flakes	8	VP-40066 (MRID 50312804)	KCA 6.1/05
	Potato chips	8	VP-40066 (MRID 50312804)	KCA 6.1/05
Analytes: 1'-CH_2OH-S-2840-A & 1'-CH_2OH-S-2840-B				
High water content	Cucumber	22	S16-05016 (TES-1614L)	KCA 6.1/02
	Apples	16	VP-39115 (MRID 50312811)	KCA 6.1/04
	Maize/corn Forage	19	VP-39115 (MRID 50312811)	KCA 6.1/04
	Wet apple pomace	5	VP-40066 (MRID 50312804)	KCA 6.1/05
High acid content	Grape	22	S16-05016 (TES-1614L)	KCA 6.1/02
High oil content	Soyabean seed	19 22	VP-39115 (MRID 50312811) S16-05016 (TES-1614L)	KCA 6.1/04 KCA 6.1/02
	Maize/corn oil	3	VP-40066 (MRID 50312804)	KCA 6.1/05
Dry / High starch content	Wheat grain	22	S16-05016 (TES-1614L)	KCA 6.1/02
	Potatoes	20	VP-39115 (MRID 50312811)	KCA 6.1/04
	Maize/corn Grain	20	VP-39115 (MRID 50312811)	KCA 6.1/04
	Potato starch	14	S18-03229 (TES-1816L)	KCA 6.1/03
	Polished rice	5	VP-40066 (MRID 50312804)	KCA 6.1/05
	Maize/corn starch	3	VP-40066 (MRID 50312804)	KCA 6.1/05
Dry / High protein content	Field bean	22	S16-05016 (TES-1614L)	KCA 6.1/02
Miscellaneous	Potato crisps	14	S18-03229 (TES-1816L)	KCA 6.1/03
	Wheat flour	17	S18-03229 (TES-1816L)	KCA 6.1/03
	Wheat bread	17	S18-03229 (TES-1816L)	KCA 6.1/03
	Maize/corn stover	19	VP-39115 (MRID 50312811)	KCA 6.1/04
	Soyabean hulls	2	VP-40066 (MRID 50312804)	KCA 6.1/05
	Rice hulls	5	VP-40066 (MRID 50312804)	KCA 6.1/05
	Rice bran	5	VP-40066 (MRID 50312804)	KCA 6.1/05
	Peanut meal	3	VP-40066 (MRID 50312804)	KCA 6.1/05
	Wheat germ	10	VP-40066 (MRID 50312804)	KCA 6.1/05
	Sugar beet dried pulp	8	VP-40066 (MRID 50312804)	KCA 6.1/05
	Sugar beet sugar	2	VP-40066 (MRID 50312804)	KCA 6.1/05
	Sugar beet molasses	2	VP-40066 (MRID 50312804)	KCA 6.1/05
	Potato flakes	8	VP-40066 (MRID 50312804)	KCA 6.1/05
	Potato chips	8	VP-40066 (MRID 50312804)	KCA 6.1/05
Analytes: 1'-COOH-S-2840-A & 1'-COOH-S-2840-B				
High water content	Cucumber	22	S16-05016 (TES-1614L)	KCA 6.1/02
	Maize/corn Forage	19	VP-39115 (MRID 50312811)	KCA 6.1/04
High acid content	Grape	22	S16-05016 (TES-1614L)	KCA 6.1/02
High oil content	Soyabean seed	22	S16-05016 (TES-1614L)	KCA 6.1/02
	Maize/corn oil	3	VP-40066 (MRID 50312804)	KCA 6.1/05

High starch content	Wheat grain	22	S16-05016 (TES-1614L)	KCA 6.1/02
	Potatoes	20	VP-39115 (MRID 50312811)	KCA 6.1/04
	Maize/corn Grain	20	VP-39115 (MRID 50312811)	KCA 6.1/04
	Potato starch	14	S18-03229 (TES-1816L)	KCA 6.1/03
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	Peanut meal	3	VP-40066 (MRID 50312804)	KCA 6.1/05
	Wheat germ	10	VP-40066 (MRID 50312804)	KCA 6.1/05
	Sugar beet dried pulp	8	VP-40066 (MRID 50312804)	KCA 6.1/05
	Sugar beet sugar	2	VP-40066 (MRID 50312804)	KCA 6.1/05
	Sugar beet molasses	2	VP-40066 (MRID 50312804)	KCA 6.1/05
	Potato flakes	8	VP-40066 (MRID 50312804)	KCA 6.1/05
	Potato chips	8	VP-40066 (MRID 50312804)	KCA 6.1/05

B.7.1.1.1 Study 1

Report:	**KCA 6.1/01; [redacted] (2018)**
Title:	Storage stability of S-2399 metabolites *N*-des-Me-S-2840, *N*-des-Me-1'-CH_2OH-S-2840-A and *N*-des-Me-1'-CH_2OH-S-2840-B in matrices of plant origin
Document No.:	TPR-0075 (Study No.: S17-01158)
Guidelines:	Regulation (EC) No 1107/2009 EC Guideline 7032/VI/95, rev. 5, July 1997; Appendix H of working document 1607/VI/97, rev. 2, June 1999 – Storage Stability of Residue Samples OECD 506, 2007; OECD Guideline for the Testing of Chemicals – Stability of Pesticide Residues in Stored Commodities SANCO/3029/99 rev. 4
Guideline deviations:	None

GLP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

Materials and Methods

The frozen storage of *N*-des-Me-S-2840, *N*-des-Me-1'-CH_2OH-S-2840-A and *N*-des-Me-1'-CH_2OH-S-2840-B was studied in cucumber (high water), grapes (high acid), soyabean seeds (high oil), wheat grain (high starch) and field bean (high protein) for 12 months.

Untreated homogenate cucumber, grapes, soyabean seeds, wheat grain and field bean sample matrices (20 g) were fortified with *N*-des-Me-S-2840 at a level of 0.1 mg/kg and *N*-des-Me-1'-CH_2OH-S-2840-A and *N*-des-Me-1'-CH_2OH-S-2840-B at 0.05 mg/kg (10x LOQ for the respective analytes). *N*-des-Me-S-2840 was fortified separately from *N*-des-Me-1'-CH_2OH-S-2840-A & -B but the A&B isomers were fortified jointly. The sample was mixed by brief swirling to ensure distribution of the test items in the respective matrices. The solvent was allowed to evaporate for approx. 10 min.

Three samples (two fortified and one non-fortified) per commodity matrix were kept in glass bottles with screw caps at ≤-18°C in the dark for up to 12 months. After time intervals of approximately 1, 3, 6 and 12 months, samples were removed from storage and were analysed for *N*-des-Me-S-2840, *N*-des-Me-1'-CH_2OH-S-2840-A and *N*-des-Me-1'-CH_2OH-S-2840-B.

Analysis was performed using LC-MS/MS method validation study S17-00277 (SUM-1701V). Full details and validation data for this method can be found in Vol. 3 CA B.5.1.2.5. The analytical method (SUM-1701V) was sufficiently validated in accordance with SANTE/2020/12830 rev.1. The LOQ of the method is 0.005 mg/kg for *N*-des-Me-1'-CH_2OH-S-2840-A and *N*-des-Me-1'-CH_2OH-S-2840-B and 0.01 mg/kg for *N*-des-Me-S-2840. The one procedural recovery (freshly spiked samples at 10x LOQ, i.e., 0.05 mg/kg for *N*-des-Me-1'-CH_2OH-S-2840-A and *N*-des-Me-1'-CH_2OH-S-2840-B and 0.1 mg/kg for *N*-des-Me-S-2840) for each analyte in each matrix per time point was in the acceptable range of 70 and 110%.

Results

The recoveries of *N*-des-Me-S-2840, *N*-des-Me-1'-CH_2OH-S-2840-A and *N*-des-Me-1'-CH_2OH-S-2840-B from cucumber, grapes, soyabean seeds, wheat grain and field bean matrices after the various storage periods are summarised below.

Procedural recovery values indicate that the method worked well throughout the storage stability study. Recoveries from stored samples are consistently >70% at all time points except for the result for *N*-des-Me-S-2840 at 35 and 50 days for wheat grain (63 – 75%, mean recoveries 64 and 69% respectively). These sample timings showed procedural recoveries of 71 and 82% (so not close to 100%). Additionally, given that the results at the subsequent sampling periods (91, 182 and 377) are >70%, these results at 35 and 50 days can be disregarded, and it can be concluded that residues are stable for at least 377 days.

Table 7.1.1.1-1: Storage stability of *N*-des-Me-S-2840, *N*-des-Me-1'-CH_2OH-S-2840-A and *N*-des-Me-1'-CH_2OH-S-2840-B

Matrix	Days (months) stored	*N*-des-Me-S-2840			*N*-des-Me-1'-CH_2OH-S-2840-A			*N*-des-Me-1'-CH_2OH-S-2840-B		
		Procedural recovery (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]	Procedural recovery (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]	Procedural recovery (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]
Fortification level		**0.1 mg/kg**			**0.05 mg/kg**			**0.05 mg/kg**		
Cucumber (high water content)	0	-	106, 108, 101	105	-	84, 85, 91	87	-	82, 86, 88	85
	35 (1)	103	101, 98	100	99	95, 94	95	100	94, 93	94
	91 (3)	108	93, 98	96	89	80, 79	80	81	77, 77	77
	182 (6)	105	102, 97	100	96	86, 82	84	95	82, 79	81
	379 (12)	99	89, 91	90	90	82, 85	84	90	84, 85	85
Grape (high acid content)	0	-	101, 102, 102	102	-	81, 87, 86	85	-	80, 86, 85	84
	35 (1)	106	100, 100	100	76	76, 80	78	77	79, 82	81
	91 (3)	107	96, 104	100	81	74, 76	75	84	76, 79	78
	182 (6)	103	94, 93	94	94	88, 85	87	91	94, 85	90
	379 (12)	102	99, 96	98	87	85, 91	88	85	81, 92	87
Soyabean seed (high oil content)	0	-	94, 93, 94	94	-	71, 71, 70	71	-	72, 73, 70	72
	34 (1)	90	91, 96	94	91	87, 81	84	97	88, 86	87
	90 (3)	102	92, 96	94	79	77, 76	77	83	76, 77	77
	182 (6)	99	93, 88	91	89	82, 72	77	95	83, 77	80
	378 (12)	95	98, 99	99	81	78, 79	79	82	78, 79	79
Wheat grain (high starch content)	0	-	98, 95, 95	96	-	80, 82, 78	80	-	79, 80, 77	79
	35 (1)	71	**64**, **63**	**64**	96	91, 92	92	99	95, 99	97
	50 [c]	82	**63**, 75	**69**	-	-	-	-	-	-
	91 (3)	96	81, 86	84	87	79, 74	77	91	82, 75	79
	182 (6)	82	84, 80	82	87	81, 83	82	86	84, 80	82

	377 (12)	84	78, 79	79	93	88, 83	86	96	91, 86	89
Field bean (high protein content)	0	-	90, 89, 90	90	-	92, 93, 104	96	-	94, 92, 104	97
	34 (1)	99	102, 107	105	95	96, 98	97	95	95, 97	96
	90 (3)	107	103, 106	105	94	89, 88	89	93	84, 87	86
	181 (6)	108	104, 99	102	93	92, 86	89	94	92, 80	86
	376 (12)	100	104, 101	103	90	87, 86	87	91	89, 86	88

(a) Stored recovery values have not been corrected to take account of procedural recovery values or recoveries at time zero (recovery is of the intended fortification level of 0.1 mg/kg or 0.05 mg/kg)

(b) Mean of duplicate uncorrected recoveries, as a % of the nominal fortification level

(c) 1 month reanalysis

Conclusion

N-des-Me-S-2840, *N*-des-Me-1'-CH_2OH-S-2840-A and *N*-des-Me-1'-CH_2OH-S-2840-B have been demonstrated to be stable in cucumber (high water), grapes (high acid), soyabean seeds (high oil), wheat grain (high starch) and field bean (high protein) for at least 12 months when stored at ≤-18°C in the dark.

B.7.1.1.2 Study 2

Report:	**KCA 6.1/02; [redacted] and [redacted] (2019)**
Title:	Final report: Storage stability of S-2399 and its metabolites in matrices of plant origin
Document No.:	TPR-0093 (Study No.: S16-05016)
Guidelines:	Regulation (EC) No 1107/2009 EC Guideline 7032/VI/95, rev. 5, July 1997; Appendix H of working document 1607/VI/97, rev. 2, June 1999 – Storage Stability of Residue Samples OECD 506, 2007; OECD Guideline for the Testing of Chemicals – Stability of Pesticide Residues in Stored Commodities SANCO/3029/99 rev. 4
Guideline deviations:	None
GLP/GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

Materials and Methods

The frozen storage of inpyrfluxam and its metabolites 3'-OH-S-2840, DFPA-$CONH_2$, *N*-des-Me-DFPA, DFPA, 1'-COOH-S-2840-A & -B and 1'-CH_2OH-S-2840-A & -B was studied in cucumber (high water), grapes (high acid), soyabean seeds (high oil), wheat grain (high starch) and field bean (high protein) for 22 months.

Untreated homogenate cucumber, grapes, soyabean seeds, wheat grain and field bean sample matrices (20 g) were fortified with inpyrfluxam, 3'-OH-S-2840, DFPA-$CONH_2$, *N*-des-Me-DFPA and DFPA at 0.1 mg/kg and 1'-COOH-S-2840-A & -B and 1'-CH_2OH-S-2840-A & -B at 0.05 mg/kg (10x LOQ for all the respective analytes). A&B isomers of 1'-COOH-S-2840 and 1'-CH_2OH-S-2840 were fortified jointly, while all other analytes were fortified separately.

Three samples (two fortified and one non-fortified) per commodity matrix were kept

in glass bottles with screw caps at ≤-18°C in the dark for up to 22 months. After time intervals of approximately 1, 3, 6, 12, 18 and 22 months, samples were removed from storage and were analysed for inpyrfluxam, 3'-OH-S-2840, DFPA-$CONH_2$, *N*-des-Me-DFPA, DFPA, 1'-COOH-S-2840-A & -B and 1'-CH_2OH-S-2840-A & -B.

Analysis was performed using LC-MS/MS method validation study no. S16-03371 (SUM-1601V). Full details and validation data for this method can be found in Vol. 3 CA B.5.1.2.5. The analytical method (SUM-1601V) was sufficiently validated in accordance with SANTE/2020/12830 rev.1. The limit of quantification (LOQ) of the analytical method was 0.01 mg/kg for inpyrfluxam, 3'-OH-S-2840, DFPA-$CONH_2$, *N*-des-Me-DFPA and DFPA and 0.005 mg/kg for the A&B isomers of 1'-COOH-S-2840 and 1'-CH_2OH-S-2840. The one procedural recovery (freshly spiked samples at 10x LOQ, i.e., 0.1 mg/kg for inpyrfluxam, 3'-OH-S-2840, DFPA-$CONH_2$, *N*-des-Me-DFPA and DFPA and 0.05 mg/kg for 1'-COOH-S-2840-A & -B and 1'-CH_2OH-S-2840-A & -B) for each analyte in each matrix per time point was in the acceptable range of 70 and 110% except for wheat grain at one month (61%) for inpyrfluxam.

Results

The recoveries of inpyrfluxam, 3'-OH-S-2840, DFPA-$CONH_2$, *N*-des-Me-DFPA, DFPA, 1'-COOH-S-2840-A & -B and 1'-CH_2OH-S-2840-A & -B from cucumber, grapes, soyabean seeds, wheat grain and field bean matrices after the various storage periods are summarised below.

Table 7.1.1.2-1: Storage stability of inpyrfluxam

Matrix	Days (months) stored	Inpyrfluxam		
		Procedural recovery (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]
Fortification level		0.1 mg/kg		
Cucumber (high water content)	0	-	108, 109, 104	107
	38 (1)	88	75, 98	87
	86 (3)	107	100, 105	103
	197 (6)	103	89, 95	92
	399 (12)	106	92, 106	99
	554 (18)	107	101, 100	101
	681 (22)	102	99, 99	99
Grape (high acid content)	0	-	108, 109, 109	109
	43 (1)	108	109, 99	104
	105 (3)	100	96, 90	93
	201 (6)	106	94, 96	95
	398 (12)	109	109, 103	106
	(18)	n.a.[c]	n.a.[c]	n.a.[c]
	679 (22)	94	97, 105	101
Soyabean seed (high oil content)	0	-	79, 87, 83	83
	28 (1)	104	95, 91	93
	98 (3)	91	76, 79	78
	145 (6)	91	75, 72	74
	385 (12)	87	90, 76	83
	567 (18)	89	95, 93	94

Matrix	Days (months) stored	Inpyrfluxam		
		Procedural recovery (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]
	683 (22)	91	94, 96	95
Wheat grain (high starch content)	0	-	102, 106, 104	104
	42 (1)	**61**	91, 94	93
	85 (3)	89	90, 87	89
	196 (6)	87	88, 82	85
	395 (12)	70	81, 77	79
	549 (18)	81	85, 80	83
	679 (22)	82	88, 88	88
Field bean (high protein content)	0	-	111, 112, 108	110
	27 (1)	84	81, 83	82
	88 (3)	107	104, 97	101
	167 (6)	98	88, 90	89
	382 (12)	94	100, 93	97
	550 (18)	88	80, 87	84
	672 (22)	104	103, 103	103

[a] Stored recovery values have not been corrected to take account of procedural recovery values or recoveries at time zero (recovery is of the intended fortification level of 0.1 mg/kg)

[b] Mean of duplicate uncorrected recoveries, as a % of the nominal fortification level

[c] due to a limited amount of storage samples, analysis of storage samples at a period of 18 month was not performed for inpyrfluxam

Table 7.1.1.2-2: Storage stability of 3'-OH-S-2840, DFPA-CONH$_2$, *N*-des-Me-DFPA and DFPA

Matrix	Days (months) stored	3'-OH-S-2840			DFPA-CONH$_2$			*N*-des-Me-DFPA			DFPA		
		Procedural recovery (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]	Procedural recovery (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]	Procedural recovery (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]	Procedural recovery (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]
Fortification level		**0.1 mg/kg**			**0.1 mg/kg**			**0.1 mg/kg**			**0.1 mg/kg**		
Cucumber (high water content)	0	-	111, 109, 110	107	-	106, 107, 101	110	-	84, 89, 90	79	-	88, 88, 84	110
	38 (1)	96	78, 80	81	94	93, 92	85	85	87, 79	89	104	103, 104	103
	86 (3)	106	95, 99	96	106	84, 85	99	88	72, 81	87	98	80, 85	87
	197 (6)[c]	99	93, 92	99	106	91, 96	100	87	109, 85	96	96	86, 82	89
	399 (12)[d]	96	99, 105	102	94	108, 103	89	80	81, 89	85	97	90, 92	94
	554 (18)	105	96, 93	98	104	77, 87	97	95	76, 80	93	105	81, 74	91
	681 (22)	94	106, 104	97	96	107, 108	94	93	89, 91	90	100	91, 88	94
Grape (high acid content)	0	-	110, 109, 109	109	-	111, 109, 108	109	-	91, 87, 85	88	-	117, 109, 108	111
	43 (1)	108	96, 93	95	107	104, 105	105	97	72, 80	76	110	96, 95	96
	105 (3)[e]	99	83, 87	85	102	100, 99	100	83	82, 75	79	76	80, 79	80
	201 (6)[f]	105	92, 88	90	109	105, 107	106	89	83, 86	85	87	78, 65	72
	398 (12)	105	98, 92	95	100	105, 102	104	80	83, 80	82	100	90, 85	88
	553 (18)	-	n.a.[i]	-	-	n.a.[i]	-	-	n.a.[i]	-	86	88, 83	86
	679 (22)	95	79, 81	80	94	98, 98	98	88	94, 88	91	86	96, 91	94
Soyabean seed (high oil content)	0	-	87, 76, 81	81	-	89, 88, 83	87	-	79, 74, 70	74	-	87, 86, 87	87
	28 (1)[g]	99	96, 95	96	94	88, 88	88	79	83, 80	82	78	71, 71	71
	98 (3)[g]	84	69, 72	71	90	83, 80	82	71	74, 75	75	76	81, 82	82
	145 (6)[g]	90	79, 79	79	97	83, 81	82	70	73, 75	74	81	77, 74	76
	385 (12)[g]	86	81, 86	84	88	84, 87	86	71	83, 74	79	86	94, 92	93
	567 (18)[g]	89	98, 88	93	92	85, 95	90	75	74, 75	75	87	81, 82	82

	683 (22)	88	90, 87	89	92	82, 82	82	79	70, 75	73	71	76, 72	74
Wheat grain (high starch content)	0	-	106, 113, 115	111	-	106, 109, 105	107	-	81, 82, 77	80	-	94, 103, 115	104
	42 (1)	74	98, 97	98	77	90, 111	101	86	93, 99	96	93	90, 92	91
	85 (3)	86	90, 92	91	94	102, 94	98	78	77, 81	79	97	80, 93	87
	196 (6)	109	105, 102	104	105	103, 103	103	76	82, 84	83	82	76, 77	77
	395 (12)	82	79, 79	79	84	73, 90	82	110	108, 84	96	73	80, 72	76
	549 (18)	95	87, 92	90	96	99, 96	98	91	97, 82	90	107	75, 74	75
	679 (22)	89	87, 78	83	91	95, 87	91	78	71, 75	73	102	84, 88	86
Field bean (high protein content)	0	-	111, 109, 110	110	-	106, 107, 101	105	-	84, 89, 90	88	-	88, 88, 84	87
	27 (1)	79	78, 80	79	93	93, 92	93	85	87, 79	83	97	103, 104	104
	88 (3)	106	95, 99	97	107	84, 85	85	81	72, 81	77	93	80, 85	83
	167 (6)[(h)]	96	93, 92	93	97	91, 96	94	112	109, 85	97	100	86, 82	84
	382 (12)	97	99, 105	102	95	108, 103	106	81	81, 89	85	79	90, 92	91
	550 (18)	86	96, 93	95	88	77, 87	82	76	76, 80	78	81	81, 74	78
	672 (22)	102	106, 104	105	97	107, 108	108	89	89, 91	90	93	91, 88	90

[(a)] Stored recovery values have not been corrected to take account of procedural recovery values or recoveries at time zero (recovery is of the intended fortification level of 0.1 mg/kg)

[(b)] Mean of duplicate uncorrected recoveries, as a % of the nominal fortification level

[(c)] 159 days for DFPA

[(d)] 407 days for DFPA

[(e)] 88 days for *N*-des-Me-DFPA and DFPA

[(f)] 202 days for *N*-des-Me-DFPA

[(g)] Intervals for DFPA were 31, 87, 182, 374 and 500 days

[(h)] 175 days for *N*-des-Me-DFPA and DFPA

[(i)] due to a limited amount of storage samples, analysis of storage samples at a period of 18 month was not performed for 3'-OH-S-2840, DFPA-$CONH_2$ and *N*-des-Me-DFPA

Table 7.1.1.2-3: Storage stability of 1'-COOH-S-2840-A, 1'-COOH-S-2840-B, 1'-CH_2OH-S-2840-A and 1'-CH_2OH-S-2840-B

	Days (months) stored	1'-COOH-S-2840-A			1'-COOH-S-2840-B			1'-CH_2OH-S-2840-A			1'-CH_2OH-S-2840-B		
		Procedural recovery (%)	Stored recoveries (%)[a]	Mean stored recovery(%)[b]	Procedural recovery (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]	Procedural recovery (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]	Procedural recovery (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]
Fortification level		**0.05 mg/kg**			**0.05 mg/kg**			**0.05 mg/kg**			**0.05 mg/kg**		
Cucumber (high water content)	0	-	115, 100, 90	102	-	113, 98, 88	100	-	105, 103, 106	105	-	107, 103, 105	105
	38 (1)	97	102, 103	103	96	101, 100	101	98	102, 102	102	96	101, 99	100
	86 (3)	92	82, 82	82	98	77, 81	79	101	95, 93	94	102	95, 93	94
	197 (6)	90	87, 83	85	91	87, 85	86	86	77, 77	77	87	79, 79	79
	399 (12)[c]	92	90, 90	90	88	84, 91	88	100	92, 101	97	95	95, 96	96
	554 (18)	94	86, 88	87	92	82, 90	86	92	85, 84	85	94	85, 86	86
	681 (22)	103	98, 100	99	108	96, 99	98	94	96, 96	96	95	102, 103	103
Grape (high acid content)	0	-	103, 109, 109	107	-	103, 107, 109	106	-	106, 102, 105	104	-	105, 100, 100	102
	43 (1)	93	85, 91	88	98	83, 89	86	101	89, 91	90	97	88, 87	88
	105 (3)[d]	78	92, 84	88	81	93, 86	90	82	90, 88	89	80	88, 89	89
	201 (6)	84	80, 81	81	92	82, 83	83	74	83, 80	82	85	83, 81	82
	398 (12)	89	95, 86	91	87	91, 94	93	95	94, 92	93	96	100, 105	103
	553 (18)	78	88, 84	86	74	85, 83	84	89	95, 93	94	92	98, 91	95
	679 (22)	93	87, 92	90	96	80, 89	85	86	91, 88	90	89	95, 95	95
Soyabean seed (high oil content)	0	-	87, 88, 89	88	-	81, 87, 83	84	-	80, 77, 80	79	-	79, 72, 79	77
	28 (1)	83	88, 90	89	87	86, 87	87	88	89, 89	89	89	92, 92	92
	98 (3)	84	77, 77	77	80	74, 77	76	84	80, 79	80	81	79, 77	78
	145 (6)	83	72, 72	72	80	68, 68	68	74	66, 67	67	76	66, 66	66
	385 (12)	75	82, 73	78	79	87, 75	81	80	82, 83	83	82	84, 83	84

	567 (18)	78	87, 76	82	90	90, 85	88	80	72, 71	72	92	78, 72	75
	683 (22)	99	87, 81	84	93	89, 84	87	93	78, 81	80	93	77, 81	79
Wheat grain (high starch content)	0	-	112, 102, 102	105	-	108, 102, 98	103	-	113, 109, 110	111	-	112, 108, 112	111
	42 (1)	77	83, 80	82	80	83, 81	82	78	84, 78	81	77	84, 77	81
	85 (3)	106	102, 100	101	107	95, 96	96	104	104, 111	108	102	100, 112	106
	196 (6)	84	70, 84	77	83	76, 80	78	87	77, 75	76	85	78, 79	79
	395 (12)	79	77, 77	77	76	75, 75	75	78	80, 90	85	77	89, 88	89
	549 (18)	87	85, 79	82	87	87, 78	83	86	78, 79	79	91	82, 87	85
	679 (22)	98	84, 72	78	106	82, 74	78	102	78, 84	81	106	78, 86	82
Field bean (high protein content)	0	-	101, 99, 99	100	-	98, 96, 99	98	-	101, 99, 102	101	-	101, 94, 97	97
	27 (1)	94	103, 104	104	97	103, 102	103	96	101, 107	104	96	102, 104	103
	88 (3)	91	77, 82	80	92	79, 82	81	94	89, 85	87	97	93, 90	92
	167 (6)[(e)]	91	87, 82	85	94	89, 83	86	93	85, 86	86	89	85, 85	85
	382 (12)	77	90, 89	90	79	90, 89	90	83	91, 96	94	80	92, 99	96
	550 (18)	76	75, 79	77	75	75, 77	76	73	71, 72	72	75	71, 73	72
	672 (22)	94	90, 90	90	97	87, 88	88	82	82, 78	80	84	86, 84	85

[(a)] Stored recovery values have not been corrected to take account of procedural recovery values or recoveries at time zero (recovery is of the intended fortification level of 0.05 mg/kg)

[(b)] Mean of duplicate uncorrected recoveries, as a % of the nominal fortification level

[(c)] 407 days for 1'-COOH-S-2480-A and -B, and 1'-CH_2OH-S-2840-A and -B

[(d)] 88 days for 1'-COOH-S-2480-A and -B, and 1'-CH_2OH-S-2840-A and -B

[(e)] 175 days for 1'-COOH-S-2480-A and -B, and 1'-CH_2OH-S-2840-A and -B

Conclusion

Inpyrfluxam, 3'-OH-S-2840, DFPA-$CONH_2$, *N*-des-Me-DFPA, DFPA, 1'-COOH-S-2840-A & -B and 1'-CH_2OH-S-2840-A & -B have been demonstrated to be stable in cucumber (high water), grapes (high acid), soyabean seeds (high oil), wheat grain (high starch) and field bean (high protein) for at least 22 months when stored at ≤-18°C in the dark.

B.7.1.1.3 Study 3

Report:	**KCA 6.1/03; [redacted] and [redacted] (2019)**
Title:	Final report: Storage stability of S-2399 and its metabolites in processed food matrices of plant origin
Document No.:	TPR-0101 (Study No.: S18-03229)
Guidelines:	Regulation (EC) No 1107/2009 EC Guideline 7032/VI/95, rev. 5, July 1997; Appendix H of working document 1607/VI/97, rev. 2, June 1999 – Storage Stability of Residue Samples OECD 506, 2007; OECD Guideline for the Testing of Chemicals – Stability of Pesticide Residues in Stored Commodities SANCO/3029/99 rev. 4
Guideline deviations:	None
GLP/GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

Materials and Methods

The frozen storage of inpyrfluxam and its metabolites 3'-OH-S-2840, DFPA-$CONH_2$, *N*-des-Me-DFPA, DFPA, 1'-COOH-S-2840-A & -B and 1'-CH_2OH-S-2840-A & -B was studied in potato (starch and crisps) and wheat (flour and bread) for 14 and 17 months respectively.

5 g of untreated homogenate potato starch and crisps and wheat flour sample matrices and 20 g of untreated homogenate wheat flour were fortified with inpyrfluxam, 3'-OH-S-2840, DFPA-$CONH_2$, *N*-des-Me-DFPA and DFPA at 0.1 mg/kg and 1'-COOH-S-2840-A & -B and 1'-CH_2OH-S-2840-A & -B at 0.05 mg/kg (10x LOQ for all the respective analytes). A&B isomers of 1'-COOH-S-2840 and 1'-CH_2OH-S-2840 were fortified jointly, while all other analytes were fortified separately.

Three samples (two fortified and one non-fortified) per commodity matrix were kept in glass bottles with screw caps at ≤-18°C in the dark for up to 17 months. After time

intervals of approximately 7 or 10 and 14 or 17 months, samples were removed from storage and were analysed for inpyrfluxam, 3'-OH-S-2840, DFPA-$CONH_2$, *N*-des-Me-DFPA, DFPA, 1'-COOH-S-2840-A & -B and 1'-CH_2OH-S-2840-A & -B.

Analysis was performed using LC-MS/MS method validation study no. S16-03371 (SUM-1601V). Full details and validation data for this method can be found in Vol. 3 CA B.5.1.2.5. The analytical method (SUM-1601V) was sufficiently validated in accordance with SANTE/2020/12830 rev.1. The limit of quantification (LOQ) of the analytical method was 0.01 mg/kg for inpyrfluxam, 3'-OH-S-2840, DFPA-$CONH_2$, *N*-des-Me-DFPA and DFPA and 0.005 mg/kg for the A&B isomers of 1'-COOH-S-2840 and 1'-CH_2OH-S-2840. The one procedural recovery (freshly spiked samples at 10x LOQ, i.e., 0.1 mg/kg for inpyrfluxam, 3'-OH-S-2840, DFPA-$CONH_2$, *N*-des-Me-DFPA and DFPA and 0.05 mg/kg for 1'-COOH-S-2840-A & -B and 1'-CH_2OH-S-2840-A & -B) for each analyte in each matrix per time point was in the acceptable range of 70 and 110% except for potato crisps at 7 months (68%) for *N*-des-Me-DFPA.

Results

The recoveries of inpyrfluxam, 3'-OH-S-2840, DFPA-$CONH_2$, *N*-des-Me-DFPA, DFPA, 1'-COOH-S-2840-A & -B and 1'-CH_2OH-S-2840-A & -B from potato starch and crisps and wheat flour and bread matrices after the various storage periods are summarised below.

Table 7.1.1.3-1: Storage stability of inpyrfluxam

Matrix	Days (months) stored	Inpyrfluxam		
		Procedural recovery (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]
Fortification level		0.1 mg/kg		
Potato starch	0	-	102, 107, 109	106
	217 (7)	83	86, 79	83
	425 (14)	108	100, 99	100
Potato crisps	0	-	107, 110, 110	109
	217 (7)	82	87, 94	91
	428 (14)	108	101, 99	100
Wheat flour	0	-	81, 70, 71	74
	298 (10)	86	74, 91	83
	518 (17)	106	93, 106	100
Wheat bread	0	-	105, 107,110	107
	220 (7)	109	100, 101	101
	518 (17)	108	98, 96	97

[a] Stored recovery values have not been corrected to take account of procedural recovery values or recoveries at time zero (recovery is of the intended fortification level of 0.1 mg/kg)

[b] Mean of duplicate uncorrected recoveries, as a % of the nominal fortification level

Table 7.1.1.3-2: Storage stability of 3'-OH-S-2840, DFPA-CONH$_2$, *N*-des-Me-DFPA and DFPA

Matrix	Days (months) stored	3'-OH-S-2840			DFPA-CONH$_2$			*N*-des-Me-DFPA			DFPA		
		Procedural recovery (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]	Procedural recovery (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]	Procedural recovery (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]	Procedural recovery (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]
Fortification level		**0.1 mg/kg**			**0.1 mg/kg**			**0.1 mg/kg**			**0.1 mg/kg**		
Potato starch	0	-	107, 102, 110	106	-	106, 102, 108	105	-	83, 91, 96	90	-	105, 107, 110	107
	217 (7)	89	97, 96	97	79	88, 85	87	70	77, 71	74	98	96, 93	95
	425 (14)	101	105, 101	103	108	99, 103	101	86	82, 70	76	110	94, 103	99
Potato crisps	0	-	110, 110, 110	110	-	110, 105, 115	110	-	79, 72, 81	77	-	108, 107, 110	108
	217 (7)	97	98, 103	101	106	99, 92	96	**68**	84, 80	82	122	100, 99	100
	428 (14)	104	109, 101	105	109	106, 105	106	83	78, 83	81	110	95, 96	96
Wheat flour	0	-	91, 94, 95	93	-	98, 108, 90	99	-	70, 71, 72	71	-	92, 92, 93	92
	298 (10)	99	86, 91	89	90	85, 95	90	75	**64**, 73	**69**	101	90, 95	93
	518 (17)	106	105, 101	103	107	104, 106	105	85	78, 78	78	109	97, 94	96
Wheat bread	0	-	101, 99, 104	101	-	95, 98, 102	98	-	80, 87, 84	84	-	84, 89, 84	86
	220 (7)	106	102, 102	102	107	106, 101	104	70	75, 76	76	108	98, 96	97
	519 (17)	107	99, 102	101	108	105, 102	104	100	98, 93	96	99	95, 92	94

[a] Stored recovery values have not been corrected to take account of procedural recovery values or recoveries at time zero (recovery is of the intended fortification level of 0.1 mg/kg)

[b] Mean of duplicate uncorrected recoveries, as a % of the nominal fortification level

Table 7.1.1.3-3: Storage stability of 1'-COOH-S-2840-A, 1'-COOH-S-2840-B, 1'-CH_2OH-S-2840-A and 1'-CH_2OH-S-2840-B

Matrix	Days (months) stored	1'-COOH-S-2840-A			1'-COOH-S-2840-B			1'-CH_2OH-S-2840-A			1'-CH_2OH-S-2840-B		
		Procedural recovery (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]	Procedural recovery (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]	Procedural recovery (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]	Procedural recovery (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]
Fortification level		**0.05 mg/kg**			**0.05 mg/kg**			**0.05 mg/kg**			**0.05 mg/kg**		
Potato starch	0	-	94, 94, 95	94	-	93, 93, 95	94	-	103, 100, 99	101	-	91, 91, 89	90
	217 (7)	81	79, 77	78	85	81, 79	80	90	81, 77	79	86	76, 75	76
	425 (14)	94	81, 76	79	96	88, 82	85	95	87, 87	87	95	77, 78	78
Potato crisps	0	-	98, 98, 97	98	-	99, 100, 100	100	-	99, 104, 104	102	-	101, 95, 101	99
	217 (7)	87	87,77	82	89	99, 86	93	88	76, 83	80	86	78, 78	78
	428 (14)	99	89, 88	89	99	90, 92	91	95	85, 89	87	95	88, 85	87
Wheat flour	0	-	94, 88, 85	89	-	91, 85, 88	88	-	87, 83, 92	87	-	80, 86, 84	83
	298 (10)	76	71, 77	74	86	75, 74	75	83	81, 94	88	84	81, 88	85
	518 (17)	85	81, 84	83	88	84, 86	85	85	88, 89	89	87	92, 90	91
Wheat bread	0	-	95, 97, 95	96	-	99, 101, 97	99	-	96, 96, 94	95	-	101, 99, 98	99
	220 (7)	99	94, 81	88	99	99, 90	95	103	97, 87	92	102	96, 91	94
	519 (17)	93	87, 85	86	97	89, 86	88	99	97, 87	93	95	94, 93	94

[a] Stored recovery values have not been corrected to take account of procedural recovery values or recoveries at time zero (recovery is of the intended fortification level of 0.05 mg/kg)

[b] Mean of duplicate uncorrected recoveries, as a % of the nominal fortification level

Conclusion

Inpyrfluxam and its metabolites 3'-OH-S-2840, DFPA-$CONH_2$, *N*-des-Me-DFPA, DFPA, 1'-COOH-S-2840-A & -B and 1'-CH_2OH-S-2840-A & -B have been demonstrated to be stable in potato (starch and crisps) and wheat (flour and bread) when stored at ≤-18°C in the dark for 14 and 17 months respectively.

B.7.1.1.4 Study 4

Report:	**KCA 6.1/04; [redacted] (2017a)**
Title:	S-2399: Freezer Storage Stability of S-2399 and Metabolites in Crops
Document No.:	TPR-0067 (Study No.: VP-39115)
Guidelines:	US EPA Residue Chemistry Guideline OCSPP 860.1380 “Storage Stability Data”
Guideline deviations:	None
GLP/GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

Materials and Methods

The frozen storage of inpyrfluxam and its metabolites 3'-OH-S-2840, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B and DFPA-$CONH_2$ in apple, soyabean seed, potato, maize/corn grain, maize/corn forage and maize/corn stover for up to 20 months. Additionally, the stability of the metabolites 1'-COOH-S-2840-A and 1'-COOH-S-2840-B were investigated in potato, maize/corn grain, maize/corn forage and maize/corn stover for up to 20 months.

10 g of untreated homogenate apples, soyabean seeds, potatoes, com grain, and com forage sample matrices and 2.5 g of untreated homogenate maize/corn stover were fortified with inpyrfluxam, 3'-OH-S-2840, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B, DFPA-$CONH_2$ at a level of 0.1 mg/kg (10x LOQ). 10 g of untreated homogenate potatoes and 2.5 g of untreated homogenate maize/corn stover were fortified with 1'-COOH-S-2840-B at a level of 0.1 mg/kg (10x LOQ) and with 1'-COOH-S-2840-A at a level of 0.129 mg/kg.

Four samples (two fortified and two non-fortified) per commodity matrix were kept in glass bottles with screw caps at ≤-20°C in the dark for up to 20 months. A deviation occurred for apple samples intended to show stability of DFPA-$CONH_2$, 3’-OH-S-2840, 1’-CH_2OH-S-2840-A, and 1’-CH_2OH-S-2840-B for 126 days, as one set of samples

were not fortified. Therefore, only one stored sample is available for this time interval. This is not deemed to affect the overall conclusion of stability as this was the first time interval investigated and all later timepoints have two stored sample results that provide results that support the conclusion of overall stability in the study.

At least 4 timepoints samples were removed from storage and were analysed for inpyrfluxam, 3'-OH-S-2840, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B, DFPA-$CONH_2$, 1'-COOH-S-2840-A and 1'-COOH-S-2840-B.

Analysis was performed using LC-MS/MS method RM-50C-1. Full details and validation data for this method can be found in Vol. 3 CA B.5.1.2.5. The analytical method (RM-50C-1) was sufficiently validated in accordance with SANTE/2020/12830 rev.1. The limit of quantification (LOQ) of the analytical method was 0.01 mg/kg for all analytes. The procedural recoveries of most samples (freshly spiked samples at 0.1 mg/kg, 10x LOQ, with inpyrfluxam, 3'-OH-S-2840, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B, DFPA-$CONH_2$ and 1'-COOH-S-2840-B and at 0.129 mg/kg with 1′-COOH-S-2840-A) were in the acceptable range of 70 and 110%. Those that fell outside that range are discussed below and highlighted in bold in the tables.

Results

The recoveries of 3'-OH-S-2840, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B and DFPA-$CONH_2$ from apple, soyabean seed, potato, maize/corn grain, maize/corn forage and maize/corn stover matrices and 1'-COOH-S-2840-A and 1'-COOH-S-2840-B from potato, maize/corn grain, maize/corn forage and maize/corn stover matrices after the various storage periods are summarised below.

Analysis was not performed at day 0, the first time period studied is after 4 months of storage. This is not generally considered a suitable approach for determining storage stability on compounds which are analysed for in magnitude of residues studies, as the sample fortification levels are not reported in the day 0 data. In the other studies, the recovery of storage has not been corrected for the recoveries at time zero, recovery is a % of the intended fortification level for each analyte. Therefore, this study is still considered valid despite the lack of day 0 results.

Procedural recovery values indicate that the method worked reasonably well throughout the storage stability study, noting that there were some low (60 – 70%) and some high individual procedural recoveries (up to 132.1%); these were generally accompanied by stored recoveries of similar magnitudes.

Individual and mean stored recoveries found between 60 – 70% (highlighted in bold in the tables) are all followed by recoveries >70% at subsequent sampling periods. The procedural recoveries at the same time periods where low stored recoveries were

observed were generally <70%, which may have impacted the stored recoveries. These results of <70% can therefore be disregarded and it can be concluded that residues of inpyrfluxam and its metabolites are stable for at least 16 months.

There were some high individual and mean stored recoveries (>100 – 206.7). These were generally accompanied by procedural recoveries >110%. Whilst unusual compared to the results of the other studies, this does not impact the conclusions on stability.

Table 7.1.1.4-1 Storage stability of inpyrfluxam

Matrix	Days (months) stored	Inpyrfluxam		
		Procedural recoveries (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]
Fortification level		0.1 mg/kg		
Apples	132 (4)	75.9, 99.6	79.0, 71.8	75.4
	253 (8)	100.0, 104.6	95.3, 89.0	92.2
	377 (12)	82.8, 91.9	84.9, 81.8	83.4
	514 (16)	95.4, 92.4	93.3, 87.6	90.5
Soyabean seed	132 (4)	**64.6**, 70.0	**68.9**, **64.0**	**66.5**
	275 (9)	92.5, 91.3	91.2, 95.0	93.1
	422 (13)	81.9, 86.6	77.0, 74.7	75.9
	440 (14)	87.6, 88.0	79.6, 82.9	81.3
	513 (16)	87.7, 99.7	80.1, 85.0	82.6
	594 (19)	94.2, 94.4	84.8, 87.8	86.3
Potato	141 (4)	85.4, 82.2	79.4, 72.8	76.1
	275 (9)	95.4, 90.9	86.3, 88.3	87.3
	393 (13)	93.2, 93.5	84.0, 90.4	87.2
	513 (16)	95.3, 96.0	92.3, 88.4	90.4
	623 (20)	91.2, 90.5	88.4, 90.2	89.3
Maize/corn grain	141 (4)	80.6, 87.2	77.2, 77.7	77.5
	294 (9)	87.3, 88.0	75.9, 83.2	79.6
	356 (11)	90.6, 89.2	84.2, 85.0	84.6
	420 (13)	91.1, 93.5	92.1, 86.4	89.3
	510 (16)	96.1, 96.8	89.8, 85.9	87.9
	630 (20)	91.4, 94.2	93.1, 83.0	88.1
Maize/corn forage	155 (5)	79.1, 84.4	86.4, 85.9	86.2
	253 (8)	89.5, 89.6	83.7, 86.5	85.1
	343 (11)	82.1, 85.0	80.6, 78.1	79.4
	429 (14)	91.9, 82.9	73.3, 77.0	79.4
	491 (16)	97.1, 95.8	80.5, 84.1	82.3
	597 (19)	92.5, 91.0	87.0, 82.7	84.9
Maize/corn stover	149 (4)	**63.5**, 74.8	**65.1**, **65.5**	**65.3**
	336 (11)	81.2, 83.7	80.0, 82.0	81.0
	423 (14)	82.5, 80.5	82.8, 82.9	82.9
	505 (16)	85.1, 86.0	85.7, 87.1	86.4
	532 (17)	91.5, 92.6	83.0, 86.8	84.9
	591 (19)	104.8, 100.2	88.1, 92.1	90.1

[a] Stored recovery values have not been corrected to take account of procedural recovery values. The recovery is of the intended fortification level of 0.1 mg/kg

[b] Mean of duplicate uncorrected recoveries, as a % of the nominal fortification level

Table 7.1.1.4-2: Storage stability of 3'-OH-S-2840, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B and DFPA-$CONH_2$

Matrix	Days (months) stored	3'-OH-S-2840			1'-CH_2OH-S-2840-A			1'-CH_2OH-S-2840-B			DFPA-$CONH_2$		
		Procedural recoveries (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]	Procedural recoveries (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]	Procedural recoveries (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]	Procedural recoveries (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]
Fortification level: 0.1 mg/kg													
Apples	126 (4)	86.9, 102.3	81.6	81.6	96.5, 99.1	83.1	83.1	101.7, 104.2	87.2	87.2	96.3, 89.8	87.0	87.0
	247 (8)	**112.4**, **122.5**	106.8, 100.5	103.7	92.0, 94.1	88.0, 88.3	88.2	94.2, 90.5	91.8, 89.7	90.8	93.6, 89.6	93.6, 96.6	95.1
	371 (12)	84.4, 96.0	86.4, 85.5	86.0	88.0, 94.5	89.1, 89.8	89.5	90.0, 89.5	101.8, 101.3	101.6	88.5, 93.4	92.1, 97.0	94.6
	508 (16)	99.1, 94.0	91.2, 91.4	91.3	100.1, 98.5	100.9, 99.5	100.2	96.2, 93.2	97.6, 94.0	95.8	**122.9**, 107.2	**206.7**, 111.1	**158.9**
Soyabean seed	126 (4)	77.3, 79.5	72.2, 68.0	70.1	87.1, 89.5	74.5, 72.3	73.4	87.0, 83.0	71.0, **65.6**	**68.3**	80.2, 78.2	80.7, 75.6	78.2
	269 (8)	106.2, 107.5	105.0, 120.1	112.6	90.7, 88.0	88.7, 101.0	94.85	84.5, 87.0	94.8, 92.7	93.8	79.5, 83.1	79.0, 87.0	83.0
	416 (13)	90.5, 91.5	79.1, 83.4	81.3	86.9, 87.1	82.7, 82.7	82.7	87.3, 87.8	86.7, 86.6	86.7	79.7, 78.4	84.1, 89.0	86.6
	434 (14)	87.8, 90.7	83.1, 89.8	86.5	83.9, 85.6	79.2, 83.1	81.2	86.8, 87.0	87.4, 92.5	89.0	83.6, 86.8	92.9, 90.2	91.6
	507 (16)	92.2, 99.6	82.9, 90.7	86.8	94.0, 100.1	86.6, 97.7	92.2	87.0, 94.6	93.5, 95.4	94.5	109.6, 108.3	104.0, 97.0	100.5
	588 (19)	96.5, 94.9	90.9, 90.2	90.6	96.0, 100.1	96.2, 96.5	96.4	90.2, 88.6	91.6, 92.9	92.3	**118.8, 115.2**	**120.0, 118.4**	**119.2**
Potato	128 (4)	99.7, 100.7	**69.8**, 84.6	77.2	88.9, 83.5	73.1, 82.1	77.6	94.8, 92.1	71.2, 86.0	78.6	97.8, 81.3	**64.2**, 76.4	70.3
	262 (8)	100.9, 95.4	94.8, 90.3	92.6	94.2, 98.6	93.0, 87.6	90.3	97.3, 89.5	96.2, 92.7	94.5	89.6, 85.5	88.1, 90.4	89.3

	380 (12)	95.3, 98.1	88.4, 91.0	89.7	92.8, 94.2	90.4, 95.4	92.9	90.7, 91.6	93.0, 96.7	94.9	96.6, 93.6	90.0, 97.3	93.7
	500 (16)	102.4, 99.9	98.9, 90.5	94.7	102.8, 89.5	104.3, 95.4	99.9	97.9, 99.1	101.9, 97.0	99.5	**128.1, 121.7**	**131.2, 124.3**	**127.4**
	610 (20)	95.1, 93.5	88.0, 83.1	85.6	89.5, 91.4	100.0, 92.5	96.3	90.5, 93.4	99.0, 94.0	96.5	92.6, 95.4	99.1, 89.8	94.5
Maize/corn grain	127 – 135 (4)	95.6, 97.8	83.8, 82.9	83.4	86.4, 88.6	80.4, 79.7	80.1	94.2, 95.3	85.9, 81.4	83.7	82.4, 79.6	81.3, 82.0	81.7
	280 – 288 (9)	92.4, 94.9	94.8, 97.8	96.3	89.4, 88.0	90.9, 93.3	92.1	90.5, 90.4	101.0, 101.2	101.1	83.6, 80.0	90.8, 96.0	93.4
	342 – 350 (11)	94.7, 89.2	88.9, 87.3	88.1	92.8, 86.8	86.2, 76.5	81.4	92.4, 88.1	85.0, 75.9	80.5	78.9, 80.6	89.1, 88.1	88.6
	406 – 414 (13)	90.9, 95.2	88.3, 92.2	90.3	93.1, 94.4	89.3, 94.9	92.1	92.2, 90.8	89.8, 92.9	91.4	83.5, 90.4	91.9, 86.4	89.2
	496 – 504 (16)	97.4, 96.4	91.1, 96.3	93.7	94.8, 94.2	92.8, 98.0	95.4	87.8, 88.2	94.9, 97.7	96.3	90.8, 89.4	94.2, 91.8	93.0
	616 – 624 (20)	94.0, 91.3	89.1, 88.0	88.6	92.3, 88.3	98.4, 99.4	98.9	90.0, 89.5	101.8, 101.3	101.6	89.2, 86.5	92.3, 89.4	90.9
Maize/corn forage	142 – 149 (4)	77.4, 88.1	72.6, 69.9	71.3	82.1, 84.4	81.8, 84.2	83.0	81.9, 84.9	71.4, 74.4	72.9	87.0, 96.4	84.1, 83.9	84.0
	240 – 247 (8)	91.5, 93.9	90.0, 98.4	94.2	92.8, 93.5	94.2, 101.3	97.8	89.0, 91.4	97.2, 105.6	101.4	89.8, 92.9	90.8, 84.2	87.5
	330 – 337 (11)	82.4, 86.1	81.2, 84.2	82.7	83.6, 82.1	77.8, 83.4	80.6	81.3, 84.6	85.5, 89.7	87.6	83.6, 87.9	88.7, 87.0	87.9
	416 – 423 (13)	90.8, 87.6	85.7, 84.0	84.9	91.5, 85.2	82.4, 85.9	84.2	93.0, 83.2	88.3, 88.7	88.5	92.0, 85.6	80.2, 80.0	80.1
	478 – 485 (15)	98.9, 96.5	87.1, 80.6	83.9	100.9, 98.0	86.5, 85.1	85.8	98.3, 93.4	90.5, 86.5	88.5	99.2, 91.5	96.4, 89.7	93.1
	584 – 591 (19)	92.2, 94.6	90.5, 89.4	90.0	97.2, 98.0	97.9, 92.8	95.4	92.5, 90.1	94.0, 96.5	95.3	**115.9, 121.6**	**126.7, 115.3**	**121**
Maize/corn stover	142 – 145 (4)	96.3, 93.9	80.4, 77.7	79.1	94.7, 85.4	84.6, 81.1	82.9	90.0, 91.3	76.1, 75.7	75.9	97.7, 94.9	91.1, 104.6	97.9
	329 – 332 (11)	81.1, 87.2	80.1, 77.1	78.6	79.8, 85.7	83.1, 81.6	82.4	80.4, 85.0	86.2, 86.1	86.2	77.6, 79.8	77.3, 89.4	83.4

	416 – 419 (13)	91.0, 90.6	87.1, 86.3	86.7	90.4, 97.1	93.4, 94.3	93.9	93.0, 96.1	100.8, 101.7	101.3	92.9, 96.0	94.6, 97.8	96.2
	498 – 501 (16)	95.7, 96.0	89.2, 83.2	86.2	104.8, 102.6	102.1, 91.9	97.0	99.3, 100.9	102.6, 96.7	99.7	**126.1, 116.4**	**114.5, 125.7**	**120.1**
	525 – 528 (17)	96.4, 89.2	84.8, 86.2	85.5	87.0, 91.9	84.1, 85.4	84.8	88.1, 92.6	92.0, 91.5	91.8	87.8, 83.3	88.2, 87.3	87.8
	584 – 587 (19)	99.4, 99.6	85.7, 89.7	87.7	110.4, 106.2	94.7, 100.4	97.6	96.1, 96.9	95.7, 93.1	94.4	**132.1, 120.7**	**122.5, 131.1**	**126.8**

(a) Stored recovery values have not been corrected to take account of procedural recovery values. Recovery is of the intended fortification level of 0.1 mg/kg

(b) Mean of duplicate uncorrected recoveries, as a % of the nominal fortification level

Table 7.1.1.4-3: Storage stability of 1'-COOH-S-2840-A and 1'-COOH-S-2840-B

Matrix	Days (months) stored	1'-COOH-S-2840-A			1'-COOH-S-2840-B		
		Procedural recoveries (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]	Procedural recoveries (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]
Fortification level		0.129 mg/kg			0.1 mg/kg		
Potato	128 (4)	93.0, 94.8	82.5, 86.5	84.5	90.5, 87.5	76.6, 81.4	79.0
	262 (8)	77.4, 71.8	78.2, 72.7	75.5	90.8, 97.3	81.4, 73.2	77.3
	380 (12)	91.8, 97.9	97.8, 107.1	102.5	88.7, 93.6	84.3, 90.9	87.6
	500 (16)	92.0, 93.8	94.9, 90.1	92.5	106.6, 106.9	98.5, 94.6	96.6
	610 (20)	92.0, 93.2	77.9, 73.3	75.6	87.9, 91.3	75.8, 72.5	74.2
Maize/corn grain	127 (4)	90.4, 84.8	82.6, 84.1	83.4	85.4, 84.3	84.9, 82.9	83.9
	280 (8)	83.7, 87.6	97.2, 102.5	99.9	81.2, 82.8	81.3, 85.8	83.6
	342 (11)	96.3/79.7, 87.1/80.7	88.6, 96.1	92.4	93.6/79.3, 83.4/81.3	75.1, 84.4	79.8
	406 (13)	88.0, 93.6	89.0, 98.1	93.6	84.7, 94.9	75.7, 83.6	79.7
	496 (16)	96.5, 93.4	94.1, 94.8	94.5	91.7, 88.1	81.8, 85.6	83.7
	616 (20)	90.4, 85.3	81.1, 77.3	79.2	91.0, 88.9	77.2, 76.3	76.8
Maize/corn forage	142 (4)	84.7, 87.9	81.9, 83.8	82.9	83.4, 87.3	84.8, 85.9	83.4
	240 (8)	87.5, 84.8	92.6, 92.2	92.4	86.7, 85.6	86.3, 80.7	83.5
	330 (11)	**63.8, 66.4**	**68.3**, **69.6**	**69.0**	80.5, 82.4	73.7, 76.4	75.1
	416 (13)	**65.5, 65.3**	71.4, 70.5	71.0	82.6, 82.4	76.5, 70.1	73.3
	478 (15)	97.2, 92.7	87.6, 86.9	87.3	95.4, 90.1	74.9, 73.9	74.4
	584 (19)	87.6, 87.1	89.4, 86.7	88.1	95.2, 100.7	86.7, 90.2	88.5
Maize/corn stover	142 (4)	84.5, 88.6	84.7, 87.3	86.0	86.7, 85.0	87.2, 82.2	84.7
	329 (11)	78.0, 80.6	82.6, 86.8	84.7	81.2, 82.1	74.2, 77.9	76.1
	416 (13)	84.0, 81.9	89.8, 89.7	89.8	83.2, 85.9	81.4, 79.8	80.6
	498 (16)	89.4, 83.9	85.3, 83.1	84.2	101.4, 93.3	84.2, 81.0	82.6
	525 (17)	84.4, 85.3	79.1, 80.8	80.0	82.7, 87.8	75.6, 73.3	75.5
	584 (19)	87.6, 89.9	90.6, 80.5	85.6	104.1, 101.6	88.5, 89.3	88.9

[a] Stored recovery values have not been corrected to take account of procedural recovery values. Recovery is of the intended fortification level of 0.129 or 0.1 mg/kg

[b] Mean of duplicate uncorrected recoveries, as a % of the nominal fortification level

Conclusion

Inpyrfluxam, 3'-OH-S-2840, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B and DFPA-$CONH_2$ have been demonstrated to be stable when stored at ≤-18°C in apple (high water) for 16 months, in soyabean seed (high oil) for 19 months, in potato (high starch) for 20 months, in maize/corn grain (high starch) for 20 months, in maize/corn forage (high water) for 19 months and in maize/corn stover (miscellaneous) for 19 months.

1'-COOH-S-2840-A and 1'-COOH-S-2840-B have been demonstrated to be stable when stored at ≤-18°C in in potato (high starch) for 20 months, in maize/corn grain

(high starch) for 20 months, in maize/corn forage (high water) for 19 months and in maize/corn stover (miscellaneous) for 19 months.

B.7.1.1.5 Study 5

Report:	**KCA 6.1/05; [redacted] (2017b)**
Title:	Inpyrfluxam: Freezer Storage Stability of S-2399 and Metabolites in Processed Fractions
Document No.:	TPR-0065 (Study No.: VP-40066)
Guidelines:	US EPA Residue Chemistry Guideline OCSPP 860.1380 “Storage Stability Data”
Guideline deviations:	None
GLP/GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

Materials and Methods

The frozen storage of inpyrfluxam and its metabolites 3'-OH-S-2840, DFPA-$CONH_2$, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B in soyabean hulls, rice hulls, rice bran, polished rice, wet apple pomace, com starch, com oil, peanut meal, wheat germ, sugar beet dried pulp, sugar beet sugar, sugar beet molasses, potato flakes, and potato chips. Additionally, the frozen storage of metabolites 1'- COOH-S-2840-A, and 1'- COOH-S-2840-B were also investigated in wet apple pomace, maize/corn starch, maize/corn oil, peanut meal, wheat germ, sugar beet dried pulp, sugar beet sugar, sugar beet molasses, potato flakes and potato chips.

Homogenised samples (2.5 g) of soyabean hulls, rice hulls, rice bran, polished rice, wet apple pomace, maize/corn starch, maize/corn oil, peanut meal, wheat germ, sugar beet dried pulp, sugar beet sugar, sugar beet molasses, potato flakes, and potato chips were weighed into 50 mL polypropylene centrifuge tubes. 30 samples from each matrix were left untreated as untreated control and for fresh fortification samples. Storage stability test samples were fortified with inpyrfluxam, 3'-OH-S-2840, DFPA-$CONH_2$, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B and 1'- COOH-S-2840-B at 0.1 mg/kg (20x LOQ for 1’- COOH-S-2840-B and 10x LOQ for the other analytes) and with 1′-COOH-S-2840-A at a level of 0.129 mg/kg.

Four samples (two fortified and two non-fortified) per commodity matrix were kept in glass bottles with screw caps at ≤-20°C in the dark for up to 10 months. For soyabean hulls, sugar beet sugar and sugar beet molasses, 2 timepoint samples and for the remaining matrices 4 timepoint samples were removed from storage and analysed for inpyrfluxam and its metabolites 3'-OH-S-2840, DFPA-$CONH_2$, 1'-CH_2OH-S-2840-A and 1'-CH_2OH-S-2840-B.

In each analytical set, an untreated control sample and two freshly fortified untreated control samples (fortified freshly with all the metabolites) were analysed concurrently with the storage stability samples (two stored samples at each time point). After fortification of stored fortification samples, all samples were capped and placed in the freezer for storage (except day 0 samples) and maintained at approximately -20°C.

Analysis was performed using LC-MS/MS method Valent method RM-50C-1. Full details and validation data for this method can be found in Vol. 3 CA B.5.1.2.5. The analytical method (RM-50C-1) was sufficiently validated in accordance with SANTE/2020/12830 rev.1. The limit of quantification (LOQ) of the analytical method was 0.01 mg/kg for inpyrfluxam, 3'-OH-S-2840, DFPA-$CONH_2$, *N*-des-Me-DFPA and DFPA and 0.005 mg/kg for the A&B isomers of 1'-COOH-S-2840 and 1'-CH_2OH-S-2840. The procedural recoveries (freshly spiked samples at 0.1 mg/kg, 10x LOQ, with inpyrfluxam, 3'-OH-S-2840, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B, DFPA-$CONH_2$, at 0.1 mg/kg, 20x LOQ, with 1'-COOH-S-2840-B and at 0.129 mg/kg with 1′-COOH-S-2840-A) were in the acceptable range of 70 and 110%. Those that fell outside that range are discussed below and highlighted in bold in the tables.

Results

The recoveries of inpyrfluxam, 3'-OH-S-2840, DFPA-$CONH_2$, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B, in soyabean hulls, rice hulls, rice bran, polished rice, wet apple pomace, maize/corn starch, maize/corn oil, peanut meal, wheat germ , sugar beet dried pulp, sugar beet sugar, sugar beet molasses, potato flakes, and potato chips and 1'-COOH-S-2840-A, and 1'-COOH-S-2840-B were also investigated in wet apple pomace, maize/corn starch, maize/corn oil, peanut meal, wheat germ, sugar beet dried pulp, sugar beet sugar, sugar beet molasses, potato flakes, and potato chips matrices after the various storage periods are summarised below.

Procedural recovery values indicate that the method worked reasonably well throughout the storage stability study, noting that there were some low (60-70%) individual procedural recoveries for the particular timepoint of 49 days for sugar beet pulp analysis. Recoveries from stored samples are consistently >70% at all time points except for the following results:

At 49 days for sugar beet dried pulp inpyrfluxam had a mean recovery of 52.4% (individual recoveries of 59.8 and 45.0%) 3'-OH-S-2840 was found at 55.0% (58.5 and 51.5%), 1'-CH_2OH-S-2840-A at 62.9% (68.0 and 57.7%), DFPA-$CONH_2$ at 55.25% (52.8 and 57.7%), 1'-COOH-S-2840-A at 63.15% (76.0 and 50.3%), and 1'-COOH-S-2840-B at 55.45% (67.2 and 43.7%). The procedural recoveries at this time period for

all compounds with low stored recoveries was generally <70%, which may have impacted the stored recoveries observed. As the mean procedural recoveries at the subsequent sampling periods (84 and 263) are >70%, these results at 49 days can be disregarded and it can be concluded that residues of inpyrfluxam and its metabolites are stable for at least 263 days. As the mean stored recovery results at all other sugar beet dried pulp timepoints and for all timepoints for the other matrices are >70%, this low recovery is considered unusual.

Table 7.1.1.5-1: Storage stability of inpyrfluxam

Matrix	Days (months) stored	Inpyrfluxam		
		Procedural recoveries (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]
Fortification level		0.1 mg/kg		
Soyabean hulls	0 (0)	82.5, 88.3, 88.7	86.0, 102.1	94.0
	45 (1)	**62.7**, 72.7	71.2, 75.6	73.4
	70 (2)	100.1, 97.1	99.8, 101.0	100.4
Rice hulls	0 (0)	86.2, 79.4	99.4, 77.6	88.5
	55 (1)	102.3, 99.3	105.0, 104.7	104.85
	104 (3)	97.5, 100.1	89.2, 95.3	92.25
	175 (5)	98.1, 99.1	93.3, 90.2	91.75
Rice bran	0 (0)	101.6	76.6, 89.1	82.85
	70 (2)	86.1, 70.8	86.5, 82.3	84.4
	114 (3)	87.8, 86.8	91.2, 85.9	88.55
	174 (5)	89.5, 95.1	91.6, 92.4	92.0
Polished rice	0 (0)	87.0, 93.3	80.5, 92.9	86.7
	71 (2)	104.2, 90.8	94.6, 99.4	97.0
	115 (3)	98.2, 96.5	95.3, 93.9	94.6
	175 (5)	89.9, 92.0	92.4, 97.7	95.05
Wet apple pomace	0 (0)	90.2, 116.7	**69.9**, 91.7	80.8
	50 (1)	89.9, 91.3	86.9, 91.2	89.05
	113 (3)	92.8, 91.8	92.9, 87.9	90.4
	155 (5)	96.0, 89.3	89.3, 78.3	83.8
Maize/corn starch	0 (1)	-	97	97.0
	37 (1)	78.3, 85.5	77.7, 84.6	81.15
	65 (2)	91.0, 92.5	93.2, 96.4	94.8
	98 (3)	91.1, 91.1	85.1, 80.5	82.8
Maize/corn oil	0 (0)	-	69.5, 72.6	71.05
	49 (1)	73.8, 76.0	77.0, 71.2	74.1
	79 (2)	80.2, 89.9	91.6, 96.4	94.0
	115 (3)	97.1, 96.5	90.4, 103.1	96.8
Peanut meal	0 (0)	-	86.0, 78.3	82.2
	44 (1)	**67.5**, 74.0	74.4, 79.6	77.0
	80 (2)	96.0, 93.0	82.2, 93.0	87.6
	119 (3)	91.7, 94.3	91.8, 91.3	91.6
Wheat germ	0 (0)	88.7, 100.5	88.0, 98.8	93.4
	97 (3)	84.8, 88.3	85.8, 90.7	88.3
	222 (7)	92.5, 105.9	85.8, 101.1	93.5
	313 (10)	98.2, 98.6	92.1, 89.2	90.7

Matrix	Days (months) stored	Inpyrfluxam		
		Procedural recoveries (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]
Sugar beet dried pulp	0 (0)	78.0	80.1, 80.4	80.3
	49 (2)	**64.4**, **66.1**	**59.8**, **45**	**52.4**[c]
	84 (2)	78.1, 75.8	81.1, 75.2	78.2
	263 (8)	91.6, 92.5	89.4, 81.2	85.3
Sugar beet sugar	0 (0)	82.8	94.9, 88.8	91.9
	49 (2)	87.9, 85.5	87.9, 92.6	90.3
	84 (2)	90.9, 88.6	89.3, 88.8	89.1
Sugar beet molasses	0 (0)	93.2 84.1	75.7 **66.6**	71.2
	50 (1)	95.0 97.6	90.4 96.5	93.5
	85 (2)	82.4 87.9	86.3 74.9	80.6
Potato flakes	0 (0)	82.7 82.4	78.1 79.7	78.9
	119 (3)	82.1 80.2	83.1 83.2	83.2
	179 (5)	87.8 89.7	88.3 91.1	89.7
	253 (8)	89.5 91.8	79.0 84.6	81.8
Potato chips	0 (0)	-	85.8 92.1	89.0
	118 (3)	83.3 82.3	88.2 87.8	88.0
	178 (5)	88.2 90.0	87.8 87.4	87.6
	256 (8)	100.9 89.4	86.1 89.9	88.0

[a] Stored recovery values have not been corrected to take account of procedural recovery values or recoveries at time zero (recovery is of the intended fortification level of 0.1 mg/kg)

[b] Mean of duplicate uncorrected recoveries, as a % of the nominal fortification level

[c] mean stored recovery of 52.4% below acceptable recovery of 70%.

Table 7.1.1.5-2: Storage stability of 3'-OH-S-2840, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B and DFPA-$CONH_2$

Matrix	Days (months)	3'-OH-S-2840			1'-CH_2OH-S-2840-A			1'-CH_2OH-S-2840-B			DFPA-$CONH_2$		
		Procedural recoveries (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]	Procedural recoveries (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]	Procedural recoveries (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]	Procedural recoveries (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]
Fortification level		**0.1 mg/kg**			**0.1 mg/kg**			**0.1 mg/kg**			**0.1 mg/kg**		
Soyabean hulls	0 (0) 45 (1) 70 (2)	86.9 93.0 76.0 87.9 96.6 90.1	89.2 82.8 86.8 88.2 93.2 92.5	86.0 87.5 92.9	84.3 88.1 68.6 76.7 96.1 94.3	84.8 78.3 94.9 83.5 92.8 98.4	81.6 89.2 95.6	84.7 89.9 65.2 73.2 95.4 91.2	86.5 81.4 83.9 78.1 101.2 99.9	84.0 81.0 100.6	73.5 70.6 79.1 72.0 72.6 98.0	72.7 88.0 81.9 79.6 115.0 92.5	80.35 80.75 103.75
Rice hulls	0 (0) 55 (2) 104 (3) 175 (5)	85.7 81.7 112.8 116.7 91.9 93.5 95.4 95.0	91.9 66.0 110.0 119.1 78.9 92.4 83.9 88.4	79.0 114.6 85.7 86.2	87.1 84.7 87.9 89.2 91.5 86.7 89.9 94.2	89.2 67.6 93.5 97.8 81.5 94.1 88.9 95.1	78.4 95.7 87.8 92.0	89.1 83.9 92.7 92.9 94.1 90.6 93.9 97.5	89.8 68.3 95.7 107.0 87.2 101.4 91.7 99.8	79.1 101.4 94.3 95.8	86.7 80.2 96.9 88.7 86.1 87.2 93.2 97.3	73.6 77.0 113.4 112.7 94.1 90.0 98.9 100.0	75.3 113.05 92.05 99.45
Rice bran	0 (0) 70 (2) 114 (3) 174 (5)	101.6 86.1 70.8 87.8 86.8 89.5 95.1	76.6 89.1 86.5 82.3 91.2 85.9 91.6 92.4	82.9 84.4 88.6 92.0	107.8 104.0 87.0 84.6 86.4 89.5 93.4	88.1 104.4 100.5 105.7 82.3 88.1 93.6 91.1	96.3 103.1 85.2 92.4	104.8 94.3 72.2 86.1 89.4 90.9 94.6	93.6 106.4 85.6 97.6 89.0 93.1 90.1 95.3	100.0 91.6 91.1 92.7	98.9 90.4 75.5 87.5 90.4 86.6 95.5	87.6 98.9 83.7 90.3 95.0 99.5 96.9 96.2	93.25 87.0 97.25 96.55
Polished rice	0 (0) 71 (2) 115 (3) 175 (5)	86.4 96.0 113.3 106.4 100.2 98.5 92.8 92.3	87.3 88.5 100.8 116.6 96.8 94.9 93.5 89.0	87.9 108.7 95.9 91.3	87.0 94.3 94.0 90.5 98.6 93.6	87.8 86.2 88.9 103.0 97.8 96.7 96.7 92.8	87.0 96.0 97.3 94.8	90.4 93.0 88.1 95.5 98.9 96.6	89.6 88.5 91.9 105.8 106.0 101.8 95.4 95.8	89.1 89.9 103.9 95.6	86.5 85.6 83.4 89.2 93.0 95.0	89.5 89.3 98.6 123.7 94.3 98.2 94.3 96.3	89.4 111.15 96.25 95.3

					88.6 87.8			91.3 87.7			93.3 86.8		
Wet apple pomace	0 (0) 50 (1) 113 (3) 155 (5)	91.2 114.7 90.9 90.5 97.1 94.5 96.4 95.9	105.1 76.0 95.6 96.5 87.4 86.4 93.0 89.3	90.6 96.1 86.9 91.2	84.9 113.5 89.9 91.1 94.5 91.9 93.9 88.3	100.4 75.1 98.9 98.2 92.6 90.2 94.5 85.1	87.8 98.6 91.4 89.8	93.8 115.4 88.4 91.1 96.9 96.5 97.9 91.8	104.4 77.6 97.0 104.7 100.3 95.6 101.1 93.0	91.0 100.9 98.0 97.1	78.0 76.9 85.9 96.2 83.0 81.8 78.6 88.6	90.5 90.9 101.6 99.9 90.2 104.1 99.8 94.3	90.7 100.75 97.15 97.05
Maize/corn starch	0 (0) 37 (1) 65 (2) 98 (3)	- 82.4 86.5 81.9 88.6 84.6 84.0	113.0 91.1 81.6 80.9 93.4 94.8 85.8 81.0	102.1 81.3 94.1 83.4	- 83.1 85.4 91.2 92.5 87.9 88.1	87.6 93.1 82.7 80.8 94.3 98.3 90.6 92.5	90.4 81.8 96.3 91.6	- 86.9 78.1 87.9 99.5 89.2 97.7	89.6 97.5 78.1 77.7 99.5 99.2 97.7 97.2	93.6 77.9 99.4 97.5	- 84.1 85.1 73.2 85.7 83.9 86.2	86.4 84.3 82.9 80.8 68.6 100.9 99.4 97.2	85.35 81.85 84.75 98.3
Maize/corn oil	0 (0) 49 (1) 79 (2) 115 (3)	- 79.4 86.8 84.3 87.3 96.5 99.7	81.3 78.7 86.6 82.4 85.2 87.5 92.6 87.5	80.0 84.5 86.4 90.1	- 90.7 93.4 83.8 88.5 93.6 100.7	109.5 104.6 97.7 93.2 94.6 93.9 98.5 92.9	107.1 95.5 94.3 95.7	- 92.2 91.7 82.6 86.6 95.6 100.0	90.8 90.9 106.1 95.3 97.3 93.5 102.4 94.0	90.9 100.7 95.4 98.2	- 86.6 87.5 91.3 91.8 99.4 92.1	83.9 84.7 95.6 93.2 106.1 97.4 100.4 95.4	84.3 94.4 101.75 97.9
Peanut meal	0 (0) 44 (1) 80 (2) 119 (3)	- 83.7 96.4 95.6 93.6 97.4	85.6 91.1 90.3 96.7 100.1 96.8 87.5 87.7	88.4 93.5 98.5 87.6	- 88.2 91.6 92.3 90.6 95.1 100.0	79.9 91.6 93.6 90.6 100.3 100.0 91.4	79.9 92.6 95.5 95.7	- 87.6 94.6 95.6 93.4 90.2 94.6	80.3 85.1 102.0 102.2 107.1 100.7 96.6 93.5	82.7 102.1 103.9 95.1	- 85.6 84.5 112.1 89.2 87.5 96.2	82.4 78.5 100.9 107.3 101.6 103.9 86.4 99.0	80.45 104.1 102.75 92.7
Wheat germ	0 (0) 97 (3) 222 (7) 313 (10)	96.7 84.4 88.3 99.1 103.3 99.2 99.0	113.5 88.8 88.0 84.6 96.8 101.3 96.8 96.8	101.2 86.3 99.1 96.8	96.6 88.4 92.4 96.1 97.8	111.4 91.2 90.5 87.1 97.2 99.7 98.8 94.8	101.3 88.8 98.5 96.8	101.7 87.8 93.1 94.3 100.9	117.9 95.2 97.3 91.7 100.2 103.7 100.7 96.8	106.6 94.5 102.0 98.8	94.0 84.9 87.8 95.0 93.1	95.0 95.5 98.8 90.7 98.0 103.1 95.6 96.0	95.25 94.75 100.55 95.8

					96.0 97.0			98.0 100.7			92.6 90.7		
Sugar beet dried pulp	0 (0) 49 (1) 84 (2) 263 (8)	73.9 **66.2 68.4** 76.2 78.0 93.3 90.3	94.4 82.3 **58.5 51.5** 83.9 64.1 77.9 83.5	88.4 **55.0** 74.0 80.7	73.4 **65.5** **65.9** 77.2 82.4 91.6 91.0	87.1 78.6 **68.0 57.7** 94.3 69.7 81.6 88.7	92.9 **62.9** 82.0 85.1	126.5 71.3 **67.8** 81.5 78.6 89.5 90.2	98.3 100.4 77.7 **63.2** 91.3 74.0 83.6 91.8	99.4 70.5 82.7 87.7	79.6 72.0 **65.2** 76.2 77.1 87.4 88.9	93.5 89.4 **52.8 57.7** 88.2 88.5 85.8 91.4	91.45 **55.25** 88.35 88.6
Sugar beet sugar	0 (0) 49 (1) 84 (2)	85.5 92.4 87.2 91.5 86.2	90.0 81.4 91.9 95.9 95.8 95.6	85.7 93.9 95.7	82.5 87.6 85.2 97.0 89.9	86.8 81.6 98.3 97.1 94.5 99.4	84.2 97.7 97.0	96.0 87.7 89.2 94.6 89.2	97.5 88.9 104.6 109.0 104.7 103.8	93.2 106.8 104.3	77.9 86.6 86.1 91.9 90.2	88.8 88.4 102.1 86.5 107.9 104.7	88.6 94.3 106.3
Sugar beet molasses	0 (0) 50 (1) 85 (2)	91.7 85.9 89.8 93.0 81.8 91.9	79.8 81.1 84.1 88.6 89.2 72.9	80.5 86.4 81.1	87.4 85.5 93.4 93.6 91.5 86.9	77.5 80.0 94.7 98.3 98.1 82.6	78.8 96.5 90.4	90.6 88.3 97.4 98.3 91.0 92.5	80.5 82.1 99.0 105.5 104.2 92.6	81.3 102.3 98.4	75.4 87.4 92.4 97.6 76.1 90.0	95.6 79.6 96.3 104.4 84.4 87.8	87.6 100.35 86.1
Potato flakes	0 (0) 119 (3) 179 (5) 253 (8)	80.9 85.4 76.6 78.8 85.1 88.7 94.4 92.4	85.1 91.7 82.9 82.9 92.9 86.8 94.0 92.8	88.4 82.9 89.9 93.4	80.0 86.0 78.5 81.3 86.7 85.3 94.2 95.6	87.4 86.3 87.8 88.9 91.3 86.3 99.0 98.6	86.9 88.35 88.8 98.8	83.6 90.3 83.0 84.0 87.3 86.8 94.6 95.3	87.8 89.4 94.4 98.7 102.1 93.3 103.9 99.9	88.6 96.6 97.7 101.9	87.5 88.7 76.4 75.8 84.3 86.4 93.2 93.5	82.5 89.2 97.6 92.7 91.2 93.6 103.3 96.6	85.85 95.15 92.4 99.95
Potato chips	0 (0) 118 (3) 178 (5) 256 (8)	- 85.9 85.7 88.5 93.4 88.7 98.4	95.8 97.1 86.3 86.6 92.3 93.6 94.7 91.3	96.5 86.5 93.0 93.0	- 86.4 85.3 85.0 91.9 84.2 87.8	92.1 103.6 92.5 88.8 93.7 94.7 86.1 86.1	97.9 90.7 94.2 86.1	- 87.3 89.2 85.9 91.1 96.9 93.9	94.4 106.3 95.8 96.5 103.8 102.8 95.1 94.1	100.4 96.2 103.3 94.6	- 83.9 87.2 86.1 86.2 88.2 88.2	88.2 115.3 92.5 100.5 95.6 95.6 93.5 97.6	101.75 96.5 95.6 95.55

[(a)] Stored recovery values have not been corrected to take account of procedural recovery values or recoveries at time zero (recovery is of the intended fortification level of 0.1 mg/kg)

(b) Mean of duplicate uncorrected recoveries, as a % of the nominal fortification level

Table 7.1.1.5-3: Storage stability of 3'-OH-S-2840, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B and DFPA-$CONH_2$

Matrix	Days stored (months)	1'-COOH-S-2840-A			1'-COOH-S-2840-B		
		Procedural recoveries (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]	Procedural recoveries (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]
Fortification level		**0.129 mg/kg**			**0.1 mg/kg**		
Maize/corn starch	0 (0)	83.6 87.5	92.1	92.1	71.8 75.2	82.3	82.3
	37 (1)	76.4 77.7	92.8 80.5	86.65	73.1 77.3	86.4 80.5	83.45
	65 (2)	90.1 86.7	99.6 95.5	97.55	78.9 89.9	79.3 82.0	80.65
	98 (3)	81.5 84.2	91.8 93.9	92.85	84.3 91.7	85.8 85.5	85.65
Maize/corn oil	0 (0)	88.3 86.5	95.9 92.8	94.35	83.9 88.1	89.0 90.9	89.95
	49 (1)	88.3 91.6	101.5 99.1	100.3	88.4 84.9	85.3 85.1	85.2
	79 (2)	78.7 90.2	97.7 97.6	97.65	76.9 89.2	83.4 84.7	84.05
	115 (3)	89.4 93.4	100.2 83.5	91.85	92.6 91.4	83.7 74.8	79.25
Peanut meal	0 (0)	77.4 86.1	81.4 85.7	83.55	76.1 82.2	78.8 82.1	80.45
	44 (1)	93.0 82.7	89.6 91.3	90.45	87.4 74.2	78.3 83.7	81.0
	80 (2)	122.1 87.3	104.9 100.8	102.85	114.8 94.6	94.8 87.7	91.25
	119 (3)	87.9 92.2	95.5 97.5	96.5	88.7 92.8	87.9 85.8	86.85
Wheat germ	0 (0)	95.5	91.0 97.9	94.45	82.6	83.0 86.1	84.55
	97 (3)	84.9 81.4	91.7 90.0	90.85	82.4 87.0	80.9 78.7	79.8
	222 (7)	88.5 85.6	92.9 91.6	92.25	90.2 84.2	81.7 79.1	80.4
	313 (10)	94.5 95.0	94.3 99.6	96.95	92.1 90.3	81.3 86.7	84.0
Sugar beet dried pulp	0 (0)	86.6	85.5 87.8	86.65	78.0	76.5 80.9	78.7
	49 (1)	78.5 68.0	76.0 50.3	63.15	73.1 68.9	67.2 43.7	55.45
	84 (2)	72.8 74.0	88.2 75.5	81.85	72.6 75.8	77.1 66.4	71.75
	263 (8)	86.9 85.8	90.8 91.0	90.9	85.5 85.9	78.7 77.5	78.1
Sugar beet sugar	0 (0)	100.5	106.8 100.4	103.6	85.2	92.4 87.1	89.75
	49 (1)	92.7 90.1	105.9 110.5	108.2	95.3 84.3	88.2 96.7	92.45
	84 (2)	91.3 89.5	108.0 91.4	99.7	90.7 88.0	97.5 82.3	89.9
Sugar beet molasses	0 (0)	76.8 96.8	88.5 93.9	91.2	79.7 93.2	85.3 92.7	89.0
	50 (1)	93.4 95.4	96.7 99.4	98.05	92.9 99.6	90.4 86.3	88.35
	85 (2)	89.7 92.7	94.8 99.8	97.3	89.5 94.8	81.6 85.3	83.45
Potato flakes	0 (0)	93.6 96.3	100.2 98.3	99.25	98.6 99.0	103.7 104.7	104.2
	119 (3)	74.5 74.1	89.1 93.1	91.1	74.9 74.4	76.2 77.6	76.9
	179 (5)	79.1 81.6	93.1 98.9	96.0	84.0 88.1	82.2 80.6	81.4
	253 (8)	80.7 86.0	96.6 97.3	96.95	84.2 88.1	84.7 81.9	83.3
Potato chips	0 (0)	-	74.0 91.2	82.6	-	71.9 78.9	75.4
	118 (3)	83.0 82.5	90.2 99.7	94.95	83.9 83.4	80.4 80.4	80.4
	178 (5)	81.8 84.7	98.3 96.2	97.25	83.3 88.2	86.6 78.3	82.45
	256 (8)	84.0 87.0	95.8 94.4	95.1	83.5 90.4	83.9 80.8	82.35

[a] Stored recovery values have not been corrected to take account of procedural recovery values or recoveries at time zero (recovery is of the intended fortification level of 0.129 or 0.1 mg/kg)

[b] Mean of duplicate uncorrected recoveries, as a % of the nominal fortification level

Conclusion

Inpyrfluxam, 3'-OH-S-2840, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B and DFPA-$CONH_2$ have been demonstrated to be stable when stored at ≤-18°C in soyabean hulls (miscellaneous) for 2 months, in rice hulls (miscellaneous) for 5 months, in rice

bran (miscellaneous) for 5 months, in polished rice (high starch) for 5 months and in apple pomace (high water) for 5 months.

Inpyrfluxam, 3'-OH-S-2840, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B and DFPA-$CONH_2$,1'-COOH-S-2840-A and 1'-COOH-S-2840-B have been demonstrated to be stable when stored at ≤-18°C in maize/corn starch (high starch) for 3 months, in maize/corn oil (high oil) for 3 months, in peanut meal for 3 months (miscellaneous), in wheat germ (miscellaneous) for 10 months, in sugar beet dried pulp (miscellaneous) for 8 months, in sugar beet sugar (miscellaneous) for 2 months, in sugar beet molasses (miscellaneous) for 2 months, in potato flakes (miscellaneous) for 8 months and in potato chips (miscellaneous) for 8 months.

B.7.1.2 Animal matrices

Storage stability studies on animal commodities were conducted within the feeding studies on poultry and ruminants and have been evaluated here.

B.7.1.2.1 Poultry

Report:	**KCA 6.4.1/01; [redacted] 2017**
Title:	Magnitude of S-2399 and Metabolites 1'-CH_2OH-S-2840 A&B (including conjugate) and 1'-COOH-S-2840 A&B Residues in Laying Hen Tissues and Eggs from a 28-Day Feeding Study
Document No.:	TPR-0015 (Study No.: 2815W)
Guidelines:	OPPTS 860.1480, PMRA DIR-98-02, Section 8 OECD Guidelines for the testing of chemicals: Residues in Livestock 505 (January 8, 2007) MAFF in Japan (12-Nousa*N*-No. 8147, 3-2-1, 2000)
Guideline deviations:	None
GLP/GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

Materials and Methods

The frozen storage of inpyrfluxam and its metabolites 1'-COOH-S-2840-A, 1'-COOH-S-2840-B, 1'-CH_2OH-S-2840-A and 1'-CH_2OH-S-2840-B was studied for 90 days in eggs, 40 days in muscle and liver and 49 days in fat.

Untreated homogenate egg (egg yolk + egg white), muscle, liver and fat sample matrices (5 g for egg and liver, 10 g for muscle and fat) were fortified with inpyrfluxam at a level of 0.1 mg/kg and 1'-COOH-S-2840-A, 1'-COOH-S-2840-B, 1'-CH_2OH-S-2840-A and 1'-CH_2OH-S-2840-B at 0.05 mg/kg (10x LOQ for the respective analytes). The A&B isomers were fortified jointly for 1'-COOH-S-2840 and 1'-CH_2OH-S-2840.

Four samples (two fortified and two non-fortified) per commodity matrix were kept in glass bottle with screw cap stored in freezers at <0°C in the dark for up to 90 days. After time intervals of 30, 49 (egg and fat) and 90 days (egg only) and 21 and 40 days (liver and muscle), samples were removed from storage and were analysed for inpyrfluxam, 1'-COOH-S-2840-A, 1'-COOH-S-2840-B, 1'-CH_2OH-S-2840-A and 1'-CH_2OH-S-2840-B.

Analysis was performed using an LC-MS/MS method validated within the study report. Full details and validation data for this method can be found in Vol. 3 CA B.5.1.2.5. The method can be considered sufficiently validated in accordance with SANTE/2020/12830 rev.1.

The LOQ of the method is 0.01 mg/kg for inpyrfluxam and 0.005 mg/kg for the metabolites 1'-COOH-S-2840-A & -B, 1'-CH_2OH-S-2840-A & -B. The procedural recoveries (freshly spiked samples at 10x LOQ, i.e. 0.1 mg/kg for inpyrfluxam and 0.05 mg/kg for the metabolites 1'-COOH-S-2840-A & -B and 1'-CH2OH-S-2840-A & -B) for each analyte in each matrix per time point were mostly in the acceptable range of 70 and 110%. Those that fell outside that range are discussed below and highlighted in bold in the tables.

Results

The recoveries of inpyrfluxam and its metabolites 1'-COOH-S-2840-A, 1'-COOH-S-2840-B, 1'-CH_2OH-S-2840-A and 1'-CH_2OH-S-2840-B from egg (egg yolk + egg white), muscle, liver and fat matrices after the various storage periods are summarised below.

Procedural recovery values indicate that the method worked reasonably well throughout the storage stability study, noting that there were some low (57 – 70%) procedural recoveries; these were generally accompanied by stored recoveries of similar magnitudes. Some individual stored recoveries were <70% (lowest 66%) but all mean procedural recoveries were ≥70%, so stability for the duration of the study can be assumed. Mean stored recoveries for 1'-COOH-S-2840-B in egg were 70% for 49 and 90 days, with individual results between 66 and 73%. The mean recovery at time 0 was 79%, so even though the results at days 49 and 90 are quite low, very little change in recovery over storage was observed.

Table 7.1.2.1-1 Storage stability of inpyrfluxam

Matrix	Days stored	Inpyrfluxam		
		Procedural recoveries (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]
Fortification level		0.1 mg/kg		
Egg	0	-	87, 86	87
	30	74, 77	74, 89	82

Matrix	Days stored	Inpyrfluxam		
		Procedural recoveries (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]
	49	71, 83	76, 77	77
	90	90, 98	84, 71	78
Muscle	0	-	90	-
	21	91, 88	91, 88	90
	40	92, 91	89, 81	85
Liver	0	-	71, 75	73
	21	81, 85	75, 74	75
	40	81, 83	80, 77	79
Fat	0	-	88, 89	89
	30	78, 82	73, 78	76
	49	81, 82	81, 77	79

[a] Stored recovery values have not been corrected to take account of procedural recovery values or recoveries at time zero (recovery is of the intended fortification level of 0.1 mg/kg)

[b] Mean of duplicate uncorrected recoveries, as a % of the nominal fortification level

Table 7.1.2.1-2: Storage stability of 1'-COOH-S-2840-A, 1'-COOH-S-2840-B, 1'-CH_2OH-S-2840-A and 1'-CH_2OH-S-2840-B

Matrix	Days	1'-COOH-S-2840-A			1'-COOH-S-2840-B			1'-CH_2OH-S-2840-A			1'-CH_2OH-S-2840-B		
		Procedural recoveries (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]	Procedural recoveries (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]	Procedural recoveries (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]	Procedural recoveries (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]
Fortification level		**0.05 mg/kg**			**0.05 mg/kg**			**0.05 mg/kg**			**0.05 mg/kg**		
Egg	0	-	81, 87	84	-	79, 78	79	-	88, 91	90	-	88, 88	88
	30	72, 78	77, 88	83	**68**, 74	73, 85	79	85, 85	92, 104	98	84, 83	85, 103	94
	49	**68**, 81	73, 75	74	70, 78	68, 71	70	88, 89	82, 89	86	82, 86	84, 87	86
	90	82, 96	78, 70	74	80, 83	73, 66	70	93, 101	90, 81	86	90, 99	85, 81	83
Muscle	0	-	85	-	-	87	-	-	78	-	-	72	-
	21	90, 87	89, 85	87	93, 89	90, 91	91	85, 80	84, 76	80	80,79	82, 70	76
	40	91, 93	90, 85	88	95, 92	89, 85	87	87, 82	80, 74	77	82, 82	71, 67	69
Liver	0	-	87, 91	89	-	83, 90	87	-	78, 82	80	-	72, 79	76
	21	89, 100	84, 86	85	94, 101	87, 90	89	87, 95	85, 78	82	83, 93	84, 75	80
	40	93, 88	91, 88	90	93, 90	91, 88	90	99, 88	87, 86	87	96, 88	83, 81	82
Fat	0	-	89, 93	91	-	97, 101	99	-	76, 86	81	-	73, 89	81
	30	93, 97	88, 94	91	94, 94	91, 96	94	83, 88	86, 90	88	83, 85	82, 84	83
	49	93, 93	94, 87	91	95, 89	95, 92	94	**61**, 81	71, 85	78	**57**, 77	70, 84	77

[a] Stored recovery values have not been corrected to take account of procedural recovery values or recoveries at time zero (recovery is of the intended fortification level of 0.05 mg/kg)

[b] Mean of duplicate uncorrected recoveries, as a % of the nominal fortification level

Conclusion

Inpyrfluxam, 1'-COOH-S-2840-A, 1'-COOH-S-2840-B, 1'-CH_2OH-S-2840-A and 1'-CH_2OH-S-2840-B have been demonstrated to be stable when stored in freezers (<0°C) for 90 days in eggs, 40 days in muscle and liver and 49 days in fat.

B.7.1.2.2 Ruminants

Report:	**KCA 6.4.2/01; [redacted] 2016**
Title:	Magnitude of S-2399 and Metabolites 1'-CH_2OH-S-2840 A&B (including conjugate) and 1'-COOH-S-2840 A&B Residues in Bovine Tissues and Milk from a 28-Day Feeding Study
Document No.:	TPR-0013 (Study No.: 2814W)
Guidelines:	OPPTS 860.1480, PMRA DIR-98-02, Section 8 OECD Guidelines for the testing of chemicals: Residues in Livestock 505 (January 8, 2007) MAFF in Japan (12-Nousa*N*-No. 8147, 3-2-1, 2000)
Guideline deviations:	None
GLP/GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

Materials and Methods

The frozen storage of inpyrfluxam and its metabolites 1'-COOH-S-2840-A, 1'-COOH-S-2840-B, 1'-CH_2OH-S-2840-A and 1'-CH_2OH-S-2840-B was studied for 75 days in milk, 29 days in muscle, liver and kidney and 31 days in fat.

Untreated homogenate milk, muscle, liver, kidney and fat sample matrices (10 g) were fortified with inpyrfluxam at a level of 0.1 mg/kg and 1'-COOH-S-2840-A, 1'-COOH-S-2840-B, 1'-CH_2OH-S-2840-A and 1'-CH_2OH-S-2840-B at 0.05 mg/kg (10x LOQ for the respective analytes). The A&B isomers were fortified jointly for 1'-COOH-S-2840 and 1'-CH_2OH-S-2840.

Four samples (two fortified and two non-fortified) per commodity matrix were kept in glass bottle with screw cap stored in freezers at <0°C in the dark for up to 75 days. After time intervals of 29 and 75 days (milk), 29 days only (muscle, liver and kidney) and 31 days only (fat), samples were removed from storage and were analysed for inpyrfluxam, 1'-COOH-S-2840-A, 1'-COOH-S-2840-B, 1'-CH_2OH-S-2840-A and 1'-CH_2OH-S-2840-B.

Analysis was performed using an LC-MS/MS method validated within the study report. Full details and validation data for this method can be found in Vol. 3 CA B.5.1.2.5. The method can be considered sufficiently validated in accordance with SANTE/2020/12830 rev.1.

The LOQ of the method is 0.01 mg/kg for inpyrfluxam and 0.005 mg/kg for the metabolites 1'-COOH-S-2840-A & -B, 1'-CH_2OH-S-2840-A & -B. The procedural recoveries (freshly spiked samples at 10x LOQ, i.e. 0.1 mg/kg for inpyrfluxam and 0.05 mg/kg for the metabolites 1'-COOH-S-2840-A & -B and 1'-CH_2OH-S-2840-A & -B) for each analyte in each matrix per time point were all the acceptable range of 70 and 110%.

Results

The recoveries of inpyrfluxam and its metabolites 1'-COOH-S-2840-A, 1'-COOH-S-2840-B, 1'-CH_2OH-S-2840-A and 1'-CH_2OH-S-2840-B from milk, muscle, liver, kidney and fat matrices after the various storage periods are summarised below.

Procedural recovery values indicate that the method worked well throughout the storage stability study. One recovery of inpyrfluxam in kidney at time 0 was 69%, but the mean was still >70%. All other individual and mean recoveries were >70%.

Table 7.1.2.2-1 Storage stability of inpyrfluxam

Matrix	Days stored	Inpyrfluxam		
		Procedural recoveries (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]
Fortification level		0.1 mg/kg		
Milk	0	-	74, 85	80
	29	78, 83	78, 82	80
	75	100, 101	82, 83	83
Muscle	0	-	72, 76	74
	29	94, 89	87, 83	85
Liver	0	-	81, 80	81
	29	88, 79	77, 78	78
Kidney	0	-	69, 73	71
	29	91, 85	81, 75	78
Fat	0	-	83, 72	78
	31	98, 100	94, 89	92

[a] Stored recovery values have not been corrected to take account of procedural recovery values or recoveries at time zero (recovery is of the intended fortification level of 0.1 mg/kg)

[b] Mean of duplicate uncorrected recoveries, as a % of the nominal fortification level

Table 7.1.2.2-2: Storage stability of 1'-COOH-S-2840-A, 1'-COOH-S-2840-B, 1'-CH_2OH-S-2840-A and 1'-CH_2OH-S-2840-B

Matrix	Days	1'-COOH-S-2840-A			1'-COOH-S-2840-B			1'-CH_2OH-S-2840-A			1'-CH_2OH-S-2840-B		
		Procedural recoveries (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]	Procedural recoveries (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]	Procedural recoveries (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]	Procedural recoveries (%)	Stored recoveries (%)[a]	Mean stored recovery (%)[b]
Fortification level		**0.05 mg/kg**			**0.05 mg/kg**			**0.05 mg/kg**			**0.05 mg/kg**		
Milk	0	-	87, 96	92	-	86, 99	93	-	95, 96	96	-	92, 94	93
	29	91, 94	91, 93	92	93, 97	93, 93	93	89, 90	83, 87	85	87, 88	81, 85	83
	75	109, 114	88, 92	90	112, 112	90, 90	90	99, 86	82, 89	86	93, 77	74, 81	78
Muscle	0	-	80, 87	84	-	81, 88	85	-	95, 100	98	-	93, 96	95
	29	103, 95	95, 87	91	99, 96	97, 87	92	87, 91	85, 86	86	83, 88	84, 83	84
Liver	0	-	92, 94	93	-	93, 88	91	-	93, 90	92	-	86, 85	86
	29	101, 90	85, 85	85	99, 91	89, 89	89	84, 77	77, 77	77	80, 71	74, 73	74
Kidney	0	-	84, 86	85	-	81, 87	84	-	90, 92	91	-	87, 86	87
	29	103, 97	87, 85	86	100, 97	91, 88	90	88, 86	77, 77	77	86, 79	71, 72	72
Fat	0	-	94, 82	88	-	91, 83	87	-	87, 79	83	-	80, 74	77
	31	99, 103	95, 91	93	98, 101	95, 88	92	87, 86	85, 77	81	87, 85	84, 75	80

[a] Stored recovery values have not been corrected to take account of procedural recovery values or recoveries at time zero (recovery is of the intended fortification level of 0.05 mg/kg)

[b] Mean of duplicate uncorrected recoveries, as a % of the nominal fortification level

Conclusion

Inpyrfluxam, 1'-COOH-S-2840-A, 1'-COOH-S-2840-B, 1'-CH_2OH-S-2840-A and 1'-CH_2OH-S-2840-B have been demonstrated to be stable when stored in freezers (<0°C) for 75 days in milk, 29 days in muscle, liver and kidney and 31 days in fat.

B.7.2. Metabolism, distribution and expression of residues

The applicant submitted studies investigating the metabolism of inpyrfluxam in plants: apple (foliar treatment), rice (foliar and early application granular treatment), soyabean (foliar treatment) and maize, sorghum, oilseed rape, and potato as seed treatments.

Studies on metabolism in animals have been submitted using poultry and goat.

The submitted metabolism studies are compliant with current OECD guidelines.

The structure of inpyrfluxam is shown in Figure 7.2-1; the label positions are also shown.

The metabolism and distribution of inpyrfluxam in plants and livestock was investigated using the active substance labelled in either the phenyl ring or the pyrazolyl ring. This is considered appropriate to elucidate sufficient information on the metabolic pathway.

All mg/kg expressions of the results in the metabolism studies are as mg/kg parent equivalents (mg eq./kg).

Please refer to the start of Vol. 1 section 2.7.2 for a discussion on the storage stability of residues in a metabolism study context. This discussion brings together data from the studies on storage stability of residues in the non-radiolabelled studies (those evaluated in section B.7.1) as well as evidence from the metabolism studies. Overall, the data provide reassurance that the metabolism studies can be used to derive the required knowledge on nature of the residues assessment of these studies, supporting the consumer risk assessment.

Please refer to the start of Vol. 1 section 2.7.2 for a discussion on the location of plant metabolism studies (USA) for these studies assessed as suitable for the determination of nature of residues, in order to address the consumer risk for the currently assessed uses.

Isomers: Parent (inpyrfluxam)

In some of the primary crop plant metabolism studies, where detectable residues were analysed and residues of parent inpyrfluxam were extracted, these were

analysed using chiral methodology to determine whether the residues were found as R-isomer (inpyrfluxam) or whether any S-isomer was found. These reports showed that only R-isomer was present (apple rinse, apple peels, apple pulp, rice forage and straw (in the granular study), rice foliage, straw, hulls and grain (in the rice foliar study) and in soya bean matrices (forage, hay, edamame pods, and mature pods)). In the radiolabelled hydrolysis study (see section on the nature of the residues over processing. Vol. 3 CA B.7.5.1) the solutions at time 0 and termination of the study were analysed and shown not to contain any S-isomer. This potential for conversion from R-isomer (inpyrfluxam) to S-isomer was not assessed in the rotational crop metabolism samples (section Vol. 3 CA B.7.6.1). The evidence taken together indicates that R-isomer of inpyrfluxam is stable and does not convert to S-isomer, either in terms of high temperature hydrolysis conditions or during metabolism.

In animal metabolism samples (hen fat: hexane extracts, and goat liver: hexane extracts) chiral methodology was used to confirm the stability of the R-isomer. Again, there was no marked conversion from the R-isomer. The hen fat extracts were mainly R-isomer (3 – 4% S-isomer). The goat liver extracts were 100% R-isomer.

Therefore, for consumer risk assessment purposes, no conversion factor needs to be applied to either products of plant or animal origin to account for the potential of isomer conversion of inpyrfluxam in plants or animals.

Isomers: Metabolites

In the metabolism studies (and nature of hydrolysis studies) some metabolites were specifically analysed as the ‘A’ and ‘B’ isomers of the various metabolites. Commonly whilst these were specifically determined as ‘A’ and ‘B’ they were often summed for the purpose of summary of residues results.

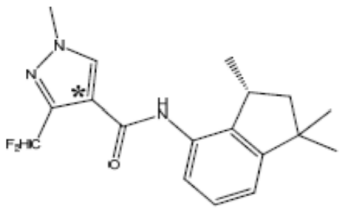
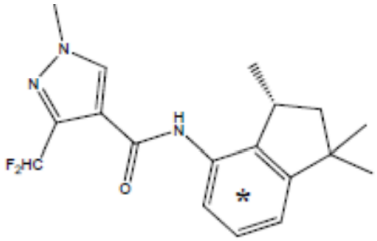
The applicant has explained that (N5 document) “For metabolites substituted on the 1-methyl groups of the indane (1’-CH_2OH-S-2840, 1’-COOH-S-2840), a second chiral centre was introduced, and this is expected to be racemic. Two diastereomer pairs could be separated on standard RP-HPLC columns, resulting in “A” and “B” compounds. These were summed together to ensure the total racemic mixture was quantified for risk assessment purposes.”

As examples, please see section B.7.5.1 for pictorial depictions of the 4 isomers of 1’-CH_2OH-S-2840, comprising 1’-CH_2OH-S-2840-A (an isomer pair) and 1’-CH_2OH-S-2840-B (an isomer pair). Likewise for 1’-COOH-S-2840-A and 1’-COOH-S-2840-B.

Considering the prevalence of ‘A’ and ‘B’ forms for both 1’-COOH-S2480 and 1’-CH_2OH-S2480 there are variable results in the metabolism data, but both feature (in plants and animals). There is no evidence that there is just a shift in only one direction in terms of formation in the metabolism studies.

Additionally in the magnitude of residues trials on wheat and barley, the 'A' and the 'B' isomer pairs for 1'-CH_2OH-S-2840 ~~-A~~ and 1'-COOH-S-2840 were reported separately (see Vol. 3 CA B.7.3), and the amounts in wheat and barley grain and straw are similar across the 'A' and the 'B' forms.

Figure 7.2-1 Structure of inpyrfluxam (S-2399), with label positions

[Pyrazolyl-4-^{14}C]S-2399	[Phenyl-U-^{14}C]S-2399
 * Indicates position of ^{14}C label.	 * Indicates position of ^{14}C label.

B.7.2.1. Plants

B.7.2.1.1 Apple (foliar treatment)

Metabolism studies were conducted in apple after foliar application with [pyrazolyl-4-^{14}C] and [phenyl-U-^{14}C] inpyrfluxam.

Table 7.2.1.1-1: Overview of apple metabolism studies

Plant	Application	Target application rate	BBCH at application	Days prior to harvest	Reference
Apple	Three foliar spray applications, pyrazolyl-labelled inpyrfluxam & phenyl-labelled inpyrfluxam	3 x ~220 g a.s./ha	77 – 79, 79 – 81 & 81 – 83	35, 24 & 14	KCA 6.2.1/01 TPM-0013

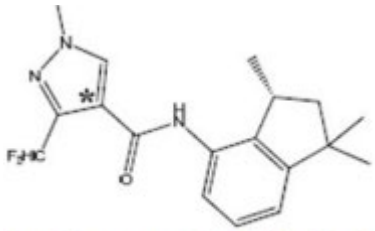
Report:	**KCA 6.2.1/01; [redacted] and [redacted] Amended Final Report #2 2018**
Title:	A Metabolism Study of [^{14}C] S-2399 (2 Radiolabels) in Apple (Malus domesticus)
Document No.:	TPM-0013 (Study No.: 2507W)
Guidelines:	USE EPA OPPTS 860.1300 OECD/OCDE Guideline 501 EU Guideline 7028/VI/95 JMAFF 12-Nohsan No. 8147
Guideline deviations:	None
GLP/GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

The metabolism of inpyrfluxam was investigated in apple fruits after three spray applications. For each of the three foliar applications the test items [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam were formulated as a 40% SC spray. The applications were performed 35, 24 and 14 days before harvest with individual application rates of approximately 200 g a.s./ha.

[Pyrazolyl-4- ^{14}C] inpyrfluxam

Chemical name [Pyrazolyl-4- ^{14}C] inpyrfluxam

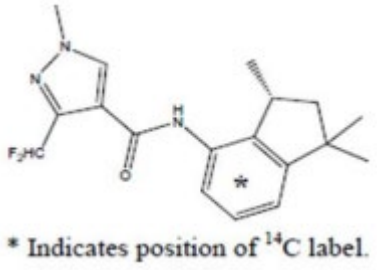
Chemical structure



* Indicates position of ^{14}C label.

Radiolabel position	[pyrazolyl-4- ^{14}C]
Specific radioactivity	2.22 GBq/mmol
Radiochemical purity	≥97.4% (determined pre and post application)
Chemical purity	≥96.7% (determined pre and post application)

[Phenyl-U- ^{14}C] inpyrfluxam

Chemical name	[Phenyl-U- ^{14}C] inpyrfluxam
Chemical structure	 * Indicates position of ^{14}C label.
Radiolabel position	[phenyl-U- ^{14}C]
Specific radioactivity	4.48 GBq/mmol
Radiochemical purity	≥98.1% (determined pre and post application)
Chemical purity	≥98.0% (determined pre and post application)

The apple trees were in test plots (one apple tree per plot in the ground) located outdoors in Madera, California, USA and included one control plot and two treated plots (one for each radiolabel). Each treated plot was 1 m^2 in area and contained loamy sand soil. The apple plants (*Malus domesticus*), variety “FUJI”, were treated at 35 day PHI, 24 day PHI and 14 day PHI for each plot.

The formulated test compound (as a 40% SC spray formulation) was applied to the apple trees at a target rate of 3 x 200 g a.s./ha.

The apple trees were treated with 200 mL of spray onto the foliage using a manually operated, trigger-pumped, pump sprayer. During application, plastic barriers were used to prevent contamination to the soil and between the plots. For [pyrazolyl-4-^{14}C] inpyrfluxam, the application rates corresponded to 214, 214 and 221 g a.s./ha for the

first, second and third application, respectively, resulting in a total application rate of 649 g a.s./ha. For [phenyl-U-^{14}C] inpyrfluxam, the application rates corresponded to 220, 218 and 220 g a.s./ha for the first, second and third application, respectively, resulting in a total application rate of 658 g a.s./ha.

Samples of apple fruit and leaves were collected at mature harvest, 14 days after last application. Samples were transferred in coolers on ice to the analytical laboratory, where they were rinsed with acetonitrile and a portion separated into peel and pulp. The specimens of peel and pulp were homogenised (in the presence of dry ice) and then stored in the freezer. The samples and extracts of fruits were stored deep frozen for a maximum of 47 days. Samples of leaf were not analysed and are not mentioned further in this evaluation.

The total radioactive residues (TRR) were initially determined by combustion of solid matrix/LSC of all samples using an approximately 100 – 250 mg aliquot. The resultant $^{14}CO_2$ was trapped in 15 mL of scintillation cocktail and then radioassayed by LSC.

Samples of the peel and pulp with residues > 0.01 mg eq./kg were extracted sequentially two times with acetonitrile:water (1:1, v/v) and once using 100% acetonitrile. For each extraction, the solutions were shaken for 30 minutes and then centrifuged and the supernatants removed. Triplicate samples were then radioassayed directly by liquid scintillation counting to determine the concentration of inpyrfluxam equivalents. The three extracts were combined and were radioassayed a second time. Remaining solids were analysed by combustion and LSC.

For each specific sample analysed solvent extraction solubilised ≥89% of the TRR with 7 – 10% TRR remaining associated with post extraction solids (max 0.01 mg eq./kg). Therefore, further extraction and analysis of the PES was not undertaken.

The initial TRR, determined by combustion, of the apple fruit was calculated by summation of the radioactive residues in apple rinse, peel and pulp samples. Peel and pulp TRRs were also determined by the summation of extracted and solid residues.

The results of the total radioactive residue determinations are presented below in Table 7.2.1.1-2.

By summing the extracted and post-extraction solid residues, the TRR in apple rinse, peel and pulp amounted to 0.300 mg eq./kg for [pyrazolyl-4-^{14}C] inpyrfluxam and 0.249 mg eq./kg for [phenyl-U-^{14}C] inpyrfluxam. For both [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam, the TRR was highest in the rinse (0.192 mg eq./kg and 0.145 mg eq./kg, respectively) followed by peel (0.094 mg eq./kg and 0.093 mg eq./kg, respectively) and pulp (0.014 mg eq./kg and 0.011 mg eq./kg, respectively).

Table 7.2.1.1-2: TRR in apple matrices after foliar application of [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam

Matrix	Timing and application	PHI (days)[(a)]	TRR (mg eq./kg)[(a)]	TRR (mg eq./kg)[(b)]
[pyrazolyl-4-^{14}C] inpyrfluxam				
Apple rinses	3 spray applications: 3 x 220 g a.s./ha	35, 24 and 14	0.192	0.192
Apple peel			0.102	0.094
Apple pulp			0.013	0.014
[phenyl-U-^{14}C] inpyrfluxam				
Apple rinses	3 spray applications: 3 x 220 g a.s./ha	35, 24 and 14	0.145	0.145
Apple peel			0.101	0.093
Apple pulp			0.009	0.011

(a) TRR values based on combustion of solid matrix/LSC
(b) TRR values calculated from sum of extracted and post-extraction solid residues

Considering the overall TRR associated with the fruits, the majority was associated with the surface rinse (64% (pyrazolyl label) or 58% (phenyl label)), then the peel (31% (pyrazolyl label) or 37% (phenyl label)), then pulp (4.7% (pyrazolyl label) or 4.1% (phenyl label)).

The amounts of radioactivity extracted are presented in Table 7.2.1.1-3.

Table 7.2.1.1-3: Distribution of radioactivity in the extracts of apple matrices after three foliar applications of [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam

Sample	Apple rinse		Apple peel		Apple pulp	
	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg
[pyrazolyl-4-^{14}C] inpyrfluxam						
TRR (combustion of solid matrix)	**N/A**	**N/A**	**-**	**0.102**	**-**	**0.013**
TRR (sum of extracted and solid residues)	**64.0**	**0.192**	**31.3**	**0.094**	**4.7**	**0.014**

ACN/H_2O Extract 1 **Acetonitrile: water 1:1, v/v, Extract #1**	*N/A*	*N/A*	*17.3*	*0.052*	*2.7*	*0.008*
ACN/H_2O Extract 2 **Acetonitrile: water 1:1, v/v, Extract #2**	*N/A*	*N/A*	*8.0*	*0.024*	*1.3*	*0.004*
ACN Extract 3	*N/A*	*N/A*	*2.7*	*0.008*	*0.3*	*0.001*
Total extracted	**64.0**	**0.192**	**28.0**	**0.084**	**4.3**	**0.013**
Post extraction solids (PES)	**N/A**	**N/A**	**3.3**	**0.010**	**0.3**	**0.001**
Accountability[(a)]	**NA**		**92.2%**		**107.7%**	
[phenyl-U-^{14}C] inpyrfluxam						
TRR (combustion of solid matrix)	**N/A**	**N/A**	**-**	**0.101**	**-**	**0.009**
TRR (sum of extracted and solid residues)	**58.2**	**0.145**	**37.3**	**0.093**	**4.4**	**0.011**
ACN/H_2O Extract 1 **Acetonitrile: water 1:1, v/v, Extract #1**	*N/A*	*N/A*	*22.1*	*0.055*	*2.0*	*0.005*
ACN/H_2O Extract 2 **Acetonitrile: water 1:1, v/v, Extract #2**	*N/A*	*N/A*	*9.6*	*0.024*	*1.6*	*0.004*
ACN Extract 3	*N/A*	*N/A*	*2.0*	*0.005*	*0.4*	*0.001*
Total extracted	**58.2**	**0.145**	**33.7**	**0.084**	**4.0**	**0.010**
Post extraction solids (PES)	**N/A**	**N/A**	**3.6**	**0.009**	**0.4**	**0.001**
Accountability[(a)]	**NA**		**92.1%**		**122.2%**	

% TRR are based on the sum of extracted and solid residues in whole apple i.e. apple rinse plus apple peel plus apple pulp

[(a)] Accountability = [(sum of ERR + PES)(/initial TRR)] x 100, determined separately for apple peel and apple pulp

No radioactivity above 2x background level was detected for all control samples.

For [pyrazolyl-4-^{14}C] inpyrfluxam treated apple, the extraction rates were high and amounted to 89% (0.084 mg eq./kg) of the TRR for peel and 93% (0.013 mg eq./kg)

of the TRR for pulp. For [phenyl-U-^{14}C] inpyrfluxam treated apple, the extraction rates were also high and amounted to 90% (0.084 mg eq./kg) of the TRR for peel and 91% (0.010 mg eq./kg) of the TRR for pulp. The maximum amount of radioactivity in the post extraction solids was 0.01 mg eq./kg, for the peel. The remaining solids, the PES, across all the samples of peel or pulp individually represented 7 – 11% of the TRR (sum of ERR and PES).

Residues in the combined extracts (extract fractions 1 – 3) of apple fruit were identified using HPLC, LC-MS and TLC. The retention times of co-eluting standards were used and confirmed by 1D TLC and in selected samples by LC-MS. A range of reference standards were used to seek to identify the residues, namely: inpyrfluxam, DFPA, 3'-OH-S-2840, *N*-des-Me-S-2840, *N*-des-Me-DFPA, DFPA-$CONH_2$, ATMI, Glc-NDM-S2399A, Glc-NDM-S2399B, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B, 1'-COOH-S-2840-A and 1'-COOH-S-2840-B.

The results of the distribution of the residues found (parent and metabolites) are presented in Tables 7.2.1.1-4 (pyrazolyl label) and Table 7.2.1.1-5 (phenyl label).

Table 7.2.1.1-4: Distribution of parent and metabolites in the extracts of apple matrices after three foliar applications of [pyrazolyl-4-^{14}C] inpyrfluxam

	Apple rinse		Apple peel		Apple pulp		Total	
	% TRR[(a)]	mg eq./kg	% TRR[(a)]	mg eq./kg	% TRR[(a)]	mg eq./kg	% TRR[(a)]	mg eq./kg
Inpyrfluxam	57.2	0.171	20.4	0.061	1.5	0.004	79.1	0.236
3'-OH-S-2840	6.8	0.021	4.4	0.013	0.3	0.001	11.5	0.035
1'-CH_2OH-S-2840	ND	ND	3.2	0.010	1.4	0.004	4.6	0.014
Total identified							**95.2**	**0.286**
PES	**NA**	**NA**	**3.3**	**0.010**	**0.3**	**0.001**	**3.6**	**0.011**

ND not detected

[(a)] % TRR are based on the sum of extracted and solid residues in whole apple i.e. apple rinse plus apple peel plus apple pulp (0.3 mg eq./kg in total)

Table 7.2.1.1-5: Distribution of parent and metabolites in the extracts of apple matrices after three foliar applications of [phenyl-U-^{14}C] inpyrfluxam

	Apple rinse		Apple peel		Apple pulp		Total	
	% TRR[(a)]	mg eq./kg	% TRR[(a)]	mg eq./kg	% TRR[(a)]	mg eq./kg	% TRR[(a)]	mg eq./kg
Inpyrfluxam	53.5	0.133	23.3	0.058	1.0	0.002	77.8	0.193
3'-OH-S-2840	4.7	0.012	6.1	0.015	0.2	0.001	11.0	0.028
1'-CH_2OH-S-2840	ND	ND	4.3	0.011	1.3	0.003	5.6	0.014
Total identified							**94.4**	**0.235**
PES	**NA**	**NA**	**3.6**	**0.009**	**0.4**	**0.001**	**4.0**	**0.010**

ND not detected

[(a)] % TRR are based on the sum of extracted and solid residues in whole apple i.e. apple rinse plus apple peel plus apple pulp (0.249 mg eq./kg in total)

The parent substance inpyrfluxam represented the most prominent residue component in all samples of rinse and peel. In pulp, the levels of parent were lower and found in amounts that were broadly comparable to the level of 1'-CH_2OH-S-2840 found in the pulp (where overall, the levels of radioactivity found were much lower).

Based on a consideration of the TRR in the rinse, pulp and peel, parent substance inpyrfluxam accounted for 53 – 57% of the TRR in the rinse, a further 20 – 23% of the TRR in the peel and 1.0 – 1.5% of the TRR in the pulp.

In relation to overall total TRR amounts, across rinse, peel, and pulp, the primary component of the residues in both radiolabels was parent inpyrfluxam, (78 – 79% of the TRR). There were two other metabolites detected, 3'-OH-S-2840 present at approx. 11% of TRR and 1'-CH_2OH-S-2840 present at 5 – 6% of TRR. In the pulp the individual residues (metabolites or parent) were present only at up to 0.004 mg eq./kg. The maximum amount of an individual metabolite in peel was 0.015 mg eq./kg (3'-OH-S-2840).

Overall, identification rates and extractability of the residues was sufficient, no label specific metabolites were observed, and the results were similar across the pyrazolyl, and phenyl labelled study.

Metabolism proceeds via oxidation of inpyrfluxam forming hydroxylation products 3'-OH-S-2840 and 1'-CH_2OH-S-2840.

Based on these results, the metabolic pathway of [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam in apple is adequately understood and a pathway is proposed (see Figure 7.2-2).

Chiral analysis

Chiral HPLC analysis were performed to determine the ratio of enantiomers of inpyrfluxam in plant extracts. Sample extracts of rinse, peel and pulp characterised as containing parent compound were partitioned between hexane and water to selectively isolate the parent compound into the hexane fraction. The hexane fraction was concentrated under nitrogen or using a rotary evaporator prior to further analysis using a chiral HPLC method (resolution into early eluting “S” and later eluting “R” enantiomers) using a reference standard where the R- and S- isomers were resolved. In apple rinse, peels and pulp (both labels), the samples contained 100% R-isomer and 0% S-isomer. This demonstrated that there was no stereoisomeric conversion of the parent R isomer to the S isomer over the course of the study.

Storage stability

The initial profiles of the combined extracts (extract fractions 1 – 3) of apple peel and pulp as well as the apple rinses were analysed within 47 days after harvest of the apple plant raw material. The samples of peel and pulp were stored homogenised and frozen during this period. Given all extraction experiments of samples and the first HPLC analyses were performed well within six months after harvest of the samples, storage stability data are not required.

Nonetheless, the stability of the residues in stored extracts of apple peel was investigated following storage at -27°C for 665 days.

The comparison of the respective HPLC chromatograms revealed that the profiles of the apple peel extracts did not significantly change after storage. Therefore, it can be concluded the residues in the extracts when stored were stable.

Summary of inpyrfluxam metabolism in apple

The metabolism of inpyrfluxam in apple was investigated after three foliar applications 35, 24 and 14 days prior to harvest. The apple trees were treated three times with either [pyrazolyl-4-^{14}C] inpyrfluxam or [phenyl-U-^{14}C] inpyrfluxam formulated as a 40% SC formulation at a nominal individual application rate of 200 g a.s./ha (actual: 214 – 221 g a.s./ha) corresponding to a total nominal application rate of 600 g a.s./ha (actual: 649 – 658 g a.s./ha). Apple fruits and leaves were harvested 14 days after the last application.

Residues in the apple pulp were significantly lower than those in the peel and apple rinse. The extraction rates in rinse, peel and pulp were high. Overall, identification

rates in apple rinse, peel and pulp were sufficient. In [pyrazolyl-4-^{14}C] inpyrfluxam treated apples, parent compound inpyrfluxam was the main residue component for all matrices. In [phenyl-U-^{14}C] inpyrfluxam treated apples, parent compound inpyrfluxam was the main residue component in apple rinse and apple peel. In apple pulp, the metabolite 1'-CH_2OH-S-2840-B was the main residue component.

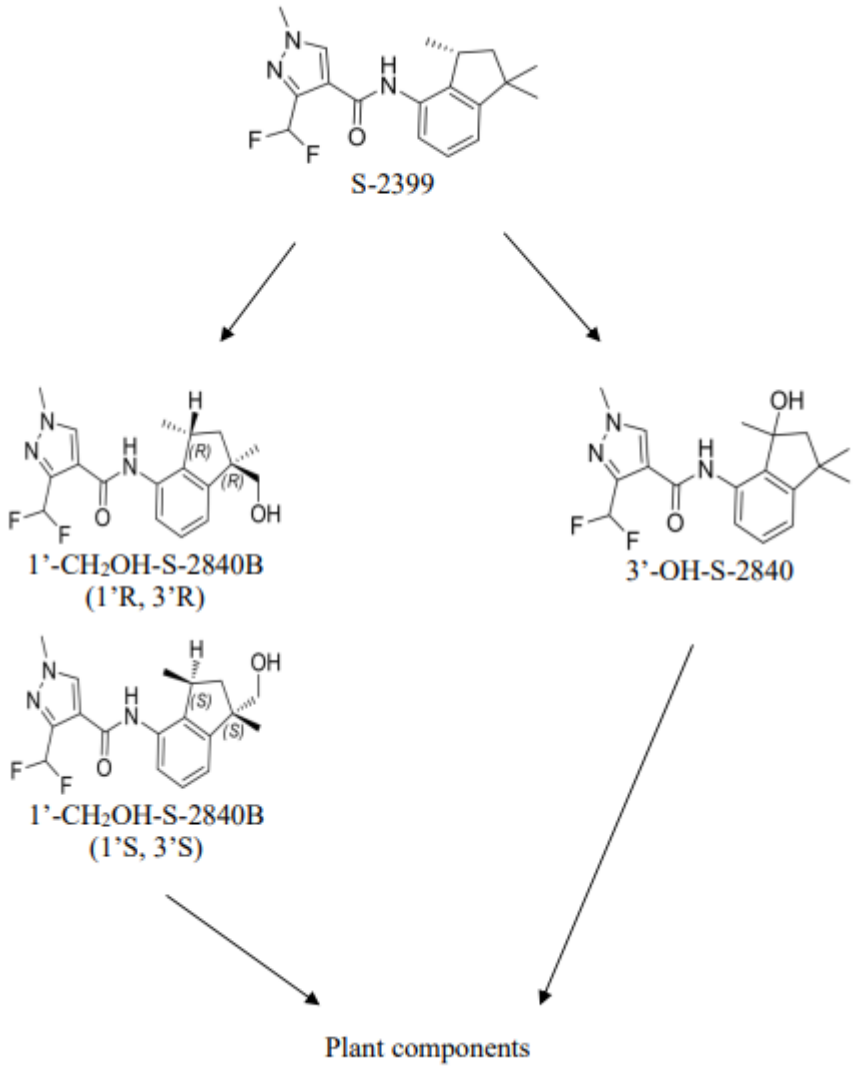
The results from the studies involving [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam are in close agreement. No label specific metabolites were observed.

Inpyrfluxam was found to be moderately metabolised in apple after three applications. The main metabolic reaction is listed below:

- Oxidation of inpyrfluxam forming hydroxylation products 3'-OH-S-2840 and 1'-CH_2OH-S-2840 (≥ 94% of the TRR in apples).

Based on these results, the metabolic pathway of inpyrfluxam in apple is adequately understood and is proposed in the figure below:

Figure 7.2-2: Proposed metabolic pathway of inpyrfluxam in apple



B.7.2.1.2 Rice (foliar treatment)

Table 7.2.1.2-1: Overview of rice metabolism studies

Plant	Application	Target application rate	BBCH at application	Days prior to final harvest	Reference
Rice	One foliar spray application, pyrazolyl-labelled inpyrfluxam & phenyl-labelled inpyrfluxam	1 x ~100 g a.s./ha	77	14 (immature plants) 28 (grain, straw, hulls)	KCA 6.2.1/04 TPM-0014

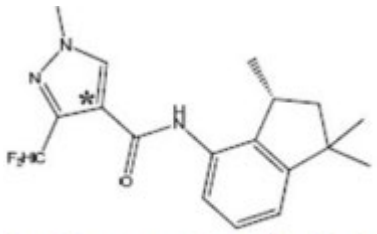
Report:	**KCA 6.2.1/04; [redacted] and [redacted] 2017**
Title:	A Metabolism Study of [^{14}C] S-2399 (2 Radiolabels) in Rice (oryza sativa L.) With Foliar Treatment
Document No.:	TPM-0014 (Study No.: 2508W)
Guidelines:	US EPA OPPTS 860.1300 OECD/OCDE Guideline 501 EU Guideline 7028/VI/95 JMAFF 12-Nohsan No. 8147
Guideline deviations:	None
GLP/GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

The metabolism of inpyrfluxam was investigated in rice after one foliar spray application 28 days before final harvest. The spray of [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam was formulated as a 40% SC spray formulation and the achieved application rates using a hand operated trigger pump sprayer were 95.0 g a.s./ha and 108.1 g a.s./ha respectively. This metabolism study is the one that is most relevant to the currently intended uses on wheat and barley, by way of application rate. For the current intended GAPs on these crops the application rates correspond to circa 1.1N or 1.2N. The mimic of the agricultural practice of growing paddy rice, by flooding the plots is not how cereal crops are typically grown in the UK.

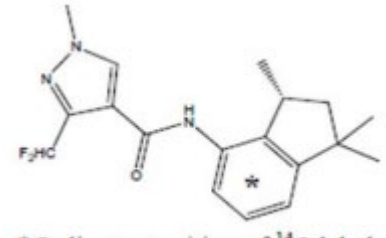
The tests material used to make the formulated spray were:

[Pyrazolyl-4- ^{14}C] inpyrfluxam

Chemical name [Pyrazolyl-4- ^{14}C] inpyrfluxam

Chemical structure	 * Indicates position of ^{14}C label.
Radiolabel position	[pyrazolyl-4- ^{14}C]
Specific radioactivity	2.22 GBq/mmol
Radiochemical purity	≥96.5% (before and after application)
Chemical purity	95.8%

[Phenyl-U- ^{14}C] inpyrfluxam

Chemical name	[Phenyl-U- ^{14}C] inpyrfluxam
Chemical structure	 * Indicates position of ^{14}C label.
Radiolabel position	[phenyl-U- ^{14}C]
Specific radioactivity	4.48 GBq/mmol
Radiochemical purity	≥96.5% (before and after application)
Chemical purity	95.8%

Conducted in the USA (California) in 2013, the experiment was conducted with rice plants (variety “CM205”) transplanted into the test plots at approximately 4 leaf stage of development. The rice plants were grown in flooded plots to mimic normal application to paddy rice. Test plots consisted of outdoor boxes (lined with a heavy gauge plastic liner) filled with clay soil and included one control plot and two treated plots (one for each radiolabel). Each treated plot was 1 m^2 in area with the soil approximately 38 cm deep. The plants were treated at BBCH 77 for each plot, with

plastic barriers in place at just the time of application to prevent wind transfer of spray. Rice plants had developed with grain heads formed prior to test substance application. The actual amount of radiolabelled active substance applied was calculated based on the radioactivity added to the individual dosing bottles minus any residual radioactivity remaining after application.

Immature rice plants were harvested 14 days after application (BBCH 87) by cutting plants above about 2.5 cm the soil line. Rice straw, rice grain and rice hulls were harvested 28 days after application. To do this heads were cut from the standing plants with pruning shears. The standing plants were cut 2.5 – 5 cm above the soil line and collected as the straw sample. Brown rice was separated from seed heads by use of threshing blocks, then the hulls were separated from the brown rice. Samples were placed in the freezer prior to transfer to the analytical laboratory on dry ice, where the specimens were processed with dry ice and then stored deep frozen for a maximum of 50 days before extraction prior to analysis.

The total radioactive residues (TRR) of each matrix were determined by combustion of solid matrix and LSC. The results are presented in Table 7.2.1.2-2.

Table 7.2.1.2-2: TRR in rice matrices after foliar application of [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam

Matrix	Application	PHI (days)	TRR (mg eq./kg)[(a)]	TRR (mg eq./kg)[(b)]
[pyrazolyl-4-^{14}C] inpyrfluxam				
Immature rice plants	1 spray application: 1 x 95 g a.s./ha (1.1N)	14	0.323	0.285
Rice straw		28	0.848	0.851
Rice grain			0.053	0.064
Rice hulls			1.576	1.527
[phenyl-U-^{14}C] inpyrfluxam				
Immature rice plants	1 spray application: 1 x 108 g a.s./ha (1.2N)	14	0.391	0.379
Rice straw		28	0.805	0.927
Rice grain			0.044	0.049

Rice hulls			1.430	1.680

(a) TRR values based on combustion of solid matrix/LSC
(b) TRR values calculated from sum of extracted and post-extraction solid residues

The results show that the total radioactive residues (TRRs) [combustion] were similar across the two different radiolabels: immature rice plants was 0.32 – 0.39 mg eq./kg, straw was 0.8 – 0.85 mg eq./kg, rice grain was 0.04 – 0.05 mg eq./kg, and hulls was 1.4 – 1.6 mg eq./kg.

Residue transfer from application to grain was relatively low whereas transfer was high for hulls. This is likely attributed to the surface areas of the plant part directly intercepting the spray application in addition to the surface area to low sample mass of rice hulls. The residues in the aerial portion of the rice plants were lower in harvest 1 (immature crop) than harvest 2 (maturity) likely because of the desiccation of the plant as it matured.

Radioactive residues from the rice matrices were extracted twice using acetonitrile and water (1:1 , v/v) and then once using 100% acetonitrile, prior to analysis by LSC. Extracts were combined and stored frozen. A portion of the post-extraction solids (PES) was combusted to determine the remaining radiocarbon levels.

The results for the TRR based on sum of ERR and PES were also quite similar to the results obtained for combustion. The accountability levels ranged from 100 – 121%, aside for immature rice plants, where the accountability was lower at 88%. Accountability% = [(sum of ERR + PES)(/initial TRR)] x 100. Overall, the data demonstrate that aside from some variability that is expected, there were no marked losses in the extraction and work up of the samples.

The distribution of residues and extractability with solvents is presented in Table 7.2.1.2-3. The solvent extraction rates were high and in the range of 81.5 – 95.9% across all the sample types and both radiolabels.

The remaining solids after solvent extraction (PES1) represented: For immature rice plants, 10.2% (0.029 mg eq./kg) of the TRR [pyrazolyl-4-^{14}C] inpyrfluxam and 12.9% (0.049 mg eq./kg) of the TRR for [phenyl-U-^{14}C] inpyrfluxam. For rice straw 14% (0.119 mg eq./kg) of the TRR for [pyrazolyl-4-^{14}C] inpyrfluxam and 18.6% (0.172 mg eq./kg) of the TRR for [phenyl-U-^{14}C] inpyrfluxam. For rice hulls, 14.5% (0.221 mg eq./kg) of the TRR for [pyrazolyl-4-^{14}C] inpyrfluxam and 17% (0.285 mg eq./kg) of the TRR for [phenyl-U-^{14}C] inpyrfluxam. For rice grain, 4.7% (0.003 mg eq./kg) of the TRR for [pyrazolyl-4-^{14}C] inpyrfluxam and 4.1% (0.002 mg eq./kg) of the TRR for [phenyl-U-^{14}C] inpyrfluxam.

Solids remaining after solvent extraction (PES) of rice matrices (solids with TRR >10%) were then sequentially extracted using acidified acetonitrile (0.1 M HCl in

acetonitrile), followed by 50 mM ethylene glycol bis-(2-aminoeythl ester)-N,N,N',N'-tetraacetic acid (EGTA), shaken in DMSO for 66 hours, DMSO for 1 hour at 80°C, strong base (24% KOH) and finally strong acid (6M H_2SO_4).

The final PES (PES2) was combusted to determine the remaining residual radiocarbon levels (comprising 0.6 – 3.2% TRR, maximum 0.054 mg eq./kg in rice hulls).

The applicant postulated that the further work up of the PES characterised the residues as a mixture of pectin, lignin, hemicelluloses and cellulose fractions (≤ 5% TRR for each) according to the following applicant proposals: Post extraction solids with TRR > 10% were further extracted sequentially with acidified acetonitrile, ethylene glycol bis-(2- aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) which (proposed) solubilises plant pectins; dimethyl sulfoxide (DMSO) which (proposed) solubilizes plant lignins; potassium hydroxide (KOH) which (proposed) dissolves plant hemicelluloses and sulfuric acid (H_2SO_4) which (proposed) dissolves plant cellulose. HSE notes that no enzymes were used to demonstrate association with plant macromolecules, although these further sequential extractions performed on PES1 (which comprised 4.1 – 18.6%TRR) enabled the PES to be extensively characterised (in terms of the release of radioactivity following different 'treatments').

The residues in the solids after further extraction (PES2) were determined by combustion for both [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam treated rice. The maximum amount remaining in PES2 was for rice hulls which was 3.2% (0.054 mg eq./kg) of the TRR for [phenyl-U-^{14}C] inpyrfluxam. No further extraction of the remaining radioactivity was undertaken.

The distribution of the radioactive residues is shown in the following table.

Table 7.2.1.2-3: Distribution of radioactivity in the extracts of rice matrices after one foliar application of [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam

Sample	Immature rice plants		Rice straw		Rice hulls		Rice grain	
	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg
[pyrazolyl-4-^{14}C] inpyrfluxam								
TRR (combustion of solid matrix)	-	0.323	-	0.848	-	1.576	-	0.053
TRR (extracted	100	0.285	100	0.851	100	1.527	100%	0.064

and solid residues)								
ACN/H_2O Extract 1	*50.2*	*0.143*	*51.6*	*0.439*	*45.6*	*0.696*	*64.1*	*0.041*
ACN/H_2O Extract 2	*28.1*	*0.080*	*23.3*	*0.198*	*28.2*	*0.431*	*26.6*	*0.017*
ACN Extract 3	*11.6*	*0.033*	*11.2*	*0.095*	*11.7*	*0.179*	*4.7*	*0.003*
ACN/H_2O combined	89.8	0.256	86.0	0.732	85.5	1.306	95.3	0.061
PES 1[(a)]	10.2	0.029	14.0	0.119	14.5	0.221	4.7	0.003
0.1M HCl in ACN	3.9	0.011	5.1	0.043	3.9	0.060	N/A	N/A
EGTA	1.4	0.004	1.9	0.016	1.1	0.017	N/A	N/A
DMSO 66hr	1.4	0.004	1.1	0.009	1.6	0.025	N/A	N/A
DMSO 1hr 80°C	0.7	0.002	1.5	0.013	1.2	0.018	N/A	N/A
24% KOH overnight	1.8	0.005	2.8	0.024	4.2	0.064	N/A	N/A
72% H_2SO_4 4hr	0.7	0.002	0.9	0.008	0.5	0.007	N/A	N/A
PES 2[(b)]	0.7	0.002	0.6	0.005	2.0	0.030	N/A	N/A
Accountability[(c)]	**88.3%**		**100.4%**		**96.9%**		**120.8%**	
[phenyl-U-^{14}C] inpyrfluxam								
TRR (combustion of solid matrix)	**-**	**0.391**	**-**	**0.805**	**-**	**1.430**	**-**	**0.044**
TRR (extracted and solid residues)	**100**	**0.379**	**100**	**0.927**	**100**	**0.049**	**100**	**1.680**

ACN/H_2O Extract 1	*48.3*	*0.183*	*42.7*	*0.396*	*N/A[(d)]*	*N/A[(d)]*	*40.1*	*0.674*
ACN/H_2O Extract 2	*27.4*	*0.104*	*26.9*	*0.249*	*N/A[(d)]*	*N/A[(d)]*	*28.9*	*0.486*
ACN Extract 3	*11.4*	*0.043*	*11.9*	*0.110*	*N/A[(d)]*	*N/A[(d)]*	*14.0*	*0.235*
ACN/H_2O combined	**87.1**	**0.330**	**81.5**	**0.755**	**83.0**	**1.395**	**95.9**	**0.047**
PES 1[(a)]	12.9	0.049	18.6	0.172	17.0	0.285	4.1	0.002
0.1M HCl in ACN	5.0	0.019	4.8	0.044	4.1	0.068	N/A	N/A
EGTA	1.6	0.006	2.9	0.027	1.6	0.026	N/A	N/A
DMSO 66hr	1.6	0.006	1.8	0.017	1.9	0.032	N/A	N/A
DMSO 1hr 80°C	1.1	0.004	1.6	0.015	1.3	0.021	N/A	N/A
24% KOH overnight	2.1	0.008	3.1	0.029	4.4	0.073	N/A	N/A
72% H_2SO_4 4hr	0.8	0.003	1.5	0.014	0.6	0.010	N/A	N/A
PES 2[(b)]	0.8	0.003	2.8	0.026	3.2	0.054	N/A	N/A
Accountability[(c)]	**96.9%**		**115.2%**		**117.5%**		**111.4%**	

[(a)] PES 1 refers to the post extraction solids remaining following the initial extraction with acetonitrile and water (1:1, v/v) twice and once with 100% acetonitrile

[(b)] PES 2 refers to the post extraction solids remaining following further extraction with HCl, EGTA, DMSO, strong base and strong acid

[(c)] Accountability = [(sum of ERR + PES)(/initial TRR)] x 100

[(d)] Individual extracts were combined prior to radioanalysis

Chiral analysis

Where parent inpyrfluxam was detected in solvent fractions, chiral HPLC analyses were performed to determine the ratio of enantiomers of inpyrfluxam. Sample extracts characterised as containing parent compound were partitioned between hexane and water (x2) to selectively isolate the parent compound into the hexane fraction. A combined hexane fraction was analysed by chiral HPLC to resolve

inpyrfluxam into early eluting “S” and later eluting “R” enantiomers. All the sample types were analysed and showed that no isomerisation on/in the rice plants (100% R-isomer and no S-isomer).

Identification

Residues in the conventional extracts of rice matrices (combined) were identified by reverse phase HPLC and using co-chromatography with reference standards. The identities of inpyrfluxam, 3’-OH-S-2840, 1’-CH_2OH-S-2840 and trace amounts of DFPA-$CONH_2$ metabolites were also confirmed by TLC analyses. The solvent system used for TLC was Chloroform: Methanol (9:1) [one dimensional TLC]. Example chromatograms for the HPLC and TLC work showed the chromatographic separation to be of a suitable quality. The following metabolites were identified: 3’-OH-S-2840, 1’-CH_2OH-S-2840, and trace amounts of *N*-des-Me-S-2840, Gly-1’-CH_2OH-S-2840 and DFPA-$CONH_2$. Overall, identification rates were high (82 – 93%). The maximum individual unidentified component represented 2.9% (0.049 mg eq./kg) of the TRR in rice hulls. The maximum individual unidentified component in rice grain represented 1.2% (≤0.001 mg eq./kg) of the TRR. The ‘unknowns’ are not considered further in this evaluation.

The identification and the distribution of parent and metabolites in rice matrices following treatment by [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam are shown in the following tables.

Table 7.2.1.2-4: Distribution of parent and metabolites in the extracts of rice matrices after one foliar application of [pyrazolyl-4-^{14}C] inpyrfluxam (total amounts from the solvent extracts plus the 0.1 M HCl extract, the amounts released by 0.1 M HCl were small e.g. parent inpyrfluxam up to 0.007 mg/kg in immature rice and 0.029 mg/kg in rice hulls)

	Immature rice plants		Rice straw	Rice hulls			Rice grain	
	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg
Inpyrfluxam	86.7	0.247	67.7	0.576	41.8	0.639	60.6	0.039
***N*-des-Me-S-2840**	0.2	≤0.001	0.4	0.003	ND	ND	ND	ND
3’-OH-S-2840	5.6	0.016	12.0	0.102	5.8	0.088	5.9	0.004
1’-CH_2OH-S-2840-B	0.2	≤0.001	0.3	0.003	11.9	0.181	ND	ND

1'-CH_2OH-S-2840-A	ND	ND	0.4	0.003	22.0	0.336	ND	ND
Gly-1'-CH_2OH-S-2840[(a)]	ND	ND	5.2	0.040	7.2	0.110	16.0	0.010
DFPA-$CONH_2$	ND	ND	4.6	0.039	ND	ND	ND	ND
Total identified	**92.7**	**0.265**	**90.6**	**0.766**	**88.7**	**1.354**	**82.5**	**0.053**

ND not detected

(a) Undifferentiated peak region consisting of multiple sugar conjugates of 1'-CH_2OH-S-2840, based on the results of a rice metabolism study with granule application (Vol. 3 CA B7.2.1.2).

Table 7.2.1.2-5: Distribution of parent and metabolites in the extracts of rice matrices after one foliar application of [phenyl-U-^{14}C] inpyrfluxam (total amounts from the solvent extracts plus the 0.1 M HCl extract, the amounts released by 0.1 M HCl were small e.g. parent inpyrfluxam up to 0.013 mg/kg and in immature rice and 0.055 mg/kg in rice hulls)

	Immature rice plants		Rice straw	Rice hulls			Rice grain	
	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg
Inpyrfluxam	81.2	0.308	77.8	0.721	52.5	0.881	78.6	0.038
***N*-des-Me-S-2840**	0.5	0.002	0.5	0.005	ND	ND	ND	ND
3'-OH-S-2840	7.1	0.027	6.0	0.055	5.6	0.087	7.0	0.003
1'-CH_2OH-S-2840-B	1.1	0.004	0.2	0.002	6.0	0.093	ND	ND
1'-CH_2OH-S-2840-A	1.9	0.007	1.3	0.012	12.0	0.184	ND	ND
Gly-1'-CH_2OH-S-2840[(a)]	ND	ND	ND	ND	7.1	0.118	3.1	0.002
Total identified	**91.8**	**0.348**	**85.8**	**0.795**	**83.2**	**1.363**	**88.7**	**0.043**

ND not detected

(a) Undifferentiated peak region consisting of multiple sugar conjugates of 1'-CH_2OH-S-2840, based on the results of a rice metabolism study with granule application (Vol. 3 CA B.7.2.1.3).

The parent substance inpyrfluxam represented the most prominent residue component in all matrices for both radiolabels (42 – 87% TRR).

The next prevalent metabolites were 3'-OH-S-2840 at 5 – 12% TRR, 1'-CH_2OH-S-2840-A at 0.4 – 22% TRR and 1'-CH_2OH-S-2840-B at 0.2 – 12% TRR. Together with postulated conjugated material of 1'-CH_2OH-S-2840 as 'Gly-1'-CH_2OH-S-2840', 1'-CH_2OH-S-2840 was found in low amounts in straw, grain and immature plants, and large amounts in rice hulls especially (combined 39% TRR in the pyrazolyl labelled study in rice hulls); the latter 'Gly-1'-CH_2OH-S-2840'was proposed as an undifferentiated peak region consisting of multiple sugar conjugates of 1'-CH_2OH-S-2840. Profiles of the rice matrices showed some major differences across the individual matrices. *N*-des-Me-S-2840 was found in only trace amounts (max 0.005 mg eq./kg). A pyrazolyl specific metabolite was found in rice straw: DFPA-$CONH_2$ at up to 5%TRR (0.039 mg eq./kg).

Storage stability

Samples were stored frozen for a maximum of 50 days (after harvest) before extraction prior to analysis. All HPLC profiles of conventional extracts of the RACs were performed within 64 days after harvest. This comprised most of the analytical work determining levels of metabolites in the solvent extracted material. The PES1 was worked on after 414 days (post harvest 1).

The stability of the residues was demonstrated in immature rice samples by re-extraction (of immature foliage) followed by HPLC after one year of frozen storage in a freezer (-17^{o}C). The comparison of the respective HPLC chromatograms revealed that the profiles of the extracts did not significantly change after storage of the material and the TRR values from the initial extraction of immature rice plants were similar (within 5%). The chromatograms showed similar amounts of inpyrfluxam for both labels and a decrease (8 to 3% TRR) of 3'-OH-S-2840 in extracts of immature rice plants for the phenyl label after one year of frozen storage. The decrease in the corresponding pyrazolyl sample was less (6 to 5% TRR).

Additional stability analysis was repeated on the remaining rice matrices (straw, grain and hull) for both labels, by HPLC reanalysis of the initially analysed HPLC extracts, 22 months later. For all three matrices, both labels showed similar agreement to the initial analysis.

This storage stability work confirms the validity of the study.

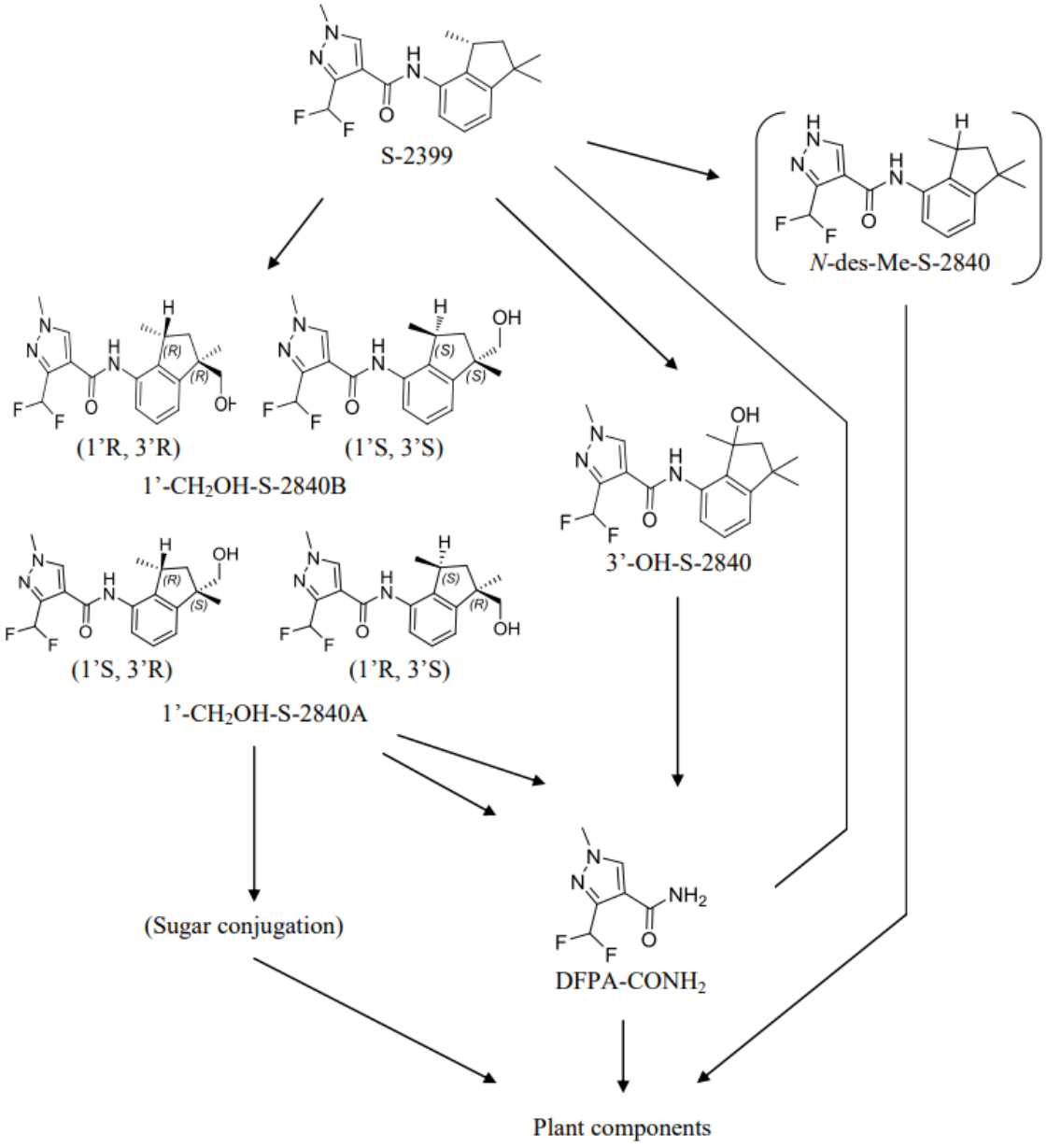
Summary of inpyrfluxam metabolism in rice (foliar treatment)

Inpyrfluxam was found to be moderately metabolised in rice after one application. Metabolism proceeds via oxidation of inpyrfluxam forming hydroxylation metabolites

3'-OH-S-2840 and 1'-CH_2OH-S-2840 (the latter thought to be forming sugar conjugates). Furthermore, de-methylation of inpyrfluxam forming *N*-des-Me-S-2840

Based on these results, the metabolic pathway of inpyrfluxam in rice is adequately understood and is proposed in the figure below:

Figure 7.2.1.2-1: Proposed metabolic pathway of inpyrfluxam in rice



B.7.2.1.3 Rice (granular treatment)

Table 7.2.1.3-1: Overview of rice metabolism studies

Plant	Application	Target application rate	BBCH at application	Days prior to final harvest	Reference
Rice	One granular application, pyrazolyl-labelled inpyrfluxam & phenyl-labelled inpyrfluxam	1 x ~400 (391 or 357) g a.s./ha	13 – 14	30 (immature plants) 132 (grain, straw, hulls)	KCA 6.2.1/05 TPM-0016

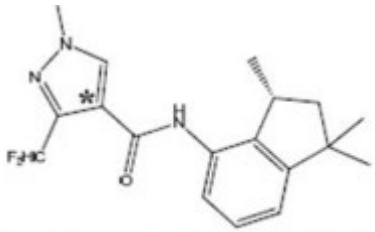
Report:	**KCA 6.2.1/05; [redacted] and [redacted] (Final Report), [redacted] (Amended Final Report); 2016 (Final Report), 2017 (Amended Final Report)**
Title:	A Metabolism Study of [^{14}C]S-2399 (2 Radiolabels) in Rice (*Oryza sativa L.*) With Granular Application
Document No.:	TPM-0016 (Study No.: 2509W)
Guidelines:	US EPA OPPTS 860.1300 OECD/OCDE Guideline 501 EU Guideline 7028/VI/95 JMAFF 12-Nohsan No. 8147
Guideline deviations:	None
GLP/GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

The metabolism of inpyrfluxam was investigated in rice after one granular application 132 days before final harvest. The test items [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam were formulated as a 4% granular formulation and the

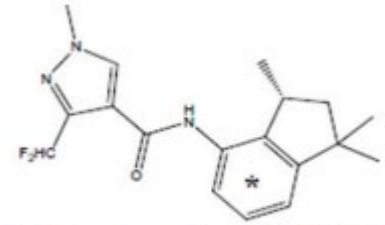
achieved application rates using a hand operated shaker were 391 g a.s./ha and 357 g a.s./ha, respectively.

The test material used to make the granular formulation were:

[Pyrazolyl-4- ^{14}C] inpyrfluxam

Chemical name	[Pyrazolyl-4- ^{14}C] inpyrfluxam
Chemical structure	 * Indicates position of ^{14}C label.
Radiolabel position	[pyrazolyl-4- ^{14}C]
Specific radioactivity	2.22 GBq/mmol
Radiochemical purity	97.1% (before and after application)
Chemical purity	95.8%

[Phenyl-U- ^{14}C] inpyrfluxam

Chemical name	[Phenyl-U- ^{14}C] inpyrfluxam
Chemical structure	 * Indicates position of ^{14}C label.
Radiolabel position	[phenyl-U- ^{14}C]
Specific radioactivity	4.48 GBq/mmol
Radiochemical purity	95.9% (before and after application)
Chemical purity	95.8%

Conducted in the USA (California) in 2013, the experiment was conducted with rice plants (variety “CM205”) transplanted into the test plots at approximately 3 – 4 leaf stage of development. The rice plants were grown in flooded plots to mimic normal application to paddy rice. Test plots consisted of outdoor boxes (lined with a heavy gauge plastic liner) filled with clay soil and included one control plot and two treated plots (one for each radiolabel). Each treated plot was 1 m^2 in area with the soil approximately 38 cm deep. The plants were treated at BBCH 13 – 14 for each plot using a small bottle with holes in the cap to sprinkle the granules at the base of each plant site. The surface was then covered with soil. The actual amount of radiolabelled active substance applied was calculated based on the radioactivity added to the individual dosing bottles minus any residual radioactivity remaining after application.

Immature rice plants were harvested 30 days after application (BBCH 30) by cutting the plants about 2.5 cm above the soil line. Mature rice plants were harvested 132 days after application (BBCH 89). To do this heads were cut from the standing plants with pruning shears. The standing plants were cut 2.5 – 5 cm above the soil line and collected as the straw sample. Brown rice was separated from seed heads by use of threshing blocks, then the hulls were separated from the brown rice. Samples were placed in the freezer prior to transfer to the analytical laboratory on dry ice, where the specimens were processed with dry ice and then stored deep frozen for a maximum of 50 days before extraction or 100 days before analysis.

The total radioactive residues (TRR) of each matrix were determined by combustion of solid matrix and LSC. The results are presented in Table 7.2.1.3-2.

Table 7.2.1.3-2: TRR in rice matrices after granular application of [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam

Matrix	Application	PHI (days)	TRR (mg/kg)[(a)]	TRR (mg/kg)[(b)]
[pyrazolyl-4-^{14}C] inpyrfluxam				
Immature rice plants	1 granular application: 1 x 391 g a.s./ha	30	3.783	3.888
Rice straw		132	1.548	1.582
Rice grain			0.010	0.009
Rice hulls			0.171	0.175
[phenyl-U-^{14}C] inpyrfluxam				

Immature rice plants	1 granular application: 1 x 357 g a.s./ha	30	1.909	1.887
Rice straw		132	1.095	1.072
Rice grain			0.015	0.015
Rice hulls			0.158	0.156

(a) TRR values based on combustion of solid matrix/LSC
(b) TRR values calculated from sum of extracted and post-extraction solid residues

The results show that the total radioactive residues (TRRs) [combustion] were broadly similar across the two different radiolabels for rice straw (1.10 – 1.55 mg eq./kg), rice grain (0.01 – 0.015 mg eq./kg) and rice hulls (0.16 – 0.17 mg eq./kg). A greater difference between the radiolabels was evident for immature rice plants (1.91 – 3.78 mg eq./kg).

Residue transfer from application to grain was relatively low whereas transfer was high for hulls. This is likely attributed to the surface areas of the plant part directly intercepting the spray application in addition to the surface area to low sample mass of rice hulls.

Radioactive residues from the rice matrices were extracted twice using acetonitrile and water (1:1 , v/v) and then once using 100% acetonitrile, prior to analysis by LSC. Extracts were combined and stored frozen. A portion of the post-extraction solids (PES) was combusted to determine the remaining radiocarbon levels.

The results for the TRR based on sum of ERR and PES were also quite similar to the results obtained for combustion. The accountability levels ranged from 98 – 103%, aside for rice grain ([pyrazolyl-4-^{14}C] inpyrfluxam), where the accountability was lower at 90%. Accountability% = [(sum of ERR + PES)(/initial TRR)] x 100. Overall, the data demonstrate that aside from some variability that is expected, there were no marked losses in the extraction and work up of the samples.

The distribution of residues and extractability with solvents is presented in table 7.2.1.3-3. The solvent extraction rates were in the range of 33 – 64% across all the sample types and both radiolabels.

The remaining solids after solvent extraction (PES1) represented: For immature rice plants, 48.7% (1.895 mg eq./kg) of the TRR [pyrazolyl-4-^{14}C] inpyrfluxam and 41.5% (0.783 mg eq./kg) of the TRR for [phenyl-U-^{14}C] inpyrfluxam. For rice straw 36% (0.569 mg eq./kg) of the TRR for [pyrazolyl-4-^{14}C] inpyrfluxam and 37.6% (0.403 mg eq./kg) of the TRR for [phenyl-U-^{14}C] inpyrfluxam. For rice grain, 44.4% (0.004 mg

eq./kg) of the TRR for [pyrazolyl-4-^{14}C] inpyrfluxam and 66.7% (0.010 mg eq./kg) of the TRR for [phenyl-U-^{14}C] inpyrfluxam. For rice hulls, 37.1% (0.065 mg eq./kg) of the TRR for [pyrazolyl-4-^{14}C] inpyrfluxam and 39.7% (0.062 mg eq./kg) of the TRR for [phenyl-U-^{14}C] inpyrfluxam.

Solids remaining after solvent extraction (PES) of rice matrices (solids with TRR > 10%) were then sequentially extracted using acidified acetonitrile (0.1 M HCl in acetonitrile), followed by 50 mM ethylene glycol bis-(2-aminoeythl ester)-N,N,N',N'-tetraacetic acid (EGTA), shaken in DMSO for 48 – 72 hours, DMSO for 1 hour at 80°C, strong base (24% KOH) and finally strong acid (72% H_2SO_4). The strong base extracts containing > 10% TRR were partitioned a further three times with ethyl acetate.

The final PES (PES2) was combusted to determine the remaining residual radiocarbon levels. No radioactive residues remained for immature rice plants and straw. Rice hulls had remaining radioactive levels of 6.9 – 9.6% TRR, maximum 0.015 mg eq./kg. Rice grain had remaining radioactive levels for phenyl labelled inpyrfluxam at 60% TRR (0.009 mg eq./kg).

The applicant postulated that the further work up of the PES characterised the residues as a mixture of pectin, lignin, hemicelluloses and cellulose fractions according to the following applicant proposals: Post extraction solids with TRR >10% were further extracted sequentially with acidified acetonitrile, ethylene glycol bis-(2-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) which (proposed) solubilises plant pectins; dimethyl sulfoxide (DMSO) which (proposed) solubilises plant lignins; potassium hydroxide (KOH) which (proposed) dissolves plant hemicelluloses and sulfuric acid (H_2SO_4) which (proposed) dissolves plant cellulose. HSE notes that no enzymes were used to demonstrate association with plant macromolecules, although these further sequential extractions performed on PES1 (which comprised 36 – 66.7% TRR) enabled the PES to be extensively characterised (in terms of the release of radioactivity following different 'treatments').

The residues in the solids after further extraction (PES2) were determined by combustion for both [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam treated rice. The maximum amount remaining in PES2 was for rice hulls at 9.6% (0.015 mg eq./kg) of the TRR for [phenyl-U-^{14}C] inpyrfluxam. No further extraction of the remaining radioactivity was undertaken. Although grain remaining radioactivity for PES2 was 0.009 mg eq./kg representing a low level of radioactivity, this is noted to represent 60%TRR (phenyl) however these samples were only subjected to the 0.1M HCL treatment and not the follow on more extensive treatments due to the low level of remaining residues involved. The corresponding samples for pyrazolyl label weren't further worked on as the pyrazolyl grain characterisation had stopped at PES1 (containing only 0.004 mg eq./kg representing 44%TRR).

The distribution of the radioactive residues is shown in the following table.

Table 7.2.1.3-3: Distribution of radioactivity in the extracts of rice matrices after one foliar application of [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam

Sample	Immature rice plants		Rice straw	Rice hulls			Rice grain	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
[pyrazolyl-4-^{14}C] inpyrfluxam								
TRR (combustion of solid matrix)	**-**	**3.783**	**-**	**1.548**	**-**	**0.171**	**-**	**0.010**
TRR (extracted and solid residues)	**100**	**3.888**	**100**	**1.582**	**100**	**0.175**	**100**	**0.009**
ACN/H_2O Extract 1	*36.3*	*1.410*	*38.6*	*0.611*	*37.7*	*0.066*	*44.4*	*0.004*
ACN/H_2O Extract 2	*12.0*	*0.465*	*19.1*	*0.302*	*19.4*	*0.034*	*11.1*	*0.001*
ACN Extract 3	*3.0*	*0.118*	*6.3*	*0.100*	*5.7*	*0.010*	-	*<LOQ*
ACN/H_2O combined	51.3	1.993	64.0	1.013	62.9	0.110	55.6	0.005
PES 1[(a)]	48.7	1.895	36.0	0.569	37.1	0.065	44.4	0.004
0.1M HCl in ACN	8.9	0.345	3.9	0.061	4.0	0.007	N/A	N/A
EGTA	4.8	0.185	5.5	0.087	5.1	0.009	N/A	N/A
DMSO 66hr	1.3	0.050	1.0	0.016	1.7	0.003	N/A	N/A
DMSO 1hr 80°C	0.5	0.018	1.0	0.016	1.1	0.002	N/A	N/A

24% KOH overnight	31.1	1.208	19.2	0.303	18.3	0.032	N/A	N/A
Aqueous	*25.5*	*0.992*	*14.5*	*0.230*	*10.9*	*0.019*	*N/A*	*N/A*
Organic	*5.6*	*0.216*	*4.6*	*0.073*	*7.4*	*0.013*	*N/A*	*N/A*
72% H_2SO_4 4hr	2.3	0.089	5.4	0.086	-	-	N/A	N/A
PES 2[(b)]	-	-	-	-	6.9	0.012	N/A	N/A
Accountability[(c)]	**102.8%**		**102.2%**	**102.3%**			**90.0%**	
[phenyl-U-^{14}C] inpyrfluxam								
TRR (extracted and solid residues)	**-**	**1.909**	**-**	**1.095**	**-**	**0.158**	**-**	**0.015**
TRR (combustion of solid matrix)	**100**	**1.887**	**100**	**1.072**	**100**	**0.156**	**100**	**0.015**
ACN/H_2O Extract 1	*38.4*	*0.724*	*36.6*	*0.392*	*-*	*-*	*26.7*	*0.004*
ACN/H_2O Extract 2	*15.5*	*0.292*	*18.7*	*0.200*	*-*	*-*	*6.7*	*0.001*
ACN Extract 3	*4.7*	*0.088*	*7.2*	*0.077*	*-*	*-*	*-*	*<LOQ*
ACN/H_2O combined	58.5	1.104	62.4	0.669	60.3	0.094	33.3	0.005
PES 1[(a)]	41.5	0.783	37.6	0.403	39.7	0.062	66.7	0.010
0.1M HCl in ACN	4.1	0.077	4.3	0.046	5.8	0.009	6.7	0.001
EGTA	11.1	0.210	6.8	0.073	3.9	0.006	N/A	N/A
DMSO 66hr	2.2	0.041	1.2	0.013	1.3	0.002	N/A	N/A

DMSO 1hr 80°C	1.5	0.028	1.4	0.015	1.3	0.002	N/A	N/A
24% KOH overnight	18.8	0.355	19.2	0.206	18.6	0.029	N/A	N/A
Aqueous	*14.2*	*0.267*	*15.4*	*0.165*	*11.5*	*0.018*	*N/A*	*N/A*
Organic	*4.7*	*0.088*	*3.8*	*0.041*	*7.1*	*0.011*	*N/A*	*N/A*
72% H_2SO_4 4hr	3.8	0.071	4.7	0.050	-	-	N/A	N/A
PES 2[(b)]	-	-	-	-	9.6	0.015	60.0	0.009
Accountability[(c)]	**98.8%**		**97.9%**	**98.7%**			**100%**	

[(a)] PES 1 refers to the post extraction solids remaining following the initial extraction with acetonitrile and water (1:1, v/v) twice and once with 100% acetonitrile

[(b)] PES 2 refers to the post extraction solids remaining following further extraction with HCl, EGTA, DMSO, strong base and strong acid

[(c)] Accountability = [(sum of ERR + PES)(/initial TRR)] x 100

Chiral analysis

Where parent inpyrfluxam was detected in solvent fractions, chiral HPLC analyses were performed to determine the ratio of enantiomers of inpyrfluxam. Sample extracts characterised as containing parent compound were partitioned between hexane and water (x2) to selectively isolate the parent compound into the hexane fraction. A combined hexane fraction was analysed by chiral HPLC to resolve inpyrfluxam into early eluting “S” and later eluting “R” enantiomers. All the sample types were analysed and showed that no isomerisation on/in the rice plants (100% R-isomer and no S-isomer).

Identification

Residues in the conventional extracts of rice matrices (combined) were identified by reverse phase HPLC and using co-chromatography with reference standards. The identities of inpyrfluxam, 3’-OH-S-2840, 1’-CH_2OH-S-2840 and trace amounts of DFPA-$CONH_2$ metabolites were also confirmed by TLC analyses. The solvent systems used for TLC were: Chloroform: Methanol (9:1, v/v); Ethyl Acetate: Acetic Acid: Water (35:2:1, v/v/v); and Toluene: Ethyl Acetate: Acetic Acid (5:7:1, v/v/v). Example chromatograms for the HPLC and TLC work showed the chromatographic separation to be of a suitable quality. The following metabolites were identified: 3’-OH-S-2840, 1’-CH_2OH-S-2840, *N*-des-Me-DFPA, Gly-1’-CH_2OH-S-2840 and DFPA-$CONH_2$. Overall, identification rates were between 7 – 67%. The maximum individual

unidentified component represented 0.4% (0.006 mg eq./kg) of the TRR in rice straw. The 'unknowns' are not considered further in this evaluation.

The identification and the distribution of parent and metabolites in rice matrices following treatment by [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam are shown in the following tables.

Table 7.2.1.3-4: Distribution of parent and metabolites in the extracts of rice matrices after one granular application of [pyrazolyl-4-^{14}C] inpyrfluxam

	Immature rice plants		Rice straw		Rice hulls		Rice grain	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Inpyrfluxam	20.3	0.788	2.0	0.032	ND	ND	ND	ND
3'-OH-S-2840	3.6	0.142	0.6	0.009	ND	ND	ND	ND
1'-CH_2OH-S-2840-B	3.5	0.134	10.1	0.159	4.8	0.008	ND	ND
1'-CH_2OH-S-2840-A	3.7	0.145	13.1	0.206	35.3	0.062	4.7	≤0.001
Gly-1'-CH_2OH-S-2840[(a)]	26.0	1.010	31.7	0.498	ND	ND	ND	ND
DFPA-$CONH_2$	2.2	0.086	2.1	0.034	17.5	0.031	1.5	≤0.001
***N*-des-Me-DFPA**	ND	ND	1.6	0.025	5.3	0.009	23.1	0.002
Total identified	**59.3**	**2.305**	**61.2**	**0.963**	**62.9**	**0.11**	**29.3**	**0.004**

ND not detected

[(a)] Undifferentiated peak region consisting of multiple sugar conjugates of 1'-CH_2OH-S-2840

Table 7.2.1.3-5: Distribution of parent and metabolites in the extracts of rice matrices after one foliar application of [phenyl-U-^{14}C] inpyrfluxam

	Immature rice plants		Rice straw		Rice hulls		Rice grain	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Inpyrfluxam	38.4	0.724	2.8	0.031	ND	ND	ND	ND
3'-OH-S-2840	1.2	0.023	0.1	≤0.001	ND	ND	ND	ND

1'-CH_2OH-S-2840-B	3.0	0.056	13.1	0.141	10.0	0.016	ND	ND
1'-CH_2OH-S-2840-A	3.3	0.062	12.6	0.135	43.2	0.068	6.8	≤0.001
Gly-1'-CH_2OH-S-2840[(a)]	16.7	0.315	38.0	0.407	9.4	0.015	ND	ND
Total identified	**62.6**	**1.18**	**66.6**	**0.716**	**62.6**	**0.098**	**6.8**	**≤0.001**

ND not detected

(a) Undifferentiated peak region consisting of multiple sugar conjugates of 1'-CH_2OH-S-2840

The parent substance inpyrfluxam represented the most prominent residues component in immature rice plants for both radiolabels (20 – 38% TRR). The metabolite Gly-1'-CH_2OH-S-2840 was the most prominent residue component in rice straw for both radiolabels (32 – 38% TRR). The metabolite 1'-CH_2OH-S-2840 was the most prominent residue component in rice hulls for both radiolabels (40 – 53% TRR). The metabolite *N*-des-Me-DFPA was the most prominent residue component in rice grain for the pyrazolyl labelled inpyrfluxam (23% TRR) and 1'-CH_2OH-S-2840 was the most prominent for phenyl labelled inpyrfluxam (6.8% TRR). The parent substance inpyrfluxam was not detected in rice grain and hulls for both radiolabels.

Storage stability

Samples were stored frozen for a maximum of 51 days (after harvest) before extraction prior to analysis. All HPLC profiles of conventional extracts of the RACs were performed within 97 days after harvest. This comprised most of the analytical work determining levels of metabolites in the solvent extracted material.

Initial chromatographic profiling of the [pyrazolyl-4-^{14}C] inpyrfluxam residue components from immature rice was performed 61 days after harvest. Enzyme (beta-glucosidase) hydrolysis of this extract performed 307 days after the initial analysis showed a similar magnitude of inpyrfluxam to the 61 day extract, demonstrating stability of inpyrfluxam when stored frozen for up to 307 days. These comparative chromatograms also indicated stability of DFPA-$CONH_2$ and partial stability (3.6%TRR declined to 1.9%TRR) of 3'-OH-S-2480.

Samples of immature rice were re-extracted. Comparison of chromatograms showed broad similarity of peaks inpyrfluxam, 3-OH-S-2840, 1′-CH_2OH-S-2840 and Gly-1'-CH_2OH-S-2840 after 429 days (pyrazolyl) and 196 days (phenyl) of frozen storage

Additional stability analysis (comparative chromatograms) was performed on straw, hulls and grain extracts for both radiolabels. Samples were reanalysed 555 days

(straw), 517 days (hulls), and 428 days (grain) of frozen storage. In these samples there was broad similarity of peaks (inpyrfluxam, 1′-CH_2OH-S-2840, Gly-1'-CH_2OH-S-2840, *N*-des-Me-DFPA and DFPA).

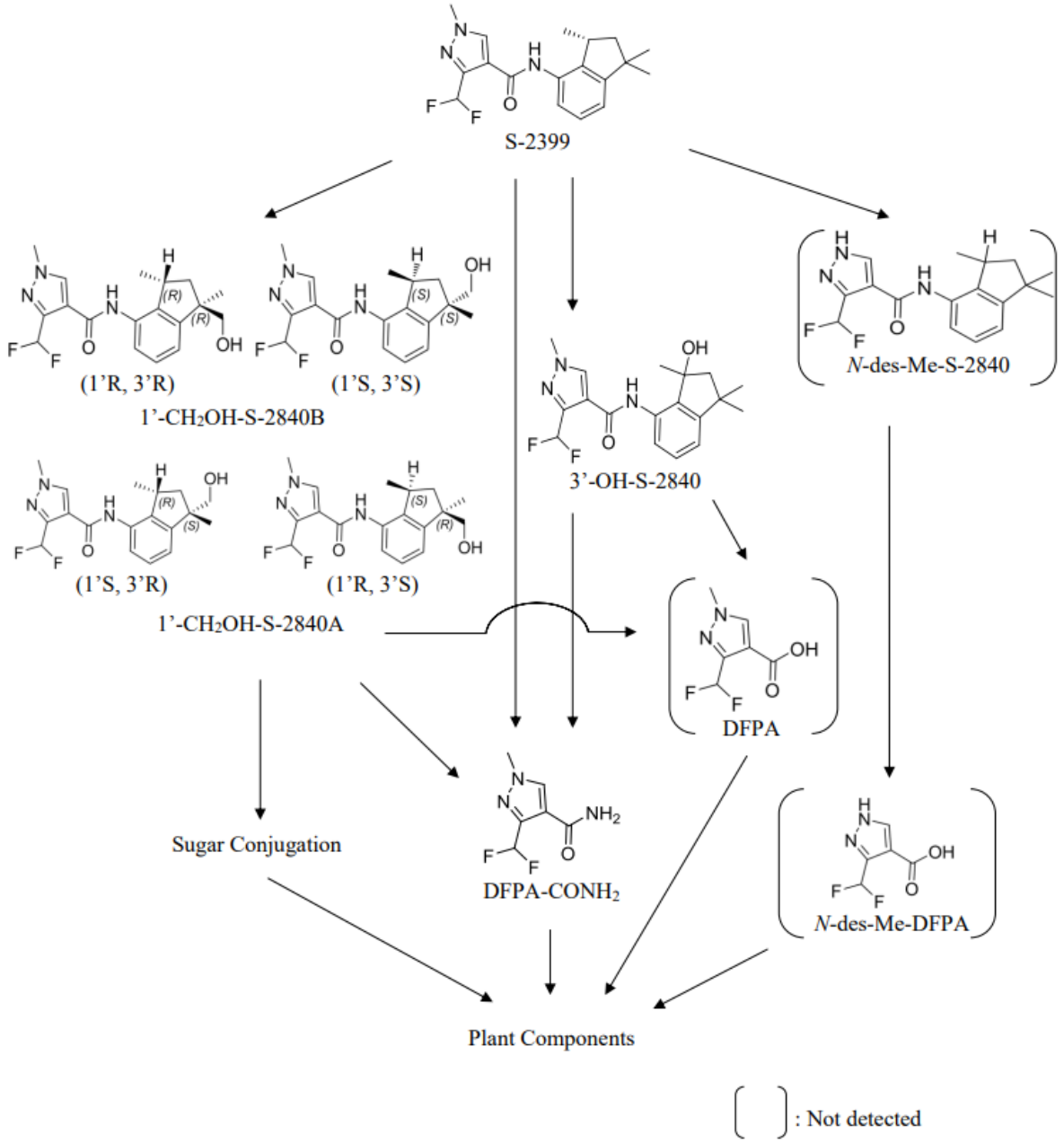
This storage stability work confirms the validity of the study.

Summary of inpyrfluxam metabolism in rice (granular treatment)

Inpyrfluxam was found to be extensively metabolised in rice after one granular application. Metabolism proceeds via oxidation of inpyrfluxam forming hydroxylation metabolites 3'-OH-S-2840 and 1'-CH_2OH-S-2840 (the latter thought to be forming sugar conjugates). An additional pathway was the cleavage of the amide bond in inpyrfluxam to form DFPA-$CONH_2$ which can undergo further transformation by *N*-demethylation on the pyrazolyl ring to form *N*-des-Me-S-2840. In rice, 1'-CH_2OH-S-2840 undergoes phase II transformation pathways forming glycoside conjugates. The applicant has proposed that radioactivity might have also become associated with plant constituents such as pectin, lignin, hemicellulose and cellulose.

Based on these results, the metabolic pathway of inpyrfluxam in rice is adequately understood and is proposed in the figure below:

Figure 7.2.1.3-1: Proposed metabolic pathway of inpyrfluxam in rice following granular application



B.7.2.1.4 Canola/Oilseed rape (seed treatment)

Table 7.2.1.4-1: Overview of canola/oilseed rape metabolism studies

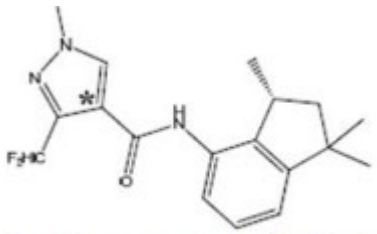
Plant	Application	Application rate	Reference
Canola/oilseed rape	One seed treatment, pyrazolyl-labelled inpyrfluxam & phenyl-labelled inpyrfluxam	1 x ~5.0 g a.s./100 kg of seeds	KCA 6.2.1/06 TPM-0031

Report:	**KCA 6.2.1/06; [redacted] and [redacted]**
Title:	Residues in Canola/oilseed rape Crops Grown from Seeds Treated with [Phenyl-^{14}C]S-2399 and [Pyrazolyl-^{14}C]S-2399
Document No.:	TPM-0031 (Study No.: VP-38998)
Guidelines:	US EPA Residue Chemistry Guideline 860.1300 "Nature of the Residue"
Guideline deviations:	None
GLP/GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

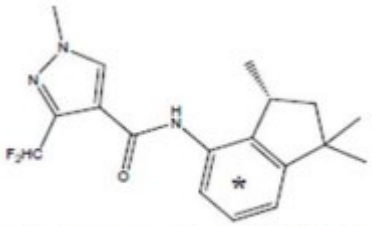
The metabolism of [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam was investigated in canola/oilseed rape after one seed treatment application formulated as an FS (flowable concentrate) containing radiolabel test substance and formulation blank. The application rates achieved were 5.13 g a.s./100 kg of seeds (pyrazolyl) and 4.68 g a.s./100 kg of seeds (phenyl). The actual treatment rates were determined by LSC analysis of acetonitrile rinsing (x2) following on from the treatment of seeds. The details of the radiolabel test materials are presented below.

[Pyrazolyl-4- ^{14}C] inpyrfluxam

Chemical name [Pyrazolyl-4- ^{14}C] inpyrfluxam

Chemical structure	 * Indicates position of ^{14}C label.
Radiolabel position	[pyrazolyl-4- ^{14}C]
Specific radioactivity	2.11 GBq/mmol
Radiochemical purity	95.4%

[Phenyl-U- ^{14}C] inpyrfluxam

Chemical name	[Phenyl-U- ^{14}C] inpyrfluxam
Chemical structure	 * Indicates position of ^{14}C label.
Radiolabel position	[phenyl-U- ^{14}C]
Specific radioactivity	4.51 GBq/mmol
Radiochemical purity	97.7% (before and after application)

Conducted in the USA (California) in 2015/2016, the treated canola/oilseed rape seeds variety “Star” were planted into sandy loam soil in plastic shielded outdoor boxes comprising one control plot and two treated plots (one for each radiolabel). Each treated plot was approximately 3 ft x 5 ft and filled with soil to within approximately 6 inches from the top. Each box was planted with 212 or 217 seeds. During the growing period, crops were hand irrigated and fertilised as required.

Canola/oilseed rape pods containing mature canola/oilseed rape seeds were harvested at BBCH 97 – 99. Seeds were carefully removed from the pod, the pods discarded and seed samples were transferred to the analytical laboratory and placed in frozen storage.

The total radioactive residues (TRR) of triplicate samples of seed (per radiolabel) were determined by combustion of solid matrix/LSC of all samples. Due to the very low radioactivity in each of the samples no extraction using solvents and no further characterisation (or identification) of residues was performed.

All samples were analysed within 2 months of harvest after frozen storage, and therefore storage stability was assumed.

The total radioactive residues (TRR) in the seeds were of the [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam were <0.005 mg eq./kg for each radiolabel.

B.7.2.1.5 Maize/corn and sorghum crops (seed treatment)

Table 7.2.1.5-1: Overview of maize/corn and sorghum metabolism studies

Plant	Application	Application rate	Reference
Maize/corn	One seed treatment, pyrazolyl-labelled inpyrfluxam & phenyl-labelled inpyrfluxam	1 x ~0.018 mg a.s./seed (= 6.6 g a.s./100 kg seed)	KCA 6.2.1/03 TPM-0017
Sorghum		1 x ~6.3 g a.s./100 kg seed	

Report:	**KCA 6.2.1/03; [redacted] and [redacted] 2016**
Title:	Residues in Maize/corn and Sorghum Crops Grown from Seeds Treated with [phenyl-^{14}C]S-2399 and [pyrazolyl-^{14}C]S-2399, Study No. VP-38699, MRID 49706047
Document No.:	TPM-0017 (Study No.: VP-38699)
Guidelines:	US EPA Residue Chemistry Guideline 860. 1300 “Nature of the Residue”
Guideline deviations:	None
GLP/GEP:	Yes

Acceptability:	Yes
Study relied upon:	Yes

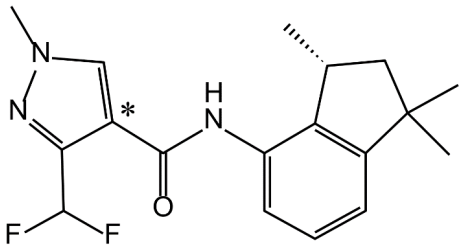
The metabolism of [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam was investigated in maize/corn and sorghum after one seed treatment application. Maize (corn) seeds were uniformly treated with formulation blank (an FS formulation) and then the seeds were spiked with the radiolabelled test solution. Sorghum seeds were seed treated with a mixture of the radiolabelled dosing solution and formulation blank (FS formulation).

The application rates achieved were 0.018 – 0.019 mg a.s./seed (maize) [or 6.6 g a.s./100 kg seed] and 6.2 – 6.3 g a.s./100 kg of seeds (sorghum). The actual treatment rates were determined by LSC analysis of acetonitrile rinsing (x2) following on from the treatment of seeds. The details of the radiolabel test materials are presented below.

[Pyrazolyl-4- ^{14}C] inpyrfluxam

Chemical name [Pyrazolyl-4- ^{14}C] inpyrfluxam

Chemical structure



* Labelled at position 4 in the pyrazolyl ring

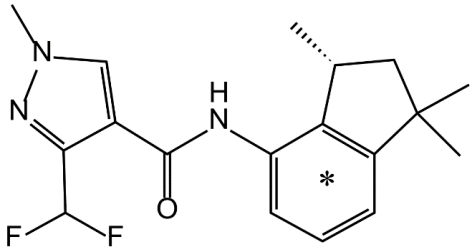
Radiolabel position [pyrazolyl-4- ^{14}C]

Specific radioactivity 2.11 GBq/mmol

Radiochemical purity 97.8%

[Phenyl-U- ^{14}C] inpyrfluxam

Chemical name [Phenyl-U- ^{14}C] inpyrfluxam

Chemical structure	 * Uniformly labelled in the phenyl ring
Radiolabel position	[phenyl- U-^{14}C]
Specific radioactivity	4.51 GBq/mmol
Radiochemical purity	99.0%

Conducted in the USA (California) in 2014, the treated Maize/corn, variety “TA 304-02 ND” and sorghum, variety “GA 3545” were planted into loamy sand soil in plastic shielded outdoor boxes comprising one control plot and two treated plots (one for each radiolabel). Each treated plot was approximately 3 ft x 5 ft and filled with soil to within approximately 6 inches from the top. Each box was planted with 12 seeds per plot for maize (corn) and 56 seeds per plot for sorghum. During the growing period, crops were hand irrigated and fertilised as required.

For maize/corn, the first harvest took place at the late dough/early dent stage and collected maize/corn forage. The second harvest took place at the milk/succulent stage and collected kernels plus cob with husks removed (also referred to as ‘kernels + cob’) to mimic the RAC of sweet maize/corn. The remaining maize/corn plant was harvested at maturity, when the grain was separated from the cob. The mature grain free cobs were added to the stalks and processed to maize/corn stover.

For sorghum, the first harvest took place at the soft dough to hard dough stage and collected sorghum forage. The remining sorghum plants were harvested at maturity and separated into sorghum grain and sorghum stover.

All samples were transferred to the analytical laboratory on dry ice and processed on receipt with dry ice using a blender. Homogenised samples were placed in the freezer.

The total radioactive residues (TRR) of triplicate samples of maize/corn (forage, ‘kernels + cob’, stover and grain) and sorghum (forage, grain and stover) were determined by combustion of solid matrix/LSC of all samples. Due to the very low radioactivity in each of the samples no extraction using solvents and no further characterisation (or identification) of residues was performed.

All samples were analysed within 4 months of harvest after frozen storage, and therefore storage stability was assumed.

The total radioactive residues (TRR) in all plant samples of the [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam were <0.005 mg eq./kg for each radiolabel.

B.7.2.1.6 Potato (seed treatment)

Table 7.2.1.6-1: Overview of potato metabolism studies

Plant	Application	Target application rate	Reference
Potato	One seed treatment, pyrazolyl-labelled inpyrfluxam & phenyl-labelled inpyrfluxam	1 x ~5.0 g a.s./100 kg of seed	KCA 6.2.1/07 TPM-0042

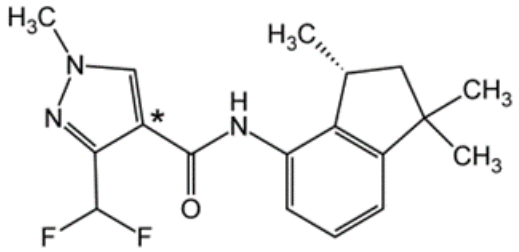
Report:	**KCA 6.2.1/07; [redacted] 2017**
Title:	Nature of Residues of [Phenyl-^{14}C]S-2399 and [Pyrazolyl-^{14}C]S-2399 in Potatoes Grown from Treated Seeds
Document No.:	TPM-0042 (Study No.: VP-38692)
Guidelines:	860.1300
Guideline deviations:	None
GLP/GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

The metabolism of [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam was investigated in potato after one seed treatment application. Potato pieces containing some sprouting ‘eyes’ were cut into uniform pieces (35 g) and were uniformly treated with formulation blank (an FS formulation) and then the seed potato pieces were spiked with the radiolabelled test solution.

The application rates achieved were 4.9 – 5.0 g a.s./100 kg of seed potato. The actual treatment rates were determined on treated potatoes (prior to planting) by LSC analysis of acetonitrile rinsing, acetonitrile extract of the treated potatoes and combustion analysis of the post-extraction solids following on from the treatment of seeds. The LSC levels form each of these results was combined to determine the treatment rates. The details of the radiolabel test materials are presented below.

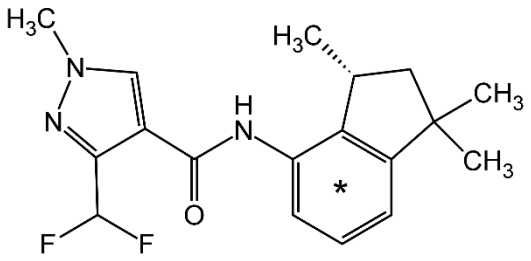
As well as a control, an unlabelled higher rate (25 g a.s./100 kg of seed potato) was also treated. This sample was designated as a surrogate plot to aid metabolite identification, however the results presented by the applicant (and therefore presented in this evaluation) only used the radiolabel treated samples. These additional surrogate samples are not mentioned further in the assessment.

[Pyrazolyl-4- ^{14}C] inpyrfluxam

Chemical name	[Pyrazolyl-4- ^{14}C] inpyrfluxam
Chemical structure	 Radiolabelled Position (*): Labelled at the 4-position in the pyrazolyl ring.
Radiolabel position	[pyrazolyl-4- ^{14}C]
Specific radioactivity	2.11 GBq/mmol (before isotopic dilution)
Radiochemical purity	97.9%

[Phenyl-U- ^{14}C] inpyrfluxam

Chemical name	[Phenyl-U- ^{14}C] inpyrfluxam

Chemical structure	 Radiolabelled Position (*): Uniformly labelled in the phenyl ring.
Specific radioactivity	4.51 GBq/mmol (before isotopic dilution)
Radiochemical purity	98.7%
Chemical purity	96.0%

Conducted in the USA (California) in 2014, the treated seed potato pieces, variety “Red La Soda” were planted into loamy sand soil in plastic shielded outdoor boxes comprising one control plot and three treated plots (one for each radiolabel, and one for the exaggerated (25 g a.s./100 kg) treatment using non-radiolabelled inpyrfluxam). Each treated plot was approximately 3 ft x 5 ft and filled with soil to within approximately two inches from the top. Each box was planted with 10 potato seeds, in a single row with 4 inch spacing. During the growing period, crops were hand irrigated and fertilised as required and were observed to have 100% germination.

Potato foliage samples were collected at growth stage of BBCH 48 (80% of the expected tuber diameter reached and 70 days after planting) by cutting leaf material from within the plots.

Potato tubers samples were taken at maturity at growth stage of BBCH 49 (skin set complete in 95% of tubers and 83 days after planting). Any adhering soil was carefully removed using paper towels.

All samples were transferred after harvest to the analytical laboratory on dry ice and processed with dry ice using a blender. All samples (either samples or extracts) were analysed within 6 – 7 months of harvest (virtually all work completed within 6 months). All samples and extracts were kept in frozen storage during the course of the study. Storage stability was also assessed by re-extracting potato tubers at the end of the of the study, after about 6.5 months from the initial extraction. Overall recoveries of total residue were similar in both extractions (before and after storage) for the potato tubers. The HPLC analysis profiles of the pre- and post-storage

sample extracts were compared and showed similar HPLC profiles, indicating that samples were stable over the course of the study, validating the results presented in this study.

The total radioactive residues (TRR) determined by combustion are presented in Table 7.2.1.6-2. Having determined the amount applied to the plots, the applicant determined that the % of the AR applied (radioactivity) recovered in the tubers represented 0.32% AR (phenyl plot) and 1.39% (pyrazolyl plot).

Table 7.2.1.6-2 Total radioactive residues (mg eq./kg) determined by combustion

	Tuber TRR (mg eq./kg)	Foliage TRR (mg eq./kg)
[pyrazolyl-4-^{14}C] inpyrfluxam	0.040	0.385
[phenyl-U-^{14}C] inpyrfluxam	0.012	0.151

Samples of potato tuber were extracted with acetone (x2) and the extracts were separated from the solids by centrifugation. The extract was designated as the 'acetone extract'. The remaining residue was further extracted twice by acetone and water (60:40, v/v) leaving the post-extractions solids (PES). The supernatants from these extractions were combined as designated as the 'aqueous extract'.

The 'acetone extracts' contained residues > 0.01 mg eq./kg and were further analysed to determine the nature of residues. Residues in the aqueous extracts of tubers were very low for both labels (0.001 mg eq./kg for the phenyl label and 0.003 mg eq./kg for the pyrazolyl label) therefore no further analysis of these was carried out.

The remaining PES was analysed by combustion and LSC. The unextracted residues in the PES were very low for both radiolabels (0.001 mg eq./kg and 0.003 mg eq./kg for [phenyl-U-^{14}C] inpyrfluxam and [pyrazolyl-4-^{14}C] inpyrfluxam, respectively), therefore no further analysis was carried out on these samples.

A subsample of potato tuber was rinsed with acetone to determine the surface radioactivity. Aliquots of the surface rinses were analysed using LSC. Residues in the acetone rinses from potato tubers (surface residue) treated with [pyrazolyl-4-^{14}C] and [phenyl-U-^{14}C] inpyrfluxam were very low (≤0.001 mg eq./kg) so the rinses were not analysed further.

Chiral HPLC analyses were performed to determine the ratio of enantiomers of inpyrfluxam in the treatment solutions. This confirmed R-isomer at >97% and only small amounts of S-isomer. No chiral analysis was performed for the treated samples of potatoes.

For identification of radioactive ingredients in acetone extract from potato tubers, the retention times of co-eluting standards were used and confirmed by 2D TLC.

The acetone extract solutions for both radiolabels were evaporated to dryness and the residue dissolved in acetonitrile and water (50:50, v/v). The mixture was centrifuged and the supernatant separated into an acetonitrile phase and an aqueous phase. Triplicate aliquots of each phase were analysed by LSC. Both fractions were further analysed by HPLC and 2D TLC.

For both radiolabels, components were present in both fractions of the acetone extract that did not match standards in either HPLC or TLC analysis. The applicant noted that the residues were mostly polar and showed poor separation in the HPLC method. 2D TLC analysis of the extract fractions showed better separation of the radio-residues and was used to determine the final percentage distribution of the metabolites in the extracts (by radioactive software).

To further determine the identity of the proposed polar and possibly conjugated residues, the extract fractions underwent acid hydrolysis (2 M HCl at 100°C for 2 hrs). Hydrolysates were partitioned with ethyl acetate were analysed by HPLC and 2D TLC. There was poor resolution on 2D TLC which the applicant considered was due to interference from matrix.

To identify the composition of metabolites in the hydrolysates, an aliquot from pyrazolyl label was subjected to preparative HPLC and individual metabolites were separated by fraction collection. This hydrolysate fraction contained all the metabolites observed in the phenyl label extract in addition to the metabolites specific to pyrazolyl label. The separated HPLC peaks were collected and further analysed by 2D TLC. The separated metabolite fractions were identified based on the retention time comparison with the standards in the HPLC method, and co-migration with standards in the 2D TLC method.

Overall, the following metabolites were identified: 3'-OH-S-2840 (free and conjugated), 1'-CH_2OH-S-2840 (free and conjugated), 1'-COOH-S-2840 (free and conjugated), DFPA (free and conjugated) and *N*-des-Me-DFPA (free and conjugated). Free residues of metabolites were those present in the extract before hydrolysis and those designated as conjugated residues were those released after acid hydrolysis.

The TRR values of the potato tubers determined in two different ways are compared in Table 7.2.1.6-3. One method is from the combustion analysis of the sample prior to extraction and the other approach considers the sum of the extractable radioactivity and that in the PES (TRR of sum of ERR and PES).

Table 7.2.1.6-3: TRR in potato tuber after seed treatment of [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam

Matrix	Timing and Application	BBCH growth stage	Sum of total radioactive residues TRR (mg eq./kg)	
			TRR based on combustion of solid matrix/LSC	TRR based on sum of extracted and post-extraction solid residues [a]
[pyrazolyl-4-^{14}C] inpyrfluxam				
Mature potato tubers	1 seed treatment: 1 x ~5.0 g a.s./100 kg of seed	BBCH 49	0.040	0.041
[phenyl-U-^{14}C] inpyrfluxam				
Mature potato tubers	1 seed treatment: 1 x ~5.0 g a.s./100 kg of seed	BBCH 49	0.012	0.012

[a] The extraction TRR value was obtained by summation of the residues in acetone extract (including surface residue), aqueous extract and post-extraction solid (PES) fraction

By comparing the TRRs determined by the two methods we can note that accountability for the tuber samples analysed, as presented in Table 7.2.1.6-4 below is good: 103% (both labels).

The recovery by solvent extraction indicates that extractability was fairly high. The distribution of the radioactive residues is shown in the following Table 7.2.1.6-4.

Most of the radioactivity was recovered in the ‘acetone extract’ 85.5% TRR for pyrazolyl label and 80.3% TRR for phenyl label. The ‘aqueous extract’ comprised 7.9% (pyrazolyl) and 7.5% (phenyl).

Table 7.2.1.6-4: Distribution of radioactivity in the extracts of potato tubers after one seed treatment of [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam

Sample	Mature potato tubers	
	% TRR	µg eq./kg
[pyrazolyl-4-^{14}C] inpyrfluxam		
TRR (combustion of solid matrix)	**-**	**0.040**
TRR (extracted and solid residues)	**100%**	**0.041**
Acetone extract	85.5%	0.035
Aqueous extract	7.9%	0.003
Total extracted	**93.4%**	0.039
PES	6.6%	0.003
Accountability[(a)]	**102.6%**	
[phenyl-U-^{14}C] inpyrfluxam		
TRR (extracted and solid residues)	**-**	**0.012**
TRR (combustion of solid matrix)	**100%**	**0.012**
Acetone extract	80.3%	0.008
Aqueous extract	7.5%	0.001
Total extracted	**87.9%**	0.011
PES	12.1%	0.001
Accountability[(a)]	**103%**	

[(a)] Accountability = [(sum of ERR + PES)(/initial TRR)] x 100

Identification

[pyrazolyl-4-^{14}C] inpyrfluxam label

Overall, identification rates were sufficient taking account of the low levels of radioactivity involved. For [pyrazolyl-4-^{14}C] Inpyrfluxam treated potatoes, 52.7% of the TRR (0.021 mg eq./kg) was identified. The maximum individual unidentified component represented 7.8% (0.003 mg eq./kg) of the TRR.

Free residues of metabolites were those present in the extract before hydrolysis and those designated as conjugated residues were those released after acid hydrolysis.

The metabolite 1'-COOH-S-2840 (conjugated) was the major component representing 18.5% of the TRR (0.008 mg eq./kg). A further 3.7% was as the free 1'-COOH-S-2840. The parent inpyrfluxam represented 5.8% of the TRR (0.002 mg eq./kg). The metabolites 3'-OH-S-2840 (free), 1'-CH_2OH-S2840 (free), 1'-CH_2OH-S2840 (conjugated), 1'-COOH-S-2840 (free), 1'-COOH-S-2840 (conjugated), DFPA (free), DFPA (conjugated) *N*-des-Me-DFPA (free) and *N*-des-Me-DFPA (conjugated) represented 1.6, 0.9, 2.6, 3.7, 18.5, 4.7, 4.5, 10.1 and 0.1% of the TRR corresponding to 0.001, <0.001, 0.001, 0.002, 0.008, 0.002, 0.002, 0.004 and <0.001 mg eq./kg, respectively. The metabolite 3'-OH-S-2840 (conjugate) was not detected.

The TRR and the distribution of parent and metabolite in potato tuber following treatment by [pyrazolyl-4-^{14}C] inpyrfluxam are shown in the following table.

Table 7.2.1.6-5: Distribution of parent and metabolites in the extracts of potato tubers after one seed treatment of [pyrazolyl-4-^{14}C] inpyrfluxam

	Mature potato tuber	
	% TRR	mg eq./kg
Inpyrfluxam	5.8%	0.002
3'-OH-S-2840 (free)	*1.6%*	*0.001*
3'-OH-S-2840 (conjugated)	*ND*	*ND*
3'-OH-S-2840 (total)	1.6%	0.001
1'-CH_2OH-S2840 (free)	*0.9%*	*<0.001*
1'-CH_2OH-S2840 (conjugated)	*2.6%*	*0.001*
1'-CH_2OH-S2840 (total)	3.4%	0.001
1'-COOH-S-2840 (free)	*3.7%*	*0.002*
1'-COOH-S-2840 (conjugated)	*18.5%*	*0.008*
1'-COOH-S-2840 (total)	22.3%	0.009
DFPA (free)	*4.7%*	*0.002*
DFPA (conjugated)	*4.5%*	*0.002*
DFPA (total)	9.2%	0.004
N-des-Me-DFPA (free)	*10.1%*	*0.004*

N-des-Me-DFPA (conjugated)	*0.1%*	*<0.001*
***N*-des-Me-DFPA (total)**	10.2%	0.004
Total identified	**52.7%**	**0.021**
Total characterised/unidentified[(a)]:	**40.8%**	**0.017**
PES	**6.6%**	**0.003**
Accountability:	**100%**	

ND: not detected

[(a)] Number of unidentified compounds was >8, largest component 7.8% of TRR (0.003 mg eq./kg)

[phenyl-U-^{14}C] inpyrfluxam label

Overall, identification rates were sufficient taking account of the low levels of radioactivity involved. In [phenyl-4-^{14}C] inpyrfluxam treated potatoes, 38.2% of the TRR (0.005 mg eq./kg) was identified. The maximum individual unidentified component represented 4.9% of the TRR (0.001 mg eq./kg).

Free residues of metabolites were those present in the extract before hydrolysis and those designated as conjugated residues were those released after acid hydrolysis.

For [phenyl-U-^{14}C] inpyrfluxam treated potato, 38.2% of the TRR (0.005 mg eq./kg) was identified in total. The parent compound was the major component representing 15.0% of the TRR (0.002 mg eq./kg). The metabolites 3'-OH-S-2840 (free), 3'-OH-S-2840 (conjugated), 1'-CH_2OH-S2840 (free), 1'-CH_2OH-S2840 (conjugated), 1'-COOH-S-2840 (free) and 1'-COOH-S-2840 (conjugated), represented 3.6, 2.4, 1.8, 1.0, 5.3 and 9.2% of the TRR corresponding to <0.001, <0.001, <0.001, <0.001, 0.001 and 0.001 mg eq./kg, respectively. The metabolites DFPA (free), DFPA (conjugated), *N*-des-Me-DFPA (free) and *N*-des-Me-DFPA (conjugated) were not detected.

The TRR and the distribution of parent and metabolite in potato tuber following treatment by [phenyl-U-^{14}C] inpyrfluxam are shown in the following table.

Table 7.2.1.6-6: Distribution of parent and metabolites in the extracts of potato tuber after one seed treatment of [phenyl-U-^{14}C] inpyrfluxam

	Mature potato tubers	
	% TRR	mg eq./kg
Inpyrfluxam	15.0%	0.002

3'-OH-S-2840 (free)	*3.6%*	*<0.001*
3'-OH-S-2840 (conjugated)	*2.4%*	*<0.001*
3'-OH-S-2840 (total)	6.0%	0.001
1'-CH_2OH-S2840 (free)	*1.8%*	*<0.001*
1'-CH_2OH-S2840 (conjugated)	*1.0%*	*<0.001*
1'-CH_2OH-S2840 (total)	2.7%	<0.001
1'-COOH-S-2840 (free)	*5.3%*	*0.001*
1'-COOH-S-2840 (conjugated)	*9.2%*	*0.001*
1'-COOH-S-2840 (total)	14.5%	0.002
DFPA (free)	*NA*	*NA*
DFPA (conjugated)	*NA*	*NA*
DFPA (total)	NA	NA
N-des-Me-DFPA (free)	*NA*	*NA*
N-des-Me-DFPA (conjugated)	*NA*	*NA*
***N*-des-Me-DFPA (total)**	NA	NA
Total identified	**38.2%**	**0.005**
Total characterised/unidentified[(a)]:	**49.7%**	**0.006**
PES:	**12.1%**	**0.001**
Accountability:	**100%**	

NA: not applicable

[(a)] Number of unidentified compounds was >6, largest component 4.9% of TRR (0.001 mg eq./kg). PES and aqueous extracts not analysed further due to very low residue levels.

Summary of inpyrfluxam metabolism in potato (seed treatment)

The metabolism of inpyrfluxam in potato was investigated after one seed treatment.

The extraction rates from solvent extraction were 87.9% and 93.4% for [phenyl-U-^{14}C] and [pyrazolyl-4-^{14}C] inpyrfluxam treated potatoes, respectively. Overall, identification rates in the potato tuber varied. Identification rate was lowest for

[phenyl-U-^{14}C] inpyrfluxam treated potato, with 38.2% of the TRR identified. In [pyrazolyl-4-^{14}C] inpyrfluxam treated potato, 52.5% of the TRR was identified. The metabolite 1'-COOH-S-2840 (conjugated) was the main residue component in potato tubers following seed treatment with [pyrazolyl-4-^{14}C] inpyrfluxam (18.5% of the TRR, 0.008 mg eq./kg). The parent compound represented 5.8% of the TRR (0.002 mg eq./kg). For [phenyl-U-^{14}C] inpyrfluxam treated potato, the parent compound was the major component at 15.0% of the TRR, corresponding to 0.002 mg eq./kg.

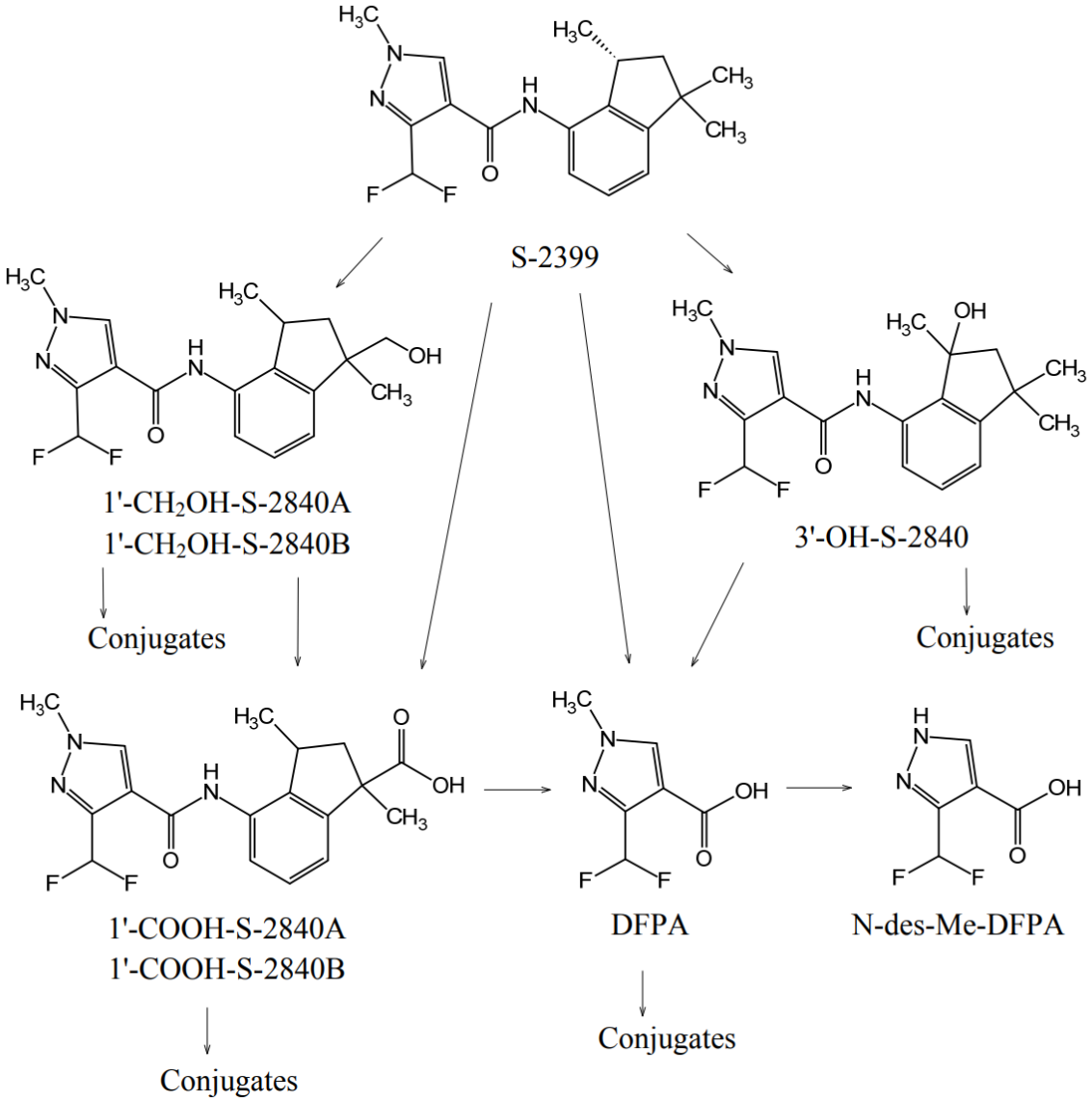
The metabolites DFPA (free and conjugated) and *N*-des-Me-DFPA (free and conjugated) were found to be present in potato tubers treated with [pyrazolyl-4-^{14}C] inpyrfluxam but not [phenyl-U-^{14}C] inpyrfluxam.

Inpyrfluxam was found to be extensively metabolised in potato after one application. The main metabolic reactions are listed below:

- Oxidation of inpyrfluxam forming hydroxylation products 3'-OH-S-2840, 1'-CH_2OH-S-2840 and 1'-COOH-S-2840.
- Cleavage of the amide bond and subsequent loss of indenyl moiety of inpyrfluxam, 3'-OH-S-2840, 1'-CH_2OH-S-2840 or 1'-COOH-S-2840 forming DFPA
- DFPA can undergo further transformation by *N*-methylation on the pyrazolyl ring forming *N*-des-Me-S-2840.

Based on these results, the total radioactive residues identified and characterised are in accordance with OECD 501. The metabolic pathway of inpyrfluxam in potato following seed treatment is adequately understood and is proposed in the figure below:

Figure 7.2.1.6-1: Proposed metabolic pathway of inpyrfluxam in potato following seed treatment



B.7.2.1.7 Soyabean (foliar treatment)

Table 7.2.1.7-1: Overview of soyabean metabolism studies

Plant	Application	Target application rate	BBCH at application	Days prior to final harvest	Reference
Soyabean	Two foliar spray applications,	2 x ~110 g a.s./ha	60 & 75	53 & 89	KCA 6.2.1/02

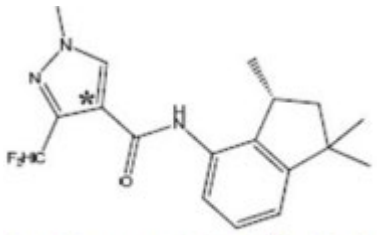
	pyrazolyl-labelled inpyrfluxam & phenyl-labelled inpyrfluxam				TPM-0015

Report:	**KCA 6.2.1/02; [redacted] and [redacted] 2017**
Title:	A Metabolism Study of [^{14}C]S-2399 (2 Radiolabels) in Soyabean (*Glycine Max*)
Document No.:	TPM-0015 (Study No.: 2506W)
Guidelines:	US EPA OPPTS 860.1300 OECD/OCDE Guideline 501 EU Guideline 7028/VI/95 JMAFF 12-Nohsan No. 8147
Guideline deviations:	None
GLP/GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

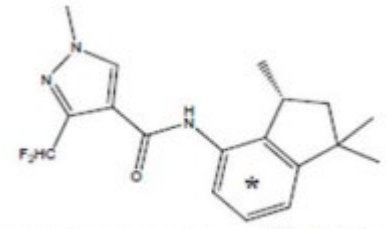
The metabolism of inpyrfluxam was investigated in soyabean after two foliar spray applications performed 89 and 53 days before final harvest. The applied [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam was formulated as a 40% SC spray formulation. For [pyrazolyl-4-^{14}C] inpyrfluxam, the achieved application rates using a manually operated trigger pump sprayer were 108 and 113 g a.s./ha for the first and second application, respectively, resulting in a total application rate of 221 g a.s./ha. For [phenyl-U-^{14}C] inpyrfluxam, the achieved application rates using a manually operated trigger pump sprayer were 107 and 111 g a.s./ha for the first and second application, respectively, resulting in a total application rate of 218 g a.s./ha.

The test material used to make the formulated spray were:

[Pyrazolyl-4- ^{14}C] inpyrfluxam

Chemical name	[Pyrazolyl-4-^{14}C] inpyrfluxam
Chemical structure	 * Indicates position of ^{14}C label.
Radiolabel position	[pyrazolyl-4-^{14}C]
Specific radioactivity	2.22 GBq/mmol
Radiochemical purity	≥95.4% (determination pre and post application)
Chemical purity	95.8% (technical grade, purity of the [pyrazolyl-4-^{14}C] inpyrfluxam was acceptable as received)

[Phenyl-U- ^{14}C] inpyrfluxam

Chemical name	[Phenyl-U-^{14}C] inpyrfluxam
Chemical structure	 * Indicates position of ^{14}C label.
Radiolabel position	[phenyl-U-^{14}C]
Specific radioactivity	4.48 GBq/mmol
Radiochemical purity	≥95.1%(determination pre and post application)
Chemical purity	95.8%(98.8% purified [phenyl-U-^{14}C] inpyrfluxam test substance)

Conducted in the USA (California) in 2013, the experiment was conducted with soyabean plants (variety “Mycogen 5N451R2”) grown from seed in test plots of a

sandy loam soil. Test plots consisted of outdoor boxes (lined with a heavy gauge plastic liner) filled with soil and included one control plot and two treated plots (one for each radiolabel). Each treated plot was 1 m^2 in area with the soil approximately 30 cm deep. The applications were made at BBCH 60 and 75 for each plot, with plastic barriers in place at just the time of application to prevent wind transfer of spray. The actual amount of radiolabelled active substance applied was calculated based on the radioactivity added to the individual dosing bottles minus any residual radioactivity remaining after application.

Soyabean forage was harvested 20 days after the first application (16 days before the second application) (BBCH 65) by cutting plants 2.5 cm above the soil line. Soyabean hay was harvested 33 days after the first application (3 days before the second application) (BBCH 75) by cutting fresh plants 2.5 cm above the soil line and placing on racks to dry. The hay was sampled following 4 days of drying. Immature soyabean (edamame) pods and seeds were harvested 11 days after the second application (47 days after the first application) (BBCH 77) by collecting green pods and opening along the seam to collect the seeds. Mature soyabeans were harvested 53 days after the second application (89 days after the first application) (BBCH 89) by removing pods from the standing field-dried plants and opening along the seam to collect the seeds. Samples were placed in the freezer prior to transfer to the analytical laboratory on dry ice, where the specimens were processed with dry ice and then stored deep frozen for a maximum of around 90 days before extraction (1 – 12 days) prior to analysis.

The total radioactive residues (TRR) of each matrix were determined by combustion of solid matrix and LSC. The results are presented in Table 7.2.1.7-2.

Table 7.2.1.7-2: TRR in soyabean matrices after foliar application of [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam

Matrix	Timing and Application	BBCH growth stage	TRR (mg eq./kg)[(a)]	TRR (mg eq./kg)[(b)]
[pyrazolyl-4-^{14}C] inpyrfluxam				
Soyabean forage	2 spray applications: 2 x ~110 g a.s./ha	BBCH 60 & 75	1.652	1.391
Soyabean hay			2.088	2.378
Soyabean edamame seed			0.120	0.109
Soyabean edamame pods			0.715	0.710
Soyabean mature seeds			0.210	0.219

Soyabean mature pods (unrinsed)			1.250	NA
Soyabean mature pods (rinse)			0.065	0.065
Soyabean mature pods (post-rinse)			1.120	1.136
Soyabeans mature pods (total (rinse + post-rinse))			1.185	1.201
[phenyl-U-^{14}C] inpyrfluxam				
Soyabean forage	2 spray applications: 2 x 100 g a.s./ha	BBCH 60 & 75	1.878	1.557
Soyabean hay			1.942	2.241
Soyabean edamame seed			0.024	0.022
Soyabean edamame pods			0.703	0.635
Soyabean mature seeds			0.038	0.038
Soyabean mature pods (unrinsed)			0.781	NA
Soyabean mature pods (rinse)			0.055	0.055
Soyabean mature pods (post-rinse)			0.657	0.687
Soyabeans mature pods (total (rinse + post-rinse))			0.712	0.742

(a) TRR values based on combustion of solid matrix/LSC
(b) TRR values calculated from sum of extracted and post-extraction solid residues

The results show that the total radioactive residues (TRRs) [combustion] were similar across the two different radiolabels, except for mature seeds and pods where residues were lower in the phenyl label: soyabean forage was 1.65 – 1.88 mg eq./kg, hay was 1.94 – 2.09 mg eq./kg, immature (edamame) seeds was 0.02 – 0.12 mg eq./kg, immature (edamame) pods was 0.70 – 0.72 mg eq./kg, mature seeds was 0.04 – 0.21 mg eq./kg and mature pods (total (rinse + post-rinse)) was 0.71 – 1.19 mg eq./kg.

Mature soyabean pods were initially rinsed with acetonitrile to solubilise surface residues. For all soyabean sample types, radioactive residues were then extracted twice using acetonitrile and water (1:1, v/v) and then once using 100% acetonitrile,

prior to analysis by LSC. Extracts were combined and stored frozen. A portion of the post-extraction solids (PES) was combusted to determine the remaining radiocarbon levels.

The results for the TRR based on sum of ERR and PES were also quite similar to the results obtained for combustion. The accountability levels ranged from 90 – 115%, aside for soyabean forage, where the accountability was lower at 83%. Accountability% = [(sum of ERR + PES)(/initial TRR)] x 100. Overall, the data demonstrate that aside from some variability that is expected, there were no marked losses in the extraction and work up of the samples.

The distribution of residues and extractability with solvents is presented in Table 7.2.1.7-3. Only 5 – 7% of the residues were removed in the rinse (mature pods rinsed only) The solvent extraction rates were in the range 58 – 96% across all the sample types and both radiolabels. Extraction rates were higher for forage and edamame pods. Extraction rates were lower in hay and mature seeds and pods (especially for the phenyl label).

The remaining solids after solvent extraction (PES1) represented: For forage, 15.3% (0.213 mg eq./kg) of the TRR for [pyrazolyl-4-^{14}C] inpyrfluxam and 15.7% (0.244 mg eq./kg) of the TRR for [phenyl-U-^{14}C] inpyrfluxam. For hay, 36.5% (0.867 mg eq./kg) of the TRR for [pyrazolyl-4-^{14}C] inpyrfluxam and 40.4% (0.906 mg eq./kg) of the TRR for [phenyl-U-^{14}C] inpyrfluxam. For edamame seeds, 3.7% (0.004 mg eq./kg) of the TRR for [pyrazolyl-4-^{14}C] inpyrfluxam and 27.3% (0.006 mg eq./kg) of the TRR for [phenyl-U-^{14}C] inpyrfluxam. For edamame pods, 16.9% (0.120 mg eq./kg) of the TRR for [pyrazolyl-4-^{14}C] inpyrfluxam and 15.3% (0.097 mg eq./kg) of the TRR for [phenyl-U-^{14}C] inpyrfluxam. For mature seeds, 11.0% (0.024 mg eq./kg) of the TRR for [pyrazolyl-4-^{14}C] inpyrfluxam and 42.1% (0.016 mg eq./kg) of the TRR for [phenyl-U-^{14}C] inpyrfluxam. For mature pods, 26.7% (0.321 mg eq./kg) of the TRR for [pyrazolyl-4-^{14}C] inpyrfluxam and 33.8% (0.251 mg eq./kg) of the TRR for [phenyl-U-^{14}C] inpyrfluxam.

Solids remaining after solvent extraction (PES) of soyabean matrices (solids with TRR > 10%) were then sequentially extracted using acidified acetonitrile (0.1 M HCl in acetonitrile), followed by 50 mM ethylene glycol bis-(2- aminoethyl ether)-N,N,N', N'-tetraacetic acid (EGTA), shaken in DMSO for 48 hours, DMSO for 1 hour at 80°C, strong base (24% KOH) and finally strong acid (72% H_2SO_4). The strong base extracts containing > 10% TRR were partitioned a further three times with ethyl acetate.

The final PES (PES2) were combusted to determine the remaining residual radiocarbon levels.

The applicant postulated that the further work up of the PES characterised the residues as a mixture of pectin, lignin, hemicelluloses and cellulose fractions

according to the following applicant proposals: Post extraction solids with TRR >10% were further extracted sequentially with acidified acetonitrile, ethylene glycol bis-(2-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid (EGTA) which (proposed) solubilises plant pectins; dimethyl sulfoxide (DMSO) which (proposed) solubilises plant lignins; potassium hydroxide (KOH) which (proposed) dissolves plant hemicelluloses and sulfuric acid (H_2SO_4) which (proposed) dissolves plant cellulose. HSE notes that no enzymes were used to demonstrate association with these plant macromolecules, although these further sequential extractions performed on PES1 (which comprised 3.7 – 42.1%TRR) enabled the PES to be extensively characterised (in terms of the release of radioactivity following different 'treatments').

No radioactive residues remained for all soyabean matrices except for mature seeds, which had remaining residual radiocarbon levels comprising 4.1 – 36.8% TRR, 0.009 – 0.014 mg eq./kg. The larger degree of radioactivity in the PES2 for mature seeds (phenyl 36.8%) reflects that due to low radioactive residues the seeds were not worked on through all of the above mentioned steps for assessment of the PES1. Please see the distributions in the Table 7.2.1.7-3 for further details.

The distribution of the radioactive residues in shown in the following table.

Table 7.2.1.7-3: Distribution of radioactivity in the extracts of soyabean matrices after two foliar applications of [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam

Sample	Soyabean forage		Soyabean hay		Soyabean edamame seed		Soyabean edamame pods		Soyabean mature seeds		Soyabean mature pods	
	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg
[pyrazolyl-4-^{14}C] inpyrfluxam												
TRR (combustion of solid matrix)	**-**	**1.652**	**-**	**2.088**	**-**	**0.120**	**-**	**0.715**	**-**	**0.210**	**-**	**1.250**
TRR (extracted and solid residues)	**100**	**1.391**	**100**	**2.378**	**100**	**0.109**	**100**	**0.710**	**100**	**0.219**	**100**	**1.201**
ACN/H_2O Extract 1	*56.0*	*0.779*	*29.4*	*0.700*	*87.2*	*0.095*	*59.0*	*0.419*	*55.7*	*0.122*	*35.8*	*0.430*
ACN/H_2O Extract 2	*19.3*	*0.268*	*22.0*	*0.523*	*8.3*	*0.009*	*19.3*	*0.137*	*26.5*	*0.058*	*24.5*	*0.294*
ACN Extract 3	*9.4*	*0.131*	*12.1*	*0.288*	*0.9*	*0.001*	*4.8*	*0.034*	*6.9*	*0.015*	*7.6*	*0.091*
ACN/H_2O combined	84.7	1.178	63.5	1.511	96.3	0.105	83.1	0.590	89.0	0.195	67.9[(d)]	0.815[(d)]
PES 1[(a)]	15.3	0.213	36.5	0.867	3.7	0.004	16.9	0.120	11.0	0.024	26.7	0.321

0.1M HCl in ACN	3.6	0.050	6.6	0.158	N/A	N/A	4.4	0.031	3.7	0.008	5.4	0.065
EGTA	1.7	0.024	4.2	0.099	N/A	N/A	0.9	0.006	2.3	0.005	3.0	0.036
DMSO	1.8	0.025	4.6	0.106	N/A	N/A	1.7	0.012	0.5	0.001	1.4	0.017
24% KOH	5.4	0.075	12.1	0.288	N/A	N/A	6.5	0.046	N/A	N/A	9.3	0.112
Aqueous	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*3.8*	*0.045*
Organic	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*5.6*	*0.067*
72% H_2SO_4	2.7	0.038	9.0	0.213	N/A	N/A	3.7	0.026	N/A	N/A	7.5	0.090
PES 2[(b)]	-	-	-	-	-	-	-	-	4.1	0.009	-	-
Accountability[(c)]	**84.2%**		**113.9%**		**90.8%**		**99.3%**		**104.3%**		**96.1%**	
[phenyl-U-^{14}C] inpyrfluxam												
TRR (combustion of solid matrix)	**-**	**1.878**	**-**	**1.942**	**-**	**0.024**	**-**	**0.703**	**-**	**0.038**	**-**	**0.781**
TRR (extracted and solid residues)	**100%**	**1.557**	**100%**	**2.241**	**100%**	**0.022**	**100%**	**0.635**	**100%**	**0.038**	**100%**	**0.742**
ACN/H_2O Extract 1	*55.8*	*0.869*	*26.0*	*0.583*	*59.1*	*0.013*	*59.7*	*0.379*	*34.2*	*0.013*	*29.7*	*0.220*

ACN/H_2O Extract 2	*19.4*	*0.302*	*21.8*	*0.489*	*9.1*	*0.002*	*19.8*	*0.126*	*18.4*	*0.007*	*21.7*	*0.161*
ACN Extract 3	*9.1*	*0.142*	*11.7*	*0.263*	*4.6*	*0.001*	*5.2*	*0.033*	*5.3*	*0.002*	*7.4*	*0.055*
ACN/H_2O combined	84.3	1.313	59.6	1.335	72.7	0.016	84.7	0.538	57.9	0.022	58.8[(d)]	0.436 [(d)]
PES 1[(a)]	15.7	0.244	40.4	0.906	27.3	0.006	15.3	0.097	42.1	0.016	33.8	0.251
0.1M HCl in ACN	3.9	0.060	8.7	0.194	N/A	N/A	3.5	0.022	5.3	0.002	4.0	0.030
EGTA	1.4	0.022	4.6	0.103	N/A	N/A	2.7	0.017	2.6	0.001	1.8	0.013
DMSO	2.1	0.032	5.4	0.121	N/A	N/A	1.6	0.010	N/A	N/A	2.3	0.017
24% KOH	5.3	0.082	12.8	0.287	N/A	N/A	4.6	0.029	N/A	N/A	14.7	0.109
Aqueous	*N/A*	*N/A*	*4.7*	*0.106*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*9.4*	*0.070*
Organic	*N/A*	*N/A*	*8.1*	*0.181*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*5.3*	*0.039*
72% H_2SO_4	3.0	0.046	8.9	0.200	N/A	N/A	3.2	0.020	N/A	N/A	10.9	0.081
PES 2[(b)]	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	36.8	0.014	**-**	**-**
Accountability[(c)]	**82.9%**		**115.4%**		**91.7%**		**90.3%**		**100%**		**95.0%**	

[(a)] PES 1 refers to the post extraction solids remaining following the initial extraction with acetonitrile and water (1:1, v/v) twice and once with 100% acetonitrile

(b) PES 2 refers to the post extraction solids remaining following further extraction with HCl, EGTA, DMSO, strong base and strong acid

(c) Accountability = [(sum of ERR + PES)(/initial TRR)] x 100

(d) Mature pods only (other samples, not applicable). A further 5 – 7% TRR was in the rinse, circa 0.06 mg eq./kg which is not included in these (sum of extracts) values

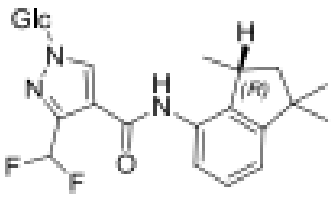
Chiral analysis

Where parent inpyrfluxam was detected in solvent fractions, chiral HPLC analyses were performed to determine the ratio of enantiomers of inpyrfluxam. Sample extracts characterised as containing parent compound were partitioned between hexane and water (x2) to selectively isolate the parent compound into the hexane fraction. A combined hexane fraction was analysed by chiral HPLC to resolve inpyrfluxam into early eluting “S” and later eluting “R” enantiomers. Soyabean forage, hay immature (edamame) pods and mature pods were analysed and showed that no isomerisation on/in the soyabean plants (100% R-isomer and no S-isomer).

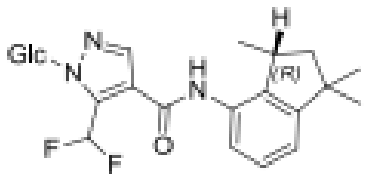
Identification

Residues in the conventional extracts of soyabean matrices were identified by reverse phase HPLC and using co-chromatography with reference standards. Some treatment of extracts using base or acid (0.06M NaOH or 1M HCL followed by beta-glucosidase, or hydrolysis of extracts with 0.1M, 1M or 6M HCL was used to further isolate metabolites in extracts). A methylation and acetylation approach were used to seek to identify an unknown Uk24 (17.5% TRR) fraction in mature seeds. Based on the hydrolysis and chromatographic work, Uk24 was considered to be a N-glycoside conjugate of *N*-des-Me-DFPA. The identity of the compounds in soyabean hay and immature (edamame) seeds were confirmed by high resolution LC-MS and TLC analysis, respectively. The solvent systems used for TLC were Chloroform: Methanol: Acetic acid (9:1:0.2, v/v/v), Chloroform: Methanol (8:2, v/v) in 0.3% Acetic Acid and Ethyl Acetate: Acetic Acid: Water (35:2:1, v/v/v). In mature seed, the *N*-des-Me-DFPA conjugate (also referred to as Uk 24) was also analysed by LC-MS/MS.

The following metabolites were identified: 3’-OH-S-2840, 1’-CH_2OH-S-2840, *N*-des-Me-S-2840 and trace amounts of Glc-NDM-S-2399 (only up to 5% TRR in hay and 0.1% TRR in mature seed, when considering the sum of the ‘A’ and ‘B’ forms and not detected in other sample types). ~~This is the only plant metabolism study with any reports of finding parent inpyrfluxam as a conjugate, showing some (limited level) conjugation of parent with glucose.~~



Glc-NDM-S-2399-A



Glc-NDM-S-2399-B

Overall, identification rates were reasonably sufficient (10 – 85%, with the lowest levels being seen in the matrices with the lowest TRR levels). The maximum individual unidentified component represented 5.0% (0.07 mg eq./kg) of the TRR in soyabean forage. The maximum individual unidentified component represented 5.0% (0.12 mg eq./kg) of the TRR in soyabean hay. The maximum individual unidentified component represented 13.1% (0.003 mg eq./kg) of the TRR in immature (edamame) seeds. The maximum individual unidentified component represented 2.7% (0.019 mg eq./kg) of the TRR in immature (edamame) pods. The maximum individual unidentified component represented 10.3% (0.004 mg eq./kg) of the TRR in mature seeds. The maximum individual unidentified component represented 5.6% (0.042 mg eq./kg) of the TRR in mature pods. The ‘unknowns’ are not considered further in this evaluation.

The identification and the distribution of parent and metabolites in soyabean matrices following treatment by [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam are shown in the following tables.

Table 7.2.1.7-4: Distribution of parent and metabolites in the extracts of soyabean matrices after two foliar applications of [pyrazolyl-4-^{14}C] inpyrfluxam

	Soyabean forage		Soyabean hay		Soyabean edamame seed		Soyabean edamame pods		Soyabean mature seeds		Soyabean mature pods (Soyabean mature pods rinse)	
	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg
Inpyrfluxam	40.3	0.561	17.8	0.424	3.0	0.003	34.0	0.241	ND	ND	6.9 (4.0)	0.083 (0.047)
1'-CH_2OH-S-2840	3.6	0.050	3.7	0.087	ND	ND	ND	ND	ND	ND	ND (ND)	ND (ND)
3'-OH-S-2840	22.1	0.308	14.7	0.349	ND	ND	9.2	0.065	ND	ND	2.1 (1.4)	0.025 (0.017)
***N*-des-Me-S-2840**	2.3	0.032	2.3	0.054	ND	ND	4.6	0.032	ND	ND	2.0 (0.1)	0.024 (0.001)
***N*-des-Me-DFPA conjugate (Uk 24)**	ND	ND	ND	ND	9.0	0.010	ND	ND	17.5	0.038	1.8 (ND)	0.022 (ND)
Total identified	**68.3**	**0.951**	**38.5**	**0.914**	**12.0**	**0.013**	**47.8**	**0.338**	**17.5**	**0.038**	**18.3[a]**	**0.219[a]**
Polars[b]	NA	NA	NA	NA	61.6	0.067	26.8	0.191	63.8	0.140	48.9 (ND)	0.588 (ND)

Max other single	5.0	0.070	5.0	0.120	4.6	0.005	2.7	0.019	2.2	0.005	2.7 (ND)	0.005 (ND)

ND not detected

(a) Total of Pods+Rinse Components

(b) Un-retained in the HPLC column and revealed as undifferentiated multiples by TLC analysis

Table 7.2.1.7-5: Distribution of parent and metabolites in the extracts of soyabean matrices after two foliar applications of [phenyl-U-^{14}C] inpyrfluxam

	Soyabean forage		Soyabean hay		Soyabean edamame seed		Soyabean edamame pods		Soyabean mature seeds		Soyabean mature pods (Soyabean mature pods rinse)	
	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg
Inpyrfluxam	50.5	0.786	22.1	0.495	9.8	0.002	65.2	0.414	2.0	≤0.001	23.0 (6.2)	0.170 (0.046)
1'-CH_2OH-S-2840	3.7	0.058	ND	ND	ND	ND	4.0	0.026	5.2	0.002	2.8 (ND)	0.021 (ND)
3'-OH-S-2840	15.3	0.238	14.3	0.321	ND	ND	9.0	0.057	0.8	≤0.001	11.6 (1.1)	0.086 (0.008)
***N*-des-Me-S-2840**	2.8	0.044	2.4	0.053	4.6	≤0.001	6.6	0.042	ND	ND	3.9 (ND)	0.029 (ND)
***N*-des-Me-DFPA**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND (ND)	ND (ND)

conjugate (Uk 24)												
Glc-NDM-S-2399-B	ND	ND	1.3	0.028	ND	ND	ND	ND	ND	ND	ND (ND)	ND (ND)
Glc-NDM-S-2399-A	ND	ND	3.8	0.085	ND	ND	ND	ND	1.6	≤0.001	ND (ND)	ND (ND)
Total identified	**72.3**	**1.126**	**43.9**	**0.982**	**14.4**	**0.003**	**84.7**	**0.539**	**9.6**	**0.005**	**48.6[(a)]**	**0.360[(a)]**
Polars[(b)]	ND	ND	ND	ND	ND	ND	ND	ND	11.7	0.004	ND (ND)	ND (ND)
Max single other	2.3	0.036	5.1	0.114	13.1	0.003	ND	ND	10.3	0.004	5.6 (ND)	0.042 (ND)

ND not detected

[(a)] Total of Pods+Rinse Components

[(b)] Un-retained in the HPLC column and revealed as undifferentiated multiples by TLC analysis

The parent substance inpyrfluxam represented the most prominent residue component in all matrices for [pyrazolyl-4-^{14}C] inpyrfluxam (6.9 – 40.3% TRR), except for immature (edamame) seeds and mature seeds in which the most prominent residue component was *N*-des-Me-DFPA conjugate (Uk 24) (9.0% and 17.5% TRR, respectively).

The parent substance inpyrfluxam represented the most prominent residue component in all matrices for [phenyl-U-^{14}C] inpyrfluxam (9.8 – 65.2% TRR), except for mature seeds in which the most prominent residue component was 1'-CH_2OH-S-2840 (5.2% TRR).

The next prevalent metabolite was 3'-OH-S-2840 with a maximum content of 22.1% TRR. Profiles of the soyabean matrices showed some differences across the individual matrices. A ~~proposed~~ low amount of the conjugate ~~glucose conjugate of parent~~ Glc-NDM-S-2399 was found in soyabean hay and mature seeds: Glc-NDM-S-2399 at up to 5.1% TRR (0.113 mg eq./kg) and 1.6%TRR (up to 0.001 mg eq./kg) respectively in the phenyl label study

Storage stability

Samples were stored frozen for a maximum of 86 days (after harvest) before extraction prior to analysis. All HPLC profiles of conventional extracts of the RACs were performed within 90 days after harvest.

Since there was further work up of the samples after initial analysis, the storage stability of the residues was demonstrated by repeat analysis of the initial extracts later in the study.

These investigations were performed for phenyl labelled soyabean hay and pyrazolyl labelled immature (edamame) pods and mature seed samples by HPLC re-analysis after 146, 186 and 624 days of storage in a freezer (-17°C), respectively. The comparison of the respective HPLC chromatograms revealed that the profiles of the extracts did not significantly change after storage of the material and the TRR values from the initial analysis were similar (within 11%). The chromatograms showed variation in the amount of inpyrfluxam for phenyl labelled soyabean hay (13.6 – 22.1% TRR) and pyrazolyl labelled immature (edamame) pods (22.7 – 34.0% TRR). Other slight variations were observed. A slight increase (13.3 to 14.3% TRR) of 3'-OH-S-2840 and *N*-des-Me-S-2840 (1.1 to 2.4% TRR) in extracts of phenyl labelled soyabean hay were reported. A slight increase (8.7 to 9.2% TRR) of 3'-OH-S-2840 in extracts of pyrazolyl labelled immature (edamame) pods was reported, whereas a slight decrease was reported for *N*-des-Me-S-2840 (4.8 to 4.6% TRR).

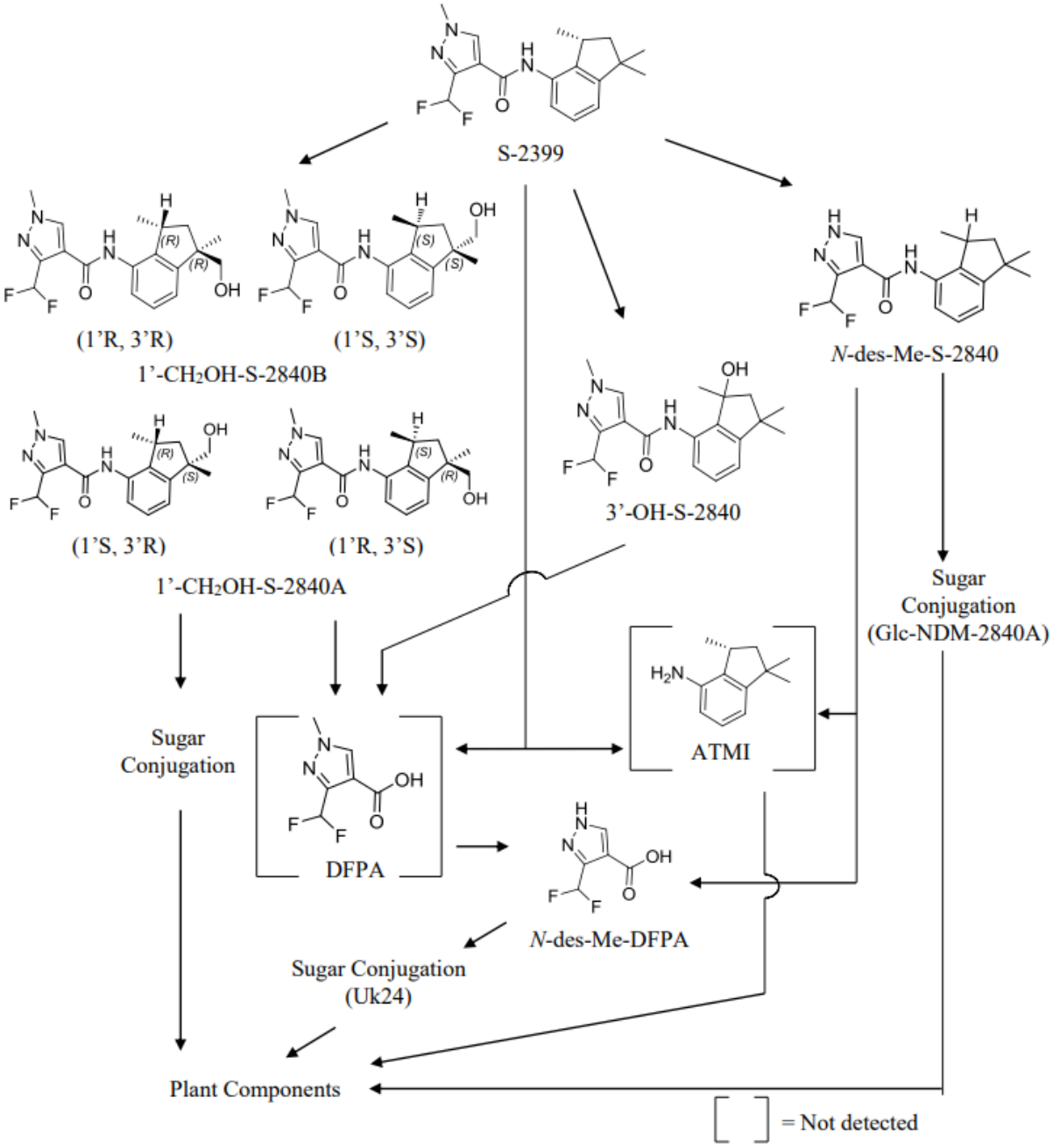
This storage stability work confirms the validity of the study.

Summary of inpyrfluxam metabolism in soyabean (foliar treatment)

Inpyrfluxam was found to be moderately metabolised in soyabeans after two applications. The metabolism proceeds via oxidation of inpyrfluxam forming hydroxylation metabolite 3'-OH-S-2840 along with lower amounts of *N*-des-Me-S-2840 and 1'-CH_2OH-S-2840. As the soyabean matures, the transformation products observed from [pyrazolyl-^{14}C] inpyrfluxam and [phenyl-^{14}C] inpyrfluxam start to differ indicating cleavage of the pyrazolyl ring from the indane ring most likely by hydrolysis of the amide bond. The possibly released pyrazolyl moiety (*N*-des-Me-DFPA) was rapidly conjugated with sugar (Uk 24) or further metabolized into multiple high-polar components.

Based on these results, the metabolic pathway of inpyrfluxam in soyabean is adequately understood and is proposed in the figure below:

Figure 7.2.1.7-1: Proposed metabolic pathway of inpyrfluxam in soyabean



B.7.2.2. Poultry

The metabolism of inpyrfluxam was investigated in laying hens after oral administration of either phenyl or pyrazolyl labelled inpyrfluxam.

Table 7.2.2-1 – Overview of poultry metabolism studies

Poultry	Application		Dose
Laying hens **(*Gallus gallus domesticus*)**	Group A	7 daily administrations of [pyrazolyl-4-^{14}C] inpyrfluxam in cellulose-filled gelatine capsules	12.44mg a.s./kg feed (0.86 mg a.s./kg bw/d) (14.12 dry matter mg/kg/day)
	Group B	7 daily doses of [phenyl-U-^{14}C] inpyrfluxam in cellulose-filled gelatine capsules	13.13mg a.s./kg feed (0.83 mg a.s./kg bw/d) (14.90 dry matter mg/kg/day)

Report:	**KCA 6.2.2/01; [redacted] T, 2017;**
Title:	Metabolism of [^{14}C] S-2399 (2 radiolabels) in Laying Hens 21-SEP-2016 (Final Report) 26-APR-2017 (Amended Final Report)
Document No.:	TPM-0025 (Study No.: 2453W)
Guidelines:	EPA Residue Chemistry Test Guidelines; OPPTS 860.1300, Nature of the Residue-Livestock; OECD/OCDE 503, Metabolism in Livestock January 2007
Guideline deviations:	None
GLP/GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

Summary

The metabolism of inpyrfluxam labelled either in the pyrazolyl ring or the phenyl ring was investigated in laying hens (*Gallus gallus domesticus*; breed "*Hyline Brown*").

Hens were housed individually and were judged to be healthy and suitable for testing during the acclimatisation period (16 days). Egg production was monitored prior to dosing and was considered acceptable. The hens were provided with game bird ration formulated to [redacted] specifications, with an additional 5% of limestone added. Feed was provided *ad libitum* during acclimatisation, then at 200 g per bird per day during dosing. Tap water (mains supply) was given *ad libitum* throughout.

The test compound was administered orally to a total of twenty hens (10 hens per radiolabel) in cellulose-filled gelatine capsules at the intended dose rate of 10 mg a.s./kg feed per day (actual dose: 12.44 mg a.s./kg feed for [pyrazolyl-4-^{14}C] label and 13.13 mg a.s./kg feed per day for the [phenyl-U-^{14}C] inpyrfluxam label). The test material contents were suitably verified. The test substances used to dose hens showed >99.30% of the R-enantiomer.

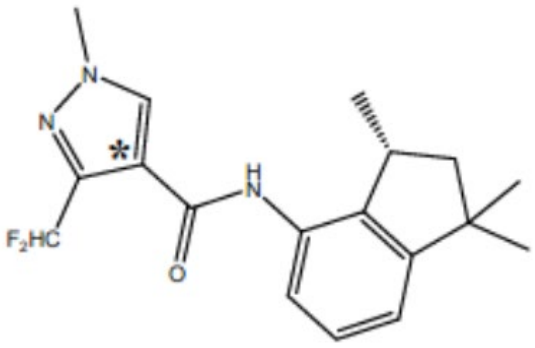
Based on the daily feed consumption and body weight of the birds, the dose level corresponds to 14.12 mg a.s./kg dry matter per day for birds dosed with [pyrazolyl-4-^{14}C] inpyrfluxam and 14.90 mg a.s./kg dry matter/day for birds dosed with [phenyl-U-^{14}C] inpyrfluxam. This gives a dose of 0.855 and 0.835 mg/kg bw/day, calculated assuming a group mean body weight day 1 – 7 of 1730 and 1760 g / animal, for the pyrazolyl and phenyl label respectively. The hens received one dose per day for 7 consecutive days, administered after the morning collection of eggs and excreta.

A control group was not used as pre-treatment sampling of eggs and excreta served to establish background values.

Materials

[pyrazolyl-4-^{14}C] inpyrfluxam

Chemical Structure

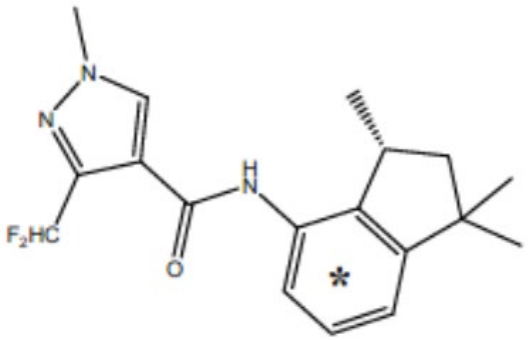


* denotes the ^{14}C-label position

Radiolabelled test material	[pyrazolyl-4-^{14}C] inpyrfluxam
Specific radioactivity	Delivered sample before radio dilution: 2.11 GBq/mmol (57 mCi/mmol) = 3.78X10^8 dpm/mg After isotopic dilution: 1.51X10^8 dpm/mg
Radiochemical purity	99.3%
Chemical purity	99.3%
Dose level	7 doses of 12.44 mg/kg feed/day

[phenyl-U-^{14}C] inpyrfluxam

Chemical Structure



* denotes the ^{14}C-label position

Radiolabelled test material	[phenyl-U-^{14}C] inpyrfluxam
Specific radioactivity	Delivered sample before radio dilution: 4.51 GBq/mmol (122 mCi/mmol) = 8.03X10^8 dpm/mg After isotopic dilution: 1.49X10^8 dpm/mg
Radiochemical purity	99.4%
Chemical purity	99.5%
Dose level	7 doses of 13.13 mg/kg feed/day

Sampling

Eggs and excreta were collected prior to the initial dosing to establish a base line and then twice daily, once in the morning prior to dosing and once in the afternoon at least 8 hours later.

Eggs from all pens with eggs present were composited by collection interval. Approximately 10% of the sample weight of each sample interval (Day 2 AM through Day 7 PM) was taken to form the composite samples. The eggs were scrubbed with a soft brush using soap and rinsed with water. Whole eggs (yolk and whites) were beaten and placed in storage containers.

Excreta from all pens were composited by collection interval.

The surfaces of the cage were rinsed with acetonitrile (ACN) at the end of the study. The rinses were stored frozen prior to shipment.

The animals were euthanised approximately 6 hours after receiving the final dose. The following tissues samples were collected: breast muscle, thigh muscle, liver (whole), abdominal fat, subcutaneous fat, entire gastro-intestinal tract, including the contents. Individual samples were collected from each bird and pooled together based on sample type. All samples were homogenized in the presence for dry ice and stored frozen (approximately –20°C) prior to shipping for analysis.

Analytical procedures

Radioactive measurement of liquid samples (cage wash and sample extracts) was done by liquid scintillation counting (LSC). All sample count rates were corrected for the background of LSC cocktail.

The radioactive residues in muscle, liver, fat and egg samples were measured by tissue digestion of sub-samples with tissue solubilizer (Soluene). Acetic acid and scintillation cocktail were added and the ^{14}C contents of the sample measured by LSC.

Matrices not amenable to direct radio analysis measurement using LSC were radio assayed by oxidation of the organic content into $^{14}CO_2$ using a biological oxidizer. The $^{14}CO_2$ was trapped with carbon 14 cocktail and the ^{14}C content determined by LSC.

Portions of muscle, liver, excreta and composite egg samples were extracted twice with acetonitrile/water (1:1, v/v) and then once with acetonitrile using a wrist action shaker for 45 minutes followed by separation of the solid and liquid phase by centrifugation (10 min at 10,000 rpm). The radiocarbon level of each extract was measured by LSC. The post-extraction solids (PES) were combusted (triplicate aliquots) and radiocarbon measured by LSC analysis. The acetonitrile/water extracts of muscle, liver and excreta were combined and concentrated prior to characterisation using chromatography. For egg, the acetonitrile/water extracts were combined, concentrated before being partitioned with three portions of hexane. The aqueous layer and the hexane layer were radio assayed by LSC analysis. The water extracts were concentrated for chromatographic characterization.

Fat subsamples were extracted once with hexane/acetone (4:1, v/v) and then twice with acetone using a wrist action shaker for 45 minutes followed by separation of the solid and liquid phase by centrifugation (10 min at 10,000 rpm). Additional sub-samples extracted once with hexane/acetone (1:4, v/v) and then once with acetone. The radiocarbon of each extract was measured by LSC. PES were combusted and the radiocarbon of these also measured by LSC analysis. The first two extracts were combined, concentrated and partitioned three times with ACN. The radioactivity of the ACN and hexane layers was measured with LSC. The ACN layers were concentrated for characterisation with chromatography.

Sample extracts characterised as containing parent compound (abdominal and subcutaneous fat tissues) were used to examine the isomeric composition. The extracts from the pyrazolyl-labelled abdominal and subcutaneous fat samples were combined and the phenyl-labelled abdominal and subcutaneous fat samples were combined for chromatographic characterisation. Analysis was done using isocratic normal phase HPLC.

Radiolabelled residues were characterized by HPLC using co-chromatography with reference standards. The identity of residues was assigned based on HPLC retention times. Identities of components were confirmed by TLC using comparison of Rf values to standards which were analysed with the sample or by LC-MS.

Parent compounds and metabolites were quantified in the extracts using linear gradient reverse phase HPLC. The identity of the residues was assigned based on HPLC retention times. Identities of components were confirmed by TLC using comparison of R_f values to standards which were analysed with the sample or by LC-MS.

Results and discussion

All birds were normal in appearance and normal behaviour was observed throughout the study. No effects on egg production or body weight were observed throughout the study. Average feed consumption did reduce during the dosing period based on 7 day average during acclimation (140 g/day) vs 7 day average during dosing (112 – 119 g/day). No deviations from the study plan were reported.

The total recovery of the administered dose in tissue, excreta and cage wash was 82.68% for hens treated with [pyrazolyl-4-^{14}C] inpyrfluxam and 84.63% for [phenyl-U-^{14}C] inpyrfluxam. No explanation was provided for the remaining amount of radioactivity (17.3 and 15.4% for pyrazolyl and phenyl label respectively) which was not recovered.

The majority of the administered dose was recorded in the excreta samples (≥ 80.25% of dose). The overall distribution of the percentage dose excreted for hens was similar in [phenyl-U-^{14}C] inpyrfluxam and [pyrazolyl-4-^{14}C] inpyrfluxam dosages.

Small amounts of the radioactivity were recovered in the GI tract and the cage wash (≤ 2.48% of dose).

The dose % recovered in tissues and eggs was <1% for both Group A and Group B. Radioactive residue levels in egg samples were similar for both dosage regimes. For both labels the residue levels in eggs continued to rise up to day 5 of the regime, and by day 7 the residues in eggs were 0.033mg eq./kg in hens dosed with [pyrazolyl-4-^{14}C] inpyrfluxam and 0.031 mg eq./kg in hens dosed with [phenyl-U-^{14}C] inpyrfluxam.

Table 7.2.2.1-1 Distribution of residues in matrices of laying hens following oral administration of 7 daily doses of [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam

Radioactivity Balance				
^{14}C-Inpyrfluxam	Pyrazolyl-^{14}C Label		Phenyl-^{14}C Label	
Tissues	% of Total Dosed radioactivity	mg eq./kg[(a)]	% of Total Dosed radioactivity	mg eq./kg[(a)]
Liver	0.22	0.526	0.11	0.268
Eggs	0.06	0.025	0.06	0.020
Thigh Muscle	0.01	0.013	0.01	0.012
Breast Muscle	0.01	0.012	0.02	0.022
Abdominal Fat	0.01	0.069	0.03	0.107
Subcutaneous Fat	0.01	0.109	0.01	0.086
Excreta	80.25		81.70	
G.I Tract	0.78	2.124	1.12	2.478
Cage Wash	1.33	0.510	1.57	0.594
Total	**82.68**		**84.63**	

[(a)] The TRR values (mg eq./kg) are derived from initial LSC analysis.

The majority of residues were efficiently extracted using the neutral solvents. In all matrices, ≥ 90% of the [^{14}C] inpyrfluxam residues were extracted, excluding thigh muscle samples from hens dosed with [phenyl-U-^{14}C] inpyrfluxam where only 80% of the TRR (0.012 mg eq./kg) was extracted. In the phenyl label thigh sample, although the PES represented 20% of the TRR, the absolute residues were very low (≤ 0.003 mg eq./kg) so no further characterisation was needed.

The extractability and accountability of residues in summarised in Table 7.2.2.1-2.

The highest TRR in edible matrices, based on the sum of extracted residue and PES, was found in the liver (0.317 mg eq./kg; 0.22% of the total dose for [pyrazolyl-4-^{14}C] inpyrfluxam, 0.255 mg eq./kg; 0.11% of the total dose for [phenyl-U-^{14}C] inpyrfluxam) showing the significance of this organ for the metabolism and excretion of inpyrfluxam. For the pyrazolyl label, this does show a poor comparison with the TRR by combustion which was 0.526 mg eq./kg (*ca.* 60% accountability). This only seems to be the case for the liver sample for the pyrazolyl label, whereas accountability for liver for the phenyl label was 95.1%. It is not known whether this is due to sample heterogeneity within the sample, or an unexplained loss was observed. Accountability in all other matrices was acceptable, with > 87.9% of the TRR accounted for in solvent extracts and TRR remaining in PES.

The TRR in eggs ranged from 0 mg eq./kg at day one to 0.033 mg eq./kg on day seven. The residue level followed an approximately linear increase over the 7 day dosing period. The TRR measured in composite egg accounted for 0.06% of the total dose; for [pyrazolyl-4-^{14}C] inpyrfluxam the TRR was 0.025mg eq./kg and for hens dosed with [phenyl-U-^{14}C] inpyrfluxam the TRR was 0.020 mg eq./kg. The residues extracted in eggs over the 7 day dosing period can be found in Table 7.2.2.1-3.

Lower % TRR was detected in other tissues. In hens dosed with [pyrazolyl-4-^{14}C] inpyrfluxam 0.01% of the total dose was found in all muscle and fat samples. In hens dosed with [phenyl-U-^{14}C] inpyrfluxam slightly higher levels were found in breast muscle samples (0.02%) and abdominal fat samples (0.03%).

Table 7.2.2.1-2 Extraction efficiency of poultry samples following oral administration of 7 daily doses of [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam

[pyrazolyl-4-^{14}C] inpyrfluxam									
Tissue	Extract 1	Extract 2	Extract 3	Total Extracted Radioactive Residue (ERR)		Post Extract Solids		TRR[a]	Accountability[b]
	% Extracted			mg eq./kg	%ERR	mg eq./kg	%TRR	mg eq./kg	%
Liver	90.64	9.36	ND	0.299	94.32	0.018	5.68	0.317	60.3
Egg[c]	71.43	19.05	9.52	0.021	91.30	0.002	8.70	0.023	92.0
Breast Muscle	72.73	18.18	9.09	0.011	91.67	0.001	8.33	0.012	100.0
Thigh Muscle	75.00	16.67	8.33	0.012	92.31	0.001	7.69	0.013	100.0
Abdominal Fat	100.00	ND	ND	0.063	98.44	0.001	1.56	0.064	92.8
Subcutaneous Fat	91.09	8.91	ND	0.101	99.02	0.001	0.98	0.102	93.6
Excreta	75.70	20.47	3.83	13.475	98.22	0.244	1.78	13.719	NC
[phenyl-U-^{14}C] inpyrfluxam									
	Extract 1	Extract 2	Extract 3	Total Extracted Radioactive Residue (ERR)		Post Extract Solids		TRR[a]	Accountability[b]
	% Extracted			mg eq./kg	%ERR	mg eq./kg	%TRR	mg eq./kg	%

Liver	90.13	9.87	ND	0.233	91.37	0.022	8.63	0.255	95.1
Egg [(c)]	72.22	16.67	11.11	0.018	90.00	0.002	10.00	0.020	100.0
Breast Muscle	76.19	19.05	4.76	0.021	91.30	0.002	8.70	0.023	104.5
Thigh Muscle	75.00	16.67	8.33	0.012	80.00	0.003	20.00	0.015	125.0
Abdominal Fat	83.52	16.48	ND	0.091	96.81	0.003	3.19	0.094	87.9
Subcutaneous Fat	89.97	10.13	ND	0.079	97.53	0.002	2.47	0.081	94.2
Excreta	77.61	18.60	3.79	20.872	98.16	0.391	1.84	21.263	NC

ND = not detected - sample was < 2X background values

NC = Not calculated

(a) = TRR determined by sum of fractions (extracts 1 – 3 + PES = TRR) used for distribution of residues characterised by chromatographic methods.

(b) = accountability calculated from the [(sum of ERR + PES)/(initial TRR)] x 100

(c) = representative composite samples

Table 7.2.2.1-3 Time course of radioactivity in eggs following oral administration of 7 daily doses of [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam

Sampling interval	Hours	[pyrazolyl-4-^{14}C] inpyrfluxam (mg eq./kg)	[phenyl-U-^{14}C] inpyrfluxam (mg eq./kg)
Day 1 AM	0	0.000	0.000
Day 1 PM	12	0.000	0.000
Day 2 AM	24	0.024	0.012
Day 2 PM	36	0.012	0.014
Day 3 AM	48	-[(a)]	0.007
Day 3 PM	60	0.019	0.017
Day 4 AM	72	0.021	0.012
Day 4 PM	84	0.020	0.022
Day 5 AM	96	0.032	0.017
Day 5 PM	108	0.022	0.028
Day 6 AM	120	-[(a)]	0.019
Day 6 PM	132	0.031	0.033
Day 7 AM	144	0.032	0.023
Day 7 PM	156	0.033	0.031

[(a)] no eggs were laid for time-point collection

Parent compounds and metabolites were quantified using reverse phase HPLC. The residues were identified relative to co-injected reference standards with assignments made by retention times of the co-eluting peaks (radioisotope compared to UV detection). Identities of components were confirmed by TLC using comparison of R_f values to standards which were analysed with the sample by LC-MS.

Both the pyrazolyl- and phenyl-labelled metabolites were isolated from the liver for analysis by high resolution mass spectrometry. Sample extracts were cleaned up using a solid phase extraction (SPE) cartridge pre-conditioned with methanol followed by water. The cartridge was eluted twice with sodium acetate and followed by three portions of ammonium hydroxide in methanol. The ammonium hydroxide fractions were combined and concentrated. The SPE purified residues were further

purified using HPLC. The isolated peaks were then concentrated and analysed by high resolution LC-MS. A chemically hydrolysed product of one of the isolated metabolites was also analysed using LC-MS/MS.

The liver extracts were additionally treated with β-glucuronidase, sulfatase and 1M HCl. The largest metabolites in liver extract were not changed by treatment with β-glucuronidase or sulfatase. Treatment with 1M HCl (100°C, 4 hours), hydrolysed the metabolite to the mixture of aglycones confirmed by TLC to be 1'-CH_2OH-S-2840 A&B.

A summary of all identified residues can be seen in Table 7.2.2.1-4 and 7.2.2.1-5.

Metabolites in liver

Hens dosed with [pyrazolyl-4-^{14}C] inpyrfluxam (Group A)

The TRR determined by the sum of fractions (ERR + PES) was 0.317 mg eq./kg in liver collected from hens treated with [pyrazolyl-4-^{14}C] inpyrfluxam. Extraction performed with neutral solvents solubilised 94.32% TRR equivalent to 0.299 mg eq./kg. 5.68% TRR remained in post extraction solids, since this was < 10% TRR and < 0.05 mg eq./kg (0.018 mg eq./kg, 5.68% TRR) no further characterisation of the non-extractable residues was performed in line with OECD guidance. The metabolites eluting at Rt 37 and 39 accounting for 22.17 and 29.52% TRR respectively were identified in liver samples as sulphate conjugates of 1′-CH_2OH-S-2840 and termed sulphate 37 and 39. Also identified in extracts from the liver were *N*-des-Me-S-2480 (4.62% TRR), 1'-COOH-S-2840-A (3.30% TRR) and 1'-COOH-S-2840-B (3.21% TRR). Up to 20.66% of the TRR was not identified. This was made up of 4 peaks, with the largest individual peak accounting for 8.87% TRR equivalent to 0.028 mg eq./kg. As this is < 10% TRR and < 0.05 mg eq./kg no further consideration is required.

Analysis of the total extract by TLC confirmed the presence of *N*-des-Me-S-2480 and 1'-COOH-S-2840 A&B isomers.

Hens dosed with [phenyl-U-^{14}C] inpyrfluxam (Group B)

The TRR determined by the sum of fractions was 0.255 mg eq./kg in liver collected from hens treated with [phenyl-U-^{14}C] inpyrfluxam. Solvent extraction solubilised 91.37% TRR with 8.63% TRR remaining in post extraction solids (0.022 mg eq./kg), since the levels in the post extraction solids were < 10% TRR and < 0.05 mg eq./kg no further characterisation was performed in line with the OECD guidance.

Chromatographic characterisation by reverse phase HPLC showed the largest peaks at Rt 37 and Rt 39 with residue levels of 27.59% and 16.45% TRR respectively. The Rt 37 and Rt 39 peaks were identified as sulphate conjugates of 1'-CH_2OH-S-2840.

Also identified were *N*-des-Me-S-2480 (9.50% TRR), 1'-COOH-S-2840-B (6.58% TRR) and 1'-COOH-S-2840-A (4.39% TRR). Up to 17.82% of the TRR was not identified. This was made up of 4 peaks, with the largest individual peak accounting for 5.94% TRR (0.015 mg eq./kg). As this is < 10% TRR and < 0.05 mg eq./kg no further consideration is required.

Analysis of the total extract by TLC confirmed the presence of *N*-des-Me-S-2480 and 1'-COOH-S-2840 A&B isomers.

Metabolites in eggs

Hens dosed with [pyrazolyl-4-^{14}C] inpyrfluxam (Group A)

A composite sample of eggs was collected from hens dosed with [pyrazolyl-4-^{14}C] inpyrfluxam. The determined TRR was 0.023 mg eq./kg. Extraction with a neutral solvent solubilised 91.30% of the TRR with 8.70% of the TRR remaining in the post extracted solids. Since the post extraction solids was < 10% of the TRR and < 0.05 mg eq./kg (0.002 mg eq./kg, 8.70% TRR) no further characterisation was performed in line with OECD guidance.

Chromatographic characterisation was done by reverse phase HPLC. The largest peak was equivalent to 25.02% of the TRR and co-eluted with 1'-CH_2OH-S-2840-B. The other identifiable peak was equivalent to 10.50% of the TRR and co-eluted with inpyrfluxam. Up to 29.94% of the TRR was not identified. This was made up of 5 peaks, with the largest individual peak accounting for 12.6% TRR (0.003 mg eq./kg). As this is < 0.05 mg/kg no further consideration is required.

TLC confirmed the presence of inpyrfluxam and a mixture of 1'-CH_2OH-S-2840-B isomers in the egg extracts.

Hens dosed with [phenyl-U-^{14}C] inpyrfluxam (Group B)

A composite sample of eggs was collected from hens dosed with [phenyl-U-^{14}C] inpyrfluxam. The TRR determined from the sum of fractions was 0.020 mg eq./kg. Extraction with neutral solvents extracted 90% of the TRR. Since post extractable solids was < 0.05 mg eq./kg (0.002 mg eq./kg, 10% TRR) no further characterisation was performed in line with OECD guidance.

The largest classified peak accounted for 36.67% of the TRR and co-eluted with 1'-CH_2OH-S-2840-B. Identification of inpyrfluxam accounted for 10.89% of the TRR. Trace amounts of metabolites 1'-COOH-S-2840-B, 3'-OH-S-2840 and *N*-des-Me-S-2480 were detected accounting for <5.58% TRR.

Up to 17.73% of the TRR was not identified. This was made up of 4 peaks, with the largest individual peak accounting for 6.21% TRR (0.001 mg eq./kg). As this is < 10% TRR and < 0.05 mg eq./kg no further consideration is required.

Metabolites in muscle (thigh and breast)

Hens dosed with [pyrazolyl-4-^{14}C] inpyrfluxam (Group A)

Thigh muscle samples from hens dosed with [pyrazolyl-4-^{14}C] inpyrfluxam had a TRR determined from the sum of fractions of 0.013 mg eq./kg. Extraction with neutral solvents solubilised 92.31% of the TRR, no further characterisation was performed on the post extraction solids (0.001 mg eq./kg, 7.69% TRR) in line with OECD guidance as the trigger of 0.05 mg eq./kg or 10% TRR was not met. The largest components characterised represented 14.49% of the TRR and co-eluted with DFPA-$CONH_2$. The other components characterised were 1'-COOH-S-2840-A (4.34% TRR), 1'-COOH-S-2840-B (5.45% TRR), 1'-CH_2OH-S-2840-B (11.08%) and inpyrfluxam (4.89%). The sulphate conjugates Sulphate 37 and Sulphate 39 were detected between 4.43 – 6.92% of the TRR.

Breast muscle samples from hens dosed with [pyrazolyl-4-^{14}C] inpyrfluxam had a TRR of 0.012 mg eq./kg. Extraction solubilised 91.67% with no further characterisation being performed as post extraction solids were < 10% of the TRR (0.01 mg eq./kg, 8.33% TRR) in line with OECD guidance as the trigger of 0.05 mg eq./kg or 10% TRR was not met. Reverse phase HPLC of the soluble residues showed metabolites 1'-COOH-S-2840-A (4.86% TRR), 1'-COOH-S-2840-B (6.14% TRR), Sulphate 37 (4.49%), Sulphate 39 (5.86%), 1'-CH_2OH-S-2840-B (5.59%) and DFPA-$CONH_2$ (11.83%).

Up to 25.21% of the TRR was not identified. This was made up of up to 5 peaks, with the largest individual peak accounting for 11.37% TRR (0.001 mg eq./kg). As this is < 0.05 mg eq./kg no further consideration is required.

Hens dosed with [phenyl-U-^{14}C] inpyrfluxam (Group B)

Thigh muscle samples from hens dosed with [phenyl-U-^{14}C] inpyrfluxam had a TRR determined from the sum of fractions of 0.015 mg eq./kg. Extraction with neutral solvents solubilised 80% of the TRR with 20% of the TRR being in post extraction solids. The 20% of the TRR in post extraction solids was equivalent to ≤0.003 mg eq./kg therefore no further characterisation was performed as the trigger of 0.05 mg eq./kg in accordance with the OECD guidance was not met. The largest component detected in reverse phase HPLC accounted for 11.20% of the TRR and co-eluted with 1'-COOH-S-2840-B. Sulphate 37 and Sulphate 39 were detected (11.60 – 13.60%). The other components characterised were 1'-COOH-S-2840-A (5.20%), 1'-CH_2OH-S-2840-A (5.20%), 1'-CH_2OH-S-2840-B (5.60%) and inpyrfluxam (2.16%).

In breast muscle, the TRR determined by the sum of fractions was 0.023 mg eq./kg. Extraction solubilised 91.30% TRR, no further characterisation was performed as the PES did not account for > 10% of the TRR in line with OECD guidance (0.002 mg eq./kg, 8.70% TRR). The largest peak identified via chromatographic

characterisation accounted for 35.33% of the TRR and corresponded to Sulphate 39, followed by a peak accounting for 12.33% TRR corresponding to Sulphate 37. The other components characterised were 1'-COOH-S-2840-B (7.76%), 1'-COOH-S-2840-A (2.86%), 1'-CH_2OH-S-2840-B (3.38%).

Up to 25.44% of the TRR was not identified. This was made up of up to 4 peaks, with the largest individual peak accounting for 15.28% TRR (0.002 mg eq./kg). As this is < 0.05 mg eq./kg no further consideration is required.

Metabolites in fat (abdominal and subcutaneous)

Hens dosed with [pyrazolyl-4-^{14}C] inpyrfluxam (Group A)

The TRR determined by the sum of fractions was 0.064 mg eq./kg in abdominal fat samples. Extractions solubilised 98.44% with no further characterisation of the post extraction solids being performed as, in line with OECD guidance, < 10% TRR and < 0.05 mg eq./kg remained. The largest component characterised co-eluted with inpyrfluxam and accounted for 69.89% of the TRR. Other components characterised were 1'-CH_2OH-S-2840-B (2.66%), 3'-OH-S-2840 (2.66%) and *N*-des-Me-S-2480 (3.25%). TLC confirmed identification of inpyrfluxam with trace amounts of radioactivity co-eluting with *N*-des-Me-S-2480.

Subcutaneous fat samples had a TRR of 0.102 mg eq./kg. Extraction solubilised 99.02% with no further characterisation performed as, in line with OECD guidance, < 10% TRR and < 0.05 mg eq./kg remained. The largest components accounted for 73.67% of the TRR and co-eluted with inpyrfluxam. The other components identified in subcutaneous fat samples were 1'-CH_2OH-S-2840-B (2.57%), 3'-OH-S-2840 (2.18%) and *N*-des-Me-S-2480 (3.17%). TLC confirmed the identification of inpyrfluxam with trace amounts of radioactivity co-eluting with *N*-des-Me-S-2480.

Up to 8.02% of the TRR was not identified. This was made up of up to 7 peaks, with the largest individual peak accounting for 2.46% TRR (0.002 mg eq./kg). As this is < 10% TRR and < 0.05 mg eq./kg no further consideration is required.

Hens dosed with [phenyl-U-^{14}C] inpyrfluxam (Group B)

Abdominal fat samples had a TRR of 0.094 mg eq./kg. Extraction with neutral solvents solubilised 96.81% TRR with no further extraction of the post extraction solids as, in line with OECD guidance, < 10% TRR and < 0.05 mg eq./kg remained. Characterisation by reverse phase HPLC of the soluble residues showed the largest component co-eluted with inpyrfluxam and accounted for 54.99% of the TRR. The other components characterised in abdominal fat samples from hens dosed with [phenyl-U-^{14}C] inpyrfluxam were 1'-COOH-S-2840-B (3.19%), 1'-CH_2OH-S-2840-B (2.32%) and *N*-des-Me-S-2480 (2.52%). TLC confirmed identification of inpyrfluxam

in the soluble fat samples with trace amounts of radioactivity co-eluting with *N*-des-Me-S-2480.

Subcutaneous fat residues in hens dosed with [phenyl-U-^{14}C] Inpyrfluxam had a TRR of 0.081 mg eq./kg. Extraction solubilised 97.53% with no further characterisation being performed as, in line with OECD guidance, < 10% TRR and < 0.05 mg eq./kg remained. The largest components co-eluted with inpyrfluxam and accounted for 80.66% TRR. A further HPLC component contributing towards 16.87% of the TRR that was characterised did not match any available reference compounds but had an elution time similar to sulphate conjugate of 1'-CH_2OH-S-2840 (sulphate 37).

TLC confirmed identification of inpyrfluxam with trace amounts of *N*-des-Me-S-2480.

Up to 12.48% of the TRR was not identified. This was made up of up to 5 peaks, with the largest individual peak accounting for 4.55% TRR (0.004 mg eq./kg). As this is < 10% TRR and < 0.05 mg eq./kg no further consideration is required.

Metabolites in excreta

Hens dosed with [pyrazolyl-4-^{14}C] inpyrfluxam (Group A)

The TRR determined by the sum of fractions was 13.719 mg eq./kg in excreta (Day 4 PM). Extraction with neutral solvents solubilised 98.22% so no further extraction was required as, in line with OECD guidance, < 10% TRR and < 0.05 mg eq./kg remained. The largest component identified corresponds to the previously identified Sulphate 39 (37.80%). The other components characterised were Sulphate 37 (11.57%), 1'-COOH-S-2840-A (4.78%), 1'-COOH-S-2840-B (24.45%), 1'-CH_2OH-S-2840-A (1.16%), 1'-CH_2OH-S-2840-B (0.89%).

Up to 17.56% of the TRR was not identified. This was made up of up to 10 peaks, with the largest individual peak accounting for 3.21% TRR (0.441 mg eq./kg). As > 80% of the TRR has been identified and the largest unknown peak represents < 10% TRR no further consideration is required.

Hens dosed with [phenyl-U-^{14}C] inpyrfluxam (Group B)

The TRR determined by sum of excreta fractions was 21.263 mg eq./kg (Day 6 PM). Extraction with neutral solvents solubilised 98.16% and therefore no further characterisation was required as, in line with OECD guidance, < 10% TRR and < 0.05 mg eq./kg remained. The largest identified component accounted for 35.04% TRR and corresponded to Sulphate 39. The second largest component (30.20%) co-eluted with 1'-COOH-S-2840-B. The other components characterised were Sulphate 37 (14.23%) and 1'-COOH-S-2840-A (5.01%).

Up to 13.68% of the TRR was not identified. This was made up of up to 5 peaks, with the largest individual peak accounting for 7.76% TRR (1.651 mg eq./kg). As > 84%

of the TRR has been identified and the largest unknown peak represents < 10% TRR no further consideration is required.

Chiral analysis

Portions of abdominal and subcutaneous fat extract residues from [pyrazolyl-4-^{14}C] inpyrfluxam treated hens were combined and concentrated. The concentrated residues were solubilised in hexane, characterisation of the hexane soluble residues was performed using a chiral chromatographic separation of inpyrfluxam technical grade compound containing 3 – 4% of the S-isomer. The major component in the mixture co-chromatographed with the inpyrfluxam (R) isomer.

Similarly, the [phenyl-U-^{14}C] inpyrfluxam residues of abdominal fat extract combined with the subcutaneous fat extract were concentrated and then reconstituted in hexane with a small amount of 2-proponal. Characterisation of the technical grade inpyrfluxam containing 3 – 4% S-isomer found the major component in the mixture to be inpyrfluxam (R) isomer.

Storage Stability

All samples were homogenized in the presences of dry ice before shipping and analysis. Initial chromatographic characterization of all the tissue extracts (liver, muscle, and fat) was conducted within 40 days of sacrifice. Selected excreta samples (pyrazolyl-label Day 4 PM and phenyl-label Day 6 PM) were also extracted and analysed within 40 days of sacrifice.

Identification of some major metabolites in hen liver extracts were performed after 6 months; therefore, chromatographic profiles of extracts were compared to the profiles of extracts that had been stored in a freezer (-20°C) for at least 286 days to cover the storage period of the sample extracts. Further HPLC isolation and enzyme hydrolysis took place up to 338 days after sacrifice. Storage stability of sample extracts was checked after 583 days. The distribution of components in the chromatograms were similar between time points.

Table 7.2.2.1-4 Distribution of residues in hens dosed with [pyrazolyl-4-^{14}C] inpyrfluxam

Description	Liver		Egg		Thigh		Breast		Abdominal Fat		Subcutaneous Fat		Excreta (Day 4)	
	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR
Combined Extracts [a]	0.299	94.32	0.021	91.30	0.012	92.31	0.011	91.67	0.063	98.44	0.101	99.02	13.48	98.22
PES [b]	0.018	5.68	0.002	8.70	0.001	7.69	0.001	8.33	0.001	1.56	0.001	0.98	0.244	1.78
TRR[c]	0.317	100.00	0.023	100.00	0.013	100.00	0.012	100.00	0.064	100.00	0.102	100.00	13.719	100
DFPA-CONH$_2$	ND	ND	0.001	5.02	0.002	14.49	0.001	11.83	ND	ND	ND	ND	ND	ND
1'-COOH-S-2840-A	0.010	3.30	ND	ND	0.001	4.34	0.001	4.86	ND	ND	ND	ND	0.656	4.78
1'-COOH-S-2840-B	0.010	3.21	ND	ND	0.001	5.45	0.001	6.14	0.001	1.18	ND	ND	3.354	24.45
Sulphate 37 [d]	0.070	22.17	<0.001	1.73	0.001	4.43	0.001	4.49	0.001	1.08	ND	ND	1.587	11.57
Sulphate 39 [d]	0.094	29.52	0.001	3.38	0.001	6.92	0.001	5.68	ND	ND	0.003	3.17	5.187	37.80
1'-CH$_2$OH-S-2840-A	ND	ND	0.002	6.57	ND	ND	ND	ND	ND	ND	ND	ND	0.159	1.16
1'-CH$_2$OH-S-2840-B	ND	ND	0.006	25.02	0.001	11.08	0.001	5.59	0.002	2.66	0.003	2.57	0.123	0.89
3'-OH-S-2840	ND	ND	≤0.001	1.92	ND	ND	ND	ND	0.002	2.66	0.002	2.18	ND	ND
***N*-des-Me-S-2840**	0.015	4.62	0.001	5.02	ND	ND	ND	ND	0.002	3.25	0.003	3.17	ND	ND
Inpyrfluxam	ND	ND	0.002	10.50	0.001	4.89	≤0.001	2.93	0.045	69.89	0.075	73.67	ND	ND

Unknown [e]	0.066	20.66	0.008	29.94	0.002	14.68	0.005	25.21	0.006	7.67	0.008	8.02	2.409	17.56

ND = not detected
(a) Residues in extracts chromatographically characterised
(b) Post Extraction Solids
(c) TRR = Sum of combined extracts + PES
(d) Metabolites are sulphate conjugates of 1′-CH2OH-S-2840 isomers
(e) Sum of all unknowns measured

Table 7.2.2.1-5 Distribution of residues in hens dosed with [phenyl-U-^{14}C] inpyrfluxam

Description	Liver		Egg		Thigh		Breast		Abdominal Fat		Subcutaneous Fat		Excreta (Day 6)	
	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR
Combined Extracts [a]	0.233	91.37	0.018	90.00	0.012	80.00	0.021	91.30	0.091	96.81	0.079	97.53	20.87	98.16
PES [b]	0.022	8.63	0.002	10.00	0.003	20.00	0.002	8.70	0.003	3.19	0.002	2.47	0.39	1.84
TRR [c]	0.255	100.00	0.020	100.00	0.015	100.00	0.023	100.00	0.094	100.00	0.081	100.00	21.26	100
DFPA-CONH$_2$	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1'-COOH-S-2840-A	0.011	4.39	<0.001	2.16	0.001	5.20	0.001	2.83	ND	ND	ND	ND	1.06	5.01
1'-COOH-S-2840-B	0.017	6.58	<0.001	2.52	0.002	11.20	0.002	7.76	0.003	3.19	ND	ND	6.42	30.20
Sulphate 37 [d]	0.070	27.59	<0.001	3.06	0.002	11.60	0.003	12.33	0.003	2.71	0.014	16.87	3.03	14.23

Sulfate39 [(d)]	0.042	16.45	0.001	6.12	0.002	13.60	0.008	35.33	0.005	5.71	ND	ND	7.45	35.04
1'-CH_2OH-S-2840-A	ND	ND	0.001	6.12	0.001	5.20	ND	ND	ND	ND	ND	ND	ND	ND
1'-CH_2OH-S-2840-B	ND	ND	0.005	23.67	0.001	5.60	0.001	3.38	0.002	2.32	ND	ND	ND	ND
3'-OH-S-2840	ND	ND	<0.001	2.52	ND	ND	ND	ND	0.001	1.45	ND	ND	ND	ND
***N*-des-Me-S-2840**	0.024	9.50	0.001	5.58	ND	ND	ND	ND	0.002	2.52	ND	ND	ND	ND
Inpyrfluxam	ND	ND	0.002	10.89	<0.001	2.16	ND	ND	0.052	54.99	0.065	80.66	ND	ND
Unknown [(e)]	0.046	17.82	0.004	17.73	0.005	25.44	0.005	20.81	0.011	12.48	ND	ND	2.91	13.68

ND = not detected

(a) Residues in extracts chromatographically characterised

(b) Post Extraction Solids

(c) TRR = Sum of combined extracts + PES

(d) Metabolites are sulphate conjugates of 1′-CH_2OH-S-2840 isomers

(e) Sum of all unknowns

Summary of inpyrfluxam metabolism in poultry

The metabolism of inpyrfluxam labelled either in the pyrazolyl ring or the phenyl ring was investigated in twenty laying hens (10 hens per radiolabel) over 7 days with an intended dose rate of 10 mg a.s./kg feed per day (actual dose: 12.44 and 13.13 mg a.s./kg feed per day for the pyrazolyl and phenyl label respectively). Greater than 80% of the dose was recovered in excreta and GI tract. Less than 1% of the dose was found in representative liver, muscle, fat or egg matrices. No explanation was provided for the 15.4 – 17.4% of the dose which was not recovered.

The majority of residues were efficiently extracted using the neutral solvents. In all matrices, ≥ 90% of the [^{14}C] inpyrfluxam residues were extracted, excluding thigh muscle samples for the phenyl label were 80% of the TRR (0.012 mg eq./kg) was extracted. Although the % TRR remaining in PES for thigh muscle for the phenyl label was relatively high by comparison to other matrices, the absolute measured residue in terms of mg eq./kg was very low (0.003 mg eq./kg) so this is considered acceptable.

The maximum egg residues were 0.033 mg eq./kg. Neutral solvent solubilised at least 90% of the radioactive residue in egg samples. Characterisation of the soluble residues showed the largest component was 1'-CH_2OH-S-2840-A&B (0.006 – 0.008 mg eq./kg, 29.79 – 31.59% TRR) with trace amounts of inpyrfluxam (0.002 mg eq./kg, 10.50 – 10.89% TRR) and *N*-des-Me-S-2840 (0.001 mg eq./kg, 5.58% TRR).

Residues in liver were between 0.268 – 0.526 mg eq./kg based on LSC / initial combustion. The TRR based on extractable residue and PES is 0.255 – 0.317 mg eq./kg. Accountability was relatively low (*ca.* 60%) for the pyrazolyl label; however, it was acceptable for the phenyl label (95.1%). It is not known whether this is due to heterogeneity within the sample, or an unexplained loss was observed. Accountability in all other matrices was acceptable; therefore, this low result is not considered to invalidate the results of the study. The major residues in extract of liver samples were 0.112 – 0.164 mg eq./kg (as sum of two isomers) identified as sulphate conjugates of 1'-CH_2OH-S-2840. Other metabolites identified in liver extracts were metabolites *N*-des-Me-S-2840 and 1'-COOH-S-2840 A&B.

The fat samples (abdominal and subcutaneous) residues ranged between 0.064 – 0.102 mg eq./kg. Residues of inpyrfluxam were identified at levels between 0.045 – 0.075 mg eq./kg. Chiral separation of inpyrfluxam isolated from extracts of fat samples showed only the R-enantiomer. No epimerisation of the chiral carbon in inpyrfluxam occurs in the hen.

The residues in muscle samples (thigh and breast) were between 0.012 – 0.023 mg eq./kg in hens after being dosed for 7 days. Chromatographic characterisation of the neutral solvent soluble residues showed multiple components. The largest

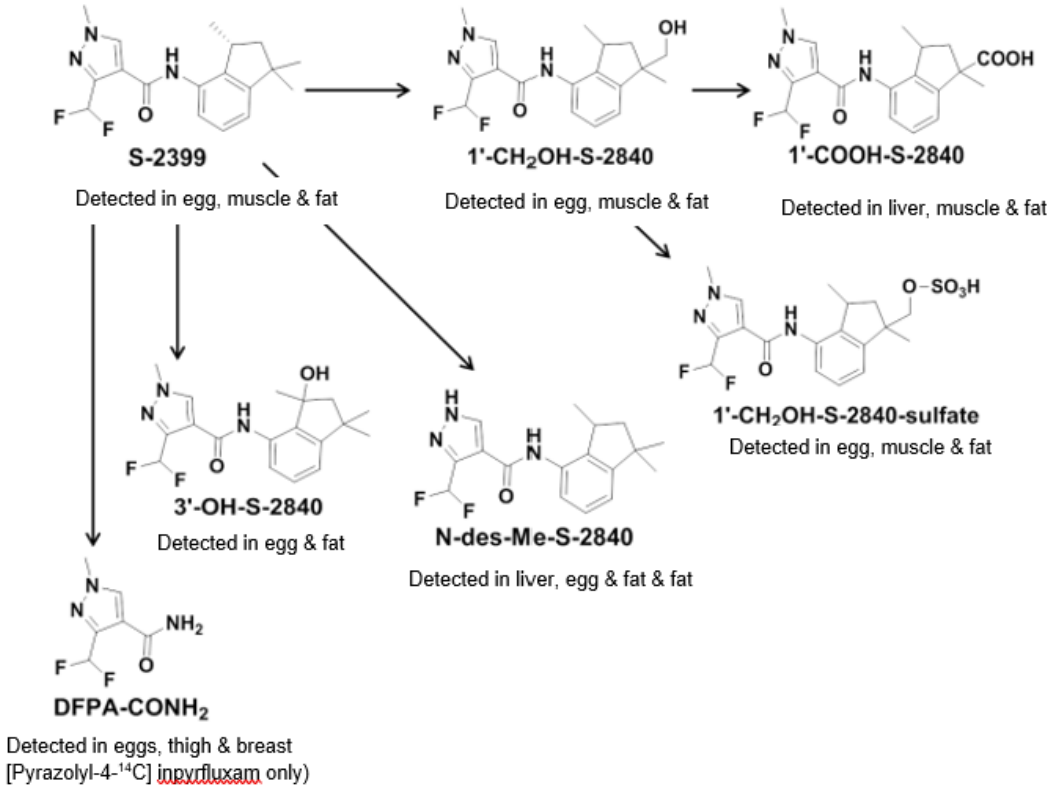
metabolites were characterised as sulphate conjugates of 1'-CH_2OH-S-2840 at levels ≤0.008 mg eq./kg. Other metabolites identified at low levels (≤ 0.003 mg eq./kg) included inpyrfluxam, 1'-COOH-S-2840-A&B and DFPA-$CONH_2$.

In the hen metabolism samples (hen fat: hexane extracts) chiral methodology was used to confirm the stability of the R-isomer. There was no marked conversion from the R-isomer (to the S-isomer). The hen fat extracts were mainly R-isomer (S-isomer: 3 – 4%).

The metabolism of inpyrfluxam in hens following the oral administration of inpyrfluxam proceeds via the oxidation to from the hydroxylated component 1'-CH_2OH-S-2840 (isomers). This metabolite is further transformed by oxidation to the carboxylic acid metabolite 1'-COOH-S-2840. The primary alcohol of 1'-CH_2OH-S-2840 forms the corresponding sulphate conjugates. *N*-demethylation on the pyrazolyl ring forms the metabolite *N*-des-Me-S-2840. *N*-demethylation or cleavage of the amide bond of inpyrfluxam form the metabolites *N*-des-Me-S-2840 and DFPA-$CONH_2$ respectively.

Based on the results the metabolism of both [phenyl-U-^{14}C] inpyrfluxam and [pyrazolyl-4-^{14}C] inpyrfluxam in laying hens is considered understood and a metabolic pathway in laying hens is proposed (see figure 7.2.2.1-1).

Figure 7.2.2.1-1 Proposed metabolic pathway of inpyrfluxam in laying hens



B.7.2.3. Lactating ruminants

The metabolism of inpyrfluxam was investigated in lactating goats after oral administration with either phenyl or pyrazolyl labelled inpyrfluxam.

Table 7.2.3-1 Overview of ruminant metabolism studies

Ruminant	Application		Dose
Lactating goat	[pyrazolyl-4-^{14}C] inpyrfluxam	5 daily administrations of [pyrazolyl-4-^{14}C] inpyrfluxam in cellulose-filled gelatine capsules	13.74 mg a.s./kg feed/day (0.51 mg a.s./kg bw/d)
	[phenyl-U-^{14}C] inpyrfluxam	5 daily doses of [phenyl-U-^{14}C] inpyrfluxam in cellulose-filled gelatine capsules	15.74 mg a.s./kg feed/day (0.64 mg a.s./kg bw/d)

Report:	**KCA 6.2.3/01; [redacted] and [redacted] 2016;**
Title:	Metabolism of [^{14}C]S-2399 (2 radiolabels) in the lactating goat 20-Sep-2016 (Final report) 26-Apr-2017 (Amended Final report)
Document No.:	TPM-0024 (Study No.: 2452W)
Guidelines:	OPPTS 860.1300 Nature of the Residue—Plants, Livestock, August 1996 OECD/OCDE 503 Metabolism in Livestock, January 2007 JMAFF 2-4-2 Livestock Metabolism, December 2014
Guideline deviations:	None
GLP/GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

Summary

The metabolism of inpyrfluxam labelled either in the pyrazolyl ring (pyrazolyl label) or the phenyl ring (phenyl label) was investigated in lactating goats (*Capra hircus*; breed "*Alpine*").

Goats were housed individually and were determined to be in good health by a veterinarian prior to the start of the study. Milk production was monitored prior to dosing and was considered acceptable. The goats were provided with a diet typical of commercial goat husbandry and fresh potable water *ad libitum* as well as Sprout Sweet Pellet 16% Goat Chow and alfalfa hay via feed bins.

The test compound was administered orally to two goats (1 goat per radiolabel) in cellulose-filled gelatine capsules at the intended dose of 10.5 mg/kg feed/day based on dry matter in the diet (actual dose 13.74 mg/kg dry feed/day for [pyrazolyl-4-^{14}C] inpyrfluxam and 15.74 mg/kg dry feed/day for [phenyl-U-^{14}C] inpyrfluxam). This equates to 0.51 and 0.63 mg/kg body weight/day for the goat treated with [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam respectively.

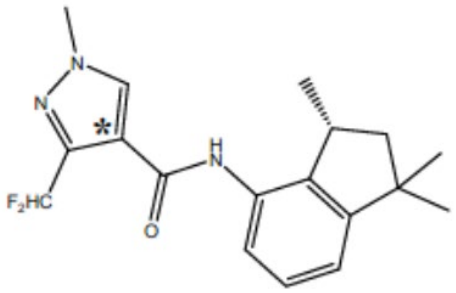
The average daily consumption of feed over the 5 treatment days and the average body weights on day of receipt of animal, study day 1 and day 5 were used to calculate the actual dose levels. The test material contents were suitably verified. The test substance used to dose goats showed >99.9% of the R-enantiomer. Each goat received one dose per day for 5 consecutive days, administered after the morning collection of milk, urine and excreta.

Urine, faeces, and milk were collected from the animals prior to the administration of the first dose to show the absence of ^{14}C (above background levels) in the test animals.

Materials

[pyrazolyl-4-^{14}C] inpyrfluxam

Chemical Structure



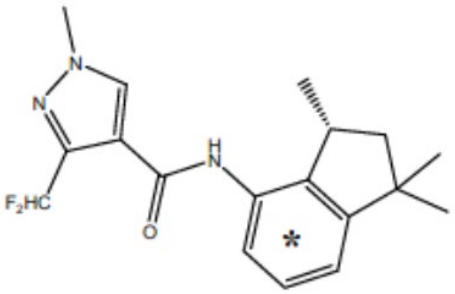
* denotes the ^{14}C-label position

Radiolabelled test material [pyrazolyl-4-^{14}C] inpyrfluxam

Specific radioactivity	Delivered sample before radio dilution: 2.11 GBq/mmol (57 mCi/mmol) = 3.78X10^8 dpm/mg	After isotopic dilution: 1.57X10^8 dpm/mg
Radiochemical purity	99.3%	
Chemical purity	99.3%	
Dose level	5 doses of 13.74 mg/kg feed/day	

[phenyl-U-^{14}C] inpyrfluxam

Chemical Structure



* denotes the ^{14}C-label position

Radiolabelled test material	[phenyl-U-^{14}C] inpyrfluxam	
Specific radioactivity	Delivered sample before radio dilution: 4.51 GBq/mmol (122 mCi/mmol) = 8.03X10^8 dpm/mg	After isotopic dilution: 1.61X10^8 dpm/mg

Radiochemical purity	99.4%
Chemical purity	99.5%
Dose level	5 doses of 15.74 mg/kg feed/day

Sampling

Milk was collected twice daily at approximately 12 hour intervals. After the completion of the in-life phase of the study, portions of the milk samples were separated by centrifugation to milk fat and skimmed milk to be analysed separately.

Urine and faeces were collected twice daily, prior to the morning dosing and approximately 11 hours later. For urine, a small amount of acetonitrile (ACN) was added to the collection vessel as a preservative. Urine composites were prepared by combining 10% (by weight) of each urine sample from Day 2 – 6. For faeces, the samples were composited by combining 5% (by weight) of each faeces sample from Day 2 – 6.

At the conclusion of the in-life phase, the faeces and urine collection chambers were rinsed with ACN. This cage wash was collected for analysis.

The animals were sacrificed approximately 7 hours after the last dose. The following tissue samples were collected from each animal: the bile from the bile ducts, the entire liver, both kidneys, loin muscle, flank muscle, omental fat, subcutaneous fat and blood. The edible tissue samples (liver, kidney, muscle and fat) were rinsed with water and blotted dry before being homogenized in the presence of dry ice.

Blood was placed into containers with anticoagulant. Urine found in the bladder at the time of the sacrifice was added to the last collected urine sample.

The entire GI tract was removed and homogenized in the presence of dry ice. Three equal sub-samples were collected from different locations.

All samples were placed in frozen storage after collection and processing until shipment.

Analytical procedures

The measurement of the [^{14}C] content of the samples was carried out by liquid scintillation counting. All sample count rates were corrected for the background of LSC cocktail.

Sub-samples of skim-milk, urine and cage wash were analysed directly by LSC at least in triplicate. The radioactive residues in milkfat, muscle, fat, liver and kidney

samples were measured by tissue digestion of sub-samples with a tissue solubilizer (Soluene). Acetic acid and scintillation cocktail were added and the ^{14}C contents of the samples were measured by LSC.

Matrices not amenable to direct radio analysis measurement using LSC were radio assayed by oxidation of the organic content into $^{14}CO_2$ using a biological oxidizer. The $^{14}CO_2$ was trapped with carbon 14 cocktail and the ^{14}C content determined by LSC. Portions of the blood, faeces, bile and homogenized gastrointestinal tracts (typically five aliquots) were subjected to combustion analysis/LSC.

Faeces, Muscle, liver and kidney portions were extracted twice with acetonitrile:water (1:1, v/v) and once with ACN using a wrist action shaker for 45 minutes followed by separation of the solid and liquid phase by centrifugation (10 min at 10,000 rpm). The radioactive contents of the extracts were measured by LSC. The extracts were combined and concentrated prior to chromatographic characterization. PES was analysed by combustion analysis/LSC.

Milk fat was extracted twice with hexane:acetone (4:1, v/v) and once with acetone using a wrist action shaker for 45 minutes followed by separation of the solid and liquid phase by centrifugation (10 min at 4,000 rpm). Aliquots of each extract were taken for LSC analysis. The PES for both labels were negligible (≤ 0.001 mg eq./kg, ≤ 5.56% TRR) and were not analysed. The hexane:acetone extracts were reduced in volume using a rotary evaporator to remove the acetone. The concentrated extract was transferred to separating funnel and partitioned between hexane and three portions of ACN. The hexane and combined ACN partition fractions were radio assayed by LSC. The combined ACN extracts were concentrated prior to chromatographic characterisation.

Skimmed milk samples were extracted once with acetone, followed by acetone:water (1:1, v/v) and again with acetone using a wrist action shaker for 45 minutes followed by separation of the solid and liquid phase by centrifugation (10 min at 10,000 rpm). Extracts were aliquoted for LSC analysis. PES were analysed by combustion analysis/LSC. The extracts were concentration for HPLC analysis.

Omental, subcutaneous and renal fat portions were extracted once with hexane:acetone (4:1, v/v) and twice with acetone using a wrist action shaker for 45 minutes followed by separation of the solid and liquid phase by centrifugation (10 min at 10,000 rpm). PES were analysed by combustion analysis/LSC. The hexane:acetone extracts were combined, concentrated and transferred to a separatory funnels. The residues were partitioned between hexane and three portions of acetonitrile The radioactive contents of the acetonitrile and hexane layers were measured by LSC. The acetonitrile fraction was concentrated for chromatographic characterisation.

Radiolabelled residues were characterised by linear gradient reverse phase HPLC using co-chromatography with reference standards. The identity of the residues was assigned based on HPLC retention times. Identity of components were confirmed by TLC using comparison of Rf values to standards analysed with the sample or by LC-MS.

Sample extracts characterized as containing parent compound were partitioned between hexane and water to selectively isolate parent compound into the hexane fraction prior to HPLC chiral separation.

Results and discussion

During the study all goats were examined twice a day for clinical abnormalities and any overt changes in health. The goats were observed to remain healthy throughout the course of the study and milk production remained normal based on the goats age, stage of lactation and time of year. A decrease in the average daily feed consumption was observed for both goats during the dosing phase of the study (average during dosing:1929.5 – 2148.5 g feed / day vs. average prior to dosing, during quarantine period: 2517.8 – 2534.3 g feed / day) which was attributed by the applicant to be related to the dosing activity. A decrease in body weight occurred during the study (maximum change of 1.4 kg was recorded over the 5 treatment days). No deviations from the study plan were reported.

The total recovery of the administered dose in excreta and tissues was 96.97% for [pyrazolyl-4-^{14}C] inpyrfluxam and 97.11% for [phenyl-U-^{14}C] inpyrfluxam.

The majority of the administered dose was recorded in excreta (urine and faeces) accounting for >76% of the dose. The overall distribution of the percentage dose excreted for goats was similar in [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam dosed goats. The amounts remaining in the GI tracts at sacrifice were > 18.61% of the initial dose. A small amount of radioactivity was recovered from the cage wash (< 0.09%).

The dose % recovered in tissues and milk was < 1% for both radiolabelled substances. The TRR values (mg eq./kg) were similar between both radioactive labels. Radioactive residue levels in milk (skimmed and fat) were similar in both labels. In whole milk, day 1 residues were between 0.031 – 0.035 mg eq./kg and by day 5 they had only risen slightly to be 0.040 mg eq./kg for both radiolabelled test substances.

Table 7.2.3.1-1 Distribution of residues in matrices of lactating goats following oral administration of 5 daily doses of [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam

Radioactivity Balance				
^{14}C-inpyrfluxam	Pyrazolyl-^{14}C Label		Phenyl-^{14}C Label	
Tissues	% of Total Dosed radioactivity	mg eq./kg[(a)]	% of Total Dosed radioactivity	mg eq./kg[(a)]
Liver	0.24	0.334	0.26	0.350
Kidney	0.02	0.169	0.02	0.166
Flank Muscle	≤0.01	0.015	≤0.01	0.024
Loin Muscle	≤0.01	0.011	0.01	0.016
Omental Fat	≤0.01	0.007	≤0.01	0.024
Subcutaneous Fats	≤0.01	0.017	≤0.01	0.029
Renal Fats	≤0.01	0.009	≤0.01	0.040
Skimmed Milk	0.11	NA	0.08	NA
Milk Fat	0.01	NA	0.01	NA
Faeces	41.12	5.880	44.61	6.004
G.I Tract	19.80	1.678	18.61	1.893
Cage wash[1]	0.09	0.541	0.07	0.437
Urine	35.35	4.336	33.38	6.184
Bile	0.23	9.196	0.05	12.406
Blood	≤0.01	0.039	≤0.01	0.048
Total	**96.97**		**97.11**	

[(a)] The TRR values (mg eq./kg) are derived from initial LSC analysis.
NA = not applicable due to multi-day collection

The majority of residues were efficiently extracted using the neutral solvents. In all matrices, > 90% of the [^{14}C] Inpyrfluxam residues were extracted, excluding omental, subcutaneous and renal fat for the pyrazolyl label were 83.33, 83.33 and 71.43% TRR were extracted respectively. For these fat matrices, PES were < 30% TRR,

however the absolute residues were very low (≤ 0.002 mg eq./kg) so no further characterisation was needed.

The extractability and accountability of residues in summarised in Table 7.2.3.1-2.

Accountability in all matrices was > 85%, apart from subcutaneous and renal fat in the pyrazolyl label were it was 70 and 77%. This is considered acceptable given that the overall TRR, determined by LSC for both subcutaneous and renal fat was low (≤ 0.017 mg eq./kg).

The highest TRR in edible matrices, based on the sum of extracted residue and PES, was found in the liver (0.313 mg eq./kg for the pyrazolyl-label and 0.344 mg eq./kg for the phenyl-label). Kidney had a TRR of 0.162 mg eq./kg for the pyrazolyl label and 0.170 mg eq./kg for the phenyl-label. Radioactive residues in fat and muscle tissues were ≤ 0.041 mg eq./kg with [phenyl-U-^{14}C] inpyrfluxam qualitatively similar to the [pyrazolyl-4-^{14}C] inpyrfluxam residues in tissues.

Radioactive residue levels in milk (skimmed and fat) were similar in both labels. On day 1 the residues in whole milk were between 0.031 – 0.035 mg eq./kg, by day 5 residues in milk were 0.040 mg eq./kg for both radiolabelled test substances. The residues extracted in milk over the 5 day dosing period can be found in Table 7.2.3.1-3.

Table 7.2.3.1-2 Extraction efficiency of ruminant samples following 5 daily doses of [pyrazolyl-4-^{14}C] inpyrfluxam or [phenyl-U-^{14}C] inpyrfluxam

[Pyrazolyl-^{14}C] inpyrfluxam									
Tissue	**Extract 1**	**Extract 2**	**Extract 3**	**Total Extracted Radioactive Residue (ERR)**		**Post Extract Solids**		**TRR[a]**	**Accountability[b]**
	% Extracted			**mg eq./kg**	**%ERR**	**mg eq./kg**	**%TRR**	**mg eq./kg**	**%**
Liver	88.07	10.88	1.05	0.285	91.05	0.028	8.95	0.313	93.7
Kidney	84.91	13.21	1.89	0.159	98.15	0.003	1.85	0.162	95.9
Flank muscle	85.71	14.29	≤0.001	0.014	100.00	≤0.001	≤0.10	0.014	93.3
Loin muscle	81.82	18.18	≤0.001	0.011	91.67	0.001	8.33	0.012	109.1
Omental fat	80.00	20.00	≤0.001	0.005	83.33	0.001	16.67	0.006	85.7
Subcutaneous fat	60.00	40.00	≤0.001	0.010	83.33	0.002	16.67	0.012	70.6
Renal fat	40.00	40.00	20.00	0.005	71.43	0.002	28.57	0.007	77.8
Skimmed milk	94.12	5.88	≤0.001	0.034	100.00	≤0.001	≤0.10	0.034	NC
Milk fat	72.41	20.69	6.90	0.029	100.00	≤0.001	≤0.10	0.029	NC
Faeces	74.61	20.98	4.41	5.152	91.04	0.507	8.96	5.659	96.2

[Phenyl-^{14}C] inpyrfluxam									
Tissue	**Extract 1**	**Extract 2**	**Extract 3**	**Total Extracted Radioactive Residue (ERR)**		**Post Extract Solids**		**TRR[a]**	**Accountability[b]**
	% Extracted			**mg eq./kg**	**%ERR**	**mg eq./kg**	**%TRR**	**mg eq./kg**	**%**
Liver	89.39	9.65	0.96	0.311	90.41	0.033	9.59	0.344	98.3
Kidney	85.54	13.25	1.20	0.166	97.65	0.004	2.35	0.170	102.4
Flank muscle	85.00	15.00	≤0.001	0.020	95.24	0.001	4.76	0.021	87.5
Loin muscle	85.71	14.29	≤0.001	0.014	93.33	0.001	6.67	0.015	93.8
Omental fat	71.43	23.81	4.76	0.021	87.50	0.003	12.50	0.024	100.0
Subcutaneous fat	60.71	35.71	3.57	0.028	96.55	0.001	3.45	0.029	100.0
Renal fat	64.87	32.43	2.70	0.037	90.24	0.004	9.76	0.041	102.5
Skimmed milk	90.00	7.50	2.50	0.040	100.00	≤0.001	≤0.10	0.040	NC
Milk fat	52.94	41.18	5.88	0.017	94.44	0.001	5.56	0.018	NC
Faeces	76.53	19.29	4.18	5.568	91.52	0.516	8.48	6.084	101.3

NC = Not calculated

[a] TRR determined by sum of fractions (extracts 1 – 3 + PES = TRR) used for distribution of residues characterised by chromatographic methods.

[b] accountability calculated from the [(sum of ERR + PES) / (initial TRR)] x 100

Table 7.2.3.1-3 Time course of radioactivity in milk following oral administration of 5 daily doses of [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam

Sample	Hours	[pyrazolyl-4-^{14}C] inpyrfluxam mg eq./kg			[phenyl-U-^{14}C] inpyrfluxam mg eq./kg		
		Skimmed Milk	Milk Fat	Whole Milk	Skimmed Milk	Milk Fat	Whole Milk
Day 1 AM	0	0.000	0.000	0.000	0.000	0.000	0.000
Day 1 PM	12	0.032	0.022	0.031	0.036	0.032	0.035
Day 2 AM	24	0.014	0.011	0.014	0.014	0.014	0.014
Day 2 PM	36	0.034	0.024	0.033	0.034	0.032	0.034
Day 3 AM	48	0.015	0.013	0.015	0.014	0.014	0.014
Day 3 PM	60	0.033	0.025	0.032	0.034	0.033	0.034
Day 4 AM	72	0.017	0.013	0.017	0.015	0.015	0.015
Day 4 PM	84	0.038	0.027	0.037	0.039	0.037	0.039
Day 5 AM	96	0.016	0.013	0.016	0.013	0.013	0.013
Day 5 PM	108	0.041	0.030	0.040	0.039	0.042	0.040

Parent compounds and metabolites were characterised using HPLC co-chromatography with reference standards. The identity of the residues was assigned based on HPLC retention times. Identities of components were confirmed by TLC using comparison of R_f values to standards which were analysed with the sample or by LC-MS.

To further investigate metabolites in liver and kidney extracts hydrolysis experiments were performed with the addition of β-glucuronidase and 0.5M, 1M and 2M HCl to concentrated samples.

Furthermore, isolation and identification of particular chromatographic components was performed. A mixture of 7 components were found to elute between 30 – 45 min in milk samples. Urine samples showed the same components eluting between 29 – 37 minutes. Radioactive residues in these peaks had 10 – 20 times higher concentration in urine than tissues therefore urine was used to isolate the two major components (> 0.050 mg eq./kg) to confirm their identity. A sample of the urine was purified by solid phase extraction followed by separation by HPLC.

Some samples contain high %TRR of unknown compounds however, the absolute residue values (mg eq./kg) remain low so further consideration of these where not required.

A summary of identified metabolites can be seen in Table 7.2.3.1-4 and 7.2.3.1-5 for edible matrices and Table 7.2.3.1-6 for urine, faeces and bile.

Metabolites in liver

Goats dosed with [pyrazolyl-4-^{14}C] inpyrfluxam

The TRR determined by the sum of extractable residue plus post extract solids was 0.313 mg eq./kg in liver collected from goat treated with [pyrazolyl-4-^{14}C] inpyrfluxam. Extraction with neutral solvent solubilised 91.05% TRR (0.285 mg eq./kg) with 8.95% TRR remaining in the post extraction solids. Since residues in the post extraction solids were < 10% TRR and < 0.05 mg eq./kg (0.028 mg eq./kg) no further characterisation was performed in line with OECD guidance.

The largest chromatographic peak accounted for 31.70% TRR which co-chromatographed with 1'-COOH-S-2480A. Also characterised in liver extracts were glucuronide conjugates of 1'-CH_2OH-S-2480 (Glu-1'-CH_2OH-S-2480 with a total of 15.88% TRR, relating to 0.050 mg eq./kg. Trace amounts of DFPA-$CONH_2$ (2.54% TRR, 0.008 mg eq./kg), 1'-CH_2OH-S-2840-A (4.92% TRR, 0.015 mg eq./kg) and inpyrfluxam (5.94% TRR, 0.019 mg eq./kg) were detected.

Only the R-enantiomer of inpyrfluxam was present in the hexane partition fraction. The analysis of the hydrolysed extract showed the largest component was a peak consistent with 1'-COOH-S-2840-A (Rt 30). The Rt 32 peak was no longer present, and the increased peaks at Rt 45 and Rt 46 appeared consistent with 1'-CH_2OH-S-2840-A and 1'-CH_2OH-S-2840-B. Results from the acid hydrolysis are consistent with the presence of glucuronide conjugates of 1'-CH_2OH-S-2840 in the soluble residues from liver including the Rt 32 component.

Up to 19.67% of the TRR was not identified. This was made up of 5 peaks, with the largest individual peak accounting for 5.09% TRR (0.016 mg eq./kg). As this is < 10% TRR and < 0.05 mg eq./kg no further consideration is required.

Goats dosed with [phenyl-U-^{14}C] inpyrfluxam

The TRR from extraction and post-extraction solids was 0.344 mg eq./kg in liver samples from a goat dosed with [phenyl-U-^{14}C] inpyrfluxam. Extraction with neutral solvents solubilised 90.41% TRR with 9.59% TRR remaining in the post extraction solids. Post extraction solids were < 10% TRR which accounts for < 0.05 mg eq./kg so no further characterisation of the residues on post extraction solids was performed.

Reverse phase HPLC of the combined extracts. The major peak co-eluted with the reference compound 1'-COOH-S-2840-A (30.20% TRR, 0.104 mg eq./kg). Also characterised were Glu-1'-CH_2OH-S-2480 (19.17% TRR, 0.066 mg eq./kg), 1'-COOH-S-2840-B (5.13% TRR, 0.018 mg eq./kg), 1'-CH_2OH-S-2840-A (6.27% TRR, 0.022 mg eq./kg) and inpyrfluxam (4.94% TRR, 0.017 mg eq./kg).

Up to 24.68% of the TRR was not identified. This was made up of 4 peaks, with the largest individual peak accounting for 7.59% TRR (0.026 mg eq./kg). As this is < 10% TRR and < 0.05 mg eq./kg no further consideration is required.

Metabolites in Kidney

Goats dosed with [pyrazolyl-4-^{14}C] inpyrfluxam

Extraction with neutral solvents solubilised 98.15% TRR (0.159 mg eq./kg) with 1.85% TRR remaining in the post extraction solids. The TRR determined by the sum of fractions in kidney was 0.162 mg eq./kg.

HPLC characterisation of the kidney soluble residues showed the largest chromatographic peak was 39.12% TRR co-eluting with 1'-COOH-S-2840-A. Other peaks characterised were Glu-1'-CH_2OH-S-2480 (24.49% TRR, 0.040 mg eq./kg (sum of isomers)), 1'-COOH-S-2840-B (10.56% TRR, 0.017 mg eq./kg) and 3'-OH-S-2840 (3.11% TRR, 0.005 mg eq./kg). The peak eluting at 34.45 minutes accounting for 9.18% TRR was consistent with the reference compound 1',1'-bis(CH_2OH)-S-2480 but was revealed by TLC to be comprised of 3 separate components, including1′,1′-bis-(CH_2OH)-S-2840-B, Glu-1′-CH_2OH-S-2840 and an unknown.

Up to 19.81% of the TRR was not identified (unknown + other). This was made up of 5 peaks, with the largest individual peak accounting for 6.06% TRR (0.010 mg eq./kg). As this is < 10% TRR and < 0.05 mg eq./kg no further consideration is required.

Goats dosed with [phenyl-U-^{14}C] inpyrfluxam

The TRR determined by extraction including post extraction solids was 0.170 mg eq./kg. Neutral solvent solubilised 97.65% TRR with only 2.35% remaining in the PES solids.

HPLC analysis of the combined extracts of kidney from goats treated with [phenyl-U-^{14}C] inpyrfluxam. Inpyrfluxam was not found. The highest residue was metabolite 1'-COOH-S-2840-A (34.42% TRR, 0.059 mg eq./kg) along with Glu-1'-CH_2OH-S-2840 (20.68% TRR, 0.035 mg eq./kg), 1'-COOH-S-2840-B (10.94% TRR, 0.019 mg eq./kg), 1'-CH_2OH-S-2840-A (3.36% TRR, 0.006 mg eq./kg) and Glu-1'-CH_2OH-S-2840 (12.77% TRR, 0.022 mg eq./kg) and 3'-OH-S-2840 (1.84% TRR, 0.003 mg eq./kg).

Up to 13.63% of the TRR was not identified. This was made up of 3 peaks, with the largest individual peak accounting for 6.82% TRR (0.012 mg eq./kg). As this is < 10% TRR and < 0.05 mg eq./kg no further consideration is required.

Metabolites in muscle

For both labels, the TRR in muscle samples were low between 0.011 – 0.020 mg eq./kg. Neutral solvent solubilised > 91% TRR in muscle samples with post extraction residues acquainting to ≤ 0.001 mg eq./kg, as these are below the level included in the OECD guidance (0.05 mg eq./kg) no further characterisation is required.

Up to 21.46% of the TRR was not identified. This was made up of 4 peaks, with the largest individual peak accounting for 9.01% TRR (0.002 mg eq./kg). As this is < 10% TRR and < 0.05 mg eq./kg no further consideration is required.

Flank muscle

Goats treated with [pyrazolyl-4-^{14}C] inpyrfluxam

The TRR determined by sum of fractions was 0.014 mg eq./kg. The TRR in the neutral solvent extract was 100.0% TRR (0.014 mg eq./kg) with ≤ 0.001 mg eq./kg remaining in the post extraction solids.

Reverse phase HPLC of the soluble residues show components characterised as 1'-COOH-S-2840-A (20.17% TRR, 0.003 mg eq./kg), 1'-COOH-S-2840-B (23.61% TRR, 0.003 mg eq./kg). Glu-1'-CH_2OH-S-2840 (10.09% TRR, 0.001 mg eq./kg), 1'-CH_2OH-S-2840-A (8.37% TRR, 0.001 mg eq./kg) and DFPS-$CONH_2$ (11.16% TRR, 0.002 mg eq./kg). Inpyrfluxam was not detected.

Goats treated with [phenyl-U-^{14}C] inpyrfluxam

The TRR determined by the sum of fractions was 0.021 mg eq./kg. Neutral solvent solubilised 95.24% TRR with 4.76% TRR (0.001 mg eq./kg) remaining in the post extraction solids.

Chromatographic characterisation of the soluble residue showed 1'-COOH-S-2840-B (28.67% TRR, 0.006mg/kg), 1'-COOH-S-2840-A (17.77% TRR, 0.004 mg eq./kg), Glu-1'-CH_2OH-S-2840 (24.40% TRR, 0.005 mg eq./kg – sum of isomers) and 1'-CH_2OH-S-02840-A (7.82% TRR, 0.002 mg eq./kg).

Loin muscle

Goats treated with [pyrazolyl-4-^{14}C] inpyrfluxam

The TRR determined by the sum of fractions was 0.012 mg eq./kg with 91.67% TRR extracted with neutral solvents and 8.33% (0.001 mg eq./kg) in the post extraction solids.

Chromatographic characterisation of the soluble residues from loin muscle did not show any inpyrfluxam. The highest residues were 1'-COOH-S-2840-A (18.87% TRR, 0.002 mg eq./kg) and 1'-COOH-S-2840-B (14.73% TRR, 0.002 mg eq./kg). Other metabolites identified were Glu-1'-CH_2OH-S-2840 (16.02% TRR, 0.002 mg eq./kg – sum of isomers) and 1'-CH_2OH-S-2840-A (5.57% TRR, 0.001 mg eq./kg).

Goats treated with [phenyl-U-^{14}C] inpyrfluxam

The TRR determined by extraction and post extraction solids was 0.015 mg eq./kg. Extraction with neutral solvents solubilised 93.33% TRR (0.014 mg eq./kg) with only 6.67% (0.001 mg eq./kg) TRR remaining in the post extraction solids.

Chromatographic characterisation of the soluble residues showed no presence of inpyrfluxam. The highest residue was Glu-1'-CH_2OH-S-2840 (28.56% TRR, 0.004 mg eq./kg). Other metabolites identified were 1'-COOH-S-2840-A (17.08% TRR, 0.003 mg eq./kg), 1'-COOH-2840-B (9.99% TRR, 0.001 mg eq./kg) and 1'-CH_2OH-S-2840-A (6.63% TRR, 0.001 mg eq./kg).

Metabolites in fat

For both labels, the TRR in fat samples were low (≤ 0.041 mg eq./kg). Neutral solvent solubilised ≥ 83.3% TRR in fat samples with post extraction residues acquainting to ≤ 0.004 mg eq./kg, as these are below the level included in the OECD guidance (0.05 mg eq./kg) no further characterisation is required.

Up to 37.25% of the TRR was not identified. This was made up of 10 peaks, with the largest individual peak accounting for 10.5% TRR (0.005 mg eq./kg). As overall absolute residues in fat are low (< 0.05 mg eq./kg) and the largest unknown peak is < 0.05 mg eq./kg no further consideration is required.

Omental fat

Goats treated with [pyrazolyl-4-^{14}C] inpyrfluxam

The TRR determined by extraction and post extracted solids was 0.006 mg eq./kg. Due to the low levels detected no further characterisation was performed in line with OECD guidance.

Goats treated with [phenyl-U-^{14}C] inpyrfluxam

The TRR determined by the sum of fractions was 0.024 mg eq./kg with 87.50% TRR in the soluble residues and 12.50% TRR (0.003 mg eq./kg) remaining in the post

extraction solids as this is below the triggers included in the OECD guidance no further characterisation is required.

The distribution of components by HPLC showed chromatographic peaks corresponding to inpyrfluxam (15.84% TRR, 0.004 mg eq./kg), 1'-COOH-S-2480A (29.40% TRR, 0.007 mg eq./kg), 1'-COOH-S-2840-B (4.38% TRR, 0.001 mg eq./kg) and 1'-CH_2OH-S-2840-A (8.58% TRR, 0.002 mg eq./kg).

Subcutaneous fat

Goats treated with [pyrazolyl-4-^{14}C] inpyrfluxam

The TRR determined by the sum of fraction was 0.012 mg eq./kg. 83.33% TRR was extracted in the neutral solvents with 16.67% TRR (0.002 mg eq./kg) remaining in the post extraction solids, although the PES are > 10% TRR as the absolute residues are < 0.05mg eq./kg in line with the OECD guidance no further characterisation is required.

Distribution of the components demonstrated by HPLC showed the largest components were 1'-COOH-S-2840-A (27.17% TRR, 0.003 mg eq./kg) and 1'-CH_2OH-S-2840-A (6.08% TRR, 0.001 mg eq./kg). Trace amounts of inpyrfluxam (≤0.001 mg eq./kg) were detected.

Goats treated with [phenyl-U-^{14}C] inpyrfluxam

The total TRR was 0.029 mg eq./kg. The soluble residues accounted for 96.55% TRR while post extraction solids accounted for 3.54% (0.001 mg eq./kg), as residues in the PES are < 10% TRR and 0.05 mg eq./kg no further characterisation is required in line with the guidance.

The distribution of radioactivity determined by HPLC analysis showed the highest residue was 1'-COOH-S-2840-A (32.25% TRR, 0.009 mg eq./kg). Other metabolites identified were 1'-CH_2OH-S-2840-A (10.43% TRR, 0.003 mg eq./kg) and inpyrfluxam (6.37% TRR, 0.002 mg eq./kg).

Renal fat

Goats dosed with [pyrazolyl-4-^{14}C] inpyrfluxam

The TRR determined by the sum of fractions was 0.007 mg eq./kg. Due to the residues being < 0.01 mg eq./kg no further characterisation was performed in line with the guidance.

Goats dosed with [phenyl-U-^{14}C] inpyrfluxam

The TRR determined by the sum of fractions was 0.041 mg eq./kg. 90.24% TRR was extracted in the neutral solvent and 9.76% TRR remaining in the post extraction solids, as the PES TRR is < 10% no further characterisation was done in line with the OECD guidance triggers.

Analysis by HPLC showed components that co-chromatographed with 1'-COOH-S-2840-A (36.10% TRR, 0.0146 mg eq./kg), 1'-COOH-S-2840-B (3.61% TRR, 0.002 mg eq./kg) and inpyrfluxam (8.27% TRR, 0.004 mg eq./kg).

Metabolites in Milk

For both labels, samples of skimmed milk and milk fat from day 4 PM were used for characterisation of the residues as these samples contained a high level of residue.

Skimmed milk

Up to 80.1% of the TRR was not identified. This was made up of 9 peaks, with the largest individual peak accounting for 21.22% TRR (0.008 mg eq./kg). As overall absolute residues in skim milk are low (< 0.05 mg eq./kg) and the largest unknown peak is < 0.05 mg eq./kg no further consideration is required.

Goats dosed with [pyrazolyl-4-^{14}C] inpyrfluxam

The TRR for skimmed milk was 0.034 mg eq./kg. One peak co-chromatographed with 1'-COOH-S-2840-A (12.10% TRR, 0.004 mg eq./kg) and another with DFPA-$CONH_2$ (2.10% TRR, 0.001 mg eq./kg). Inpyrfluxam was not detected.

Goats dosed with [phenyl-U-^{14}C] inpyrfluxam

The TRR was 0.040 mg eq./kg. Metabolites co-chromatographed with standards of 1'-COOH-S-2840-A (9.94% TRR, 0.004 mg eq./kg) and 1'-COOH-S-2840-B (5.963% TRR, 0.002 mg eq./kg). Inpyrfluxam was not detected in skimmed milk.

Milk fat

Up to 91.2% of the TRR was not identified. This was made up of 7 peaks, with the largest individual peak accounting for 32.90% TRR (0.01 mg eq./kg). As overall absolute residues in milk fat are low (≤ 0.029 mg eq./kg) and the largest unknown peak is < 0.05 mg eq./kg no further consideration is required.

Goats dosed with [pyrazolyl-4-^{14}C] inpyrfluxam

Extraction of the Day 4 PM sample solubilised 100% TRR (0.029 mg eq./kg) with ≤ 0.001 mg eq./kg remaining in the post extraction solids. Only 8.80% of the TRR (0.003 mg eq./kg) was identified as 1'-COOH-S-2840-A. Although the % TRR unknown is high, the absolute amounts (in mg eq./kg) of the individual components

are very low so it is considered acceptable that further identification has not been carried out.

Goats dosed with [phenyl-U-^{14}C] inpyrfluxam

Day 4 PM milk fat sample had a total residue of 0.017 mg eq./kg. HPLC separation showed peaks corresponding to inpyrfluxam (9.07% TRR, 0.002 mg eq./kg), 1'-CH_2OH-S-2840-A (3.02% TRR, 0.001 mg eq./kg) and 1'-COOH-S-2840-A (5.84% TRR, 0.001 mg eq./kg). Given the low absolute levels of the individual components in the unknown it is considered acceptable that further identification was not considered.

Metabolites in Faeces

Goats dosed with [pyrazolyl-4-^{14}C] inpyrfluxam

The TRR determined by the sum of fractions was 5.659 mg eq./kg. Extraction solubilised 91.04% TRR with 8.96% TRR (0.507 mg eq./kg) remaining in the post extraction solids. The major metabolite identified was 1'-COOH-S-2840-A (63.69% TRR, 3.604 mg eq./kg). Several other small peaks were identified to be 1'-COOH-S-2840-B (2.72% TRR, 0.154 mg eq./kg), 1'-CH_2OH-S-2840-A (7.85% TRR, 0.444 mg eq./kg) and 1'-CH_2OH-S-2840-B (4.22%, 0.239 mg eq./kg).

Goats dosed with [phenyl-U-^{14}C] inpyrfluxam

A composite sample was determined to have a total radioactive residue level of 6.084 mg eq./kg. Neutral solvents solubilised 91.52% TRR with 8.48% TRR (0.516 mg eq./kg) remaining in the post extraction solids.

The major component identified was 1'-COOH-S-2840-A (65.60% TRR, 3.991 mg eq./kg). Other components identified were 1'-COOH-S-2840-B (0.90% TRR, 0.055 mg eq./kg), 1'-CH_2OH-S-2840-A (6.08% TRR, 0.370 mg eq./kg) and 1'-CH_2OH-S-2840-B (3.25% TRR, 0.198 mg eq./kg).

Metabolites in Urine

Goats dosed with [pyrazolyl-4-^{14}C] inpyrfluxam

A composite urine samples was prepared; the summation of residues was 4.336 mg eq./kg. The two major components isolated were identified to be 1'-COOH-S-2840-A (46.20% TRR, 2.003 mg eq./kg) and an isomer of Glu-1'-CH_2OH-S-2840 (14.80% TRR, 0.642 mg eq./kg). Other components found at lower levels were 1'-COOH-S-2840-B (5.30% TRR, 0.230 mg eq./kg), another isomer of Glu-1'-CH_2OH-S-2840 (8.90% TRR, 0.386 mg eq./kg) and DFPA (1.90% TRR, 0.082 mg eq./kg).

Goats dosed with [phenyl-U-^{14}C] inpyrfluxam

A composite urine sample was used. The sum of residues was 6.184 mg eq./kg. The same pattern of chromatographic components was observed for components eluting between 20 – 40 minutes in goats dosed with [phenyl-U-^{14}C] inpyrfluxam and [pyrazolyl-4-^{14}C] inpyrfluxam. The phenyl labelled residues were characterised to be 1'-COOH-S-2840-A (38.10% TRR, 2.536 mg eq./kg), an isomer of Glu-1'-CH_2OH-S-2840 (19.20% TRR, 1.187 mg eq./kg), 1'-COOH-S-2840-B (5.30% TRR, 0.328 mg eq./kg) and another isomer of Glu-1'-CH_2OH-S-2840 (9.90% TRR, 0.612 mg eq./kg).

Metabolites in Bile

Goats dosed with [pyrazolyl-4-^{14}C] inpyrfluxam

The total radioactive residue in bile was 9.196 mg eq./kg. Chromatographic characterisation by HPLC gave a mixture of components including 1'-COOH-S-2840-A (10.71% TRR, 0.985 mg eq./kg), 1'-COOH-S-2840-B (35.39% TRR, 3.254 mg eq./kg) and DFPA-$CONH_2$ (1.25% TRR, 0.115 mg eq./kg).

Goats dosed with [phenyl-U-^{14}C] inpyrfluxam

Total residues in bile were 12.406 mg eq./kg. Characterisation showed a mixture of 1'-COOH-S-2840-A (5.57% TRR, 0.691 mg eq./kg), 1'-COOH-S-2840-B (23.91% TRR, 2.966 mg eq./kg) and 1'-CH_2OH-S-2840-B (0.45% TRR, 0.056 mg eq./kg).

Chiral analysis

Partitioning the liver extract with hexane allowed the selective extraction of inpyrfluxam. Chiral analysis showed only the R-enantiomer was present indicating no epimerisation of the chiral carbon had occurred in goats.

Storage Stability

All samples were homogenized in the presences of dry ice before shipping and analysis. Initial characterisation of all the tissue extracts were conducted within 60 days of sacrifice. Composite urine and faeces samples were also extracted and analysed within 30 days of sacrifice. Metabolites were identified relative to co-injected reference standards with assignments made based on retention time of the co-eluting peaks.

For confirmation of metabolites, further extraction from kidney and liver samples was performed 431 days after the initial extraction, all samples were stored at ≤ -17°C during this time. The chromatographic profiles in the initial extracts and the re-extracted samples after 431 days of storage in liver and kidney show similar extractability (within 15%) and a similar distribution of peaks. This is considered sufficient to support the analysis in the ruminant metabolism study.

Fat solubility

Parent inpyrfluxam has an octanol-water partition coefficient of 3.65, suggesting potential accumulation in fat.

Parent and 1′-CH_2OH-S-2840 residues were predominantly found in liver and kidney commodities. In goats, concentrations between fat and muscle were close to the LOQ without clear tendency for accumulation in the fat.

In poultry metabolism studies, fat contained approximately 30x higher residue concentrations compared to muscle. However, no accumulation was observed in milk fat or egg yolk, In the feeding studies, residues were generally < LOQ, not allowing estimation of ratios between fatty and non-fatty tissues.

Overall, it is considered that inpyrfluxam is not fat-soluble.

Table 7.2.3.1-4 Distribution of residues in edible matrices in goat dosed with [pyrazolyl-4-^{14}C] inpyrfluxam

Description	Liver		Kidney		Flank Muscle		Loin Muscle		Omental Fat		Subcutaneous Fat		Renal Fat		Skimmed milk		Milk Fat	
	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR
Combine extracts [(a)]	0.285	91.05	0.159	98.15	0.014	100	0.011	91.67	-	-	0.010	83.33	-	-	0.034	100	0.029	100
PES	0.028	8.95	0.003	1.85	≤0.001	-	0.001	8.33	-	-	0.002	16.67	-	-	≤0.001	-	≤0.001	-
TRR [(b)]	0.313	100	0.162	100	0.014	100	0.012	100	0.006	-	0.012	100	0.007	-	0.034	100	0.029	100
DFPA-$CONH_2$	0.008	2.54	ND	ND	0.002	11.16	ND	ND	-	-	ND	ND	-	-	0.001	2.10	ND	ND
1'-COOH-S-2840-A	0.099	31.70	0.063	39.12	0.003	20.17	0.002	18.87	-	-	0.003	27.17	-	-	0.004	12.10	0.003	8.80
1'-COOH-S-2840-B	0.033	10.40	0.017	10.56	0.003	23.61	0.002	14.73	-	-	<0.001	1.67	-	-	ND	ND	ND	ND
Glu-1'-CH_2OH-S-2840 (sum of isomers)	0.05	15.88	0.04	24.49	0.003	22.11	0.002	16.02	-	-	ND	ND	-	-	ND	ND	ND	ND

1′-CH_2OH-S-2840-A	0.015	4.92	ND	ND	0.001	8.37	0.001	5.57	-	-	0.001	6.17	-	-	ND	ND	ND	ND
1′-CH_2OH-S-2840-B	ND	ND	ND	ND	ND	ND	ND	ND	-	-	0.001	6.08	-	-	ND	ND	ND	ND
3′-OH-S-2840	ND	ND	0.005	3.11	ND	ND	ND	ND	-	-	<0.001	<1.0	-	-	ND	ND	ND	ND
Inpyrfluxam	0.019	5.94	ND	ND	ND	ND	ND	ND	-	-	≤0.001	3.08	-	-	ND	ND	ND	ND
1′,1′-bis-(CH_2OH)-S2840-B	ND	ND	0.002 (c)	1.07 (c)	ND	ND	ND	ND	-	-	ND	ND	-	-	ND	ND	ND	ND
Unknown (d)	0.063	19.67	0.031	19.05	0.002	14.59	0.003	20.6	-	-	0.002	15.58	-	-	0.028	80.1	0.027	91.2
Other	-	-	0.001	0.76	-	-	-	-	-	-	0.003	23.58	-	-	-	-	-	-

(a) Residues in extract chromatographically characterised

(b) TRR = Sum of combined extracts + PES

(c) Distribution of components in HPLC peak determined by TLC

(d) Combined amounts of all unknown compounds

Table 7.2.3.1-5 Distribution of residues in edible matrices in goat dosed with [phenyl-U-^{14}C] inpyrfluxam

Description	Liver		Kidney		Flank Muscle		Loin Muscle		Omental Fat		Subcutaneous Fat		Renal Fat		Skimmed milk		Milk Fat	
	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR
Combine extracts [a]	0.311	90.41	0.166	97.65	0.020	95.24	0.014	93.33	0.021	87.50	0.028	96.55	0.037	90.24	0.040	100	0.017	94.44
PES	0.033	9.59	0.004	2.35	0.001	4.76	0.001	6.67	0.003	12.50	0.001	3.45	0.004	9.76	≤0.001	-	0.001	5.56
TRR [b]	0.344	100	0.170	100	0.021	100	0.015	100	0.024	100	0.029	100	0.041	100	0.040	100	0.018	100
DFPA-CONH$_2$	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1'-COOH-S-2840-A	0.104	30.20	0.059	34.42	0.004	17.77	0.003	17.08	0.007	29.40	0.009	32.25	0.016	36.10	0.004	9.94	0.001	5.48
1'-COOH-S-2840-B	0.018	5.13	0.019	10.94	0.006	28.67	0.001	9.99	0.001	4.38	ND	ND	0.002	3.61	0.002	5.93	ND	ND
Glu-1'-CH2OH-S-2840	0.066 [c]	19.17 [c]	0.057 [c]	33.45 [c]	0.005 [c]	24.4	0.004	28.56	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

1′-CH_2OH-S-2840-A	0.022	6.27	0.006	3.36	0.002	7.82	0.001	6.63	0.002	8.58	0.003	10.43	ND	ND	ND	ND	0.001	3.02
1′-CH_2OH-S-2840-B	ND	ND	ND	ND	ND	ND	<0.001	0.37	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3′-OH-S-2840	ND	ND	0.003	1.84	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Inpyrfluxam	0.017	4.94	ND	ND	ND	ND	ND	ND	0.004	15.84	0.002	6.37	0.004	8.21	ND	ND	0.002	9.07
1′,1′-bis-(CH_2OH)-S2840-B	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Unknown [(d)]	0.084	24.68	0.024	13.63	0.004	17.07	0.004	21.46	0.006	19.43	0.013	37.25	0.018	36.82	0.03	77.92	0.013	68.47

[(a)] Residues in extract chromatographically characterised

[(b)] TRR = Sum of combined extracts + PES

[(c)] Combined amounts of all detected metabolite

[(d)] Combined amounts of all unknown compounds

Table 7.2.3.1-6 Distribution of residues in urine, faeces, and bile matrices for goats dosed with [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam

Description	[pyrazolyl-4-^{14}C] inpyrfluxam						[phenyl-U-^{14}C] inpyrfluxam					
	Urine		Faeces		Bile		Urine		Faeces		Bile	
	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR
Combine extracts [(a)]	-	-	5.152	91.04	-	-	-	-	5.568	91.52	-	-
PES	-	-	0.507	8.96	-	-	-	-	0.516	8.48	-	-
TRR [(b)]	4.336	100	5.659	100	9.196	100	6.184	100	6.084	100	12.406	100
DFPA-CONH$_2$	ND	ND	ND	ND	0.115	1.25	ND	ND	ND	ND	ND	ND
DFPA	0.082	1.90	-	-	-	-	-	-	-	-	-	-
1'-COOH-S-2840-A	2.003	46.20	3.604	63.69	0.985	10.71	2.356	38.10	3.991	65.60	0.691	5.57
1'-COOH-S-2840-B	0.230	5.30	0.154	2.72	3.254	35.39	0.328	5.30	0.055	0.91	2.966	23.91
Glu-1'-CH$_2$OH-S-2840	1.028	23.7	ND	ND	ND	ND	1.799*	29.1*	ND	ND	ND	ND
1'-CH$_2$OH-S-2840-A	ND	ND	0.444	7.85	ND	ND	ND	ND	0.370	6.08	ND	ND
1'-CH$_2$OH-S-2840-B	ND	ND	0.239	4.22	ND	ND	ND	ND	0.198	3.25	0.056	0.45
3'-OH-S-2840	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Inpyrfluxam	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1′,1′-bis-(CH_2OH)-S2840-B	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Unknown [(c)]	0.997	23	0.71	12.56	4.842	52.63	1.694	27.4	0.954	15.7	8.566	69.06

[(a)] Residues in extract chromatographically characterised

[(b)] TRR = Sum of combined extracts + PES

[(c)] Combined amounts of all unknown compounds

Conclusion

The metabolism of Inpyrfluxam radiolabelled in the pyrazolyl or phenyl ring was investigated was administered orally to two goats over for 5 days with an intended dose of 10 mg/kg feed/day (actual dose rate: 13.74 mg/kg feed or 15.74 mg/kg feed). The overall post dose recovery was 96.97% for goats treated with [pyrazolyl-4-^{14}C] inpyrfluxam and 97.11% for goats treated with [phenyl-U-^{14}C] inpyrfluxam.

The majority of residues were efficiently extracted using the neutral solvents. In all matrices, > 90% of the [^{14}C] Inpyrfluxam residues were extracted, excluding omental, subcutaneous and renal fat for the pyrazolyl label were 83.33, 83.33 and 71.43% TRR were extracted respectively. For these fat matrices, PES were < 30% TRR, however the absolute residues were very low (≤ 0.002 mg eq./kg) so no further characterisation was needed.

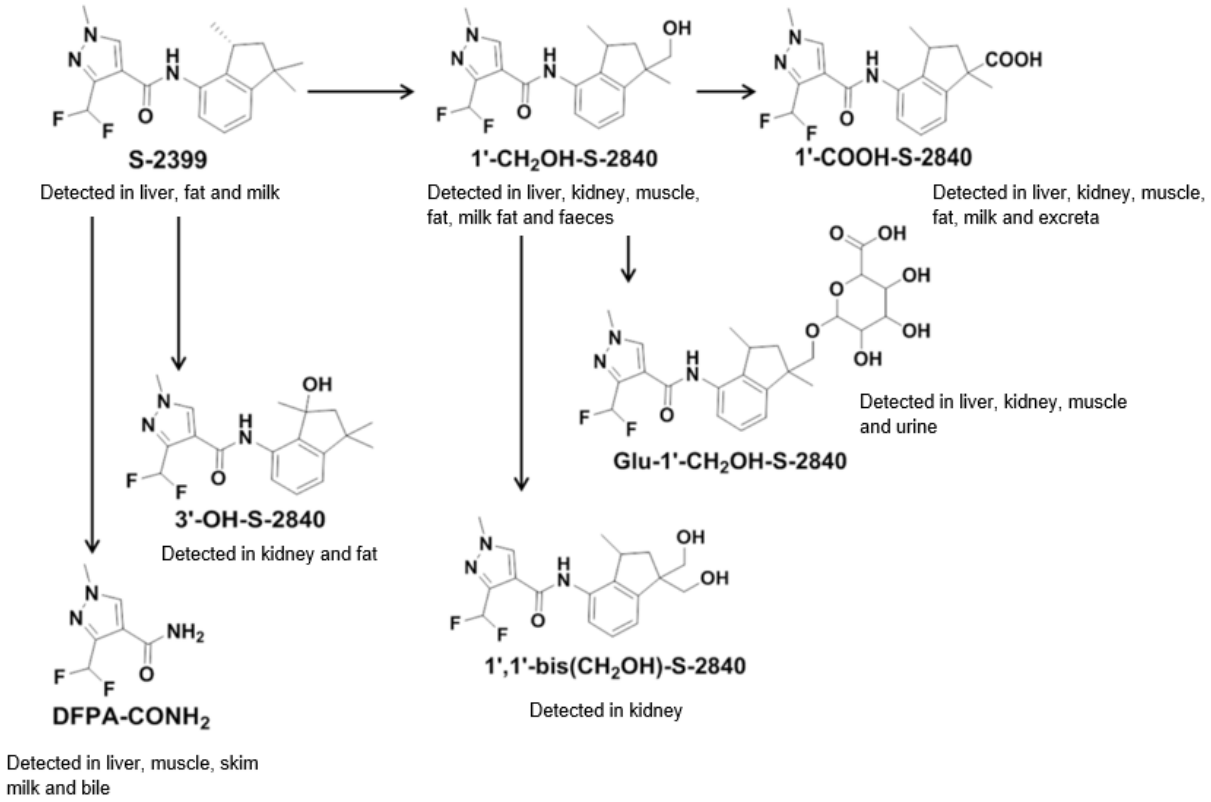
The highest tissue residues were in liver and kidney. Residues in the muscle and fat tissues were qualitatively similar to the pyrazolyl-label compared to the phenyl-label. The results showed that inpyrfluxam was extensively metabolised. Parent compound was determined in samples of liver and subcutaneous fat for both labels. Analysis of milk samples showed a greater number of components than observed in liver, kidney, muscle or fat samples. The major metabolites in the tissue samples were 1'-COOH-S-2840, glucuronide conjugates of 1'-CH_2OH-S-2840, and 1'-CH_2OH-S-2840. Minor amounts of 3'-OH-S-2840 and DFPA-$CONH_2$ were also observed in this study.

In goat metabolism samples (goat liver: hexane extracts) chiral methodology was used to confirm the stability of the R-isomer. There was no marked conversion from the R-isomer (to the S-isomer). In the goat liver extracts only the R-isomer was detected.

The metabolism of inpyrfluxam in goats after oral administration is thought to proceed via oxidation to form the alcohols 1'-CH_2OH-S-2840-A & -B. The 1'-CH_2OH-S-2840 alcohols are then further transformed to glucuronic acid conjugates or oxidised to carboxylic acids 1'-COOH-S-2840-A & -B. A minor pathway is also observed with the formation of 1',1'-bis-(CH_2OH)-S-2840 by oxidation of 1'-CH_2OH-S-2840. *N*-demethylation or cleavage of the amide bond of Inpyrfluxam form the metabolites *N*-des-Me-S-2840 and DFPA-$CONH_2$ respectively.

Based on the results the metabolism of both [pyrazolyl-4-^{14}C] inpyrfluxam and [phenyl-U-^{14}C] inpyrfluxam in lactating goats is considered understood and a metabolic pathway is proposed (See Figure 7.2.3.1-1).

Figure 7.2.3.1-1 Proposed metabolic pathway of inpyrfluxam in lactating goats



B.7.2.4. Pigs

As the metabolic pathway observed in hens and goats is similar to the metabolism observed in rats (Vol. 3 CA B6, Section 6.1), an assessment of the metabolism in pigs is not required.

B.7.2.5. Fish

At present there is no agreed guidance on how to conduct fish metabolism studies to determine the residue definition for risk assessment and enforcement and there are no agreed guidance documents on how to conduct a fish feeding study. The EU guidance: SANCO/10181/2013– rev. 5, 12 June 2019, Guidance Document For Applicants On Preparing Dossiers For The Approval Of A Chemical New Active Substance And For The Renewal Of Approval Of A Chemical Active Substance According To Regulation (EU) No 283/2013 and Regulation (EU) No 284/2013 states:

"In some cases, agreed test methods or guidance documents are not yet available for particular data requirements. In these cases, the non-submission of particular studies required by the EU legislation should be thoroughly justified and statements (often referred to as 'position papers') must be substantiated with data or information provided by the applicant in the dossier. Applicants should follow on a routine basis

the current developments, e.g. activities of the European Food Safety Authority for guidance documents and in particular publications in the Official Journal and the updates of the Commission Communications 2013/C 95/01 and 2013/C 95/02"

It is noted that the data requirements under Regulation (EU) No 283/2013 make it clear that bioaccumulation studies can be considered to address this data requirement. However, at the PRAS meeting in December 2017, for the expert discussion on Spinosad, it was agreed by the experts and EFSA that the use of such a study could not be considered at this time as it was not clear how the study design was applicable to assessing residues for consumer exposure and agreement on the approach to the dietary assessment for fish was required. This conclusion is applicable to the current evaluation.

Guidance on residues in fish (metabolism studies and feeding studies) has been under development in the EU. The OECD programme on residue guidelines has not yet considered guidelines applicable to fish.

Since no agreed guidance is available at this time, it is considered that the above requirements do not need to be addressed in the current evaluation.

B.7.3. Magnitude of Residue Trials in Plants

The present dossier supports the use of the representative formulation 'S-2399 60 g/L EC' (Emulsifiable Concentrate (EC) formulation containing 60 g/L inpyrfluxam) in Great Britian. The representative uses are for cereal crops (wheat and barley).

The requested GAPs are presented in Table 7.3-1 below.

Table 7.3-1: Requested GAPs

Member states/ zones	Crop	Application				PHI (days)
		Method/ kind	Growth stage of crop (BBCH)	Number of applications	Rate per application (g a.s./ha)	
GB	Barley	Foliar spray	30 – 71	1	90	35
GB	Wheat	Foliar spray	30 – 71	1	90	35

In support of the representative uses in GB, the notifier has provided northern Europe magnitude of residue trials in the following crops: wheat and barley. The uses on barley are covered by one cGAP assessment (see section B.7.3.1). The uses on wheat are covered by a second cGAP assessment (see section B.7.3.2).

Formulations

The representative formulation at active substance approval is ‘S-2399 60 g/L EC’ (EC formulation containing 60 g/L inpyrfluxam). In the residue trials on wheat and barley presented in the sections below, two formulations were used:

- S-2399 60 g/L EC, i.e., the representative formulation
- S-2399 40 SC, an SC formulation containing 400 g/L inpyrfluxam

Hence, some of the residue trials have been conducted using a different formulation type. In accordance with the GB Extrapolation Guidance 2024, *‘experience shows that EC, WP, WG, and SC formulations usually produce comparable residues (especially if the last application is more than seven days prior to harvest)’*. The proposed GAPs result in an interval between application and harvest of > 7 days. Therefore, it is considered that residue trials conducted with SC and EC formulations are likely to produce comparable residue levels.

Basic core acceptability criteria

Trials details

Crop variety	
Location, position and year of trial	-acceptable spread of location/season
Formulations used	-formulation reported/as proposed
Application/dilution rate	-reported/as specified on label
Maximum number of treatments	-reported/applicable
Method of application	-reported/applicable
Growth stage of the crop at treatment or Pre-harvest interval	-appropriate to proposed GAP
Geo-climate information	-reported/applicable
Residue level (control and treated)	

Analytical aspects

Method specified and submitted

Storage of samples prior to analysis (conditions and time period)

Limit of determination at an acceptable level

Acceptable recovery (means 70 – 110%).

B.7.3.1. Cereals: Barley

The requested GAP for barley is presented below (Table 7.3.1-1). The GB cGAP for winter and spring barley consists of one spray application at a rate of 1 x 90 g a.s./ha when the crop has reached the growth stage BBCH 30 – 71.

Residue trial data for barley have been submitted in support of this GAP.

Table 7.3.1-1: Requested GAP for barley

Member states/ zones	Crop	Application				PHI (days)
		Method/ kind	Growth stage of crop (BBCH)	Number of applications	Rate per application (g a.s./ha)	
GB	Winter and spring barley	Foliar spray	30 – 71	1	90	35

A summary of the trials submitted to support the GB cGAP on barley are given in table 7.3.1-2.

Barley is a major crop. A minimum of eight residue trials that reflect the agronomic and climatic conditions of GB are required. The applicant has submitted 14 trials relevant to GB. As the applicant has provided 14 trials, and a minimum of eight trials are required, this is sufficient to meet the data requirements.

As outlined in the table below, the 5 trials carried out during the 2019 growing season were undertaken at an application rate of 90 g a.s./ha. The remaining 9 trials carried out in 2016 were undertaken at an overdosed application rate of 200 g a.s./ha.

The submitted dossier also includes trials data from southern Europe. These trials do not reflect the agronomic and climatic conditions of the UK and were therefore not used in support of the representative use for approval.

Table 7.3.1-2: Number of residue trials relevant to GB and vegetation period

Crop	Region	No. of independent trials[a] (application rate)			Report No (Formulation)	Document Number	Reference
		Veget. Period		Total			
		2016	2019				
Barley	NEU	9 (200 g a.s/ha)	5 (90 g a.s./ha)	14	380-2019 (S-2399 60 g/L EC) 258-2016 (S-2399 60 g/L EC)	TPR-0139 TPR-0073	KCA 6.3.4/01 KCA 6.3.5/01

S-2399 60 g/L EC: Containing 60 g/L inpyrfluxam applied at 90 g a.s/ha and 120 g a.s./ha

S-2399 60 g/L EC: Containing 400 g/L inpyrfluxam applied at 200 g a.s./ha

[a] In some cases, S-2399 60 g/L EC formulation was tested in side-by-side plots at the same location using different application rates; where this is the case the trials are not considered as independent and the residue data from the application rate relevant to the GB cGAP (90 g a.s./ha) has been used to support the GB use.

The samples from all trials were analysed for the parent compound and the metabolites 3'-OH-S-2840, DFPA-$CONH_2$, *N*-des-Me-DFPA, DFPA, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B, 1'-COOH-S-2840-A and 1'-COOH-S-2840-B.

As highlighted above, a number of the residue trials were overdosed compared to the GAP. These trials were conducted at approximately 200 g a.s./ha. As the trials represent approximately 2.2 N, compared to the GAP, and all other trial parameters were the same as the GAP, the proportionality principle can be applied. The scaling factor for the overdosed trials is approximately 0.45 but varied based on the specific application rate used in the trials (See Vol. 1, 2.7.4).

Northern Europe trials

Report:	**KCA 6.3.4/01; [redacted] 2021**
Title:	Magnitude of the residue of S-2399 and its metabolites in barley Raw Agricultural Commodity in Northern Europe - 2019
Document No.:	TPR-0139 (Study No.: 380-2019)
Guidelines:	OECD Test Guideline 509;

	Commission Regulation (EU) no 283/2013 setting out the data requirements for active substances, in accordance with Regulation (EC) no 1107/2009
Guideline deviations:	None
GLP/GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

Report:	**KCA 6.3.5/01; [redacted] 2018**
Title:	Magnitude of the residue of S-2399 and its metabolites in barley Raw Agricultural Commodity in Northern Europe - 2016
Document No.:	TPR-0073 (Study No.: 258-2016)
Guidelines:	OECD Test Guideline 509; Commission Regulation (EU) no 283/2013 setting out the data requirements for active substances, in accordance with Regulation (EC) no 1107/2009
Guideline deviations:	None
GLP/GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

Materials and Methods

Fourteen independent field trials, each applying inpyrfluxam formulated into an EC or SC, were conducted on barley (both spring and winter varieties) in northern Europe in 2016 and 2019.

Study report 380-2019: 5 trials (HU, PL, DE, FR(N), DK; 2019) were conducted on spring or winter barley ([redacted] 2021) using one spray application of 1.5 L/ha 'S-2399 60 g/L EC' (equivalent to 90 g a.s./ha) at BBCH 61 – 75. The formulation was diluted with 75 – 300 L water/ha prior to spraying. For the 5 trials, 'S-2399 60 g/L EC' was tested in side-by-side plots at the same locations using a higher application rate (120 g a.s./ha). These trials are not considered independent and the residue data from the application rate relevant to the GB cGAP (90 g a.s./ha) has been used for assessment purposes.

Study report 258-2016: 9 trials (UK, FR(N), PL, DK, BE, HU, AT, NL, SE; 2016) were conducted on winter and spring barley ([redacted] 2018) using one foliar application of 0.5 L/ha 'S-2399 40 SC' (equivalent to 200 g a.s./ha) at BBCH 69 – 83. The formulation was diluted with 75 – 300 L water/ha prior to spraying.

Seven of these trials were decline trials where samples of green material (whole plants without roots) were taken immediately after application and at several further intervals up to 21 – 22 days (when samples of ears were also taken). In all trials, samples of grain and straw were collected at commercial harvest (BBCH 89). Grain samples containing at least 1.0 kg and straw samples containing at least 0.5 kg were collected from both treated and control plots.

Each field sample was stored at ≤-18°C within 24 hours of sampling and remained at this temperature, including during transportation to the analytical laboratory, until sample preparation. The frozen field samples were homogenised with dry ice in a cutter.

All samples were analysed for the residues of inpyrfluxam parent compound as well as the metabolites 3'-OH-S-2840, DFPA-$CONH_2$, *N*-des-Me-DFPA, DFPA, 1'-COOH-S-2840-A, 1'-COOH-S-2840-B, 1'-CH_2OH-S-2840-A and 1'-CH_2OH-S-2840-B. Samples were stored for a maximum of 370 days prior to analysis for parent inpyrfluxam and its metabolites. This length is supported by storage stability data on wheat grain which shows these analytes are considered to be stable for 22 months (see B.7.1).

The maximum storage interval between extraction and analysis was 33 days. Despite this being a long period to store analytical extracts prior to analysis, stability of residues in sample extracts has been satisfactorily addressed in the residue trials, as matrix-matched procedural recovery samples were extracted and stored for the same length of time and under the same conditions as the test sample extracts. Therefore, acceptable procedural recovery results validate the extract storage periods.

The samples were analysed for inpyrfluxam and its metabolites using LC-MS/MS method S16-03371, each expressed as the respective compound. The method was sufficiently validated on wheat grain and whole plant in accordance with

SANTE/2020/12830 rev.1 (see Section B.5.1.2.5). The LOQ was 0.01 mg/kg for parent, 3'-OH-S-2840, DFPA-$CONH_2$, *N*-des-Me-DFPA and DFPA and 0.005 mg/kg for 1'-COOH-S-2840-A, 1'-COOH-S-2840-B, 1'-CH_2OH-S-2840-A and 1'-CH_2OH-S-2840-B. It should be noted that for the metabolite DFPA, a confirmatory mass transition could not be validated due to matrix peaks at the retention time of interest. Therefore, a second, confirmatory LC-MS/MS method was validated instead (method 2).

Acceptable procedural recoveries were obtained from whole plant without roots, ear, grain and straw samples spiked with the relevant analytes at 0.005 – 10.0 mg/kg, which covers the LOQ, and residue levels found in treated samples; see the tables below.

Table 7.3.1-3: Procedural recoveries for inpyrfluxam, 3'-OH-S-2840, DFPA-CONH$_2$ and *N*-des-Me-DFPA in barley (method S16-03371)

Portion	n	Fortification level (mg/kg)	Inpyrfluxam		3'-OH-S-2840		DFPA-CONH$_2$		*N*-des-Me-DFPA	
			Recoveries (%)	Mean (%)	Recoveries (%)	Mean (%)	Recoveries (%)	Mean (%)	Recoveries (%)	Mean (%)
Study 380-2019										
Whole plant without roots	4	0.01	108, 110, 103, 98	105	100, 105, 110, 96	103	107, 97, 99, 107	103	89, 75, 80, 77	80
	4	0.1	105, 109, 102, 98	104	101, 102, 100, 98	100	97, 100, 98, 95	98	85, 80, 70, 70	76
Whole plant w/o roots and ears	2	0.01	101, 99	100	95, 102	99	105, 106	106	72, 67	70
	2	0.1	97, 103	100	98, 100	99	98, 101	100	68, 71	70
Ears	2	0.01	92, 94	93	93, 93	93	89, 89	89	110, 101	106
	2	0.1	86, 93	90	92, 94	93	92, 96	94	106, 102	104
Straw	2	0.01	95, 104	100	106, 97	102	101, 113	107	83, 74	79
	2	0.1	100, 98	99	100, 94	97	100, 98	99	87, 70	79
Grain	2	0.01	87, 77	82	96, 96	96	89, 94	92	76, 72	74
	2	0.1	83, 86	85	91, 93	92	88, 87	88	67, 73	70
Study 258-2016										
Whole plant without roots	5	0.01	92, 98, 96, 110, 103	100	103, 103, 98, 98, 102	101	88, 83, 80, 107, 108	93	86, 77, 74, 110, 87	87

	4	0.1	103, 108, 109, 105	106	102, 94, 93, 95	96	104, 112, 108, 107	108	70, 85, 90, 85	83
	3	10	108, 98, 77	94	107, 103, 83	98	-	-	-	-
Whole plant w/o roots and ears	3	0.01	98, 108, 109	105	105, 101, 103	103	102, 108, 93	101	76, 70, 78	75
	3	0.1	101, 97, 98	99	100, 97, 98	98	95, 94, 94	94	82, 79, 84	82
	3	10	82, 97, 103	94	91, 92, 98	94	107, 100, 103	103	-	-
Ears	3	0.01	111, 114, 99	108	112, 110, 91	104	109, 110, 98	106	97, 107, 122	109
	3	0.1	88, 73, 77	79	96, 95, 95	95	87, 86, 86	86	80, 100, 95	92
	3	10	81, 88, 77	82	79, 94, 94	89	82, 86, 90	86	-	-
Grain	3	0.01	76, 81, 82	80	85, 87, 95	89	80, 87, 90	86	63, 76, 71	70
	3	0.1	84, 93, 95	91	83, 95, 99	92	84, 100, 99	94	71, 72, 73	72
	3	1.0	87, 83, 90	87	75, 90, 100	88	107, 107, 109	108	-	-
Straw	3	0.01	100, 90, 90	93	100, 97, 112	103	98, 99, 91	96	77, 79, 76	77
	3	0.1	108, 110, 105	108	108, 109, 107	108	108, 108, 105	107	79, 75, 72	75
	3	10	70, 94, 65	76	66, 95, 88	83	68, 101, 93	87	-	-

Table 7.3.1-4: Procedural recoveries for 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B, 1'-COOH-S-2840-A and 1'-COOH-S-2840-B in barley (method S16-03371)

Portion	n	Fortification level (mg/kg)	1'-CH_2OH-S-2840-A		1'-CH_2OH-S-2840-B		1'-COOH-S-2840-A		1'-COOH-S-2840-B	
			Recoveries (%)	Mean (%)	Recoveries (%)	Mean (%)	Recoveries (%)	Mean (%)	Recoveries (%)	Mean (%)
Study 380-2019										
Whole plant without roots	4	0.005	102, 103, 95, 103	101	103, 104, 98, 100	101	97, 94, 87, 93	93	92, 99, 82, 99	93
	4	0.05	97, 101, 97, 95	98	96, 97, 98, 99	98	96, 96, 96, 94	96	102, 104, 98, 99	101
Whole plant w/o roots and ears	2	0.005	84, 88	86	97, 89	93	81, 84	83	84, 83	84
	2	0.05	80, 76	78	76, 76	76	80, 77	79	85, 81	83
Ears	2	0.005	82, 74	78	86, 72	79	87, 75	81	94, 76	85
	2	0.05	82, 84	83	85, 84	85	89, 88	89	92, 91	92
Straw	2	0.005	100, 97	99	99, 92	96	100, 99	100	98, 93	96
	2	0.05	90, 88	89	90, 90	90	90, 91	91	93, 91	92
Grain	2	0.005	73, 78	76	74, 78	76	81, 83	82	83, 81	82
	2	0.05	82, 80	81	81, 87	84	86, 86	86	87, 88	88
Study 258-2016										

Whole plant without roots	5	0.005	82, 85, 90, 103, 82	88	86, 94, 96, 100, 82	92	75, 73, 75, 106, 75	81	83, 71, 81, 94, 82	82
	4	0.05	81, 84, 85, 88	85	83, 89, 88, 90	88	65, 83, 86, 88	81	66, 95, 89, 94	86
	3	0.5	88, 101, 88	92	92, 102, 87	94	-	-	-	-
Whole plant w/o roots and ears	3	0.005	95, 85, 92	91	83, 90, 63	79	92, 83, 76	84	84, 88, 72	81
	3	0.05	83, 85, 79	82	80, 84, 82	82	77, 79, 79	78	76, 78, 79	78
	3	0.5	83, 82, 82	82	87, 84, 85	85	83, 83, 80	82	85, 85, 82	84
Ears	3	0.005	110, 106, 105	107	113, 112, 101	109	107, 108, 109	108	101, 106, 111	106
	3	0.05	95, 94, 91	93	94, 98, 93	95	91, 89, 82	87	92, 90, 85	89
	4	0.5	91, 88, 78, 88	86	94, 90, 86, 88	90	82, 90, 83, 85	85	90, 95, 88, 88	90
Grain	3	0.005	107, 91, 84	94	103, 87, 85	92	98, 85, 89	91	99, 86, 83	89
	3	0.05	81, 85, 84	83	79, 83, 85	82	83, 88, 90	87	76, 83, 83	81
	3	0.5	81, 78, 72	77	85, 82, 75	81	87, 84, 77	83	83, 81, 75	80
Straw	3	0.005	112, 107, 106	108	116, 111, 99	109	109, 106, 107	107	103, 104, 111	106
	3	0.05	79, 90, 90	86	86, 97, 93	92	81, 92, 91	88	90, 100, 98	96
	3	0.5 (5.0 for 1'-COOH-S-2840-B and 1'-CH OH-S-2840-B)	106, 114, 107	109	102, 107, 97	102	107, 111, 102	107	109, 108, 102	106

Table 7.3.1-5: Procedural recoveries for DFPA in barley (method S16-03371)

Study	Portion	n	Fortification level (mg/kg)	Individual recoveries (%)	Mean (%)
Method 2					
380-2019	Whole plant without roots	4	0.01	86, 92, 90, 88	89
		4	0.1	87, 77, 92, 91	87
	Whole plant without roots and ears	2	0.01	115, 104	110
		2	0.1	103, 107	105
	Straw	2	0.01	111, 107	109
		2	0.1	98, 102	100
Method 1					
380-2019	Ears	2	0.01	99, 87	93
		2	0.1	97, 88	93
	Grain	2	0.01	94, 82	88
		2	0.1	86, 88	87
258-2016	Whole plant without roots	5	0.01	107, 95, 94, 108, 86	98
		4	0.1	79, 101, 104, 102	97
		3	1.0	89, 107, 100	99
	Whole plant without roots and ears	3	0.01	70, 76, 70	72
		3	0.1	74, 82, 75	77
		3	1.0	90, 91, 87	89
	Ears	3	0.01	110, 103, 100	104
		3	0.1	96, 99, 92	96
		4	1.0	79, 92, 84, 89	86
	Grain	3	0.01	95, 82, 82	86
		3	0.1	74, 81, 80	78
		3	1.0	77, 78, 70	75

	Straw	3	0.01	100, 104, 102	102
		3	0.1	79, 84, 86	83
		3	10	88, 87, 87	87

Results

Appropriate representative chromatograms were provided and no residues above the LOQ were found in any control samples.

In trials where the decline of residues in whole plant without roots was investigated, residues of inpyrfluxam parent compound were observed to dissipate in whole plant without roots in most instances, from 1.1 – 6.5 mg/kg on Day +0 to 0.14 – 3.4 mg/kg on Day 21 – 22.

At harvest, unscaled residues of inpyrfluxam parent in grain ranged from <0.01 – 0.26 mg/kg and residues in straw ranged from 0.09 – 7.1 mg/kg.

At harvest, unscaled residues of the metabolite 3'-OH-S-2840 in grain ranged from <0.01 – 0.03 mg/kg and residues in straw ranged from <0.01 – 0.30 mg/kg.

At harvest, unscaled residues of the metabolite DFPA-$CONH_2$ in grain and straw were <0.01 mg/kg.

At harvest, unscaled residues of the metabolite *N*-des-Me-DFPA in grain ranged from <0.01 – 0.06 mg/kg and residues in straw ranged from <0.01 – 0.03 mg/kg.

At harvest, unscaled residues of the metabolite DFPA in grain ranged from <0.01 – 0.06 mg/kg and residues in straw ranged from 0.04 – 0.30 mg/kg.

At harvest, unscaled residues of the metabolite 1'-COOH-S-2840-A in grain ranged from <0.005 – 0.024 mg/kg and residues in straw ranged from 0.006 – 0.043 mg/kg.

At harvest, unscaled residues of the metabolite 1'-COOH-S-2840-B in grain ranged from <0.005 – 0.034 mg/kg and residues in straw ranged from <0.005 – 0.039 mg/kg.

At harvest, unscaled residues of the metabolite 1'-CH_2OH-S-2840-A in grain ranged from 0.017 – 0.073 mg/kg and residues in straw ranged from 0.065 – 0.74 mg/kg.

At harvest, unscaled residues of the metabolite 1'-CH_2OH-S-2840-B in grain ranged from 0.017 – 0.14 mg/kg and residues in straw ranged from 0.078 – 1.5 mg/kg.

The detailed results are summarised in Table 7.3.1-6 and are not corrected for procedural recoveries.

Table 7.3.1-6: Residue trials on barley in northern Europe

References:	**KCA 6.3.4/01; [redacted] 2021; 380-2019; KCA 6.3.5/01; [redacted] 2018; 258-2016**		
GLP:	Yes	**Sample storage conditions:**	Frozen (-18 °C), max 370 days
Crop/crop group:	Barley	**Analytical method:**	S16-03371
Indoor/Outdoor:	Northern Europe Outdoor	**Limit of Quantification (mg/kg):**	Parent, 3'-OH-S-2840, DFPA-$CONH_2$, *N*-des-Me-DFPA, DFPA: 0.01 1'-COOH-S-2840-A, 1'-COOH-S-2840-B, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B: 0.005
Formulation:	S-2399 60 g/L EC or S-2399 40 SC		
Content of active substance (g/L):	60 g/L or 400 g/L	**Residues calculated as:**	Inpyrfluxam and its metabolites

Commodity (Variety)	Date of 1. Sowing 2. Flowering 3. Harvest	Application rate			Treatment date (formulation, method)	BBCH at treatment	Portion analysed	Residues (mg/kg)[(a)]									PHI (days)
		g a.s./ha	Water (L/ha)	g a.s./hL				Inpyrfluxam	3-OH-S-2840	DFPA-$CONH_2$	*N*-des-Me-DFPA	DFPA	1-COOH-S-2840-A	1-COOH-S-2840-B	1-CH_2OH-S-2840-A	1-CH_2OH-S-2840-B	
380-2019 HU01 Hungary Northern Europe 2019 PLOT T1																	

Winter barley (Monique)	1. 28/09/18 2. 07/05/19 to 20/05/19 3. 27/06/19 to 01/07/19	93.9	260	36.1	21/05/19 (S-2399 60 g/L EC, foliar)	71	WP WP WP WP Ears Straw Grain	2.4 0.36 0.13 0.30 0.05 <u>0.18</u> <u>0.02</u>	<0.01 0.02 0.02 0.08 <0.01 0.06 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	0.03 0.01 <0.01 0.07 0.02 0.06 <0.01	<0.005 0.009 0.009 0.017 0.024 0.038 <0.005	<0.005 <0.005 <0.005 0.008 <0.005 0.01 <0.005	0.038 0.14 0.19 0.27 0.23 0.13 0.037	0.024 0.082 0.12 0.20 0.14 0.10 0.028	0 7 14 21 21 37 37
380-2019 HU01 Hungary Northern Europe 2019 PLOT T2																	
Winter barley (Monique)	1. 28/09/18 2. 07/05/19 to 20/05/19 3. 27/06/19 to 01/07/19	121.9	253	48.2	21/05/19 (S-2399 60 g/L EC, foliar)	71	WP WP WP WP Ears Straw Grain	3.1 0.54 0.16 0.40 0.37 0.26 0.02	0.01 0.04 0.02 0.12 0.10 0.10 <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 <0.01	0.03 <0.01 <0.01 0.10 0.02 0.09 <0.01	<0.005 0.012 0.011 0.02 0.027 0.055 <0.005	<0.005 <0.005 <0.005 0.01 <0.005 0.018 <0.005	0.035 0.16 0.16 0.41 0.27 0.22 0.041	0.024 0.10 0.10 0.29 0.17 0.19 0.030	0 7 14 21 21 37 37

380-2019 PL02 Poland Northern Europe 2019 PLOT T1																	
Spring barley (Kus Dante)	1. 23/03/19 2. 11/06/19 to 15/06/19 3. 29/07/19	88.2	293	30.1	24/06/19 (S-2399 60 g/L EC, foliar)	75	WP	1.6	<0.01	<0.01	<0.01	0.02	<0.005	<0.005	<0.005	<0.005	0
							WP	1.6	0.11	<0.01	<0.01	0.03	0.009	<0.005	0.17	0.10	7
							WP	1.4	0.11	<0.01	<0.01	0.03	0.008	<0.005	0.20	0.13	14
							WP	2.4	0.22	<0.01	<0.01	0.19	<0.005	<0.005	0.12	0.15	21
							Ears	1.1	0.16	<0.01	<0.01	0.05	0.011	<0.005	0.17	0.10	21
							Straw	1.7	0.30	<0.01	<0.01	0.21	0.006	<0.005	0.19	0.19	35
							Grain	0.20	0.02	<0.01	0.01	0.01	<0.005	<0.005	0.047	0.030	35
380-2019 PL02 Poland Northern Europe 2019 PLOT T2																	
Spring barley (Kus Dante)	1. 23/03/19 2. 11/06/19 to 15/06/19 3. 29/07/19	120.0	299	40.1	24/06/19 (S-2399 60 g/L EC, foliar)	75	WP	3.2	<0.01	<0.01	<0.01	0.02	<0.005	<0.005	<0.005	<0.005	0
							WP	2.8	0.20	<0.01	<0.01	0.03	0.006	<0.005	0.14	0.078	7
							WP	2.8	0.26	<0.01	<0.01	0.03	<0.005	<0.005	0.13	0.081	14
							WP	3.3	0.34	<0.01	<0.01	0.25	<0.005	<0.005	0.13	0.17	21
							Ears	1.6	0.25	<0.01	<0.01	0.05	0.008	<0.005	0.15	0.12	21
							Straw	2.8	0.42	<0.01	<0.01	0.30	<0.005	<0.005	0.21	0.21	35
							Grain	0.28	0.04	<0.01	<0.01	0.02	<0.005	<0.005	0.048	0.029	35

380-2019 GE03 Germany Northern Europe 2019 PLOT T1																	
Winter barley (Quadriga)	1. 10/10/18 2. 12/05/19 to 23/05/19 3. 27/06/19	89.7	298	30.1	23/05/19 (S-2399 60 g/L EC, foliar)	69	WP	1.1	<0.01	<0.01	<0.01	0.01	<0.005	<0.005	<0.005	<0.005	0
							WP	0.33	0.02	<0.01	<0.01	<0.01	0.011	<0.005	0.15	0.11	7
							WP	0.15	0.02	<0.01	<0.01	<0.01	0.011	<0.005	0.15	0.11	14
							WP	0.14	0.03	<0.01	<0.01	0.03	<0.005	<0.005	0.092	0.14	22
							Ears	0.03	<0.01	<0.01	<0.01	0.01	0.020	<0.005	0.14	0.076	22
							Straw	0.09	0.03	<0.01	<0.01	0.04	0.012	<0.005	0.13	0.13	35
							Grain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.005	<0.005	0.042	0.028	35
380-2019 GE03 Germany Northern Europe 2019 PLOT T2																	
Winter barley (Quadriga)	1. 10/10/18 2. 12/05/19 to 23/05/19 3. 27/06/19	121.6	303	40.1	23/05/19 (S-2399 60 g/L EC, foliar)	69	WP	3.1	<0.01	<0.01	<0.01	0.02	<0.005	<0.005	0.029	0.022	0
							WP	0.77	0.06	<0.01	<0.01	0.01	0.022	0.007	0.24	0.17	7
							WP	0.44	0.05	<0.01	<0.01	0.01	0.017	0.006	0.27	0.21	14
							WP	0.32	0.05	<0.01	<0.01	0.06	0.008	<0.005	0.18	0.21	22
							Ears	0.07	<0.01	<0.01	<0.01	<0.01	0.016	<0.005	0.16	0.10	22
							Straw	0.49	0.12	<0.01	<0.01	0.14	0.035	0.016	0.52	0.52	35

							Grain	0.02	<0.01	<0.01	<0.01	<0.01	<0.005	<0.005	0.081	0.057	35
380-2019 FR04 Northern France Northern Europe 2019 PLOT T1																	
Winter barley (Orwell)	1. 10/11/18 2. 22/05/19 to 29/05/19 3. 29/06/19 to 06/07/19	94.4	209	45.2	22/05/19 (S-2399 60 g/L EC, foliar)	61	Straw Grain	0.28 <0.01	0.05 <0.01	<0.01 <0.01	<0.01 <0.01	0.06 0.06	0.006 <0.005	0.005 <0.005	0.16 0.034	0.14 0.024	35 35
380-2019 FR04 Northern France Northern Europe 2019 PLOT T2																	
Winter barley (Orwell)	1. 10/11/18 2. 22/05/19 to 29/05/19 3. 29/06/19 to 06/07/19	128.2	213	60.2	22/05/19 (S-2399 60 g/L EC, foliar)	61	Straw Grain	0.54 <0.01	0.09 <0.01	<0.01 <0.01	<0.01 <0.01	0.10 <0.01	0.011 <0.005	0.010 <0.005	0.31 0.042	0.27 0.032	35 35
380-2019 DK05 Denmark Northern Europe 2019 PLOT T1																	
Winter barley (Frigg)	1. 01/10/18 2. 16/05/19 to 24/05/19 3. 15/07/19 to 01/08/19	93.9	208	45.1	14/06/19 (S-2399 60 g/L EC, foliar)	73	Straw Grain	0.09 0.06	0.01 <0.01	<0.01 <0.01	<0.01 <0.01	0.04 <0.01	0.019 <0.005	0.011 <0.005	0.16 0.041	0.15 0.028	33 33

380-2019 DK05 Denmark Northern Europe 2019 PLOT T2																	
Winter barley (Frigg)	1. 01/10/18 2. 16/05/19 to 24/05/19 3. 15/07/19 to 01/08/19	124.0	206	60.2	14/06/19 (S-2399 60 g/L EC, foliar)	73	Straw Grain	0.15 0.03	0.02 <0.01	<0.01 <0.01	<0.01 <0.01	0.05 <0.01	0.022 <0.005	0.008 <0.005	0.25 0.058	0.16 0.052	33 33
258-2016 UK01 United Kingdom Northern Europe 2016																	
Spring barley (Kws Irina)	1. 16/03/16 2. 03/06/16 to 21/06/16 3. 09/08/16	213.5	208	102.6	05/07/16 (S-2399 40 SC, foliar)	77	WP WP WP WP Ears Straw Grain	2.9 2.8 1.3 2.4 1.7 <u>0.80</u> <u>0.13</u>	<0.01 0.07 0.10 0.23 0.24 0.15 0.02	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 0.01 0.02 0.01 0.03 <0.01	0.15 0.15 0.13 0.12 0.08 0.06 0.01	<0.005 0.009 0.027 0.054 0.025 0.034 0.005	<0.005 <0.005 0.010 0.018 0.006 0.017 <0.005	<0.005 0.12 0.26 0.34 0.38 0.21 0.061	<0.005 0.20 0.47 0.84 0.42 0.49 0.080	0 7 14 21 21 35 35
258-2016 FR02 Northern France Northern Europe 2016																	
Spring barley	1. 01/03/16 2. Not recorded	204.3	199	102.7	23/06/16 (S-2399 40 SC, foliar)	71 – 73	WP WP	3.3 1.4	0.01 0.03	<0.01 <0.01	<0.01 <0.01	0.20 0.10	<0.005 0.006	<0.005 <0.005	<0.005 0.068	<0.005 0.093	0 7

(Sunshine)	3. 01/08/16						WP	1.2	0.06	<0.01	<0.01	0.08	0.009	<0.005	0.14	0.20	14
							WP	3.4	0.22	<0.01	0.01	0.18	0.021	0.016	0.43	0.86	22
							Ears	0.65	0.11	<0.01	<0.01	0.06	0.029	0.007	0.31	0.31	22
							Straw	7.1	0.70	<0.01	0.02	0.30	0.028	0.023	0.74	1.5	35
							Grain	0.26	0.03	<0.01	<0.01	0.01	<0.005	<0.005	0.073	0.14	35
258-2016 PL03 Poland Northern Europe 2016																	
Spring barley (Kucyk)	1. 20/03/16 2. 18/06/16 to 23/06/16 3. 07/08/16	210.7	308	68.4	05/07/16 (S-2399 40 SC, foliar)	83	WP	5.9	0.01	<0.01	<0.01	0.24	<0.005	<0.005	0.009	0.014	0
							WP	1.6	0.07	<0.01	<0.01	0.05	0.007	<0.005	0.10	0.16	7
							WP	0.80	0.05	<0.01	0.01	0.03	0.010	0.006	0.078	0.10	15
							WP	1.2	0.13	<0.01	0.01	0.05	0.024	0.015	0.13	0.22	22
							Ears	0.25	0.05	<0.01	<0.01	0.02	0.008	<0.005	0.075	0.069	22
							Straw	0.65	0.09	<0.01	<0.01	0.04	0.016	0.009	0.065	0.12	33
							Grain	0.12	0.02	<0.01	<0.01	<0.01	0.007	<0.005	0.046	0.038	33
258-2016 DK05 Denmark Northern Europe 2016																	
Spring barley	1. 28/03/16 2. 17/06/16 to 25/06/16	215.5	210	102.6	07/07/16 (S-2399 40 SC, foliar)	73	WP	6.5	0.02	<0.01	<0.01	0.19	<0.005	<0.005	0.007	0.007	0
							WP	0.40	0.02	<0.01	0.01	0.03	0.016	0.008	0.17	0.24	7

(Quench)	3. 12/08/16						WP	0.43	0.03	<0.01	0.02	0.04	0.022	0.013	0.21	0.27	14
							WP	0.38	0.05	<0.01	0.02	0.04	0.026	0.020	0.20	0.42	21
							Ears	0.19	0.03	<0.01	<0.01	0.03	0.025	0.009	0.22	0.20	21
							Straw	0.25	0.05	<0.01	0.01	0.04	0.032	0.026	0.24	0.38	35
							Grain	0.03	<0.01	<0.01	<0.01	<0.01	<0.005	<0.005	0.027	0.038	35
258-2016 BE06 Belgium Northern Europe 2016																	
Spring barley (Hilford)	1. 18/03/16 2. 15/06/16 to 19/06/16 3. 26/07/16 to 01/08/16	211.8	258	82.1	21/06/16 (S-2399 40 SC, foliar)	73	Straw	0.50	0.07	<0.01	<0.01	0.04	0.043	0.039	0.25	0.41	35
							Grain	0.02	<0.01	<0.01	<0.01	<0.01	<0.005	<0.005	0.035	0.030	35
258-2016 HU07 Hungary Northern Europe 2016																	
Spring barley (Hansi)	1. 08/03/16 2. 03/06/16 to 10/06/16 3. 21/07/16	211.4	309	68.4	14/06/16 (S-2399 40 SC, foliar)	72	Straw	0.67	0.13	<0.01	<0.01	0.04	0.015	0.012	0.26	0.25	36
							Grain	0.07	0.02	<0.01	0.01	<0.01	<0.005	<0.005	0.060	0.057	36
258-2016 AU08 Austria Northern Europe 2016																	

Winter barley (Sandra)	1. 02/10/15 2. 13/05/16 to 22/05/16 3. 25/06/16 to 26/06/16	214.6	314	68.3	21/05/16 (S-2399 40 SC, foliar)	69	Straw Grain	0.50 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	0.011 <0.005	0.008 <0.005	0.085 0.029	0.082 0.021	34 34
258-2016 NL09 The Netherlands Northern Europe 2016																
Winter barley (Antonella)	1. 10/10/15 2. 07/05/16 to 21/05/16 3. 12/07/16	203.8	298	68.4	07/06/16 (S-2399 40 SC, foliar)	79	Straw Grain	0.18 0.06	0.02 <0.01	<0.01 <0.01	0.01 0.01	0.02 0.01	0.015 0.024	0.025 0.034	0.065 0.049	0.29 0.14	35 35
258-2016 SW10 Sweden Northern Europe 2016																
Spring barley (Filippa)	1. 12/04/16 2. 16/06/16 to 23/06/16 3. 09/08/16	202.1	197	102.6	04/07/16 (S-2399 40 SC, foliar)	73	Straw Grain	0.14 0.02	0.02 <0.01	<0.01 <0.01	<0.01 <0.01	0.02 <0.01	0.009 <0.005	0.006 <0.005	0.082 0.017	0.078 0.017	36 36

(a) Inpyrfluxam residues expressed as inpyrfluxam, 3'-OH-S-2840 residues expressed as 3'-OH-S-2840, DFPA-$CONH_2$ residues expressed as DFPA-$CONH_2$, *N*-des-Me-DFPA residues expressed as *N*-des-Me-DFPA, DFPA residues expressed as DFPA, 1'-COOH-S-2840-A residues expressed as 1'-COOH-S-2840-A, 1'-COOH-S-2840-B residues expressed as 1'-COOH-S-2840-B, 1'-CH_2OH-S-2840-A residues expressed as 1'-CH_2OH-S-2840-A and 1'-CH_2OH-S-2840-B residues expressed as 1'-CH_2OH-S-2840-B

B.7.3.2. Cereals: Wheat

The requested GAPs for wheat are presented below (Table 7.3.2-1). The GB cGAP for winter and spring wheat consists of one spray application at a rate of 1 x 90 g a.s./ha when the crop has reached the growth stage BBCH 30 – 71.

Residue trial data for wheat have been submitted in support of this GAP.

Table 7.3.2-1: Requested GAPs for wheat

Member states/ zones	Crop	Application				PHI (days)
		Method/ kind	Growth stage of crop (BBCH)	Number of applications	Rate per application (g a.s./ha)	
GB	Wheat	Foliar spray	30 – 71	1	90	35

A summary of the trials submitted to support the GB cGAP on wheat is given in Table 7.3.2-2.

Wheat is a major crop. A minimum of eight residue trials that reflect the agronomic and climatic conditions of GB are required. The applicant has submitted 15 trials relevant to GB. As the applicant has provided 15 trials, and a minimum of eight trials are required, this is sufficient to meet the data requirements.

As outlined in the table below, the 5 trials carried out during the 2019 growing season were undertaken at an application rate of 90 g a.s./ha. The remining 10 trials carried out in 2016 were undertaken at an overdosed application rate of 200 g a.s./ha and require scaling.

The submitted dossier also includes trials data from southern Europe. These trials do not reflect the agronomic and climatic conditions of the UK and were therefore not used in support of the representative use for approval.

Table 7.3.2-2: Number of residue trials relevant to GB and vegetation period

Crop	Region	No. of independent trials[(a)] (application rate)			Report No (Formulation)	Document Number	Reference
		Veget. Period		Total			
		2016	2019				

Wheat	NEU	10 (200 g a.s./ha)	5 (90 g a.s./ha)	15	376-2019 (S-2399 60 g/L EC) 256-2016 (S-2399 40 SC)	TPR-0137 TPR-0076	KCA 6.3.1/01 KCA 6.3.2/01

S-2399 60 g/L EC: Containing 60 g/L inpyrfluxam applied at 90 g a.s/ha and 120 g a.s./ha

S-2399 40 SC: Containing 400 g/L inpyrfluxam applied at 200 g a.s./ha

(a) In some cases, S-2399 60 g/L EC formulation was tested in side-by-side plots at the same location using different application rates; where this is the case the trials are not considered as independent and the residue data from the application rate relevant to the GB cGAP (90 g a.s./ha) has been used to support the GB use.

The samples from all trials were analysed for the parent compound and the metabolites 3'-OH-S-2840, DFPA-$CONH_2$, *N*-des-Me-DFPA, DFPA, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B, 1'-COOH-S-2840-A and 1'-COOH-S-2840-B.

As highlighted above, a number of the residue trials were overdosed compared to the GAP. These trials were conducted at approximately 200 g a.s./ha. As this application rate represents approximately 2.2 N compared to the GAP, and all other trial parameters were the same as the GAP, the proportionality principle can be applied. The scaling factor for the overdosed trials is approximately 0.45 but varied based on the specific application rate used in the trials (See Vol. 1, 2.7.4).

Northern Europe trials

Report:	**KCA 6.3.1/01; [redacted] 2021**
Title:	Magnitude of the residue of S-2399 and its metabolites in wheat Raw Agricultural Commodity in Northern Europe - 2019
Document No.:	TPR-0137 (Study No.: 376-2019)
Guidelines:	OECD Test Guideline 509; Commission Regulation (EU) no 283/2013 setting out the data requirements for active substances, in accordance with Regulation (EC) no 1107/2009
Guideline deviations:	None
GLP/GEP:	Yes

Acceptability:	Yes
Study relied upon:	Yes

Report:	**KCA 6.3.2/01; [redacted] 2018**
Title:	Magnitude of the residue of S-2399 and its metabolites in wheat Raw Agricultural Commodity in Northern Europe - 2016
Document No.:	TPR-0076 (Study No.: 256-2016)
Guidelines:	OECD Test Guideline 509; Commission Regulation (EU) no 283/2013 setting out the data requirements for active substances, in accordance with Regulation (EC) no 1107/2009
Guideline deviations:	None
GLP/GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

Materials and Methods

Fifteen independent field trials, each applying inpyrfluxam formulated into an EC or SC, were conducted on wheat (both spring and winter varieties) in northern Europe in 2016 and 2019.

Study report 376-2019: 5 trials (HU, PL, DE, FR(N); 2019) were conducted on winter wheat ([redacted] 2021) using one spray application of 1.5 L/ha 'S-2399 60 g/L EC' (equivalent to 90 g a.s./ha) at BBCH 69 – 77. The formulation was diluted with 75 – 300 L water/ha prior to spraying. For the 5 trials, 'S-2399 60 g/L EC' was tested in side-by-side plots at the same locations using a higher application rate (120 g a.s./ha). These trials are not considered independent and the residue data from the application rate relevant to the GB cGAP (90 g a.s./ha) has been used.

Study report 256-2016: 10 trials (UK, FR(N), PL, DE, DK, BE, HU, AT, NL, SE; 2016) were conducted on winter and spring wheat ([redacted] 2018) using one foliar application of 0.5 L/ha 'S-2399 40 SC' (equivalent to 200 g a.s./ha) at BBCH 73 – 83. The formulation was diluted with 75 – 300 L water/ha prior to spraying.

Eight of these trials were decline trials where samples of green material (whole plants without roots) were taken immediately after application and at several further intervals up to 20 – 22 days (when samples of ears were also taken). In all trials, samples of grain and straw were collected at commercial harvest (BBCH 89), 33 – 38 days after last application. Grain samples containing at least 1.0 kg and straw samples containing at least 0.5 kg were collected from both treated and control plots.

Each field sample was stored at ≤-18°C within 24 hours of sampling and remained at this temperature, including during transportation to the analytical laboratory, until sample preparation. The frozen field samples were homogenised with dry ice in a cutter.

All samples were analysed for the residues of inpyrfluxam parent compound as well as the metabolites 3'-OH-S-2840, DFPA-$CONH_2$, *N*-des-Me-DFPA, DFPA, 1'-COOH-S-2840-A, 1'-COOH-S-2840-B, 1'-CH_2OH-S-2840-A and 1'-CH_2OH-S-2840-B. Samples were stored for a maximum of 455 days prior to analysis for parent inpyrfluxam and its metabolites. This length is supported by storage stability data on wheat grain which shows these analytes are considered to be stable for 22 months (see B.7.1).

The maximum storage interval between extraction and analysis was 12 days. Despite this being a long period to store analytical extracts prior to analysis, stability of residues in sample extracts has been satisfactorily addressed in the residue trials, as matrix-matched procedural recovery samples were extracted and stored for the same length of time and under the same conditions as the test sample extracts. Therefore, acceptable procedural recovery results validate the extract storage periods.

The samples were analysed for inpyrfluxam and its metabolites using LC-MS/MS method S16-03371, each expressed as the respective compound. The method was sufficiently validated on wheat grain and whole plant in accordance with SANTE/2020/12830 rev.1 (see Section B.5.1.2.5). The LOQ was 0.01 mg/kg for parent, 3'-OH-S-2840, DFPA-$CONH_2$, *N*-des-Me-DFPA and DFPA and 0.005 mg/kg for 1'-COOH-S-2840-A, 1'-COOH-S-2840-B, 1'-CH_2OH-S-2840-A and 1'-CH_2OH-S-2840-B. It should be noted that for the metabolite DFPA, a confirmatory mass transition could not be validated due to matrix peaks at the retention time of interest. Therefore, a second, confirmatory LC-MS/MS method was validated instead (method 2).

Acceptable procedural recoveries were obtained from whole plant without roots, ear, grain and straw samples spiked with the relevant analytes at 0.005 – 10.0 mg/kg, which covers the LOQ, and residue levels found in treated samples; see the tables below.

Table 7.3.2-3: Procedural recoveries for inpyrfluxam, 3'-OH-S-2840, DFPA-CONH$_2$ and *N*-des-Me-DFPA in wheat (method S16-03371)

Portion	n	Fortification level (mg/kg)	Inpyrfluxam		3'-OH-S-2840		DFPA-CONH$_2$		*N*-des-Me-DFPA	
			Recoveries (%)	Mean (%)	Recoveries (%)	Mean (%)	Recoveries (%)	Mean (%)	Recoveries (%)	Mean (%)
Study 376-2019										
Whole plant without roots	4	0.01	98, 89, 89, 83	90	100, 106, 100, 97	101	97, 104, 95, 89	96	73, 73, 72, 67	71
	4	0.1	91, 89, 86, 92	90	101, 92, 94, 90	94	88, 98, 96, 91	93	77, 81, 76, 71	76
	3	5.0	118, 109, 103	110	115, 110, 101	109	-	-	-	-
Whole plant without roots and ears	2	0.01	108, 90	99	117, 99	108	90, 79	85	70, 70	70
	2	0.1	105, 99	102	104, 106	105	97, 90	94	79, 81	80
	3	5.0	86, 88, 87	87	82, 83, 89	85	-	-	-	-
Ears	2	0.01	81, 75	78	96, 99	98	98, 96	97	77, 78	78
	2	0.1	85, 71	78	102, 88	95	101, 87	94	83, 76	80
	3	2.0	84, 89, 77	83	93, 89, 84	89	-	-	-	-
Straw	2	0.01	103, 103	103	98, 101	100	105, 86	96	82, 86	84
	2	0.1	87, 106	97	79, 97	88	73, 92	83	90, 98	94

	3	5.0	93, 81, 82	87	85, 78, 78	80	-	-	-	-
Grain	2	0.01	74, 71	73	92, 82	87	98, 90	94	73, 70	72
	2	0.1	74, 70	72	79, 74	77	75, 79	77	70, 69	70
Study 256-2016										
Whole plant without roots	3	0.01	103, 80, 116	100	102, 83, 100	95	102, 85, 108	98	113, 99, 98	103
	3	0.1	67, 84, 84	78	62, 77, 85	75	63, 82, 88	78	108, 108, 94	103
	3	10	109, 87, 110	102	93, 85, 107	95	90, 91, 107	96	-	-
Whole plant without roots and ears	2	0.01	117, 103	110	108, 104	106	112, 105	109	79, 61	70
	1	0.1	104	-	106	-	101	-	79	-
	3	10	85, 104, 99	96	80, 100, 96	92	83, 103, 101	96	-	-
Ears	2	0.01	97, 95	96	79, 82	81	90, 88	89	91, 72	82
	1	0.1	96	-	92	-	99	-	77	-
	3	1.0	81, 80, 78	80	86, 85, 85	85	91, 86, 91	89	-	-
Straw	3	0.01	119, 99	109	93, 93	93	97, 104	101	92, 93	93
	3	0.1	112, 107, 106	108	112, 106, 108	109	105, 101, 94	100	64, 78, 72	71

	3	10	100, 99, 92	97	94, 91, 88	91	94, 95, 91	93	-	-
Grain	2	0.01	72, 74	73	84, 84	84	99, 92	96	82, 87	85
	3	0.1	68, 77, 80	75	79, 77, 86	81	96, 91, 92	93	72, 71	72

Table 7.3.2-4: Procedural recoveries for 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B, 1'-COOH-S-2840-A and 1'-COOH-S-2840-B in wheat (method S16-03371)

Portion	n	Fortification level (mg/kg)	1'-CH_2OH-S-2840-A		1'-CH_2OH-S-2840-B		1'-COOH-S-2840-A		1'-COOH-S-2840-B	
			Recoveries (%)	Mean (%)	Recoveries (%)	Mean (%)	Recoveries (%)	Mean (%)	Recoveries (%)	Mean (%)
Study 376-2019										
Whole plant without roots	4	0.005	90, 87, 83, 73	83	89, 87, 80, 74	83	84, 88, 82, 86	85	91, 82, 83, 82	85
	4	0.05	88, 91, 86, 89	89	87, 89, 81, 84	85	90, 91, 85, 84	88	94, 99, 95, 89	94
	3	1.0	80, 85, 74	79	80, 87, 77	81	-	-	-	-
Whole plant without roots and ears	2	0.005	84, 82	83	88, 88	88	90, 86	88	99, 98	99
	2	0.05	80, 83	82	82, 82	82	85, 82	84	88, 83	86
	3	1.0	87, 78, 80	82	85, 83, 75	81	-	-	-	-
Ears	2	0.005	80, 87	84	75, 84	80	80, 82	81	92, 83	89

	2	0.05	78, 84	81	81, 88	85	81, 82	82	84, 94	89
	3	0.5	81, 84, 80	82	84, 83, 79	82	-	-	-	-
Straw	2	0.005	92, 82	87	95, 105	100	91, 90	91	101, 108	105
	2	0.05	89, 78	84	88, 83	86	84, 84	84	92, 88	90
	3	1.5	78, 75, 70	74	74, 75, 72	74	-	-	-	-
Grain	2	0.005	89, 80	85	91, 96	94	89, 78	84	86, 81	84
	2	0.05	102, 105	104	101, 106	104	95, 101	98	93, 104	99
Study 256-2016										
Whole plant without roots	3	0.005	92, 96, 100	96	85, 91, 108	95	81, 76, 96	84	77, 80, 94	84
	3	0.05	87, 90, 92	90	87, 88, 93	89	82, 79, 89	83	81, 80, 86	82
	3	0.5	81, 78, 79	79	73, 71, 72	72	87, 81, 81	83	77, 70, 68	72
	3	5.0	111, 100, 100	104	110, 98, 96	101	-	-	-	-
Whole plant without roots and ears	2	0.005	104, 100	102	110, 100	105	104, 102	103	112, 104	108
	1	0.05	110	-	108	-	110	-	110	-
	3	0.5	72, 73, 73	73	77, 79, 77	78	78, 77, 80	78	79, 82, 81	81
	3	5.0	99, 112, 104	105	95, 105, 96	99	-	-	-	-

Ears	2	0.005	83, 82	83	94, 103	99	97, 94	96	94, 87	91
	1	0.05	92	-	98	-	96	-	91	-
	3	0.5	72, 73, 74	73	70, 68, 72	70	79, 82, 80	80	68, 72, 70	70
Straw	2	0.005	100, 102	101	106, 106	106	106, 102	104	108, 106	107
	3	0.05	94, 96, 94	95	100, 100, 94	98	92, 102, 98	97	92, 98, 98	96
	3	0.5	78, 78, 73	76	79, 81, 76	79	79, 80, 76	78	81, 83, 79	81
	3	5.0	107, 100, 106	104	100, 97, 106	101	-	-	-	-
Grain	3	0.005	101, 102, 102	102	105, 112, 104	107	108, 111, 105	108	111, 112, 105	109
	2	0.05	90, 79	85	92, 78	85	93, 84	89	92, 91	92
	1	0.5	93	-	105	-	88	-	94	-

Table 7.3.2-5: Procedural recoveries for DFPA in wheat (method S16-03371)

Study	Portion	n	Fortification level (mg/kg)	Recovery (%)			
				Individual recoveries	Min.	Max.	Mean
Method 2							
376-2019	Whole plant without roots	4	0.01	105, 98, 84, 87	84	105	94
		4	0.1	81, 87, 95, 89	81	95	88
	Whole plant without roots and ears	2	0.01	101, 104	101	104	103
		2	0.1	93, 92	92	93	93
		3	2.0	91, 82, 80	80	91	84
	Straw	2	0.01	109, 103	103	109	106
		2	0.1	100, 97	97	100	99
		3	3.0	84, 86, 92	84	92	87
Method 1							
376-2019	Ears	2	0.01	102, 86	86	102	94
		2	0.1	90, 91	90	91	91
	Grain	2	0.01	89, 72	72	89	81
		2	0.1	97, 86	86	97	92
256-2016	Whole plant without roots	3	0.01	85, 82, 105	82	105	91
		3	0.1	115, 103, 101	101	115	106
		3	1.0	73, 70, 73	70	73	72
	Whole plant without roots and ears	2	0.01	77, 65	65	77	71
		1	0.1	87	-	-	-
		3	1.0	94, 90, 91	90	94	92
	Ears	2	0.01	103, 113	103	113	108

		1	0.1	100	-	-	-
		3	1.0	77, 80, 81	77	81	79
	Straw	2	0.01	74, 78	74	78	76
		3	0.1	74, 72, 72	72	74	73
		3	1.0	99, 96, 92	92	99	96
	Grain	3	0.01	102, 118, 94	94	118	105
		2	0.1	95, 90	90	95	93
		1	1.0	91	-	-	-

Results

Appropriate representative chromatograms were provided and no residues above the LOQ were found in any control samples.

In trials where the decline of residues in whole plant without roots was investigated, residues of inpyrfluxam parent compound were observed to dissipate in whole plant without roots in most instances, from 1.3 – 8.1 mg/kg on Day +0 to 0.15 – 4.0 mg/kg on Day 20 – 22.

At harvest, unscaled residues of inpyrfluxam parent in grain ranged from <0.01 – 0.02 mg/kg and residues in straw ranged from 0.23 – 3.0 mg/kg.

At harvest, unscaled residues of the metabolite 3'-OH-S-2840 in grain were <0.01 mg/kg and residues in straw ranged from 0.02 – 0.77 mg/kg.

At harvest, unscaled residues of the metabolite DFPA-$CONH_2$ in grain were <0.01 mg/kg and residues in straw ranged from <0.01 – 0.01 mg/kg. At harvest, unscaled residues of the metabolite *N*-des-Me-DFPA in grain were <0.01 mg/kg and residues in straw ranged from <0.01 – 0.02 mg/kg.

At harvest, unscaled residues of the metabolite DFPA in grain were <0.01 mg/kg and residues in straw ranged from 0.03 – 0.32 mg/kg.

At harvest, unscaled residues of the metabolite 1'-COOH-S-2840-A in grain ranged from <0.005 – 0.009 mg/kg and residues in straw ranged from <0.005 – 0.46 mg/kg.

At harvest, unscaled residues of the metabolite 1'-COOH-S-2840-B in grain ranged from <0.005 – 0.010 mg/kg and residues in straw ranged from <0.005 – 0.64 mg/kg.

At harvest, unscaled residues of the metabolite 1'-CH_2OH-S-2840-A in grain ranged from <0.005 – 0.011 mg/kg and residues in straw ranged from <0.005 – 0.58 mg/kg.

At harvest, unscaled residues of the metabolite 1'-CH_2OH-S-2840-B in grain ranged from <0.005 – 0.041 mg/kg and residues in straw ranged from <0.005 – 1.1 mg/kg.

The detailed results are summarised in Table 7.3.2-6 and are not corrected for procedural recoveries.

Table 7.3.2-6: Residue trials on wheat in northern Europe

References:	**KCA 6.3.1/01; [redacted] 2021; 376-2019; KCA 6.3.2/01; [redacted] 256-2018**		
GLP:	Yes	**Sample storage conditions:**	Frozen (-18 °C), max 455 days
Crop/crop group:	Wheat	**Analytical method:**	S16-03371
Indoor/Outdoor:	Northern Europe Outdoor	**Limit of Quantification (mg/kg):**	Parent, 3'-OH-S-2840, DFPA-$CONH_2$, *N*-des-Me-DFPA, DFPA: 0.01 1'-COOH-S-2840-A, 1'-COOH-S-2840-B, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B: 0.005
Formulation:	S-2399 60 g/L EC or S-2399 40 SC		
Content of active substance (g/L):	60 g/L or 400 g/L	**Residues calculated as:**	Inpyrfluxam and its metabolites

Commodity (Variety)	Date of 1. Sowing 2. Flowering 3. Harvest	Application rate			Date of treatment (formulation, method)	Growth stage at treatment (BBCH)	Portion analysed	Residues (mg/kg)[(a)]									PHI (days)
		g a.s./ha	Water (L/ha)	g a.s./hL				Inpyr-fluxam	3' -OH-S-2840	DFPA-$CONH_2$	*N*-des-Me-DFPA	DFPA	1' -COOH-S-2840-A	1' -COOH-S-2840-B	1' -CH_2OH-S-2840-A	1' -CH_2OH-S-2840-B	
376-2019 HU01 Hungary Northern Europe 2019 PLOT T1																	

Winter wheat (Astardo)	1. 01/10/18 2. 22/05/19 to 03/06/19 3. 09/07/19 to 11/07/19	91.0	252	36.1	03/06/19 (S-2399 60 g/L EC, foliar)	69	WP WP WP WP Ears Straw Grain	2.5 0.49 0.48 0.15 0.03 <u>0.23</u> <u><0.01</u>	0.01 0.04 0.09 0.04 <0.01 0.08 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	0.03 0.02 0.03 0.04 0.01 0.07 <0.01	0.064 0.25 0.58 0.36 0.12 0.46 <0.005	0.062 0.26 0.036 0.42 0.17 0.64 <0.005	<0.005 <0.005 <0.005 0.006 <0.005 0.020 <0.005	<0.005 <0.005 0.008 0.007 <0.005 0.011 <0.005	0 7 14 22 22 36 36
376-2019 HU01 Hungary Northern Europe 2019 PLOT T2																	
Winter wheat (Astardo)	1. 01/10/18 2. 22/05/19 to 03/06/19 3. 09/07/19 to 11/07/19	120.9	251	48.2	03/06/19 (S-2399 60 g/L EC, foliar)	69	WP WP WP WP Ears Straw Grain	3.1 0.78 0.55 0.29 0.11 0.72 <0.01	0.02 0.10 0.11 0.07 0.05 0.28 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 0.01 0.02 <0.01 0.01 0.01 <0.01	0.08 0.04 0.07 0.07 0.03 0.11 <0.01	0.073 0.44 0.59 0.48 0.28 0.82 <0.005	0.059 0.54 0.69 0.51 0.37 1.1 <0.005	<0.005 <0.005 <0.005 0.008 <0.005 0.025 <0.005	<0.005 0.006 0.007 0.008 <0.005 0.012 <0.005	0 7 14 22 22 36 36
376-2019 PL02 Poland Northern Europe 2019 PLOT T1																	

Winter wheat (Linus)	1. 01/10/18 2. 04/06/19 to 16/06/19 3. 26/07/19 to 27/07/19	91.8	305	30.1	22/06/19 (S-23996 0 g/L EC, foliar)	75 – 77	WP WP WP WP Ears Straw Grain	1.9 2.2 2.1 4.0 1.3 <u>3.0</u> <u>0.02</u>	<0.01 0.26 0.36 0.70 0.33 0.77 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	0.02 0.04 0.04 0.16 0.07 0.15 <0.01	0.012 0.047 0.049 0.25 0.094 0.41 0.006	0.011 0.057 0.065 0.31 0.14 0.44 0.008	<0.005 <0.005 <0.005 <0.005 <0.005 0.006 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005 <0.005	0 7 14 20 20 34 34
376-2019 PL02 Poland Northern Europe 2019 PLOT T2																	
Winter wheat (Linus)	1. 01/10/18 2. 04/06/19 to 16/06/19 3. 26/07/19 to 27/07/19	120.0	304	40.1	22/06/19 (S-23996 0 g/L EC, foliar)	75 – 77	WP WP WP WP Ears Straw Grain	2.8 3.0 2.2 2.8 1.4 3.6 0.03	0.01 0.35 0.45 0.05 0.34 0.88 0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 0.01 0.01 0.01 <0.01 0.01 <0.01	0.08 0.13 0.11 0.15 0.06 0.12 0.01	0.012 0.081 0.083 0.18 0.048 0.45 0.006	0.012 0.12 0.14 0.24 0.075 0.54 0.008	<0.005 <0.005 <0.005 <0.005 <0.005 0.01 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005 <0.005	0 7 14 20 20 34 34

376-2019 GE03 Germany Northern Europe 2019 PLOT T1																	
Winter wheat (Kamerad 13)	1. 20/10/18 2. 05/06/19 to 11/06/19 3. 31/07/19	91.8	305	30.1	26/06/19 (S-2399 60 g/L EC, foliar)	77	WP	1.3	<0.01	<0.01	<0.01	0.02	0.012	0.008	<0.005	<0.005	0
							WP	0.70	0.08	<0.01	<0.01	0.02	0.096	0.071	<0.005	<0.005	7
							WP	0.65	0.10	<0.01	<0.01	0.02	0.11	0.081	<0.005	<0.005	14
							WP	0.62	0.14	<0.01	<0.01	0.07	0.14	0.13	<0.005	<0.005	21
							Ears	0.25	0.07	<0.01	<0.01	0.03	0.077	0.092	<0.005	<0.005	21
							Straw	2.8	0.46	<0.01	0.01	0.14	0.37	0.39	<0.005	0.006	35
							Grain	<0.01	<0.01	<0.01	<0.01	<0.01	0.009	0.010	<0.005	<0.005	35
376-2019 GE03 Germany Northern Europe 2019 PLOT T2																	
Winter wheat (Kamerad 13)	1. 20/10/18 2. 05/06/19 to 11/06/19 3. 31/07/19	119.2	297	40.1	26/06/19 (S-2399 60 g/L EC, foliar)	77	WP	1.6	<0.01	<0.01	<0.01	0.04	0.014	0.009	<0.005	<0.005	0
							WP	0.63	0.10	<0.01	<0.01	0.04	0.14	0.079	<0.005	<0.005	7
							WP	0.69	0.12	<0.01	<0.01	0.05	0.18	0.12	<0.005	<0.005	14
							WP	0.66	0.15	<0.01	0.02	0.07	0.220	0.15	<0.005	<0.005	21
							Ears	0.12	0.04	<0.01	<0.01	0.01	0.075	0.068	<0.005	<0.005	21
							Straw	2.5	0.51	<0.01	0.02	0.15	0.64	0.53	0.005	0.009	35
							Grain	0.01	<0.01	<0.01	<0.01	<0.01	0.011	0.011	<0.005	<0.005	35

376-2019 FR04 Northern France Northern Europe 2019 PLOT T1																	
Winter wheat (Filon)	1. 20/11/18 2. 20/05/19 to 28/05/19 3. 10/07/19 to 25/07/19	95.3	211	45.2	13/06/19 (S-2399 60 g/L EC, foliar)	73	Straw Grain	2.4 0.02	0.50 <0.01	0.01 <0.01	<0.01 <0.01	0.15 <0.01	0.24 <0.005	0.43 <0.005	<0.005 <0.005	0.006 <0.005	33 33
376-2019 FR04 Northern France Northern Europe 2019 PLOT T2																	
Winter wheat (Filon)	1. 20/11/18 2. 20/05/19 to 28/05/19 3. 10/07/19 to 25/07/19	122.8	204	60.2	15/06/19 (S-2399 60 g/L EC, foliar)	73	Straw Grain	2.8 0.01	0.61 <0.01	0.02 <0.01	<0.01 <0.01	0.15 <0.01	0.24 <0.005	0.45 <0.005	<0.005 <0.005	0.005 <0.005	33 33
376-2019 BE05 Belgium Northern Europe 2019 PLOT T1																	

Winter wheat (Reflexion)	1. 05/10/18 2. 03/06/19 to 10/06/19 3. 24/07/19 to 05/08/19	86.2	191	45.1	20/06/19 (S-2399 60 g/L EC, foliar)	71	Straw Grain	0.96 <0.01	0.20 <0.01	<0.01 <0.01	<0.01 <0.01	0.08 <0.01	0.13 <0.005	0.39 <0.005	0.013 <0.005	0.010 <0.005	35 35
376-2019 BE05 Belgium Northern Europe 2019 PLOT T2																	
Winter wheat (Reflexion)	1. 05/10/18 2. 03/06/19 to 10/06/19 3. 24/07/19 to 05/08/19	120.4	200	60.2	20/06/19 (S-2399 60 g/L EC, foliar)	71	Straw Grain	1.1 <0.01	0.22 <0.01	<0.01 <0.01	<0.01 <0.01	0.10 <0.01	0.15 <0.005	0.49 0.006	0.019 <0.005	0.016 <0.005	35 35
256-2016 UK01 UK Northern Europe 2016																	
Spring wheat (Mulika)	1. 16/03/16	204.2	199	102.6	14/07/16 (S-2399	73	WP WP	2.3 1.3	<0.01 0.06	<0.01 <0.01	<0.01 <0.01	0.14 0.12	<0.005 <0.005	<0.005 <0.005	<0.005 0.12	<0.005 0.16	0 7

	2. 17/06/16 to 07/07/16 3. 17/08/16 to 18/08/16				40 SC, foliar)		WP WP Ears Straw Grain	0.89 0.94 0.52 2.1 <0.01	0.08 0.13 0.12 0.50 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01	0.02 0.01 0.01 0.02 <0.01	0.14 0.03 0.05 0.32 <0.01	<0.005 0.014 <0.005 0.019 <0.005	0.007 0.015 <0.005 0.021 <0.005	0.22 0.23 0.13 0.54 <0.005	0.39 0.59 0.24 0.89 0.005	13 21 21 34 34
256-2016 FR02 Northern France Northern Europe 2016																
Winter wheat (Calabro)	1. 13/11/15 2. 23/05/16 to 05/06/16 3. 20/07/16	206.3	201	102.6	09/06/16 (S-2399 40 SC, foliar)	73	WP WP WP Ears Straw Grain	2.3 0.26 0.02 0.22 0.12 0.20 <0.01	<0.01 <0.01 0.01 0.02 0.01 0.02 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	0.06 0.01 0.01 <0.01 0.01 0.03 <0.01	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 0.007 <0.005 0.006 <0.005	<0.005 0.030 0.040 0.065 0.041 0.038 <0.005	<0.005 0.037 0.051 0.11 0.054 0.089 <0.005	0 7 14 21 21 34 34
256-2016 PL03 Poland Northern Europe 2016																

Spring wheat (Izera)	1. 06/04/16 2. 16/06/16 to 25/06/16 3. 12/08/16	207.3	303	68.4	08/07/16 (S-2399 40 SC, foliar)	77	WP	4.1	<0.01	<0.01	<0.01	0.10	<0.005	<0.005	<0.005	<0.005	0
							WP	1.1	0.06	<0.01	0.01	0.04	0.007	0.008	0.080	0.16	7
							WP	1.3	0.10	<0.01	0.02	0.05	0.021	0.025	0.12	0.26	14
							WP	1.2	0.13	<0.01	0.02	0.03	0.035	0.031	0.18	0.47	20
							Ears	0.54	0.07	<0.01	0.01	0.03	<0.005	<0.005	0.10	0.17	20
							Straw	<u>0.57</u>	0.12	<0.01	<0.01	0.07	0.047	0.054	0.10	0.27	35
							Grain	<u>0.02</u>	<0.01	<0.01	<0.01	<0.01	<0.005	<0.005	<0.005	0.010	35
256-2016 GE04 Germany Northern Europe 2016																	
Winter wheat (Elixer)	1. 01/10/15 2. 26/05/16 to 09/06/16 3. 09/08/16	207.3	303	68.4	05/07/16 (S-2399 40 SC, foliar)	83	WP	8.1	0.02	<0.01	<0.01	0.16	<0.005	<0.005	<0.005	<0.005	0
							WP	1.1	0.06	<0.01	<0.01	0.05	0.017	0.021	0.14	0.44	7
							WP	1.2	0.09	<0.01	0.01	0.06	0.028	0.035	0.18	0.63	14
							WP	1.2	0.12	<0.01	0.01	0.03	0.080	0.15	0.21	0.77	20
							Ears	0.65	0.06	<0.01	0.01	0.04	<0.005	<0.005	0.11	0.45	20
							Straw	<u>0.50</u>	0.11	<0.01	<0.01	0.12	0.044	0.063	0.17	0.75	35
							Grain	<u>0.02</u>	<0.01	<0.01	<0.01	<0.01	<0.005	<0.005	0.011	0.041	35

256-2016 DK05 Denmark Northern Europe 2016																
Spring wheat (Trappe)	1. 21/04/16 2. 25/06/16 to 04/07/16 3. 24/08/16	200.0	195	102.6	20/07/16 (S-2399 40 SC, foliar)	73	WP WP WP Ears Straw Grain	4.3 1.3 0.69 0.51 0.38 0.37 <0.01	0.02 0.09 0.09 0.11 0.07 0.11 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 0.02 0.02 0.03 0.02 0.02 <0.01	0.10 0.09 0.06 0.05 0.04 0.25 <0.01	<0.005 0.015 0.019 0.040 0.008 0.071 <0.005	<0.005 0.016 0.021 0.044 0.009 0.071 <0.005	0.009 0.25 0.27 0.51 0.18 0.58 0.006	0.015 0.73 0.74 1.3 0.40 1.1 0.014	0 7 13 20 20 35 35
256-2016 BE06 Belgium Northern Europe 2016																
Spring wheat (Triso)	1. 18/03/16 2. 15/06/16 to 24/06/16 3. 01/08/16 to 17/08/16	211.0	257	82.1	28/06/16 (S-2399 40 SC, foliar)	77	Straw Grain	3.3 <0.01	0.40 <0.01	<0.01 <0.01	<0.01 <0.01	0.22 <0.01	0.008 <0.005	0.010 <0.005	0.19 <0.005	0.22 <0.005	34 34
256-2016 HU07 Hungary Northern Europe 2016																

Winter wheat (Antonius)	1. 13/10/15 2. 29/05/16 to 07/06/16 3. 20/07/16	207.3	303	68.4	11/06/16 (S-2399 40 SC, foliar)	73	Straw Grain	0.25 <0.01	0.07 <0.01	<0.01 <0.01	<0.01 <0.01	0.04 <0.01	<0.005 <0.005	<0.005 <0.005	0.11 <0.005	0.13 <0.005	38 38
256-2016 AU08 Austria Northern Europe 2016																	
Winter wheat (Lukullus)	1. 28/10/15 2. 26/05/16 to 05/06/16 3. 19/07/16 to 21/07/16	207.3	303	68.4	14/06/16 (S-2399 40 SC, foliar)	73	Straw Grain	0.06 <0.01	0.02 <0.01	<0.01 <0.01	<0.01 <0.01	0.04 <0.01	0.015 <0.005	0.014 <0.005	0.19 <0.005	0.20 <0.005	35 35
256-2016 NL09 The Netherlands Northern Europe 2016																	
Spring wheat (Nobless)	1. 18/03/16 2. 06/06/16	200.4	293	68.4	08/07/16 (S-2399 40 SC, foliar)	75	Straw Grain	0.17 <0.01	0.04 <0.01	<0.01 <0.01	<0.01 <0.01	0.04 <0.01	<0.005 <0.005	<0.005 <0.005	0.048 <0.005	0.098 <0.005	37 37

	to 21/06/16 3. 14/08/16																
256-2016 SW10 Sweden Northern Europe 2016																	
Winter wheat (Brons)	1. 20/09/15 2. 13/06/16 to 22/06/16 3. 09/08/16 to 10/08/16	208.3	203	102.6	04/07/16 (S-2399 40 SC, foliar)	73	Straw Grain	0.20 <0.01	0.04 <0.01	<0.01 <0.01	<0.01 <0.01	0.03 <0.01	0.012 <0.005	0.008 <0.005	0.13 <0.005	0.19 <0.005	36 36

(a) Inpyrfluxam residues expressed as inpyrfluxam, 3'-OH-S-2840 residues expressed as 3'-OH-S-2840, DFPA-$CONH_2$ residues expressed as DFPA-$CONH_2$, *N*-des-Me-DFPA residues expressed as *N*-des-Me-DFPA, DFPA residues expressed as DFPA, 1'-COOH-S-2840-A residues expressed as 1'-COOH-S-2840-A, 1'-COOH-S-2840-B residues expressed as 1'-COOH-S-2840-B, 1'-CH2OH-S-2840-A residues expressed as 1'-CH2OH-S-2840-A and 1'-CH2OH-S-2840-B residues expressed as 1'-CH2OH-S-2840-B

B.7.4. Feeding Studies

B.7.4.1. Dietary burden calculations

The representative use of inpyrfluxam is on wheat, triticale and barley which may be fed to livestock.

The dietary burdens for different groups of livestock have been estimated using the OECD feeding tables using the excel spreadsheet proposed by EFSA (pesticide_mrl_guidlines_animal_model_2017). These are presented in the Volume 1.

The dietary burden calculations exceed the trigger of 0.004 mg/kg bw/day for all relevant groups except swine therefore further consideration of feeding studies is required.

Livestock feeding studies were conducted in laying poultry (hens) and lactating ruminants (cows).

B.7.4.2. Poultry

Report:	**KCA 6.4.1/01; [redacted] 2017**
Title:	Magnitude of S-2399 and Metabolites 1'-CH_2OH-S-2840 A&B (including conjugate) and 1'-COOH-S-2840 A&B Residues in Laying Hen Tissues and Eggs from a 28-Day Feeding Study
Document No.:	TPR-0015 (Study No.: 2815W)
Guidelines:	OPPTS 860.1480, PMRA DIR-98-02, Section 8 OECD Guidelines for the testing of chemicals: Residues in Livestock 505 (January 8, 2007) MAFF in Japan (12-Nousa*N*-No. 8147, 3-2-1, 2000)
Guideline deviations:	None
GLP/GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

Materials and methods

The acclimatisation period started 14 days prior to the anticipated start date of dosing and continued until dosing began. During the acclimatisation period the overall health of 175 hens were monitored and 60 were deemed acceptable to be used for the study. Of the 60 birds they were randomly assigned to one of the five treatment groups: Control (12 hens in 3 subgroups of 4 hens: 1A, 1B and 1C), low-dose (12 hens in 3 subgroups of 4 hens: 2A, 2B and 2C), mid-dose (12 hens in 3 subgroups of 4 hens each: 3A, 3B and 3C) and high-dose (24 hens in 6 subgroups of 4 hens each: 4A, 4B, 4C, 4D, 4E and 4F). After acclimatisation sixty egg laying hens (*Gallus gallus domesticus*) were dosed orally with gelatine capsules for 28 consecutive days with inpyrfluxam at dose rates of 0 mg (Group 1: control), 1 mg (Group 2: low-dose), 3 mg (Group 3: mid-dose) and 10 mg (Group 4: high-dose) inpyrfluxam/kg diet (dry weight basis). One control subgroup (1C) and three subgroups for the high-dose group (4D, 4E and 4F) were assigned for use in the depuration of the study, additional samples were collected on 3 separate days throughout the depuration phase, study days 32, 36 and 43. The other birds were terminated within 6 hours of receiving the final dose.

Dose capsules were prepared on a weekly basis based on the mean daily feed consumption levels (dry weight basis) of the dose group hens during the previous week. Dose capsules were prepared by adding calculated amounts of inpyrfluxam into gelatine capsules. Extra doses were prepared each week to allow for dose verification or loss during dosage. Dose capsules were stored in a freezer prior to use. Doses were verified by [redacted].

The hens were fed with 16% layer poultry ration [redacted]) throughout the study. The birds were offered *ad libitum* access to fresh food daily. Daily feed consumption was calculated from the start of acclimatisation until termination. Hens were allowed *ad libitum* access to water.

Given the amounts of test substance added to the dose capsules, the chemical purity of the test substance and the mean feed consumption levels observed during the treatment period, the actual average doses received by the low, medium and high dose groups were 1.031, 3.113 and 10.399 mg a.s./kg feed, respectively.

The dose rates used in the study are summarised in the table below.

Table 7.4.2-1 Summary of inpyrfluxam dose administration

Dose groups	Sub-groups	Number of hens	Dose level		
			mg Inpyrfluxam / Capsule[(a)]	mg Inpyrfluxam / kg bw / Capsule[(b)]	Actual average dose mg a.s. / kg feed

Control	1A, 1B, 1C	12	0	0	0
Low-dose	2A, 2B, 2C	12	0.12	0.063	1.031
Mid-dose	3A, 3B, 3C	12	0.36	0.19	3.113
High-dose	4A, 4B, 4C, 4D, 4E, 4F	24	1.20	0.63	10.339

(a) Based on the average daily feed consumption of 120 g dry m
atter per day.
(b) Based on the average body weight of 1.9 kg.

The hens were dosed daily for 28 consecutive days via capsules given orally. The time of dosing was approximately the same each day. The control animal receiving placebos were dosed first followed by the treatment groups in increasing dosage levels.

Eggs were collected twice daily (AM and PM). Egg collection started at the beginning of the acclimation and continued until termination for each bird. Collection for sampling began on day -1 and continued on select intervals during the dosing. At the AM collection the eggs were placed into labelled containers and refrigerated. At the PM collection the eggs from the AM and PM collection were composited by subgroup and the sample weight recorded. Each pooled sample was thoroughly mixed, divided into two portions and stored frozen. The pooled egg samples on Days 14 and 28 were separated into egg yolk and egg white from a single control (group 1, subgroup 1C), three subgroups in the low-dose dosing group (group 2, subgroups 2A, 2B, and 2C), and three subgroups in the high-dose dosing group (group 4, subgroup 4D, 4E, and 4F). Egg yolk and egg white samples from each dose group were stored frozen after pooling. One sub-sample at the appropriate time points was shipped frozen to EAG laboratories.

On the day of the final dose (within 6 hours) the birds were terminated. Muscle, Liver, fat (abdominal and subcutaneous) samples were collected and pooled by subgroup. Specimens were frozen within one hour of collection and were homogenized in the presence of dry ice. Each specimen was divided, and half of the collected specimen was shipped frozen to [redacted].

Samples were analysed for residues of inpyrfluxam, 1’-COOH-S-2840-A, 1’-COOH-S-2840-B, 1'-CH_2OH-S2840-A and 1'-CH_2OH-S2840-B by LC-MS/MS chromatography. The method validated within the study report on egg, egg white, egg yolk, muscle, liver, and fat with an LOQ of 0.01 mg/kg for inpyrfluxam and 0.005 mg/kg for the metabolites 1’-COOH-S-2840-A&B, 1’-CH_2OH-S-2840-A&B.

The dosages contained in the dose capsules where suitably verified.

During the in-life phase the hens appeared normal and healthy. Group mean daily feed consumption levels were comparable in the acclimation period and the treatment period. Egg production of all groups remained consistent throughout. Group mean body weight changes in all groups were comparable between all dose groups, Post-mortem examination did not reveal any significant tissue abnormalities.

Procedural fortification recoveries were performed for samples of egg and tissue.

Results and discussion

Egg samples were analysed in 14 sets. Each set had two procedural fortifications with the control being spiked just prior to extraction, the fortification levels were at LOQ and 50X LOQ. The average recovery for the procedural fortification samples were in the range of 60 – 120% when fortified at LOQ and 70 – 110% when fortified at 50X LOQ.

No residues greater than LOQ of inpyrfluxam, 1'-COOH-S-2840-A, 1'-COOH-S-2840-B or 1-CH_2OH-S-2840-A were detected in any of the samples of egg during the dosing period. It is noted that residues of inpyrfluxam and 1'-COOH-S-2840-A were detected in the control group on Day-1 at a level lower than the LOQ (0.00181 and 0.000741 mg/kg respectively). No explanation of these residues was provided. No residues of 1-CH_2OH-S-2840-B were detected above LOQ in the control group, the low-dose group or the mid-dose group. However, 1-CH_2OH-S-2840-B was detected in the high-dose group greater than LOQ on treatment days 7, 14, 17, 21, 24, and 28. No residues above LOQ were found in the depuration phase suggesting that these metabolites are quickly excreted. As residues were < LOQ at all time points a plateau could not be determined. Recovery results and residue levels are presented in table 7.4.2-2 and 7.4.2-3 below.

Table 7.4.2-2 Procedural recovery data in egg samples

Fortification level	% Recovery				
	Inpyrfluxam	1'-COOH-S2840-A	1'-COOH-S2840-B	1'-CH_2OH-S2840-A	1'-CH_2OH-S2840-B
LOQ (Inpyrfluxam: 0.01 mg/kg, Metabolites: 0.005mg/kg)	70, 72, 74, 78, 81, 82, 83, 86, 88, 89, 94, 94, 95, 95	68, 68, 68, 68, 74, 74, 75, 76, 81, 82, 83, 85, 86, 87	64, 64, 66, 70, 71, 73, 73, 74, 77, 77, 78, 82, 82	71, 72, 72, 77, 78, 80, 82, 83, 84, 87, 87, 936, 94, 110	65, 68, 70, 75, 78, 78, 80, 82, 82, 85, 89, 95, 96, 108

Average	81	75	73	82	78
% RSD (n=14)	10	12	11	13	14
50 X LOQ (Inpyrfluxam: 0.5mg/kg, Metabolites: 0.25mg/kg)	70, 72, 74, 78, 81, 82, 83, 86, 88, 89, 94, 94, 95, 95	68, 68, 68, 68, 74, 74, 75, 76, 81, 82, 83, 85, 86, 87	64, 64, 66, 70, 71, 73, 73, 74, 77, 77, 77, 78, 82, 82	71, 72, 72, 77, 78, 80, 82, 83, 84, 87, 87, 93, 94, 110	65, 68, 70, 75, 78, 78, 80, 82, 82, 85, 89, 95, 96, 108
Average	84	77	73	84	82
% RSD (n=14)	10	9	8	13	14

Table 7.4.2-3 Residue levels in eggs

Group Dose	Sampling day	Residue levels of individual analytes (mg/kg)				
		Inpyrfluxam	1'-COOH-S2840-A	1'-COOH-S2840-B	1'-CH_2OH-S2840-A	1'-CH_2OH-S2840-B
Group 1 – Control group	Day -1	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 1	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 3	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 7	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 10	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 14	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 17	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 21	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 24	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 28	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005

	3 days post dose	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	7 days post dose	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	14 days post dose	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
Group 2 – low-dose	Day -1	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 1	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 3	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 7	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 10	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 17	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 21	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 24	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
Group 3 – mid-dose	Day -1	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 1	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 3	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 7	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 10	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 14	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 17	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 21	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 24	<0.01 <0.01	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005

		<0.01	<0.005	<0.005	<0.005	<0.005
	Day 28	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
Group 4 – high-dose	Day -1	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005
	Day 1	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005
	Day 3	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005
	Day 7	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 0.00713 0.00625 <0.005 0.00502
	Day 10	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005
	Day 14	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 17	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 0.00649 <0.005 <0.005 <0.005
	Day 21	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 0.00543 <0.005 <0.005
	Day 24	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 0.00792 0.00865 0.00757 0.00676 0.00920
	Day 28	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005

	3 days post dose	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	7 days post dose	<0.01 <0.01	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005
	14 days post dose	<0.01	<0.005	<0.005	<0.005	<0.005

Egg white was analysed in two sets, each set included two procedural fortifications, LOQ and 50X LOQ. The average recovery was in the acceptable range of 60 – 120% when fortified at LOQ. Average recoveries for 1'-COOH-S-2840-A & -B metabolites were below the acceptable level of recovery (64% – 67%) but no residues of these analytes were detected in egg white samples. No residues > LOQ of inpyrfluxam or metabolites were detected in egg white samples of the control dosage group, low- and mid-dose group. In the high-dose group residues of 1'-CH_2OH-S-2840-B were measured above LOQ in the 28-day sample. Recovery results and residue levels are presented in table 7.4.2-4 and 7.4.2-5.

Table 7.4.2-4 Procedural recovery data in egg white samples

Fortification level	% Recovery				
	Inpyrfluxam	1'-COOH-S2840-A	1'-COOH-S2840-B	1'-CH_2OH-S2840-A	1'-CH_2OH-S2840-B
LOQ (Inpyrfluxam: 0.01mg/kg, Metabolites: 0.005mg/kg)	66, 76	64, 66	61, 67	83, 68	81, 69
Average	71	65	64	76	75
% RSD (n=2)	10	2	7	14	11
50 X LOQ (Inpyrfluxam: 0.5mg/kg, Metabolites: 0.25mg/kg)	77, 72	71, 63	68, 60	80, 66	78, 64
Average	75	67	64	73	71
% RSD (n=2)	5	8	9	14	14

Table 7.4.2-5 Residue levels in egg white samples

Group Dose	Sampling day	Residue levels of individual analytes (mg/kg)				
		Inpyrfluxam	1'-COOH-S2840-A	1'-COOH-S2840-B	1'-CH_2OH-S2840-A	1'-CH_2OH-S2840-B
Group 1 – Control group	Day 14	<0.01	<0.005	<0.005	<0.005	<0.005
	Day 28	<0.01	<0.005	<0.005	<0.005	<0.005
Group 2 – Low-dose	Day 14	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 28	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
Group 4 – High-dose	Day 14	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 28	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	0.00525 0.00761 0.00533

Egg yolk was analysed in 2 sample sets with two procedural fortifications at LOQ and 50X LOQ. The average recovery was in the acceptable ranges. No residues > LOQ of inpyrfluxam or metabolites were detected in the egg yolk samples of the control, low-dose group or mid-dose group. In the high-dose group residues of 1-CH_2OH-S-2840-B were measured above LOQ in the day 14 and day 28 sample. Recovery results and residue levels are presented in table 7.4.2-6 and 7.4.2-7.

Table 7.4.2-6 Procedural recovery results in egg yolk samples

Fortification level	% Recovery				
	Inpyrfluxam	1'-COOH-S2840-A	1'-COOH-S2840-B	1'-CH_2OH-S2840-A	1'-CH_2OH-S2840-B
LOQ (Inpyrfluxam: 0.01mg/kg, Metabolites: 0.005mg/kg)	116, 90	103, 92	108, 90	87, 85	90, 87
Average	103	98	99	86	89

% RSD (n=2)	18	8	13	2	2
50 X LOQ (Inpyrfluxam: 0.5mg/kg, Metabolites: 0.25mg/kg)	104, 85	104, 89	102, 87	81, 89	82, 88
Average	95	97	95	85	85
% RSD (n=2)	14	11	11	7	5

Table 7.4.2-7 Residue levels in egg yolk samples

Group Dose	Sampling day	Residue levels of individual analytes (mg/kg)				
		Inpyrfluxam	1'-COOH-S2840-A	1'-COOH-S2840-B	1'-CH_2OH-S2840-A	1'-CH_2OH-S2840-B
Group 1 – Control group	Day 14	<0.01	<0.005	<0.005	<0.005	<0.005
	Day 28	<0.01	<0.005	<0.005	<0.005	<0.005
Group 2 – Low-dose	Day 14	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 28	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
Group 4 – High-dose	Day 14	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	0.00735 0.00764 0.00742
	Day 28	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	0.00103 0.00120 0.00117

Muscle samples were analysed in 3 subgroups with each subgroup including two procedural fortifications (LOQ and 50X LOQ). The average recovery was within acceptable ranges. No residues > LOQ were detected of inpyrfluxam or the metabolites in any of the muscle samples. Recovery results and residue levels are presented in table 7.4.2-8 and 7.4.2-9.

Table 7.4.2-8 Procedural recovery results in muscle samples

Fortification level	% Recovery				
	Inpyrfluxam	1'-COOH-S2840-A	1'-COOH-S2840-B	1'-CH_2OH-S2840-A	1'-CH_2OH-S2840-B
LOQ (Inpyrfluxam: 0.01mg/kg, Metabolites: 0.005mg/kg)	81, 100	90, 102	87, 98	74, 77	70, 74
Average	91	96	93	76	72
% RSD (n=2)	15	9	8	3	4
50 X LOQ (Inpyrfluxam: 0.5mg/kg, Metabolites: 0.25mg/kg)	85, 88	89, 92	88, 88	73, 75	72, 72
Average	87	91	88	74	72
% RSD (n=2)	2	2	0	2	0

Table 7.4.2-9 Residue levels in muscle samples

Group Dose	Sampling day	Residue levels of individual analytes (mg/kg)				
		Inpyrfluxam	1'-COOH-S2840-A	1'-COOH-S2840-B	1'-CH_2OH-S2840-A	1'-CH_2OH-S2840-B
Group 1 – Control group	Day 28	<0.01 <0.01	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005
Group 2 – Low-dose	Day 28	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
Group 3 – Mid-dose	Day 28	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 28	<0.01 <0.01	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005

Group 4 – High-dose		<0.01	<0.005	<0.005	<0.005	<0.005
	3 days post dose	<0.01	<0.005	<0.005	<0.005	<0.005
	7 days post dose	<0.01	<0.005	<0.005	<0.005	<0.005
	14 days post dose	<0.01 <0.01	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005

Liver samples were analysed in two subgroups also, each set included two procedural fortifications one at LOQ and one at 50X LOQ. Recovery levels for both fortification levels were in the acceptable ranges. No residues > LOQ of inpyrfluxam, 1'-COOH-S-2840-A or 1'-CH2OH-S-2840-A were detected in the liver samples analysed at any sampling period. 1'-COOH-S-2840-B was detected in one of three sample repetitions from the mid-dose group at the 28-day timepoint. For the high-dose group 1'-CH_2OH-S-2840-B was detected in all three liver samples. Control group and low-dose group liver samples had no quantifiable residues. No residues of 1'-COOH-S-2840-B or 1'-CH_2OH-S-2840-B were detected in the depuration phase. Recovery results and residue levels are presented in table 7.4.2-10 and 7.4.2-11.

Table 7.4.2-10 Procedural recovery levels in liver samples

Fortification level	**% Recovery**				
	Inpyrfluxam	**1'-COOH-S2840-A**	**1'-COOH-S2840-B**	**1'-CH_2OH-S2840-A**	**1'-CH_2OH-S2840-B**
LOQ (Inpyrfluxam: 0.01mg/kg, Metabolites: 0.005mg/kg)	76, 84	82, 87	85, 89	71, 82	61, 80
Average	80	85	87	77	71
% RSD (n=2)	7	4	3	10	19
50 X LOQ (Inpyrfluxam: 0.5mg/kg, Metabolites: 0.25mg/kg)	83, 109	82, 88	82, 92	77, 80	74, 77
Average	96	85	87	79	76

% RSD (n=2)	19	5	8	3	3

Table 7.4.2-11 Residue levels in liver samples

Group Dose	Sampling day	Residue levels of individual analytes (mg/kg)				
		Inpyrfluxam	1'-COOH-S2840-A	1'-COOH-S2840-B	1'-CH_2OH-S2840-A	1'-CH_2OH-S2840-B
Group 1 – Control group	Day 28	<0.01 <0.01	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005
Group 2 – Low-dose	Day 28	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
Group 3 – Mid-dose	Day 28	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 0.0081
Group 4 – High-dose	Day 28	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 0.0081	<0.005 <0.005 <0.005	0.011 0.014 0.016
	3 days post dose	<0.01	<0.005	<0.005	<0.005	<0.005
	7 days post dose	<0.01	<0.005	<0.005	<0.005	<0.005
	14 days post dose	<0.01 <0.01	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005

Fat samples were analysed in two subgroups in two procedural fortifications (LOQ and 50X LOQ). The average recovery was within the acceptable range for both fortification levels. No residues > LOQ of the metabolites were detected in any of the fat samples. Inpyrfluxam residues were found in all three samples of the high-dose group. No residues of inpyrfluxam or the metabolites were detected in the depuration phase. Recovery results and residue levels are presented in table 7.4.2-12 and 7.4.2-13.

Table 7.4.2-12 Procedural recovery results in fat samples

Fortification level	% Recovery				
	Inpyrfluxam	1'-COOH-S2840-A	1'-COOH-S2840-B	1'-CH_2OH-S2840-A	1'-CH_2OH-S2840-B
LOQ (Inpyrfluxam: 0.01mg/kg, Metabolites: 0.005mg/kg)	71, 85	91, 93	81, 85	64, 84	68, 77
Average	78	92	83	74	73
% RSD (n=2)	13	2	3	19	9
50 X LOQ (Inpyrfluxam: 0.5mg/kg, Metabolites: 0.25mg/kg)	80, 84	87, 90	84, 89	68, 70	68, 71
Average	82	89	87	69	70
% RSD (n=2)	3	2	4	2	3

Table 7.4.2-13 Residue levels in fat samples

Group Dose	Sampling day	Residue levels of individual analytes (mg/kg)				
		Inpyrfluxam	1'-COOH-S2840-A	1'-COOH-S2840-B	1'-CH_2OH-S2840-A	1'-CH_2OH-S2840-B
Group 1 – Control group	Day 28	<0.01 <0.01	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005
Group 2 – Low-dose	Day 28	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
Group 3 – Mid-dose	Day 28	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 28	0.015 0.018	<0.005	<0.005	<0.005	<0.005

		0.018				
Group 4 – High-dose	3 days post dose	<0.01	<0.005	<0.005	<0.005	<0.005
	7 days post dose	<0.01	<0.005	<0.005	<0.005	<0.005
	14 days post dose	<0.01 <0.01	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005

Conclusions

The feeding study was conducted with inpyrfluxam on poultry to determine the levels of relevant residues in poultry tissue and in eggs.

Inpyrfluxam was administered orally (via gelatine capsules) to laying hens for 28 consecutive days at average dose rates of 0mg (Control), 1 mg (low-dose), 3 mg (mid-dose) and 10 mg (high-dose) inpyrfluxam/kg diet (dry weight basis). Feed consumption, body weights and egg production were not adversely affected, and post-mortem examinations did not relevant any significant tissue abnormalities.

Eggs and tissues were analysed for the residues of inpyrfluxam, 1'-COOH-S-2840-A, 1'-COOH-S-2840-A, 1-CH_2OH-S-2840-A and 1-CH_2OH-S-2840-A.

Residues in eggs (composite white and yolk samples) were found to be <LOQ for inpyrfluxam, 1'-COOH-S-2840-A, 1'-COOOH-S-2840-A and 1-CH_2OH-S-2840-A at all dosage rates and time points. 1-CH_2OH-S-2840-A was detected in the high-dose group from day 7 onwards but quickly depreciated to < LOQ during the depuration stage.

Residues in egg white and yolk were found < LOQ for inpyrfluxam, 1'-COOH-S-2840-A, 1'-COOH-S-2840-A and 1-CH_2OH-S-2840-A at all dosage rates and time points. Residues of 1-CH_2OH-S-2840-A were detected in the high-dose group at Day 14.

Residues in fat were all < LOQ, except inpyrfluxam which was detected above LOQ in the high-dose group.

Residues in muscle were all < LOQ.

Residues in liver were all < LOQ expect for 1'-COOH-S-2840-A which was found in the mid-dose group and 1'-CH_2OH-S2840-A which was found in the mid- and high-dose group.

After the depuration phase of 3, 7 and 14 days all measured residues of inpyrfluxam and its metabolites were below LOQ in eggs and tissues.

B.7.4.3. Ruminants

Report:	**KCA 6.4.2/01; [redacted] 2016**
Title:	Magnitude of S-2399 and Metabolites 1'-CH_2OH-S-2840 A&B (including conjugate) and 1'-COOH-S-2840 A&B Residues in Bovine Tissues and Milk from a 28-Day Feeding Study
Document No.:	TPR-0013 (Study No.: 2814W)
Guidelines:	OPPTS 860.1480, PMRA DIR-98-02, Section 8 OECD Guidelines for the testing of chemicals: Residues in Livestock 505 (January 8, 2007) MAFF in Japan (12-Nousa*N*-No. 8147, 3-2-1, 2000)
Guideline deviations:	None
GLP/GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

Materials and methods

The acclimatisation period started 14 days prior to the start of dosing and continued until dosing began. After acclimatisation fourteen lactating dairy cows (Holstein cows) were dosed orally with gelatine capsules containing inpyrfluxam for 28 consecutive days at the dose rates of 0 mg (control group), 2 mg (low-dose), 6 mg (mid-dose) and 20 mg (high-dose) inpyrfluxam/kg diet (dry weight basis). During the acclimatisation period the cows were monitored to ensure they were healthy enough for the trial, suitable animals those not exhibiting behavioural or physical abnormalities were randomly selected for each dose group. Fourteen lactating cows were randomly assigned to the four treatment groups: control (2 cows), low-dose (3 cows), mid-dose (3 cows) and high-dose (6 cows). One control and three animals from the high-dose group were randomly assigned for use in the depuration phase where samples were collected on three separate days throughout the depuration phase (days 31, 35 and 42). The other cows were terminated within 24 hours of the final dose.

Dose capsules were prepared on a weekly basis with daily dosages based on the mean daily feed consumption (dry weight basis) of the dose group cows during the first five days of the previous week. Dose capsules were prepared by transferring pre-calculated amounts of inpyrfluxam into gelatine capsules. Three extra dose capsules were prepared each week to allow for dose verification or loss during dosing. Dose capsules were stored in a freezer prior to use. Empty capsules were prepared weekly for the untreated control cows. Doses were verified by LC-UV analysis.

The cows were fed 6 g of grain, 6 kg baled hay and approximately 20 kg corn silage per day with feedings taking place twice daily at the AM and PM milking. The quantity of corn silage was adjusted based on consumption. Feed consumption was measured daily prior to the AM feeding. The cows were offered fresh potable well water *ad libitum*. The dose rates used the trial are summarised in the table below:

Table 7.4.3-1 Summary of inpyrfluxam dose administration

Dose groups	Number of cows	Target dose (mg/kg feed)	Capsule dose level		
			mg inpyrfluxam / Capsule[(a)]	mg inpyrfluxam / kg bw / Capsule[(b)]	mg a.s. / kg feed
Control	2	0	0	0	0
Low-dose	3	2	35.5	0.07	1.99
Mid-dose	3	6	106.4	0.20	6.01
High-dose	6	20	354.8	0.61	19.84

(a) Based on the average daily feed consumption.
(b) Based on the average body weight.

The cows were dosed for 28 consecutive days via capsules given orally. The time of dosing should be roughly the same each day. The control animal received the placebo concurrently with the treated animals.

The cows were milked twice daily. Collection started on acclimation day -1 and continued throughout the study, the milk from each treated animal over a 24 hour period was pooled. A 400 g composite sample of milk was prepared based on the calculated percentage of AM and PM milk production for each animal on that study day. The pooled samples were mixed thoroughly and divided into two portions and stored frozen. One subsample was shipped frozen for analysis at [redacted].

Extra pooled milk from days 14 and 28 were separated into cream and skim milk from the two control groups, three animals from the low-dose group and three animals from high-dose group. The samples of cream and skim milk were shipped for analysis by [redacted].

Within 24 hours of the final dose animals were terminated. Samples of muscle (round, flank and loin), liver, kidney and fat (perirenal, omental and subcutaneous) approximately 500 g were taken. The samples were frozen within an hour of collection. Cow tissues were homogenised and divided with approximately half the specimen shipped to [redacted] for analysis.

Samples were analysed for residues of inpyrfluxam, 1'-COOH-S-2840-A, 1'-COOH-S-2840-B, 1'-CH_2OH-S2840-A and 1'-CH_2OH-S2840-B by LC-MS/MS chromatography. The method was validated within the study report on milk, liver, kidney, muscle, fat and eggs with an LOQ of 0.010 mg/kg for inpyrfluxam and 0.005mg/kg for all other metabolites.

To verify the dosages the representative dose capsules from each dose level were shipped to [redacted]. Two dose capsules from each dose group were selected for each week of dosing. The capsules were opened and the inpyrfluxam rinsed with methanol, with the rinse collected in a volumetric flask. The flask was made to volume with methanol and analysed by HPLC-UV. The dose capsules were found to be accurate and stable over the course of the study.

Upon receipt all animals received a physical examination, no cows were excluded from the study upon initial examination. The animals were observed for a minimum of twice daily for clinical abnormalities from receipt until termination. Any problems were noted and if any observation highlighted health related issues animals were re-examined by a licensed veterinarian. Animals were otherwise normal and healthy; no indication of treatment-related effects were observed. Animal weights were recorded throughout the study and no significant changes were observed. Feed consumption for all groups throughout the study was considered normal for dairy cows. Milk production remained consistent throughout the study.

Procedural fortification recoveries were performed for samples of milk and tissue.

Results and discussion

Milk samples were analysed at two procedural fortifications (one at LOQ and one at 50X LOQ). The average procedural recovery fortifications were within the acceptable range of 60 – 120% when fortified at LOQ and 70 – 110% when fortified at 50X LOQ. No residues > LOQ of inpyrfluxam or the metabolites were detected in any of the treated or control milk samples at any time. As such, a plateau could not be observed in milk. Recovery results and residue levels are presented in table 7.4.3-2 and 7.4.3-3 below.

Table 7.4.3-2 Procedural recovery results for milk samples

Fortification level	% recovery				
	Inpyrfluxam	1'-COOH-S2840-A	1'-COOH-S2840-B	1'-CH_2OH-S2840-A	1'-CH_2OH-S2840-B
LOQ (Inpyrfluxam: 0.01 mg/kg, Metabolites: 0.005 mg/kg)	63, 65, 66, 67, 68, 69, 73, 73, 74, 75, 78, 80, 81, 83,85	63, 67, 69, 73, 78, 79, 80, 82, 85, 85, 86, 91, 92, 93, 120	70, 74, 76, 78, 79, 81, 84, 85, 88, 89, 92, 92, 93, 95	74, 75, 75, 75, 78, 78, 83, 83, 84, 85, 85, 87, 90, 100, 105	69, 72, 72, 73, 76, 76, 79, 79, 80 , 83, 83, 86, 90, 92, 99
Average	**73**	**83**	**84**	**84**	**81**
% RSD (n=15)	**9**	**17**	**9**	**11**	**10**
50 X LOQ (Inpyrfluxam: 0.5 mg/kg, Metabolites: 0.25 mg/kg)	70, 72, 75, 78, 80, 82, 83, 84, 85, 86, 87, 89, 89, 92, 93	76, 80, 86, 90, 93, 94, 95, 95, 96, 97, 98, 99, 102, 102, 104	79, 81, 85, 91, 92, 92, 94, 97, 97, 98, 98, 102, 104, 106, 108	83, 84, 85, 86, 86, 87, 93, 93, 94, 94, 96, 97, 101, 101, 112	82, 83, 84, 85, 86, 90, 91, 92, 93, 96, 96, 98, 101, 101, 110
Average	**83**	**94**	**95**	**93**	**93**
% RSD (n=15)	**8**	**8**	**9**	**9**	**9**

Table 7.4.3-3 Residue levels in milk samples

Group Dose	Sampling day	Residue levels of individual analytes (mg/kg)				
		Inpyrfluxam	1'-COOH-S2840-A	1'-COOH-S2840-B	1'-CH_2OH-S2840-A	1'-CH_2OH-S2840-B
Group 1 – Control group	Day -1	<0.01 <0.01	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005
	Day 1	<0.01 <0.01	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005
	Day 3	<0.01 <0.01	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005
	Day 7	<0.01 <0.01	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005
	Day 10	<0.01 <0.01	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005

	Day 14	<0.01 <0.01	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005
	Day 17	<0.01 <0.01	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005
	Day 21	<0.01 <0.01	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005
	Day 24	<0.01 <0.01	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005
	Day 28	<0.01 <0.01	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005
	3 days post dose	<0.01	<0.005	<0.005	<0.005	<0.005
	7 days post dose	<0.01	<0.005	<0.005	<0.005	<0.005
	14 days post dose	<0.01	<0.005	<0.005	<0.005	<0.005
Group 2 – Low-dose	Day -1	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 1	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 3	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 7	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 10	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 14	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 17	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 21	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 24	<0.01	<0.005	<0.005	<0.005	<0.005

		<0.01 <0.01	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005
	Day 28	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	3 days post dose	<0.01	<0.005	<0.005	<0.005	<0.005
	7 days post dose	<0.01	<0.005	<0.005	<0.005	<0.005
	14 days post dose	<0.01	<0.005	<0.005	<0.005	<0.005
Group 3 – Mid-dose	Day -1	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 1	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 3	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 7	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 10	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 14	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 17	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 21	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 24	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 28	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005

Group 4 – High-dose	Day -1	<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
	Day 1	<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
	Day 3	<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
	Day 7	<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
	Day 10	<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
	Day 14	<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
	Day 17	<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
		<0.01	<0.005	<0.005	<0.005	<0.005
	Day 21	<0.01	<0.005	<0.005	<0.005	<0.005

		<0.01 <0.01 <0.01 <0.01 <0.01	<0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005
	Day 24	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005
	Day 28	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005
	3 days post dose	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	7 days post dose	<0.01 <0.01	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005
	14 days post dose	<0.01	<0.005	<0.005	<0.005	<0.005

Skimmed milk samples were analysed in 2 sample sets. The average recovery and RSD for the procedural fortifications at LOQ were within the acceptable range as was the recoveries for the 50X LOQ samples. No residues > LOQ of inpyrfluxam or any of the metabolites were detected in any of the skimmed milk samples in any dose group. Recovery and residue results are presented in Table 7.4.3-4 and 7.4.3-5 below.

Table 7.4.3-4 Procedural recovery results for skimmed milk

Fortification level	% Recovery				
	Inpyrfluxam	1'-COOH-S2840-A	1'-COOH-S2840-B	1'-CH_2OH-S2840-A	1'-CH_2OH-S2840-B
LOQ (Inpyrfluxam: 0.01 mg/kg, Metabolites: 0.005 mg/kg)	65, 69	68, 79	76, 79	74, 82	72, 72

Average	**67**	**74**	**78**	**78**	**72**
% RSD (n=2)	**2.8**	**7.8**	**2.1**	**5.7**	**0**
50 X LOQ (Inpyrfluxam: 0.5 mg/kg, Metabolites: 0.25 mg/kg)	78, 82	88, 97	85, 95	91, 96	90, 96
Average	**80**	**93**	**90**	**94**	**93**
% RSD (n=2)	**3**	**6**	**7**	**4**	**4**

Table 7.4.3-5 Residue levels in skimmed milk

Group Dose	Sampling day	Residue levels of individual analytes (mg/kg)				
		Inpyrfluxam	1'-COOH-S2840-A	1'-COOH-S2840-B	1'-CH_2OH-S2840-A	1'-CH_2OH-S2840-B
Group 1 – Control group	Day 14	<0.01	<0.005	<0.005	<0.005	<0.005
	Day 28	<0.01	<0.005	<0.005	<0.005	<0.005
Group 2 – Low-dose	Day 14	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 28	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
Group 4 – High-dose	Day 14	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 28	<0.01 <0.01 <0.01 <0.01	<0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005

Cream was analysed in 2 sample sets. All recoveries and RSDs reported for samples at LOQ and 50X LOQ were within the acceptable ranges. No residues > LOQ of inpyrfluxam or any of the metabolites analysed for were detected in the cream samples analysed. Recovery results and residue levels are presented in table 7.4.3-6 and 7.4.3-7 below.

Table 7.4.3-6 Procedural recovery results for cream

Fortification level	% Recovery				
	Inpyrfluxam	1'-COOH-S2840-A	1'-COOH-S2840-B	1'-CH_2OH-S2840-A	1'-CH_2OH-S2840-B
LOQ (Inpyrfluxam: 0.01 mg/kg, Metabolites: 0.005 mg/kg)	70, 92	77, 96	78, 90	86, 93	75, 88
Average	**81**	**87**	**84**	**90**	**82**
% RSD (n=2)	**16**	**13**	**8**	**5**	**9**
50 X LOQ (Inpyrfluxam: 0.5 mg/kg, Metabolites: 0.25 mg/kg)	77, 102	82, 108	83, 111	91, 92	84, 91
Average	**90**	**95**	**97**	**92**	**88**
% RSD (n=2)	**18**	**18**	**20**	**1**	**5**

Table 7.4.3-7 Residue levels in cream

Group Dose	Sampling day	Residue levels of individual analytes (mg/kg)				
		Inpyrfluxam	1'-COOH-S2840-A	1'-COOH-S2840-B	1'-CH_2OH-S2840-A	1'-CH_2OH-S2840-B
Group 1 – Control group	Day 14	<0.01	<0.005	<0.005	<0.005	<0.005
	Day 28	<0.01	<0.005	<0.005	<0.005	<0.005
Group 2 – Low-dose	Day 14	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 28	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	Day 14	<0.01 <0.01	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005

Group 4 – High-dose		<0.01	<0.005	<0.005	<0.005	<0.005
	Day 28	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005

Muscle samples were analysed in 3 sample sets. Two procedural fortifications at LOQ and 50X LOQ were analysed. All recoveries were within the acceptable ranges for the respective fortification level. No residues > LOQ were detected of inpyrfluxam or any metabolites in any of the samples. Recovery results and residue levels are presented in table 7.4.3-8 and 7.4.3-9 below, results are taken from flank and loin muscle samples.

Table 7.4.3-8 Procedural recovery results for muscle samples

Fortification level	% Recovery				
	Inpyrfluxam	1'-COOH-S2840-A	1'-COOH-S2840-B	1'-CH_2OH-S2840-A	1'-CH_2OH-S2840-B
LOQ (Inpyrfluxam: 0.01 mg/kg, Metabolites: 0.005 mg/kg)	68, 71, 78	70, 72, 90	75, 84, 94	71, 82, 84	69, 75, 83
Average	**72**	**77**	**84**	**79**	**76**
% RSD (n=3)	**7**	**14**	**11**	**9**	**9**
50 X LOQ (Inpyrfluxam: 0.5 mg/kg, Metabolites: 0.25 mg/kg)	84, 87, 88	89, 102, 107	90, 98, 104	83, 91, 90	82, 91, 92
Average	**86**	**99**	**97**	**88**	**88**
% RSD (n=3)	**2**	**9**	**7**	**5**	**6**

Table 7.4.3-9 Residue levels in muscle samples

Group Dose	Sampling day	Residue levels of individual analytes (mg/kg)				
		Inpyrfluxam	1'-COOH-S2840-A	1'-COOH-S2840-B	1'-CH_2OH-S2840-A	1'-CH_2OH-S2840-B
	Day 28	<0.01 <0.01	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005

Group 1 – Control group	14 days post dose	<0.01 <0.01	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005
Group 2 – Low-dose	Day 28	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005
Group 3 – Mid-dose	Day 28	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005
Group 4 – High-dose	Day 28	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005
	3 days post dose	<0.01 <0.01	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005
	7 days post dose	<0.01 <0.01	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005
	14 days post dose	<0.01 <0.01	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005	<0.005 <0.005

Liver samples were analysed in 2 sets with all average recoveries being within the acceptable ranges. No residues (> LOQ) of inpyrfluxam, 1'-COOH-S-2840-A, 1'-COOH-S-2840-B or 1-CH_2OH-S-2840-A were detected in any of the liver samples analysed. One of the three repetition samples from day 28 mid-dose group had a quantifiable level of 1-CH_2OH-S-2840-B, the remaining day 28 residues for that dose group were < LOQ, which gives a mean residue of 0.0061 mg/kg. For the high-dose group samples all reptations gave quantifiable residues of 1-CH_2OH-2840-B with an average of 0.0127 mg/kg. The control and low-dose group had no quantifiable residues. 1-CH_2OH-2840-B was not detected in any of the depuration phase samples. Recoveries and residue levels in liver samples are reported in tables 7.4.3-10 and 7.4.3-11 below.

Table 7.4.3-10 Procedural recovery results for liver samples

Fortification level	% Recovery				
	Inpyrfluxam	1'-COOH-S2840-A	1'-COOH-S2840-B	1'-CH_2OH-S2840-A	1'-CH_2OH-S2840-B
LOQ (Inpyrfluxam: 0.01 mg/kg, Metabolites: 0.005 mg/kg)	62, 79	74, 83	67, 86	71, 71	64, 73
Average	**71**	**79**	**77**	**71**	**69**
% RSD (n=3)	**12**	**6**	**13**	**0**	**9**
50 X LOQ (Inpyrfluxam: 0.5 mg/kg, Metabolites: 0.25 mg/kg)	71, 78	90, 97	86, 100	80, 89	78, 86
Average	**75**	**94**	**93**	**85**	**82**
% RSD (n=3)	**5**	**5**	**10**	**6**	**6**

Table 7.4.3-11 Residue levels in liver samples

Group Dose	Sampling day	Residue levels of individual analytes (mg/kg)				
		Inpyrfluxam	1'-COOH-S2840-A	1'-COOH-S2840-B	1'-CH_2OH-S2840-A	1'-CH_2OH-S2840-B
Group 1 – Control group	Day 28	<0.01	<0.005	<0.005	<0.005	<0.005
	14 days post dose	<0.01	<0.005	<0.005	<0.005	<0.005
Group 2 – Low-dose	Day 28	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
Group 3 – Mid-dose	Day 28	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 0.0084
	Day 28	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	0.0156 0.0080 0.0146

Group 4 – High-dose	3 days post dose	<0.01	<0.005	<0.005	<0.005	<0.005
	7 days post dose	<0.01	<0.005	<0.005	<0.005	<0.005
	14 days post dose	<0.01	<0.005	<0.005	<0.005	<0.005

Kidney samples were analysed in two sets at LOQ and 50X LOQ. Kidney set #2 was not analysed at 50X due to loss of sample during analysis. Average recovery levels were in the acceptable range of 60 – 120% and 70 – 110% for LOQ and 50X LOQ fortification levels respectively. Results are presented in table 7.4.3-12 below.

No quantifiable residues (> LOQ) were detected of inpyrfluxam, 1'-COOH-S-2840-A, 1'-COOH-S-2840-B or 1-CH_2OH-S-2840-A. 1'-CH_2OH-S-2840-B was quantifiable in kidney samples. Residues of 1'-CH_2OH-S-2840-B were detected in all repetitions of the day 28 mid-dose group at 0.0117 mg/kg and high-dose group 0.0215mg/kg. Results are presented in table 7.4.3-13 below.

Table 7.4.3-12 Procedural recoveries results for kidney samples

Fortification level	**% Recovery**				
	Inpyrfluxam	**1'-COOH-S2840-A**	**1'-COOH-S2840-B**	**1'-CH_2OH-S2840-A**	**1'-CH_2OH-S2840-B**
LOQ (Inpyrfluxam: 0.01 mg/kg, Metabolites: 0.005 mg/kg)	61, 95	69, 91	75, 95	63, 85	61, 78
Average	**78**	**80**	**85**	**76**	**70**
% RSD (n=3)	**24**	**16**	**14**	**16**	**12**
50 X LOQ (Inpyrfluxam: 0.5 mg/kg, Metabolites: 0.25 mg/kg)	71	88	90	81	78
Average	**71**	**88**	**90**	**81**	**78**
% RSD (n=3)	**-**	**-**	**-**	**-**	**-**

Table 7.4.3-13 Residue results in kidney samples

Group Dose	Sampling day	Residue levels of individual analytes (mg/kg)				
		Inpyrfluxam	1'-COOH-S2840-A	1'-COOH-S2840-B	1'-CH_2OH-S2840-A	1'-CH_2OH-S2840-B
Group 1 – Control group	Day 28	<0.01	<0.005	<0.005	<0.005	<0.005
	14 days post dose	<0.01	<0.005	<0.005	<0.005	<0.005
Group 2 – Low-dose	Day 28	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
Group 3 – Mid-dose	Day 28	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	0.0140 0.00753 0.0135
Group 4 – High-dose	Day 28	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	0.0180 0.0147 0.0319
	3 days post dose	<0.01	<0.005	<0.005	<0.005	<0.005
	7 days post dose	<0.01	<0.005	<0.005	<0.005	<0.005
	14 days post dose	<0.01	<0.005	<0.005	<0.005	<0.005

Fat samples were analysed in 4 sets. All recoveries were within the respectable ranges. No residues of inpyrfluxam or any metabolites were detected in the fat samples. Recovery results and residue levels are presented in table 7.4.3-14 and 7.4.3-15 below.

Table 7.4.3-14 Procedural recoveries results for fat samples

Fortification level	% Recovery				
	Inpyrfluxam	1'-COOH-S2840-A	1'-COOH-S2840-B	1'-CH_2OH-S2840-A	1'-CH_2OH-S2840-B

LOQ (Inpyrfluxam: 0.01 mg/kg, Metabolites: 0.005 mg/kg)	73	56	57	75	73
	216[(a)]	69	67	63	60
	74	66	68	65	66
	75	71	81	69	68
Average	**74**	**66**	**68**	**68**	**67**
% RSD (n=4)	**1**	**10**	**14**	**8**	**8**
50 X LOQ (Inpyrfluxam: 0.5 mg/kg, Metabolites: 0.25 mg/kg)	90	69	66	82	82
	83	80	79	67	66
	82	76	74	74	71
	77	72	70	70	70
Average	**83**	**74**	**72**	**73**	**72**
% RSD (n=4)	**6**	**6**	**8**	**9**	**9**

[(a)] Sample identified as an outlier through statistical tests, the method has been shown to be validated in the matrices of fat and therefore the three acceptable procedural recoveries are considered sufficient.

Table 7.4.3-15 Residue levels in fat samples

Group Dose	Sampling day	Fat type	Residue levels of individual analytes (mg/kg)				
			Inpyrfluxam	1'-COOH-S2840-A	1'-COOH-S2840-B	1'-CH_2OH-S2840-A	1'-CH_2OH-S2840-B
Group 1 – Control group	Day 28	Perirenal	<0.01	<0.005	<0.005	<0.005	<0.005
		Omental	<0.01	<0.005	<0.005	<0.005	<0.005
		Subcutaneous	<0.01	<0.005	<0.005	<0.005	<0.005
	14 days post dose	Perirenal	<0.01[(a)]	<0.005[(a)]	<0.005[(a)]	<0.005[(a)]	<0.005[(a)]
		Omental	<0.01	<0.005	<0.005	<0.005	<0.005
		Subcutaneous	<0.01	<0.005	<0.005	<0.005	<0.005
Group 2 –	Day 28	Perirenal	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005

Low-dose		Omental	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
		Subcutaneous	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
Group 3 – Mid-dose	Day 28	Perirenal	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
		Omental	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
		Subcutaneous	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
Group 4 – High-dose	Day 28	Perirenal	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
		Omental	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
		Subcutaneous	<0.01 <0.01 <0.01	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005	<0.005 <0.005 <0.005
	3 days post dose	Perirenal	<0.01	<0.005	<0.005	<0.005	<0.005
		Omental	<0.01	<0.005	<0.005	<0.005	<0.005
		Subcutaneous	<0.01	<0.005	<0.005	<0.005	<0.005
	7 days post dose	Perirenal	<0.01	<0.005	<0.005	<0.005	<0.005
		Omental	<0.01	<0.005	<0.005	<0.005	<0.005
		Subcutaneous	<0.01	<0.005	<0.005	<0.005	<0.005
	14 days post dose	Perirenal	<0.01	<0.005	<0.005	<0.005	<0.005
		Omental	<0.01	<0.005	<0.005	<0.005	<0.005
		Subcutaneous	<0.01	<0.005	<0.005	<0.005	<0.005

[(a)] Samples were analysed using the muscle extraction method, this was noted as a protocol deviation. No impact on the study is expected as no residues were detected in samples 3 days post dose or 7 days post dose therefore, no residues are expected 14 days post dose.

Conclusion

The feeding study was conducted with inpyrfluxam on ruminants to determine the levels of relevant residues in ruminants' milk and tissue.

Inpyrfluxam was administered orally (via gelatine capsules) for 28 consecutive days at the dose rates of 0 mg (Group 1: control group), 2 mg (Group 2: Low-dose), 6mg (Group 3: Mid-dose) and 20 mg (Group 4: High-dose) inpyrfluxam/kg diet (dry weight basis). Feed consumption, body weight and milk production were monitored throughout, and no adverse effects were noted.

Milk and tissues were analysed for residues of inpyrfluxam, 1'-COOH-S-2840-A, 1'-COOH-S-2840-B, 1-CH_2OH-S-2840-A and 1-CH_2OH-S-2840-B.

No quantifiable residues (> LOQ) were detected of 1'-COOH-S-2840-A, 1'-COOH-S-2840-B, 1-CH_2OH-S-2840-A in any samples at any time point. Residues of 1-CH_2OH-S-2840-B were detected in samples of liver and kidney from the mid- and high-dose groups in the day 28 samples.

After the depuration phase of 31, 35 and 42 days all measured residues of inpyrfluxam and its metabolites were below LOQ in tissue and milk samples.

B.7.4.4. Pigs

The maximum dietary burden for pigs is 0.003 mg/kg bw per day which is below the trigger of 0.004mg/kg bw therefore no feeding studies for pigs is required. Additionally, the metabolism in rats is sufficiently similar to that in poultry and ruminants so no further consideration of swine is required (see B.7.2.4).

B.7.4.5. Fish

No residues study on fish was conducted.

Currently no test method or guidance is available for conducting residue studies on fish. In this case it is acceptable not to address the need for fish studies in accordance with the "Guidance document for applicants on preparing dossiers for the approval of a chemical new active substance and the renewal of approval of the chemical active substance according to Regulation (EU) No. 283/2013 and Regulation (EU) No. 284/2013" (SANCO/10181/2013-rev.2 of 2-May-2013).

B.7.5. Effects of Processing

B.7.5.1. Nature of the residue

Four separate studies were evaluated investigating the nature of residues over processing (under representative high temperature hydrolysis conditions to consider

the stability of the substances over processing) for the 4 substances: inpyrfluxam, 1'-COOH-S-2840, 1'-CH_2OH-S-2840 and 3'-OH-S-2840.

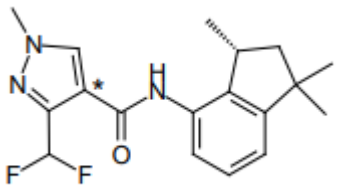
B.7.5.1.1 Inpyrfluxam

Report:	**KCA 6.5.1/01; [redacted] 2016**
Title:	[^{14}C]S-2399 – Nature of residues in processed commodities – High temperature hydrolysis
Document No.:	TPM-0022 (Study No.: 13048.6947)
Guidelines:	OECD Test Guidelines 507
Guideline deviations:	None
GLP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

Materials and Methods

The material used for testing in a hydrolysis study (according to OECD guideline 507) was [pyrazolyl-4-^{14}C] inpyrfluxam. Although there is only one radiolabel form tested (unlike the plant metabolism studies) this is acceptable as the current study showed full recovery of the test material and the material is stable, without any cleavage of the molecule.

Chemical structure



*Denotes position of ^{14}C label

Radiolabel position [pyrazolyl-4-^{14}C] inpyrfluxam

Radiochemical purity 99.3% (HPLC)

Chemical purity (%) 99.3% (HPLC)

Specific activity	2.11 GBq/mmol, 57 mCi/mmol (377,506 dpm/µg)

Buffers were suitably prepared at pH 4, pH 5 and pH 6 for use in the study.

An isotopically diluted stock solution was prepared by combining purified primary radiolabelled stock solution (checked for specific activity) with non-radiolabelled stock solution and making to volume with acetonitrile. Final checks of specific activity and concentration were made prior to preparing the test samples.

Individual samples of 10 mL of dosed buffer solution were prepared in test vials. The intended concentration was 1.00 µg/mL (in line with the OECD guidance) and the measured concentrations were close to this with levels of 1.02 µg/mL (pH 4), 1.02 µg/mL (pH 5) and 1.03 µg/mL (pH 6) [pyrazole-4-^{14}C] inpyrfluxam. The test vials were flushed with nitrogen and then capped.

In line with OECD guideline 507, tests were carried out at three pH levels and temperatures as shown in the table below.

Table 7.5.1.1-1: Hydrolysis study test conditions

pH	Process represented	Temperature (°C)	Incubation time (min)
4	Pasteurisation	90	20
5	Baking, Brewing and Boiling	100	60
6	Sterilisation	120	20

Two samples for each buffer solution were extracted immediately after T0 for analysis. An additionally 8 samples were incubated in the thermostatic mineral oil baths in the dark set at 90°C, 100°C and 120°C respectively. The incubation temperature was monitored using a data logger.

Buffer solutions were placed in a closed system of sealed crimp-caped glass vials. A sealed glass vials placed in a 120°C bath is auto-pressurising by the vapour pressure of water at this temperature. Providing the vessel withstands these conditions this is considered to be a pressurised system as required for the sterilisation procedure.

Over the course of the tests, all incubation temperatures were within ±5°C of the target temperature and minimal effects of dosing on the sample pH occurred. Results of sterility tests indicated that all samples remained sterile.

Duplicate samples were analysed at each sampling interval. Samples were removed from the thermostatic baths and allowed to cool for approximately 30 seconds. An aliquot of acetonitrile was then added to halt any further hydrolysis. Samples not immediately analysed were stored frozen, all samples were generally analysed within one day of sampling.

Samples were vortexed for approximately 10 seconds, the volume measured and analysed by LSC allowing quantification of the radioactivity. Samples were then analysed by HPLC/RAM (Radiodetection) for determination of the distribution of [pyrazole-4-^{14}C] inpyrfluxam. Chiral HPLC was used to determine the R-isomer as well as any potential residues of the S-isomer of inpyrfluxam. There were no degrades to quantify.

Quantification and identification was achieved by co-chromatography with reference standards (available for inpyrfluxam (pure R-isomer) and the S-isomer of inpyrfluxam), prepared in a mixed standard.

Results and discussion

Based on the measurements after hydrolysis the mean % applied recovery was > 97.6% under all conditions. This indicates that there was no significant precipitation, adsorption to glassware or volatile degradation products formed.

Results of measured radioactivity are given in table 7.5.1.1-2 below. These reflect the content of parent substance (inpyrfluxam) as in the tests almost complete recovery of the parent compound was observed (> 97%). No further characterisation of the identity of residues was done, aside from chiral analysis performed to determine the distribution of chiral forms of [pyrazole-4-^{14}C] inpyrfluxam by HPLC. Inpyrfluxam (S-isomer) was not detected at time 0 or at study termination. There was no observed isomerisation in any of the samples and the *R* isomer accounted for all radioactive residues.

Table 7.5.1.1-2: Material balance of radioactivity in all samples

Test item	Processing conditions	% Applied recovery time 0 (Average)	% Applied recovery termination (average)	Recovery %[(a)]
	pH 4, 90°C, 20 mins	97.1, 97.7 (97.4)	97.5, 98.2 (97.9)	100.5

[pyrazole-4-^{14}C] inpyrfluxam	pH 5, 100°C, 60 mins	93.9, 95.9 (94.9)	97.1, 98.7 (97.9)	103.2
	pH 6, 120°C, 20 mins	94.9, 98.9 (96.9)	97.4, 97.7 (97.6)	100.7

(a) Recovery calculated between time 0 and termination

Conclusion

Degradation products were not observed under any of the representative conditions, and the recovery of parent inpyrfluxam was high. Under the representative conditions inpyrfluxam is considered to be stable. The parent material, the R-isomer inpyrfluxam did not convert to the S-isomer, as none was detected.

B.7.5.1.2 1'-COOH-S-2840

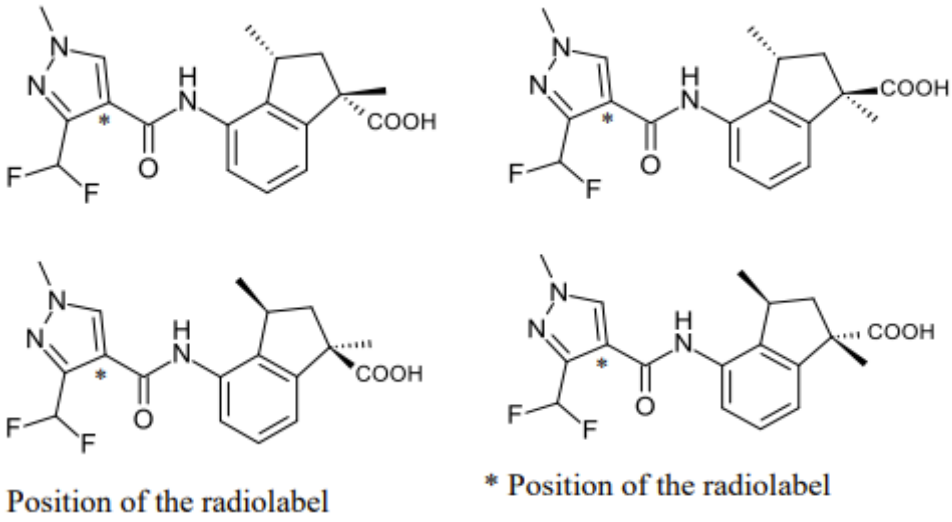
Report:	**KCA 6.5.1/04; [redacted] 2020**
Title:	[^{14}C]1'-COOH-S-2840: OECD 507 Nature of residue – High temperature hydrolysis
Document No.:	TPM-0067 (Study No.: 3202780)
Guidelines:	OECD Guideline 507
Guideline deviations:	None
GLP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

Materials and methods

The material used for testing in a hydrolysis study (according to OECD guideline 507) was [pyrazole-4-^{14}C] 1'-COOH-S-2840-A and 1'-COOH-S-2840-B. Although there is only one radiolabel form (pyrazole-4-^{14}C) tested (unlike the plant metabolism studies) this is acceptable as the current study showed full recovery of the test material and the material is stable, without any cleavage of the molecule.

The metabolite 1'-COOH-S-2840 exists as four isomers, two for [pyrazolyl-4-^{14}C] 1'-COOH-S-2840-A and two for [pyrazolyl-4-^{14}C] 1'-COOH-S-2840-B.

Chemical structure



* Position of the radiolabel

* Position of the radiolabel

Radiolabel position	[Pyazolyl-4-^{14}C] 1'-COOH-S-2840-A	[Pyazolyl-4-^{14}C] 1'-COOH-S-2840-B
Radiochemical purity	93.9%	94.6%
Chemical purity (%)	99.7%	99.8%
Specific activity	5.81MBq/mg, 2.11 GBq/mmol	5.81MBq/mg, 2.11 GBq/mmol

Buffers were suitably prepared at pH level 4, pH 5 and pH 6 for use in the study.

Radiolabelled stock solutions (checked) of [pyrazole-4-^{14}C] 1'-COOH-S-2840-A and [pyrazole-4-^{14}C] 1'-COOH-S-2840-B were added to the prepared pH buffers to obtained solutions with a concentration of 1.00 mg/L in line with OECD guidance (1.054 mg/L for 'A' and 1.063 mg/L for 'B').

In line with OECD guideline 507, tests were carried out under three different conditions as shown in the table below.

Table 7.5.1.2-1: Hydrolysis study test conditions

pH	Process represented	Temperature (°C)	Incubation time (min)
4	Pasteurisation	90	20

5	Baking, Brewing and Boiling	100	60
6	Sterilisation	120	20

For each of the ‘pH’ treatment conditions (see above summary), four vessels were prepared with [pyrazole-4-^{14}C] 1’-COOH-S-2840-A and four with [pyrazole-4-^{14}C] 1’-COOH-S-2840-B. Two vessels of each substance were analysed at T0 and the two other vessels incubated for the specified time period.

Incubations were performed in the dark in a bath of polyethylene glycol (PEG). Temperatures were recorded throughout incubation.

For each pH treatment, two further vessels with non-radiolabelled 1’-COOH-S-2840-A and 1’-COOH-S-2840-B were used to check the pH after incubation. Plain buffer was used to check the pH prior to incubation.

Buffer solutions were placed in a closed system of sealed crimp-caped glass vials. Sealed glass vials placed in a 120°C bath are auto-pressurising by the vapour pressure of water at this temperature. Providing the vessel withstands these conditions this is considered to be a pressurised system as required for the sterilisation procedure.

Over the course of the test, all incubation temperatures were within 5±°C of the target temperature and minimal effects of the dosing on the sample pH occurred. No sterilisation procedures were used due to the high temperatures and short incubation times used in the study.

Duplicate samples for each test substance at each pH were removed at selected times. Those removed from incubations were cooled prior to analysis. Duplicate portions of each incubate were added to the scintillant and assayed for radioactivity by liquid scintillation counting (LSC).

Vessel washes were performed with acetonitrile and analysed by LSC to account for any radioactivity not in the solution. All vessels contained < 2.1% applied radioactivity therefore the wash was combined with the buffer for each sample prior to an aliquot being taken for HPLC and TLC analysis.

HPLC analysis was performed within one day of sample collection. Samples were co-chromatographed using a non-radiolabelled reference standard mix of 1’-COOH-S-2840-A and 1’-COOH-S-2840-B; this enabled the potential for isomerisation between the 1’-COOH-S-2840-A and 1’-COOH-S-2840-B isomer pairs to be checked. The identity of the analysed compounds was confirmed in selected samples by TLC.

Results and discussion

The recovery of the applied radioactivity ranged between 92.6 – 106.7%. The majority of the radioactivity was present in the buffer solutions, with only a small amount being present in the vessel washes. This shows there was no significant precipitation, adsorption to glassware or volatile degradation products. Full results are given in the table 7.5.1.2-2 below.

Table 7.5.1.2-2: Breakdown of recovered applied radioactivity prior to and after simulated food processing

Test item	Processing conditions	Sample	% Applied radioactivity time 0 (Average)	% Applied radioactivity at termination	Recovery %[(a)]
[pyrazole-4-^{14}C] 1'-COOH-S-2840-A	pH 4, 90°C, 20 mins	Buffer	94.2, 97.1 (95.7)	96.5, 95.7 (96.1)	-
		Wash	1.2, 0.9 (1.1)	1.0, 1.7 (1.4)	
		Total	95.4, 98 (96.7)	97.5, 97.4 (97.5)	101
	pH 5, 100°C, 60 mins	Buffer	100.4, 100.2 (100.3)	100.4, 101.3 (100.9)	-
		Wash	0.6, 0.9 (0.8)	1.0, 0.9 (1.0)	
		Total	101, 101.1 (101.1)	101.4, 102.2 (101.8)	101
	pH 6, 120°C, 20 mins	Buffer	101.6, 101.9 (101.8)	103.5, 105.8 (104.7)	-
		Wash	1.3, 0.8 (1.1)	1.1, 0.9 (1.0)	
		Total	102.9, 102.7 (102.8)	104.6, 106.7 (105.7)	103
[pyrazole-4-^{14}C] 1'-COOH-S-2840-B	pH 4, 90°C, 20 mins	Buffer	91.3, 97.0 (94.2)	97.5, 94.9 (96.2)	-
		Wash	1.3, 1.5 (1.4)	1.9, 2.1 (2.0)	
		Total	92.6, 98.5 (95.6)	99.4, 97.0 (98.2)	103

	pH 5, 100°C, 60 mins	Buffer	98.6, 100.7 (99.7)	100.8, 99 (99.9)	-
		Wash	1.0, 1.1 (1.1)	1.0, 1.6 (1.3)	
		Total	99.6, 108.1 (100.7)	101.8, 100.6 (101.2)	100
	pH 6, 120°C, 20 mins	Buffer	102.2, 104.8 (103.5)	101.8, 103.9 (102.9)	-
		Wash	1.0, 1.2 (1.1)	1.1, 1.2 (1.2)	
		Total	103.2, 106 (104.6)	102.9, 105.1 (104)	99

(a) Recovery calculated between time 0 and termination

No degradation was observed in buffer solutions as a result of the different pH environments.

Under the simulated condition of **pasteurisation**, **baking, brewing and boiling and sterilisation** total [pyrazole-4-^{14}C] 1'-COOH-S-2840-A ranged from 97.4 – 106.7% and total [^{14}C] 1'-COOH-S-2840-B ranged from 97.0 – 105.1%. No degradation products > 0.3% applied radioactivity were detected in any of the buffer samples. The identity of 1'-COOH-S-2840 was confirmed by TLC.

No degradation was observed under any of the representative conditions and no isomerisation between 1'-COOH-S-2840-A and 1'-COOH-S-2840-B isomer pairs were detected in any of the samples. In each of the respective samples, the other isomer was not detected (< 0.001mg/L). Unknown peaks were either fully absent or present (in the 1'-COOH-S-2840-A pH 5 post incubation samples) at 0.001 mg/L. Unresolved background material was minimal (max 0.003 mg/L).

Conclusion

Under the simulated conditions of pasteurisation, baking, brewing and boiling degradation of 1'-COOH-S-2840 was not observed. No isomerisation between the two isomer pairs were detected in any of the samples. Therefore, under the conditions both isomer 1'-COOH-S-2840-A and 1'-COOH-S-2840-B can be considered hydrolytically stable.

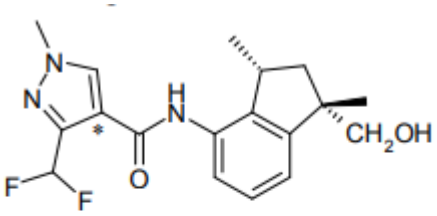
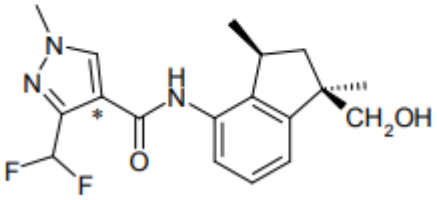
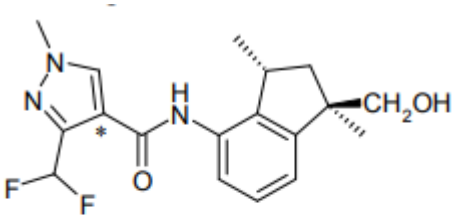
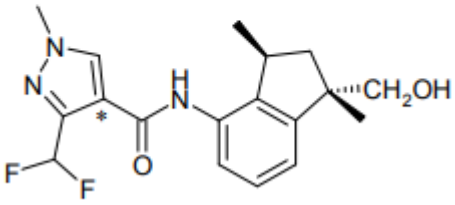
B.7.5.1.3 1'-CH_2OH-S-2840

Report:	**KCA 6.5.1/03; [redacted] 2017**
Title:	[^{14}C]1'-CH_2OH-S-2840: OECD 507 Nature of residue – High temperature Hydrolysis
Document No.:	TPM-0055 (Study No.: 3201988)
Guidelines:	OECD Guideline 507
Guideline deviations:	None
GLP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

Materials and methods

The material used for testing in the hydrolysis study (according to OECD guideline 507) was [pyrazole-4-^{14}C] 1'-CH_2OH-S-2840-A and [pyrazole-4-^{14}C] 1'-CH_2OH-S-2840-B. Although there is only one radiolabel form (pyrazole-4-^{14}C) tested (unlike the plant metabolism studies) this is acceptable as the current study showed full recovery of the test material and the material is stable, without any cleavage of the molecule.

The metabolite 1'-CH_2OH-S-2840 exists as four isomers, two for [pyrazolyl-4-^{14}C] 1'-CH_2OH-S-2840-A and two for [pyrazolyl-4-^{14}C] 1'-CH_2OH-S-2840-B that were tested as a mixture of the two isomers.

Chemical structure	  * Position of the radiolabel	  * Position of the radiolabel
	The supplied test substance was a mixture of the two isomers.	The supplied test substance was a mixture of the two isomers.
Radiolabel position	[Pyrazolyly-4-^{14}C] 1'-CH_2OH-S-2840-A	[Pyrazolyly-4-^{14}C] 1'-CH_2OH-S-2840-B
Radiochemical purity	99.8% (HPLC) 99.0% (TLC)	99.6% (HPLC) 99.2% (TLC)
Chemical purity (%)	99.7%	100%
Specific activity	6.04 MBq/mg, 2.11 GBq/mmol	6.04 MBq/mg, 2.11 GBq/mmol

Citrate buffers were suitably prepared at pH 4, pH 5 and pH 6 for use in the hydrolysis study.

Two separate (checked) stock solutions of [pyrazolyl-4-^{14}C] 1'-CH_2OH-S-2840-A and [pyrazolyl-4-^{14}C] 1'-CH_2OH-S-2840-B were combined to form a single application solution containing approximately equal concentrations of each isomer.

[pyrazole-4-^{14}C]1'-CH_2OH-S-2840 was added to the buffer solutions to obtain a test solution with the concentration of 1 mg/L (1.023 mg/L).

In line with OECD guideline 507 tests were carried out under the following conditions as shown in the table below.

Table 7.5.1.3-1: Hydrolysis study test conditions

pH	Process represented	Temperature (°C)	Incubation time (min)
4	Pasteurisation	90	20
5	Baking, Brewing and Boiling	100	60
6	Sterilisation	120	20

For each of the 'pH' treatment conditions (see above summary), four vessels were prepared with pH 4 buffer and [pyrazole-4-^{14}C]1'-CH_2OH-S-2840. Two vessels were analysed at T0 and two vessels incubated for the specified treatments and periods in the summary table above.

Incubations were performed in the dark in a bath of polyethylene glycol (PEG). Temperatures were recorded throughout incubation.

For each pH treatment, two further vessels with non-radiolabelled 1'-CH_2OH-S-2840-A and 1'-CH_2OH-S-2840-B were used to check the pH after incubation. Plain buffer was used to check the pH prior to incubation.

Buffer solutions were placed in a closed system of sealed crimp-caped glass vials. Sealed glass vials placed in a 120°C bath are auto-pressurising by the vapour pressure of water at this temperature. Providing the vessel withstands these conditions this is considered to be a pressurised system as required for the sterilisation procedure.

All incubation temperatures were within ±5°C of the target temperature and minimal effects on the of the dosing on the pH was observed. No sterilisation techniques were used due to the high temperatures and short incubation times.

Duplicate samples at each pH level were removed. Samples were cooled prior to analysis. Duplicate portions of each incubate were added directly to scintillant and assayed for radioactivity by liquid scintillation counting (LSC).

Vessel washes were performed acetonitrile and analysed by LSC to account for any radioactivity not in the solution. All vessels contained only up to 3.4% applied radioactivity, and no further analysis was performed with vessel washes.

HPLC analysis of the buffer solutions was performed on the same day as sample collection. Samples were co-chromatographed using a non-radiolabelled reference

standard mix of 1'-CH_2OH-S-2840-A and 1'-CH_2OH-S-2840-B. The identity of 1'-CH_2OH-S-2840 was confirmed in selected samples by TLC.

Results and discussion

The overall recovery of the applied radioactivity ranged from 95.1 – 107.7% showing there was no significant precipitation, adsorption to glassware or volatile degradation products formed. The majority of the radioactivity was present in the buffer solutions with only a small percentage (≤ 3.4%) being found in the vessel washes. Full results are given in table 7.5.1.3-2 below.

Table 7.5.1.3-2: Breakdown of recovered applied radioactivity prior to and after simulated food processing.

Test item	Processing conditions	Sample	% Applied radioactivity time 0 (Average)	% Applied radioactivity at termination (Average)	Recovery %[(a)]
[pyrazole-4-^{14}C] 1'-CH_2OH-S-2840	pH 4, 90°C, 20 mins	Buffer	95.4, 96.9 (96.0)	96.4, 99.6 (98.0)	-
		Wash	1.2, 1.1 (1.2)	0.8, 1.0 (0.9)	
		Total	96.6, 97.7 (97.2)	97.2, 100.6 (98.9)	102
	pH 5, 100°C, 60 mins	Buffer	101.9, 99 (100.5)	97.1, 94.2 (95.7)	-
		Wash	0.7, 1.2 (1.0)	0.8, 0.9 (0.9)	
		Total	100.5, 1.0 (101.4)	97.9, 95.1 (96.5)	95
	pH 6, 120°C, 20 mins	Buffer	104.8, 101.7 (103.3)	101.7, 104.3 (103.0)	-
		Wash	0.4, 1.1 (0.8)	1.3, 3.4 (2.4)	
		Total	105.2, 102.8 (104.0)	103, 107.7 (105.4)	101

(a) Recovery calculated between time 0 and termination

No degradation was observed in buffer solutions as a result of the different pH environments.

Under the simulated condition of **pasteurisation**, **baking, brewing and boiling and sterilisation** total 1'-CH_2OH-S-2840 ranged from 95.1 – 107.7%. No degradation products were present in any buffer sample. The identity of 1'-CH_2OH-S-2840 was confirmed by TLC.

The distribution of [pyrazole-4-^{14}C] 1'-CH2OH-S-2840 given in the table below. As the samples involved treatment with a mix of radiolabelled material of 1'-CH_2OH-2840-A and 1'-CH_2OH-2840-B, only a basic consideration of the potential isomerisation changes can be made. Despite this, the samples did show very similar results between T0 and the end of the incubation periods for each of 'A' and 'B' indicating that no marked isomerisation changes had occurred.

Table 7.5.1.3-3: Distribution of [pyrazole-4-^{14}C] 1'-CH_2OH-S-2840

Processing conditions	Compound	Time 0 (average of two results)		Termination (average of two results)	
		%TRR[(a)]	mg/L	% TRR[(a)]	mg/L
pH 4, 90°C, 20 mins	1'-CH_2OH-S-2840-A	48.7	0.4979	48.0	0.4910
	1'-CH_2OH-S-2840-B	51.0	0.5220	51.8	0.5296
	Total 1'-CH_2OH-S-2840	99.7	1.0199	99.8	1.0206
	Unknown	ND	ND	ND	ND
	Unresolved background	0.3	0.0031	0.2	0.0024
	Total	100	1.0230	100	1.0230
pH 5, 100°C, 60 mins	1'-CH_2OH-S-2840-A	49.8	0.5096	49.3	0.5046
	1'-CH_2OH-S-2840-B	49.9	0.5108	50.4	0.5159
	Total 1'-CH_2OH-S-2840	99.7	1.0204	99.8	1.0205
	Unknown	ND	ND	ND	ND

	Unresolved background	0.3	0.0026	0.2	0.0025
	Total	100	1.0230	100	1.0230
pH 6, 120°C, 20 mins	1'-CH_2OH-S-2840-A	49.2	0.5028	48.8	0.4997
	1'-CH_2OH-S-2840-B	50.6	0.5176	50.9	0.5208
	Total 1'-CH_2OH-S-2840	99.8	1.0205	99.8	1.0205
	Unknown	ND	ND	ND	ND
	Unresolved background	0.2	0.0025	0.2	0.0025
	Total	100	1.0230	100	1.0230

ND = Not detected, no peaks for any degradation products

(a) %TRR: The % recovery of radioactivity present as parent test substance was normalised to 100% radioactivity found in the buffer samples.

Conclusion

Under the simulated conditions of pasteurisation, baking, brewing, boiling and sterilisation no degradation was observed. Therefore, under the representative conditions 1'-CH_2OH-S-2840 can be considered hydrolytically stable. There is no evidence of marked isomer changes ('A' and 'B') in the study.

B.7.5.1.4 3'-OH-S-2840

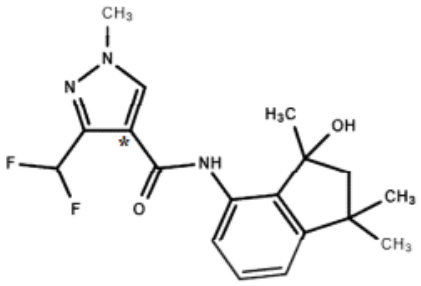
Report:	**KCA 6.5.1/02; [redacted] 2018**
Title:	[^{14}C]3'-OH-S-2840: OECD 507 Nature of Residue – High Temperature Hydrolysis
Document No.:	TPM-0054 (Study No.: 3201987)
Guidelines:	OECD Guideline 507
Guideline deviations:	None
GLP:	Yes

Acceptability:	Yes
Study relied upon:	Yes

Materials and methods

The material used for testing in a hydrolysis study (according to OECD guideline 507) was [pyrazole-4-^{14}C] 3'-OH-S-2840. Although there is only one radiolabel form (pyrazole-4-^{14}C) tested (unlike the plant metabolism studies) this is acceptable as the current study showed full recovery of the test material and main product formed (3'-OH-S-2840 dehydrate) has not formed as a result of cleavage of the molecule (the bridge between the two main ring systems remained intact). Other minor degradants were not identified in the study but were found only at very low levels (see below).

Chemical structure



* indicates position of radiolabel

Radiolabel position	[pyrazolyl-4-^{14}C]-3'-OH-S-2840
Radiochemical purity	99.3%
Chemical purity (%)	100%
Specific activity	6.35 MBq/mg, 2.22GBq/mmol

Buffers were suitably prepared at pH level 4, 5 and 6 for use in the hydrolysis study.

[pyrazole-4-^{14}C]3'-OH-S-2840 from radiolabelled stock solution was checked and was added to the buffers to obtain a test solution concentration of 1 mg/L (1.021 mg/L).

In accordance with OECD guideline 507, tests were carried out under three different conditions as shown in the table below.

Table 7.5.1.4-1: Hydrolysis study test conditions

Process represented	pH	Temperature (°C)	Incubation time (min)
Pasteurisation	4	90	20
Baking, Brewing and Boiling	5	100	60
Sterilisation	6	120	20

For each of the 'pH' treatment conditions (see above summary), four vessels were prepared with pH 4 buffer and radiolabelled [pyrazole-4-^{14}C]3'-OH-S-2840. Two vessels were analysed at T0 and two vessels incubated for the above specified periods. Incubations were performed in the dark in a bath of polyethylene glycol (PEG). Temperatures were recorded throughout incubation.

At each pH treatment, two further vessels were prepared with non-radiolabelled 3'-OH-S-2840 used to check the pH after incubation. Plain buffer was used to check the pH prior to incubation.

All incubation temperatures were within ±5°C of the target temperature and minimal effects of the dosing on the pH were observed. No sterilisation procedures were carried out due to the short incubation times and high temperatures used.

Buffer solutions were placed in a closed system of sealed crimp-caped glass vials. Sealed glass vials placed in a 120°C bath are auto-pressurising by the vapour pressure of water at this temperature. Providing the vessel withstands these conditions this is considered to be a pressurised system as required for the sterilisation procedure.

The incubated solutions were cooled by planning them in cool water and duplicate portions of each incubate were analysed by liquid scintillation counting (LSC).

Vessel washes were made by adding acetonitrile to the original vials and analysed by LSC to account for any radioactivity not in the buffer solutions. The maximum amounts of applied radioactivity in the wash were 7.7% (one of the pH 6 duplicate results). The washes were not analysed further.

Samples were analysed by reverse-phase HPLC. Degradant compound peaks were observed in the initial HPLC analyses and further work was required to develop HPLC conditions to separate the degradant peaks from the parent test substance. During this time (21 – 22 days), the original samples were stored at <-10°C in a freezer in the dark. Results were given in the form of HPLC chromatograms (before

and after storing for this period) indicating that no new peaks were formed after storage).

Samples were co-chromatographed with non-radiolabelled 3'-OH-S-2840 and 3'-OH-S-2840 dehydrate. Other minor peaks were not identified (see table 7.5.1.4-3 for relative levels of these peaks).

TLC analysis was used to confirm the presence of the parent compound and 3'-OH-S-2840 dehydrate.

Results and discussion

The mean recovery of the applied radioactivity (AR) at termination was 91.7 – 93.2%. No more than 7.7% of the applied radioactivity was detected in the wash. Full results are given in table 7.5.1.4-2 below. The results of recovered radioactivity are below 95% this is likely due to not pooling the vessel washes with the buffer solutions, so the %AR is reflective of the buffer solutions only. The applicant has postulated (see below) that the vessel washes might be excess solution rather than material that absorbed onto the vessel walls.

Table 7.5.1.4-1: Breakdown of recovered applied radioactivity prior to and after simulated food processing.

Test item	Processing conditions	Sample	% Applied radioactivity time 0 (Average)	% Applied radioactivity at termination (Average)	Recovery %[(a)]
[pyrazole-4-^{14}C] 3'-OH-S-2840	pH 4, 90°C, 20 mins	Buffer	87.8, 86 (86.9)	91.6, 89.5 (90.6)	-
		Wash	2.8, 4.4 (3.6)	3, 1.9 (2.5)	
		Total	90.6, 90.4 (90.5)	94.6, 91.4 (93.0)	103
	pH 5, 100°C, 60 mins	Buffer	90.9, 91.9 (91.4)	90.5, 90.1 (90.3)	-
		Wash	4.4, 2.0 (3.2)	3.3, 2.5 (2.9)	
		Total	95.3, 93.9 (94.6)	93.8, 92.6 (93.2)	99
		Buffer	90.1, 92.4 (91.3)	84.2, 88.6 (86.4)	-

	pH 6, 120°C, 20 mins	Wash	1.9, 2.8 (2.4)	7.7, 2.9 (5.3)	
		Total	92, 95.2 (93.6)	91.9, 91.5 (91.7)	98

(a) Recovery calculated between time 0 and termination
No degradation was observed in buffer solutions for the different pH environments.

The %TRR levels observed in table 7.5.1.4-3 and the text that follows here are calculated values when normalising the overall amount of applied radioactivity recovered to 100%.

Under the simulated condition of **pasteurisation** 3'-OH-S-2840 degraded to form 3'-OH-S-2840 dehydrate [13% TRR] as well as a few other degradation products (maximally comprised of 8 components in which the largest peak was quantified to be 1.9% TRR, 0.19 mg/L).

The conditions of **baking, brewing and boiling** conditions degraded 3'-OH-S-2840 to form 3'-OH-S-2840 dehydrate [9% TRR] as well as a small number of other degradation products (maximally comprised of 6 components in which the largest peak was quantified to be 2.8% TRR, 0.029 mg/L).

The final condition of **sterilisation** leads to no significant degradation of 3'-OH-S-2840 [9% TRR] (unknown degradants were maximally comprised of 2 components in which the largest peak was quantified to be 1.0% TRR, 0.010 mg/L).

The TLC of wash solutions showed that it mainly compromised of 3'-OH-S-2840 and small amounts of 3'-OH-S-2840 dehydrate. The TLC chromatography of wash solutions was like those of the buffer solutions and therefore the applicant suggested they contained excess solution rather than material that absorbed onto the vessel walls.

The distribution of [pyrazole-4-^{14}C]3'-OH-S-2840 under the representative conditions is given below:

Table 7.5.1.4-3: Distribution of [pyrazole-4-^{14}C] 3'-OH-S-2840

Processing conditions	Compound	% Recovered Radioactivity			
		Time 0 (average of two results)		Termination (average of two results)	
		%TRR	mg/L	% TRR	mg/L
	3'-OH-S-2840	97.8	0.999	82.8	0.846

pH 4, 90°C, 20 mins	3'-OH-S-2840 dehydrate	ND	ND	13.0	0.133
	Unknown[(a)]	1.4	0.015	2.9	0.029
	Unresolved background	0.8	0.008	1.3	0.013
	Total	100	1.021	100	1.021
pH 5, 100°C, 60 mins	3'-OH-S-2840	98.5	1.005	87.6	0.895
	3'-OH-S-2840 dehydrate	ND	ND	9.0	0.092
	Unknown[(b)]	1.0	0.011	2.9	0.030
	Unresolved background	0.5	0.005	0.5	0.005
	Total	100	1.021	100	1.021
pH 6, 120°C, 20 mins	3'-OH-S-2840	99.0	1.011	96.7	0.987
	3'-OH-S-2840 dehydrate	ND	ND	1.7	0.017
	Unknown[(c)]	0.2	0.002	1.0	0.010
	Unresolved background	0.8	0.009	0.6	0.006
	Total	100	1.021	100	1.021

ND = Not detected (<0.21% TRR/<0.002 mg/L)

The % recovery of radioactivity was normalised to 100%, calculated by dividing the amount of applied radioactivity determined by the total recovery for that sample and multiplying by 100. The normalised values were used as total recovered radioactivity when evaluating chromatograms and calculating the concentration of [pyrazole-4-^{14}C]3'-OH-S-2840 derived residues.

(a) Unknown degradants were maximally comprised of 8 components in which the largest peak was quantified to be 1.9% TRR (0.019 mg/L)

(b)Unknown degradants were compromised of 6 compounds with the single largest peak 2.8% TRR (0.029 mg/L)

(c)Unknown degradants were maximally comprised of 2 components in which the largest peak was quantified to be 1.0% TRR (0.010 mg/L)

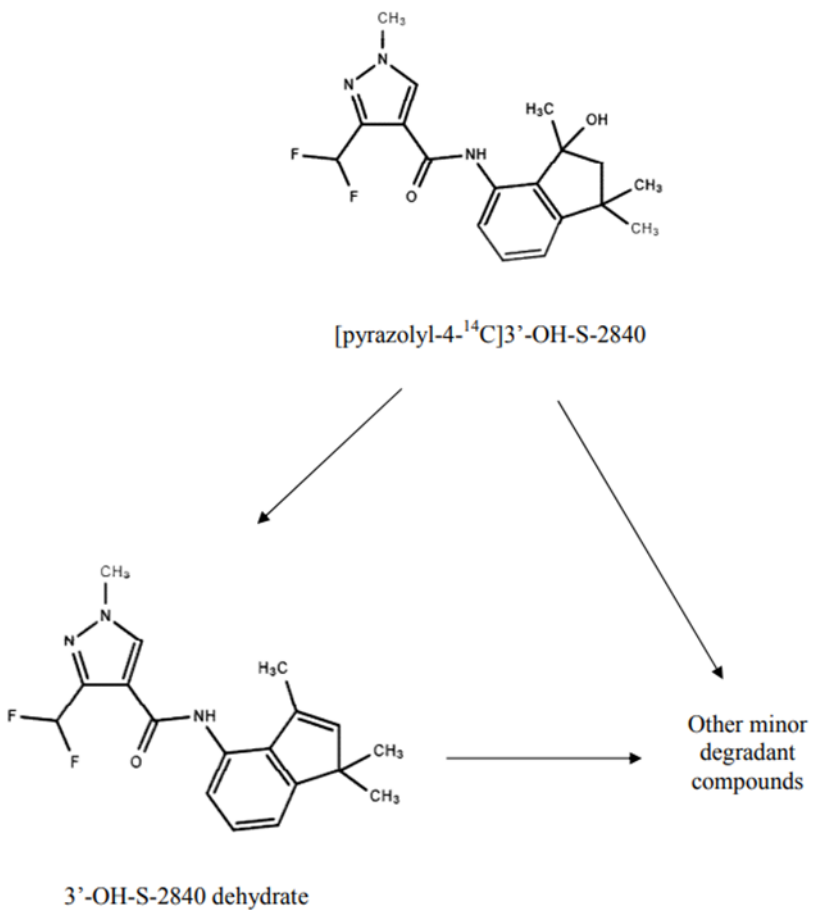
Conclusion

Under the simulated conditions of pasteurisation, baking, brewing and boiling degradation of 3'-OH-S-2840 was observed to form 3'-OH-S-2840 dehydrate (in the various tests at %TRR levels of 9 – 13% with the highest level in the simulated pasteurisation conditions of pH 4, 90°C, 20 mins). A number of other low level unidentified degradation products were formed, but these are not considered significant. The proposed degradation route is included in figure 7.5.1.4-1.

Under the condition of sterilisation there was no significant degradation and 3'-OH-S-2840 can be considered hydrolytically stable.

The total radioactive recoveries were between 98 – 103% showing that there was no significant precipitation, adsorption to glassware or volatile degradation products formed.

Figure 7.5.1.4-1: Proposed route of degradation of 3'-OH-S-2840



B.7.5.2. Distribution of the residue in inedible peel and pulp

The distribution of residues in inpyrfluxam between peel and pulp is not relevant for the proposed representative use.

B.7.5.3. Magnitude of residues in processed commodities

Two magnitude of residues studies were submitted for evaluation to support the intended uses, one on barley and one on wheat. These involved generating field incurred residues (from supervised field trials) and then subjecting the grain to processing operations which simulated industrial processes for the processing of barley and wheat, into beer and food and animal feed item products.

B.7.5.3.1 Barley

Report:	**KCA 6.5.3/02, [redacted] 2018**
Title:	Magnitude of the residue of S-2399 and its metabolites in barley processed fractions in Northern and Southern Europe – 2016
Document No.:	TPR-0082 (Study No.: 262-2016)
Guidelines:	OECD Test Guideline 509; OECD Test Guideline 508; Commission Regulation (EU) No 283/2013 of 1 March 2013; OECD ENV/JM/MONO(2008)23; US EPA OCSPP 860.1500; US EPA OCSPP 860.1520
Guideline deviations:	None
GLP/GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

The study included two supervised residue trials with barley, conducted in the field in southern Europe (Italy) and northern Europe (Germany) in the 2016 season in order to determine the magnitude of the residues of inpyrfluxam in/on barley grain and its processed fractions for the processing of pearled (pot) barley, malting and brewing.

Field part

In the field trials the formulation ‘S-2399 40 SC’ was sprayed once at growth stage of BBCH 73 – 83 at a rate of 1000 g a.s./ha (water volumes of 284 and 300 L/ha).

Barley grain samples were collected at BBCH 89, 36 and 37 days after application (DAA) in trials GE01 and IT02, respectively.

Barley grain samples from GE01 and IT02 weighed 37 kg and > 50 kg, respectively. The samples weighing < 50 kg were due to limited grain being available. As sufficient grain samples were available for the processing study, no adverse impact is expected. The specimens for processing were sent on the day of harvest or the day after to the processing test site under ambient conditions.

Processing procedures

The processing simulated industrial practice at a laboratory scale. The processing of spring barley grain into the processed fractions (malt sprouts; brewer’s malt; brewer’s grain (fresh); brewer’s grain (dried); floc (hops draff); brewer’s yeast; beer; pearled (pot) barley; bran and flour) was performed simulating the common industrial processes.

The below descriptions of the processing procedures are supported by the flow chart schemes in Figures 7.5.3.1-1 (pot barley) and 7.5.3.1-2 (beer production).

Cleaning

All field specimens for processing were cleaned which allows the separation of soil particles and other contaminations from the grain in a steady air flow.

Before processing, the corresponding fresh grain samples were deep-frozen, identified as grain stored (RAC) samples and stored deep frozen at <-18°C until analysis.

Pearled (pot) barley

Conditioning

Before beginning of pearled (pot) barley production, an optimal moisture content of barley grain of approximately 14% should be achieved. In order to obtain acceptable milling results a moisture content of up to 16% was possible. Therefore, the grain with non-optimal moisture content was damped.

Hulling

Samples were hulled until an abrasion of 20 – 25% for pearled barley was reached. The degree of abrasion was determined by the proportion of pearled barley with

respect to the total portion of cleaned grain used for the hulling process. Abrasion was sieved to bran and flour.

Pearled (pot) barley, bran and flour were sampled.

Malting

The malting process involved the following steps:

Sieving (mesh 2.5 mm)
Steeping (about 2 days)
Germination (about 5 days)
Kiln-drying (about 1 day)
Removal of malt sprouts

Brewer’s malt and malt sprouts were sampled.

The remining malt was stored at room temperature.

Brewing

Milling

The malt was dry milled in a special malt mill.

Mashing

The ground malt was mixed with water and heated for approximately 2 hours.

Lautering: wort extraction and separation

After mash boiling, the wort was separated from the insoluble malt components (brewer’s grain). The remaining extract in the brewer’s grain was extracted by washing with hot water. The wort separation was done using a refining vat and took 2 – 3 hours.

The brewer’s grain (fresh) was sampled.

For the production of brewer’s grain (dried), brewer’s grain (fresh) was dried at 50°C until a dry matter content of <10% was reached.

The brewer’s grain (dried) was sampled.

Wort boiling and conditioning

After the addition of hop pellets, the separated wort was boiled (for approximately 90 minutes).

After boiling, the flocs (hops draff) were separated in a whirlpool causing the sludge to deposit on the bottom. For cooling and ventilating the wort, an intra-plant circulation was used. Oxygen was added (intra-plant circulation), creating the conditions for the start of the fermentation.

Flocs (hops draff) were sampled.

Fermentation and maturation

Yeast is added and the classical primary fermentation (low fermentation) was carried out in bottom fermentation containers. The fermentation temperature was 9°C and lasted between 7 and 10 days.

The maturation time began when the extract content of the fermented young beer was 2% higher than the final attenuation. Before maturation, the young beer was cooled.

Brewer's yeast, from the bottom of the tank, was sampled.

The young beer was stored at room temperature (warm maturation) in casks for 2 days.

The young beer was then stored under pressure (1.0 bar) at 2°C (cold maturation) for 3 – 4 weeks.

The beer was filtered and all organisms harming the beer (bacteria and yeast) were removed and sludge particles were separated.

The final product of beer was sampled.

During processing of barley grain, laboratory samples of the various processing fractions taken at specific processing steps were deep frozen and stored deep frozen until dispatch to the analytical laboratory. Upon reception, samples of grain and processed commodities were stored frozen in the dark at -18°C or below. For the preparation of samples, the samples of malt sprouts, brewer's malt, brewer's grain (fresh and dried), flocs (hops draff), bran and pearled (pot) barley were thoroughly homogenised in a knife mill or a cutter with dry ice. The samples of brewer's yeast and flour were already homogenised and were therefore thoroughly mixed by hand before taking aliquots for analysis. The samples of beer were shaken thoroughly before sampling for analysis.

Residue analysis

The samples were analysed for the parent compound and the metabolites 3'-OH-S-2840, DFPA-$CONH_2$, *N*-des-Me-DFPA, DFPA, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B, 1'-COOH-S-2840-A and 1'-COOH-S-2840-B using analytical method S16-

03371. The method was sufficiently validated on wheat grain and whole plant in accordance with SANTE/2020/12830 rev. 1 (see Vol. 3 CA B.5.1.2.5).

Due to the matrix similarity, the analytical method is therefore considered validated for barley grain, malt sprouts, brewer's malt, brewer's grain (fresh), brewer's grain (dried), flocs (hops draff), brewer's yeast, pearl barley, bran and flour. In solid matrices, the LOQ was 0.01 mg/kg for the parent compound and the metabolites 3'-OH-S-2840, DFPA-$CONH_2$, *N*-des-Me-DFPA and DFPA and was 0.005 mg/kg for the metabolites 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B, 1'-COOH-S-2840-A and 1'-COOH-S-2840-B. At least one procedural recovery was performed at the LOQ per analytical set for all analytes in barley grain, malt sprouts, brewer's malt, brewer's grain (fresh), brewer's grain (dried), flocs (hops draff), brewer's yeast, bran and flour. Additionally, 10 x LOQ and higher level fortification procedural recoveries were generated when needed. When there were at least 2 recoveries, the average recoveries were within the acceptable range of 70 – 110%. Single procedural recoveries were in the range of 60 – 120% each, with the exception of the recovery at LOQ level for the metabolite 3'-OH-S-2840 in flour which was 123%. As the residues of 3'-OH-S-2840 are covered by the acceptable procedural recovery (107%) at 10 x LOQ level, this deviation is not expected to have an adverse impact. The RSD values (calculated only where there were three or above results generated) were all below 20%.

Due to the difference in matrix between beer and wheat grain/whole plant, on which the analytical method was validated (see Vol. 3 CA B.5.1.2.5), additional validation recoveries were conducted for beer. The LOQ was 0.01 mg/L for the parent compound, 3'-OH-S-2840, DFPA-$CONH_2$, *N*-des-Me-DFPA and DFPA in beer and was 0.005 mg/L for the metabolites 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B, 1'-COOH-S-2840-A and 1'-COOH-S-2840-B in beer. Five recoveries were performed at fortification levels of LOQ and 10 x LOQ for all analytes at both quantification and confirmation mass transitions. The data are acceptable with mean recoveries between 70 – 110% and all RSD values below 20%, as reported in Vol. 3 CA B.5.1.2.5.

Storage stability

The analyses were done after a maximum frozen storage period of samples of 375 days. The time between the beginning of the sample preparation and the sample analysis did not exceed 18 days. Sample extracts were stored between 1 – 10 °C. Stability of residues in sample extracts has been satisfactorily addressed as the matrix-matched standards used for quantification were always prepared on the same day (using control untreated sample fortified with solvent standards) as the production of the sample extracts for residues analysis; these matrix matched standards were also stored at 1 – 10 °C. The procedural recoveries were handled and stored in the same way and for the same time period as the sample extracts that were prepared within the same analytical set.

Please refer to Vol 1 section 2.7.1 for conclusions regarding storage stability of samples and extracts.

Figure 7.5.3.1-1: The simulated industrial processing of grain to pearled (pot) barley

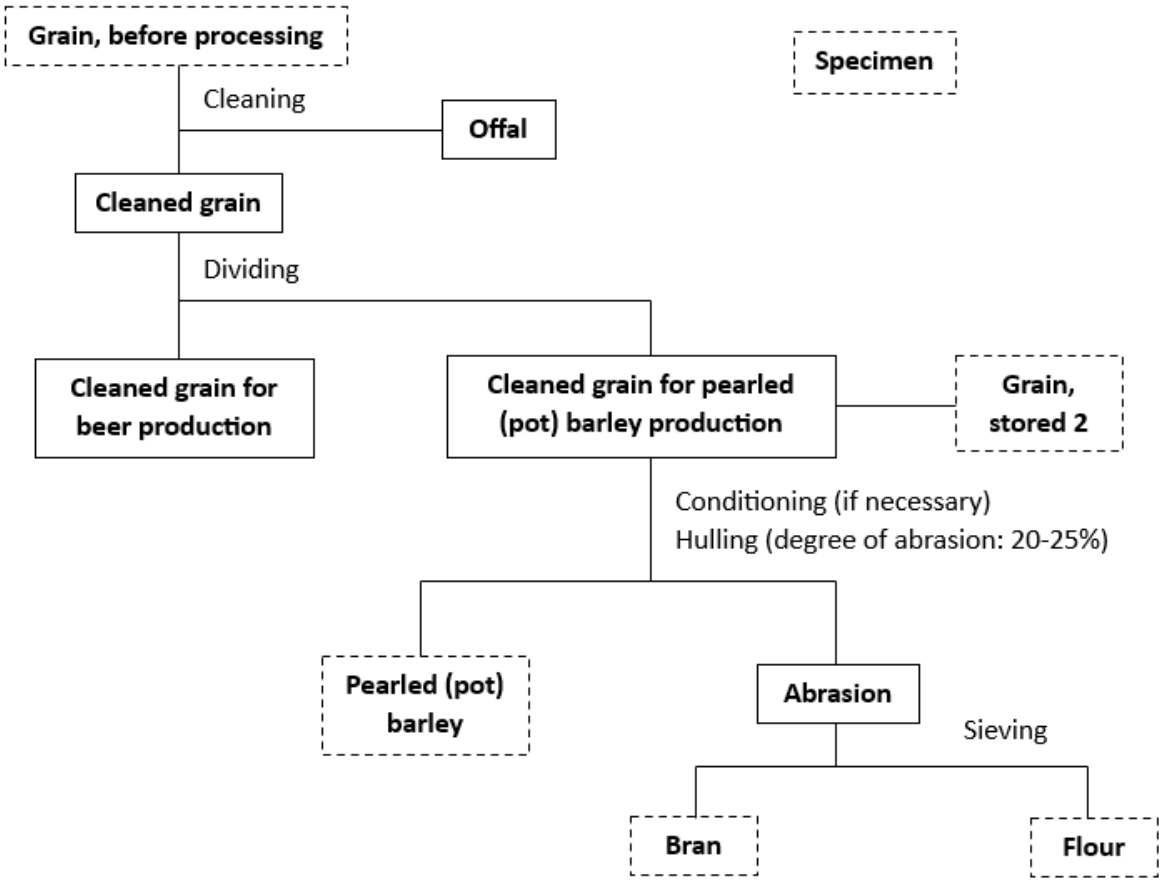
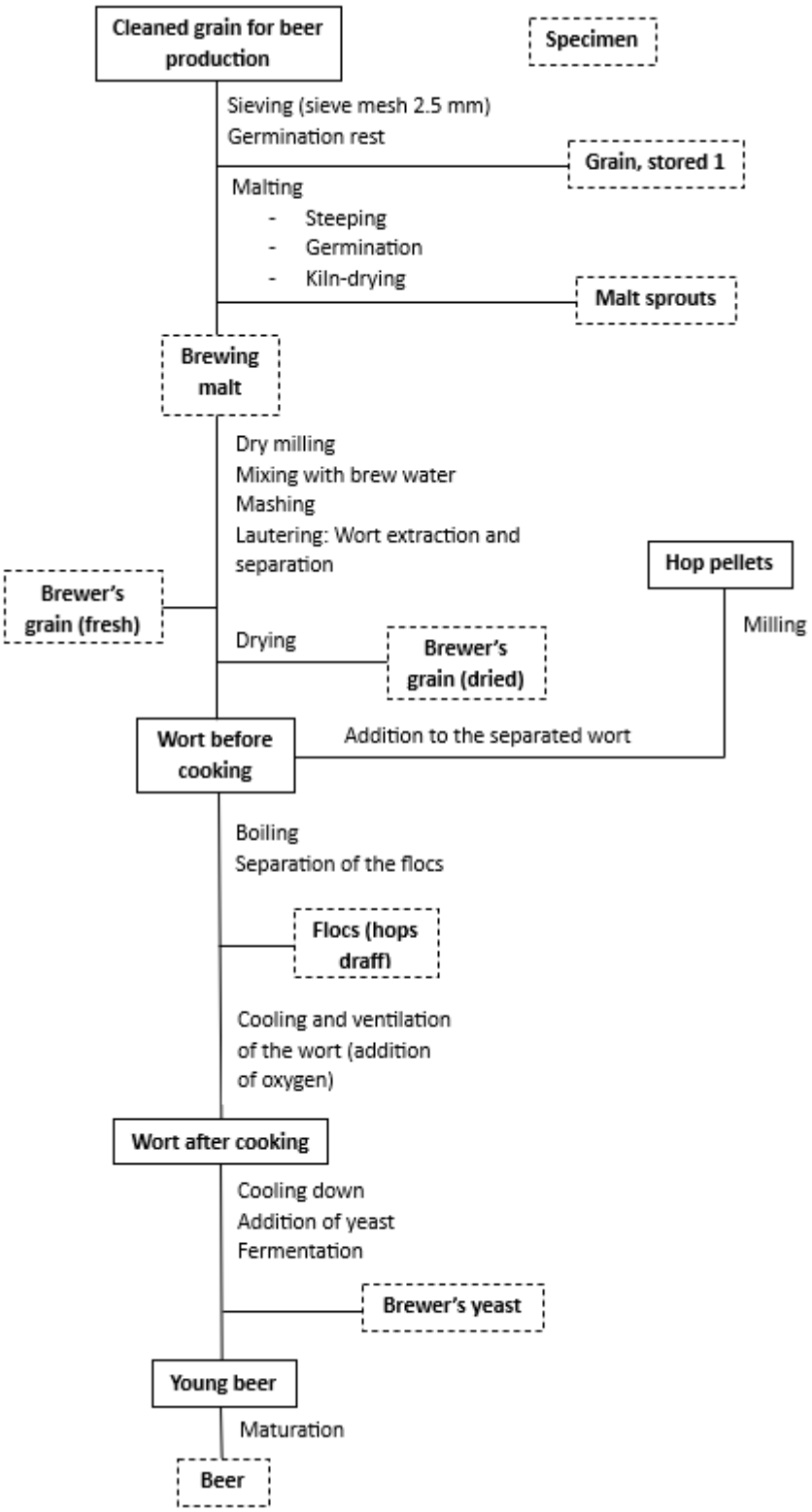


Figure 7.5.3.1-2: The simulated industrial processing of grain for beer production



Results

The levels of residues of inpyrfluxam and the metabolites 3'-OH-S-2840, DFPA-$CONH_2$, *N*-des-Me-DPFA, DFPA, 1'-COOH-S-2840-A, 1'-COOH-S-2840-B, 1'-CH_2OH-S-2840-A and 1'-CH_2OH-S-2840-B in the treated samples are summarised in Table 7.5.3.1-2. No residues above the LOQ were found in the control samples. The results were not corrected for procedural recoveries.

Residues of parent inpyrfluxam in barley grain before processing (RAC) were found to be 0.42 mg/kg in the first trial (Germany) and 0.17 mg/kg in the second trial (Italy).

The levels of residues of parent inpyrfluxam from the first trial (Germany) were 0.37, 0.28, 0.28, 0.22, 0.61, 0.31, 0.08, <0.01, 0.44, 0.07, 3.0 and 2.5 mg/kg in stored grain 1, malt sprouts, brewer's malt, brewer's grain (fresh), brewer's grain (dried), flocs (hops draff), brewer's yeast, beer, stored grain 2, pearled (pot) barley, bran and flour, respectively.

The levels of residues of parent inpyrfluxam from the second trial (Italy) were 0.11, 0.09, 0.06, 0.06, 0.12, 0.06, 0.03, 0.14, 0.03, 0.81 and 0.64 mg/kg in stored grain 1, malt sprouts, brewer's malt, brewer's grain (fresh), brewer's grain (dried), flocs (hops draff), brewer's yeast, stored grain 2, pearled (pot) barley, bran and flour, respectively. Inpyrfluxam was found at <LOQ in beer.

The results are summarised below in Tables 7.5.3.1-2 to -5.

For the presentation of the setting of processing factors for the currently intended uses please refer to section 2.7.8 of the Vol 1.

Conclusions

Two residue trials were conducted in northern and southern Europe in 2016. Barley was treated at a growth stage of BBCH 73 – 83 with a dose rate of 1 x 1000 g a.s./ha.

Barley grain was processed in order to obtain pearled (pot) barley and beer. The samples (RAC and processed fractions) were analysed for the residues of inpyrfluxam parent compound as well as the metabolites 3'-OH-S-2840, DFPA-$CONH_2$, *N*-des-Me-DFPA, DFPA, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B, 1'-COOH-S-2840-A and 1'-COOH-S-2840-B.

The results of the study indicate that generally residues of inpyrfluxam are diluted by the brewing process, other than in brewer's grain (dried) which results in slightly concentrated residues.

For the pearled (pot) barley process, the results of the study indicate that generally residues of inpyrfluxam are concentrated in bran and flour but are diluted in pearl (pot) barley.

Processing factors have been calculated and presented in Volume 1, section 2.7.6.

Table 7.5.3.1-2: Detailed results of the barley processing studies for inpyrfluxam, 3'-OH-S-2840, DFPA-CONH$_2$ and *N*-des-Me-DPFA in beer

Trial No. / Location / Year	Commodity / Variety	Date of 1. Sowing 2. Flowering 3. Harvest	Application rate per treatment			Date of treatment	Growth stage at last treatment or date	Portions analysed	Residues mg/kg				PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL				Inpyrfluxam	3' -OH-S-2840	DFPA-CONH$_2$	*N*-des-Me-DFPA		
04827 / [redacted], Saxony, Germany, Northern Europe / 2016	Spring barley / SOLIST	1. 15/04/16 2. 15/06/16 to 23/06/16 3. 17/08/16	1.005	294	0.3418	12/07/16	BBCH 83	Grain before processing	0.42	0.10	<0.01	0.01	36	Untreated specimen: <LOQ LC-MS/MS Method validation: S16-003371 (wheat plant and grain, potato, grape, soyabean) 258-2016
								Grain after ambient storage	0.37	0.10	<0.01	0.01		
								Malt sprouts	0.28	0.09	<0.01	<0.01		
								Brewing malt	0.28	0.07	<0.01	0.01		
								Brewer's grain (fresh)	0.22	0.03	<0.01	0.01		
								Brewer's grain (dried)	0.61	0.11	<0.01	<0.01		

								Flocs (hops draff)	0.31	0.07	<0.01	<0.01		(barley plant and grain) 262-2016 (beer) Max. storage between sampling and extraction / extraction and analyses over the whole study: 375 / 18 days
								Brewer's yeast	0.08	0.02	<0.01	<0.01		
								Beer	<0.01	0.01	0.04	<0.01		
40024 / [redacted], Terme, Italy, Southern Europe / 2016	Winter barley / LUTECE	1. 27/10/15 2. 15/04/16 to 27/04/16	1.026	300	0.3420	10/05/16	BBCH 73 – 75	Grain before processing	0.17	0.03	<0.01	0.01	36	Untreated specimen: <LOD LC-MS/MS Method validation:
								Grain after storage	0.11	0.02	<0.01	0.01		
								Malt sprouts	0.09	0.02	<0.01	<0.01		

		3. 16/06/16						Brewing malt	0.06	0.01	<0.01	0.01		S16-003371 (wheat plant and grain, potato, grape, soyabean) 258-2016 (barley plant and grain) 262-2016 (beer) Max. storage between sampling and extraction / extraction and analyses over the whole study: 375 / 18 days
								Brewer's grain (fresh)	0.06	<0.01	<0.01	<0.01		
								Brewer's grain (dried)	0.12	0.01	<0.01	<0.01		
								Flocs (hops draff)	0.06	0.01	<0.01	<0.01		
								Brewer's yeast	0.03	<0.01	<0.01	<0.01		
								Beer	<0.01	<0.01	<0.01	<0.01		

Table 7.5.3.1-3: Detailed results of the barley processing studies for DFPA, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B, 1'-COOH-S-2840-A and 1'-COOH-S-2840-B in beer

Trial No. / Location / Year	Commodity / Variety	Date of 1. Sowing 2. Flowering 3. Harvest	Application rate per treatment			Date of treatment	Growth stage at last treatment or date	Portions analysed	Residues mg/kg					PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL				DFPA	1' -COOH-S-2840-A	1' -COOH-S-2840-B	1' -CH_2OH-S-2840-A	1' -CH_2OH-S-2840-B		
04827 / [redacted]i [redacted], Saxony, Germany, Northern Europe / 2016	Spring barley / SOLIST	1. 15/04/16 2. 15/06/16 to 23/06/16 3. 17/08/16	1.005	294	0.3418	12/07/16	BBCH 83	Grain before processing	0.06	0.020	0.008	0.20	0.30	36	Untreated specimen: <LOQ LC-MS/MS Method validation: S16-003371 (wheat plant and grain, potato, grape, soyabean) 258-
								Grain after ambient storage	0.05	0.022	0.009	0.19	0.24		
								Malt sprouts	0.05	0.026	0.024	0.18	0.21		
								Brewing malt	0.07	0.031	0.013	0.22	0.19		
								Brewer's grain (fresh)	0.03	<0.005	<0.005	0.017	0.019		

								Brewer's grain (dried)	0.09	0.018	0.008	0.11	0.12		2016 (barley plant and grain) 262-2016 (beer) Max. storage between sampling and extraction / extraction and analyses over the whole study: 375 / 18 days
								Flocs (hops draff)	0.05	0.009	<0.005	0.062	0.074		
								Brewer's yeast	0.01	0.009	<0.005	0.041	0.037		
								Beer	<0.01	<0.005	<0.005	0.031	0.027		
40024 / [redacted], Terme, Italy, Southern Europe / 2016	Winter barley / LUTECE	1. 27/10/15 2. 15/04/16 to 27/06/16	1.026	300	0.3420	10/05/16	BBCH 73 – 75	Grain before processing	0.02	0.012	0.006	0.14	0.11	36	Untreated specimen: <LOD LC-MS/MS Method validation
								Grain after ambient storage	0.01	0.007	<0.005	0.13	0.093		

		3. 16/06/16						Malt sprouts	0.01	0.026	0.009	0.15	0.10		: S16-003371 (wheat plant and grain, potato, grape, soyabean) 258-2016 (barley plant and grain) 262-2016 (beer) Max. storage between sampling and extraction / extraction and analyses over the whole study: 375 / 18 days
								Brewing malt	0.02	0.018	0.008	0.10	0.061		
								Brewer's grain (fresh)	<0.01	<0.005	<0.005	0.006	<0.005		
								Brewer's grain (dried)	0.01	0.008	0.006	0.037	0.026		
								Flocs (hops draff)	<0.01	<0.005	<0.005	0.032	0.027		
								Brewer's yeast	<0.01	0.005	<0.005	0.018	0.013		
								Beer	<0.01	<0.005	<0.005	0.013	0.009		

Table 7.5.3.1-4: Detailed results of the barley processing studies for inpyrfluxam, 3'-OH-S-2840, DFPA-CONH$_2$ and *N*-des-Me-DPFA in flour

Trial No. / Location / Year	Commodity / Variety	Date of 1. Sowing 2. Flowering 3. Harvest	Application rate per treatment			Date of treatment	Growth stage at last treatment or date	Portions analysed	Residues mg/kg				PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL				Inpyrfluxam	3' -OH-S-2840	DFPA-CONH$_2$	*N*-des-Me-DFPA		
04827 / [redacted], Saxony, Germany, Northern Europe / 2016	Spring barley / SOLIST	1. 15/04/16 2. 15/06/16 to 23/06/16 3. 17/08/16	1.005	294	0.3418	12/07/16	BBCH 83	Grain before processing	0.42	0.10	<0.01	0.01	36	Untreated specimen: <LOD LC-MS/MS Method validation: S16-003371 (wheat plant and grain, potato,
								Grain after storage	0.44	0.10	<0.01	<0.01		
								Pearled (pot) barley	0.07	0.01	<0.01	<0.01		

								Bran	3.0	0.65	0.01	0.02		grape, soyabean) 258-2016 (barley plant and grain) 262-2016 (beer) Max. storage between sampling and extraction / extraction and analyses over the whole study: 375 / 18 days
								Flour	2.5	0.40	<0.01	0.04		
40024 / [redacted], Terme, Italy, Southern Europe / 2016	Winter barley / LUTECE	1. 27/10/15 2. 15/04/16 to 27/04/16	1.026	300	0.3420	10/05/16	BBCH 73 – 75	Grain before processing	0.17	0.03	<0.01	0.01	36	Untreated specimen: <LOD LC-MS/MS Method validation: S16-
								Grain after storage	0.14	0.02	<0.01	<0.01		

		3. 16/06/16						Pearled (pot) barley	0.03	<0.01	<0.01	<0.01		003371 (wheat plant and grain, potato, grape, soyabean) 258-2016 (barley plant and grain) 262-2016 (beer) Max. storage between sampling and extraction / extraction and analyses over the whole study: 375 / 18 days
								Bran	0.81	0.09	<0.01	0.01		
								Flour	0.64	0.07	<0.01	0.03		

Table 7.5.3.1-5: Detailed results of the barley processing studies for DFPA, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B, 1'-COOH-S-2840-A and 1'-COOH-S-2840-B in flour

Trial No. / Location / Year	Commodity / Variety	Date of 1. Sowing 2. Flowering 3. Harvest	Application rate per treatment			Date of treatment	Growth stage at last treatment or date	Portions analysed	Residues mg/kg					PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL				DFPA	1' -COOH-S-2840-A	1' -COOH-S-2840-B	1' -CH_2OH-S-2840-A	1' -CH_2OH-S-2840-B		
04827 / [redacted] Saxony, Germany, Northern Europe / 2016	Spring barley / SOLIST	1. 15/04/16 2. 15/06/16 to 23/06/16 3. 17/08/16	1.005	294	0.3418	12/07/16	BBCH 83	Grain before processing	0.06	0.020	0.008	0.20	0.30	36	Untreated specimen: <LOD LC-MS/MS Method validation: S16-003371 (wheat plant and grain, potato, grape, soyabean) 258-2016 (barley plant and grain) 262-2016 (beer)
								Grain after storage	0.05	0.018	0.008	0.18	0.25		
								Pearled (pot) barley	<0.01	0.006	<0.005	0.043	0.037		
								Bran	0.56	0.051	0.032	0.91	1.8		
								Flour	0.37	0.079	0.032	0.83	0.94		

															Max. storage between sampling and extraction / extraction and analyses over the whole study: 375 / 18 days
40024 / [redacted], Terme, Italy, Southern Europe / 2016	Winter barley / LUTECE	1. 27/10/15 2. 15/04/16 to 27/04/16 3. 16/06/16	1.026	300	0.3420	10/05/16	BBCH 73 – 75	Grain before processing	0.02	0.012	0.006	0.14	0.11	36	Untreated specimen: <LOD LC-MS/MS Method validation: S16-003371 (wheat plant and grain, potato, grape, soyabean) 258-2016 (barley
								Grain after storage	0.01	0.011	0.007	0.13	0.10		
								Pearled (pot) barley	<0.01	<0.005	<0.005	0.034	0.021		
								Bran	0.07	0.027	0.018	0.59	0.44		

								Flour	0.08	0.038	0.021	0.46	0.30		plant and grain) 262-2016 (beer) Max. storage between sampling and extraction / extraction and analyses over the whole study: 375 / 18 days

B.7.5.3.2 Wheat

Report:	**KCA 6.5.3/01, [redacted] 2018**
Title:	Magnitude of the residue of S-2399 and its metabolites in wheat processed fractions in Northern and Southern Europe – 2016
Document No.:	TPR-0081 (Study No.: 261-2016)
Guidelines:	OECD Test Guideline 509; OECD Test Guideline 508; Commission Regulation (EU) No 283/2013 of 1 March 2013; OECD ENV/JM/MONO(2008)23; US EPA OCSPP 860.1500; US EPA OCSPP 860.1520
Guideline deviations:	None
GLP/GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

The study included two supervised residue trials with wheat, conducted in the field in southern Europe (Italy) and northern Europe (Germany) in the 2016 season in order to determine the magnitude of the residues of inpyrfluxam in/on wheat grain and their processed fractions for the processing of flour (type 550), whole-meal flour/whole-grain bread, wheat germs, starch and gluten.

Field part

In the field trials the formulation ‘S-2399 40 SC’ was sprayed once at a growth stage of BBCH 71 – 75 at a rate of 750 g a.s./ha (water volumes of 298 and 306 L/ha).

Wheat grain samples were collected at BBCH 89, 36 and 35 days after application (DAA) in trials 04827 and 71042, respectively.

Wheat grain samples from trial 04827 and 71042 weighed 41.33 – 48.5 kg and >50 kg, respectively. The specimens were sent on the day of harvest or the day after to the processing test site under ambient conditions.

Processing procedures

The processing simulates industrial practice at a laboratory scale. The processing of wheat grain into the processed fractions (grain, bran, shorts, whole-meal flour, whole-grain bread, germs, milled by-products, gluten and gluten feed meal) was performed simulating the common industrial processes.

The below descriptions of the processing procedures are supported by the flow chart schemes in Figures 7.5.3.2-1 (flour (type 550) and whole-meal flour), Figure 7.5.3.2-2 (whole-meal flour and baking of whole-grain bread), Figure 7.5.3.2-3 (wheat germs) and Figure 7.5.3.2-4 (starch and gluten).

Cleaning/Division/Conditioning

Just before processing start the corresponding RAC specimen “grain, stored” were sampled.

Each of the delivered samples for processing was cleaned. Aspirated grain fractions were sampled. Cleaned grain of each field specimen for processing was separated dependent on the processing destination and the respective final products for 4 special processing parts as follows:

- Flour (type 550) process
- Whole-meal flour and whole meal-grain bread process
- Wheat germs process
- Starch and gluten process

All samples were then kept in frozen storage at or below -18°C until processing.

Flour (type 550) process

The grain was milled to straight flour, bran (coarse bran) and middlings (fine bran) in a closed system with different pairs of smooth rollers and sifter passages of the ‘Bühler Mahlautomat’.

Samples of bran (coarse bran) and middlings (fine bran) were taken.

Bran (coarse bran) and middlings (fine bran) were mixed together and low grade meal was separated using a centrifuge/scouring machine. This process results in shorts (total bran) and low grade meal. Samples of short (total bran) were taken.

The mineral content of straight flour and low grade meal was determined before both fractions were mixed together to make the final product of white flour (type 550) until the mineral content of the flour reached 510 – 630 g/100 kg flour.

Samples of the flour were taken.

Whole-meal flour and whole-meal grain bread process

The same process as used for the production of flour type 550 was used to generate whole-meal flour and whole-grain bread.

After milling, the shorts were cracked with an impact mill to smaller pieces. All milling products of the process were used completely for the whole meal and mixed homogeneously in a special flour mixer.

Samples of the whole-meal flour were collected.

For baking whole-meal bread, whole meal (1.0 kg), yeast (40 g), salt (20 g) and water (0.70 L) were mixed. The resulting dough was kneaded for 7 minutes and fermented for approximately 20 minutes. It was then moved for 10 minutes and a second rest for fermentation followed (40 minutes in a baking tin). The baking process was conducted at 210°C.

Samples of whole-grain bread were taken.

Wheat germs process

The grain was broken to bruised grain in a special mill (roller mill with 0.5 mm roller distance). The fraction 400 – 1000 µm was collected and the fraction above 1000 µm was broken once more (0.3 mm roller distance). This process was repeated for a total of three times, with a final roller distance of 0.2 mm. The fractions obtained below 400 µm and the last fraction above 1000 µm were excluded from further processing and were used for mixing to milled by-products.

The fraction 400 – 1000 µm, a mixture of bran, middlings and germs, were loaded to a special separator. Due to differences in weight, the middlings/germ mixture separates from most parts of the bran. Bran was used for mixing to milled by-products.

Subsequently, in a mill with a pair of smooth rollers the middlings/germ mixture was milled to flour, bran and small wheat germ discs. The mixture was then sieved to allow separation of the various fractions. The first sieving step resulted in flour and a bran/germ fraction. Flour was used for mixing to milled by-products. The bran/germ fraction was sieved once again to fine bran/germ fraction and coarse bran/germ fraction.

From the separated germ discs small parts of bran were removed manually. At first coarse bran/germ mixture and in case of insufficient material fine bran/germ mixture were used. The output of coarse bran and fine bran was dependent on the wheat (variety, moisture content etc.).

Samples of wheat germs were taken.

Bran was used for mixing to milled by-products. Afterwards, the components for milled by-products were mixed in the ratio in which they were generated.

Samples of milled by-products were taken.

Starch and gluten process

The grain was milled to straight flour, bran (coarse bran) and middlings (fine bran).

Further process steps involved mixing 1 kg of straight flour and 1.2 kg of water (adapted on quality) to obtain a hydrated dough. The dough was separated by centrifugation into wet starch, process water 1 and gluten (containing starch).

A sample of process water 1 was taken.

Subsequently, the starch was washed out (washing 1) with 1.5 kg water and was separated by centrifugation into starch A, process water 2 and gluten. This process was repeated. The starch was washed out (washing 2) with 1.5 kg of water and was separated by centrifugation into starch A, process water 3 and gluten.

The gluten (containing starch) was washed out with the process water 2 and resulted in gluten after washing and process water 4 (containing starch B and fibre). This process was repeated. The gluten (containing starch) was then washed out with the process water 3 and resulted in gluten after washing and process water 5 (containing starch B and fibre).

Remaining process water 4 and 5 was separated by centrifugation into starch B, fibre and process water 6. Subsequently, the fibre was washed out with process water 6 into wet starch B, fibre and process water 7.

Samples of the process water 7 were taken.

Fibre was then dried at 60°C and was milled.

Wet starch (A&B) was dried at approximately 60°C and wet gluten was dried by freeze drying. After the drying process the dried products were milled. Dried starch A and dried starch B were mixed in the ratio in which they were generated.

Samples of starch and gluten were taken.

The three dried fractions (fibre, starch B and gluten) were mixed, in the ratio in which they were generated after milling, to gluten feed meal.

Samples of gluten feed meal were taken.

Residue analysis

The samples were analysed for the parent compound and the metabolites 3'-OH-S-2840, DFPA-$CONH_2$, *N*-des-Me-DFPA, DFPA, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B, 1'-COOH-S-2840-A and 1'-COOH-S-2840-B using analytical method S16-03371. The method was sufficiently validated on wheat grain and whole plant in accordance with SANTE/2020/12830 rev.1 (see Vol. 3 CA B.5.1.2.5).

Due to the matrix similarity, the analytical method is therefore considered validated for wheat bran, shorts, germs, milled by-products, starch, gluten and gluten feed meal. In solid matrices, the LOQ was 0.01 mg/kg for the parent compound and the metabolites 3'-OH-S-2840, DFPA-$CONH_2$, *N*-des-Me-DFPA and DFPA and was 0.005 mg/kg for the metabolites 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B, 1'-COOH-S-2840-A and 1'-COOH-S-2840-B.

Further analytical validations were performed, within this MOR processing study on wheat, for all analytes at LOQ and 10 x LOQ in wheat flour and whole-grain bread at both the quantification and confirmation mass transitions. All mean recoveries were in the acceptable range of 70 – 110% and all RSD values were below 20%, with the exception of the HPLC confirmatory method recovery for DFPA in wheat flour at LOQ which had an RSD value of 24%. HSE notes that the mean recoveries and %RSD for the analysis of DFPA in wheat flour for the HPLC quantification method were acceptable. All the additional validation results for wheat flour and whole grain bread are presented in Vol. 3 CA B.5.1.2.5.

In addition, typically procedural recovery was performed at the LOQ and 10 x the LOQ per analytical set for all analytes in wheat grain, bran, shorts, flour, whole-grain bread, germs, milled by-products, starch, gluten and gluten feed meal. Virtually all the individual procedural recoveries were in the range of 70 – 110% (and all were within the range of 60 – 120%, except for wheat germ (DFPA at the LOQ, see below). The recoveries at LOQ level (0.01 mg/kg) for 3'-OH-S-2840 in in gluten feed meal was 118% and DFPA in wheat starch was 114%. The recovery at 10 x LOQ level (0.1 mg/kg) for the metabolite DFPA-$CONH_2$ in wheat bran was 114%. As all reported residues of DFPA-$CONH_2$ in wheat bran were <LOQ, the results are covered by the acceptable procedural recovery at LOQ (106%). For the metabolite DFPA in wheat germs, the applicant reported that a reasonable recovery at LOQ could not be obtained, but 10 x LOQ only. The applicant has noted that the (DFPA procedural) recoveries at 10xLOQ for wheat (germs) and also recoveries for wheat (shorts) at LOQ and 10xLOQ were all acceptable (shorts and germs were analysed in the same analytical set). All these aspects only reflect slight uncertainties in the results for the overall study and will hardly impact derivation of processing factors (the Pfs are presented in Vol 1 section 2.7.8). Due to the similarity in matrix between the process waters and beer, the additional validation data provided for beer in the processing study on barley (262-2016) is considered applicable to the process waters. The LOQ was 0.01 mg/L for the parent compound, 3'-OH-S-2840, DFPA-

$CONH_2$, *N*-des-Me-DFPA and DFPA in beer and was 0.005 mg/L for the metabolites 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B, 1'-COOH-S-2840-A and 1'-COOH-S-2840-B in liquid matrices. Within the processing study on wheat, one procedural recovery was performed at the LOQ and one at 10 x the LOQ per analytical set for all analytes in the process waters. The individual level of the procedural recoveries for process water were all within 84 – 110%.

Storage stability

RAC and processed samples were extracted after a maximum frozen storage period of 519 days.

The maximum time between last extraction and last analysis was 14 days where final sample extracts were stored at typically 1 °C – 10 °C. Stability of residues in sample extracts has been satisfactorily addressed as the matrix-matched standards used for quantification were always prepared on the same day (using control untreated sample fortified with solvent standards) as the production of the sample extracts for residues analysis; these matrix matched standards were also stored at 1 – 10 °C. Extract stability was also confirmed by the corresponding procedural recovery samples. Procedural recoveries were handled and stored in the same way and for the same time period as the sample extracts that were prepared within the same analytical set. Please refer to Vol 1 section 2.7.1 on the conclusions regarding storage stability of samples and extracts.

Figure 7.5.3.2-1: The simulated industrial processing of grain to flour (type 550) and whole-meal flour

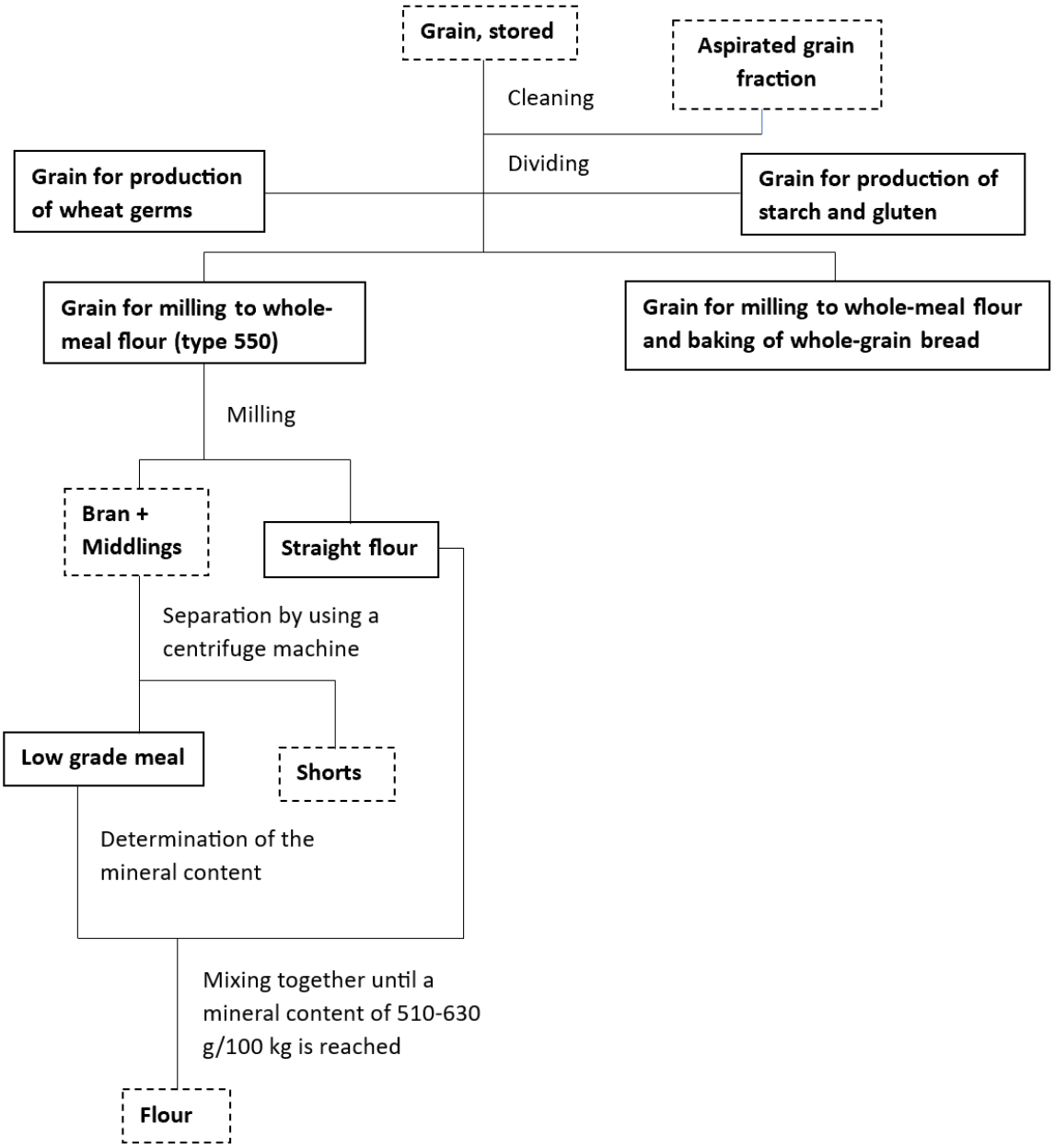


Figure 7.5.3.2-2: The simulated industrial processing of grain for milling to whole-meal flour and baking of whole-grain bread

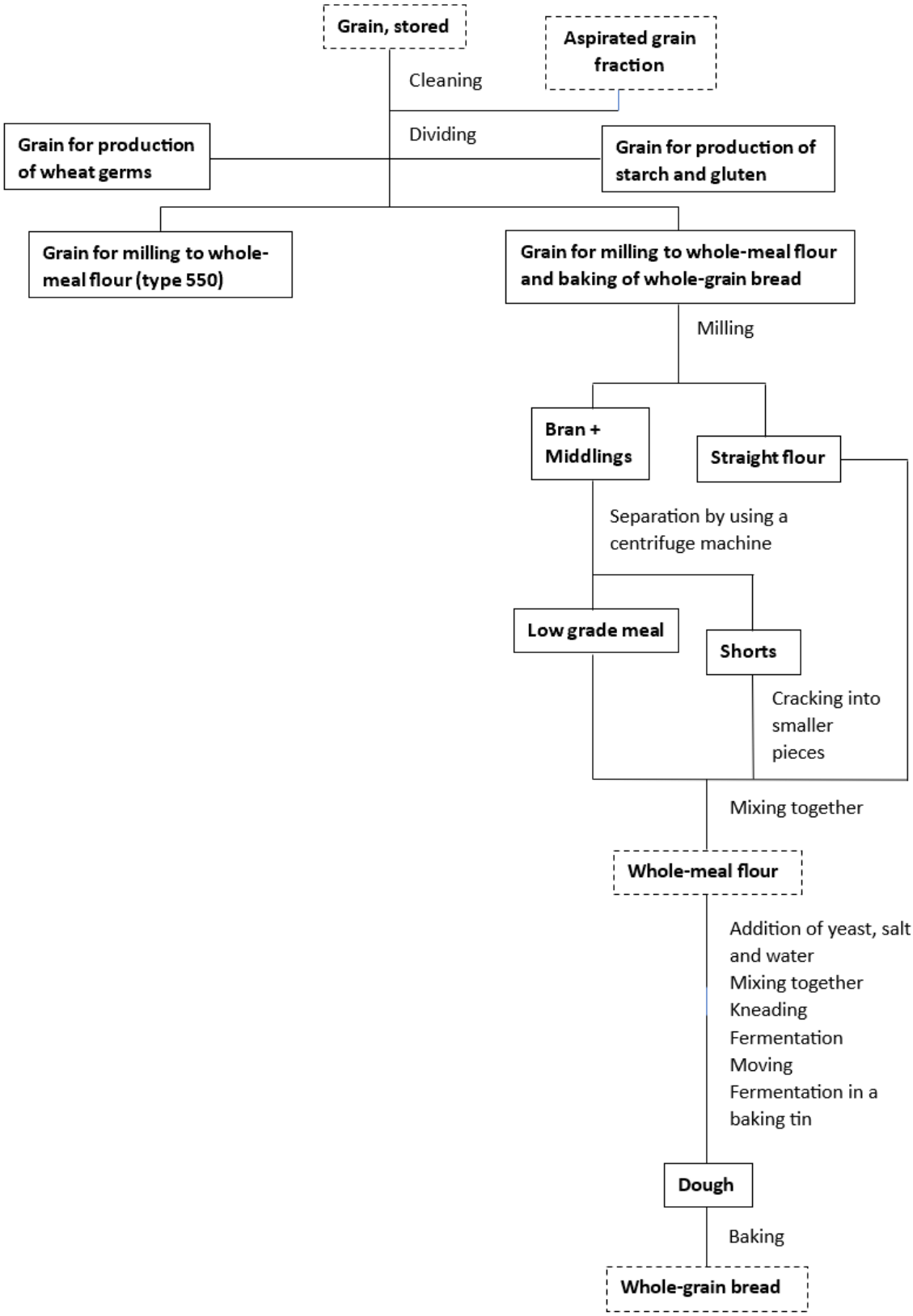


Figure 7.5.3.2-3: The simulated industrial processing of grain for production of wheat germs

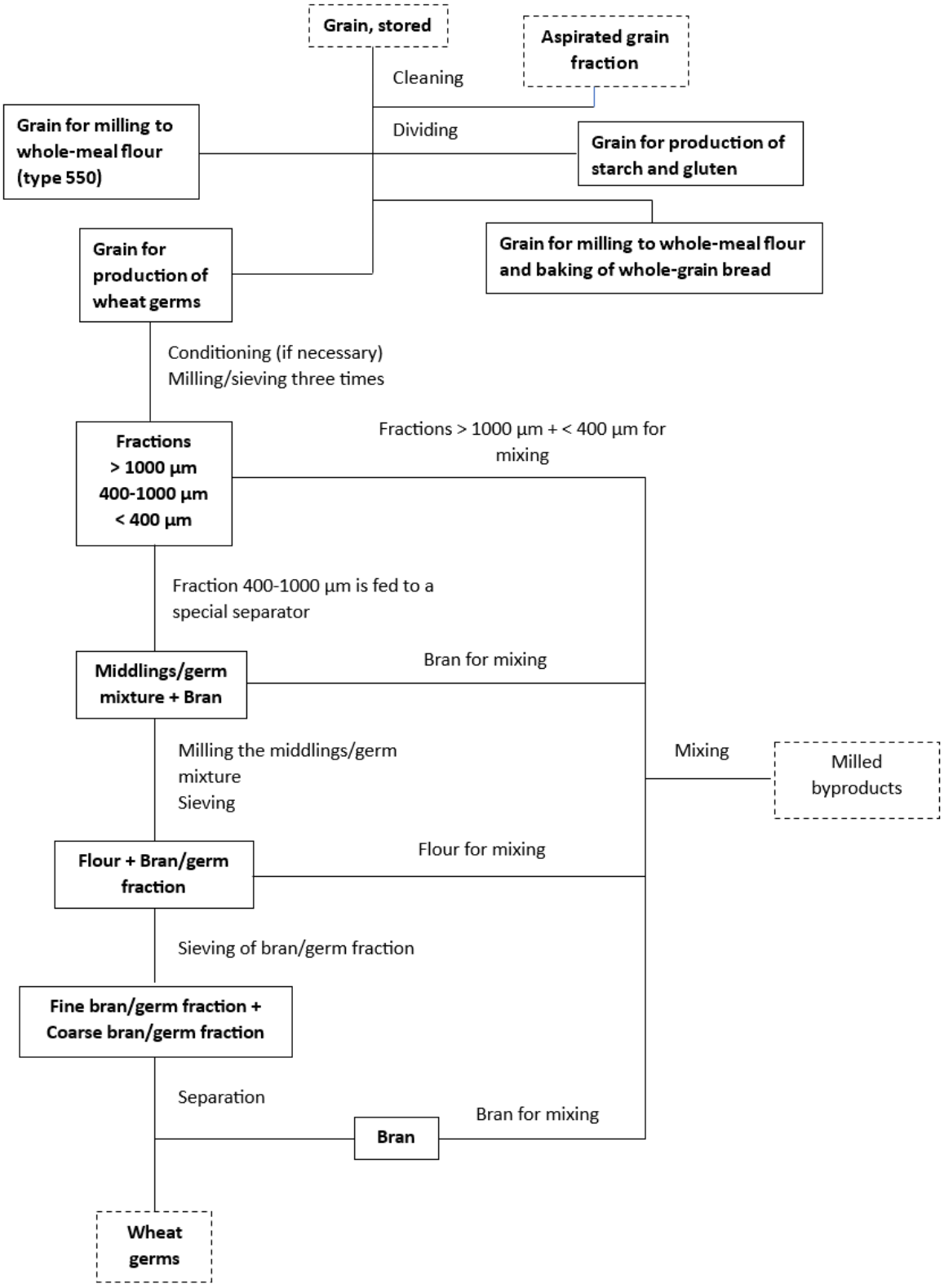
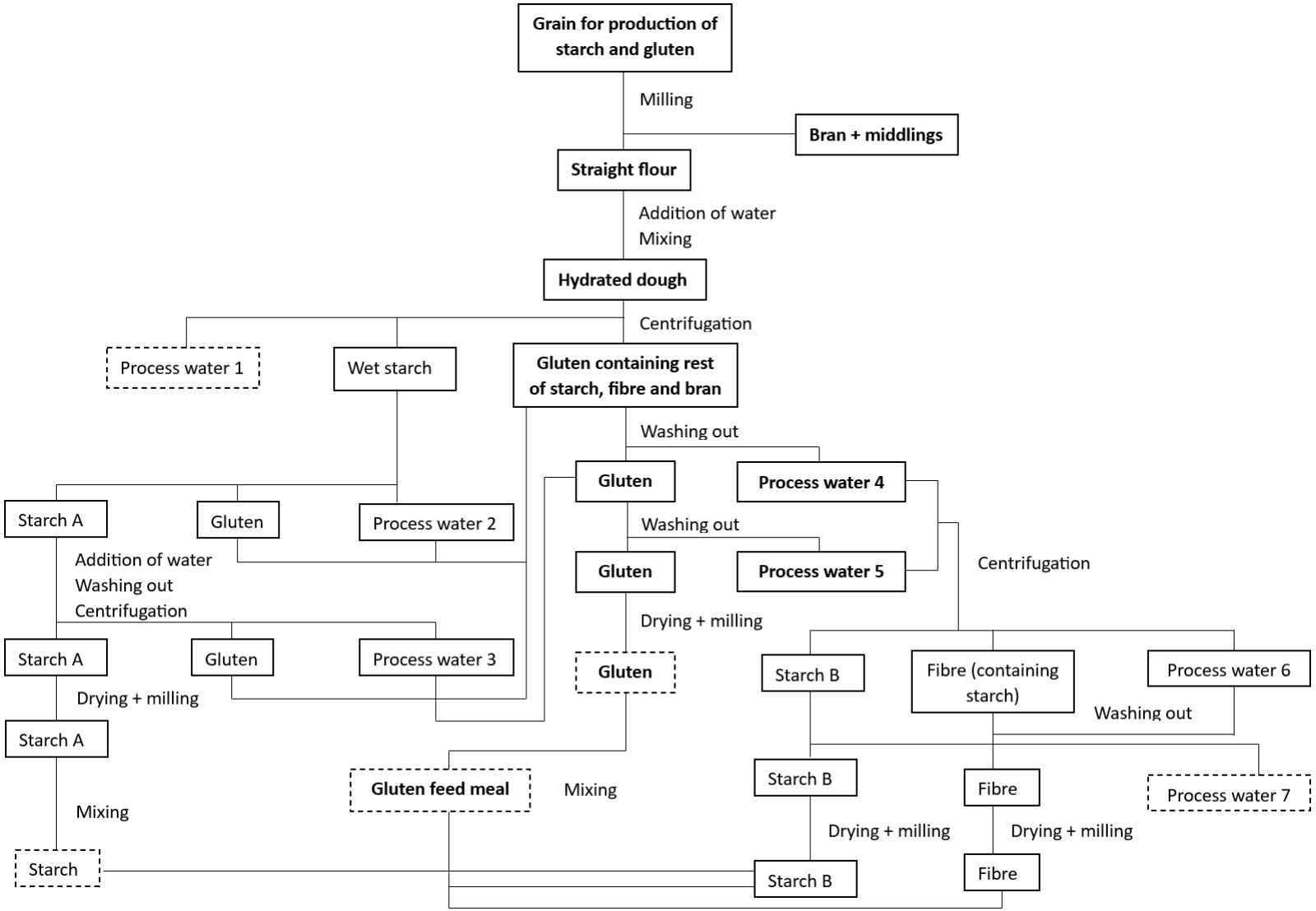


Figure 7.5.3.2-4: The simulated industrial processing of grain for production of starch and gluten



Results

No residues above the LOQ were found in the control samples. The results were not corrected for procedural recoveries.

Residues of parent inpyrfluxam in wheat grain before processing (RAC) were found to be 0.11 mg/kg in the first trial (Germany) and 0.27 mg/kg in the second trial (Italy).

The levels of residues of parent inpyrfluxam from the first trial (Germany) were 0.77, 0.42, 0.23, 0.45, 0.02, 0.08, 0.07, 0.08, 0.1, 0.02 and 0.02 mg/kg in aspirated grain fractions, bran, middlings, shorts, flour, whole-meal flour, whole-grain bread, wheat germs, milled by-products, gluten and gluten feed meal, respectively. Inpyrfluxam were found at < LOQ in process water 1, process water 7 or starch.

The levels of residues of parent inpyrfluxam from the second trial (Italy) were 0.47, 1.3, 0.4, 0.72, 0.05, 0.27, 0.16, 0.12, 0.28, 0.01, <0.01, 0.15 and 0.12 mg/kg in aspirated grain fractions, bran, middlings, shorts, flour, whole-meal flour, whole-grain bread, wheat germs, milled by-products, process water 1, starch, gluten and gluten feed meal, respectively. Inpyrfluxam was not detected in process water 7.

The results are summarised below in Table 7.5.3.2-2 and 7.5.3.2-3

For the presentation of the setting of processing factors for the currently intended use please refer to section 2.7.8 of the Volume 1.

Conclusions

Two residue trials were conducted in northern and southern Europe in 2016. Wheat was treated at a growth stage of BBCH 71 – 75 with a dose rate of 1 x 750 g a.s./ha.

Wheat grain was processed in order to obtain flour (type 550), whole-meal flour, whole-grain bread, wheat germ, starch and gluten. The samples (RAC and processed fractions) were analysed for the residues of inpyrfluxam parent compound as well as the metabolites 3'OH-S-2840, DFPA-$CONH_2$, *N*-des-Me-DFPA, DFPA, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B, 1'-COOH-S-2840-A and 1'-COOH-S-2840-B.

The results of the study indicate that generally residues of inpyrfluxam are concentrated in aspirated grain fractions. In the flour process, generally residues of inpyrfluxam are concentrated in bran, middlings and shorts but diluted in the final products of flour and whole-meal flour. Generally, residues of inpyrfluxam are diluted in whole-grain bread. Likewise for the production of wheat germ and gluten, generally the residues of inpyrfluxam are diluted.

Processing factors have been calculated and presented in Volume 1, section 2.7.6.

Table 7.5.3.2-2: Detailed results of the wheat processing studies for inpyrfluxam, 3'-OH-S-2840, DFPA-CONH$_2$ and *N*-des-Me-DPFA

Trial No. / Location / Year	Commodity / Variety	Date of 1. Sowing 2. Flowering 3. Harvest	Application rate per treatment			Date of treatment	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)				PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL				Inpyrfluxam	3' -OH-S-2840	DFPA-CONH$_2$	*N*-des-Me-DPFA		
04827 / [redacted], Saxony, Germany, Northern Europe / 2016	Spring wheat / KADRI LJ	1. 15/04/16 2. 30/06/16 to 08/07/16 3. 17/08/16	1.0184	298	0.3417	12/07/16	BBCH 73 – 75	grain, stored (before processing)	0.11	0.03	<0.01	<0.01	36	Untreated specimen: <LOQ LC-MS/MS Method validation reports: S16-003371 (5 matrices) 261-2016 (wheat flour and bread) 262-2016 (liquid matrix(beer)) Max. storage between sampling and
								aspirated grain fraction	0.77	0.14	<0.01	0.02		
								bran	0.42	0.12	<0.01	<0.01		
								middlings	0.23	0.06	<0.01	<0.01		
								shorts	0.45	0.14	<0.01	<0.01		
								flour	0.02	<0.01	<0.01	<0.01		
								whole-meal flour	0.08	0.03	<0.01	<0.01		
								whole-grain bread	0.07	0.02	<0.01	<0.01		
								wheat germs	0.08	0.01	<0.01	<0.01		
								milled by-products	0.10	0.03	<0.01	<0.01		

								process water 1	<0.01	<0.01	<0.01	<0.01		extraction / extraction and analyses over the whole study: 519 / 14 days
								process water 7	<0.01	<0.01	<0.01	<0.01		
								starch	<0.01	<0.01	<0.01	<0.01		
								gluten	0.02	<0.01	<0.01	<0.01		
								gluten feed meal	0.02	<0.01	<0.01	<0.01		
71042 / [redacted], Puglia, Italy, Southern Europe / 2016	Spring wheat / CORE	1. 05/01/16 2. 27/04/16 to 06/05/16 3. 13/06/16	1.0468	306	0.3421	09/05/16	BBCH 71	grain, stored (before processing)	0.27	0.03	<0.01	<0.01	36	Untreated specimen: <LOQ LC-MS/MS Method validation reports: S16-003371 (5 matrices) 261-2016 (wheat flour and bread) 262-2016 (liquid matrix(beer)) Max. storage between sampling and
								aspirated grain fraction	0.47	0.05	<0.01	<0.01		
								bran	1.3	0.13	<0.01	<0.01		
								middlings	0.40	0.05	<0.01	<0.01		
								shorts	0.72	0.10	<0.01	<0.01		
								flour	0.05	<0.01	<0.01	<0.01		
								whole-meal flour	0.27	0.03	<0.01	<0.01		
								whole-grain bread	0.16	0.02	<0.01	<0.01		
								wheat germs	0.12	0.01	<0.01	<0.01		
								milled by-products	0.28	0.03	<0.01	<0.01		

								process water 1	0.01	<0.01	<0.01	<0.01		extraction / extraction and analyses over the whole study: 519 / 14 days
								process water 7	<0.01	<0.01	<0.01	<0.01		
								starch	<0.01	<0.01	<0.01	<0.01		
								gluten	0.15	<0.01	<0.01	<0.01		
								gluten feed meal	0.12	<0.01	<0.01	<0.01		

Table 7.5.3.2-3: Detailed results of the wheat processing studies for DFPA, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B, 1'-COOH-S-2840-A and 1'-COOH-S-2840-B

Trial No. / Location / Year	Commodity / Variety	Date of 1. Sowing 2. Flowering 3. Harvest	Application rate per treatment			Date of treatment	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)					PHI (days)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL				DFPA	1' -CH_2OH-S-2840-A	1' -CH_2OH-S-2840-B	1' -COOH-S-2840-A	1' -COOH-S-2840-B		
04827 / [redacted] Saxony, Germany, Northern	Spring wheat / KADRIL J	1. 15/04/16 2. 30/06/16 to 08/07/16 3. 17/08/16	1.0184	298	0.3417	12/07/16	BBCH 73 – 75	grain, stored (before processing)	0.01	0.032	0.076	<0.005	<0.005	36	Untreated specimen: <LOQ LC-MS/MS
								aspirated grain fraction	0.08	0.21	0.52	0.011	0.006		

Europe / 2016								bran	0.04	0.10	0.27	0.008	0.006		
								middlings	0.02	0.052	0.19	<0.005	<0.005		Method validation reports: S16-003371 (5 matrices) 261-2016 (wheat flour and bread) 262-2016 (liquid matrix(beer))
								shorts	0.05	0.11	0.30	0.010	0.007		
								flour	<0.01	<0.005	0.007	<0.005	<0.005		
								whole-meal flour	0.01	0.022	0.055	<0.005	<0.005		
								whole-grain bread	<0.01	0.017	0.043	<0.005	<0.005		
								wheat germs	<0.01	0.025	0.043	<0.005	<0.005		Max. storage between sampling and extraction / extraction and analyses over the whole study: 519 / 14 days
								milled by-products	0.02	0.030	0.073	<0.005	<0.005		
								process water 1	<0.01	<0.005	<0.005	<0.005	<0.005		
								process water 7	<0.01	<0.005	<0.005	<0.005	<0.005		
								starch	<0.01	<0.005	<0.005	<0.005	<0.005		
								gluten	<0.01	<0.005	0.07	<0.005	<0.005		
								gluten feed meal	<0.01	<0.005	<0.005	<0.005	<0.005		

71042 / [redacted], Puglia, Italy Southern Europe / 2016	Spring wheat / CORE	1. 05/01/16 2. 27/04/16 to 06/05/16 3. 13/06/16	1.0468	306	0.3421	09/05/16	BBCH 71	grain, stored (before processing)	0.01	0.026	0.031	<0.005	<0.005	36	Untreated specimen: <LOQ LC-MS/MS Method validation reports: S16-003371 (5 matrices) 261-2016 (wheat flour and bread) 262-2016 (liquid matrix(beer)) Max. storage between sampling and extraction / extraction and analyses over the whole study: 519 / 14 days
								aspirated grain fraction	0.02	0.042	0.057	<0.005	<0.005		
								bran	0.08	0.088	0.17	<0.005	<0.005		
								middlings	0.02	0.030	0.034	<0.005	<0.005		
								shorts	0.04	0.10	0.11	<0.005	<0.005		
								flour	<0.01	<0.005	<0.005	<0.005	<0.005		
								whole-meal flour	0.02	0.020	0.021	<0.005	<0.005		
								whole-grain bread	<0.01	0.015	0.017	<0.005	<0.005		
								wheat germs	<0.01	0.013	0.013	<0.005	<0.005		
								milled by-products	0.03	0.023	0.023	<0.005	<0.005		
								process water 1	<0.01	<0.005	<0.005	<0.005	<0.005		
								process water 7	<0.01	<0.005	<0.005	<0.005	<0.005		

								starch	<0.01	<0.005	<0.005	<0.005	<0.005		
								gluten	0.01	0.008	0.009	<0.005	<0.005		
								gluten feed meal	<0.01	<0.005	<0.005	<0.005	<0.005		

B.7.6. Residues in Succeeding or Rotational crops

Please refer to Vol 1, section 2.7.9 for a discussion of anticipated soil exposures and the interpretation of the significance of the residues in rotational crops in the context of the intended GAP rates for the proposed uses on wheat and barley.

B.7.6.1. Metabolism in rotational crops

Report:	**KCA 6.6.1/01; [redacted] (2017)**
Title:	Confined Accumulation of [Phenyl-^{14}C]S-2399 and [pyrazolyl-4-^{14}C]S-2399 in Rotational Crops
Document No.:	TPM-0047 (Study No.: VP-38482)
Guidelines:	EPA Residue Chemistry Test Guidelines - OCSPP 860.1850 (Confined Accumulation in Rotational Crops) OECD Guideline for the Testing of Chemicals No. 502: Metabolism in Rotational Crops
Guideline deviations:	None
GLP/GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

Summary

Conducted in the USA (California) in 2014/2015, the metabolism of inpyrfluxam was investigated in confined rotational crops following one spray application with inpyrfluxam either labelled in the pyrazolyl or in the phenyl moiety onto bare soil at a rate of with ~235 g a.s./ha (this rate corresponds to 2.6 N, regarding comparison to the maximum seasonal dosage for the GB assessed uses. An exaggerated rate of study is useful for considering the potential for uptake of residues in rotational crops following year to year use and accumulation that add to soil exposure levels (see Vol 1, section 2.7.9) and for potential future extensions of uses).

Root crops are represented by radish, leafy crops by lettuce and cereals by sorghum. They were sown at plant back intervals (PBI) of 30 days (1st rotation), 120 days (2nd rotation) and 365 days (3rd rotation) after soil treatment.

A sample of immature sorghum forage was harvested at BBCH stage 85 and sorghum stover and grain samples were harvested at BBCH 89. Immature radish roots and leaves were sampled at BBCH 44 and at maturity at BBCH 49. Immature lettuce was sampled at BBCH 44 and at maturity at BBCH 49.

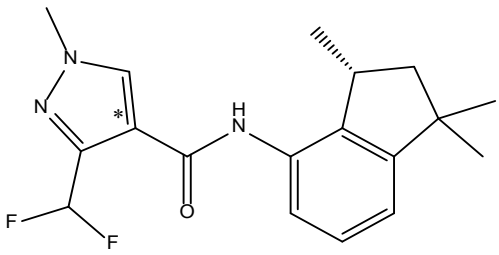
Metabolites were identified by comparison to reference standards (for the 'free' form of the metabolites). Proposed conjugates were characterised according to their behaviour (release of metabolites following acid hydrolysis). The study enabled the prevalence of the various pesticide metabolites to be considered in rotational crops, alongside the findings of inpyrfluxam, and to enable a proposed metabolic pathway to be elucidated. The possibility of the formation of pesticide metabolite conjugates and further proposed natural incorporation of residues into natural plant constituents is observed in this study.

Materials and Methods

The radiolabelled materials applied to the plots, either as [phenyl-U-^{14}C] inpyrfluxam or [pyrazolyl-4-^{14}C] inpyrfluxam, were formulated as an SC. The test materials contents were suitably verified, and the homogenous suspensions were demonstrated to deliver 94 – 100% of the calculated radioactivity.

Radiolabelled test material [pyrazolyl-4-^{14}C] inpyrfluxam

Chemical Structure

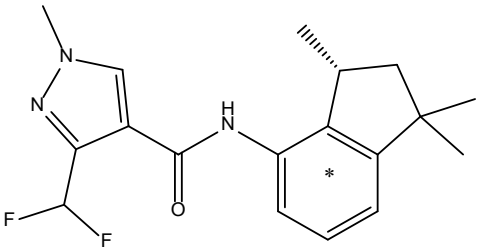


* denotes the ^{14}C-label position

Specific radioactivity 2.11 GBq/mmol (57.0 mCi/mmol or 377,700 dpm/µg)
[The specific activity was adjusted to 99,280 dpm/µg (1.66 MBq/mg) by isotopic dilution with analytical grade inpyrfluxam before application]

Radiochemical purity 98.1% (in the dosing solution)

Chemical purity	95.3%

Radiolabelled test material	[phenyl-U-^{14}C] inpyrfluxam
Chemical Structure	 * denotes the ^{14}C-label position
Specific radioactivity	4.51 GBq/mmol (122 mCi/mmol or 802,253 dpm/µg) [The specific activity was adjusted to 98,343 dpm/µg (1.64 MBq/mg) by isotopic dilution with analytical grade inpyrfluxam before application]
Radiochemical purity	98.7% (in the dosing solution)
Chemical purity	95.3%

Soil characteristics of the sandy loam soil used were provided in the study. The plot soil had no exposure to inpyrfluxam in the prior three years, and absence of soil radioactivity was confirmed by analyses prior to the study.

Post-application soil surface samples were checked, and control samples were free of radioactivity.

At all planting intervals sorghum (var. LS55), lettuce (var. Butter Crunch) and radish (var. Cherry Belle) were planted. In the control plots, sorghum, lettuce and radish were planted at 30 DAT planting interval only.

Each study plot was a 91 cm wide × 152 cm long × 38 cm deep wooden box (plot area 1.4m^2) lined with plastic sheeting. At each planting interval, one treated plot was cultivated with the leafy vegetable (lettuce) and the root crop (radish), and another plot was planted with the small grain (sorghum). For each radiolabel, the final planting at 365 days after treatment (DAT) was carried out in the same two plots used for the 30 DAT planting, and the 120 DAT planting was done in the other two plots. Two further plots were treated with a control (formulation blank) spray; these control crops were planted at 30 DAT only.

During application, the spray solution was uniformly applied to the bare soil using a hand-triggered sprayer. By taking account of residual radioactivity in spray bottles post application, the application rate was determined as approximately 235 g/ha for each label (this rate corresponds to 2.6 N, regarding comparison to the maximum seasonal dosage

for the GB assessed uses. An exaggerated rate of study is useful for considering the potential for uptake of residues in rotational crops following year to year use and accumulation that add to soil exposure levels (see Vol 1, section 2.7.9) and for potential future extensions of uses).

Sorghum forage was sampled at GS 85 by taking cuts approximately 1 – 2 inches above the soil surface. The sorghum stover and grain samples were collected at growth stage BBCH 89. The sorghum heads were removed and grain separated from the chaff; chaff was added to the remaining plant material (stover) which was cut approximately 1 inch above the soil.

The radish plants were harvested at an immature stage (BBCH 44) and also at maturity (BBCH 49). Radishes were pulled out of the ground and shaken gently to remove adhering soil. The tops were cut away and the roots were rinsed with water by hand and patted dry. This way of removing soil is acceptable for regulatory residues work (reference Annex 1 in OECD 509).

Immature lettuce samples were collected at BBCH 44, and the mature lettuce samples were harvested at BBCH 49 by cutting plants just above the soil level.

All unprocessed harvested RAC samples were stored in bags frozen (-18^{o}C) until analysis.

Plant samples were mixed with dry ice and homogenized to the consistency of a fine powder. Following sublimation of dry ice subsamples were taken for combustion analysis and extraction.

Combustion Analysis

Total radioactivity in soil and plant samples was determined by combustion using an oxidiser. The resultant $^{14}CO_2$ was trapped in 15 mL of scintillation cocktail and then radioassayed by LSC.

Extraction

Crop samples with residues above 0.01 mg eq./kg were extracted. Samples were mixed thoroughly with diatomaceous earth and extracted sequentially with acetonitrile (x2), water (x2) and finally again with acetonitrile using an accelerated solvent extractor. The respective (x2) extracts were pooled to produce a designated acetonitrile and aqueous extract. The acetonitrile extract from the final extraction cycle was kept separate and designated as acetonitrile rinse. The extraction approach was first experimentally tested (using 2 x, 3 x and 4 x sequence) using in 30 DAT sorghum stover samples which justified using a 2x acetonitrile sequence (followed by water extraction and the acetonitrile rinse). The residue or post-extraction solids (PES) remaining was quantified by combustion and

LSC and where an appreciable amount of radioactivity remained, the PES was subjected to further analysis (see below).

Identification of Crop Metabolites

The acetonitrile and aqueous extracts of crops that contained ≥0.01 mg eq./kg of radioactive residue were analysed by both HPLC and 2D TLC. The parent compound, inpyrfluxam, and the metabolites, 3'-OH-S-2840, *N*-des-Me-S-2840, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B, 1'-COOH-S-2840-A and 1'-COOH-S-2840-B, *N*-des-Me-1'-CH_2OH-S-2840-A, *N*-des-Me1'-CH_2OH-S-2840-B, DFPA, *N*-des-Me-DFPA, DFPA-$CONH_2$ were identified in the extracts of various rotational crops. The parent and the major metabolites were isolated from extracts by preparative HPLC, and their identification was carried out by HPLC retention time correlation and 2D TLC co-migration with the reference standards.

Characterisation of the Polar Proposed Conjugated Residues (lettuce, radish and sorghum)

The major metabolite fraction in both phenyl label and pyrazolyl label crop extracts were polar in nature, and eluted at an approximate retention time of 16 min in HPLC analysis, and stayed at or near the origin in 2D TLC analysis. The nature of this proposed polar conjugated residue fraction was analysed by fractionation and chromatographic analysis using HPLC and TLC. The 16 min peak fractions of polar material were isolated by preparative HPLC from the extracts of both phenyl and pyrazolyl labels of crops. The isolated peak fraction was concentrated to a smaller volume and a portion of it was subjected to acid hydrolysis (2M HCl at ca 100ºC for 2 hours) to release multiple residues. The applicant proposed that this step released metabolites (as free exocons) from the conjugated residues (in the isolated peak fraction). Following the acid treatment, the hydrolysate was partitioned (4 x) with ethyl acetate; the aqueous and (pooled) ethyl acetate phases were analysed by LSC. The ethyl acetate fraction was concentrated and the residue dissolved in acetonitrile. Aliquots were measured by LSC and analysed by 2D TLC with co-chromatographed standards.

Acid Hydrolysis of Individual Proposed Conjugate Peaks of Sorghum Stover

A number of individual 'conjugate' peaks were isolated from the 16 min band of the aqueous extract of pyrazolyl label 30 DAT sorghum stover using preparative HPLC. The radioactivity of separated peak fractions (dried and dissolved in acetonitrile) was determined by LSC. acetonitrile) determined by LSC. Further acid treatment of dried residues (addition of 2M HCl, 100 ºC for 4 hours) followed by drying of the hydrolysate and dissolution in ethyl acetate. The peak fractions were then prepared for LSC and 2D TLC analysis.

Determination of Metabolite Profiles

The metabolite profiles of the extracts containing residues ≥ 0.01 mg eq./kg, were determined by both 2D TLC and HPLC analyses with radioactive detection. Total radioactive residues associated with individual metabolite (TLC) spots was quantified using radiographic volume quantification software. Several HPLC methods were used during the course of the study to analyse samples, to separate co-eluting metabolites and to characterise the proposed polar conjugated fraction of the extracts. 2D TLC was more successful at separating the residues, whereas some peaks were superimposed using HPLC. The reports of the levels of individual metabolites were based on the results of the TLC.

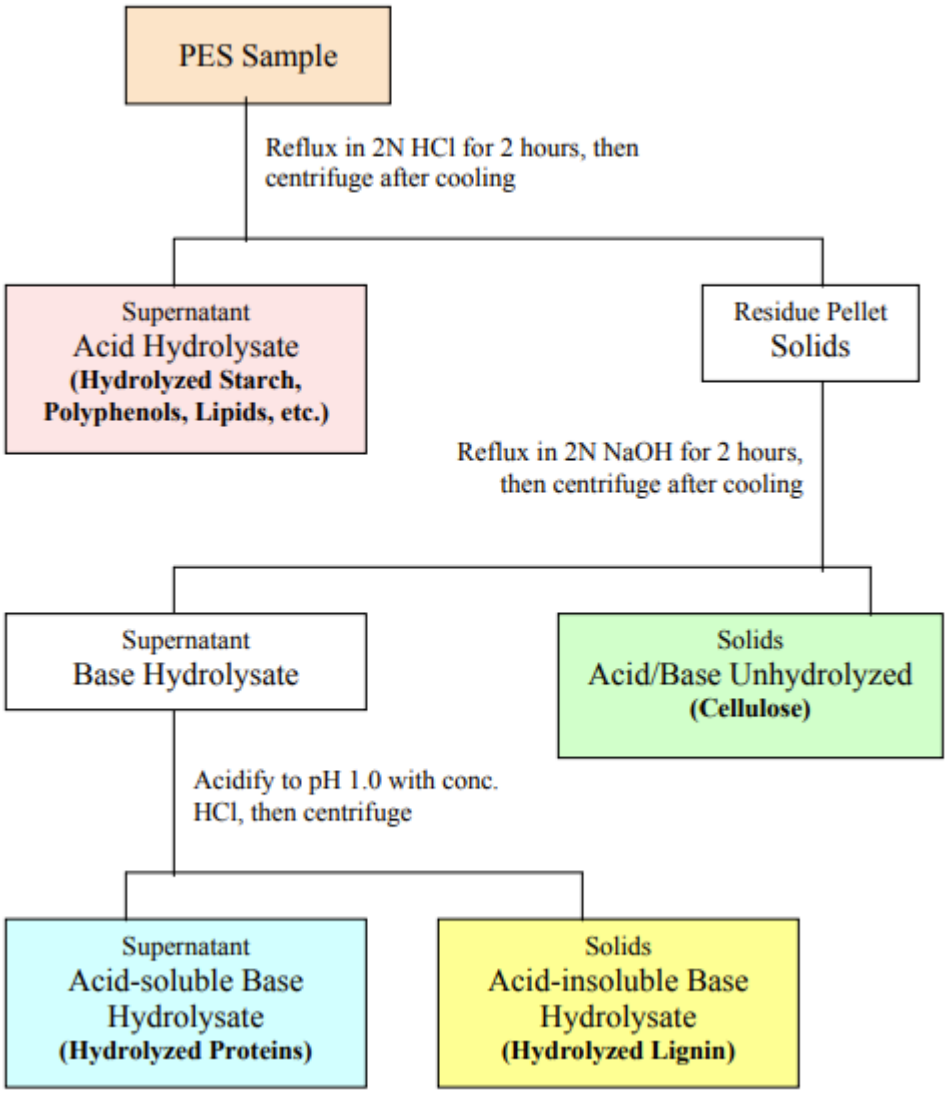
Analysis of Proposed Bound Residues

A number of PES samples of lettuce, radish and sorghum contained radioactivity above 10% of TRR, but very few of them (only sorghum stover) exceeded 0.05 mg eq./kg of residue. The nature of the proposed 'bound' residue in samples containing > 10%TRR was investigated by subjecting representative PES samples to sequential hydrolysis with acid (suspension in 50 – 100 mL of 2M HCl under reflux for 2 hours) and then base (suspension in 50 – 100mL of 2M NaOH under reflux for 2 hours). The base hydrolysate was adjusted to pH 1, refrigerated for an hour and centrifuged. Solubilised residues and the remaining PES were quantified by LSC and combustion analysis.

In their interpretation of this study, the applicant has postulated that residues in the PES have become associated with the plant macromolecular matrix. There have not been specific tailored methods (e.g. enzyme treatments) applied to establish more specifically the nature of these plant materials, however the applicant has provided the following scheme as the basis for these inferred positions. HSE noted that the acid and base treatments are at 2M HCl or 2M NaOH under reflux, so these are relatively harsh conditions applied to release these residues.

Applicant proposals for radioactive residue associations with plant macromolecular matrix:

Figure 7.6.1-1: Applicants proposals for radioactive residues associations



Applicant proposals:

The acid hydrolysate might contain pesticide residue as well as phenolic and fatty acid residues from acid-hydrolysable plant constituents such as polyphenols and lipids. But it was likely to predominantly contained simple sugars released from polymeric carbohydrates such as starch.

The insoluble pellet remaining after base hydrolysis mainly contained cellulose fibres. The base hydrolysate after adjustment to pH 1, refrigerated for an hour and centrifuged to separate out the solids (thought to be precipitated lignin fraction). The clear supernatant of the acidified base hydrolysate was considered to be predominantly amino acids and small peptides hydrolysed from plant proteins.

Storage stability

Samples were stored for a minimum of 85 days (3 months) and maximum 418 days (13 months) before extraction. All sample extracts were then analysed within 18 days. 17 – 21 months after the initial extraction, representative freezer-stored crop samples (30 DAT mature lettuce, mature radish tops, mature radish roots, sorghum stover and sorghum grain from both phenyl and pyrazolyl labels) were re-extracted and re-analysed to determine the storage stability of the samples.

The overall recoveries of total residue and metabolite compositions of 30 DAT mature lettuce, mature radish tops, mature radish roots, and sorghum stover from both phenyl and pyrazolyl labels and the aqueous extract of sorghum grain from the pyrazolyl label were approximately similar in both extractions (pre- and post-storage). The extracts of 30 DAT sorghum grain from the phenyl label could not be analysed further due to low level of residue (0.005 mg eq./kg).

These metabolite compositions in the storage stability work are reported by way of comparative TLC plate pictures in the study reports showing the position and ‘strength’ of the metabolite spots. This storage stability work indicates that the major inpyrfluxam metabolites in the rotational crop samples were likely stable for the period between the initial extractions (for some sample extractions these ‘initial’ extractions were done after a year after harvest) and later re-extraction of the samples. This provides some support for the sample materials analysis results in this study as being relevant for the assessment of nature of rotational crop residues for consumer risk assessment, since the nature of the residues hasn’t markedly changed in a long period (17 – 21 months). Please refer to the discussion in Vol 1 section 2.7.2.

Results and Discussion

Total Radioactive Residues

The total radioactive residues (TRR) in homogenised plant samples as determined by combustion analysis are presented in Table 7.6.1-1. The TRR is expressed in mg a.s. equivalents per kg sample material (abbreviated as mg eq./kg).

Table 7.6.1-1: Total radioactive residues (TRRs) in rotational crops following treatment of soil with [phenyl-^{14}C] and [pyrazolyl-^{14}C] labelled inpyrfluxam

Crop	Crop portion	TRR (mg eq./kg) [combustion analysis]					
		30 PBI		120 PBI		365 PBI	
		Phenyl	Pyrazolyl	Phenyl	Pyrazolyl	Phenyl	Pyrazolyl
Lettuce	Immature	0.045	0.080	0.052	0.103	0.023	0.039
	Mature	0.094	0.074	0.069	0.093	0.012	0.025
Radish	Immature tops	0.112	0.139	0.106	0.230	0.088	0.101
	Immature roots	0.033	0.040	0.029	0.059	0.021	0.024
	Mature tops	0.136	0.228	0.117	0.367	0.092	0.073
	Mature roots	0.044	0.065	0.030	0.108	0.028	0.022
Sorghum	Forage	0.102	0.209	0.135	0.180	0.035	0.047
	Stover	0.692	0.703	1.074	0.945	0.133	0.236
	Grain	0.012	0.048	0.012	0.058	0.014	0.014

Sorghum: The highest total radioactive residue based on combustion results was observed in sorghum stover samples, in which the total residue of ca 0.692 – 0.703 mg eq./kg at 30 DAT planting increased to ca 0.945 mg eq./kg – 1.074 mg eq./kg at 120 DAT planting, then decreased to 0.133 – 0.236 mg eq./kg at 365 DAT planting. The sorghum forage contained considerably lower residue levels than the stover samples at all planting intervals (0.102 – 0.209 mg eq./kg at 30 and 120 DAT, and 0.035 – 0.047 mg eq./kg at 365 DAT). The residue level was low (0.012 – 0.014 mg eq./kg) in the phenyl label sorghum grain from all planting periods and pyrazolyl label grain from 365 DAT planting interval, but slightly higher (0.048 – 0.058 mg eq./kg) in the pyrazolyl label grain from the two earlier planting intervals.

Radish: The immature and mature radish top samples contained total residues comparable to the sorghum forage samples (0.106 – 0.367 mg eq./kg at 30 and 120 DAT and 0.073 – 0.101 mg eq./kg at 365 DAT). The total residue in immature and mature radish roots were considerably lower; 0.029 – 0.108 mg eq./kg at 30 and 120 DAT, and 0.021 – 0.028 mg eq./kg at 365 DAT. The TRR level in the phenyl label radish tops was highest in the 30 DAT planting interval and was comparatively lower in the immature sample (0.112 mg eq./kg) than in the mature sample (0.136 mg eq./kg). The TRR levels in

the pyrazolyl label immature and mature radish roots were generally much lower than the corresponding radish tops.

Lettuce: The immature and mature lettuce also contained relatively low levels of total residue similar to the radish roots (0.045 – 0.103 mg eq./kg at 30 and 120 DAT, and 0.012 – 0.039 mg eq./kg at 365 DAT). In the phenyl label, the TRR level in lettuce samples was highest in the 30 DAT planting interval and ranged from 0.045 mg eq./kg in the immature sample to 0.094 mg eq./kg in the mature sample. In the pyrazolyl label, the TRR level in lettuce samples was highest in the 120 DAT planting interval and ranged from 0.103 mg eq./kg in the immature sample to 0.093 mg eq./kg in the mature sample.

The subsamples of lettuce, radish, and sorghum RACs showed total radioactive residues that were above 0.01 mg eq./kg. Therefore, extraction to determine the distribution of residues in the extracts and in the PES was performed.

Extractability of Residues

A summary of extraction for the phenyl label and pyrazolyl label samples is given in tables 7.6.1-2 and 7.6.1-3, respectively. The TRR by extraction in the phenyl label from all plantings and crop samples was within 86 – 118% of the combustion TRR (Accountability (%) expressed as {[(sum of ERR + PES)/(initial combustion TRR)] x 100}). Extracted residues varied from 47.4% in sorghum grain to 96.8% in immature Radish Roots. The results for extractability were broadly similar across both radiolabels.

Lettuce

The extraction results showed that 79 – 92% of the TRR in the immature and mature lettuce of all 3 planting intervals was extracted by acetonitrile (up to 0.09 mg eq./kg). 3 – 8% of TRR of the lettuce samples went into the aqueous extract up to 0.006 mg eq./kg), and ca 4 – 13% (0.002 – 0.011 mg eq./kg) remained in the PES. Approximately 87 – 96% of TRR (0.010 – 0.093 mg eq./kg) of immature and mature lettuce harvested from all three plantings was extracted.

Radish

A higher portion of the TRR in the immature and mature radish tops from various planting intervals was extracted by acetonitrile (79 – 88% of TRR, 0.062 – 0.291 mg eq./kg) compared to that extracted by water (8 – 10% of TRR, 0.006 – 0.037 mg eq./kg). Around 5 – 11% (0.004 – 0.039 mg eq./kg) remained in the PES. Approximately 90 – 95% of total radioactive residue (0.067 – 0.331 mg eq./kg) of immature and mature radish tops harvested from all three plantings was extracted.

From the radish immature and mature roots of all planting intervals, acetonitrile extracted 91 – 94% of total residue (0.019 – 0.099 mg eq./kg). In both of these planting intervals,

water extracted an additional 2 – 5% (<0.001 – 0.005 mg eq./kg), and 3 – 10% remained in the PES (0.001 – 0.004 mg eq./kg). Approximately 90 – 97% of TRR (0.019 – 0.104 mg eq./kg) of immature and mature radish roots harvested from all three plantings was extracted.

Sorghum

Approximately 77 – 86% of the TRR (0.029 – 0.171 mg eq./kg) of sorghum forage harvested from all three plantings was extracted, with acetonitrile extracting 54 – 66% (0.020 – 0.124 mg eq./kg), and the aqueous extract extracted an additional 17 – 24% (0.010 – 0.048 mg eq./kg). The PES accounted for 14 – 23% of TRR (0.007 – 0.041 mg eq./kg) in these samples.

About 77 – 82% of the TRR (0.105 – 1.027 mg eq./kg) of sorghum stover harvested from all three plantings was extracted, with acetonitrile extracting only 10 – 28% (0.038 – 0.131 mg eq./kg), and the aqueous extract contained the major potion of 46 – 67% of TRR (0.063 – 0.843 mg eq./kg). The PES accounted for 18 – 23% of TRR (0.030 – 0.236 mg eq./kg) in these samples.

In sorghum grain samples harvested from all three planting intervals, approximately 47 – 60% of the TRR (0.005 – 0.031 mg eq./kg) was extracted, almost all of which was extracted in the aqueous extract. The PES accounted for the remaining 48 – 50% of TRR (0.005 – 0.031 mg eq./kg) in these samples.

Table 7.6.1-2 Distribution of Radioactive residues in Extracts and PES of samples from Plots Treated with [Phenyl-U-^{14}C] inpyrfluxam

Matrix	Acetonitrile extract		Aqueous extract (water)		Acet. Rinse		Total extracted		PES Residue		TRR from Extraction [a]	
	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR
Rotational interval: 30 DAT												
Immature lettuce	0.039	92.1	0.001	2.9	<0.001	<0.01	0.040	95.0	0.002	5.0	0.042	100
Mature lettuce	0.089	89.3	0.004	4.0	<0.001	0.1	0.093	93.4	0.007	6.6	0.100	100
Immature Radish tops	0.093	80.7	0.010	9.0	0.001	0.6	0.104	90.3	0.011	9.7	0.115	100
Immature Radish roots	0.030	91.8	0.001 <0.001	1.6 <0.1	<0.001	<0.1	0.030	93.4	0.002	6.6	0.032	100
Mature Radish Tops	0.105	81.4	0.010	8.1	0.001	0.7	0.116	90.3	0.012	9.7	0.129	100
Mature Radish Roots	0.042	89.8	0.002	4.5	<0.001	0.3	0.045	94.6	0.003	5.4	0.047	100
Sorghum Forage	0.062	65.7	0.016	17.0	-	-	0.078	82.6	0.016	17.4	0.094	100
Sorghum Stover	0.089	13.0	0.395	57.8	0.039	5.7	0.523	76.6	0.160	23.4	0.684	100
Sorghum Grain	<0.001	<0.1	0.006	47.9	<0.001	2.0	0.006	49.9	0.006	50.1	0.012	100
Rotational interval: 120 DAT												
Immature lettuce	0.048	92.4	0.002	3.2	<0.001	<0.1	0.049	95.6	0.002	4.4	0.052	100
Mature lettuce	0.058	91.0	0.001	2.2	<0.001	<0.1	0.060	93.2	0.004	6.8	0.064	100
Immature Radish tops	0.086	84.3	0.009	8.6	0.001	0.5	0.095	93.5	0.007	6.5	0.101	100
Immature Radish roots	0.023	87.1	0.001	2.7	<0.001	<0.1	0.024	89.8	0.003	10.2	0.027	100
Mature Radish Tops	0.084	81.6	0.011	10.2	0.001	0.6	0.095	92.4	0.008	7.6	0.103	100
Mature Radish Roots	0.027	89.0	0.001	4.4	<0.001	<0.1	0.028	93.4	0.002	6.6	0.030	100
Sorghum Forage	0.073	62.2	0.024	20.5	0.002	1.8	0.100	84.5	0.018	15.5	0.118	100
Sorghum Stover	0.127	10.1	0.843	67.3	0.057	4.6	1.027	82.0	0.226	18.0	1.252	100
Sorghum Grain	<0.001	1.4	0.005	43.2	0.001	7.1	0.005	51.7	0.005	48.3	0.010	100
Rotational interval: 365 DAT												
Immature lettuce	0.019	90.4	0.001	3.2	<0.001	<0.6	0.019	93.7	0.001	6.3	0.021	100
Mature lettuce	0.009	90.3	0.0004	3.5	<0.001	<1.1	0.010	93.8	0.001	6.2	0.010	100
Immature Radish tops	0.072	85.1	0.008	9.4	0.0004	0.5	0.080	95.1	0.004	4.9	0.084	100
Immature Radish roots	0.020	92.5	0.001	3.2	<0.001	<0.5	0.021	95.7	0.001	4.3	0.022	100

Matrix	Acetonitrile extract		Aqueous extract (water)		Acet. Rinse		Total extracted		PES Residue		TRR from Extraction [a]	
	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR
Mature Radish Tops	0.072	84.6	0.009	10.1	<0.001	0.3	0.081	94.9	0.004	5.1	0.085	100
Mature Radish Roots	0.023	94.7	<0.001	2.0	<0.001	<0.5	0.023	96.7	0.01	3.3	0.024	100
Sorghum Forage	0.020	55.9	0.008	22.2	0.001	2.3	0.029	80.3	0.007	19.7	0.036	100
Sorghum Stover	0.028	27.9	0.063	46.1	0.005	3.5	0.105	77.6	0.03	22.4	0.136	100
Sorghum Grain	<0.001	<2.0	0.005	28.1	0.002	14.3	0.007	52.4	0.006	47.6	0.014	100

(a) Sum of TRRs from extracts (ERR, extractable radioactive residues) + PES. The TRR by extraction in the phenyl label from all plantings and crop samples was within 86 – 117% of the combustion TRR (Accountability (%) expressed as {[(sum of ERR + PES)/(initial combustion TRR)] x 100}).

Table 7.6.1-3 Distribution of Radioactive residues in Extracts and PES of samples from Plots Treated with [Pyrazolyl-4-^{14}C] inpyrfluxam

Matrix	Acetonitrile extract		Aqueous extract		Acet. Rinse		Total extracted		PES Residue		TRR from Extraction [a]	
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
Rotational interval: 30 DAT												
Immature lettuce	0.069	91.9	0.002	3.0	<0.001	<0.1	0.072	94.9	0.004	5.1	0.076	100
Mature lettuce	0.064	85.6	0.003	3.5	<0.001	<0.1	0.066	89.1	0.008	10.9	0.075	100
Immature Radish tops	0.117	87.7	0.009	6.5	0.001	0.4	0.126	94.6	0.007	5.4	0.133	100
Immature Radish roots	0.039	93.2	0.001	2.5	<0.001	<0.1	0.040	95.7	0.002	4.3	0.042	100
Mature Radish Tops	0.188	83.1	0.021	9.1	0.002	0.9	0.210	93.1	0.016	6.9	0.226	100
Mature Radish Roots	0.060	91.2	0.003	4.1	0.0005	0.7	0.063	96.0	0.003	4.0	0.066	100
Sorghum Forage	0.124	62.0	0.048	23.9	-	-	0.171	85.9	0.028	14.1	0.200	100
Sorghum Stover	0.131	17.4	0.410	54.6	0.036	4.8	0.577	76.7	0.175	23.3	0.752	100
Sorghum Grain	<0.001	<0.1	0.029	59.0	0.0004	0.9	0.029	59.9	0.020	40.1	0.049	100
Rotational interval: 120 DAT												
Immature lettuce	0.090	89.9	0.004	3.5	<0.001	<0.1	0.093	93.4	0.007	6.6	0.100	100
Mature lettuce	0.070	80.2	0.006	6.8	<0.001	<0.1	0.076	87.0	0.011	13.0	0.087	100
Immature Radish tops	0.184	86.2	0.017	8.1	0.001	0.5	0.202	94.8	0.011	5.2	0.213	100
Immature Radish roots	0.054	92.7	0.002	2.9	<0.001	0.3	0.056	95.8	0.002	4.2	0.059	100
Mature Radish Tops	0.291	78.5	0.037	10.1	0.003	0.9	0.331	89.5	0.039	10.5	0.037	100
Mature Radish Roots	0.099	91.0	0.005	4.2	0.001	0.8	0.104	96.1	0.004	3.9	0.108	100
Sorghum Forage	0.102	54.6	0.040	21.7	0.003	1.7	0.145	77.9	0.041	22.1	0.187	100
Sorghum Stover	0.116	10.5	0.715	64.6	0.040	3.6	0.871	78.7	0.236	21.3	1.107	100
Sorghum Grain	<0.001	0.6	0.027	43.6	0.003	5.6	0.031	49.8	0.031	50.2	0.062	100
Rotational interval: 365 DAT												
Immature lettuce	0.032	85.8	0.003	7.6	<0.001	<0.1	0.034	93.4	0.002	6.6	0.037	100
Mature lettuce	0.018	79.3	0.002	8.2	<0.001	<0.1	0.02	87.5	0.003	12.5	0.023	100
Immature Radish tops	0.079	83.6	0.009	9.8	<0.001	0.3	0.089	93.6	0.006	6.4	0.095	100
Immature Radish roots	0.021	93.9	0.001	2.9	<0.001	<0.6	0.002	96.8	0.001	3.2	0.022	100
Mature Radish Tops	0.062	86.4	0.006	7.8	<0.001	<0.2	0.067	94.2	0.004	5.8	0.072	100

Mature Radish Roots	0.019	90.5	0.001	3.8	<0.001	<0.6	0.019	94.3	0.001	5.7	0.021	100
Sorghum Forage	0.025	54.3	0.010	21.5	0.001	1.6	0.036	77.4	0.011	22.6	0.047	100
Sorghum Stover	0.057	27.0	0.101	48.0	0.008	3.7	0.165	78.7	0.045	21.3	0.210	100
Sorghum Grain	<0.001	<1.0	0.007	38.8	0.001	8.7	0.008	47.4	0.009	52.6	0.017	100

(a) Sum of TRRs from extracts (ERR, extractable radioactive residues) + PES. The TRR by extraction in the pyrazolyl label from all plantings and crop samples was within 89 – 118% of the combustion TRR (Accountability (%) expressed as {[(sum of ERR + PES)/(initial combustion TRR)] x 100}).

Characterisation, Identification and Quantification of Radioactive Residues in rotational crop matrices

A summary of the distribution of TRR in lettuce, radish tops, radish roots, sorghum forage, sorghum stover and sorghum grain is given in tables 7.6.1-2 and 7.6.1-3.

The polar material (which co-eluted at an approximate retention time of 16 min in HPLC analysis) was characterised as comprising multiple residue components, which were postulated as having formed as a result of the conjugation of various identified metabolites of inpyrfluxam with endogenous plant molecules.

Metabolites were identified by comparison to reference standards (for the 'free' form of the metabolites). Proposed conjugates were characterised according to their behaviour (release of metabolites following acid hydrolysis).

Lettuce (Immature and Mature)

All extracts contained sufficient TRR levels for further analysis, except for the proposed conjugated fractions of the acetonitrile extracts and in the aqueous extracts in all 365 PBI lettuce samples where levels were <0.01 mg eq./kg and therefore not analysed further.

For all three rotations, metabolic profiles of lettuce were found to be similar. The parent compound inpyrfluxam accounted for ca 6 – 46% of TRR (0.009 – 0.027 mg eq./kg) in the extracts of immature and mature lettuce samples harvested from both phenyl and pyrazolyl label treated plots for all PBIs.

The following metabolites were detected above 10% of the TRR in 30 day PBI samples: 3'-OH-S-2840 (4 – 12% TRR, 0.003 – 0.010 mg eq./kg), isomers of 1'-CH_2OH-S-2840 (15 – 25% TRR, 0.008 – 0.025 mg eq./kg, total of A&B),

The following metabolites were detected above 10% of the TRR in 120 day PBI samples: 3'-OH-S-2840 (5 – 18% TRR, 0.005 – 0.011 mg eq./kg), isomers of 1'-CH_2OH-S-2840 (8 – 24% TRR, 0.008 – 0.015 mg eq./kg, total of A&B), and isomers of 1'-COOH-S-2840 (4 – 15% TRR, 0.004 – 0.009 mg eq./kg, total of A&B).

The following metabolites were detected above 10% of the TRR in 365 day PBI samples: 3'-OH-S-2840 (2 – 11% TRR, 0.001 – 0.002 mg eq./kg), DFPA (10 – 16% TRR, 0.004 mg eq./kg), *N*-des-Me-DFPA (19 – 28% TRR, 0.006 – 0.007 mg eq./kg), and DFPA-$CONH_2$ (3 – 5% TRR, 0.001 – 0.002 mg eq./kg). It is noted DFPA, *N*-des-Me-DFPA, and DFPA-$CONH_2$ were only found in extracts of the pyrazolyl label.

Residues in the PES fraction of the mature lettuce 120 DAT pyrazolyl label sample (0.011 mg eq./kg, 13% of TRR) had been further investigated using sequential

hydrolysis with acid and base (according to the flowchart scheme presented in the methods section). More than half of this residue (8% TRR, 0.007 mg eq./kg) was released into the acid hydrolysate, which the applicant postulated contained hydrolysed starch and other carbohydrates, acid-labile polyphenolic compounds, as well as hydrolysed 'unextracted' metabolites. The acid hydrolysate was partitioned with ethyl acetate to transfer the hydrolysed 'unextracted' metabolites into the organic phase. Almost the entire residue (8% TRR, 0.007 mg eq./kg) transferred into the ethyl acetate fraction after partition. The 2D TLC of the ethyl acetate fraction showed DFPA (5% TRR, 0.005 mg eq./kg) and *N*-des-Me-DFPA (1% TRR, 0.001 mg eq./kg) as the major components, with traces of some other metabolites, which were also observed in the extracts of the lettuce samples. The base hydrolysed acid-soluble material (proposed by the applicant as protein and acid insoluble lignin fractions) accounted for 0.003 mg eq./kg and 0.001 mg eq./kg, respectively. The unhydrolysed solids remaining after acid and base hydrolysis were postulated by the applicant as predominantly containing cellulose. This fraction retained about 0.001 mg eq./kg of residue (0.7% TRR).

Radish Tops (Immature and Mature)

The following metabolites were detected above 10% of the TRR in 30 day PBI samples: parent inpyrfluxam 6 – 15% of TRR (0.014 – 0.019 mg eq./kg), 3'- OH-S-2840 (2 – 13% TRR, 0.003 – 0.017 mg eq./kg), *N*-des-Me-S-2840 (11 – 15% TRR, 0.015 – 0.029 mg eq./kg) and isomers of *N*-des-Me-1'-CH_2OH-S-2840 (9 – 15% TRR, 0.012 – 0.029 mg eq./kg, total of A&B). Isomers of 1'-CH_2OH-S-2840 (4 – 8% TRR, 0.005 – 0.018 mg eq./kg, total of A&B) and isomers of 1'-COOH-S-2840 (3 – 7% TRR, 0.004 – 0.014 mg eq./kg, total of A&B) were also detected.

The following metabolites were detected above 10% of the TRR in 120 day PBI samples: parent inpyrfluxam (7 – 10% of TRR, 0.009 – 0.025 mg eq./kg), 3'- OH-S-2840 (3 – 10% TRR, 0.006 – 0.015 mg eq./kg), *N*-des-Me-S-2840 (10 – 13% TRR, 0.011 – 0.038 mg eq./kg), isomers of 1'-CH_2OH-S-2840 (4 – 10% TRR, 0.004 – 0.037 mg eq./kg, total of A&B), isomers of 1'-COOH-S-2840 (7 – 16% TRR, 0.008 – 0.026 mg eq./kg, total of A&B), and isomers of *N*-des-Me-1'-CH_2OH-S-2840 (10 – 15% TRR, 0.011 – 0.056 mg eq./kg, total of A&B). Similar to the 30 DAT samples, the 120 DAT radish tops samples contained higher amounts the metabolite *N*-des-Me-1'-CH_2OH-S-2840-B (8 – 12% TRR, 0.009 – 0.044 mg eq./kg), compared to its isomer *N*-des-Me-1'- CH_2OH-S-2840-A (2 – 3% TRR, 0.002 – 0.011 mg eq./kg).

The following metabolites were detected above 10% of the TRR in 365 day PBI samples: parent compound inpyrfluxam accounted for ca 6 – 11% of TRR (0.005 – 0.009 mg eq./kg), 3'-OH-S-2840 (0.3 – 10% TRR, <0.001 – 0.009 mg eq./kg), *N*-des-Me-S-2840 (7 – 14% TRR, 0.007 – 0.012 mg eq./kg) and isomers of 1'-COOH-S-2840 (11 – 26% TRR, 0.008 – 0.022 mg eq./kg, total of A&B).

Generally, *N*-des-Me-S-2840 was present in free form whereas the major portions of other metabolites seemed to be present in conjugated form. Major portions of metabolites were released after acid hydrolysis of the apparent conjugated residue fraction.

Unextracted residues in the PES fractions of the mature radish tops from 30 DAT (phenyl label) and 120 DAT (pyrazolyl label) were investigated using sequential hydrolysis with acid and base. The 30 DAT phenyl label mature radish tops contained 0.012 mg eq./kg (10% of TRR) in the PES. Less than half of this residue (4% TRR, 0.005 mg eq./kg) was released into the acid hydrolysate, which the applicant postulated contained hydrolysed starch and other carbohydrates, acid-labile polyphenolic compounds, as well as hydrolysed ‘unextracted’ metabolites. A major part the residue (3% TRR, 0.004 mg eq./kg) of the acid hydrolysate transferred into the ethyl acetate fraction after solvent partition. The 2D TLC of the ethyl acetate fraction showed the isomers of 1'-COOH-S-2840 (1% TRR, 0.001 mg eq./kg) as the major components, with traces of some other metabolites, which were also observed in the extracts of the radish top samples. The base hydrolysed acid-soluble proposed protein and acid insoluble proposed lignin fractions accounted for 0.003 mg eq./kg and 0.002 mg eq./kg, respectively. The unhydrolysed solids remaining after acid and base hydrolysis were postulated to be predominantly containing cellulose. This fraction retained about 0.002 mg eq./kg of residue (1.5% TRR).

The 120 DAT pyrazolyl label mature radish tops contained 0.039 mg eq./kg (11% of TRR) as residue in the PES. A little less than half of this residue (5% TRR, 0.017 mg eq./kg) was released into the acid hydrolysate, which the applicant postulated contained hydrolysed starch and other carbohydrates, acid-labile polyphenolic compounds, as well as hydrolysed ‘unextracted’ metabolites. A major part the residue (4% TRR, 0.014 mg eq./kg) of the acid hydrolysate transferred into the ethyl acetate fraction after solvent partition. The 2D TLC of the ethyl acetate fraction showed DFPA (1% TRR, 0.004 mg eq./kg) as the major component, with minor amounts of *N*-des-Me-S-2840, the isomers of 1'-COOH-S-2840 and some other known and unknown metabolites, which were also observed in the extracts of the radish top samples. The base hydrolysed acid-soluble proposed protein and acid insoluble proposed lignin fractions accounted for 0.011 mg eq./kg and 0.005 mg eq./kg, respectively. The unhydrolysed solids remaining after acid and base hydrolysis were postulated to be predominantly containing cellulose. This fraction retained about 0.005 mg eq./kg of residue (1.3% TRR).

Radish Roots (Immature and Mature)

All extracts contained sufficient TRR levels for further analysis, except for the proposed conjugated fractions of the acetonitrile extracts and in the aqueous extracts of the 30 and 365 PBI samples for both labels and for phenyl label samples of 120 day PBI. PES fractions of phenyl and pyrazolyl label immature and mature

radish roots from all planting periods contained low residue levels, and were not analysed further.

The following metabolites were detected above 10% of the TRR in 30 day PBI samples: inpyrfluxam (52 – 59% of TRR, 0.019 – 0.038 mg eq./kg), 3'-OH-S-2840 (5 – 10% TRR, 0.002 – 0.004 mg eq./kg), isomers of 1'-COOH-S-2840 (3 – 10% TRR, 0.002 – 0.005 mg eq./kg, total of A&B), DFPA (4 – 11% TRR, 0.003 – 0.005 mg eq./kg).

The following metabolites were detected above 10% of the TRR in 120 day PBI samples: inpyrfluxam (35 – 47% of TRR, 0.010 – 0.045 mg eq./kg), 3'-OH-S-2840 (4 – 12% TRR, 0.003 – 0.005 mg eq./kg), DFPA (11 – 13% TRR, 0.007 – 0.014 mg eq./kg) and isomers of 1'-COOH-S-2840 (6 – 16% TRR, 0.003 – 0.015 mg eq./kg, total of A&B).

The following metabolites were detected above 10% of the TRR in 365 day PBI samples: inpyrfluxam (28 – 49% of TRR, 0.006 – 0.012 mg eq./kg), 3'-OH-S-2840 (4 – 11% TRR, 0.001 – 0.003 mg eq./kg), and 1'-COOH-S-2840-A (5 – 23% TRR, 0.001 – 0.006 mg eq./kg), DFPA (14 – 24% TRR, 0.003 – 0.005 mg eq./kg).

The metabolite DFPA was only detected in the pyrazolyl label of immature and mature radish roots.

Sorghum Forage

The following metabolites were detected above 10% of the TRR in 30 day PBI samples: isomers of 1'-CH_2OH-S-2840 (8 – 14% TRR, 0.013 – 0.016 mg eq./kg, total of A&B) and DFPA (11% TRR, 0.022 mg eq./kg). In both phenyl and pyrazolyl sample extracts, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B, 1'- COOH-S-2840-A, 1'-COOH-S-2840-B, *N*-des-Me-1'-CH_2OH-S-2840-A, and *N*-des-Me-1'-CH_2OH-S-2840-B seemed to be present only in conjugated form. These metabolites were released after acid hydrolysis of the proposed conjugate fraction (16 min peak). Among these metabolites, 1'-CH_2OH-S-2840-B was released in highest amounts from the proposed conjugate residue fractions (6 – 10% TRR, 0.010 – 0.012 mg eq./kg).

The following metabolites were detected above 10% of the TRR in 120 day PBI samples: isomers of 1'-CH_2OH-S-2840 (4 – 13% TRR, 0.006 – 0.015 mg eq./kg, total of A&B), isomers of *N*-des-Me-1'-CH_2OH-S-2840 (4 – 12% TRR, 0.007 – 0.014 mg eq./kg, total of A&B) and DFPA (13% TRR, 0.025 mg eq./kg). In both phenyl and pyrazolyl sample extracts, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B, 1'-COOH-S-2840-A, 1'-COOH-S-2840-B, *N*-des-Me-1'- CH_2OH-S-2840-A, and *N*-des-Me-1'-CH_2OH-S-2840-B seemed only to be present in conjugated form. These metabolites were released after acid hydrolysis of the proposed conjugate fraction, 1'-CH_2OH-S-2840-B was released in highest amounts from the proposed conjugate residue fractions (4 – 10% TRR, 0.006 – 0.012 mg eq./kg).

The following metabolites were detected above 10% of the TRR in 365 day PBI samples: isomers of 1'-CH_2OH-S-2840 (8 – 10% TRR, 0.003 – 0.004 mg eq./kg, total of A&B), isomers of *N*-des-Me-1'-CH_2OH-S-2840 (6 – 10% TRR, 0.003 – 0.004 mg eq./kg, total of A&B) and DFPA (11% TRR, 0.005 mg eq./kg). In both phenyl and pyrazolyl sample extracts, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B, 1'-COOH-S-2840-A, 1'-COOH-S2840-B, *N*-des-Me-1'-CH_2OH-S-2840-A, and *N*-des-Me-1'-CH_2OH-S-2840-B seemed only to be in conjugated form. These metabolites were released after acid hydrolysis of the proposed conjugate residue fractions.

At 30 and 120 day PBIs levels of parent were low (max 4% of TRR, 0.005 mg eq./kg) and it was not detected at 365 day PBI.

The 120 DAT phenyl and pyrazolyl label sorghum forage contained 0.018 mg eq./kg and 0.041 mg eq./kg (16% and 22% of TRR), respectively, as unextracted residue in PES which was investigated further. Highest portions of the unextracted residue in the PES of phenyl and pyrazolyl label sorghum forage (0.010 and 0.024 mg eq./kg, respectively) were associated with their acid hydrolysates. The major portions the residue (0.009 mg eq./kg and 0.017 mg eq./kg, respectively) of the phenyl and pyrazolyl label acid hydrolysates transferred into the ethyl acetate fractions after solvent partition. The 2D TLC of the ethyl acetate fraction of the phenyl label PES showed minor amounts (≤0.002 mg eq./kg) of the isomers of 1'-CH_2OH-S-2840, and 1'-COOH-S-2840, NDM-1'-CH_2OH-S-2840 and some other metabolites. The 2D TLC of the ethyl acetate fraction of the pyrazolyl label PES showed DFPA (3% TRR, 0.005 mg eq./kg) as the major component, with minor amounts of *N*-des-Me-DFPA, the isomers of 1'-COOH-S-2840 and 1'-CH_2OH-S-2840, and some other known and unknown metabolites, which were also observed in the extracts of the forage samples. The base hydrolysed acid-soluble proposed protein and acid insoluble proposed lignin fractions of phenyl label each accounted for 0.003 mg eq./kg, and those fractions of the pyrazolyl label accounted for 0.010 mg eq./kg and 0.004 mg eq./kg, respectively. The unhydrolysed proposed cellulose-containing fractions remaining after acid and base hydrolysis of the phenyl and pyrazolyl label PES retained about 0.002 mg eq./kg and 0.003 mg eq./kg of residue, respectively.

Sorghum Stover

The following metabolites were detected above 10% of the TRR in 30 day PBI samples: isomers of 1'-CH_2OH-S-2840 (11 – 14% TRR, 0.083 – 0.096 mg eq./kg, total of A&B and DFPA (12% TRR, 0.088 mg eq./kg). In both phenyl and pyrazolyl sample extracts, either the entire amounts or the major portions of 1'-CH_2OH-S2840-A, 1'-CH_2OH-S-2840-B, 1'-COOH-S-2840-A, 1'-COOH-S-2840-B, *N*-des-Me-1'-CH_2OH-S-2840-A, and *N*-des-Me-1'-CH_2OH-S-2840-B were present in proposed conjugated form, and were released after acid hydrolysis of the proposed conjugate fraction. Among these metabolites, 1'-CH_2OH-S-2840-B was released in highest amounts from the proposed conjugate residue fractions (8 – 11% TRR, 0.066 – 0.076 mg eq./kg).

The following metabolites were detected above 10% of the TRR in 120 day PBI samples: 3'-OH-S-2840 (5 – 10% TRR, 0.055 – 0.131 mg eq./kg), isomers of 1'-CH_2OH-S-2840 (7 – 15% TRR, 0.082 – 0.193 mg eq./kg, total of A&B) and DFPA (12% TRR, 0.129 mg eq./kg). In both phenyl and pyrazolyl label sample extracts, either the entire amounts or the major portions of 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B, 1'-COOH-S-2840-A, 1'-COOH-S2840-B, *N*-des-Me-1'-CH_2OH-S-2840-A, and *N*-des-Me-1'-CH_2OH-S-2840-B were present in proposed conjugated form. These metabolites were released after acid hydrolysis of the proposed conjugate fraction. Among these metabolites, 1'-CH_2OH-S-2840-B was released in highest amounts from the proposed conjugate residue fractions (6 – 11% TRR, 0.064 – 0.137 mg eq./kg).

The following metabolites were detected above 10% of the TRR in 365 day PBI samples: 3'-OH-S-2840 (8 – 12% TRR, 0.016 – 0.017 mg eq./kg). In both phenyl and pyrazolyl label sample extracts, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B, 1'-COOH-S-2840-A, 1'- COOH-S-2840-B, *N*-des-Me-1'-CH_2OH-S-2840-A, and *N*-des-Me-1'-CH_2OH-S-2840-B were present only in proposed conjugated forms (max 4.5%TRR, 0.006 mg eq./kg).

Generally, only extracts of the pyrazolyl label sorghum stover contained the metabolites, DFPA, *N*-des-Me-DFPA and DFPA-$CONH_2$. The majority of DFPA and *N*-des-Me-DFPA were present in proposed conjugated forms, but the entire amount of DFPA-$CONH_2$ was present in free form.

Unextracted residue in PES of the 120 DAT phenyl and pyrazolyl label sorghum stover (containing 0.226 mg eq./kg and 0.236 mg eq./kg unextracted residue (18% and 21% of TRR) respectively) were investigated further using acid hydrolysis and 2D TLC:

- Nearly one third of the unextracted residue in the PES of phenyl and pyrazolyl label sorghum stover (0.062 and 0.082 mg eq./kg, respectively) was associated with acid hydrolysates. The major portions the residue (0.047 mg eq./kg and 0.069 mg eq./kg, respectively) of the phenyl and pyrazolyl label acid hydrolysates transferred into the ethyl acetate fractions after solvent partition.

- The 2D TLC of the ethyl acetate fraction of the phenyl label PES showed 1'-CH_2OH-S-2840-B (1% TRR, 0.013 mg eq./kg) as the major component, with minor amounts (≤0.006 mg eq./kg) of 1'-CH_2OH-S-2840-A, isomers of 1'-COOH-S-2840 and some other metabolites.

- The 2D TLC of the ethyl acetate fraction of the pyrazolyl label PES showed DFPA (3% TRR, 0.030 mg eq./kg) as the major component, with minor amounts of *N*-des-Me-DFPA (0.9% TRR), the isomers of 1'-COOH-S-2840 and 1'-CH_2OH-S-2840 (max 0.4% TRR when A&B isomers

combined), and some other known and unknown metabolites (max of 0.5% TRR), which were also observed in the extracts of the forage samples.

- The base hydrolysed acid-soluble proposed protein fraction and acid insoluble proposed lignin fraction of phenyl label accounted for 0.037 mg eq./kg and 0.102 mg eq./kg, respectively, and those fractions of the pyrazolyl label accounted for 0.069 mg eq./kg and 0.061 mg eq./kg, respectively. The unhydrolysed proposed cellulose-containing fractions remaining after acid and base hydrolysis of the phenyl and pyrazolyl label PES retained about 0.026 mg eq./kg and 0.025 mg eq./kg of residue, respectively.

Sorghum Grain

Extracts contained sufficient TRR levels for further analysis, except for the acetonitrile extracts for both labels at all PBIs, in the aqueous extracts at all PBIs for the phenyl label and the aqueous extract of the 365 day PBI for the pyrazolyl label.

The following metabolites were detected above 10% of the TRR in 30 day PBI samples (pyrazolyl label, aqueous extract): DFPA accounted for 23% of TRR (0.011 mg eq./kg). *N*-des-Me-DFPA was present at 9% of TRR (0.004 mg eq./kg).

The following metabolites were detected above 10% of the TRR in 120 day PBI samples (pyrazolyl label, aqueous extract): Chromatographic analysis of the aqueous extract by HPLC and 2D TLC analysis showed that the extracted residue appeared only to be present in conjugated form. Based on the hydrolysis results obtained with the 30 DAT sample, it was assumed that DFPA and *N*-des-Me-DFPA are the likely components of the proposed conjugated material in the pyrazolyl label 120 DAT grain sample.

Total residue in the acetonitrile extract of 365 DAT sorghum grain from both labels were below 0.01 mg eq./kg and not investigated further. The following metabolites were detected above 10% of the TRR in 365 day PBI samples:

The 365 DAT phenyl and 120 DAT pyrazolyl label sorghum grain contained 0.006 mg eq./kg and 0.031 mg eq./kg (48% and 50% of TRR), respectively, as unextracted residue in PES which was investigated further using sequential hydrolysis with acid and base. About 40% of the phenyl and two third of the pyrazolyl label unextracted residue in the PES of sorghum grain (0.002 and 0.021 mg eq./kg, respectively) were associated with the acid hydrolysates. The major portions the residue (0.002 mg eq./kg and 0.013 mg eq./kg, respectively) of the phenyl and pyrazolyl label acid hydrolysates transferred into the ethyl acetate fractions after solvent partition. The ethyl acetate fraction of the acid hydrolysate of the phenyl label grain was not analysed further due to very low radioactivity. The 2D TLC of the ethyl acetate

fraction of the pyrazolyl label PES showed DFPA (14% TRR, 0.008 mg eq./kg) as the major component, with a minor amount of *N*-des-Me-DFPA (4% TRR, 0.002 mg eq./kg) and traces of a few other metabolites. The base hydrolysed acid-soluble proposed protein fraction and acid insoluble proposed lignin fraction of phenyl label accounted for <0.001 mg eq./kg and 0.002 mg eq./kg, respectively, and those fractions of the pyrazolyl label accounted for 0.007 mg eq./kg and 0.001 mg eq./kg, respectively. The unhydrolysed proposed cellulose-containing fractions remaining after acid and base hydrolysis of the phenyl and pyrazolyl label PES retained about 0.001 mg eq./kg and 0.002 mg eq./kg of residue, respectively.

Table 7.6.1-4 Summary of characterisation and identification of radioactive residues in the extracts of lettuce following bare soil treatment with 235 g as/ha inpyrfluxam

Crop/sample: Lettuce		Immature				Mature			
DAT	Metabolite/component	Phenyl-^{14}C		Pyrazolyl-^{14}C		Phenyl-^{14}C		Pyrazolyl-^{14}C	
		%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
30									
Overall TRR (mg/kg)		0.042		0.076		0.100		0.075	
	Inpyrfluxam	46.2	0.019	27.4	0.021	26.9	0.027	12.2	0.009
	3'-OH-S-2840 (free)	8.5	0.004	2.8	0.002	7.5	0.008	2.8	0.002
	3'-OH-S-2840 (conj)	3.1	0.001	0.9	0.001	2.9	0.003	0.9	0.001
	N-des-Me-S-2840 (free)	1.0	<0.001	0.7	0.001	-	-	-	-
	N-des-Me-S-2840 (conj)	2.6	0.001	2.0	0.002	2.8	0.003	2.2	0.002
	1'-CH_2OH-S-2840 (free)	2.6	0.002	1.4	0.002	3.6	0.003	3.3	0.003
	1'-CH_2OH-S-2840 (conj)	15.7	0.007	14.0	0.010	21.2	0.021	13.5	0.010
	1'-COOH-S-2840 (free)	1.2	<0.001	0.7	<0.001	1.5	0.002	1.9	0.002
	1'-COOH-S-2840 (conj)	4.2	0.002	2.8	0.003	7.9	0.008	7.3	0.005
	N-des-Me-1'-CH_2OH-S-2840 (conj)	-	-	-	-	0.9	0.001	-	-
	DFPA (free)	-	-	19.1	0.014	-	-	13.0	0.010
	DFPA (conj)	-	-	5.9	0.004	-	-	12.5	0.009
	N-des-Me-DFPA (free)	-	-	7.6	0.006	-	-	8.4	0.006
	N-des-Me-DFPA (conj)	-	-	-	-	-	-	1.2	0.001
	DFPA-$CONH_2$ (free)	-	-	3.3	0.002	-	-	3.2	0.002
	Others	9.8	0.004	6.2	0.005	18.1	0.018	6.9	0.005
	Total extracted	95.0	0.040	94.9	0.072	93.4	0.093	89.1	0.066
	Total identified	85.3	0.036	88.7	0.067	75.3	0.075	82.2	0.061
	Total unidentified[(a)]	9.8	0.004	6.2	0.005	18.1	0.018	6.9	0.005
	Total unextracted residues (PES)	5.0	0.002	5.1	0.004	6.6	0.007	10.9	0.008

(a) Contains >4 – 9 components, largest was 4.8% of TRR, 0.005 mg/kg in phenyl label mature lettuce.

Crop/sample: Lettuce		Immature				Mature			
DAT	Metabolite/component	Phenyl-^{14}C		Pyrazolyl-^{14}C		Phenyl-^{14}C		Pyrazolyl-^{14}C	
		%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
120									
Overall TRR (mg/kg)		0.052		0.100		0.064		0.087	
	Inpyrfluxam	41.8	0.022	28.9	0.029	17.4	0.011	11.4	0.010
	3'-OH-S-2840 (free)	10.9	0.006	5.2	0.005	11.7	0.008	3.7	0.003
	3'-OH-S-2840 (conj)	4.2	0.002	3.7	0.004	5.9	0.004	1.6	0.001
	N-des-Me-S-2840 (free)	0.6	<0.001	-	-	0.8	<0.001	0.6	0.001
	N-des-Me-S-2840 (conj)	-	-	-	-	-	-	-	-
	1'-CH_2OH-S-2840 (free)	2.2	0.001	0.8	0.001	9.0	0.006	-	-
	1'-CH_2OH-S-2840 (conj)	13.3	0.007	7.6	0.008	14.8	0.010	11.3	0.010
	1'-COOH-S-2840 (free)	2.9	0.002	1.2	0.001	6.0	0.004	1.9	0.002
	1'-COOH-S-2840 (conj)	3.9	0.002	3.0	0.003	8.6	0.005	8.1	0.007
	N-des-Me-1'-CH_2OH-S-2840 (conj)	-	-	-	-	-	-	-	-
	DFPA (free)	-	-	22.4	0.022	-	-	11.7	0.010
	DFPA (conj)	-	-	6.4	0.006	-	-	16.6	0.014
	N-des-Me-DFPA (free)	-	-	6.8	0.007	-	-	8.1	0.007
	N-des-Me-DFPA (conj)	-	-	-	-	-	-	-	-
	DFPA-$CONH_2$ (free)	-	-	2.7	0.003	-	-	2.5	0.002
	Others	15.7	0.008	4.8	0.005	19.1	0.012	9.6	0.008
	Total extracted	95.6	0.049	93.4	0.093	93.2	0.060	87.0	0.076
	Total identified	79.9	0.041	88.7	0.089	74.1	0.047	77.4	0.067
	Total unidentified[(b)]	15.7	0.008	4.8	0.005	19.1	0.012	9.6	0.008
	Total unextracted residues (PES)	4.4	0.002	6.6	0.007	6.8	0004	13.0	0.011

(b) Contains >5 – 13 components, largest was 1.9% of TRR, 0.001 mg/kg in phenyl label mature lettuce.

Crop/sample: Lettuce		Immature				Mature			
DAT	Metabolite/component	Phenyl-^{14}C		Pyrazolyl-^{14}C		Phenyl-^{14}C		Pyrazolyl-^{14}C	
		%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
365									
Overall TRR (mg/kg)		0.021		0.037		0.010		0.023	
	Inpyrfluxam	26.4	0.005	8.3	0.003	27.2	0.003	5.5	0.001
	3'-OH-S-2840 (free)	10.5	0.002	2.3	0.001	7.7	0.001	-	-
	3'-OH-S-2840 (conj)	-	-	-	-	-	-	-	-
	N-des-Me-S-2840 (free)	-	-	5.9	0.002	1.3	<0.001	-	-
	N-des-Me-S-2840 (conj)	-	-	-	-	-	-	-	-
	1'-CH_2OH-S-2840 (free)	5.6	0.001	-	-	2.4	<0.001	-	-
	1'-CH_2OH-S-2840 (conj)	-	-	-	-	-	-	-	-
	1'-COOH-S-2840 (free)	4.3	0.001	-	-	3.1	<0.001	-	-
	1'-COOH-S-2840 (conj)	-	-	-	-	-	-	-	-
	N-des-Me-1'-CH_2OH-S-2840 (conj)	-	-	-	-	-	-	-	-
	DFPA (free)	-	-	9.9	0.004	-	-	15.6	0.004
	DFPA (conj)	-	-	-	-	-	-	-	-
	N-des-Me-DFPA (free)	-	-	18.5	0.007	-	-	28.0	0.006
	N-des-Me-DFPA (conj)	-	-	-	-	-	-	-	-
	DFPA-$CONH_2$ (free)	-	-	4.5	0.002	-	-	3.1	0.001
	Others	46.8	0.010	44.0	0.016	51.0	0.005	35.2	0.008
	Total extracted	93.7	0.019	93.4	0.034	93.8	0.010	87.5	0.020
	Total identified	46.8	0.010	49.4	0.018	42.8	0.004	52.2	0.012
	Total unidentified[(c)]	46.8	0.010	44.0	0.016	51.0	0.005	35.2	0.008
	Total unextracted residues (PES)	6.3	0.001	6.6	0.002	6.2	0.001	12.5	0.003

(c) Contains >3 – 6 components, the proposed conjugated fractions were not analysed, unknown components were observed only in phenyl label mature lettuce, largest was 2.8% of TRR, <0.001 mg/kg.

Table 7.6.1-5 Summary of characterisation and identification of radioactive residues in the extracts of radish tops (immature & mature) following bare soil treatment with 235 g as/ha inpyrfluxam

Crop/sample: Radish tops		Immature				Mature			
DAT	Metabolite/component	Phenyl-^{14}C		Pyrazolyl-^{14}C		Phenyl-^{14}C		Pyrazolyl-^{14}C	
		%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
30									
Overall TRR (mg/kg)		0.115		0.133		0.129		0.226	
	Inpyrfluxam	15.0	0.017	14.0	0.019	12.3	0.016	6.1	0.014
	3'-OH-S-2840 (free)	3.4	0.004	1.8	0.002	3.1	0.004	0.8	0.002
	3'-OH-S-2840 (conj)	0.7	0.001	0.3	<0.001	10.2	0.013	1.0	0.002
	N-des-Me-S-2840 (free)	14.3	0.016	11.3	0.015	10.7	0.014	10.3	0.023
	N-des-Me-S-2840 (conj)	0.8	0.001	1.4	0.002	0.7	0.001	2.5	0.006
	1'-CH_2OH-S-2840 (free)	-	-	0.9	0.001	-	-	-	-
	1'-CH_2OH-S-2840 (conj)	6.2	0.007	2.9	0.004	7.9	0.010	7.9	0.018
	1'-COOH-S-2840 (free)	1.3	0.001	1.8	0.002	1.8	0.002	0.7	0.002
	1'-COOH-S-2840 (conj)	2.5	0.002	1.0	0.002	5.5	0.007	5.5	0.012
	N-des-Me-1'-CH_2OH-S-2840 (free)	1.7	0.002	2.5	0.003	1.4	0.002	-	-
	N-des-Me-1'-CH_2OH-S-2840 (conj)	13.0	0.015	6.2	0.008	9.8	0.012	13.0	0.029
	DFPA (free)	-	-	3.5	0.005	-	-	2.0	0.005
	DFPA (conj)	-	-	10.2	0.014	-	-	8.3	0.019
	N-des-Me-DFPA (free)	-	-	1.8	0.002	-	-	1.7	0.004
	N-des-Me-DFPA (conj)	-	-	3.0	0.004	-	-	4.7	0.011
	DFPA-$CONH_2$ (free)	-	-	18.5	0.025	-	-	10.3	0.023
	DFPA-$CONH_2$ (conj)	-	-	0.5	0.001	-	-	-	-
	Others	31.4	0.036	13.1	0.017	27.0	0.035	18.3	0.041
	Total extracted	90.3	0.104	94.6	0.126	90.3	0.116	93.1	0.210
	Total identified	58.9	0.068	81.5	0.108	63.3	0.081	74.8	0.169
	Total unidentified[(a)]	31.4	0.036	13.1	0.017	27.0	0.035	18.3	0.041
	Total unextracted residues (PES)	9.7	0.011	5.4	0.007	9.7	0.012	6.9	0.016

(a) Contains >6 – 8 components, largest was 4.8% of TRR, 0.006 mg/kg in phenyl label mature tops.

Crop/sample: Radish tops		Immature				Mature			
DAT	Metabolite/component	Phenyl-^{14}C		Pyrazolyl-^{14}C		Phenyl-^{14}C		Pyrazolyl-^{14}C	
		%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
120									
Overall TRR (mg/kg)		0.101		0.213		0.103		0.370	
	Inpyrfluxam	9.9	0.010	7.9	0.017	8.3	0.009	6.9	0.025
	3'-OH-S-2840 (free)	3.6	0.004	1.2	0.003	3.6	0.004	1.4	0.005
	3'-OH-S-2840 (conj)	6.5	0.007	2.0	0.004	2.5	0.003	2.7	0.010
	N-des-Me-S-2840 (free)	12.4	0.013	12.6	0.027	10.9	0.011	10.2	0.038
	N-des-Me-S-2840 (conj)	-	-	-	-	-	-	-	-
	1'-CH_2OH-S-2840 (free)	-	-	-	-	1.0	0.001	1.7	0.006
	1'-CH_2OH-S-2840 (conj)	3.8	0.004	6.5	0.013	5.4	0.005	8.3	0.030
	1'-COOH-S-2840 (free)	1.0	0.001	0.9	0.002	2.5	0.003	1.9	0.006
	1'-COOH-S-2840 (conj)	7.0	0.008	3.2	0.007	13.4	0.014	5.3	0.020
	N-des-Me-1'-CH_2OH-S-2840 (free)	2.2	0.002	2.1	0.004	2.4	0.002	2.6	0.010
	N-des-Me-1'-CH_2OH-S-2840 (conj)	8.2	0.008	12.3	0.026	11.5	0.012	12.4	0.046
	DFPA (free)	-	-	3.0	0.006	-	-	3.3	0.012
	DFPA (conj)	-	-	3.7	0.008	-	-	6.2	0.023
	N-des-Me-DFPA (free)	-	-	1.5	0.003	-	-	-	-
	N-des-Me-DFPA (conj)	-	-	2.9	0.006	-	-	3.4	0.012
	DFPA-$CONH_2$ (free)	-	-	17.1	0.037	-	-	7.2	0.027
	DFPA-$CONH_2$ (conj)	-	-	-	-	-	-	-	-
	Others	38.9	0.039	17.8	0.038	30.9	0.032	16.0	0.059
	Total extracted	93.5	0.095	94.8	0.202	92.4	0.095	89.5	0.331
	Total identified	54.6	0.055	77.0	0.164	61.5	0.063	73.5	0.272
	Total unidentified[(b)]	38.9	0.039	17.8	0.038	30.9	0.032	16.0	0.059
	Total unextracted residues (PES)	6.5	0.007	5.2	0.011	7.6	0.008	10.5	0.039

(b) Contains >9 – 14 components, largest was 4.6% of TRR, 0.0017 mg/kg in pyrazolyl label mature tops.

Crop/sample: Radish tops		Immature				Mature			
DAT	Metabolite/component	Phenyl-^{14}C		Pyrazolyl-^{14}C		Phenyl-^{14}C		Pyrazolyl-^{14}C	
		%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
365									
Overall TRR (mg/kg)		0.084		0.095		0.085		0.072	
	Inpyrfluxam	6.4	0.005	6.5	0.006	10.5	0.009	7.6	0.005
	3'-OH-S-2840 (free)	3.5	0.003	-	-	3.6	0.003	-	
	3'-OH-S-2840 (conj)	-	-	0.3	<0.001	6.4	0.005	2.0	0.001
	N-des-Me-S-2840 (free)	10.2	0.009	7.4	0.007	13.6	0.012	12.8	0.009
	N-des-Me-S-2840 (conj)	-	-	-	-	-	-	-	-
	1'-CH_2OH-S-2840 (free)	-	-	-	-	-	-	-	-
	1'-CH_2OH-S-2840 (conj)	7.7	0.007	4.6	0.005	8.4	0.007	6.4	0.005
	1'-COOH-S-2840 (free)	4.0	0.003	-	-	-	-	2.7	0.002
	1'-COOH-S-2840 (conj)	25.6	0.021	12.0	0.011	22.1	0.019	8.1	0.006
	N-des-Me-1'-CH_2OH-S-2840 (free)	-	-	-	-	-	-	-	-
	N-des-Me-1'-CH_2OH-S-2840 (conj)	7.6	0.007	5.1	0.005	7.9	0.007	5.4	0.004
	DFPA (free)	-	-	6.7	0.006	-	-	4.7	0.003
	DFPA (conj)	-	-	8.4	0.008	-	-	8.7	0.006
	N-des-Me-DFPA (free)	-	-	6.3	0.014	-	-	6.6	0.005
	N-des-Me-DFPA (conj)	-	-	1.3	0.001	-	-	2.7	0.002
	DFPA-$CONH_2$ (free)	-	-	14.3	0.013	-	-	9.9	0.007
	DFPA-$CONH_2$ (conj)	-	-	-	-	-	-	-	-
	Others	34.0	0.029	20.9	0.020	22.4	0.019	16.4	0.012
	Total extracted	95.0	0.081	93.8	0.089	94.9	0.081	94.0	0.067
	Total identified	61.0	0.052	72.9	0.069	72.5	0.062	77.6	0.055
	Total unidentified[(c)]	34.0	0.029	20.9	0.020	22.4	0.019	16.4	0.012
	Total unextracted residues (PES)	4.9	0.004	6.4	0.006	5.1	0.004	5.8	0.004
(c) contains >4 – 5 components, largest was 4.2% of TRR, 0.004 mg/kg in phenyl label mature tops.									

Table 7.6.1-6 Summary of characterisation and identification of radioactive residues in the extracts of radish roots (immature & mature) following bare soil treatment with 235 g as/ha inpyrfluxam

Crop/sample: Radish roots		Immature				Mature			
DAT	Metabolite/component	Phenyl-^{14}C		Pyrazolyl-^{14}C		Phenyl-^{14}C		Pyrazolyl-^{14}C	
		%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
30									
Overall TRR (mg/kg)		0.032		0.042		0.047		0.066	
	Inpyrfluxam	58.9	0.019	52.1	0.022	54.8	0.026	57.3	0.038
	3'-OH-S-2840 (free)	9.8	0.003	5.1	0.002	8.7	0.004	4.8	0.003
	3'-OH-S-2840 (conj)	-	-	-	-	-	-	-	-
	N-des-Me-S-2840 (free)	1.9	0.001	1.0	<0.001	2.0	0.001	1.8	0.001
	1'-CH_2OH-S-2840 (free)	2.6	0.001	2.0	0.001	2.7	0.001	2.2	0.001
	1'-CH_2OH-S-2840 (conj)	-	-	-	-	-	-	-	-
	1'-COOH-S-2840 (free)	5.8	0.002	8.7	0.004	9.8	0.005	3.1	0.003
	1'-COOH-S-2840 (conj)	-	-	-	-	-	-	-	-
	N-des-Me-1'-CH_2OH-S-2840 (free)	1.6	0.001	-	-	-	-	-	-
	N-des-Me-1'-CH_2OH-S-2840 (conj)	-	-	-	-	-	-	-	-
	DFPA (free)	-	-	11.0	0.005	-	-	4.2	0.003
	DFPA (conj)	-	-	-	-	-	-	-	-
	N-des-Me-DFPA (free)	-	-	3.3	0.001	-	-	1.1	0.001
	N-des-Me-DFPA (conj)	-	-	-	-	-	-	-	-
	DFPA-$CONH_2$ (free)	-	-	-	-	-	-	-	-
	Others	12.9	0.004	12.4	0.005	16.8	0.008	21.7	0.014
	Total extracted	93.4	0.030	95.7	0.040	94.6	0.045	96.0	0.063
	Total identified	80.5	0.026	83.3	0.035	77.8	0.037	74.4	0.049
	Total unidentified[(a)]	12.9	0.004	12.4	0.005	16.8	0.008	21.7	0.014
	Total unextracted residues (PES)	6.6	0.002	4.3	0.002	5.4	0.003	4.0	0.003

(a) Contains >3 – 5 components, largest was 6.4% of TRR, 0.002 mg/kg in phenyl label immature roots.

Crop/sample: Radish roots		Immature				Mature			
DAT	Metabolite/component	Phenyl-^{14}C		Pyrazolyl-^{14}C		Phenyl-^{14}C		Pyrazolyl-^{14}C	
		%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
120									
Overall TRR (mg/kg)		0.027		0.059		0.030		0.108	
	Inpyrfluxam	43.1	0.012	46.7	0.027	34.9	0.010	41.2	0.045
	3'-OH-S-2840 (free)	11.9	0.003	6.6	0.004	9.9	0.003	4.0	0.004
	3'-OH-S-2840 (conj)	-	-	-	-	-	-	0.2	<0.001
	N-des-Me-S-2840 (free)	1.1	<0.001	1.6	0.001	1.6	<0.001	7.6	0.008
	1'-CH_2OH-S-2840 (free)	2.3	0.001	3.0	0.002	2.3	0.001	3.5	0.004
	1'-CH_2OH-S-2840 (conj)	-	-	-	-	-	-	4.5	0.005
	1'-COOH-S-2840 (free)	11.7	0.003	6.2	0.004	15.7	0.005	6.3	0.007
	1'-COOH-S-2840 (conj)	-	-	-	-	-	-	7.1	0.008
	N-des-Me-1'-CH_2OH-S-2840 (free)	0.6	<0.001	0.8	<0.001	-	-	0.5	0.001
	N-des-Me-1'-CH_2OH-S-2840 (conj)	-	-	-	-	-	-	0.3	<0.001
	DFPA (free)	-	-	11.2	0.007	-	-	6.1	0.007
	DFPA (conj)	-	-	-	-	-	-	6.5	0.007
	N-des-Me-DFPA (free)	-	-	3.2	0.002	-	-	1.2	0.001
	N-des-Me-DFPA (conj)	-	-	-	-	-	-	0.6	0.001
	DFPA-$CONH_2$ (free)	-	-	3.2	0.002	-	-	1.4	0.001
	Others	19.1	0.005	16.4	0.010	29.1	0.009	5.1	0.006
	Total extracted	89.8	0.024	95.8	0.056	93.4	0.028	96.1	0.104
	Total identified	70.7	0.019	79.4	0.047	64.3	0.019	91.0	0.099
	Total unidentified[(b)]	19.1	0.005	16.4	0.010	29.1	0.009	5.1	0.006
	Total unextracted residues (PES)	10.2	0.003	4.2	0.002	6.6	0.002	3.9	0.004

(b) Contains >4 – 8 components, largest was 0.7% of TRR, 0.001 mg/kg in pyrazolyl label mature root.

Crop/sample: Radish roots		Immature				Mature			
DAT	Metabolite/component	Phenyl-^{14}C		Pyrazolyl-^{14}C		Phenyl-^{14}C		Pyrazolyl-^{14}C	
		%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
365									
Overall TRR (mg/kg)		0.022		0.022		0.024		0.021	
	Inpyrfluxam	40.2	0.009	28.2	0.006	48.7	0.012	33.0	0.007
	3'-OH-S-2840 (free)	11.0	0.002	4.4	0.001	11.1	0.003	4.5	0.001
	3'-OH-S-2840 (conj)	-	-	-	-	-	-	-	-
	N-des-Me-S-2840 (free)	-	-	2.1	<0.001	-	-	1.8	<0.001
	1'-CH_2OH-S-2840 (free)	-	-	-	-	-	-	-	-
	1'-CH_2OH-S-2840 (conj)	-	-	-	-	-	-	-	-
	1'-COOH-S-2840 (free)	25.7	0.006	14.3	0.003	19.1	0.005	4.6	0.001
	1'-COOH-S-2840 (conj)	-	-	-	-	-	-	-	-
	N-des-Me-1'-CH_2OH-S-2840 (free)	-	-	-	-	-	-	-	-
	N-des-Me-1'-CH_2OH-S-2840 (conj)	-	-	-	-	-	-	-	-
	DFPA (free)	-	-	23.6	0.005	-	-	13.7	0.003
	DFPA (conj)	-	-	-	-	-	-	-	-
	N-des-Me-DFPA (free)	-	-	-	-	-	-	-	-
	N-des-Me-DFPA (conj)	-	-	-	-	-	-	-	-
	DFPA-$CONH_2$ (free)	-	-	-	-	-	-	-	-
	Others	18.7	0.004	24.2	0.005	17.8	0.004	36.7	0.008
	Total extracted	95.6	0.021	96.8	0.022	96.7	0.024	94.3	0.019
	Total identified	77.0	0.017	72.6	0.017	78.9	0.020	57.6	0.012
	Total unidentified[(c)]	18.7	0.004	24.2	0.005	17.8	0.004	36.7	0.008
	Total unextracted residues (PES)	4.3	0.001	3.2	0.001	3.3	0.001	5.7	0.001

(c) Contains >3 components, largest was 20.9% of TRR, 0.004 mg/kg (pyrazolyl label mature root).

Table 7.6.1-7 Summary of characterisation and identification of radioactive residues in the extracts of Sorghum forage following bare soil treatment with 235 g as/ha inpyrfluxam

Crop/sample: Sorghum		Forage			
DAT	Metabolite/component	Phenyl-^{14}C		Pyrazolyl-^{14}C	
		%TRR	mg/kg	%TRR	mg/kg
30					
Overall TRR (mg/kg)		0.094		0.200	
	Inpyrfluxam	3.6	0.003	3.4	0.007
	3'-OH-S-2840 (free)	2.7	0.003	3.1	0.006
	3'-OH-S-2840 (conj)	3.1	0.003	0.5	0.001
	N-des-Me-S-2840 (free)	0.3	<0.001	0.4	0.001
	1'-CH_2OH-S-2840 (free)	-	-	-	-
	1'-CH_2OH-S-2840 (conj)	13.8	0.013	7.8	0.016
	1'-COOH-S-2840 (free)	-	-	-	-
	1'-COOH-S-2840 (conj)	4.4	0.004	1.7	0.003
	N-des-Me-1'-CH_2OH-S-2840 (free)	-	-	-	-
	N-des-Me-1'-CH_2OH-S-2840 (conj)	9.0	0.009	7.5	0.015
	DFPA (free)	-	-	1.8	0.004
	DFPA (conj)	-	-	9.3	0.019
	N-des-Me-DFPA (free)	-	-	0.7	0.001
	N-des-Me-DFPA (conj)	-	-	7.6	0.015
	DFPA-$CONH_2$ (free)	-	-	1.8	0.004
	Others	45.7	0.043	40.4	0.081
	Total extracted	82.6	0.078	85.9	0.171
	Total identified	36.9	0.035	45.5	0.091
	Total unidentified[(a)]	45.7	0.043	40.4	0.081
	Total unextracted residues (PES)	17.4	0.016	14.1	0.028

(a) Contains >9 – 27 components, largest single component was 4.6% of TRR, 0.031 mg/kg in phenyl label stover.

Crop/sample: Sorghum	Forage				
Metabolite/component		**Phenyl-^{14}C**		**Pyrazolyl-^{14}C**	
		%TRR	**mg/kg**	**%TRR**	**mg/kg**
120					
Overall TRR (mg/kg)		0.118		0.187	
	Inpyrfluxam	4.1	0.005	1.7	0.003
	3'-OH-S-2840 (free)	4.4	0.005	3.8	0.007
	3'-OH-S-2840 (conj)	-	-	-	-
	N-des-Me-S-2840 (free)	-	-	0.3	0.001
	1'-CH_2OH-S-2840 (free)	-	-	-	-
	1'-CH_2OH-S-2840 (conj)	13.0	0.015	3.5	0.006
	1'-COOH-S-2840 (free)	-	-	-	-
	1'-COOH-S-2840 (conj)	1.6	0.002	0.6	0.002
	N-des-Me-1'-CH_2OH-S-2840 (free)	-	-	-	-
	N-des-Me-1'-CH_2OH-S-2840 (conj)	12.3	0.015	3.9	0.007
	DFPA (free)	-	-	2.2	0.004
	DFPA (conj)	-	-	11.1	0.021
	N-des-Me-DFPA (free)	-	-	1.4	0.003
	N-des-Me-DFPA (conj)	-	-	7.1	0.013
	DFPA-$CONH_2$ (free)	-	-	2.9	0.006
	Others	49.1	0.058	39.3	0.073
	Total extracted	84.5	0.100	77.9	0.145
	Total identified	35.4	0.042	38.6	0.072
	Total unidentified[(b)]	49.1	0.058	39.3	0.073
	Total unextracted residues (PES)	15.5	0.018	22.1	0.041

(b) Contains >8 – 17 components, largest was 4.8% of TRR, 0.060 mg/kg in phenyl label stover

Crop/sample: Sorghum	Forage			
Metabolite/component		**Phenyl-^{14}C**		**Pyrazolyl-^{14}C**	
		%TRR	**mg/kg**	**%TRR**	**mg/kg**
365					
Overall TRR (mg/kg)		0.036		0.047	
	Inpyrfluxam	-	-	0.5	<0.001
	3'-OH-S-2840 (free)	1.4	0.001	1.4	0.001
	3'-OH-S-2840 (conj)	3.6	0.001	3.6	0.002
	N-des-Me-S-2840 (conj)	-	-	-	-
	1'-CH_2OH-S-2840 (free)	-	-	-	-
	1'-CH_2OH-S-2840 (conj)	9.7	0.003	7.8	0.003
	1'-COOH-S-2840 (free)	-	-	-	-
	1'-COOH-S-2840 (conj)	4.6	0.002	3.9	0.002
	N-des-Me-1'-CH_2OH-S-2840 (free)	-	-	3.8	0.002
	N-des-Me-1'-CH_2OH-S-2840 (conj)	10.1	0.004	2.5	0.001
	DFPA (free)	-	-	0.7	<0.001
	DFPA (conj)	-	-	9.8	0.005
	N-des-Me-DFPA (free)	-	-	-	-
	N-des-Me-DFPA (conj)	-	-	5.8	0.003
	DFPA-$CONH_2$ (free)	-	-	1.8	0.001
	Others	51.0	0.018	35.8	0.017
	Total extracted	80.3	0.028	77.4	0.036
	Total identified	29.3	0.010	41.7	0.020
	Total unidentified[(c)]	51.0	0.018	35.8	0.017
	Total unextracted residues (PES)	19.7	0.007	22.6	0.011

(c) Contains >10 – 12 single components. Largest single component was 2.3% of TRR, 0.005 mg/kg in pyrazolyl label stover.

Table 7.6.1-8 Summary of characterisation and identification of radioactive residues in the extracts of Sorghum Stover following bare soil treatment with 235 g as/ha inpyrfluxam

Crop/sample: Sorghum		Stover			
DAT	Metabolite/component	Phenyl-^{14}C		Pyrazolyl-^{14}C	
		%TRR	mg/kg	%TRR	mg/kg
30					
Overall TRR (mg/kg)		0.684		0.752	
	Inpyrfluxam	0.8	0.006	1.0	0.008
	3'-OH-S-2840 (free)	1.1	0.008	1.2	0.009
	3'-OH-S-2840 (conj)	5.5	0.038	4.3	0.032
	N-des-Me-S-2840 (free)	<0.1	<0.001	0.1	<0.001
	1'-CH_2OH-S-2840 (free)	0.4	0.003	0.7	0.004
	1'-CH_2OH-S-2840 (conj)	13.5	0.092	10.3	0.078
	1'-COOH-S-2840 (free)	-	-	-	
	1'-COOH-S-2840 (conj)	3.7	0.025	2.4	0.018
	N-des-Me-1'-CH_2OH-S-2840 (free)	0.3	0.002	0.4	0.003
	N-des-Me-1'-CH_2OH-S-2840 (conj)	7.6	0.051	4.5	0.041
	DFPA (free)	-	-	0.7	0.005
	DFPA (conj)	-	-	11.0	0.083
	N-des-Me-DFPA (free)	-	-	0.3	0.003
	N-des-Me-DFPA (conj)	-	-	4.8	0.036
	DFPA-$CONH_2$ (free)	-	-	0.3	0.002
	Others	43.5	0.297	33.7	0.253
	Total extracted	76.6	0.523	76.7	0.577
	Total identified	33.1	0.226	43.0	0.324
	Total unidentified[(a)]	43.5	0.297	33.7	0.253
	Total unextracted residues (PES)	23.4	0.160	23.3	0.175

(a) Contains >9 – 27 components, largest single component was 4.6% of TRR, 0.031 mg/kg in phenyl label stover.

120					
Overall TRR (mg/kg)		1.252		1.107	
	Inpyrfluxam	0.9	0.011	1.8	0.020
	3'-OH-S-2840 (free)	2.6	0.033	1.6	0.017
	3'-OH-S-2840 (conj)	7.8	0.098	3.4	0.038
	N-des-Me-S-2840 (free)	-	-	-	-
	1'-CH_2OH-S-2840 (free)	2.2	0.027	0.3	0.003
	1'-CH_2OH-S-2840 (conj)	13.3	0.167	7.2	0.080
	1'-COOH-S-2840 (free)	-	-	-	-
	1'-COOH-S-2840 (conj)	3.8	0.048	3.4	0.038
	N-des-Me-1'-CH_2OH-S-2840 (free)	0.5	0.006	-	-
	N-des-Me-1'-CH_2OH-S-2840 (conj)	8.9	0.111	4.2	0.046
	DFPA (free)	-	-	0.3	0.004
	DFPA (conj)	-	-	11.6	0.129
	N-des-Me-DFPA (free)	-	-	0.2	0.002
	N-des-Me-DFPA (conj)	-	-	4.5	0.059
	DFPA-$CONH_2$ (free)	-	-	1.4	0.016
	Others	42.1	0.528	38.9	0.430
	Total extracted	81.9	1.029	78.8	0.871
	Total identified	39.8	0.501	39.9	0.441
	Total unidentified[(b)]	42.1	0.528	38.9	0.430
	Total unextracted residues (PES)	18.0	0.226	21.3	0.236
(b) Contains >8 – 17 components, largest was 4.8% of TRR, 0.060 mg/kg in phenyl label stover					
365					
Overall TRR (mg/kg)		0.136		0.210	
	Inpyrfluxam	1.3	0.002	1.0	0.002
	3'-OH-S-2840 (free)	3.5	0.005	2.1	0.004
	3'-OH-S-2840 (conj)	8.6	0.012	5.5	0.012
	N-des-Me-S-2840 (conj)	2.1	0.003	1.6	0.003
	1'-CH_2OH-S-2840 (free)	-	-	-	-
	1'-CH_2OH-S-2840 (conj)	3.7	0.005	3.1	0.006
	1'-COOH-S-2840 (free)	-	-	-	-
	1'-COOH-S-2840 (conj)	4.5	0.006	2.2	0.005
	N-des-Me-1'-CH_2OH-S-2840 (free)	-	-	-	-
	N-des-Me-1'-CH_2OH-S-2840 (conj)	3.9	0.005	2.3	0.005
	DFPA (free)	-	-	-	-
	DFPA (conj)	-	-	6.1	0.013
	N-des-Me-DFPA (free)	-	-	-	-
	N-des-Me-DFPA (conj)	-	-	3.8	0.008
	DFPA-$CONH_2$ (free)	-	-	1.4	0.003
	Others	49.9	0.068	49.7	0.104
	Total extracted	77.6	0.105	78.7	0.165
	Total identified	27.7	0.038	28.9	0.061
	Total unidentified[(c)]	49.9	0.068	49.7	0.104
	Total unextracted residues (PES)	22.4	0.030	21.3	0.045
(c) Contains >10 – 12 single components. Largest single component was 2.3% of TRR, 0.005 mg/kg in pyrazolyl label stover.					

Table 7.6.1-9 Summary of characterisation and identification of radioactive residues in the extracts of Sorghum Grain following bare soil treatment with 235 g as/ha inpyrfluxam

Crop/sample: Sorghum		Grain			
DAT	Metabolite/component	Phenyl-^{14}C		Pyrazolyl-^{14}C	
		%TRR	mg/kg	%TRR	mg/kg
30					
Overall TRR (mg/kg)		0.012		0.049	
	Inpyrfluxam	-	-	-	-
	DFPA (conj)	-	-	22.6	0.011
	N-des-Me-DFPA (conj)	-	-	8.6	0.004
	Others	49.9	0.006	28.7	0.014
	Total extracted	49.9	0.006	59.9	0.029
	Total identified	0.0	0.000	31.2	0.016
	Total unidentified[(a)]	49.9	0.006	28.7	0.014
	Total unextracted residues (PES)	50.1	0.006	40.1	0.020
120					
Overall TRR (mg/kg)		0.010		0.062	
	Inpyrfluxam	-	-	-	-
	DFPA (conj)	-	-	-	-
	N-des-Me-DFPA (conj)	-	-	-	-
	Others	51.7	0.005	49.8	0.031
	Total extracted	51.7	0.005	49.8	0.031
	Total identified	0.0	0.000	0.0	0.000
	Total unidentified[(a)]	51.7	0.005	49.8	0.031
	Total unextracted residues (PES)	48.3	0.005	50.2	0.031
365					
Overall TRR (mg/kg)		0.014		0.017	
	Inpyrfluxam	-	-	-	-
	DFPA (conj)	-	-	-	-
	N-des-Me-DFPA (conj)	-	-	-	-
	Others	52.4	0.007	47.4	0.008
	Total extracted	52.4	0.007	47.4	0.008
	Total identified	0.0	0.000	0.0	0.000
	Total unidentified[(a)]	52.4	0.007	47.4	0.008
	Total unextracted residues (PES)	47.6	0.006	52.6	0.009

(a) Contains only the proposed conjugated polar components

Conclusions and metabolic pathway

The metabolism of the fungicide inpyrfluxam was investigated in confined rotational crops after one spray application onto bare soil. The application rate amounted to 235 g a.s./ha and the test compounds were ^{14}C-radiolabelled either in the phenyl or pyrazolyl moiety.

Generally, there was a considerable decrease in the TRR values between the first and second rotations and this decreased further for the third rotation.

The highest proportion of parent inpyrfluxam was found in radish roots (52 – 59% TRR), and this deceased in the subsequent planting periods of 120 DAT and 365 DAT. The TRR percentage, as well as the actual residue levels of inpyrfluxam in 30 DAT and 120 DAT lettuce (11 – 46% TRR, 0.009 – 0.027 mg eq./kg) were lower than those in radish roots. The residue levels of inpyrfluxam were also low in the radish top samples (6 – 15% TRR, 0.005 – 0.025 mg eq./kg), and very low in the sorghum forage and stover samples (0 – 4% TRR, 0.0 – 0.020 mg eq./kg). Inpyrfluxam was not detected in sorghum grain samples harvested from all planting periods. The level of accumulation of the parent inpyrfluxam in the plant samples might be impacted by both the rate of its uptake from the soil, and the rate of its metabolism by the plant. The results indicated that transfer of inpyrfluxam into sorghum grain did not occur.

The qualitative and quantitative nature of the radioactive residues observed in this study indicated that inpyrfluxam was extensively metabolised into a large number of metabolites, many of which seemed to form complex conjugates with indigenous compounds and became potentially incorporated as ‘bound’ residues in various plant constituents. The parent inpyrfluxam underwent a number of transformation processes including oxidation, des-methylation, amide bond cleavage, as well as combinations of these processes.

The primary oxidation products of inpyrfluxam were:

- **(i)** 3'-OH-S-2840, in which the site of oxidation was the 3'-position in the fused cyclopentenyl ring
- **(ii)** 1'-CH_2OH-S-2840, in which one of the two CH_3 groups attached to the 1'-position of the same ring was oxidized to CH_2OH
- **(iii)** 1'-COOH-S-2840, in which one of the CH_3 groups attached to the 1'-position of this ring was oxidized to COOH

The amide bond cleavage of the parent inpyrfluxam and its metabolites produced DFPA and DFPA-$CONH_2$. The metabolites, *N*-des-Me-S-2840, *N*-des-Me-1'-CH_2OH-S-2840 (isomers A&B, observed in both free and possibly conjugated forms), and *N*-des-Me-DFPA were produced as a result of the loss of the *N*-methyl group in the pyrazolyl ring of the parent inpyrfluxam, its oxidation products and the cleavage product, respectively.

The primary oxidation product, 3'-OH-S-2840, was present in free form in almost all rotational crop samples. A portion of 3'-OH-S-2840 also seemed to be present in conjugated form. When the proposed conjugated residue fractions from the various crop samples were hydrolysed with 2N HCl, 3'-OH-S-2840 was released in free form, but being seemingly unstable in acidic conditions, it appeared to be quickly degraded to its dehydrate, and also to a few other minor degradants. The 3'-OH-S-2840 dehydrate was also formed under representative high temperature hydrolysis conditions (in the most acidic samples) in the 3'-OH-S-2840 processing hydrolysis study (see section B.7.5.1.4).

The isomer mixtures A&B of the oxidation products, 1'-CH_2OH-S-2840 and 1'-COOH-S-2840, were also proposed to exist in both free and conjugated forms in almost all rotational crop samples. When the proposed conjugated residue fractions from various crop samples were hydrolysed with 2N HCl, the metabolites released in free form. The isomer mixture 1'-CH_2OH-S-2840-B was present in higher concentrations than 1'-CH_2OH-S-2840-A in most crop samples. The isomer 1'-COOH-S-2840-A was present in higher concentration than 1'-COOH-S-2840-B in these crop samples.

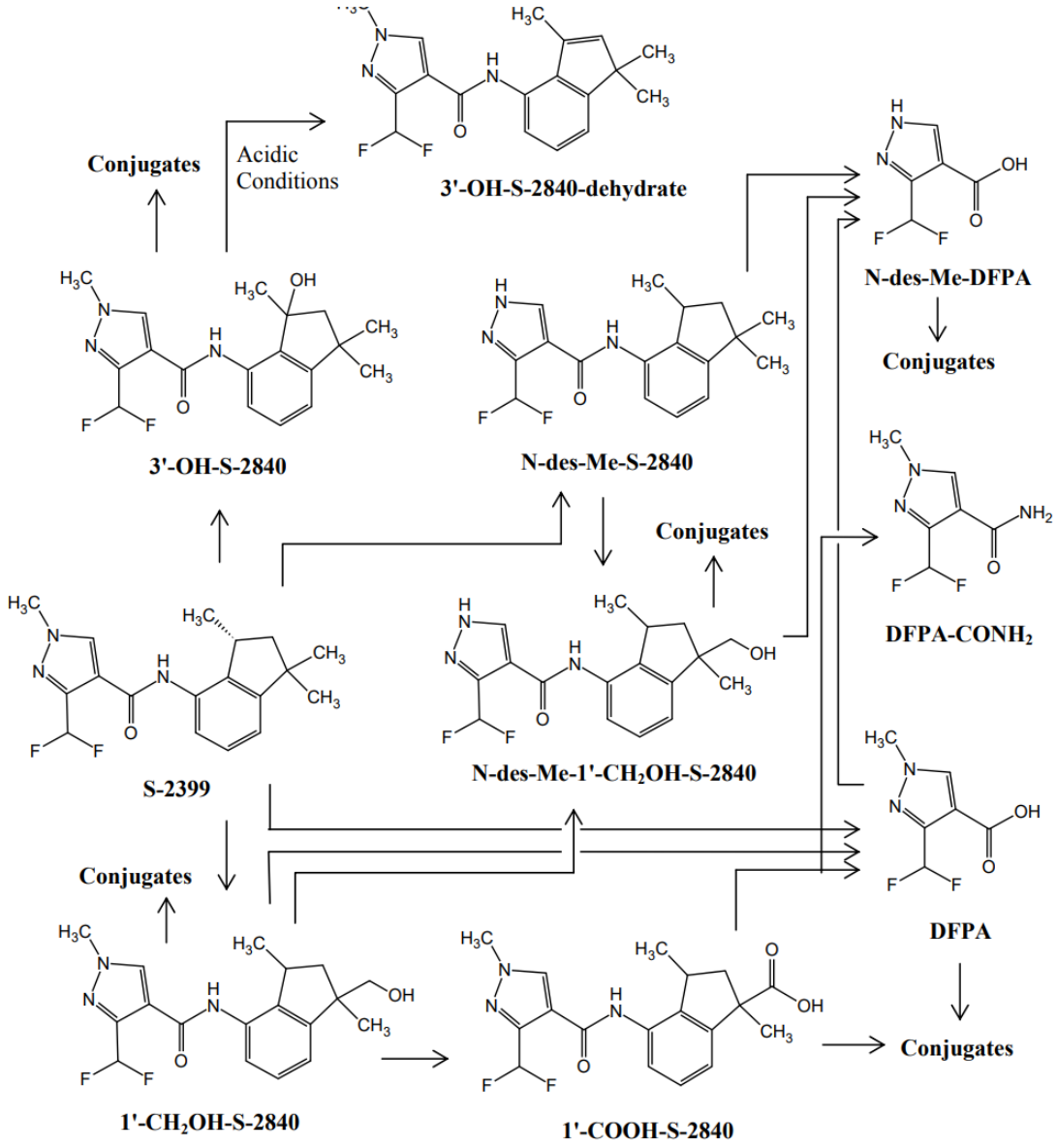
The metabolites 1'-COOH-S-2840 and 3'- OH-S-2840 were degradation products of inpyrfluxam in soil under aerobic conditions ([redacted] 2017; see part B8.1.1.3). Therefore, these metabolites might have been taken up from soil by the plant.

The metabolites, DFPA, *N*-des-Me-DFPA, and DFPA-$CONH_2$, were present in almost all crop samples, the former (DFPA) was always in higher amounts. The TRR percentage of DFPA was highest (up to 29%) in lettuce samples.

The metabolites of inpyrfluxam and their degradation products appeared to combine with various endogenous compounds to form the major polar fraction and potentially 'bound' residues in crop plants. Analysis of these residues in PES samples of sorghum, lettuce and radish samples showed that a number of metabolites including 1'-COOH-S-2840, 1'-CH_2OH-S2840, *N*-des-Me-S-2840, DFPA and *N*-des-Me-DFPA, seemed to form acid hydrolysable covalent bonds with the solids remaining after extraction. The metabolite residues might have degraded further into simpler compounds and potentially became incorporated into plant constituents such as starch, protein, lignin, cellulose and other natural components of plants. A significant part of the unextracted residue in sorghum grain was present in the proposed hydrolysed starch fraction, indicating that metabolites in grain might have essentially degraded and incorporated into starch.

Based on these results, the metabolism of inpyrfluxam in confined rotational crops is defined and the following metabolic pathway is proposed.

Figure 7.6.1-1: Proposed metabolic pathway of inpyrfluxam in rotational crops



B.7.6.2. Magnitude of residues in rotational crops

Report:	**KCA 6.6.2/01; [redacted] (2018)**
Title:	Rotational field-crops residue study after application of S-2399 to cereal crops in northern and southern Europe - 2016.
Document No.:	TPR-0080 (Study No.: 260-2016)
Guidelines:	Regulation (EC) No 1107/2009 Regulation (EU) No 283/2013 of 1 March 2013 Appendix B - General recommendations for the design, preparation and realization of residue trials, SANCO 7029/VI/95-rev 5, 22.07.97 Testing of plant protection products in rotational crops, 7524/VI/95 rev.2, 22/07/1997 OECD Guidelines No. 504, Guidelines for the Testing of Chemicals, Residues in Rotational Crops (Limited Field Studies), 08/01/2007 OECD (2009),Test No. 509: Crop Field Trial, OECD Guidelines for the Testing of Chemicals, Section 5, OECD Publishing, 07/09/2009 SANCO/3029/99 rev.4, 11 July 2000 SANCO/825/00 rev 8, 16/11/2010 ENV/JM/MONO(2007)17
Guideline deviations:	None
GLP/GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

Summary

The study included two supervised residue trials conducted in Northern Europe (Germany, soil type loam) and Southern Europe (Italy, soil type sandy loam) initiated during the 2016 season.

The purpose of study was to determine the magnitude of residues of inpyrfluxam and its metabolites 3'-OH-S-2840, DFPA-$CONH_2$, DFPA, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B, 1'COOH-S-2840-A, 1'-COOH-S-2840-B, *N*-des-Me-S-2840, *N*-des-Me-1'-CH_2OH-S-2840-A and *N*-des-Me-1'-CH_2OH-S-2840-B in/on rotated field crops (succeeding crops) after one spray application with the product 'S-2399 40 SC'.

Materials and methods

One application was made to the preceding crop (winter barley at BBCH 30 – 65) at a nominal application rate of 240 g a.s/ha (2.7N). The preceding crop was destroyed to simulate a crop failure and incorporated in the soil while preparing the soil for the rotational crops within 13 – 14 days after application. OECD TG 504 (2007) allows for the presence of a primary crop prior to planting of rotational crops. The OECD Guidance document on Rotational Crops (2018) now recommends that all rotational crops studies are performed with bare soil application rather than using an application to crops (because the envisaged soil concentrations can be more easily achieved). However, the study was completed in 2016 and 2017 prior to the publication of this guidance; the approach taken in the study (which has aimed to consider potential crop failure followed by incorporation of the preceding treated crop into the soil) is acceptable.

The actual application rates achieved were within +/-5% of 240 g as/ha across the various field plots treated.

In the NEU trial (Germany), the preceding crop was incorporated into the soil by mulching and then tilling with a rotary tiller at approx. 20 cm depth. This happened 13 days after application.

In the SEU trial (Italy), the preceding crop was incorporated into the soil using a rotary cultivator on about 20 – 30 cm depth. This happened 13 days or 14 days after application.

Lettuce (representative of leafy vegetables), carrot (representative of root and tuber vegetables) and cereals (wheat or barley) were planted at intervals of 28 +/-2, 120 +/-5 and 350 +/-15 days after treatment.

Table 7.6.2-1: Trial parameters

Application rate (nominal) to proceeding crop	Succeeding crop		Germany (GE01) soil type loam				Italy (IT02) soil type sandy loam				Crop Part sampled at NCH
			Variety	PBI (days)	Days between planting / sowing and harvest	Days between application and harvest	Variety	PBI (days)	Days between planting / sowing and harvest	Days between application and harvest	
1 x 240 g a.s/ha to winter barley at BBCH 30 – 65 (crop then destructed and incorporation into the soil of the preceding crop)	Root and tuber vegetables	Carrot	Almaro	33	113	146	Berlicum 2	27	103	130	Root & leaves
			Almaro	115	109	224	Berlicum 2	120	160	280	
			Almaro	363	93	456	Berlicum 2	336	132	468	
	Leafy vegetables	Lettuce	Casanova	33	34	67	Canasta	27	40	67	Whole plant without roots
			Zendria	115	39	154	Nauplus	120	43	163	
			Carasco	363	47	410	Paspartu	336	53	389	
	Cereals	Cereal (barley or wheat)	Tybalt	33	197	140	Timilia	27	104	131	Straw & grain
			KWS Keeper	120	109	410	Marco Aurelio	120	272	392	
			Tybalt	349	144	493	Timilia	336	103	439	

Sampling

Specimens of soil (0 – 20 cm cores) were taken at various times. A decision was taken to do this after the set-up of the trial, so the following late stage samples were taken (in early 2017 (February) from the already harvested plots), at planting for the last PBI rotation and at commercial harvest for the last PBI for all crops and the 2nd PBI for the cereals. One sample of soil per subplot was taken for residue analysis.

Collected plant samples taken at harvest comprised an appropriate weight and minimum number of units. The following plant samples were taken from treated and untreated plots from all trials (retain samples were also taken):

- Preceding crop: Cereals whole plant (w/o roots) 1 kg min / 12 units min
- Lettuce without root: 1 kg / 12 units min
- Carrot Roots: 2 kg / 12 units min and carrot leaves + tops: 1 kg / 12 units min
- Cereals Grain: 1 kg min and Straw: 0.5 kg min

Storage and storage stability

Each field sample was stored deep-frozen (≤ -18 °C) within 24 hours after sampling.

Soil samples were stored frozen for a maximum of 404 days (~13 months) and extracts were stored (1 – 10 °C) for a maximum of 2 days. Soil was only analysed for parent inpyrfluxam. The storage stability of inpyrfluxam in soil has been demonstrated for at least 19 months of frozen storage (see section 3CA B.8.1.4). Some decline was observed in the next samples (various soils) taken at 24 months. Therefore, the results for soil presented in this MOR rotational crops study are validated based on the supported period of frozen stability.

Plant samples of the rotated crops were stored frozen for a maximum of 355 days (369 days for the primary crop that was analysed) and extracts were stored (1 – 10 °C) for a maximum of 14 days. Rotational crop plant samples were analysed for parent inpyrfluxam, 3'-OH-S-2840, DFPA-$CONH_2$, DFPA, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B, 1'-COOH-S-2840-A, 1'-COOH-S-2840-B, *N*-des-Me-S-2840, *N*-des-Me-1'-CH_2OH-S-2840-A and *N*-des-Me-1'-CH_2OH-S-2840-B. The storage stability of inpyrfluxam and these metabolites analysed in this rotational crop study has been demonstrated for up to 22 months (or 12 months in the case of *N*-des-Me-S-2840, *N*-des-Me-1'-CH_2OH-S-2840-A and *N*-des-Me-1'-CH_2OH-S-2840-B) (see Vol 1 section 2.7.1). Therefore, the results for rotational crops samples presented in this MOR rotational crops study are validated based on the supported period of frozen stability.

For both soil and crops, extract stability is supported by the way that matrix-matched standards that were used for quantification were always prepared on the same day as the work up of the specimen for residue analysis took place. Furthermore, procedural recoveries were handled and stored in the same way and for the same time period as the sample extracts that were prepared within the same analytical set.

Analysis

Soil samples were analysed for inpyrfluxam only using method validated in study S16-05522. This method has been acceptably validated in accordance with SANTE/2020/12830 rev.1 for the determination of inpyrfluxam in soil (see section Volume 3, B.5). Procedural recoveries of inpyrfluxam (n=3 at each of 0.001 mg/kg, 0.02 mg/kg and 0.2 mg/kg spiking levels) were analysed during this MOR study and are acceptable (mean recoveries of 87 – 94% and %RSD levels of 7.6 – 14%).

The plant specimens were analysed for residues of inpyrfluxam and its metabolites (3'-OH-S-2840, DFPA-$CONH_2$, DFPA, 1'-CH_2OH-S-2840-A, 1'-CH_2OH-S-2840-B, 1'-COOH-S-2840-A, 1'-COOH-S-2840-B, *N*-des-Me-S-2840, *N*-des-Me-1'-CH_2OH-S-2840-A and *N*-des-Me-1'-CH_2OH-S-2840-B) using method validated in study S16-03371. This method has been acceptably validated in accordance with SANTE/2020/12830 rev.1 for the determination of the analytes in high acid (grapes (fruit)), high water content (tomato (fruit)), high starch content (potato (tuber)), high protein content (field peas (dry seed)), high oil content (sunflower (seed)) and barley (straw) with an LOQ of 0.01 mg/kg; see Vol. 3 CA B5.1.2.5.

However, the method validation data does not cover all the plant commodities in the rotational crop trials, therefore, procedural recoveries that were conducted during sample analysis.

These represented reduced validation sets for inpyrfluxam, 3'-OH-S-2840, DFPA-$CONH_2$, *N*-des-Me-S-2840, DFPA, 1'-CH_2OH-S-2840-A & -B, 1'-COOH-S-2840-A & -B, *N*-des-Me-1'CH_2OHA&B in lettuce (w/o roots) and carrot (roots, leaves + top) in accordance with SANTE/2020/12830 rev.1. These procedural recoveries (n ≥3) were provided for inpyrfluxam and its metabolites in samples of carrots roots and carrot tops, lettuce (without roots) for both the quantification and confirmation method approach. The recovery samples were spiked at levels of LOQ and 10x LOQs (LOQ of 0.01 mg/kg (or 0.005 mg/kg for each of 'A' and 'B' isomers). The average recoveries were all within the acceptable range of 70 – 110%. The %RSD values were below or at 20% for each compound and all sample materials.

A range of further procedural recovery were generated for inpyrfluxam and its metabolites 3'-OH-S-2840, DFPA-$CONH_2$, *N*-des-Me-S-2840, DFPA, DFPA, 1'-CH_2OH-S-2840-A & -B, 1'-COOH-S-2840-A & -B, *N*-des-Me-1'CH_2OHA&B in wheat (Whole Plant, inpyrfluxam only), lettuce, carrot roots and tops and cereal grain and straw for the quantification method. At the LOQs of 0.01 mg/kg, 2, 3 or 4 procedural

recoveries were available, and at higher fortification levels of 0.1 and 10 mg/kg, 1, 2 or 3 procedural recoveries were available. Procedural recoveries were generally fully acceptable, with a small number of slight deviations in cereal grains (as explained in this paragraph), which are not expected to impact the overall validity of the results in this magnitude of residues study. In cereal grain for 3'-OH-S-2840 (quantification method) the recoveries at 0.1 mg/kg were 110 and 112%, however the recoveries were in the acceptable range at the 0.01 and 10 mg/kg levels. In cereal grain. the *N*-des-Me-S-2840 had a relatively high %RSD of 21% at the LOQ of 0.01 mg/kg, though this was considered acceptable as all the individual recoveries at 0.01, 0.1 and 10 mg/kg were within the acceptable range of 70 – 110%

All the procedural recoveries available represented a good body of data to support the quantification of residues in the rotated crops in this magnitude of residues study. Example chromatograms have been provided showing the method is specific and no significant interference (> 30% of the LOQ) is observed. Overall, the method is acceptably validated in carrots (root and top), lettuce (head), and cereals (grain and straw, parent inpyrfluxam in wheat whole plant) with LOQs of 0.01 mg/kg (crops most analytes) and 0.005 mg/kg for each of the 'A' and 'B' isomers.

Results and discussion

Soil

The detailed results obtained for soil samples are summarised in Table 7.6.2-2. No residues of inpyrfluxam above the LOQ were found in the soil matrix control samples.

Residues of parent inpyrfluxam in soil specimens (expressed in mg/kg d.w. (dry weight)) ranged from:

- < 0.002 – 0.020 mg/kg in trial 260-2016 GE01 (specimens sampled 285 – 493 days after application)
- 0.012 – 0.034 mg/kg in trial 260-2016 IT02 (specimens sampled 281 – 468 days after application)

Please note that the earliest soil samples (for the 28 and 120 DAT PBIs tested) were taken in February 2017 from the already harvested plots, as the decision to take these soil samples was made after the set-up of the study.

Residues of inpyrfluxam in soil were present above the LOQ level in all the sample timings tested and at least until the last planting/sowing event for the longest tested PBI (350 +/-15 days), meaning that all planted rotational crops were planted on a soil containing inpyrfluxam residue levels above LOQ.

Table 7.6.2-2 Residues of inpyrfluxam in soil specimens

Crop and PBI	Analytical method: S16-05522 Max storage length: sample to extraction = 404 days, extract to analysis = 2 days					
	Specimens sampled in February 2017		Specimens sampled at planting / sowing		Specimens sampled at commercial harvest	
	DAA (a)	Inpyrfluxam (mg/kg) (b)	DAA (a)	Inpyrfluxam (mg/kg) (b)	DAA (a)	Inpyrfluxam (mg/kg) (b)
Trial: GE01						
Lettuce 28 +/-2 DAA	326	0.012	-	-	-	-
Carrot 28 +/-2 DAA	326	0.016	-	-	-	-
Cereal 28 +/-2 DAA	326	0.005	-	-	-	-
Lettuce 120 +/-5 DAA	326	0.011	-	-	-	-
Carrot 120 +/-5 DAA	326	0.020	-	-	-	-
Cereal 120 +/-5 DAA	285	0.004	-	-	410	<0.002
Lettuce 350 +/-15 DAA	-	-	363	0.007	410	n.d.
Carrot 350 +/-15 DAA	-	-	363	0.008	456	< 0.002
Cereal 350 +/-15 DAA	-	-	349	0.015	493	0.010
Trial: IT02						
Lettuce 28 +/-2 DAA	308	0.019	-	-	-	-
Carrot 28 +/-2 DAA	308	0.019	-	-	-	-
Cereal 28 +/-2 DAA	308	0.034	-	-	-	-
Lettuce 120 +/-5 DAA	281	0.027	-	-	-	-
Carrot 120 +/-5 DAA	281	0.027	-	-	-	-
Cereal 120 +/-5 DAA	281	0.025	-	-	392	0.027
Lettuce 350 +/-15 DAA	-	-	336	0.027	389	0.026
Carrot 350 +/-15 DAA	-	-	336	0.012	468	0.024
Cereal 350 +/-15 DAA	-	-	336	0.023	439	0.023

(a) DAA: number of days since application

(b) Residue of inpyrfluxam expressed in mg/kg d.w. in soil horizon 0 – 20 cm (d.w. = dry weight)

Plant matrices

The detailed results obtained for rotational crop samples are summarised in Table 7.6.2-3. No residues of inpyrfluxam or its metabolites above the LOQ were found in the control samples.

In the primary crop (whole plant without roots) for both trials inpyrfluxam was detected up to 8 mg/kg.

In the rotational crop samples residues of inpyrfluxam and its metabolites were always < LOQ except for straw samples from the 1st rotation (~ 30 day PBI):

- Straw from the 1st rotation (PBI 33 days) at 140 DAA in the German trial reported 3'-OH-S-2840 at 0.01 mg/kg, DFPA at 0.09 mg/kg, 1'-COOH-S-2840 (sum of isomers) at 0.019 mg/kg and 1'-CH_2OH-S-2840 (sum of isomers) at 0.023 mg/kg.
- Straw from the 1st rotation (PBI 27 days) at 131 DAA in the Italian trial reported DFPA at 0.1 mg/kg, 1'-COOH-S-2840 (sum of isomers) at 0.017

mg/kg, 1'-CH_2OH-S-2840 (sum of isomers) at 0.023 mg/kg and *N*-des-Me-1'CH_2OH-S-2840 (sum of isomers) at 0.019 mg/kg.

Table 7.6.2-3: Rotational trial results for inpyrfluxam and its metabolites following application at ~240 g a.s./ha and three plant back intervals

Trial No./ Location/ Year	Crop (Variety)	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Application Rate g a.s./ha	Treatment date	Growth Stage at Application	Portion analysed	Residues (mg/kg)[a]								DAA (days)
							Inpyrfluxam	3'-OH-S-2840	DFPA-CONH$_2$	*N*-des-Me-S-2840	DFPA	1'-COOH-S-2840†	1'-CH$_2$OH-S-2840†	*N*-des-Me-1'CH$_2$OH-S-2840[b]	
Analytical method: SUM-1601V Max storage length: sample to extraction = 369 days, extract to analysis = 14 days															
Report: 260-2016 Trial: 265-2016 GE01 **47589 [redacted], North Rhine-Westphalia, Germany 2016/17**	Primary crop: Winter barley	1. 07.10.15 2. - 3. -	240	01.04.16	BBCH 30	Whole plant without roots	3.4-8.0	Not analysed for							0
	Rotational crop: Lettuce	1. 04.05.16 2. - 3. 07.06.16	**Plant back interval (days) 33**			Whole plant	All analytes <0.01								67
	Rotational crop: Carrot	1. 04.05.16 2. - 3. 25.08.16	**Plant back interval (days) 33**			Roots Tops	All analytes <0.01								146

	Rotational crop: Spring wheat	1. 04.05.16 2. 07.07.16 - 14.07.16 3. 19.08.16	**Plant back interval (days)** **33**	Straw Grain	<0.01 <0.01	0.01 <0.01	<0.01 <0.01	<0.01 <0.01	0.09 <0.01	0.019 <0.01	0.023 <0.01	<0.01 <0.01	140
	Rotational crop: Lettuce	1. 25.07.16 2. - 3. 02.09.16	**Plant back interval (days)** **115**	Whole plant	All analytes <0.01								154
	Rotational crop: Carrot	1. 25.07.16 2. - 3. 11.11.16	**Plant back interval (days)** **115**	Roots Tops	All analytes <0.01								224
	Rotational crop: Winter barley	1. 09.09.16 2. 12.05.17 - 28.05.17 3. 28.06.17	**Plant back interval (days)** **120**	Straw Grain	All analytes <0.01								410
	Rotational crop: Lettuce	1. 10.05.17 2. - 3. 26.06.17	**Plant back interval (days)** **363**	Whole plant	All analytes <0.01								410

	Rotational crop: Carrot	1. 10.05.17 2. - 3. 11.08.17	**Plant back interval (days)** **363**			Roots Tops	All analytes <0.01		456
	Rotational crop: Spring wheat	1. 16.03.17 2. 13.06.17 - 26.06.17 3. 07.08.17	**Plant back interval (days)** **349**			Straw Grain	All analytes <0.01		493
Report: 260-2016 Trial: 265-2016 IT02 **95010 [redacted], Sicily, Italy 2016/17**	Primary crop: Spring wheat	1. 26.02.16 2. - 3. -	240	21.04.16	BBCH 31	Whole plant without roots	5.6-7.6	Not analysed for	0
	Rotational crop: Lettuce	1. 18.05.16 2. - 3. 25.06.16 - 30.16.16	**Plant back interval (days)** **27**			Whole plant	All analytes <0.01		67
	Rotational crop: Carrot	1. 18.05.16 2. - 3. 27.08.16 - 30.08.16	**Plant back interval (days)** **27**			Roots Tops	All analytes <0.01		130

	Rotational crop: Spring wheat	1. 18.05.16 2. 15.07.16 - 19.07.16 3. 27.08.16 - 30.08.16	**Plant back interval (days)** **27**	Straw Grain	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	0.10 <0.01	0.017 <0.01	0.023 <0.01	0.019 <0.01	131
	Rotational crop: Lettuce	1. 15.09.16 2. - 3. 26.10.16 - 30.10.16	**Plant back interval (days)** **120**	Whole plant	All analytes <0.01								163
	Rotational crop: Carrot	1. 15.09.16 2. - 3. 20.02.17 - 24.02.17	**Plant back interval (days)** **120**	Roots Tops	All analytes <0.01								280
	Rotational crop: Winter wheat	1. 15.09.16 2. 24.04.17 - 04.05.17 3. 21.06.17 - 16.06.17	**Plant back interval (days)** **120**	Straw Grain	All analytes <0.01								392

	Rotational crop: Lettuce	1. 23.03.17 2. - 3. 12.05.17 - 16.05.17	**Plant back interval (days)** **336**	Whole plant	All analytes <0.01	389
	Rotational crop: Carrot	1. 23.03.17 2. - 3. 29.07.17 - 05.08.17	**Plant back interval (days)** **336**	Roots Tops	All analytes <0.01	468
	Rotational crop: Spring wheat	1. 23.03.17 2. 05.05.17 - 10.05.17 3. 02.07.17 - 04.07.17	**Plant back interval (days)** **336**	Straw Grain	All analytes <0.01	439

DAA Days between application and sampling

(a) Inpyrfluxam residues expressed as inpyrfluxam, 3'-OH-S-2840 residues expressed as 3'-OH-S-2840, DFPA-$CONH_2$ residues expressed as DFPA-$CONH_2$, *N*-des-Me-DFPA residues expressed as *N*-des-Me-DFPA, DFPA residues expressed as DFPA, 1'-COOH-S-2840 (sum of A&B isomer) residues expressed as 1'-COOH-S-2840, 1'-CH_2OH-S-2840 (sum of A&B isomer) residues expressed as 1'-CH_2OH-S-2840 and *N*-des-Me-1'-CH_2OH-S-2840 (sum of A&B isomer) residues expressed as *N*-des-Me-1'-CH_2OH-S-2840

(b) Analytically determined as two separate groups of isomers, but presented summed; 1'-COOH-S-2840 A + 1'-COOH-S-2840 B; 1'-CH_2OH-S-2840 A + 1'-CH_2OH-S-2840 B; *N*-des-Me-1'CH_2OH-S-2840 A + *N*-des-Me-1'CH_2OH-S-2840 B

B.7.7. Other Studies

B.7.7.1. Effect on the residue level in pollen and bee products

No studies on residue levels in pollen and bee products have been evaluated.

Based on the decision tree set out in SANTE/11956/2016 rev. 9, residues in honey are potentially expected where the substance is applied to melliferous crops. Wheat and barley (the currently intended uses) are not melliferous and are not expected to be foraged by bees such that the potential for honey residues would be impacted. Significant residues of inpyrfluxam are also not expected in rotational crops based on the requested GAP. Application is requested in the spring, but as only some of the non-target plants will be melliferous, the contribution of residues to honey via non-target plants is not considered to be significant for the current intended uses.

B.7.7.2. Literature Review

A literature search was conducted by the applicant in September 2018 (Exponent International Ltd., 2018 – 1403863.UK0 - 4472), and updated in January 2025 (document K-CA, section 9). The original (2018) literature review did not identify any published papers on inpyrfluxam or metabolites relevant to the residues assessment which needed to be included in the residues evaluation. The applicant did not produce any updated relevant published papers for additional evaluation by HSE for the updated literature search performed in 2025.

The review for inpyrfluxam (and its metabolites) was in accordance with the EFSA 2011 Guidance (EFSA Journal 2011; 9(2):2092). HSE considers that acceptable search criteria have been applied to this literature review when considering the residues and dietary exposure areas.

Initial literature review (2018)

Summary of methodology employed

1. A very broad search was conducted in 45 scientific source databases for inpyrfluxam and its metabolites.

2. Duplicates titles from between the data bases were automatically removed from the output.

3. A rapid assessment of the titles was conducted to remove any additional duplicates and any obviously irrelevant titles (where enough information was available from the title alone).

Although the planned intention was to continue with the points 4 – 6 below (*in italics*), this wasn't applicable in this case, since paper titles were used for the rapid assessment.

4. *A further rapid assessment was conducted using summary abstracts and any clearly irrelevant titles were removed.*

5. *A detailed assessment of the full-text documents for the remaining titles was conducted using the criteria developed for study relevance*

6. *Any relevant papers were highlighted and assessed for reliability.*

Timespan

The initial literature review search was performed in September 2018, with a search period of September 2008 – September 2018.

*Regulation 1107/2009 states "*Scientific peer-reviewed open literature, as determined by the Authority, on the active substance and its relevant metabolites dealing with side-effects on health, the environment and non-target species and published within the last 10 years before the date of submission of the dossier shall be added by the applicant to the dossier."

The date of submission of inpyrfluxam was July 2023, so the literature review does not cover the period required under Regulation 1107/2009. An updated literature review was provided to cover the period September 2018 – July 2023 and this is reported below.

Search terms

The literature review covered inpyrfluxam and the following metabolites:

- 1'-COOH-S-2840
- NDM-1'-COOH-S-2840
- 3'-OH-S-2840
- 1'-CH_2OH-S-2840
- DFPA
- DFPA-$CONH_2$
- *N*-des-Me-S-2840
- *N*-des-Me-1'-CH_2OH-S-2840
- *N*-des-Me-DFPA
- 1',1'-bis(CH_2OH)-S-2840

Methodology applied and databases used

The search strategy was conducted using bibliographic databases, with STN and Dialog as host providers. The applicant provided the search strategies and included a justification of each of the databases used. Taken together, these covered a comprehensive source for which to conduct an overall search covering residues, metabolism and consumer risk assessment.

Relevancy criteria

The applicant set out in their literature review report a series of relevance criteria pertinent to the assessment of regulatory residues studies. The relevancy criteria were those included in the AGES report[1] on case studies using EFSA's Guidance document (EFSA Journal 2011;9(2):2092). These covered each of the respective data areas represented by the residues data requirements: storage stability of residues, metabolism (primary crops, livestock and rotational crops), magnitude of the residues studies, feeding studies, processing studies (hydrolysis and magnitude of residues studies), rotational crop field studies, estimation of potential and actual exposure through diet and other sources and residues in pollen and bee products.

Results and stepwise consideration

Overall, 18 papers (excluding duplicates) were retrieved in the searches of the databases across all areas under consideration (residues, toxicology, ecotoxicology, fate and behaviour in the environment). All of these were excluded at the step of the rapid assessment when considering the paper title where rapid assessment sought to exclude those titles of 'obviously irrelevant methods' as per EFSA Journal 2011; 9(2) 2092).

Therefore, no further (more detailed) assessment for relevance were carried out, no full text was assessed by the applicant, and no assessment of reliability of studies needed to be performed.

Conclusion

HSE concludes that regarding the literature search undertaken by the applicant, it is considered that the search is acceptable in terms of databases searched and the search criteria applied. The search did not reveal any references of relevance to this residues risk assessment.

Updated literature review (2025)

Summary of methodology employed

[1] EFSA supporting publication 2013:E*N*-511

1. A very broad search was conducted in 23 scientific source databases for inpyrfluxam and its metabolites.

2. Duplicates titles from between the data bases were automatically removed from the output.

3. A rapid assessment of the titles was conducted to remove any additional duplicates and any obviously irrelevant titles (where enough information was available from the title alone).

4. A further rapid assessment was conducted using summary abstracts and any clearly irrelevant titles were removed.

5. A detailed assessment of the full-text documents for the remaining titles was conducted using the criteria developed for study relevance

6. Any relevant papers were highlighted and assessed for reliability.

Timespan

For the updated literature review a search was performed in January 2025, with a search period of July 2013 – July 2023.

*Regulation 1107/2009 states "*Scientific peer-reviewed open literature, as determined by the Authority, on the active substance and its relevant metabolites dealing with side-effects on health, the environment and non-target species and published within the last 10 years before the date of submission of the dossier shall be added by the applicant to the dossier."

The date of submission of Inpyrfluxam was July 2023, therefore the literature review sufficiently covers the period required under Regulation 1107/2009.

Search terms

The literature review covered inpyrfluxam and the following metabolites:

- 3'-OH-S-2840
- 1'-CH_2OH-S-2840
- 1'-CH_2OH-S-2840
- *N*-des-Me-S-2840
- *N*-des-Me-1'-CH_2OH-S-2840
- *N*-des-Me-1'-CH_2OH-S-2840
- 7'-OH-inpyrfluxam
- 1'-CH_2OH-3'-OH-S-2840
- glucuronide of 1'-CH_2OH-3'-OH-S-2840

- ATMI
- 1'-COOH-S-2840
- 1'-COOH-S-2840
- 1',1'-bis(CH_2OH)-S-2840
- *N*-des-Me-1',1'-bis(CH_2OH)-S-2840
- *N*-des-Me-1'-CH_2OH-3'-OH-S-2840
- *N*-des-Me-1'-COOH-S-2840
- NDM-1'-COOH-S-2840
- DFPA
- *N*-des-Me-DFPA
- DFPA-$CONH_2$
- 1'-CH_2OH-S-2840-sulfate
- Glu-1'-CH_2OH-S-2840
- 3'-OH-S-2840-dehydrate

Methodology applied and databases used

The search strategy was conducted using bibliographic databases, with CAS and Dialog as host providers. The applicant provided the search strategies and included a justification of each of the databases used. Taken together, these covered a comprehensive source for which to conduct an overall search covering residues, metabolism and consumer risk assessment.

Relevancy criteria

The relevancy criteria for the updated literature review is the same as that shown above for the original 2018 review.

Results and stepwise consideration

Overall, 352 papers (excluding duplicates) were retrieved in the searches of the databases. Of these, 349 were excluded at the step of the rapid assessment when considering the paper title where rapid assessment sought to exclude those titles of 'obviously irrelevant methods' as per EFSA Journal 2011; 9(2) 2092).

Therefore, 3 further assessments for relevance were carried out. These related to toxicology and possible effects on health) and environmental fate. They are not noted in this residues assessment.

Conclusion

HSE concludes that regarding the literature search undertaken by the applicant, it is considered that the search is acceptable in terms of databases searched and the search criteria applied. The timespan of the literature review is acceptable when the later 'top up'

January 2025 report is taken account of alongside the original September 2018 report on the literature review. Both literature reports are suitable for considering parent inpyrfluxam and metabolites that could be of interest to the residues assessment. The search did not reveal any references for evaluation and inclusion in this residues risk assessment.

B.7.8. References Relied On

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	Previous evaluation
KCA 6.1/01	[redacted]	2018	Storage Stability of S-2399 Metabolites *N*-des-Me-S-2840, *N*-des-Me-1'-CH_2OH-S-2840-A and *N*-des-Me-1'-CH_2OH-S-2840-B in Matrices of Plant Origin Eurofins Agroscience Services Chem GmbH, Germany, Study No. S17-01158 (TES-1702L) Sumitomo Chemical Co., Ltd.	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	SUM	

			Report No: TPR-0075 GLP, Unpublished					
KCA 6.1/02	[redacted] & [redacted]	2019	Final report: Storage stability of S-2399 and its metabolites in matrices of plant origin Eurofins Study No: S16-05016 (TES-1614L) Sumitomo Chemical Co., Ltd. Report No: TPR-0093 GLP, Unpublished	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	SUM	
KCA 6.1/03	[redacted] & [redacted]	2019	Final report: Storage stability of S-2399 and its metabolites in processed food matrices of plant origin Eurofins Study No: S18-03229 (TES-1816L)	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been	SUM	

			Sumitomo Chemical Co., Ltd. Report No: TPR-0101 GLP, Unpublished			protected or submitted		
KCA 6.1/04	[redacted]	2017a	S-2399: Freezer Storage Stability of S-2399 and Metabolites in Crops Valent, USA. LLC, USA. Study No. VP-39115 Sumitomo Chemical Co., Ltd. Report No:, TPR-0067 GLP, unpublished	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	SUM	
KCA 6.1/05	[redacted]	2017b	S-2399: Freezer Storage Stability of S-2399 and metabolites in Processed Fractions Valent, USA. LLC, USA. Study NO. VP-40066	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been	SUM	

			Sumitomo Chemical Co., Ltd Report No: TPR-0065, GLP, unpublished			protected or submitted		
KCA 6.2.1/01	[redacted] & [redacted]	2016	A Metabolism Study of [^{14}C]S-2399 (2 Radiolabels) in Apple (*Malus domesticus*). Amended Final Report2. PTRL West, USA, Study No. 2507W Sumitomo Chemical Co., Ltd. Report No: TPM-0013, GLP, unpublished	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	SUM	
KCA 6.2.1/02	[redacted] & [redacted]	2016a	A Metabolism Study of [^{14}C]S-2399 (2 Radiolabels) in Soybean (Glycine max). Amended Final Report2. PTRL West, USA, Study No. 2506W	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been	SUM	

			Sumitomo Chemical Co., Ltd. Report No: TPM-0015, GLP, unpublished			protected or submitted		
KCA 6.2.1/03	[redacted] & [redacted]	2016	Residues in Maize/corn and Sorghum Crops Grown from Seeds Treated with [phenyl-^{14}C]S-2399 and [pyrazolyl-^{14}C]S-2399 Valent Technical Center, USA Study No. VP-38699 Sumitomo Chemical Co., Ltd. Report No: TPM-0017 GLP, unpublished	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	SUM	
KCA 6.2.1/04	[redacted] & [redacted]	2016b	A Metabolism Study of [^{14}C]S-2399 (2 Radiolabels) in Rice (*Oryza sativa* L.) with Foliar Treatment. Amended Final	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously	SUM	

			Report. PTRL West, USA, Study No. 2508W Sumitomo Chemical Co., Ltd. Report No: TPM-0014, GLP, unpublished			been protected or submitted		
KCA 6.2.1/05	[redacted] & [redacted]	2016c	A Metabolism Study of [^{14}C]S-2399 (2 Radiolabels) in Rice (*Oryza sativa* L.) with Granular Application. Final Amended Report 2. PTRL West, USA, Study Report 2509W Sumitomo Chemical Co., Ltd. Report No: TPM-0016, GLP, unpublished	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	SUM	
KCA 6.2.1/06	[redacted] & [redacted]	2017	Residues in Canola/oilseed rape Crops Grown from Seeds Treated with	N	Y	The study is necessary for the regulatory decision, conducted	SUM	

			[Phenyl-^{14}C]S-2399 and [Pyrazolyl-^{14}C]S-2399 Valent Technical Center, USA. VP-38998 Sumitomo Chemical Co., Ltd. Report No: TPM-0031 GLP, unpublished			according to GLP and has not previously been protected or submitted		
KCA 6.2.1/07	[redacted]	2017	Nature of Residues of [Phenyl-^{14}C]S-2399 and [Pyrazolyl-^{14}C]S-2399 in Potatoes Grown from Treated Seeds Valent Technical Center, USA. Study No. VP-38692 Sumitomo Chemical Co., Ltd. Report No: TPM-0042, GLP, unpublished	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	SUM	

KCA 6.2.2/01	[redacted]	2016	Metabolism of [^{14}C]S-2399 (2 radiolabels) in Laying Hens. Amended Final Report. [redacted] Study No. 2453W Sumitomo Chemical Co., Ltd. Report No: TPM-0025. GLP, Unpublished	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	SUM	
KCA 6.2.3/01	[redacted] & [redacted]	2016d	Metabolism of [^{14}C]S-2399 (2 radiolabels) in the lactating Goat .Amended Final Report. [redacted] Study No. 2452W Sumitomo Chemical Co., Ltd. Report No: TPM-0024. GLP, Unpublished	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	SUM	
KCA 6.3.1/01	[redacted]	2021a	Magnitude of the residue of S-2399 and its metabolites	N	Y	The study is necessary for the regulatory	SUM	

			in wheat. Raw Agricultural Commodity in Northern Europe - 2019 Testapi, France. Study No. 376-2019 Sumitomo Chemical Co., Ltd. Report No. TPR-0137 GLP, Unpublished			decision, conducted according to GLP and has not previously been protected or submitted		
KCA 6.3.2/01	[redacted]	2018a	Magnitude of the residue of S-2399 and its metabolites in wheat. Raw Agricultural Commodity in Northern Europe – 2016 Testapi, France. Study No. 256-2016 Sumitomo Chemical Co., Ltd. Report No. TPR-0076 GLP, Unpublished	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	SUM	

KCA 6.3.4/01	[redacted]	2021b	Magnitude of the residue of S-2399 and its metabolites in barley. Raw Agricultural Commodity in Northern Europe - 2019 Testapi, France. Study No. 380-2019 Sumitomo Chemical Co., Ltd. Report No. TPR-0139 GLP, Unpublished	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	SUM	
KCA 6.3.5/01	[redacted]	2018a	Magnitude of the residue of S-2399 and its metabolites in barley. Raw Agricultural Commodity in Northern Europe - 2016 Testapi, France. Study No. 258-2016 Sumitomo Chemical Co., Ltd.	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	SUM	

			Report No. TPR-0073 GLP, Unpublished					
KCA 6.4.1/01	[redacted]	2017	Magnitude of S-2399 and Metabolites 1'-CH2OH-S-2840 A&B (including conjugate) and 1'-COOH-S-2840 A&B Residues in Laying Hen Tissues and Eggs from a 28-Day Feeding Study [redacted]. Study No. 2815W. Sumitomo Chemical Co., Ltd. Report No: TPR-0015 GLP, unpublished	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	SUM	
KCA 6.4.2/01	[redacted] & [redacted]	2016	Magnitude of S-2399 and Metabolites 1'-CH2OH-S-2840 A&B (including conjugate) and 1'-	Y	Y	The study is necessary for the regulatory decision, conducted according to	SUM	

			COOH-S-2840 A&B Residues in Bovine Tissues and Milk from a 28-Day Feeding Study [redacted]. Study No. 2814W. Sumitomo Chemical Co., Ltd. Report No: TPR-0013 GLP, unpublished			GLP and has not previously been protected or submitted		
KCA 6.5.1/01	[redacted]	2016	[^{14}C]S-2399 - Nature of Residues in Processed Commodities - High Temperature Hydrolysis Smithers Viscient, USA, Study No. 13048.6947 Sumitomo Chemical Co., Ltd. Report No: TPM-0022 GLP, unpublished	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	SUM	

KCA 6.5.1/02	[redacted]	2018	[^{14}C]3'-OH-S-2840: OECD507 Nature of Residues - High Temperature Hydrolysis Smithers Viscient (ESG) Ltd., UK, Study No. 3201987 Sumitomo Chemical Co., Ltd. Report No: TPM-0054 GLP, unpublished	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	SUM	
KCA 6.5.1/03	[redacted]	2017	[^{14}C]1'-CH_2OH-S-2840: OECD507 Nature of Residues - High Temperature Hydrolysis Smithers Viscient (ESG) Ltd., UK, Study No. 3201988 Sumitomo Chemical Co., Ltd. Report No: TPM-0055 GLP, unpublished	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	SUM	
KCA 6.5.1/04	[redacted]	2020	[^{14}C]1'-COOH-S-2840: OECD507 Nature of Residues	N	Y	The study is necessary for the regulatory	SUM	

			- High Temperature Hydrolysis Smithers Viscient (ESG) Ltd., UK, Study No. 3202780 Sumitomo Chemical Co., Ltd. Report No: TPM-0067 GLP, unpublished			decision, conducted according to GLP and has not previously been protected or submitted		
KCA 6.5.3/01	[redacted]	2018d	Magnitude of the residue of S-2399 and its metabolites in wheat processed fractions in Northern and Southern Europe – 2016 Testapi, France. Study No. 261-2016 Sumitomo Chemical Co., Ltd. Report No. TPR – 0081 GLP, unpublished	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	SUM	
KCA 6.5.3/02	[redacted]	2018d	Magnitude of the residue of S-2399	N	Y	The study is necessary for the regulatory	SUM	

			and its metabolites in barley processed fractions in Northern and Southern Europe – 2016 Testapi, France. Study No. 262-2016 Sumitomo Chemical Co., Ltd. Report No. TPR-0082 GLP, unpublished			decision, conducted according to GLP and has not previously been protected or submitted		
KCA 6.5.3/03	[redacted]	2018e	Magnitude of the residue of S-2399 and its metabolites in potato processed fractions in northern and southern Europe - 2016. Testapi Study No: 265-2016 Sumitomo Chemical Co., Ltd. Report No: TPR-0084 GLP, unpublished	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	SUM	

KCA 6.6.1/01	[redacted]	2017	Confined Accumulation of [Phenyl-^{14}C]S-2399 and [pyrazolyl-4-^{14}C]S-2399 in Rotational Crops Valent Technical Center, USA. Study No. VP-38482, Sumitomo Chemical Co., Ltd. Report No: TPM-0047, GLP, unpublished	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	SUM	
KCA 6.6.2/01	[redacted]	2018e	Rotational field-crops residue study after application of S-2399 to cereal crops in northern and southern Europe – 2016 Testapi, France, Study No. 260-2016 Sumitomo Chemical Co., Ltd. Report No: TPR-0080	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	SUM	

			GLP, unpublished					
KCA 9.1			Literature Review Report	N				

Further information

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